

# Identification and Occurrence of Perfect Stage and Cultural and Morphological Variants of *Colletotrichum gloeosporioides* from Guava in Puerto Rico<sup>1</sup>

Lii-Jang Liu<sup>2</sup>

## INTRODUCTION

Guava (*Psidium guajava* L.) is a common fruit in Puerto Rico and other parts of tropical America. The demand for this fruit is increasing locally. Growers now are therefore much interested in establishing commercial guava plantings. Almost all varieties and clones of guava available in Puerto Rico for this purpose are unfortunately susceptible to some degree to a fruit rot presumably caused by *Glomerella cingulata* (Ston.) Spaulding and Schrenk. The actual causal agent of the so-called *Glomerella* disease, however, has not been established. Studies were undertaken recently to isolate and identify the causal agent or agents from affected fruits. Perithecia were formed when different isolates of *Colletotrichum* from guava were placed in culture media. The sexual stage of this fungus had not been obtained previously from guava in vitro in Puerto Rico. The following description thus constitutes the first of its isolation. Variations in conidia morphology, as well as the cultural appearance and physiology of the isolates, also are included.

## REVIEW OF LITERATURE

Sheldon (4) in 1906 was the first to describe a fungus isolated from mummified fruits of guava. He gave it the name *Gleosporium psidii*. Toro (5) in 1922 reported that *Gleosporium musarum* Cooke and Masee, the causal agent of banana anthracnose, also affected a number of tropical fruits including guava. He realized however that the conidia of *Gleosporium musarum* did not correspond to those of *Gleosporium psidii* from guava. He also reported the presence of several biological forms of conidia of various sizes. Nolla (2) in 1926 reported *Colletotrichum gloeosporioides* Penz as the cause of anthracnose disease of mango, orange, grapefruit, lemon, avocado, and lime. Schrenk and Spaulding (3) in 1903 suggested the name *Glomerella* as a synonym for *Gleosporium*. *Glomerella psidii* (Del) Sheldon was listed

<sup>1</sup> Manuscript submitted to Editorial Board August 12, 1971.

<sup>2</sup> Phytopathologist, Agricultural Experiment Station, Mayagüez Campus, University of Puerto Rico, Río Piedras, P.R. Thanks are due to Mrs. Julia Rodriguez for assistance in temperature studies, Mrs. Amelia Cortes-Monllor in pH studies, and Mr. Arcángel Rodríguez in spore size measurement.

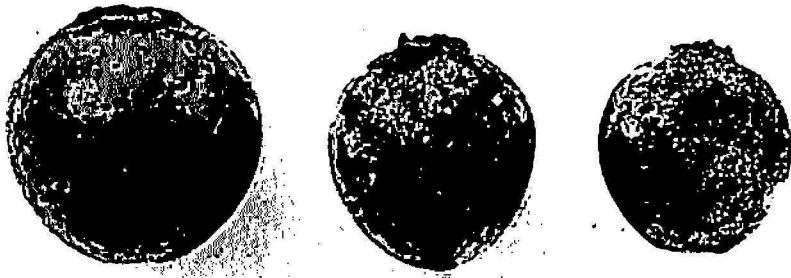


FIG. 1.—Whitish mycelial mass and black discoloration on guava fruits caused by *Colletotrichum gloeosporioides*: From left to right, healthy fruit and diseased fruits.

