Virulence Spectrum of South American Isolates of *Colletotrichum gloeosporioides* on Selected *Stylosanthes guianensis* Genotypes

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ABSTRACT

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Anthracnose, caused by *Colletotrichum gloeosporioides*, is the most important and widespread disease of *Stylosanthes*, a diverse tropical and subtropical forage legume naturally distributed in Central and South America. Although South American isolates of *C. gloeosporioides* are known to be variable in pathogenicity, no suitable differential host lines have been identified to fully characterize them. This study takes advantage of new *S. guianensis* inbred lines, cultivars, and accessions to describe the pathogenicity of South American isolates of *C. gloeosporioides* and to select potentially useful differentials. Seedlings of 23 *S. guianensis* genotypes were inoculated with 45 isolates from various regions of South America. Virulence patterns were used to select 12 differentials and determine 23 pathotypes in *C. gloeosporioides*. In contrast, the same isolates were grouped into nine pathotypes with the four Australian differentials currently in use.

Additional keyword: resistance

Stylosanthes guianensis (Aubl.) Sw. is a diverse forage legume with a wide natural distribution throughout tropical and subtropical South America (15). The species is one of the most important forage legumes in Australia (5) and South America (14).

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., is one of the major limitations to the extensive use of *S. guianensis* as a tropical forage (9,12). Dry matter losses ranging from 64 to 100% have been reported for *S. guianensis* in Colombia (2). Anthracnose symptoms on *S. guianensis* are characterized by necrosis and blight on leaves, stems, and terminal shoots. The pathogen exhibits considerable variation in morphology (3) and pathogenicity (7,11). Two separate biotypes of the pathogen, designated A and B, have been described as causing anthracnose symptoms on *Stylosanthes* species in Australia (7). Biotype A infects most species of the genus *Stylosanthes*, but biotype B infects only *S. guianensis*. Four physiologic races of biotype B have been identified on a set of differentials composed of four *S. guianensis* cultivars: Endeavour, Graham, Cook, and CPI 18750. These differentials are not very useful in characterizing South American isolates of *C. gloeosporioides*.

The center of origin of *Stylosanthes* (15), and thus the presumed center of genetic diversity of its pathogen, is in South America. Very little is known about the race composition of the South American pathogen population, primarily because appropriate differential genotypes are lacking. In *S. guianensis–C. gloeosporioides* interactions, pathogen variability studies are complicated by the host's heterogeneity and its recent domestication. Knowledge of the race composition of *C. gloeosporioides* and the geographic distribution of the various races will help in the development of effective breeding programs for anthracnose resistance and of gene deployment strategies for managing this resistance.

For this study, the *S. guianensis* inbred lines and accessions used to differentiate isolates of *C. gloeosporioides* into pathotypes were selected by an empirical method used for numerous other host–pathogen systems (4). The reactions of several accessions and inbred lines of diverse morphology to isolates of the pathogen collected from various regions were used to further select host genotypes that showed differential reactions. However, the identity (number and uniqueness) of the anthracnose resistance–conferring gene(s) in each host genotype is still unknown.

The objective of this study was to determine the variability in virulence pattern of South American isolates of *C. gloeosporioides* and thereby designate a set of *S. guianensis* genotypes that differentiate the pathogen's physiologic races.

