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Anthracnose of *Stylosanthes capitata*: Implications for Future Disease Evaluations of Indigenous Tropical Pasture Legumes

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ABSTRACT

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During field screening from 1978 to 1981 of 121 accessions of the tropical pasture legume *Stylosanthes capitata* at two sites in Colombia and at Planaltina in Brazil, 94.2% of the accessions were resistant to anthracnose (caused by *Colletotrichum gloeosporioides*) in Colombia while 85.1% were susceptible in Brazil. In comparative seedling pathogenicity studies with isolates from both countries, isolates pathogenic to a wide range of accessions of *S. capitata* were only found in Brazil. Results strongly suggest that specialized isolates of *C. gloeosporioides* pathogenic to *S. capitata* exist in Brazil (the native habitat and probable center of diversity of this legume) and not in Colombia where *S. capitata* is an exotic species. This implies the need to screen indigenous tropical pasture legumes for disease resistance in their native habitats and the need for international collaborative disease screening trials in the future.

Extensive cattle grazing is the major use of the acid infertile savannas of tropical Latin America. Because pasture productivity is limited by lack of high quality forage species, the ultimate goal of the Tropical Pastures Program of the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, is to improve year-round pasture production. The greatest need is for highly productive adapted legumes to increase total pasture production and quality and to provide nitrogen for grasses (9).

Most legumes under pasture evaluation by CIAT have been collected in Central and South America (20). Over the past 10 yr, species of the indigenous genera *Stylosanthes* Sw., *Zornia* J. Gmel., and *Centrosema* (A. P. de Candolle) Bentham have shown potential as tropical pasture legumes for this region (2). Of these, *Stylosanthes* is used most widely (10). This includes *Stylosanthes capitata* Vog., a most promising perennial pasture legume for the tropical savannas of Central and South America (9).

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Unlike many *Stylosanthes* species, *S. capitata* has a limited geographical distribution. It is known only from the acid infertile, sandy soil savannas in Brazil and Venezuela (9,19). Most collections have been made in the states of Minas Gerais and Bahia in Brazil and Monagas and Anzoategui in Venezuela (3). It has never been found in Colombia.

Tropical pasture legumes have been collected for evaluation purposes for less than 50 yr. As most collections have been made in relatively undisturbed environments, collection locations generally represent the natural habitats and probable centers of diversity of these plants. The center of diversity of *S. capitata* probably lies within the region from central Brazil to east-central Venezuela.

Anthracnose, which is caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Sacc., is the most widespread and damaging disease of *Stylosanthes* (12), occurring in all countries where this genus is under pasture evaluation (4,7,11,14,21). Dry matter losses as high as 58 and 64% have been recorded for *S. hamata* L. in southern Florida (16) and *S. guianensis* (Aubl.) Sw. in Colombia (5), respectively.

In Colombia, the first field evaluations of several accessions of *S. capitata* were begun in 1976 (1). Within 2 yr, these accessions were noted for their high resistance to anthracnose in Colombia in contrast to the susceptibility of other *Stylosanthes* species (9).

Systematic detailed disease evaluations of *S. capitata* began at Carimagua and Santander de Quilichao, Colombia, in 1978 and near Brasilia, Brazil, in 1979. To date, over 100 accessions have been evaluated in both locations.

This paper reports results of evaluations of 121 accessions and discusses the implications of these results for future disease evaluations of indigenous tropical legume species in Central and South America.

MATERIALS AND METHODS

Field evaluations. Accessions of *S. capitata* were evaluated at three sites: the Centro Nacional de Investigaciones Agropecuarias of the Instituto Colombiano Agropecuario, ICA-CIAT Research Station at Carimagua, in the eastern plains of Colombia; the CIAT Research Station at Santander de Quilichao in the Cauca Department of Colombia; and the Centro de Pesquisa Agropecuaria dos Cerrados (CPAC) of Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) near Brasilia, Brazil. Location and climatic data are given in Table 1.