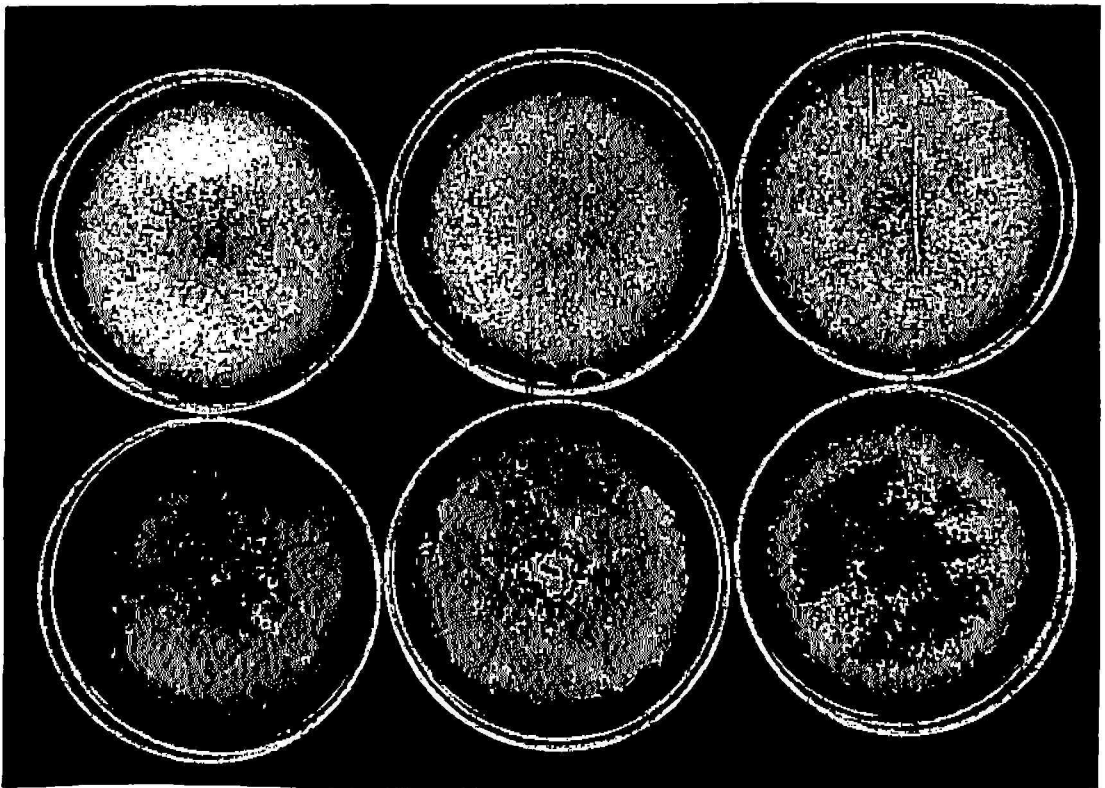


FIG. 2.—Cultural appearance of six isolates of *Colletotrichum gloeosporioides* on potato dextrose agar: From top down, left to right, 1st row, G 1, G 3, and G 5 (light strains); 2nd row, G 7, G 9 (light strains), and G 10 (dark strain).

