Morphological and Pathological Characterization of Species of *Elsinoe* Causing Scab Diseases of Citrus

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This work was supported, in part, by grant CT 417 from the Australian Horticultural Research and Development Corporation.
Florida Agricultural Experiment Station journal series R-04948.
Accepted for publication 8 July 1996.

ABSTRACT

Timmer, L. W., Priest, M., Broadbent, P., and Tan, M.-K. 1996. Morphological and pathological characterization of species of *Elsinoe* causing scab diseases of citrus. Phytopathology 86:1032-1038.

Three scab diseases have been described on citrus: (i) citrus scab caused by *Elsinoe fawcettii*, which is cosmopolitan in humid citrus-growing areas; (ii) Tryon's scab caused by *Sphaceloma fawcettii* var. *scabiosa*, which occurs in Australia and elsewhere; and (iii) sweet orange scab caused by *E. australis*, which occurs primarily in southern South America. In this study, we compared isolates from Australia, Argentina, and Florida (United States). Neither colony color nor conidial size or shape could be used to distinguish the three scab fungi on citrus. A detached-leaf assay was developed to compare pathogenicity of isolates on rough lemon, sour orange, and grapefruit. All isolates from Australia produced scab only on rough lemon, whereas all isolates from Florida produced scab on rough lemon and grapefruit and a portion of these also produced scab on sour orange. *E. australis* isolates from Argentina did not affect leaves of any species. One *E. fawcettii* isolate from Argentina produced the same reaction as Australian isolates, and two produced the same reaction as the Florida group that affected rough lemon, grapefruit, and sour orange. In greenhouse inoculations, all Australian isolates infected rough lemon, lemon, and Rangpur lime; all but a few also infected Cleopatra mandarin. In greenhouse inoculations in Florida, all isolates infected rough lemon and Cleopatra mandarin and some isolates infected sour orange. Although *E. australis* is not readily differentiated from *E. fawcettii* morphologically, we concluded that they are separate species on the basis of pathogenicity and molecular analysis. *S. fawcettii* var. *scabiosa* could be differentiated from *E. fawcettii* only by host range. We recognized four pathotypes of *E. fawcettii*: the "Broad Host Range" and "Narrow Host Range" pathotypes originally described in Florida, the "Tryon's" pathotype (formerly *S. fawcettii* var. *scabiosa*), and a new "Lemon" pathotype from northern Australia.

Three scab diseases have been described on citrus (27): (i) citrus scab, formerly known as sour orange scab, caused by *Elsinoe fawcettii* Bitancourt & Jenkins, anamorph *Sphaceloma fawcettii* Jenkins (18); (ii) Tryon's scab caused by *S. fawcettii* var. *scabiosa* (McAlp. & Tryon) Jenkins, teleomorph unknown (17); and (iii) sweet orange scab caused by *E. australis* Bitancourt & Jenkins, anamorph *S. australis* Bitancourt & Jenkins (19). The teleomorphs of *E. fawcettii* and *E. australis* have been found only in Brazil (2,3). *E. australis* and *E. fawcettii* were differentiated primarily on the basis of ascospore size; ascospores of *E. australis* are 12 to 20 × 4 to 8 µm and those of *E. fawcettii* are 10 to 12 × 5 to 6 µm (2,3,18,19). *S. fawcettii* var. *scabiosa* was separated from *E. fawcettii* on the basis of larger conidia (8 to 20 × 2 to 6 µm) and flatter, less erumpent lesions on citrus fruit (12). *E. australis* and *E. fawcettii* have been differentiated on the basis of colony color (4,6,7) and length-to-width ratios of conidia (6,7). Spindle-shaped conidia are produced by *E. fawcettii* on scab lesions (10,24), but have not been reported for *S. fawcettii* var. *scabiosa* or *E. australis*.

Citrus scab affects a broad range of hosts and is widespread in humid citrus-growing areas (18). In Florida, Whiteside (24) differentiated two biotypes of *E. fawcettii* based on host range. One affects the leaves and fruit of lemon (*Citrus limon* (L.) Burm. f.), rough lemon (*C. jambhiri* Lush.), grapefruit (*C. paradisi* Macf.), sour orange (*C. aurantium* L.), Temple and Murcott tangors (*C. sinensis* (L.) Osbeck × *C. reticulata* Blanco), and the fruit of sweet orange (*C. sinensis*). The second attacks all of the above except sour orange, Temple tangor, and sweet orange fruit. Whiteside (24) did not name the two types, but we herein designate the sour orange-infecting isolates as the "Florida Broad Host Range" (FBHR) and the other as the "Florida Narrow Host Range" (FNHR) type. *E. australis* affects the fruit but not the leaves of most sweet orange and mandarin cultivars (27). *S. fawcettii* var. *scabiosa* principally affects lemon and rough lemon rootstock seedlings in Australia, but may attack many other types of citrus in wet seasons (14). Its host range has not been determined experimentally.

The distribution and host ranges of the species and biotypes of citrus scab fungi are unclear. There are various reports of scab occurrence on the many citrus species and cultivars (4,8,9,10,28, 29) in different regions of the world, but many of these are based only on field observations.