MATERIALS AND METHODS

Isolates and culture maintenance. Isolates of *C. gloeosporioides* were collected from 1981 to 1994 (Table 1). Isolates of *C. gloeosporioides* were obtained from diseased leaves, flowers, or stems of accessions of *S. guianensis*. Small pieces of plant tissue with anthracnose lesions were surface-sterilized in 1% NaOCl solution for 3 min and in 70% ethanol for 2 min, and rinsed three times in sterile distilled water. The samples were transferred to sterile filter papers to remove excess moisture and plated onto oatmeal agar (OMA). Monoconidial isolates were derived by removing conidia from the initial isolates, placing them on the surface of water agar, and later transferring single, germinating conidia to OMA. All cultures were incubated at 28°C. Isolates collected from 1981 to 1986 were chosen from collections maintained at Centro Internacional de Agricultura Tropical (CIAT), Colombia. For short-term storage of up to 1 year, fungal disks (8 mm diameter) were removed from 4- to 6-day-old OMA cultures (10) of monoconidial isolates and maintained in screw-cap tubes of sterile distilled water at room temperature (25°C) (1). Isolates were reactivated by placing pieces of disks on plates of fresh OMA and incubating at 28°C. For long-term storage, the cultures were lyophilized in ampoules.

Development of *S. guianensis* inbred lines. The *S. guianensis* inbred lines were developed by single-seed descent over several generations, the number of which differed according to the genotype. Early-flowering genotypes had more generations than late-flowering genotypes, but no genotype was selfed fewer than five generations. Each single-seed descent line originated from a single, arbitrarily selected plant grown from seed of accessions held at CIAT. While the heritage of each plant can be traced, the genetic identity for each inbred line is not necessarily representative of the original germ plasm accession. All plants were grown in pots in a screenhouse, and the first mature seed of a plant was used to repropagate the line.

Cultivars and accessions. A total of 23 *Stylosanthes* genotypes was used in the study. Seeds of *S. guianensis* cv. Cook (CIAT 1950), cv. Endeavour (CIAT 13), cv. Graham (CIAT 15), CPI 18750, and *S.*

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scabra cv. Fitzroy were kindly provided by S. Chakraborty (CSIRO, Australia); cv. Mineirao (CIAT 2950) was provided by C. Fernandes (EMBRAPA, Brazil); and accession CIAT 184 was provided by the Seed Biology Section, Tropical Forages Program, CIAT. Inbred lines, CIAT numbers 2312, 1927, 1890, 1875, 1507, 2340, 1283, 1959, 1534, 2160, 2031, 2222, 1297, 1500, and 2023, and a bulk anthracnose-resistant population (FM 9205 P6) were all developed by the Genetics Section, Tropical Forages Program, CIAT.

Test plants. Seeds were scarified with sandpaper and surface-sterilized in 1% sodium hypochlorite (NaOCl) solution for 5 min. They were then rinsed three times with sterile deionized water and pregerminated on three layers of wet filter paper. Five-day-old seedlings were transplanted to Jiffy pots (5.7 cm high and 3.7 cm diameter) containing 100 g of steam-sterilized Oxisol field soil from the CIAT Quilichao substation (Santander de Quilichao, Department of Cauca, Colombia) and supplemented with N-P-K fertilizer (15-15-15) at a rate of 3.6 g/kg of soil. The plants were grown in a greenhouse with natural daylight and with temperatures between 19 and 30°C.

Virulence testing and disease evaluations. A total of 45 isolates of *C. gloeosporioides* from natural infections on various accessions and advanced lines of *S. guianensis* was selected. Thirty isolates were from Caquetá (the Colombian Amazon); 11 from Carimagua (Colombian savannas); 1 from Quilichao, Colombia; 1 from Paragominas, Brazil; and 2 from Pucallpa (Peruvian Amazon) (Table 1). The isolates were collected from different infected plant parts, such as stems, leaves, and flowers, and from lesions of various sizes.