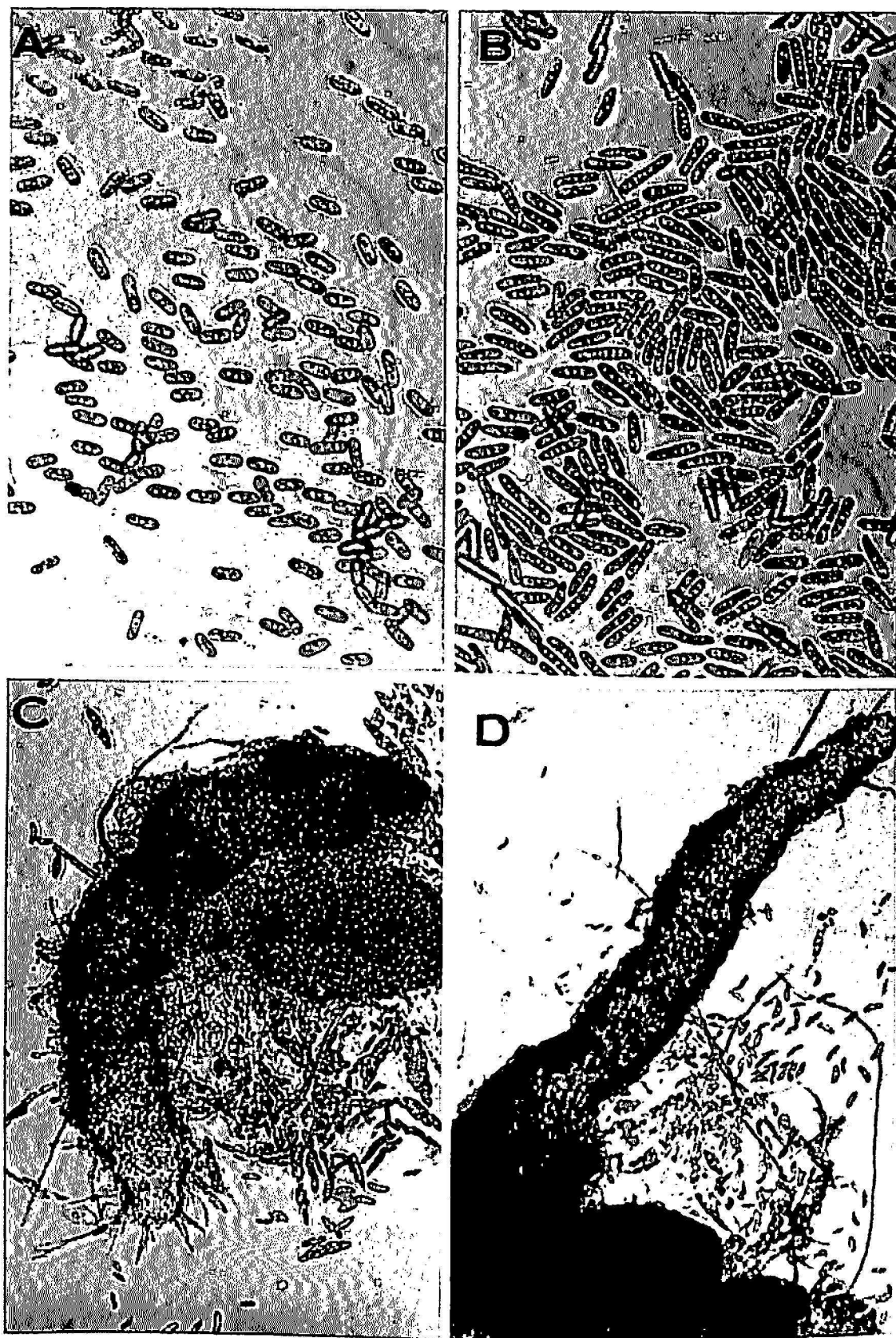


FIG. 3.—Conidia and perithecia of *Colletotrichum gloeosporioides* grown on artificial medium (potato dextrose agar): A, conidia (light strain), B, conidia (dark strain) C and D, perithecia with elongated neck.

(1) in 1960 as a synonym for *G. cingulata* (Ston.) Spaulding and Schrenk and *Colletotrichum gloeosporioides* Penz as a conidial stage of *Glomerella cingulata*. No information is available concerning the identification and occurrence of the perfect stage *Colletotrichum gloeosporioides* on Puerto Rican guava.

## METHODS AND RESULTS

### DESCRIPTION OF SYMPTOMS

In the early stage of infection, affected guava fruits show small dark brown circular spots under the skin. When the spots are one-half inch or more across, whitish mycelium appears on their surface. As the fungus pene-

TABLE 1.—Frequency of distribution and size of conidia of isolates of *Colletotrichum gloeosporioides* from guava

Dark strain (G 10)				Light strain (G 9)			
Length	Conidia	Width	Conidia	Length	Conidia	Width	Conidia
<i>Microns</i>	<i>Number</i>	<i>Microns</i>	<i>Number</i>	<i>Microns</i>	<i>Number</i>	<i>Microns</i>	<i>Number</i>
14.50-16.40	0	4.50-6.40	452	14.50-16.40	0	4.50-6.40	39
16.50-18.40	0	6.50-8.40	48	16.50-18.40	396	6.50-8.40	388
18.50-20.40	98	8.50-10.40	0	18.50-20.40	104	8.50-10.40	23
20.50-22.40	96			20.50-22.40	0		
22.50-24.40	176			22.50-24.40	0		
24.50-26.40	29			24.50-26.40	0		
26.50-28.40	39			26.50-28.40	0		
28.50-30.40	22			28.50-30.40	0		
30.50-32.40	12			30.50-32.40	0		
32.50-34.40	20			32.50-34.40	0		
34.50-36.40	2			34.50-36.40	0		
36.50-38.40	6			36.50-38.40			

trates more and more into the guava tissue, the invaded area becomes enlarged, until finally the entire fruit is involved. At this stage, the entire fruit has turned dark in color and papery in texture. Pink pustules, which are masses of conidia, appear on the surface of the affected fruits (fig. 1).

### CULTURAL AND MORPHOLOGICAL VARIANTS

Six isolates of *Colletotrichum* were obtained from affected guava fruits. Monoconidial cultures of these isolates were prepared in potato dextrose agar (PDA). Of these isolates, one dark and five light strains appeared consistently when cultured on PDA (fig. 2). The conidia produced by the dark strain were also consistently longer than those produced by the light strains (fig. 3, A, and B). The conidia of the dark strain were mostly oblong, some slightly curved, 18.50 to 38.40  $\mu$  long by 4.50 to 8.40  $\mu$  wide. Those

of the light strains were mostly cylindrical, 16.50 to 20.40  $\mu$  long by 4.50 to 10.40  $\mu$  wide (table 1).