Sweet orange scab occurs primarily in Brazil, Argentina, Paraguay, and Bolivia (19), but has been recently reported in India (15). Scab symptoms are seen commonly on fruit, but not on leaves, of sweet orange and mandarin in Uruguay (L. W. Timmer, *unpublished data*). However, Diaz et al. (5) isolated only biotypes of *E. fawcettii* from those sources. Whiteside (25,27) has suggested that *E. australis* may not be a valid taxon and that fruit infection of sweet orange in South America may be attributable to the FBHR biotype of *E. fawcettii*.

Determination of the taxonomic relationships among the described species and types of citrus scab, their distributions, and host ranges is important for regulatory purposes and in the selection of cultivars for use in various citrus areas. This study was undertaken to compare the morphology and pathology of isolates of *Elsinoe* and *Sphaceloma* from citrus from Australia, Florida,

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Publication no. P-1996-0819-01R

and Argentina. Portions of this work have been previously published (1,21,22).

MATERIALS AND METHODS

The majority of this research was conducted at the Biological and Chemical Research Institute, Rydalmere, NSW, Australia. Because of regulatory concerns, greenhouse inoculations with isolates from Florida were carried out only at the Citrus Research and Education Center, University of Florida, Lake Alfred.

Survey. Commercial orchards and nurseries in the Gosford area of New South Wales were surveyed for the presence of scab on different species and cultivars of citrus to determine the field host range of scab in Australia. Noncommercial and rootstock species were examined for scab in the arboretum at the Horticultural Research Station, NSW Agriculture, Gosford, Narara section.

Isolations and cultures. Isolates were obtained from leaves or fruit of many citrus species and cultivars in Australia and Florida (Table 1). Isolations were made by scraping scab pustules with a scalpel to deposit flakes of diseased tissue onto Whiteside's selective medium (26). Noncontaminated colonies were transferred once or twice on the selective medium and then to potato-dextrose agar (PDA) slants. Additional isolates from Australia were obtained from the collection of NSW Agriculture Plant Pathology Herbarium. These isolates were collected by R. Kennedy (13). Isolates from Argentina were supplied by E. Danos (Instituto Nacional de Técnologia Agropecúaria, Concordia, Entre Rios, Argentina) or were isolated by M. E. Palm (USDA, APHIS, Beltsville, MD) from fruit shipped from Argentina to the United States. Stock cultures were maintained on PDA slants and transferred monthly.

Production of conidia. Procedures used to produce conidia were modifications of those developed previously (13,24). Small

TABLE 1. Sources of isolates of *Elsinoe* spp. from citrus used in the study

DAR no.[a]	Host	Location	Year	Isolated by[b]
Australian isolates				
70180	Rough lemon	Mulgoa, NSW	1994	Timmer
70181	Rough lemon	Somersby, NSW	1994	Timmer
70182	Rough lemon	Somersby, NSW	1994	Timmer
70183	Rough lemon	Somersby, NSW	1994	Timmer
70184	Eureka lemon	Somersby, NSW	1994	Timmer
70185	Bergamot	Somersby, NSW	1994	Timmer
70186	Eureka lemon	Somersby, NSW	1994	Timmer
70187	Eureka lemon	Somersby, NSW	1994	Timmer
70188	Lemon	Somersby, NSW	1994	Timmer
70190	Eureka lemon	Kulnura, NSW	1994	Timmer
70023	Rangpur × Severinia	Narara, NSW	1994	Timmer
70024	*Citrus indica*	Narara, NSW	1994	Timmer
70025	Bergamot	Narara, NSW	1994	Timmer
70026	*Microcitrus*	Narara, NSW	1994	Timmer
70027	Eureka lemon	Bundaberg, Qld	1994	Timmer
57541	Bergamot	Narara, NSW	1986	Kennedy
70298	Emperor mandarin[c]	Narara, NSW	1985	Kennedy
70189	Eureka lemon	Bryon Bay, NSW	1994	Timmer
57555	Rough lemon	Nambour, Qld	1986	Kennedy
70217	Lemon	Thursday Island, Qld	1994	Timmer
70292	*Citrus* sp.	Lambell's Lagoon, NT	1994	Timmer
Florida isolates				
70033	Volkamer lemon[d]	Indiantown	1990	Timmer
70254	Marsh grapefruit	Lake Alfred	<1979	Whiteside
70258	Duncan grapefruit	Bowling Green	1993	Timmer
CC-1	Marsh grapefruit	Indiantown	1990	Timmer
70034	Sour orange	Indiantown	1990	Timmer
70035	Sweet orange	Tangerine	1988	Timmer
70255	Temple tangor	Lake Alfred	<1979	Whiteside
70256	Temple tangor	Lake Placid	1991	Timmer
70257	Temple tangor	Lake Placid	1991	Timmer
Russ-15	Temple tangor	Lake Placid	1991	Timmer
Argentine isolates				
70037	Valencia sweet orange	Entre Rios	1988	Timmer
70041	Valencia sweet orange	Entre Rios	1994	Palm
70042	Clementine mandarin	Entre Rios	1994	Palm
70210	Satsuma mandarin	Unknown	1993	Danos
70211	Valencia sweet orange	Unknown	1993	Danos
70212	Satsuma mandarin	Entre Rios	1992	Danos
70216	Valencia sweet orange	Corrientes	1993	Danos
70259	Satsuma mandarin	Entre Rios	1993	Danos
70040	Rough lemon	Entre Rios	1994	Palm
70213	Satsuma mandarin	Misiones	1993	Danos
70214	Satsuma mandarin	Misiones	1993	Danos
70036	Cleopatra mandarin	Entre Rios	1994	Timmer
70038	Navel orange	Entre Rios	1994	Palm
70039	Common mandarin	Entre Rios	1994	Palm