Virulence was tested on all 23 *S. guianensis* inbred lines, accessions, and cultivars, and on resistant *S. scabra* cv. Fitzroy. Cultivar Endeavour was the "universal suscept" included in the test. Inoculum was prepared by washing conidia from 1-week-old OMA cultures with sterile deionized water and adjusting the conidial suspension to $OD_{600} = 0.12$ (approximately 10^6 conidia per ml). From 4 to 6 weeks after transplanting, each plant was spray-inoculated with conidial suspension until leaves were dripping. The inoculated plants were then transferred to a dark room with high relative humidity (>90%) and temperatures between 21 and 29°C for 2 days. The plants were then transferred to a greenhouse with temperatures between 19 and 30°C until disease symptoms were expressed and evaluations were made. Tests were arranged in a split-plot design with each run laid out as a randomized block with three replicates. Each replicate contained six plants. Isolates CIAT 10909, 16118, 16125, 16134, 16145, and 16162 were arbitrarily chosen and included as references in each set of inoculations. Disease severity was determined 10 days after inoculation by visual estimation of leaf tissue necrosis based on a Horsfall–Barratt (6) rating scale where 0 = no visible disease symptom, 1 = 1 to 3% tissue necrotic, 2 = 4 to 6%, 3 = 7 to 12%, 4 = 13 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, 7 = 76 to 87%, 8 = 88 to 94%, and 9 = 95 to 100%. Reactions with a mean disease rating of less than or equal to 1.5 were scored as resistant, and all other reactions were rated as susceptible. Virulence tests were repeated at least once for verification.

Table 1. Number, geographic origin, collection date, and pathotypes of the 45 *Colletotrichum gloeosporioides* isolates used in this study

Isolate CIAT no.	Host acc. no.	Origin		Collection date	Pathotype
		Country	Locality		
10643	1391	Colombia	Carimagua	02/03/81	21
10909	184	Colombia	Quilichao	29/08/81	7
11372	184	Brazil	Paragominas	07/04/82	22
11932	184	Peru	Pucallpa	08/02/83	12
12622	184	Peru	Pucallpa	23/05/83	19
13366	184	Colombia	Carimagua	25/02/85	18
13373	184	Colombia	Carimagua	25/02/85	16
13393	184	Colombia	Carimagua	31/10/84	23
14101	184	Colombia	Carimagua	12/02/86	20
16064	10941	Colombia	Carimagua	28/03/94	17
16065	11062	Colombia	Carimagua	28/03/94	23
16093	1280	Colombia	Caquetá	01/04/94	5
16094	1280	Colombia	Caquetá	01/04/94	4
16112	184	Colombia	Caquetá	01/04/94	14
16113	184	Colombia	Caquetá	01/04/94	4
16114	184	Colombia	Caquetá	01/04/94	3
16118	184	Colombia	Caquetá	01/04/94	4
16119	184	Colombia	Caquetá	01/04/94	2
16122	184	Colombia	Caquetá	01/04/94	10
16124	184	Colombia	Caquetá	01/04/94	4
16125	184	Colombia	Caquetá	01/04/94	4
16128	184	Colombia	Caquetá	01/04/94	10
16131	184	Colombia	Caquetá	01/04/94	13
16132	184	Colombia	Caquetá	01/04/94	2
16133	184	Colombia	Caquetá	01/04/94	6
16134	184	Colombia	Caquetá	01/01/94	1
16135	184	Colombia	Caquetá	01/04/94	3
16137	184	Colombia	Caquetá	01/04/94	11
16140	184	Colombia	Caquetá	01/04/94	4
16141	184	Colombia	Caquetá	01/04/94	8
16145	184	Colombia	Caquetá	01/04/94	7
16146	184	Colombia	Caquetá	01/04/94	7
16147	184	Colombia	Caquetá	01/04/94	7
16162	184	Colombia	Caquetá	01/04/94	4
16166	184	Colombia	Caquetá	01/04/94	2
16172	184	Colombia	Caquetá	01/04/94	9
16173	184	Colombia	Caquetá	01/04/94	9
16176	184	Colombia	Caquetá	01/04/94	4
16179	184	Colombia	Caquetá	01/04/94	7
16181	184	Colombia	Caquetá	01/04/94	10
16182	184	Colombia	Caquetá	01/04/94	4
16191	FM104	Colombia	Carimagua	06/16/94	15
16192	FM104	Colombia	Carimagua	06/16/94	4
16197	FM104	Colombia	Carimagua	06/16/94	4
16202	FM0186P6	Colombia	Carimagua	06/16/94	23

