Morphological and Pathological Characteristics of Strains of *Colletotrichum gloeosporioides* from Citrus

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ABSTRACT

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Three strains of *Colletotrichum gloeosporioides* from citrus in Florida are described and characterized as fast-growing gray isolates from necrotic and senescent tissue (FGG), slow-growing orange isolates (SGO) associated with citrus postbloom fruit drop disease, and slow-growing isolates with deep orange pigmentation from Key limes affected by lime anthracnose (KLA) (formerly classified as *Gloeosporium limetticola*). All FGG isolates had large conidia most with both apices rounded, produced abundant setae, and had large lobulate appressoria. SGO and KLA isolates had smaller conidia, most with one fusiform apex, and rarely produced setae. The SGO isolates produced clavate, deeply pigmented appressoria, whereas the KLA isolates produced round, smaller, less pigmented appressoria. All strains had optimum temperatures for growth of 23–27 C, but FGG isolates grew better at 31 C than SGO or KLA isolates. The FGG isolates grew two to three times as rapidly as SGO isolates, and SGO isolates grew slightly faster than KLA isolates at most temperatures. The SGO strain, previously associated with postbloom fruit drop, reproduced all symptoms of the disease on sweet orange and Persian lime and was re-isolated from blighted blossoms. The KLA strain, previously associated exclusively with lime anthracnose, also reproduced all symptoms of postbloom fruit drop. The KLA strain produced typical lime anthracnose symptoms on Key lime, but SGO isolates produced only a mild mottle of Key lime leaves. The FGG isolates were not pathogenic to flowers of sweet orange or Persian lime or to foliage of Key lime. The postbloom fruit drop disease that first appeared in Florida in 1983 may have originated from Key lime affected by lime anthracnose rather than from a foreign introduction.

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. in Penz. is common on many angiosperm hosts and is ubiquitous on citrus species and relatives (20,22). The most widespread form of the fungus occurs on dead and senescent tissues on which it produces abundant acervuli. Conidia of *C. gloeosporioides* germinate on the surface of leaves and fruit and form appressoria and remain as quiescent infections (22). When tissues die or are weakened by any agent, they may be rapidly colonized by *C. gloeosporioides*, but the fungus is not usually considered a pathogen of citrus (20). However, when fruit with many quiescent infections is subjected to ethylene degreening or other stress, anthracnose symptoms develop on the fruit surface resulting in serious postharvest losses, especially on tangerines (2,3).

Lime anthracnose and withertip is a serious and widespread disease of Key lime, *Citrus aurantifolia* (L.) Swingle. The causal agent of this disease was described originally as *Gloeosporium limetticola* R. E. Clausen in 1912 (5). In most of the citrus literature, this pathogen is referred to as *G. limetticola* (21), but von Arx (19) and Sutton (18) considered this species synonymous with *C. gloeosporioides*. The fungus attacks all tender tissues of Key lime and produces necrotic spots on leaves and fruit or, if severe, blast of the entire new flush or blooms. This pathogen has been reported to affect only *C. aurantifolia* (5,10,21).

In 1979, Fagan (7) described a disease in Belize, called postbloom fruit drop that was associated with a slow-growing, orange form of *C. gloeosporioides*. This fungus infects citrus blossoms and causes orange- to peach-colored lesions on petals, fruit drop, and the formation of persistent buttons consisting of the peduncle, floral disk, calyx, and nectaries. Buttons persist for the life of the twig and are characteristic of the disease. In subsequent years, this disease has been found in most humid tropical citrus areas in Central and South America, the Caribbean (6), and was discovered in Florida in 1983 (14).

The various forms of *C. gloeosporioides* from citrus have not been well-characterized morphologically or pathologically. Burger (4) described five groups of isolates from citrus primarily on the basis of colony characteristics. Fagan (8) described three strains (*cgm*, *cgc*, and *cgp*) from citrus in Belize. The *cgm* and *cgc* strains were nonpathogenic to citrus flowers and differed from each other primarily in that *cgm* had slightly larger conidia (8). The *cgp* strain was pathogenic to citrus flowers. Fagan (8) suggested that this strain was introduced into Belize with citrus from Florida, but the postbloom fruit drop disease was unknown in Florida before the 1983 report (14). More recently, Sonoda and Pelosi (17) described two types of *C. gloeosporioides* from citrus in Florida, a fast-growing gray form and a slow-growing orange form that was associated with postbloom fruit drop. The fast-growing form was more sensitive to benomyl than was the slow-growing orange type.