[a] Five-digit numbers are those of the collection of the NSW Agriculture Plant Pathology Herbarium, Rydalmere, NSW, Australia. DAR = Department of Agriculture, Rydalmere. Other isolates not in collection.

[b] Kennedy = R. Kennedy, from doctoral dissertation research (13); Danos = E. Danos, INTA, Concordia, Entre Rios, Argentina; Palm = M. Palm, USDA, APHIS-PPQ, Beltsville, MD.

[c] Emperor mandarin, *C. reticulata*.

[d] Volkamer lemon, *C. volkameriana* Ten. and Pasq.

pieces of mycelium (5 to 10 mm^3) were removed from a 2- to 3-week-old culture on PDA, crushed in a plastic petri plate with a spatula, and the resulting fragments stirred into liquid modified Fries medium. Plates were incubated for 2 days at 27°C. Conidial production was best when the bottom of the plate was covered sparsely with microcolonies consisting of only a few hyphal strands each (24). Excessive amounts of mycelia used to inoculate plates or longer incubation times resulted in poor conidial production. Plates were washed three times with sterile, distilled water and flooded with autoclaved water collected from a farm pond (pH 6.0, electrical conductivity 0.12 dS/m). Plates were then placed under long-wavelength (366 mm) UV light for 1 h and incubated overnight in the dark at 20 to 25°C.

Morphological determinations. Cultures were transferred to PDA plates and grown for 3 weeks at 27°C in the dark to determine colony morphology and color. Plates were allowed to air-dry, and then the color of the outer edges and the center of the colonies were described using the chart presented by Rayner (16).

The length and width of 30 culture-produced conidia of arbitrarily selected isolates from all three locations were measured (Table 2). The mean and standard deviation were calculated for each isolate and means for each species were compared by analysis of variance. In addition, conidia from field-collected scab pustules in Australia were examined and compared with conidia produced in culture. Since spindle-shaped conidia of *E. fawcettii* are not produced in culture (27), we examined field-collected scab pustules on lemons and inoculated greenhouse-grown rough lemon seedlings placed outdoors for the presence of these conidia in Australia.

Detached-leaf assay. Because of quarantine restrictions, greenhouse or field inoculations with exotic isolates could not be conducted and, thus, a detached-leaf assay was developed to compare the pathogenicity of isolates of *Elsinoe* and *Sphaceloma* from Australia, Florida, and Argentina. Leaves for the assay were produced on rough lemon, Wilson sour orange, and Duncan grapefruit seedlings grown in a humid greenhouse (70 to 95% relative humidity [RH]) at 20 to 27°C. Young leaves, from 1/3 to 1/2 of full expansion were collected, disinfested for 2 min in 0.5% sodium hypochlorite and 2 min in 70% ethanol, and rinsed three times in sterile, distilled water. After blotting dry, leaves were placed

TABLE 2. Conidial lengths and width and colony colors of isolates of *Sphaceloma fawcettii* var. *scabiosa* from Australia, *Elsinoe fawcettii* from Florida, and *E. australis* from Argentina

Species	Isolate no.	Length (μm)[a]	Width (μm)[a]	Colony color[b]
S. fawcettii var. *scabiosa*	70184	4.82 ± 0.50	2.32 ± 0.33	Blood-dark vinaceous
	70185	4.88 ± 0.58	2.03 ± 0.12	Blood-dark vinaceous
	70187	5.13 ± 0.43	2.12 ± 0.22	Blood-dark vinaceous
	70024	5.02 ± 0.33	2.25 ± 0.31	Blood-dark vinaceous
	70025	5.22 ± 0.49	2.18 ± 0.33	Blood-dark vinaceous
	70026	5.14 ± 0.35	2.22 ± 0.34	Blood-dark vinaceous
	57541	5.13 ± 0.35	2.28 ± 0.31	ND
	70189	4.93 ± 0.86	2.32 ± 0.40	Blood-dark vinaceous
	57555	4.83 ± 0.71	2.33 ± 0.31	Blood-dark vinaceous
	70217	5.23 ± 0.52	2.33 ± 0.33	Blood-dark vinaceous
Means		5.03[c]	2.23[c]	
E. fawcettii	70033	4.91 ± 0.47	2.24 ± 0.25	Blood-pale vinaceous
	70254	5.30 ± 0.60	2.25 ± 0.31	Citrine-ochraceous
	70034	5.10 ± 0.30	2.08 ± 0.19	Blood-pale vinaceous
	70255	5.55 ± 0.78	2.47 ± 0.41	Citrine-livid vinaceous
	70256	5.12 ± 0.45	2.27 ± 0.31	Blood-blood
Means		5.20	2.26	
E. australis	70041	5.16 ± 0.53	2.20 ± 0.25	Blood-pale vinaceous
	70212	6.37 ± 0.81	2.37 ± 0.29	Blood-fuscous black
	70216	5.17 ± 0.46	2.45 ± 0.30	Blood-blood
	70036	4.87 ± 0.68	2.22 ± 0.28	Blood-dark vinaceous
	70039	5.20 ± 0.41	2.25 ± 0.30	Ochraceous-vinaceous buff
Means		5.35	2.30	

