Variation in Reaction to Anthracnose within Native *Stylosanthes capitata* Populations in Minas Gerais, Brazil

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ABSTRACT

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Native populations of *Stylosanthes capitata*, a promising perennial pasture legume in tropical America, were evaluated for their reaction to a virulent widespread race of *Colletotrichum gloeosporioides*. Considerable variation in anthracnose reaction among progenies from individual plants, among plants from the same site, and between populations from the same and different sites in Minas Gerais, Brazil, was found. The greatest diversity for anthracnose reaction among progeny was shown by population Diamantina B (Shannon Index of Diversity $h = 0.39$), whereas the least was found in Mendanha B ($h = 0.17$). Although the majority of progenies were susceptible, highly resistant progenies were found in all populations. Results suggest that diversity in anthracnose resistance within native *S. capitata* populations contributes to their persistence in the presence of virulent *C. gloeosporioides* and emphasize the importance of maintaining diversity in improved *S. capitata* pastures in agroecosystems where *C. gloeosporioides* is endemic.

Stylosanthes capitata Vog., native to treeless and open woodland savanna habitats in Brazil and Venezuela (16), is a promising perennial pasture legume with significant outcrossing ability (14). It has potential for the acid, low-fertility savannas of tropical America where poor quality pastures severely limit pasture productivity (10). Being well-adapted climatically and edaphically, *S. capitata* has high dry matter and seed productivity and associates well with native and introduced grasses (6–9). In 1983, the Instituto Colombiano Agropecuario (ICA) released cultivar Capica, a blend of five accessions, as a suitable pasture legume for the eastern plains of Colombia (21).

Anthracnose, caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Sacc., is the most damaging and widespread disease of *Stylosanthes* (11) and is endemic in native stands in tropical America. From 1978 to 1981, studies in Colombia and Brazil observed many evaluation plots severely affected by anthracnose in Brazil and found isolates of *C. gloeosporioides* pathogenic to a wide range of *S. capitata* accessions only in Brazil (13). This indicated the existence of specialized isolates of this pathogen in Brazil, the native habitat and probable center of diversity of *S. capitata*, and the need to study further this host pathogen system in its native habitat. This disease is regarded as the major limiting factor to the use of *S. capitata* as a legume component of improved pastures in the Brazilian cerrados (20).

Disease surveys of native *S. capitata* stands have been in progress in Brazil and Venezuela since 1979. Although anthracnose is frequently present, its severity is generally low, and epidemics have never been seen in contrast to field evaluation plots. Similar observations have been made previously for the more widely distributed species, *S. guianensis* (Aubl.) Sw., in tropical America (15).

Several recent studies have evaluated native and naturalized legume populations and their pathogens. Burdon (3) found significant variation in resistance to *Cymadothea trifolii* (Pers.) Wolf and *Pseudopeziza trifolii* (Biv.-Bernh.) Fuckel among individual members of a naturalized permanent *Trifolium repens* L. pasture in North Wales. Similarly, the occurrence of quantitative and qualitative resistance to *Phakopsora pachyrhizi* Syd. was documented in native Australian *Glycine* species with considerable variation occurring within species in a number of disease characteristics both between and within populations (4).

During studies of a natural *S. guianensis-C. gloeosporioides* host-pathogen population in Colombia, Miles and Lenné (15) found differences among host plants in reaction to anthracnose and both quantitative and qualitative differences in pathogenicity among *C. gloeosporioides* isolates. In addition, significant variation among progenies of different genotypes of the annual legume *Amphicarpa bracteata* L. Fern. in relative growth reduction caused by the host-specific pathogen *Synchytrium decipiens* Farlow have been observed (17).

In studies of small grain populations and their endemic pathogens in Israel, Browning (1974) emphasized the relevance of knowledge gained from studies of natural host-pathogen systems to the development of pest management programs in agroecosystems. Because of the existence of virulent biotypes of *C. gloeosporioides* in native habitats of *S. capitata* in Brazil (13), the following study was made to determine variation in reaction to anthracnose among native *S. capitata* populations in Minas Gerais, Brazil.