The purposes of this study were to define and characterize the forms of *C. gloeosporioides* from citrus in Florida and determine their role in associated diseases. A preliminary report of this work has been published (1).

MATERIALS AND METHODS

Isolates. Cultures of *C. gloeosporioides* were obtained from citrus trees in various locations in Florida. Isolates were tentatively grouped as rapidly growing cultures with extensive gray-pigmented mycelium (FGG); slow-growing cultures with abundant production of orange conidial masses (SGO) similar to those associated with postbloom fruit drop by Fagan (7) and Sonoda and Pelosi (17); and slow-growing colonies with deep orange pigmentation (KLA). All FGG isolates were from necrotic or senescent tissue; two were from orchards not affected by postbloom fruit drop; one was from affected blossoms in an orchard with postbloom fruit drop; and one was from blossoms on a potted nursery tree affected by postbloom fruit drop. All SGO isolates were from petals with typical postbloom fruit drop lesions. All KLA cultures were isolated from Key limes with typical symptoms of anthracnose and withertip. The sources and designations of all isolates are given in Table 1.

Single-spore isolates were derived from all of the cultures. Conidial suspensions of each isolate were prepared and stored on silica gel (15). Cultures were retrieved as needed for studies of morphology or pathology. Cultures stored on silica gel maintained the cultural characteristics of the original isolates for at least 1 yr.

Conidial characteristics. Conidial suspensions were prepared in distilled water from 7- to 10-day-old cultures of all isolates grown on potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI) at 27 C. The perimeter length and the area of at least 100 conidia per isolate were determined microscopically with an image analysis system (Micro-comp Data Acquisition System, Southern Micro Instruments, Inc., Atlanta, GA).

Conidia were divided into two types: round, with both apices of the conidium rounded; or fusiform, with one apex rounded and the other fusiform. The proportion of the two conidial types was determined by observing 100 conidia per isolate.

Setae production. Cultures of all isolates were grown under continuous light on PDA and examined for the presence of setae after 10 days. Autoclaved leaves and stems of Key lime and sweet orange (*C. sinensis* (L.) Osbeck) in petri dishes were inoculated with each of the isolates and examined for the presence of setae after 15 days.

Appressorial characteristics. We produced appressoria of each isolate by spraying two sweet orange seedlings with a suspension of about 5×10^5 conidia per milliliter prepared from 7- to 10-day-old cultures grown on PDA. Seedlings were incubated in a dew chamber with continuous dew for 48 h at 23 C in the dark. Inoculated leaves were dried, and the cuticle was removed by the technique of Fitzell et al (9). Briefly, a 50:50 mixture (v/v) of clear nail varnish and acetone was applied to the upper leaf surface and allowed to dry. The cuticle was peeled away with forceps and mounted on slides in 0.01% cotton blue in lactophenol. The shapes of at least 25 appressoria were noted for each isolate, and the lengths and widths were measured with an eyepiece micrometer.

The ascigerous stage. Various methods were used in an attempt to observe or produce the ascigerous stage of *C. gloeosporioides*. Green and dead leaves, as well as twigs and buttons, collected primarily in citrus orchards affected by postbloom fruit drop were examined extensively for the presence of perithecia typical of *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk. Cultures were grown on PDA, on PDA acidified with 7% tartaric acid, and on 2.5% water agar at room temperature under continuous light for several weeks and examined for sexual structures. Green leaves and twigs of Key lime were placed on filter paper in glass petri dishes and steam-sterilized. Leaves and twigs were inoculated by spraying them with a suspension of 5×10^5 conidia per milliliter and were incubated under continuous fluorescent light on the laboratory bench or placed in the dark in a dew chamber at 23 C and 100% relative humidity for 7 days. They were then allowed to dry for 15 days in the dark at 23 C at ambient humidity.

Temperature effects. Four-millimeter-diameter plugs from cultures of all isolates growing on PDA were transferred to PDA and incubated at 10, 16, 18, 23, 27, 31, and 34 C. Three replicate plates were used for each isolate. Colony diameters were measured daily for 7 days, and growth rates for temperatures and isolates were compared by the slope of the line as determined by linear regression. Average colony areas after 7 days were calculated. The experiment was conducted twice, and the means of the two experiments are presented.

Pathogenicity of detached blossoms. Closed blossoms were collected from potted, disease-free sweet orange trees in the screenhouse and placed in a petri dish with moist filter paper. In some experiments, blossoms were surface-disinfested with 0.525% sodium hypochlorite for 45 s and rinsed with sterile distilled water before inoculation. Two droplets of a conidial suspension of about 2×10^5 conidia per milliliter were placed on each blossom, and the plates were incubated for 3 days at room temperature. Three replicate plates of five blossoms each were used for each isolate in most experiments.