RESULTS AND DISCUSSION

Pathogenicity and disease response. Disease symptoms usually appeared 8 days after inoculation. Most of the disease reactions were at either end of the rating scale, and symptoms were markedly distinct between resistant and susceptible plants (Fig. 1). Some older isolates from the culture collection (1981 to 1986), notably CIAT 13393, 11372, 14101, 10643, 12622, and 13366, were pathogenic on fewer host genotypes and may have lost virulence during culturing and storage. The lower virulence, however, may also represent a shift toward increased virulence in the 10-year span.

Isolates CIAT 16093, 16094, 16135, 16162, 16176, 16133, 16140, 16134, and 13373 infected the supposedly resistant nonhost species *S. scabra* cv. Fitzroy. These isolates may represent a unique South American biotype. Except for isolate 13373, this group was collected from Caquetá in the Colombian Amazon. The significance of a potentially new biotype remains to be investigated.

Isolates 16119, 16132, and 16166 (pathotype 2); 16113, 16094, 16192, 16125, 16197, 16182, 16124, 16140, 16176, 16162, and 16118 (pathotype 4); 16114 and 16135 (pathotype 3); 10909,

16147, 16145, 16146, and 16179 (pathotype 7); 16173 and 16172 (pathotype 9); 16122, 16128, and 16181 (pathotype 10); and 16202, 16065, and 13393 (pathotype 23) gave the same host–pathogen interaction patterns with their respective host lines, indicating that isolates within each of the seven groups belong to the same pathotype (Table 1). However, isolates 16094, 16135, 16176, 16162, 16140 and 13373 diverge slightly within their groups, as they are among the isolates that infect the supposedly resistant control *S. scabra* cv. Fitzroy. The remaining 16 isolates belonged to 16 different pathotypes (Table 1).

Differential host selection. Individual plant reactions were examined within the same host line, and host lines that showed segregating reactions were discarded early on. Inbred lines CIAT 1500, 1959, 1534, 2160, 2031, 2023, 2222, and 1297 were discarded because of poor seed setting. Cultivar Graham and CIAT 1927 gave the same pattern of reactions to all isolates tested (data not shown). We selected cv. Graham instead of line 1927 because Graham is used as a differential for Australian isolates (8).

Although plants of FM 9205 P 6 showed no significant segregation in disease reactions, we did not select this particular line because it was developed as bulk population of highly resistant lines with similar flowering and maturity stages. Because the individual lines of this population may contain different genes for resistance and the seed lots may be variable, we did not consider this particular population as a potential differential. Twelve host genotypes (four existing Australian differential cultivars, Endeavour, Graham, Cook, and CPI 18750; one Brazilian cultivar, Mineirao; one accession that is widely grown in South America and Asia, CIAT 184; and six new inbred lines, CIAT 2312, CIAT 1890, CIAT 1875, CIAT 1507, CIAT 2340, and CIAT 1283) were selected and assembled as *S. guianensis* differentials for isolates of *C. gloeosporioides* (Table 2). Thirty-three pathotypes were obtained with all the 23 host genotypes originally tested (data not shown), as opposed to 23 pathotypes on the 12 selected host genotypes (Table 2). Although additions of five inbred lines (CIAT 1959, 1534, 2160, 2031, and 2023) to the 12 selected genotypes gave the same number of pathotypes as with all the host genotypes tested, these five lines cannot be routinely and reliably used as differentials because of poor seed setting traits.