MATERIALS AND METHODS

Field sites and collection of *S. capitata*. Two collection sites were chosen in the state of Minas Gerais, approximately 400 km north of Belo Horizonte and where relatively dense native *S. capitata* stands had been observed during the previous 3 yr. Site 1 near Diamantina at 18°20′S; 43°22′W and 700 m above sea level (m.a.s.l.) was a sandy red yellow latosol, acidic and of low fertility, with dense stands of *S. capitata, S. macrocephala, S. guianensis*, and *S. leiocarpa* among *Stylosanthes* species as well as other leguminous genera *Aeschynomene, Crotalaria, Desmodium, Zornia*, and native grasses. It was being grazed intermittently but was not fenced. Site 2 at Mendanha at 18°12′S; 43°37′W and 670 m.a.s.l. (approximately 40 km from Diamantina) was a loamy dark red latosol, also acidic and of low fertility, with dense populations of *S. capitata, S. macrocephala*, and native grasses. Other legumes were rare. It had been fenced for 5 yr, was grazed on an irregular rotational, and had been burnt three times during the previous 5 yr. Both sites were located within the well-drained isothermic savanna ecosystem or "cerrados" characterized by a 6-mo wet season with mean annual precipitation of 1,500 mm and mean annual temperature of 22 C. Both sites were sampled on 18 May 1982. Surveys of the incidence and severity of anthracnose were made at the same time.

At each time, two 1-m^2 quadrats (A and B) were placed at least

20 m from each other at representative locations of the whole site. All mature inflorescences were collected from 10 randomly selected individual plants (with respect to phenotype) in each quadrat. Seed was cleaned from inflorescences and matured in the laboratory for 5-mo.

Processing and propagation of *S. capitata*. Seed of individual plants from the four sampled *S. capitata* populations was acid-scarified and pregerminated on moist filter paper in petri dishes in the laboratory (20–25 C). Germinated seeds were planted in 5- × 5-cm jiffy pots in plastic trays with 45 pots per tray in an acid, low-fertility Oxisol soil prefertilized with the recommended rate of complete fertilizer (15).

Screening of *S. capitata* for reaction to *C. gloeosporioides*. Reaction to *C. gloeosporioides* was assessed on 35-day-old seedlings of *S. capitata* progenies (seedlings with three to four trifoliate leaves and 5 cm high). One virulent isolate of *C. gloeosporioides*, I 11339, collected near Sete Lagoas, Minas Gerais, and representative of the most common and widespread race on *S. capitata* in the Brazilian cerrados, was used. The isolate was cultured on oatmeal agar for 12 days at 28 C with a 12-hr photoperiod (12). Conidia were filtered through cheesecloth and suspended in sterile distilled water (10^6 conidia per milliliter) and sprayed onto all surviving seedling progenies of the four populations. Inoculated progenies were enclosed in moist plastic bags and incubated at 22–28 C and 12-hr photoperiod for 48 hr (12). Disease severity ratings were made 10 days after inoculation according to a 1–5 rating scale where 1 = no disease; 2 = few small lesions on leaflets and stems, no defoliation; 3 = moderately abundant small to large lesions on leaflets and stems, slight defoliation; 4 = abundant small and large lesions on leaflets and stems, considerable defoliation and dieback; and 5 = plant death.

The Shannon Index of Diversity (h), which takes account of both the number of different phenotypes and the evenness of distribution among these phenotypes, was used to assess population diversity (5,19).

RESULTS

Surveys of anthracnose made on the sampling date (18 May 1982) found low incidence and severity at both Diamantina and Mendanha. Similar observations had been made during the three previous years and have also been made subsequent to sampling until March 1986, the last survey before writing the manuscript.

Mean anthracnose severity reactions and numbers of progenies from 10 plants from each of four native *S. capitata* populations collected in Minas Gerais to *C. gloeosporioides* I 11339 are given in Table 1. Progeny numbers varied considerably among plants within populations and among populations (Table 1).

Mean anthracnose reactions of the four native *S. capitata* populations were 4.51, 4.28, 4.05, and 3.85 for Diamantina A, Diamantina B, Mendanha A, and Mendanha B, respectively (Table 1). Progenies from the two Diamantina populations were generally more susceptible to *C. gloeosporioides* I 11339 than progenies from the Mendanha populations, having almost all plant mean anthracnose reactions greater than 4.0. Diamantina A was significantly more susceptible than Mendanha A and B (Table 1).

The structure of each population is presented as frequency distributions of anthracnose reactions for progenies from each plant with five or more progeny from each population (Figs. 1–4). The majority of progenies were moderately to highly susceptible to *C. gloeosporioides* I 11339. Diamantina B showed the greatest within-plant variation in reaction to *C. gloeosporioides*, with all plants having at least two different reaction types among progenies (Fig. 2). In contrast, Mendanha B showed the least within-plant variation, with six plants having two or fewer reaction types among progenies (Fig. 4). Diamantina A (Fig. 1) and Mendanha A (Fig. 3) were composed of plants with both diverse and uniform progeny anthracnose reactions.