Both dark and light strains isolated from guava were grown in the following five different agar cultural media: malt, potato dextrose, nutrient, corn meal, and bean pod. As shown in table 2, the conidia varied in size significantly (at 1-percent level) in the different media. The largest conidia were produced in malt agar, the smallest in nutrient agar. However, data obtained from Duncan Multiple Range Test (table 3) show that conidial

TABLE 2.—*Effect of cultural media on size of conidia of Colletotrichum gloeosporioides from guava*

	Size of conidia			
	Length		Width	
	Dark strain	Light strain	Dark strain	Light strain
	<i>Microns</i>	<i>Microns</i>	<i>Microns</i>	<i>Microns</i>
Malt agar	21.42 <sup>1</sup>	17.30	6.72	8.22
Potato dextrose agar	21.75	16.35	5.85	6.60
Nutrient agar	14.95	16.60	6.52	7.80
Corn meal agar	18.82	18.80	6.37	7.57
Bean pod agar	16.80	16.62	6.17	6.92

<sup>1</sup> Average of 450 conidia.

TABLE 3.—*Statistical comparison of size of conidia of Colletotrichum gloeosporioides from guava*

Origin	Spore size	
	Length	Width
	<i>F</i>	<i>F</i>
Fungi	35.6264** <sup>1</sup>	111.9276** <sup>1</sup>
Cultural media	28.6046**	18.3103**
Fungi X media	25.1411**	2.0793

<sup>1</sup> \*\* Significant at 1-percent level.

spore size differed significantly between the dark and the light strains when cultured in different media. Although there are interactions between the length of the conidia and the cultural media (significant at 1-percent level), this same relationship was not established when the width of the conidia was used for interaction studies.

IN VITRO FORMATION OF PERFECT STAGE

Fertile perithecia were formed in 4 to 7 days at 20° to 24° C. when mono-conidial cultures of the isolates (all strains) were grown together on PDA supplemented with a 1-percent extract of guava fruit. Perithecia were also