Reactions of Australian differential cultivars and new host genotypes. We deliberately included all four Australian differentials to permit comparisons of published race classifications, both future and past, and to establish their differential usefulness. When the reaction patterns of only the four Australian differentials are used to classify the 45 South American isolates, the isolates fall into only nine pathotypes (Table 3), as opposed to 23 pathotypes when the 12 host genotypes are used (Table 2). For example, isolates that belonged to pathotypes 2, 4, 7, 8, 10, 12, and 13 were grouped in one disease reaction group when only the four Australian differentials were used (Table 3). This was expected because the larger number of host genotypes enables a finer tuning of pathotype grouping, especially with such a complex and diverse host–pathogen system as *Stylosanthes–C. gloeosporioides*.

The Australian cultivar Endeavour is the susceptible host used in Australia (8). But in its interactions with some South American isolates, Endeavour expressed resistance, indicating that it should not be considered a universal suscept. None of the host genotypes was susceptible to all of the 45 isolates of *C. gloeosporioides* used in this study. In addition, none was resistant to all of the isolates.

Six more interaction patterns were observed between the South American isolates and the four Australian differentials (Table 3) that were not seen with Australian isolates (8). South America therefore

Fig. 1. Anthracnose symptoms caused by *Colletotrichum gloeosporioides* on *Stylosanthes guianensis* 10 days after inoculations: susceptible plants (left); resistant plants (right).

Table 2. Reaction of *Stylosanthes guianensis* genotypes to pathotypes of *Colletotrichum gloeosporioides* from South America[a]

	Pathotype																						
Host no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
CIAT 2312	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	R	R	S	R	R
CIAT 184	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	R	R	R
CIAT 1890	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	R	S	R	R	R	R	R
Endeavour	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	R	R	R	R
CIAT 1875	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	R	R	R	R	R	R	R
Graham	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
CIAT 1507	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R
Cook	S	S	S	S	R	R	S	S	R	S	R	S	S	S	R	S	S	R	R	R	R	S	R
CIAT 2340	S	S	S	S	S	S	S	R	S	R	S	S	R	R	R	R	R	S	S	S	R	R	R
CIAT 1283	S	S	S	S	S	S	R	S	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R
Mineirâo	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
CPI 1875	S	R	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

[a] R = resistant, incompatible reaction, with mean disease rating less than or equal to 1.5; S = susceptible, compatible reaction, with mean disease rating greater than 1.5. Disease reaction based on visual leaf necrosis rated by the Horsfall–Barratt rating scale in which 0 = 0%, 1 = 1 to 3%, 2 = 4 to 6%, 3 = 7 to 12%, 4 = 13 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, 7 = 76 to 87%, 8 = 88 to 94%, 9 = 95 to 100%.

Table 3. Reaction of existing differential *Stylosanthes guianensis* cultivars to pathotypes of *Colletotrichum gloeosporioides* from South America

	Pathotype								
Host	3,1	2,4,7,8, 10,13,12	6	5,9, 11,15	16,17	14	18	22	19,20, 21,23
Endeavour	S	S	S	S	S	R	S	R	R
Graham	S	S	S	S	R	S	R	R	R
Cook	S	S	R	R	S	S	R	S	R
CPI 1875	S	R	S	R	R	R	R	R	R

[a] R = resistant, incompatible reaction, with mean disease rating less than or equal to 1.5; S = susceptible, compatible reaction, with mean disease rating greater than 1.5. Disease reaction based on visual leaf necrosis rated by the Horsfall–Barratt rating scale in which 0 = 0%, 1 = 1 to 3%, 2 = 4 to 6%, 3 = 7 to 12%, 4 = 13 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, 7 = 76 to 87%, 8 = 88 to 94%, 9 = 95 to 100%.

has a diverse population of *C. gloeosporioides* as well as of host genotypes.

Studies on naturally occurring variability in pathogen virulence and in host-plant resistance are widely undertaken in order to identify valuable plant germ plasm and pathogen races or pathotypes that can infect otherwise resistant cultivars. These studies assume that a genetically diverse host population leads to a genetically diverse pathogen population (13), an assumption that is borne out by our observations of the South American *C. gloeosporioides* population and its host *S. guianensis*.

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