At all sites, anthracnose evaluations were made in small (4 m^2) introduction plots of each accession established for initial germ plasm characterization, morphological evaluation, and seed collection. Accessions were exposed only to natural inoculum at all three sites. At regular intervals, samples of diseased plant material were taken from many accessions at all sites to confirm identities of the causal pathogens and for studies of isolate pathogenicity. In Carimagua and Santander de Quilichao, Colombia, accessions were evaluated at 6- to 8-wk intervals from August 1978 to October 1981. At CPAC in Brazil, from 1979 to 1981, two evaluations per year were made during the periods March to April (towards the end of the 6-mo wet season) and September to October (at the end of the dry season). A rating scale of 1–5, with half-scale divisions, was used in which 1 = no disease; 2 = few lesions, no defoliation; 3 = a moderate number of leaf and stem lesions, slight defoliation; 4 = many leaf and stem lesions, severe defoliation; 5 = plant death. In total, 121 accessions were evaluated: 110 were evaluated at all three sites; five were evaluated at CPAC, Brazil, and Santander de Quilichao, Colombia, only; while six were evaluated at Carimagua and Santander de Quilichao, only. Occurrence and severity of other diseases also was recorded.

Anthracnose reaction was summarized for each accession at each site. For Carimagua and Santander de Quilichao, Colombia, 3.5 years of 6- to 8-wk-interval evaluations were summarized. As there were no marked differences between reactions at the two sites, anthracnose reaction was summarized for this country. Three years of twice-yearly evaluations were summarized for CPAC, Brazil. All accessions with anthracnose reaction ratings of 2 or less were classed as resistant; all accessions with ratings above 2 were classed as susceptible.

Glasshouse evaluations. From periodic samples taken from diseased accessions in Carimagua in Colombia, and CPAC in Brazil, isolates of *C. gloeosporioides* were purified on oatmeal agar (OMA) according to the method of Lenné and Sonoda (13) and maintained in vials of distilled water (6). Isolates used in comparative seedling pathogenicity tests were selected from the same 10 accessions of *S. capitata* (CIAT 1019, 1097, 1315, 1324, 1405, 2250, 2254, 2260, 2263, 2565) from each location.

The pathogenicity of the 20 selected isolates of *C. gloeosporioides* was compared on 35-day-old seedlings (plants with four trifoliate leaves and 5 cm high) of 50 accessions of *S. capitata* collected from eight states of Brazil (Bahia, 8; Ceará, 2; Federal District, 3; Goiás, 7; Maranhao, 3; Minas Gerais, 8; Mato Grosso, 5; Piaui, 4), and from two states of Venezuela (Anzoategui, 8; and Monagas, 2). Isolates were cultured on OMA for 12 days on a 12-hr photoperiod at 28 C. Conidia were suspended in sterile distilled water (10^6 conidia per milliliter) and sprayed onto three replicates of 10 seedlings (13). Ten control seedlings were sprayed with sterile distilled water. Inoculated seedlings were enclosed in moist plastic bags and incubated at 22–28 C and 12 hr days for 48 hr. Disease ratings were made 10 days after inoculation according to the 1–5 rating scale: accessions rating 2 or less were classed as resistant, and accessions rating more than 2 were susceptible as described above.

RESULTS

Field evaluations. In Carimagua and Santander de Quilichao, Colombia, all accessions that showed symptoms of anthracnose were sampled at least four times during the evaluation period. A total of 73 accessions were sampled at CPAC in Brazil. From the beginning to the end of the wet season at all sites, *C. gloeosporioides* was readily isolated from all samples of plant material with symptoms of anthracnose. Toward the end of the wet season and during the dry season, *Colletotrichum truncatum* (Schw.) Andrus & Moore was isolated from stem lesions at levels of 15, 22, and 31% of samples analyzed from Carimagua and Santander de Quilichao in Colombia and at CPAC in Brazil, respectively. Seedling tests confirmed the low pathogenicity of isolates of this fungus to selected accessions of *S. capitata* in contrast to highly pathogenic isolates of *C. gloeosporioides*. Toward the end of the wet season, in addition to the dry season, the sexual state, *Glomeralla cingulata* (Stonem.) Spauld, and V. Schrenk was commonly present in cultures of *C. gloeosporioides*.