The inescapable problem of unevenness of progeny numbers

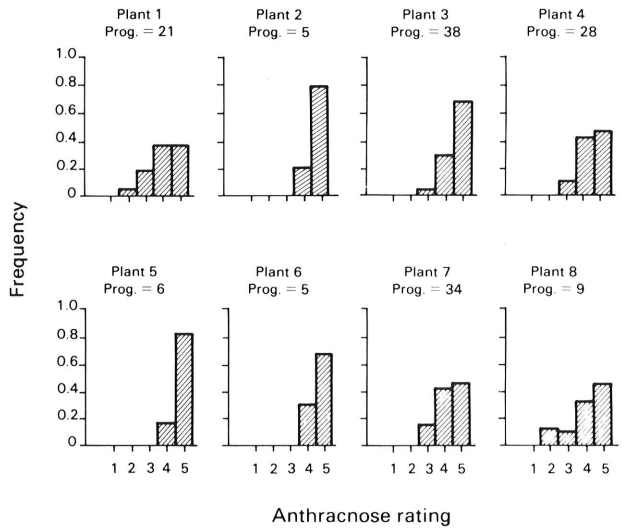

Fig. 1. Frequency distributions of anthracnose reactions of native *Stylosanthes capitata* population Diamantina A from Minas Gerais, Brazil, inoculated with *Colletotrichum gloeosporioides* I 11339. Progeny numbers for each plant are shown.

TABLE 1. Mean anthracnose reactions[a] and numbers of progenies from 10 plants from each of four native *Stylosanthes capitata* populations collected in Minas Gerais, Brazil, to *Colletotrichum gloeosporioides* I 11339

	Stylosanthes capitata population							
	Diamantina A		Diamantina B		Mendanha A		Mendanha B	
Plant number	Mean	Number	Mean	Number	Mean	Number	Mean	Number
1	4.10 (1.12)[b]	21	4.82 (0.16)	11	4.02 (0.40)	48	3.75 (0.24)	110
2	4.25 (0.92)	4	4.55 (0.36)	40	3.38 (1.44)	88	3.86 (0.12)	78
3	4.80 (0.20)	5	3.97 (0.87)	32	3.97 (0.61)	73	3.87 (0.12)	39
4	5.00 (0)	3	4.16 (0.56)	19	3.63 (0.31)	32	3.74 (0.21)	96
5	4.66 (0.34)	38	4.34 (0.83)	38	4.30 (0.77)	30	3.91 (0.08)	45
6	4.36 (0.46)	28	4.18 (1.01)	17	5.00 (0)	2	3.94 (0.06)	18
7	4.83 (0.17)	6	3.31 (0.73)	29	5.00 (0)	9	3.69 (0.54)	26
8	4.73 (0.21)	15	4.20 (0.68)	5	3.25 (0.92)	4	3.76 (0.38)	63
9	4.29 (0.52)	34	4.62 (0.45)	26	3.93 (0.87)	15	3.94 (0.06)	49
10	4.11 (1.11)	9	4.64 (0.26)	11	NP[c]		4.00 (0)	14
Population mean[d]	4.51		4.28		4.05		3.85	

[a] According to the scale 1 = no disease; 5 = plant death.
[b] Variance is shown in parentheses.
[c] NP = No progeny.
[d] Population mean variance = 0.45.

among plants and populations when using field collections can be overcome using the Shannon Index of Diversity (h) (5,19). Shannon Diversity Indices for anthracnose severity reactions of the four *S. capitata* populations are presented in Table 2. Populations from Diamantina were significantly more diverse than those from Mendanha. In addition, Diamantina B ($h = 0.39$) was significantly more diverse than A ($h = 0.35$), whereas Mendanha A ($h = 0.30$) was significantly more diverse than B ($h = 0.17$), the least diverse population. The greatest range in diversity for anthracnose reactions among plants was shown by Diamantina A where h values ranged from 0 to 0.72 (Table 2).