[a] Mean of 30 conidia ± the standard deviation.
[b] First color listed is the edge of the colony; color following the hyphen is that of the center of the colony; ND = not determined.
[c] Means among species were not significantly different according to analysis of variance, $P \leq 0.05$.

TABLE 3. Pathogenicity of Australian, Florida, and Argentine isolates of *Elsinoe* and *Sphaceloma* from citrus in detached-leaf assay on rough lemon, sour orange, and Duncan grapefruit

		Host[a]		
Country of origin	Isolate no. (no. of tests)	Rough lemon	Sour orange	Grapefruit
Australia	70180 (1)[b], 70183 (2), 70185 (1), 70187 (2), 70189 (1), 57541 (1), 57555 (3)	+++	–	–
	70188 (3), 70023 (1), 70024 (1), 70025 (2), 70026 (2), 70298 (1)	+++	–	±
	70292 (1)	++	–	–
	70027 (1)	++	±	–
United States (Florida)	70033 (2)	+++	±	+
	70254 (1), 70258 (1)	+++	±	++
	70034 (1), 70035 (2), 70255 (1), 70256 (1), 70257 (1), Russ-15 (1)	+++	++	++
Argentina	70210 (1), 70211 (1), 70212 (1), 70216 (1), 70037 (2), 70038 (1), 70041 (2), 70042 (1), 70259 (1)	–	–	–
	70040 (2)	+++	–	–
	70213 (2), 70214 (1)	+++	++	++

[a] Symptoms on detached leaves: +++ = rapid formation of erumpent scab pustules on all inoculated sites; ++ = scab pustules formed on all sites, less raised; + = scab pustules present, slower forming, less raised; ± = chlorotic reaction only, no scab pustules; – = no reaction. No reaction observed on any noninoculated control.
[b] Number of tests with each isolate. In each test, eight leaves of each host species were inoculated with about 8 to 10 inoculation sites per leaf.

abaxial side up on plates of 1.5% water agar. Four to five droplets of a conidial suspension of 2 to 6 × 10^5 conidia/ml were placed on each leaf half. For each test, two petri plates of four leaves each of rough lemon, sour orange, and grapefruit were inoculated. One plate (four leaves) of each host species was mock-inoculated with droplets of sterile, deionized water as a control. Plates were sealed with Parafilm to avoid drying and incubated at 27°C with 12 h per day of light at about 60 μE s^{-1} m^{-2}. From one to three tests were conducted with each isolate.

Greenhouse inoculations. Seedlings of various citrus species and cultivars (Tables 3 and 4) were grown to a height of 30 to 35 cm in a greenhouse maintained at 20 to 27°C and 70 to 95% RH. Plants were pruned to stimulate production of uniform flushes of new leaves and inoculated when leaves were less than one-half of mature size. A conidial suspension of 2 to 6 × 10^5 conidia/ml was applied to run-off using an atomizer. Plants were covered with plastic bags for 48 h for infection, and symptom severity was recorded after 7 days. For greenhouse inoculations conducted in Florida, plants were similarly inoculated and exposed to intermittent mist for 48 h after inoculation rather than bagged.

RESULTS

Field observations in Australia. Lisbon, Eureka, and Villafranca lemons in orchards and rough lemon seedlings in nurseries were severely attacked by scab at many sites examined in coastal New South Wales. In addition, bergamot (*C. bergamia* Risso & Poit.), Rangpur × Severinia (*C. limonia* Osbeck × *Severinia buxifolia* (Poir.) Tenore), *C. indica* Tan, and *Microcitrus australasica* (F. Muell.) Swingle were found to be affected in the arboretum (Table 1). Bergamot in the arboretum and in commercial nurseries was consistently affected by scab. Fruit and leaf symptoms were noted on all of the mentioned species, except *M. australasica* in which scab occurred only on fruit. No scab was noted in commercial orchards, nurseries, or the variety collection on grapefruit, sweet orange, sour orange or its hybrids, Cleopatra mandarin (*C. reshni* Hort. ex Tan), or most mandarins or mandarin hybrids, except some scab was found on Kara mandarin at one location.

Morphology of *Elsinoe* and *Sphaceloma*. Colonies of all Australian isolates of *S. fawcettii* var. *scabiosa* were blood-colored at the colony edge and dark vinaceous in the center (Table 2). Colonies of *E. fawcettii* from Florida and Argentina varied from pale ochraceous to dark vinaceous in the center and were generally lighter colored than Australian isolates. Argentine isolates of *E. australis* were generally highly pigmented with some becoming black near the center.