Pathogenicity of attached blossoms. Disease-free 3- to 5-yr-old budded Valencia sweet orange trees and rooted cuttings and budded trees of Persian lime (*C. latifolia* Tanaka) were used to test the pathogenicity of different isolates in a screenhouse. Because of irregular bloom on individual trees, all isolates could not be compared simultaneously. Consequently, many separate tests were conducted, often with only one or two trees per isolate. One control tree sprayed with distilled water and one tree inoculated with an FGG isolate were included in all experiments.

Conidial suspensions were prepared from 7- to 10-day-old cultures on PDA and adjusted to 5×10^5 spores per milliliter. Trees were sprayed to runoff with a hand pump sprayer and then either covered with plastic bags for 48 h or placed under a mist system to maintain surface moisture on the plants. We then moved plants to the screenhouse or greenhouse to allow disease development. Temperatures ranged from 18 to 32 C during the experiments. The percentage of the open flowers showing typical orange-pink lesions on the petals was recorded 7 days after inoculation. Three and one-half months after inoculation,

TABLE 1. Sources and designations of isolates of *Colletotrichum gloeosporioides* used in this study

Strain and location	Designation	Host	Tissue
FGG isolates[x]			
Lake Alfred, Florida	LA-1	Sweet orange	Senescent blossoms
Lake Alfred	LA-2	Sweet orange	Dead twigs
Lake Hamilton, Florida	LH	Sweet orange	Necrotic leaves
Arcadia, Florida	Ar-FGG	Sweet orange	Senescent blossoms
SGO isolates[y]			
Indiantown, Florida	In-1	Sweet orange	Blossoms
Indiantown	In-2	Sweet orange	Blossoms
La Belle, Florida	LaB	Sweet orange	Blossoms
Frostproof, Florida	Fr	Sweet orange	Blossoms
Lake Placid, Florida	LP	Sweet orange	Blossoms
Arcadia	Ar-SGO	Sweet orange	Blossoms
Homestead, Florida	Hm	Persian lime	Blossoms
Immokalee, Florida	Im	Persian lime	Blossoms
KLA isolates[z]			
Sarasota, Floridaa	Ss	Key lime	Leaves
Homestead	Hm-1	Key lime	Leaves
Homestead	Hm-2	Key lime	Leaves
Homestead	Hm-3	Key lime	Blossoms
Ft. Pierce, Florida	FtP	Key lime	Leaves

[x] Fast-growing, gray isolates; all isolates from senescent or necrotic tissues.
[y] Slow-growing, orange isolates; all isolates from blossoms showing typical postbloom fruit drop symptoms.
[z] Slow-growing, orange isolates with deep orange pigmentation; all isolates from Key lime trees showing typical symptoms of lime anthracnose.

after normal fruit drop was complete, the number of persistent buttons and the number of fruit set on each tree were counted.

Key lime. We tested the ability of the various isolates to cause lime anthracnose on potted 6-mo-old Key lime seedlings with new growth flush by spraying with conidial suspensions at 5×10^5 spores per milliliter. Plants were maintained in the greenhouse and covered with plastic bags for 48 h after inoculation. Three days after inoculation, anthracnose severity on the new flush was rated on the following scale: 0 = no disease; 1 = isolated chlorotic spots 1–3 mm in diameter; 2 = numerous small necrotic spots; 3 = large confluent necrotic areas on the leaves; and 4 = defoliation and necrosis of the shoot tip.

RESULTS

Fungal morphology. The basic growth pattern and colony type for all isolates remained constant when grown out on PDA after storage on silica gel. Within the described groups, there was some variability in the number of sporodochia formed on culture media and the amount of gray pigmentation of the mycelium. With repeated subculture on PDA, production of conidia decreased or ceased, and the gray pigmentation of the hyphae increased. SGO and KLA isolates sometimes formed nonsporulating sectors with white mycelia. Although conidial production and pigmentation changed after repeated subculture, growth rates remained consistent with the original isolates.