DISCUSSION

Considerable variation in anthracnose reaction among progenies from individual plants, among plants from the same site, and between populations from the same and from different sites was found, showing considerable genetic heterogeneity for anthracnose resistance among *S. capitata* populations in Minas Gerais. Significant variation in resistance to various specific pathogens of native and naturalized legume populations has been reported previously (3,4,15,17). At the same time, the majority of progenies obtained from plants from *S. capitata* populations were moderately to highly susceptible to a virulent *C. gloeosporioides* isolate representative of the most common and widespread race in the Brazilian cerrados. These findings fully support previous observations on a group of 121 *S. capitata* accessions collected widely in Brazil and screened for anthracnose resistance at Planaltina, also in the Brazilian cerrados. Eighty-five percent of accessions were susceptible to anthracnose (13). A considerable percentage of individuals in *S. capitata* populations in Brazil are therefore susceptible to anthracnose.

Similar observations have been made in other native population studies. Wahl (22) found 68% of *Avena sterilis* L. collected throughout Israel was susceptible to race group 264-276 of *Puccinia coronata* f. sp. *avenae*, whereas Browning (1) commented that the majority of small grain collections were susceptible to common races of serious pathogens in Israel. More than 80% of wild barleys collected in Ethiopia were susceptible to barley yellow dwarf virus (18), and 58–87% of individuals of four native *Glycine* species in Australia were susceptible (qualitatively) to *Phakopsora pachyrhizi* (4). Burdon (3), however, found considerably higher levels of resistance to *Pseudopeziza trifolii* and *Cymadothea trifolii* in a naturalized pasture of *Trifolium repens* in North Wales. As this pasture was naturalized rather than native, the original plants may have had higher levels of resistance to the two pathogens.

Variation in anthracnose reaction was high among progenies from the same plant. Only four of the 39 plants sampled in four populations showed identical progeny reactions. This is supported

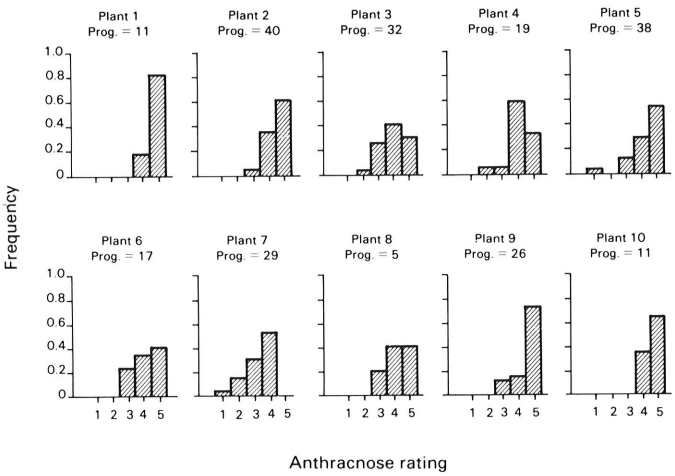

Fig. 2. Frequency distributions of anthracnose reactions of native *Stylosanthes capitata* population Diamantina B from Minas Gerais, Brazil, inoculated with *Colletotrichum gloeosporioides* I 11339. Progeny numbers for each plant are shown.

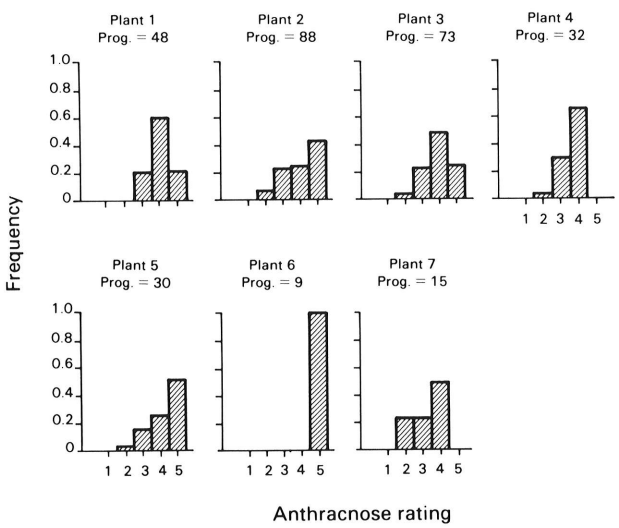

Fig. 3. Frequency distributions of anthracnose reactions of native *Stylosanthes capitata* population Mendanha A from Minas Gerais, Brazil, inoculated with *Colletotrichum gloeosporioides* I 11339. Progeny numbers for each plant are shown.