Conidia of all isolates produced in culture were hyaline, elliptical to obclavate, nonseptate, and measured 4 to 8 × 2 to 3 μm. There were small differences between individual isolates within and among species, but the means for the species did not differ significantly ($P \leq 0.05$) (Table 2). The length-to-width ratios did not differ among species. Conidia of all species were produced by small, hyaline, phialidic, conidiogenous cells on vegetative hyphae. Occasional, polyphialidic, conidiogenous cells were observed with all species.

On very young scab lesions on field-collected leaves and fruit in Australia, small, hyaline conidia of *S. fawcettii* var. *scabiosa* of the same size range as those produced in culture were produced in acervuli, which were erumpent on fruit or leaf lesions. The conidiogenous cells appeared to remain simple philalides and did not become polyphialidic. Elongated, brown conidiophores were observed arising from the acervuli and produced faintly tinted to pale brown, spindle-shaped conidia that were mostly 0 to 1 septate and measured 10 to 15 × 2.5 to 3.0 μm. On inoculated greenhouse plants placed outdoors for several days, hyaline conidia of the size produced in culture (5.0 × 2.3 μm) were observed in acervuli on young scab lesions. Faintly tinted to pale brown, spindle-shaped conidia identical to those produced on field-collected material were observed and, in some cases, were produced in chains.

TABLE 4. Pathogenicity of Australian and Florida isolates of *Elsinoe* spp. on various citrus species in the greenhouse

	Greenhouse inoculations						
	Host						No. of tests[b]
Isolate	Sour orange	Rough lemon	Rangpur lime	Eureka lemon	Cleopatra mandarin	Others[a]	
Australia							
70180	–[c]	++	++	+	++	–	1
70183	–	+++	+++	+	+++	–	2
70187	–	+++	+++	++	+++	–	1
70188	–	++	++	+	++	–	2
70023	–	++	++	+	+++	–	2
70025	–	+++	+	+	++	–	2
70026	–	++	+++	+	++	–	3
70027	ND	+++	++	+	+++	–	2
57541	–	++	++	+	++	–	3
70298	–	++	+++	+	++	–	1
57555	–	+++	+	+	–	–	2
70217	–	++	++	+	–	–	3
70024	–	+	+	+++	–	–	1
70189	ND	+++	++	+	–	–	1
70292	ND	+++	+++	++	–	–	1
Florida							
70033	–	+++			+		2
70254	–	+++			++		3
70258	–	+++			ND		2
70035	++	+++			ND		2
70034	++	+++			++		2
70255	++	+++			++		3
70256	++	+++			++		2
70257	++	+++			++		2
Russ-15	++	+++			+		2

[a] Others = Key lime, trifoliate orange, and Troyer citrange.

[b] In each test, at least three seedlings of each citrus species were inoculated.

[c] Reactions on greenhouse-inoculated seedlings: +++ = >100 pustules per plant; ++ = 10–100 pustules per plant; + = 5–10 pustules per plant; – = no reaction; and ND = not determined.

Detached-leaf assay. Detached leaves of susceptible species on agar showed chlorotic spotting 3 to 4 days after inoculation and developed typical scab lesions after 10 to 14 days (Fig. 1). Some chlorotic spotting occurred occasionally on resistant species also; with time, this either disappeared or darkened, but never developed into scab pustules. Most often, there were no symptoms on resistant species, and leaves were indistinguishable from control leaves that received only droplets of water. Scab reactions were more severe on rough lemon than on sour orange or grapefruit.

All Australian isolates (presumably *S. fawcettii* var. *scabiosa*) produced moderate to severe scab pustules on rough lemon, but no isolate produced symptoms on sour orange or grapefruit leaves (Table 3). Isolates from Florida reacted on detached leaves (Table 3) as expected from previous studies (24). The FBHR isolates of *E. fawcettii* produced scab on leaves of rough lemon, grapefruit, and sour orange, but the FNHR isolates produced only a chlorotic reaction on sour orange leaves. Most of the Argentine isolates from sweet orange or mandarins, presumably *E. australis*, produced no reactions on leaves of any of the three citrus species. One Argentine isolate from rough lemon (Table 1) produced scab only on rough lemon leaves, a reaction similar to that of the Australian isolates. Two isolates from Satsuma mandarin (*C. unshiu* (Mack.) Marc.) in Misiones, Argentina, gave reactions similar to the FBHR isolates, producing scab on all three citrus species tested.