Slight anthracnose of *S. capitata* by *C. gloeosporioides* was manifest as small (1–3 mm), dark brown to black, rounded lesions randomly distributed on leaves, stipules, and stems. At moderate to severe infection levels, dark brown to black distorting lesions developed on leaves. Stipules and petioles were severely diseased, causing wilting and death of leaves and defoliation. Lesions coalesced on stems, causing extensive blackening and occasionally plant death by stem girdling. Plant death, however, was usually caused by severe defoliation and dieback.

From 1978 to 1981, field screening of 121 accessions of *S. capitata* at both Carimagua and Quilichao in Colombia revealed few susceptible accessions. After 3.5 yr of evaluation, 94.2% of accessions had proven to be resistant. In contrast, at CPAC in Brazil, 85.1% of accessions were either moderately to severely affected or killed by anthracnose. Of the 18 (14.9%) accessions rated resistant at CPAC in Brazil, 16 originated from Anzoategui and Monagas in Venezuela, and one each from Ceara and Minas Gerais in Brazil.

Glasshouse evaluations. Results from seedling inoculations in the glasshouse showed that all 50 accessions of *S. capitata* were resistant to nine of 10 isolates collected in Carimagua in Colombia. Isolate 1097 from Colombia was virulent to eight accessions originally obtained from Bahia in Brazil. The same results were found for I 1097B collected at CPAC in Brazil. These same isolates, however, were avirulent to all other accessions including 32 from Brazil (Table 2) and 10 from Venezuela.

In contrast, only 10 to 16 accessions of *S. capitata* were resistant to nine of the 10 isolates collected at CPAC in Brazil; in all cases, these included 10 accessions collected from Anzoategui and Monagas in Venezuela. Host-pathogen specificity was found for 12 accessions and I 2265 from CPAC in Brazil, was virulent to all accessions from Brazil (Table 2).

TABLE 1. Location and climatic data of the three sites used to evaluate anthracnose resistance in *Stylosanthes capitata*

Site	Latitude	Longitude	Elevation (m)	Precipitation mean annual (mm)	Temperature mean annual (C)
Carimagua, Colombia	4° 02′ N	71° 10′ W	200	2,094	26.5
Santander de Quilichao, Colombia	3° 06′ N	76° 31′ W	990	1,845	24.8
CPAC, near Brasilia, Brazil	15° 36′ S	47° 42′ W	1,010	1,578	21.3

Other diseases. Blight, which is caused by *Sclerotium rolfsii* Sacc. was occasionally detected on several accessions of *S. capitata* during the wet seasons in both Carimagua in Colombia and CPAC in Brazil. At both sites, the few wilted or dead plants were excluded from anthracnose evaluations. Rhizopus head rot, which is caused by *Rhizopus stolonifer* (Ehreub. ex Fr.) Lind, moderately affected inflorescences of several accessions of *S. capitata* from September to October 1980, in Carimagua, Colombia.

DISCUSSION

From 1978 to 1981, field screening of 121 accessions of *S.. capitata* at two widely separated, climatically different sites in Colombia showed that 94.2% of accessions were resistant to anthracnose. At CPAC in Brazil, 85.1% of the same accessions were susceptible. As only natural inoculum was present at all sites, it is most apparent that *S. capitata* was considerably more susceptible to *C. gloeosporioides* at CPAC in Brazil, than at two sites in Colombia.