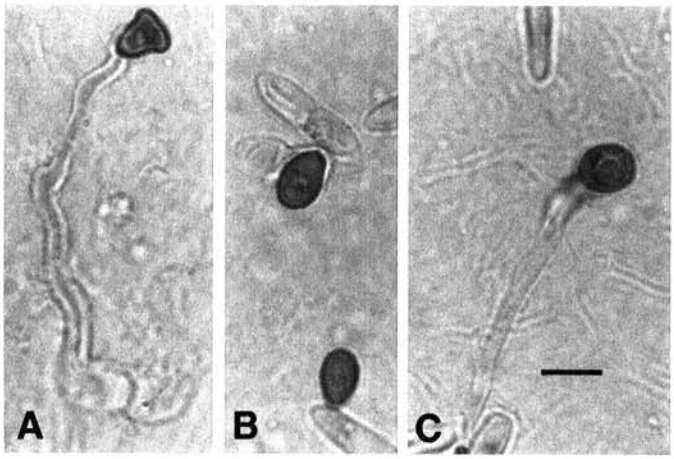

Fig. 1. Typical appressoria of the **A,** fast-growing gray (FGG); **B,** slow-growing orange (SGO); and **C,** Key lime anthracnose (KLA) strains of *Colletotrichum gloeosporioides*. Bar equals 5 μm.

FGG isolates had consistently larger conidia with greater areas and perimeter lengths than SGO and KLA isolates (Table 2). Only minor differences in conidial size were noted between SGO and KLA isolates. Most of the conidia produced by FGG isolates had rounded apices with less than 10% having one fusiform apex. In contrast, most of the conidia produced by SGO and KLA isolates had one fusiform apex.

Most FGG isolates produced setae readily and abundantly on culture media and on host tissues (Table 2). A few, sparse setae were observed on host tissue with In-2 (SGO) and Hm-1 (KLA). Most setae observed had a single septum. No conidial production from setae was observed.

All isolates readily produced appressoria from germinating conidia on sweet orange leaves. Appressoria of FGG isolates were lobulate and were larger than those of the other isolates (Table 2; Fig. 1A). Germ tubes also were longer with FGG isolates. The SGO isolates produced smaller, mostly clavate, appressoria (Fig. 1B). Occasional lobulate appressoria were observed on older leaves. The appressoria of the KLA isolates were slightly but significantly smaller than those of SGO isolates, were round rather than clavate, and were less pigmented (Fig. 1C).

Perithecia were never observed with any isolate on culture media or in any of the collections of tissue from the field. In one experiment, a few fertile perithecia of isolates LP (SGO), Ss (KLA), and LA-1 (FGG) were produced on steam-sterilized Key lime leaves. Perithecia of all three isolates were typical of *G. cingulata*. Perithecia were black, beaked, and erumpent with sparse paraphyses. Ascospores were hyaline, slightly curved with rounded apices; those of the LP and LA-1 isolates were 15.5 ± 0.5

TABLE 2. Area, perimeter, and shape of conidia; length, width, and shape of appressoria; and setae production on potato-dextrose agar (PDA) and host tissue of different isolates of *Colletotrichum gloeosporioides*

Strain and isolate	Conidia			Appressoria			Setae[x]	
	Area (μm^2)	Perimeter (μm)	Shape[v] (R/F)	Length (μm)	Width (μm)	Shape[w]	PDA	Host tissue
FGG								
LA-1	0.62 ± 0.15[y]	3.3 ± 0.4	5:1	8.7 ± 1.0	5.9 ± 0.8	Lob.	−	+
LA-2	0.61 ± 0.08	3.5 ± 0.4	15:1	8.4 ± 0.2	6.9 ± 0.3	Lob.	+	+
LH	0.62 ± 0.08	3.5 ± 0.3	11:1	8.6 ± 1.3	5.6 ± 0.6	Lob.	+	+
Ar-FGG	0.59 ± 0.05	3.4 ± 0.2	20:1	7.6 ± 0.9	5.7 ± 0.7	Lob.	+	+
Average	0.61 a[z]	3.40 a	13:1 a	8.30 a	6.0 a			
SGO								
Hm	0.52 ± 0.08	3.3 ± 0.2	0.5:1	6.4 ± 0.4	4.7 ± 0.3	Clav.	−	−
In-1	0.47 ± 0.05	3.3 ± 0.2	1.0:1	5.7 ± 0.3	4.5 ± 0.1	Clav.	−	−
In-2	0.51 ± 0.21	3.3 ± 0.3	0.3:1	5.5 ± 0.4	4.5 ± 0.1	Clav.	−	±
Ar-SGO	0.49 ± 0.05	3.3 ± 0.2	0.5:1	5.7 ± 0.4	4.5 ± 0.3	Clav.	−	−
Im	0.49 ± 0.06	3.3 ± 0.4	0.5:1	6.5 ± 0.4	5.0 ± 0.2	Clav.	−	−
Fr	0.50 ± 0.07	3.2 ± 0.2	1.0:1	5.9 ± 0.4	4.4 ± 0.1	Clav.	−	−
LaB	0.48 ± 0.08	3.1 ± 0.3	0.3:1	6.1 ± 0.3	4.5 ± 0.1	Clav.	−	ND
LP	0.48 ± 0.20	3.1 ± 0.2	0.3:1	6.6 ± 0.3	5.2 ± 0.3	Clav.	−	−
Average	0.49 b	3.21 c	0.5:1 b	6.07 b	4.7 b			
KLA								
Ss	0.48 ± 0.06	3.2 ± 0.2	0.4:1	4.8 ± 0.3	4.1 ± 0.2	Round	−	−
Hm-1	0.55 ± 0.06	3.3 ± 0.2	0.3:1	5.2 ± 0.3	4.3 ± 0.1	Round	−	±
Hm-2	0.49 ± 0.06	3.2 ± 0.2	0.5:1	5.2 ± 0.4	4.2 ± 0.2	Round	−	−
Mm-3	0.50 ± 0.15	3.2 ± 0.6	0.5:1	5.6 ± 0.3	4.5 ± 0.4	Round	−	−
FtP	0.47 ± 0.05	3.2 ± 0.2	0.5:1	4.8 ± 0.2	4.1 ± 0.2	Round	−	−
Average	0.48 b	3.24 b	0.4:1 b	5.10 b	4.24 b			