Greenhouse inoculations. In inoculations of potted seedlings with Australian isolates, rough lemon, Rangpur lime (*C. limonia*), and Eureka lemon developed mild to severe infections, whereas no infection developed on Wilson sour orange, Duncan grapefruit, Key lime (*C. aurantifolia* (Christm.) Swingle), and trifoliate orange (*Poncirus trifoliata* (L.) Raf.) (Table 4). Symptoms developed on Cleopatra mandarin with all except five isolates. In tests not included in Table 3, Dancy tangerine (*C. reticulata*) developed scab symptoms when inoculated with isolates 57541, 70025, and

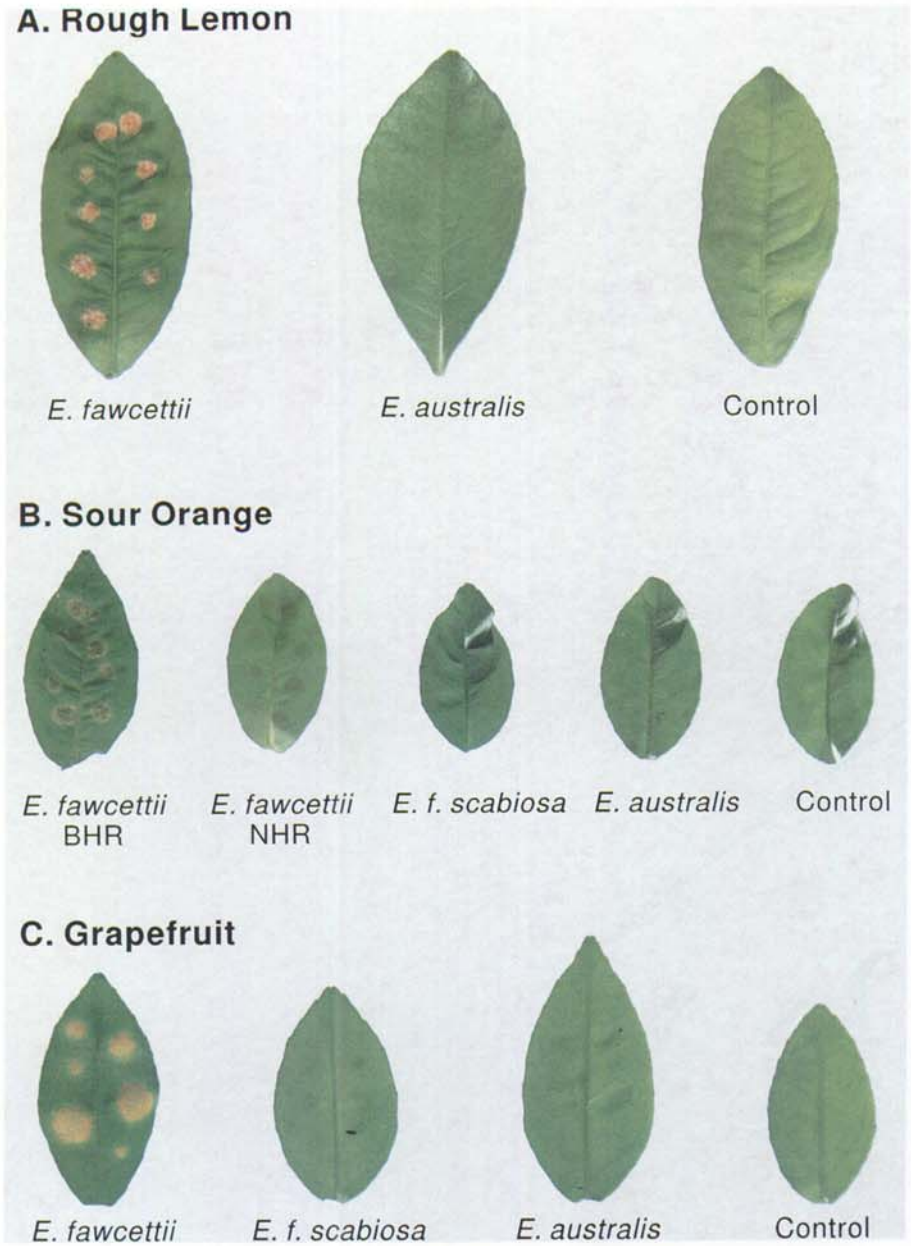

Fig. 1. Typical symptoms of isolates of *Elsinoe* and *Sphaceloma* on different hosts in a detached-leaf assay. **A,** Rough lemon: all *E. fawcettii* isolates from Argentina, Australia, and Florida gave similar reactions; no *E. australis* isolate produced symptoms on rough lemon. **B,** Grapefruit: all Florida isolates and two isolates from Argentina produced symptoms on grapefruit, but no Australian isolate nor any *E. australis* isolate from Argentina produced symptoms on grapefruit. **C,** Sour orange: only the broad host isolates from Florida produced symptoms on this host; no isolate from Australia or Argentina affected this species.

70027, but not when inoculated with isolate 70217. The above-mentioned four isolates did not produce scab when inoculated on Rough Seville (*C. aurantium* hybrid), bergamot, or Carrizo citrange (*P. trifoliata* × *C. sinensis*).

Isolates of *E. fawcettii* from Florida produced reactions in the greenhouse characteristic of the biotypes described by Whiteside (24). All isolates produced scab on rough lemon, whereas only certain isolates, the FBHR group, infected sour orange seedlings (Table 3). Isolates of both the FBHR and the FNHR groups infected Cleopatra mandarin. Pustules produced on Cleopatra mandarin by Florida isolates were atypical in that they were very small and quite raised, but lacked the chlorotic halos that surround pustules on most hosts.