Comparison of climatic data of the three sites showed greater differences between Colombia and Brazil than between the two Colombian sites with respect to mean annual precipitation and temperature (Table 1). Because these differences could cause differences in anthracnose reaction, it was essential to compare the pathogenicities of isolates of *C. gloeosporioides* from both countries. Considerable differences in pathogenicity between isolates collected in Carimagua in Colombia, and CPAC in Brazil, were obvious with all accessions being resistant to 9 of the 10 isolates from Colombia, and only 20–32% of accessions being resistant to 9 of the 10 isolates from Brazil (Table 2). Results from screening in the glasshouse correlated well with field observations, suggesting that climatic differences were not responsible for differences in anthracnose reactions of *S. capitata* accessions in Colombia and Brazil.

The CPAC screening site is in the Federal District of Brazil and within the natural distribution and probable center of diversity of *S. capitata*. Five accessions under evaluation were actually collected from this region of Brazil. In contrast, *S. capitata* has never been found in Colombia. Leppik (18) stated that the center of diversity of a plant is also the center of diversity of its specialized parasites. Results from field screening of *S. capitata* in Brazil and Colombia and seedling pathogenicity studies during the past 3 yr strongly support that specialized pathogenic isolates of *C. gloeosporioides* to *S. capitata* exist in the native habitat of this legume in Brazil and not in Colombia, where 94.2% of accessions of *S. capitata* under evaluation have remained resistant.

The accessions susceptible to anthracnose in Colombia were originally collected at Bahia in Brazil. These same accessions were also susceptible in CPAC, Brazil. In addition, the isolates collected from the Bahian accession CIAT 1097 in Brazil (I 1097B) and Colombia (I 1097Ca) were pathogenic to all Bahian accessions in seedling tests. The presence of isolates of *C. gloeosporioides* pathogenic to Bahian accessions of *S. capitata* in Colombia may be due to introduction from Brazil or mutation within the indigenous population of *C. gloeosporioides* as these isolates belong to group 2 (17), which is also pathogenic to *S. guianensis*, a species native to Colombia. Further work is necessary to determine the origin of this group.

These findings have important implications for future disease screening of indigenous tropical pasture legumes in South America. If the most pathogenic isolates of *C. gloeosporioides* to particular species of *Stylosanthes* exist in the native habitat of the

TABLE 2. Reaction of seedlings of accessions[a] of *Stylosanthes capitata* to isolates[b] of *Colletotrichum gloeosporioides* from both Brazil and Colombia

Accession number	Origin	Reaction to anthracnose[c] fungus isolate: I 1019B[d]	I 1097B	I 1097Ca[e]	I 1315B	I 1324B	I 1405B	I 2250B	I 2254B	I 2260B	I 2263B	I 2265B
2251	Ceara	+	−	−	+	+	+	+	+	−	+	+
2253	Ceara	−	−	−	+	+	−	+	+	−	+	+
1642	Fed. District	+	−	−	+	+	+	+	+	+	+	+
1990	Fed. District	+	−	−	+	+	+	+	+	+	+	+
2201	Fed. District	+	−	−	+	+	+	+	+	+	+	+
1423	Goias	+	−	−	−	−	+	+	+	+	+	+
1781	Goias	+	−	−	+	+	+	+	+	+	+	+
1986	Goias	+	−	−	+	+	+	+	+	+	+	+
2013	Goias	+	−	−	+	+	+	+	+	+	+	+
2026	Goias	+	−	−	+	+	+	+	+	+	+	+
2242	Goias	+	−	−	+	+	+	+	+	+	+	+
2565	Goias	+	−	−	+	+	+	+	+	+	+	+
1315	Maranhao	+	−	−	+	+	−	+	+	+	+	+
1318	Maranhao	+	−	−	+	+	+	+	+	+	+	+
1321	Maranhao	+	−	−	+	+	+	+	+	+	+	+
1019	Minas Gerais	+	−	−	+	+	+	+	+	+	+	+
1943	Minas Gerais	+	−	−	+	−	+	+	+	+	+	+
2249	Minas Gerais	+	−	−	+	+	+	+	+	+	+	+
2257	Minas Gerais	+	−	−	−	−	+	+	+	+	+	+
2260	Minas Gerais	+	−	−	−	−	+	+	+	+	+	+
2263	Minas Gerais	+	−	−	−	−	+	+	+	+	+	+
2265	Minas Gerais	+	−	−	+	+	+	+	+	+	+	+
2269	Minas Gerais	+	−	−	+	+	+	+	+	+	+	+
1405	Mato Grosso	+	−	−	+	+	+	+	+	+	+	+
1686	Mato Grosso	+	−	−	+	+	+	+	+	+	+	+
1691	Mato Grosso	−	−	−	+	+	+	−	−	−	−	+
1693	Mato Grosso	+	−	−	+	+	+	−	−	−	−	+
1728	Mato Grosso	−	−	−	+	+	−	+	+	−	−	+
1332	Piaui	+	−	−	+	+	+	+	+	+	+	+
1334	Piaui	+	−	−	+	+	+	+	+	+	+	+
1338	Piaui	+	−	−	+	+	+	+	+	+	+	+
2246	Piaui	+	−	−	−	−	+	+	−	+	+	+