[v] Ratio of conidia with both apices round (R) to conidia with one apex round and the other fusiform (F).
[w] Appressorial shapes: Lob. = lobulate; Clav. = clavate.
[x] Setae production on PDA or on sterile leaves or twigs of sweet orange and Key lime; + = present; − = absent; ± = scarce; ND = not determined.
[y] Standard deviation.
[z] Mean separation within columns between strains by Duncan's multiple range test ($P < 0.001$).

μm long by 4.5 $\pm$ 0.9 μm wide, and those of the Ss isolate were 15.7 $\pm$ 0.8 μm by 5.4 $\pm$ 1.1 μm.

Temperature. The FGG isolates, on the average, grew two to three times as fast as SGO and KLA isolates (Fig. 2; Table 3). Growth of the SGO isolates was slightly faster than that of KLA isolates up to 27 C but was slower than KLA isolates at 31 C. The response of individual isolates within groups to temperature was nearly identical (Fig. 2). Only Hm-2 differed slightly in its growth from other KLA isolates.

Maximum growth of all three strains was observed at 23–27 C (Fig. 2; Table 3). Growth of SGO and KLA isolates decreased substantially at 31 C, but growth of FGG decreased only slightly at that temperature.

Pathogenicity. All isolates produced necrotic spots on detached blossoms after 48 h. Necrotic lesions developed slightly less rapidly with FGG than with SGO isolates. However, necrosis eventually developed even on control blossoms, because the petals became senescent after 4–5 days.

When attached blossoms of sweet orange and Persian lime were inoculated, all SGO isolates produced peach- to orange-colored necrotic lesions on petals, typical of postbloom fruit drop on all plants. The percentage of the bloom affected ranged from 27 to 96%, depending on the isolate and host species used (Table 4). All KLA isolates produced necrotic lesions on blossoms of sweet orange and Persian lime typical of postbloom fruit drop. In all of the above inoculations, re-isolations from diseased flowers yielded cultures identical to those of the culture used for inoculations. In most cases, inoculation with FGG isolates produced no symptoms on blossoms. Occasionally, symptoms typical of postbloom fruit drop occurred on plants inoculated with FGG isolates or on control plants. However, SGO-type isolates were frequently isolated from these blossoms, and infections were probably due to contamination either during spray inoculation or during misting after inoculation.

All plants with blossom lesions typical of postbloom fruit drop also developed the characteristic persistent buttons and set relatively few fruit (Table 4). Plants inoculated with the SGO and KLA isolates had large numbers of persistent buttons. The ratio of the number of fruit to fruit + buttons was near unity on control trees and those inoculated with FGG isolates, but the ratio was 0.5 or less on most trees inoculated with the SGO and KLA isolates (Table 4).

All Key lime seedlings inoculated with KLA isolates produced typical lime anthracnose symptoms with varying degrees of severity (Table 5). Plants inoculated with SGO isolates did not produce typical lime anthracnose, but caused circular, chlorotic spots 2–3 mm in diameter. Only occasional necrosis was observed with these isolates. Cultures re-isolated from affected tissue were typical of those used for inoculation. Only a single plant inoculated with the FGG isolates showed any necrosis, and this may have been expansion of an existing wound. No control plants developed symptoms.