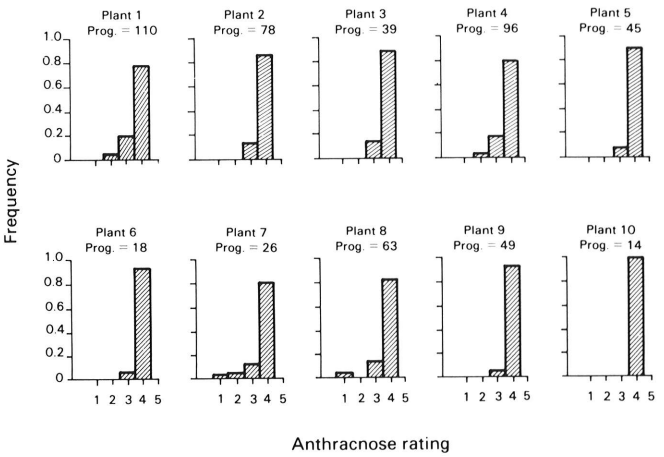

Fig. 4. Frequency distributions of anthracnose reactions of native *Stylosanthes capitata* population Mendanha B from Minas Gerais, Brazil, inoculated with *Colletotrichum gloeosporioides* I 11339. Progeny numbers for each plant are shown.

TABLE 2. Intraspecific diversity[a] in four native *Stylosanthes capitata* populations collected in Minas Gerais, Brazil, in reaction to *Colletotrichum gloeosporioides* I 11339

Plant number	*Stylosanthes capitata* population			
	Diamantina A	Diamantina B	Mendanha A	Mendanha B
1	0.52	0.21	0.41	0.26
2	0.45	0.36	0.45	0.18
3	0.22	0.37	0.50	0.17
4	0	0.43	0.33	0.25
5	0.31	0.46	0.33	0.25
6	0.42	0.47	0	0.09
7	0.20	0.48	0	0.29
8	0.25	0.46	0.45	0.24
9	0.44	0.33	0.11	0.10
10	0.72	0.29	NP[b]	0
Mean	0.35	0.39	0.30	0.17
Variance	0.02 ($P < 0.05$)			

[a] Diversity is measured by the Shannon Diversity Index (h).
[b] NP = No progeny.

by Miles' (14) findings of 19.5% outcrossing among progeny from naturally open-pollinated *S. capitata* plants.

Although the majority of *S. capitata* progenies were susceptible to the common virulent race of *C. gloeosporioides*, a small proportion of progenies was highly resistant and immune. Because potential for collecting more resistant genotypes does exist, further population sampling would be of value to locate sites with higher frequencies of resistance to anthracnose.

S. capitata progenies from the two Diamantina populations, an intermittently grazed, sandy soil site with great vegetational diversity, were generally more susceptible to *C. gloeosporioides* I 11339 than progenies from the Mendanha populations, a semi-improved loamy soil site-rotationally grazed, burnt, and fenced, with less vegetational diversity. Although any of these factors could be responsible for the difference in susceptibility, possibly semi-intensive management of the Mendanha site has lead to selection of more resistant individuals. Further consideration of the effect of grazing management on the anthracnose resistance structure of *S. capitata* populations is needed. Micro-climatic differences may also have been responsible. The frequency of resistance to crown rust in *Avena sterilis* populations in Israel (22) and that of wild *Avena* species in New South Wales, Australia (5), was higher in northern regions than southern regions. In both cases, the difference was closely related to climatic differences.

Shannon Diversity Indices for progeny anthracnose reactions for individual plants in each population generally reflected population structures shown by frequency distributions. Diamantina B with the greatest within-plant variation for reaction to *C. gloeosporioides* (Fig. 2) had the highest diversity index ($h = 0.39$), and Mendanha B with the least within-plant variation for reaction to *C. gloeosporioides* (Fig. 4) had the lowest diversity index ($h = 0.17$) (Table 2). The Shannon Diversity Index therefore was a useful statistic to summarize and compare population variability for reaction to anthracnose in this study.

Surveys of the incidence and severity of anthracnose in native *S. capitata* populations in Minas Gerais, at sampling, for 3 yr before and for 4 yr after sampling found both to be low. As found by Browning and co-workers in indigenous populations of small grains and their endemic foliar pathogens in Israel (1,2), it is most probable that the persistence and stability of native *S. capitata* populations in Brazil with high proportions of anthracnose susceptible individuals in the presence of virulent coexisting races of *C. gloeosporioides* is caused by their genetic heterogeneity. Similar results were obtained from a study of a natural *S. guianensis*-*C. gloeosporioides* host-pathogen population in Colombia (15). This implies that the persistence of improved productive perennial sown pastures of *S. capitata* in agroecosystems in the Brazilian cerrados will depend on maintenance of diversity for anthracnose resistance in the legume.

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