[a] Reactions of only 32 of the 50 accessions tested are shown. Of the others, eight accessions from Bahia were susceptible to 10 isolates from Brazil and I 1097 from Colombia while 10 accessions from Venezuela were resistant to all isolates.

[b] Only one isolate from Colombia (I 1097Ca) was virulent. the nine nonvirulent isolates are not included in the table.

[c] According to the scale 1 = no disease; 5 = plant death. + = anthracnose reaction of more than 2; − = anthracnose reaction of 2 or less.

[d] B = isolates collected in Brazil.

[e] Ca = isolates collected in Colombia.

host species, clearly each species should be screened for anthracnose reaction in its native habitat as well as in the site where it will be utilized. Surveys of *Stylosanthes* species in their native habitats, particularly *S. guianensis* in Colombia and *S. capitata* and other species in Brazil and Venezuela, have shown the widespread natural distribution of anthracnose, usually at slight to moderate levels in native stands. Natural sources of inoculum of *C. gloeosporioides* are therefore readily available for efficient screening.

The resistance of Venezuelan accessions of *S. capitata* to anthracnose in the field in both Colombia and Brazil and in glasshouse seedling tests to isolates of *C. gloeosporioides* from both countries also is noteworthy. As these accessions were just as exotic to Brazil as were the Brazilian accessions to Colombia, the need for anthracnose screening of the Venezuelan accessions in Venezuela was clear. An anthracnose screening trial planting of *S. capitata*, including all Venezuelan accessions, was recently planted at El Tigre, Anzoategui, Venezuela in collaboration with the Fondo Nacional de Investigaciones Agropecuarias (FONAIAP) to evaluate anthracnose reactions of this germ plasm in its native habitat.

Results from screening at the CPAC may not be representative of the extent of variation within the population of *C. gloeosporioides* throughout the native habitat of *S. capitata* in Brazil. Multilocational screening is essential to understand the extent of pathogenic variation and, if possible, to select germ plasm resistant to anthracnose at various locations. Recently, one *S. capitata* anthracnose screening trial was established at Acauá in Minas Gerais in Brazil, a site with native *S. capitata*, in collaboration with the Empresa de Pesquisa Agropecuaria de Minas Gerais (EPAMIG). Further trials are being considered.

Continuing research on tropical pasture production will lead to increased germ plasm exchange and seed transfer from South America to many other tropical countries. As *C. gloeosporioides* is seedborne in *Stylosanthes* species (8,15), the risk of movement of pathogenic races of this fungus from South America to other countries will increase. International collaborative disease screening trials should be established for promising tropical pasture legumes in their native habitats or centers of diversity in South America.

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