DISCUSSION

Despite the cultural variability in isolates of *C. gloeosporioides* from citrus, the three types described herein are clearly separate on the basis of morphology, growth rate, and colony characteristics. The FGG isolates differed from SGO and KLA isolates in conidium size and shape, appressorium size and shape, setae production, growth rate, and colony morphology. The SGO and KLA isolates were much more difficult to separate. There were small but significant differences in conidial size and growth, but these differences would be inadequate to identify cultures to strain. The KLA isolates produced small, round, lightly pigmented appressoria that were clearly distinguishable from the larger, clavate, highly pigmented appressoria of SGO strains.

Sonoda and Pelosi (17) originally separated Florida isolates of *C. gloeosporioides* as SGO and FGG on the basis of colony morphology and associated the SGO type with postbloom fruit drop. We found that FGG isolates are more sensitive to benomyl than are SGO isolates (L. W. Timmer, *unpublished*), confirming results of Sonoda and Pelosi (17) and Liyanage et al (13). Our SGO isolates are probably equivalent to the slow-growing orange form originally associated with postbloom fruit drop in Belize (7) and later designated *cgp* (8). The *cgp* form of Fagan (8) had small conidia and did not produce setae, but appressoria of this form were not described. The *cgm* form of Fagan (8) would probably be equivalent to our FGG strain, because *cgm* was not pathogenic to flowers and produced setae. Fagan (8) reported four-septate setae, whereas setae in all of our cultures had a single septum. The *cgc* form of Fagan (8) is similar to our FGG in not being pathogenic to flowers, but it did not produce setae. The conidia of our SGO strain resembled those of *C. gloeosporioides* var. *minor* from tropical fruits (16) and *C. gloeosporioides* type B from *Stylosanthes* spp. (12).

Liyanage et al (13) found that the type 1 strain (which cor-

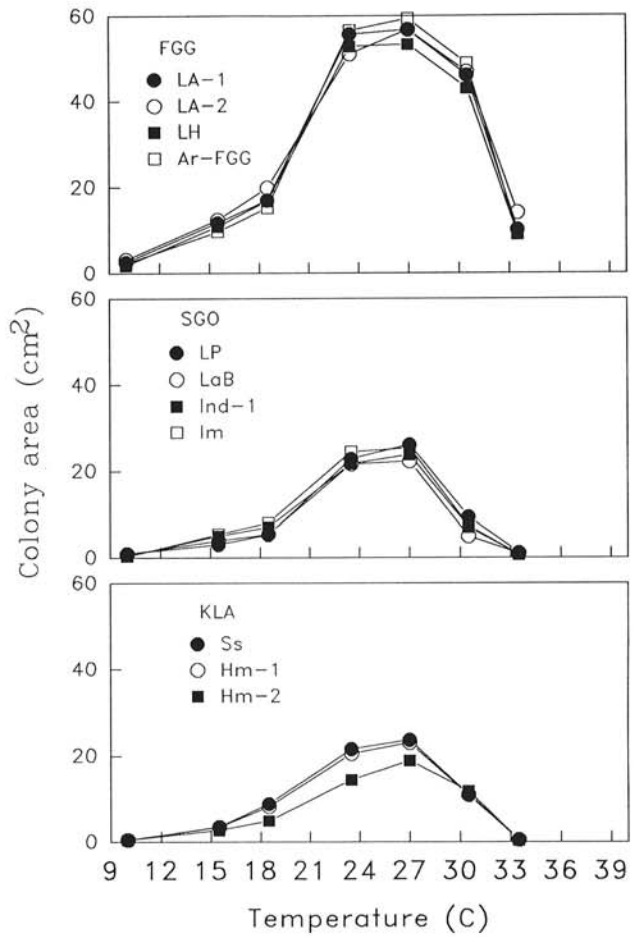

Fig. 2. Average colony area of 7-day-old cultures of strains of *Colletotrichum gloeosporioides* grown in the dark on potato-dextrose agar.

TABLE 3. Slope of regression line of colony area over time at various temperatures for strains of *Colletotrichum gloeosporioides* on potato-dextrose agar

Strain[y]	Number of isolates	Slope of regression (temperature [C])					
		15	18	23	27	31	34
FGG	4	0.12 a[z]	0.14 a	0.25 a	0.25 a	0.23 a	0.08 a
SGO	8	0.05 b	0.07 b	0.14 b	0.15 b	0.07 c	0.02 b
KLA	5	0.04 c	0.06 b	0.12 c	0.14 c	0.10 b	0.01 c

[y]FGG = fast-growing, gray isolates from senescent or necrotic tissues; SGO = slow-growing, orange isolates from blossoms showing typical postbloom fruit drop symptoms; KLA = slow-growing, orange isolates with deep orange pigmentation from Key lime trees showing typical symptoms of lime anthracnose.