DISCUSSION

Morphologically, *E. fawcettii* and *E. australis* are difficult to separate. Previous studies (6,7) indicated that hyaline conidia of *E. australis* were shorter and had a lower length:width ratio than *E. fawcettii*, but we were unable to confirm that difference. Length-to-width ratios were about 2.3:1 for both species. Hyaline conidia produced in culture or on host tissues by all isolates were similar in size and shape and compared well with those originally reported by Jenkins (10) for *E. fawcettii* and by Jenkins (11) and Bitancourt and Jenkins (4) for *E. australis*.

Hyaline conidia of *S. fawcettii* var. *scabiosa* produced in culture or on host material were no larger than those of the other two species. Jenkins (12) separated *S. fawcettii* var. *scabiosa* from *E. fawcettii* primarily on the basis of large conidia, but that does not appear to be a valid criterion.

We report here for the first time the production of spindle-shaped conidia of *S. fawcettii* var. *scabiosa* on scab lesions in Australia. The size and shape of those conidia were comparable with those produced by *E. fawcettii* in Florida (27) and Brazil (10). Spindle-shaped conidia have never been reported by investigators working with *E. australis* (3,4,6,7) and were not observed on preserved material from Brazil in recent studies (M. Priest, *unpublished data*). Apparently, the production of spindle-shaped conidia can be used to separate *E. fawcettii* from *E. australis*.

Colony color and morphology do not appear to be reliable traits for separation of species. Investigators in Brazil (4,6,7,10) noted great variability in colony color of *E. fawcettii* isolates in culture that ranged from buff to vinaceous. Isolates of *E. australis* were usually darker with some nearly black. We found that *E. fawcettii* cultures from Florida and Argentina were quite variable, whereas those of *S. fawcettii* var. *scabiosa* were consistently highly pigmented. While cultures of *E. fawcettii* may be less pigmented than *E. australis*, dark isolates of *E. fawcettii* exist and color can vary with the cultural medium (10) and colony age. Colony color is not very useful for identification purposes.

Examination of the type material of *E. fawcettii* and *E. australis* indicated that the ascocarp morphology and ascospore size were consistent with those reported by Jenkins (9) and Bitancourt and Jenkins (3) (M. Priest, *unpublished data*). However, only one specimen of the teleomorph of *E. fawcettii* exists, and the description is consistent with immature ascocarps of *E. australis*. The type material of *E. fawcettii* is on Satsuma mandarin, which is susceptible to both species. Thus, the only teleomorph of *E. fawcettii* that exists may, in reality, be an immature *E. australis*, and *E. fawcettii* may not produce a teleomorph.

Pathologically, *E. australis* is readily separable from *E. fawcettii* by its apparent inability to infect leaves. Some pathotypes of *E. fawcettii* are able to infect fruit of sweet orange and *M. australasica* without infecting leaves. However, all pathotypes of *E. fawcettii* can be differentiated from *E. australis* by their ability to infect rough lemon leaves.

Restriction analyses of the internal transcribed spacer (ITS) of ribosomal DNA indicated that *E. australis* and *E. fawcettii* belong to separate groups (20). Polymerase chain reaction amplification of segments of the ITS region and endonuclease cleavage produced different profiles for the two species (20). Nucleotide sequences of the ITS region for all isolates of *E. fawcettii* from Australia, Argentina, and Florida were virtually identical and different from *E. australis*. Thus, despite the difficulty in separation of the species morphologically, we believe that *E. australis* is a valid species. Infection of sweet orange fruit in Argentina is probably due largely to infection by *E. australis* and not to infection by the FBHR pathotype of *E. fawcettii* identified in Florida as suggested by Whiteside (25,27).

Jenkins (12) originally separated *S. fawcettii* var. *scabiosa* from other *E. fawcettii* isolates primarily on the basis of conidial size. We were unable to confirm that difference and, thus, Tryon's scab differs from other citrus scab only in its host range. DNA analysis also indicated a close relationship between these two scab fungi. Thus, we prefer to refer to the different strains of *E. fawcettii* as pathotypes. We have confirmed the presence of two pathotypes in Florida as described by Whiteside (24) and have designated them as FBHR and FNHR pathotypes. The host range of Australian isolates differs considerably from Florida isolates. The most common pathotype in Australia is provisionally designated as the "Tryon's" pathotype. The second one will be referred to as the "Lemon" pathotype, since it infects primarily lemon and close relatives. The proposed pathotypes and differential hosts for identification of them are listed in Table 5. The information on leaf infection in the table was based on work done in this study, but the ability to infect fruit was based on previous research (4,23,24). As more isolates are studied and a wider range of hosts inoculated, additional pathotypes will likely be discovered.