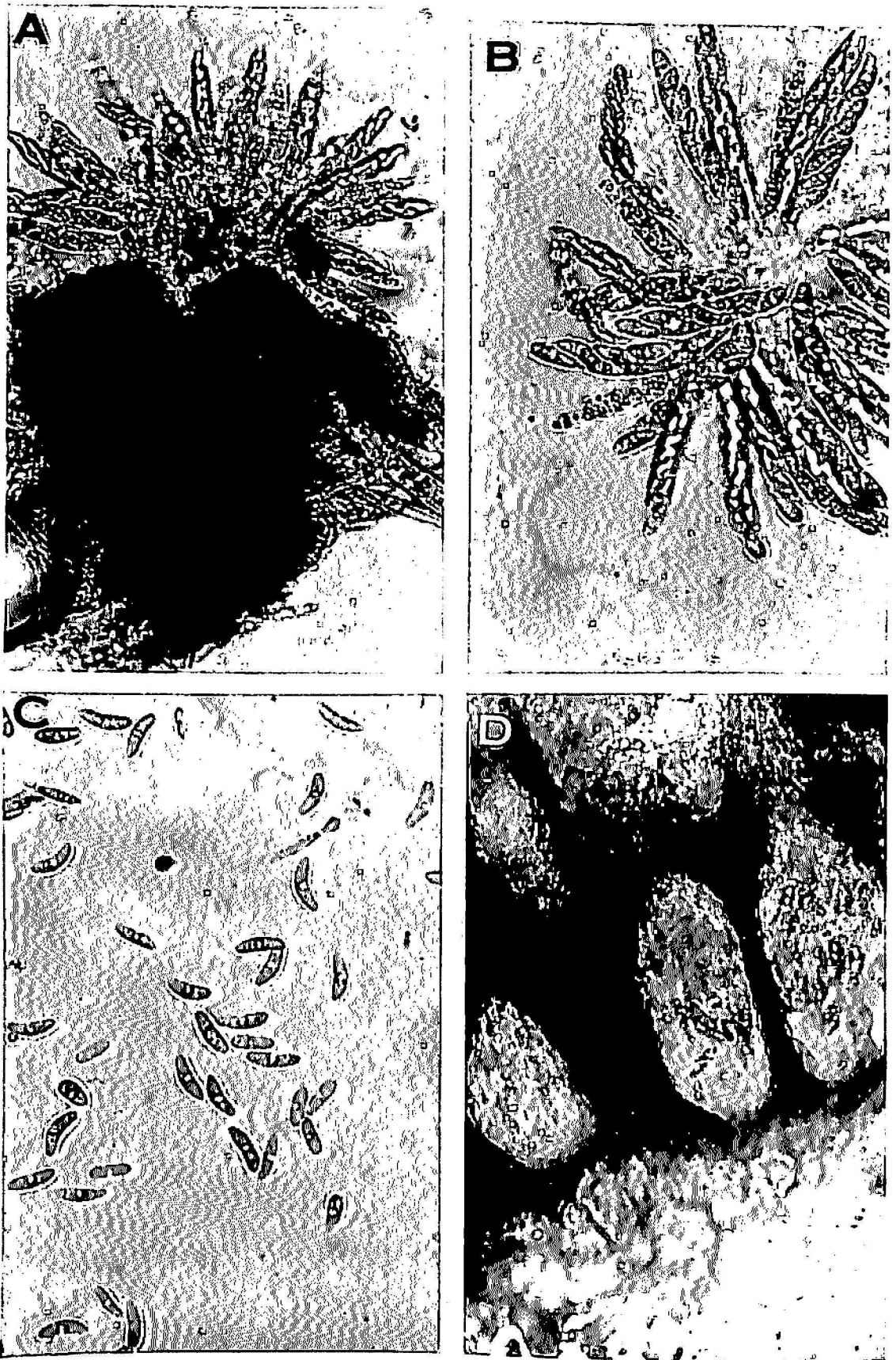


FIG. 4. —Perfect stage of *Colletotrichum gloeosporioides* grown on infected guava fruits: A, Perithecium, B, asci, C, ascospores, and D, perithecia immersed in infected tissues of guava fruits.

obtained when the same isolates were grown alone. Monoascosporic cultures of the isolates were prepared in PDA. Ascospore isolations consistently resulted in the recovery of typical *C. gloeosporioides* culture. Perithecia were immersed and more or less compounded subspherical in a 7- to 10-day old culture. The neck of the perithecia became elongated in an older artificial culture (fig. 3, C, and D). Perithecia obtained from both the dark and the light strains of *Colletotrichum* grown on guava fruits were 430  $\mu$  to 630  $\mu$  long by 157  $\mu$  to 210  $\mu$  wide. Asci were subclavate, often slightly pedicellate, 41  $\mu$  to 61  $\mu$  long by 12  $\mu$  to 12.50  $\mu$  wide. Ascospores were allantoid, hyaline, 16.4  $\mu$  to 21.3  $\mu$  long by 6.1  $\mu$  to 8.2  $\mu$  wide (fig. 4).

#### PHYSIOLOGIC CHARACTERISTICS

##### *I. Effect of Temperature on Mycelial Growth*

Two monoconidial isolates of *Colletotrichum* spp. (G 9 and 10) from guava were grown in PDA at 12°, 16°, 20°, 24°, 28°, 32°, 36°, and 40° C. For each temperature, four petri dishes containing 15 ml. of the above-mentioned medium were inoculated with 2 mm. culture discs. The discs were cut with a sterile cork borer from the advancing margin of colonies kept in potato dextrose agar. The dishes containing the inoculum were incubated at the different temperatures for 7 days. The increase in the diameter of the colonies was measured at the end of the incubation period.

The results show that the optimum temperature range for mycelial growth of one of the isolates of *Colletotrichum* (G 9) from guava lies between 28° and 32° C. on the medium used. The optimum temperature range for the other isolate (G 10) from guava lies between 24° and 28° C.

##### *II. Effect of pH on Mycelial Growth*

Two monoconidial isolates of *Colletotrichum* spp. (G 9 and 10) were grown on potato dextrose agar with the following pH: 3, 5, 5.6, 7, 9, and 11. For each pH, five petri dishes containing 15 ml. of the above-mentioned medium were inoculated with 2 mm. culture discs of the *Colletotrichum* spp., cut with a sterile cork borer from the advancing margin of the colonies kept in the same medium. The dishes containing the inoculum were incubated at 28° C. for 4 days. The increase in the diameter of the colonies was measured at the end of the incubation period.