[z]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$.

TABLE 4. Pathogenicity of strains of *Colletotrichum gloeosporioides* to flowers on potted Valencia sweet orange and Persian lime flowers

Host, strain, and isolate[w]	Number of experiments (total trees)	Flowers diseased[x] (% ± SE[y])	Number of buttons per tree ± SE	Number of fruit set per tree ± SE	Fruit set per fruit set + number of buttons [z]
Valencia orange					
SGO					
LP	3 (4)	94.4 ± 3.9[z]	55.5 ± 8.5	1.7 ± 0.8	0.04
In-1	3 (4)	69.7 ± 0.2	24.5 ± 4.9	6.5 ± 2.1	0.22
LaB	2 (3)	71.9 ± 13.1	14.6 ± 8.0	0.7 ± 0.3	0.08
Im	2 (3)	40.2 ± 5.2	28.6 ± 4.7	5.3 ± 3.3	0.12
Ar	2 (2)	89.9 ± 3.3	26.0 ± 0.1	8.0 ± 7.0	0.19
Hm	2 (2)	71.2 ± 7.5	37.0 ± 9.0	10.5 ± 6.5	0.19
KLA					
Ss	2 (3)	45.5 ± 4.5	23.3 ± 7.4	8.3 ± 4.3	0.24
Hm-1	1 (2)	54.2 ± 2.1	39.0 ± 1.0	7.0 ± 1.2	0.14
Hm-2	1 (2)	33.8 ± 0.8	53.0 ± 14.2	21.5 ± 3.5	0.14
Hm-3	1 (2)	25.7 ± 2.1	17.5 ± 1.5	41.5 ± 4.5	0.61
FtP	2 (3)	58.5 ± 14.9	49.0 ± 13.1	49.3 ± 12.9	0.51
FGG	4 (6)	7.3 ± 4.7	4.2 ± 1.9	23.3 ± 10.2	0.91
Control	4 (4)	2.3 ± 2.3	1.5 ± 2.5	20.0 ± 11.4	0.93
Persian lime					
SGO					
LP	2 (2)	26.6 ± 6.6	6.0 ± 0.2	3.5 ± 2.5	0.30
In-1	1 (2)	50.3 ± 1.6	10.0 ± 4.1	5.0 ± 2.0	0.40
Hm	1 (2)	96.0 ± 5.5	28.3 ± 3.4	4.0 ± 1.8	0.10
KLA					
Ss	1 (2)	73.2 ± 19.7	17.0 ± 5.0	6.5 ± 5.5	0.30
FGG	2 (4)	4.7 ± 4.7	0.7 ± 0.2	5.7 ± 0.6	0.90
Control	2 (3)	0.0 ± 0.6	0.5 ± 0.3	10.0 ± 1.1	0.90

[w]FGG = fast-growing, gray isolates from senescent or necrotic tissues; SGO = slow-growing, orange isolates from blossoms showing typical postbloom fruit drop symptoms; KLA = slow-growing, orange isolates with deep orange pigmentation from Key lime trees showing typical symptoms of lime anthracnose.
[x]Seven days after inoculation.
[y]Standard error.
[z]Three and one-half months after inoculation.

TABLE 5. Pathogenicity of isolates of *Colletotrichum gloeosporioides* to Key lime seedlings

Isolates[y]	Number of diseased per number of inoculated	Severity rating[z]
Control	0:6	0.00 d
FGG (4)	1:16	0.06 d
SGO		
In-1	8:8	0.80 c
In-2	6:6	1.30 c
LP	9:10	1.05 c
Hm	2:2	0.82 c
Im	3:4	0.33 d
Ar	1:2	0.30 d
LaB	2:2	0.52 d
KLA		
Ss	5:5	3.44 a
Hm-1	2:2	3.33 a
Hm-2	5:5	2.26 b
Hm-3	5:5	3.56 a
Ftp	2:2	2.15 b

[y]FGG = fast-growing, gray isolates from senescent or necrotic tissues; SGO = slow-growing, orange isolates from blossoms showing typical postbloom fruit drop symptoms; KLA = slow-growing, orange isolates with deep orange pigmentation from Key lime trees showing typical symptoms of lime anthracnose.
[z]Rated on a scale of 0 = no disease, 1 = isolated chlorotic spots 1–3 mm in diameter, 2 = numerous small necrotic spots on the new flush, 3 = large confluent necrotic areas on leaves, and 4 = defoliation and necrosis of the shoot tip.