The distribution of pathotypes of *E. fawcettii* in other citrus areas is virtually unknown, despite many reports of the occurrence of citrus scab on different citrus species and cultivars (4,8,10,28,29). As is evident from this study, pathotypes cannot be determined accurately based solely on field observations. Cleopatra mandarin was reported to be immune to citrus scab (29), and scab has not been observed in the field in Florida or Australia. Yet, when inoculated, three of the four pathotypes readily infect this species. Conidial production on scab pustules may be insufficient to sustain infections on some species. Thus, the presence of scab may indicate susceptibility to prevalent pathotypes in an area, but absence does not necessarily indicate resistance. Likewise, results of experimental inoculations must be interpreted with caution. Bergamot in the field in Australia was severely affected by scab, yet this species was not infected in greenhouse inoculations. Subsequently, we learned that the selection of bergamot

TABLE 5. Differential host range for determination of pathotypes of *Elsinoe fawcettii* and differentiation of *E. australis*

Species	Pathotype	Rough lemon	Sour orange	Cleo mandarin	Grapefruit	Sweet orange fruit[a]
E. fawcettii	FBHR[b]	+	+	+	+	+
E. fawcettii	FNHR[c]	+	–	+	+	–
E. fawcettii	Tryon's[d]	+	–	+	–	–
E. fawcettii	Lemon[e]	+	–	–	–	–
E. australis[f]	–	–	–	–	–	+

[a] Fruit susceptibility information from previous studies (4,24).
[b] FBHR = Florida broad host range isolates 70034, 70035, 70255, 70256, 70257, 70257, and Russ-15 from Florida and 70213 and 70214 from Argentina.
[c] FNHR = Florida narrow host range isolates 70254, 70258, and 70033 from Florida.
[d] Tryon's isolates 70180, 70183, 70187, 70188, 70023, 70023, 70025, 70026, 70027, 57541, and 70298 from Australia.
[e] Lemon isolates 57555, 70217, 70024, 70189, and 70292 from Australia and 70040 from Argentina.
[f] *E. australis* isolates 70210, 70211, 70212, 70216, 70037, 70038, 70041, 70042, and 70259 from Argentina.

used for greenhouse inoculations was a recent introduction from Italy and not the same source as that found in the field in Australia.

Kennedy (13) incubated cultures of *S. fawcettii* var. *scabiosa* under long-wavelength UV light overnight and obtained good conidial production. We found exposure of *E. fawcettii* and *S. fawcettii* var. *scabiosa* to long-wavelength UV was beneficial, but increasing exposure time did not seem to increase conidial production. Consistent production of conidia of *E. australis* was more problematic; abundant conidia were produced often, but at times very few or none were obtained. Fantin (6) was successful in using an extract of cornmeal as an incubation medium for the production of conidia of *E. australis*. In our experience, this was also successful at times, but at other times failed.

The detached-leaf assay functioned well for species and pathotype comparisons in this study and should be useful for preliminary evaluation of pathotypes in other citrus areas. Highly susceptible species such as rough lemon, sour orange, and grapefruit are readily infected under these conditions. It may not be possible, however, to determine the entire host range of a pathotype with the detached-leaf assay. Species that are less susceptible to Florida pathotypes, such as trifoliate orange and Key lime, failed to produce definitive results in the detached-leaf assay. In the greenhouse, very young tissue and high conidial concentrations are needed to produce lesions on these species. Leaves of some citrus species do not survive for sufficient time on agar to produce conclusive results. Further, if field-collected leaves are used in the assay, they may be heavily contaminated with other fungi and may deteriorate rapidly even after surface-disinfestation.

There is limited information on the distribution of the pathotypes we have defined. Australia has at least two of the pathotypes of *E. fawcettii* that were defined in this study. In field observations, we found no scab on grapefruit, sweet orange, sour orange, and mandarins or their hybrids, except for Kara mandarin; but scab has been reported on many of these species (14) and it is possible that other pathotypes exist in Australia. Isolates of the "Lemon" pathotype were mostly from northern Australia. Argentina has both *E. australis* and *E. fawcettii* (Table 5). In a very limited number of isolates, the FBHR pathotype and one Australian pathotype, probably the "Lemon" one (20), were identified in Argentina. Even so, infection of sour orange and grapefruit in Argentina is not commonly observed at least in Entre Rios (L. W. Timmer, *unpublished data*). The scab observed on sour orange occurred only on fruit, suggesting it may have been due to *E. australis* rather than a pathotype of *E. fawcettii*. Both of the Florida pathotypes have been identified in Uruguay (5).

Without complete knowledge of all of the pathotypes of *E. fawcettii*, and their distribution, development of rational regulations on the movement on fruit and plant materials is difficult. Certainly, efforts to avoid further distribution of *E. australis* are warranted. If airborne ascospores are produced commonly on fruit by this species, then introduction of infected fruit may constitute a significant risk. Countries such as Australia and Argentina where pathotypes of *E. fawcettii* attacking grapefruit and other important commercial cultivars apparently are currently rare or absent should make an effort to avoid introduction of those pathotypes. Additional pathotypes may exist in other citrus-producing countries that have not been widely disseminated because they occur only in dooryard plantings, citrus collections, or isolated commercial plantings. If such pathotypes are introduced into citrus nurseries, they could quickly become widely distributed. In addition to controls on international movement of infected fruit and propagating material, more control on movement of budwood and nursery stock between areas within countries may also be needed.

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