The results indicated that the optimum pH for mycelial growth of *Colletotrichum* spp. (G 10) lies between 5 and 7 (fig. 5). Similar results were obtained with isolate G 9.

#### PATHOGENICITY TESTS

Detached guava fruits of six varieties, i.e., P.R. 2 (Trujillo 2), P.R. 3 (Cibuco 3), P.R. 5 (D-18), P.R. 6 (D-20), P.R. 4 (D-13), and P.R. 7

(57-8-141) were first surface-sterilized with 95-percent alcohol for 5 minutes, then sprayed with a spore suspension of the isolates of *Colletotrichum* obtained from guava. Non-inoculated fruits were used as controls. The inoculated fruits were left in polyethylene bags at room temperature (approximately 20° to 22° C.). Two days after inoculation, small black spots appeared on the surface of the inoculated fruits of varieties P.R. 2, P.R. 3, P.R. 5, and P.R. 7. The spots enlarged until the entire fruit was covered. Pink masses appeared on the affected areas 4 days after inoculation. When inoculated fruits were left in a round polyethylene incubation chamber,

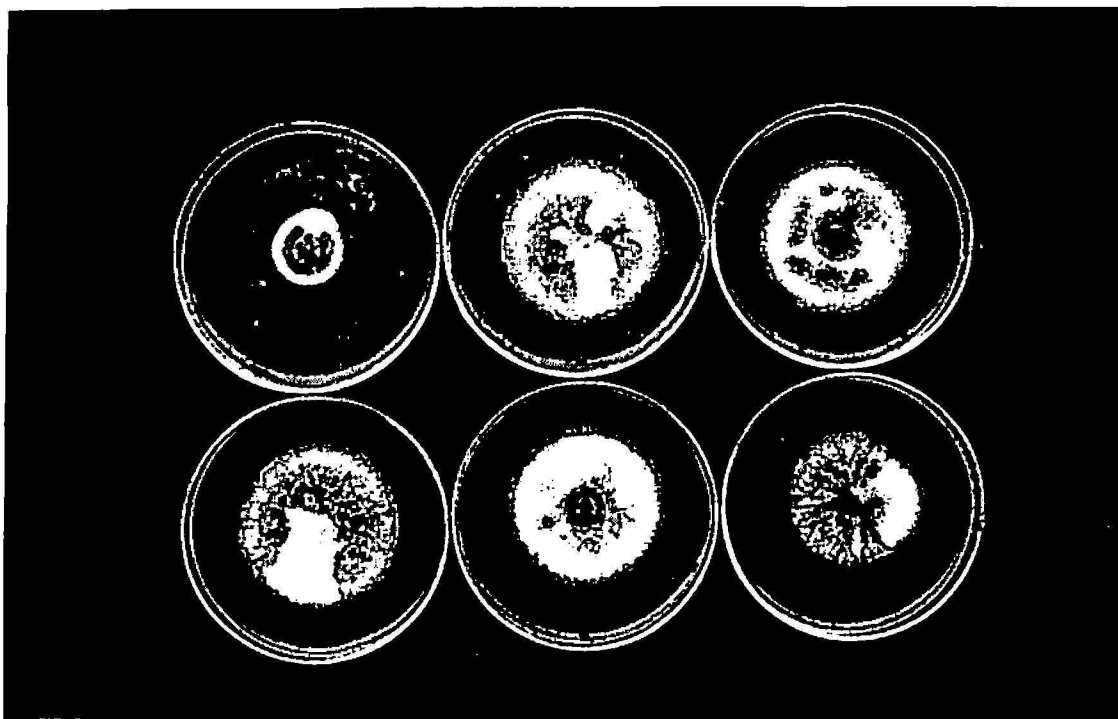


FIG. 5.—Growth of *Colletotrichum gloeosporioides* (G 10) on potato dextrose agar at various pH: From left to right, 1st row pH 3, 5.0, 5.6; 2nd row pH 7, 9, and 11, respectively.

black, immersed perithecia appeared around the pink masses 7 to 10 days after inoculation. The non-inoculated fruits remained healthy. The infection was rated by scoring each fruit from 1 to 3. A score of 1 indicated freedom from black spots and the score advanced with an increase in the severity of the infection.