responds to our SGO strain) contained a single homogenous form of ribosomal DNA, whereas the type 2 strain (which corresponds to our FGG strain) had ribosomal DNA repeats distinct from type 1. Type 1 strains had five large chromosomal DNAs and one or two smaller chromosomal DNAs, whereas type 2 strains had three large chromosomal DNAs and two to four smaller DNAs. H. D. Liyanage, W. K Köller, R. T. McMillan, Jr., and H. C. Kistler (*personal communication*) also found that type 1 and type 2 strains produced two cutinolytic esterases, but type 1 esterases had molecular weights of 20 and 26 kDa, and type 2 esterases had molecular weights of 22 and 24 kDa. Thus, genetic as well as morphological evidence indicates great diversity within this species.

In this study, strains of *C. gloeosporioides* could not be distinguished by pathogenicity to detached flowers, which confirmed the conclusions of Sonoda and Pelosi (17). Because *C. gloeosporioides* readily colonizes senescent tissue, detached petals apparently have no resistance to infection by any of the strains. With postbloom fruit drop, flowers become increasingly susceptible as they enlarge, and blooms less than 1 cm long are affected only when inoculum pressure is high (7; J. P. Agostini and L. W. Timmer, *unpublished*).

This study demonstrates conclusively that the SGO strain of *C. gloeosporioides* is the causal agent of postbloom fruit drop and reproduces all the symptoms of the disease observed in the field. In previous work, this strain had been consistently associated with the disease (7,17), but because inoculations were carried out in the field (7), some doubt remained. The few symptoms observed after inoculations with FGG isolates were probably due to cross contamination, and it is doubtful that the FGG isolates play a role in postbloom fruit drop.

Surprisingly, all KLA isolates also reproduced all the symptoms of postbloom fruit drop on sweet orange and Persian lime. There are no previous reports of any association of postbloom fruit drop and lime anthracnose. The lime anthracnose pathogen has previously been considered highly host-specific and has not been known to cause other diseases (5,10,21). Persistent buttons characteristic of postbloom fruit drop were observed on many of the Key lime trees near Homestead, Florida, where KLA isolates were collected (J. P. Agostini and L. W. Timmer, *unpublished observations*). However, KLA and SGO isolates are distinguishable by colony color of recently isolated cultures and appressorium size and shape. Also, SGO isolates do not cause anthracnose on Key lime. The small, chlorotic spots on Key lime leaves induced by inoculation with SGO isolates appear to be more

characteristic of a resistant reaction than of a disease condition.

Postbloom fruit drop may have been introduced to Florida and the Caribbean islands from Belize or elsewhere. However, it seems conceivable that SGO isolates that cause postbloom fruit drop could be variants of KLA isolates that have lost their virulence to Key lime. Key lime is widely cultivated in the American tropics, primarily as a backyard tree or in small commercial plantings. Lime anthracnose is endemic in the area and a limiting factor in commercial production. With increased commercial production of oranges and other citrus fruits in the humid tropics, the lime anthracnose pathogen may have spread to oranges from backyard lime trees initiating epidemics of postbloom fruit drop in oranges. Our recent recovery of an isolate that produces typical lime anthracnose on Key lime from a Navel orange orchard near LaBelle, which was affected by postbloom fruit drop (L. W. Timmer, *unpublished*), supports this theory. This isolate had the deep orange pigmentation typical of KLA isolates, but the closest Key lime was more than 10 km away. Comparisons of nucleic acid polymorphisms (13) and isozyme patterns (11) of SGO and KLA strains may provide further information on the origins and relationships of these organisms.

The species, *C. gloeosporioides*, is very broadly defined and encompasses a wide range of isolates with diverse characteristics, which have been recovered from many host plants (18,19). The three strains described herein most closely fit this species description, and none corresponds closely with any other described species. On the one occasion when the ascigerous stages were found for the three strains, all appeared to correspond well to *G. cingulata*, the perfect state of *C. gloeosporioides*. However, the KLA and SGO strains form a group of closely related isolates that differ in morphology, pathogenicity, nucleic acid polymorphisms (13), and in isozyme patterns (11) from the more widespread FGG strain. It may be desirable to describe these strains as a new species of *Colletotrichum*, but one must make comparisons to a range of isolates from other hosts to establish that they truly represent a separate taxon.

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