Table 4 indicates degree of resistance.

#### DISCUSSION AND CONCLUSION

The results of these investigations agree in general with those of Toro (5) in that among the isolates of *Colletotrichum* spp. there were biological forms in which conidia varied in size. Despite the fact that the size of the



conidia varies with the cultural media, the media is of importance in the separation of biologic forms on a spore-size basis. At least two morphologically and culturally distinct strains (one dark and one light) of *Colletotrichum* spp. have been isolated from affected fruits of Puerto Rican guava.

Perithecia were formed when these isolates were grown together or alone in potato dextrose agar. The perithecia, asci, and ascospores produced by both strains were typical of those of *Glomerella cingulata* as described by Schrenk and Spaulding (3). Beaked perithecia were not found in young cultures or in affected fruits. However, beaked perithecia were found in older cultures grown on potato dextrose agar (an artificial medium).

Conidia produced by the dark strain were significantly longer than those produced by the light strain but their perithecia were indistinguishable.

TABLE 4. *Pathogenicity of Colletotrichum gloeosporioides on guava fruits*

Variety	Fruits infected	Infection
	Percent	Degree
P.R. 2 (Trujillo 2)	71.1	3 <sup>1</sup>
P.R. 3 (Cibuco 3)	14.2	1
P.R. 5 (D-18)	14.2	1
P.R. 6 (D-20)	0	0
P.R. 4 (D-13)	0	0
P.R. 7 (57-8-141)	28.5	2

<sup>1</sup> Infection types: 0, no symptoms; 1, resistant (many small lesions); 2, moderately resistant (moderate amount of overlapping lesions); and 3, susceptible (many overlapping lesions).

Both strains thus have been identified as *Colletotrichum gloeosporioides* or *Glomerella cingulata*.

Perithecia of *C. gloeosporioides* have not been reported previously on guava in Puerto Rico.

Guava varieties P.R. 6 and P.R. 4 exhibit a degree of resistance to *G. gloeosporioides*. Breeding for resistant varieties thus furnishes a new approach to the possible control of this disease.

#### SUMMARY

Two strains of *Colletotrichum* spp., one dark and one light, were isolated from diseased fruits of guava in Puerto Rico. The isolates differ in cultural appearance, physiologic characteristics, and size of conidia. The optimum temperature range for mycelial growth of the dark strain lies between 24° and 28° C. The optimum temperature range for the light strain lies between 28° and 32° C. The optimum pH range for both strains lie between 5 and 7. Perithecia were produced when the dark strain was crossed with the light

strain, or grown alone in potato dextrose agar at 24° to 28° C. Perithecia obtained from both isolates were typical of *Glomerella cingulata*. Ascospore isolations consistently resulted in the recovery of typical *C. gloeosporioides* cultures. Although conidia produced by the dark strain are significantly longer than those of the light strain, their perithecia are indistinguishable. Both strains are identified as *C. gloeosporioides*, the conidial stage of *G. cingulata*. The formation of the sexual stage of *C. gloeosporioides* in vitro in Puerto Rico has not been reported hitherto.

#### RESUMEN

Se aislaron dos cepas de *Colletotrichum* spp., una oscura y una clara, de guayabas atacadas por el hongo en Puerto Rico. Las cepas aisladas diferían entre sí en cuanto a los aspectos del cultivo, características fisiológicas y tamaño de las conidiosporas. La gama de temperaturas óptimas para el crecimiento del micelio de la cepa oscura se extiende de 24° a 28° C., mientras que para el de la cepa clara se extiende de 28° a 32° C. La gama de valores pH para ambas cepas se extiende de 5 a 7. Se formaron peritecios cuando la cepa oscura se cruzó con la clara o se cultivó por sí sola en agar glucosado de papas a una temperatura de 24° a 28° C. Los peritecios formados por ambas cepas son típicos de *Glomerella cingulata*. Las ascosporas que se aislaron produjeron consistentemente cultivos típicos de *C. gloeosporioides*. Aunque las conidiosporas producidas por la cepa oscura son significativamente más largas que las de la cepa clara, los peritecios no pueden diferenciarse entre sí. Ambas cepas se identifican como *C. gloeosporioides*, estado asexual de *G. cingulata*. Esta es la primera vez que en Puerto Rico se informa la formación in vitro del estado perfecto o sexual de *C. gloeosporioides*.

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