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Sources of resistance in species of *Brachiaria* to foliar blight disease caused by *Rhizoctonia solani*

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Abstract

This study developed a fast and reliable method of inoculating *Brachiaria*, a genus of tropical forage grasses, with *Rhizoctonia solani*, the causal agent of foliar blight disease. Using this method, 42 *Brachiaria* accessions were inoculated in both field and glasshouse, and their reactions to *R. solani* evaluated. Severity of infection in the glasshouse was rated by percentage of infected leaves, percentage of leaf area infected and number of sclerotia on infected tissue, and in the field by visual estimation. Disease scores were generally higher in the glasshouse than in the field. Sclerotia numbers on infected plant tissue correlated positively with percentage of infected leaves (r = 0.67), and with percentage of leaf area infected (r = 0.72). These percentages also correlated positively with each other (r = 0.77). Three accessions of *B. humidicola* (CIAT 16868, 16886 and 6369) and the *B. dictyoneura* accession CIAT 6133 (cv. Llanero) had consistently moderate levels of resistance. Four other accessions of *B. humidicola* (CIAT 679, 16876, 16887 and 16888) and the *B. brizantha* accession CIAT 16320 had moderate-low levels of resistance. All other accessions of *B. brizantha* were susceptible.

Introduction

Brachiaria is predominantly an African genus, comprising about 100 species, some of which gave rise to forage grasses that are commercially important in tropical America, where they cover about 50 M ha (CIAT 1993). A valuable germplasm collection of *Brachiaria* spp. was assembled at CIAT during the 1980s (Schultze-Kraft *et al.* 1987). This collection contains sources of resistance to the spittlebug insect (Homoptera: Cercopidae; Lapointe *et al.* 1992), rust (S. Kelemu, unpublished field observations), and probably other diseases and pests.

Foliar blight disease is caused by *Rhizoctonia solani*. It can produce substantial foliar damage on susceptible genotypes of an extremely wide range of plant species (Baker 1970), including *Brachiaria* (CIAT 1993) and various tropical and subtropical crops (Hepperly *et al.* 1982; Galindo *et al.* 1983; Yang *et al.* 1990).

The fungus can survive for long periods in the soil or on plant debris as sclerotia, which are first seen as white masses on infected tissues. As they mature, the sclerotia turn brown and become loosely attached. They are then easily shed, thus forming the primary source of inoculum.

The use of resistant cultivars remains the cheapest and most effective method of controlling the disease. *Brachiaria* accessions react differently to foliar blight in the field, but the assessment of such differences is unreliable because of variability in environmental conditions and inoculum distribution. To identify true host-plant resistance, a uniform and reproducible inoculation and screening method is required. To meet this need, we developed a fast and effective method for inoculating *Brachiaria* accessions with *R. solani* in the field and glasshouse to identify those with resistance.

Materials and methods

Inoculum production and culture storage

Sclerotia of *R. solani* AG-1 were produced in a PSY broth, made of 20 g peptone, 20 g sucrose, 5 g yeast extract and 1 L deionised water (McCoy

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and Kraft 1984). Mycelial discs, 4 mm in diameter, were taken from a 4 to 5-day-old culture of *R. solani* CIAT 6780 grown on potato-dextrose agar (Difco). One disc was added to each of several 250-ml Erlenmeyer flasks, each containing 30 ml PSY. The flasks were wrapped with aluminium foil and incubated as still cultures at room temperature (about 25 °C) for 10 days. Sclerotia were harvested with sterile forceps that separated them from the mycelial mats. They were then air-dried overnight on sterile Whatman filter paper in a laminar flow hood. Dry sclerotia were placed in sterile glass tubes and stored at 4 °C.

Field site and inoculation

The field trial was established in Caquetá Department, situated in the Colombian Amazon (1° 51'N; altitude 560 m; mean annual rainfall 3552 mm; mean annual temperature 25 °C; mean annual relative humidity 85%). Environmental conditions (high relative humidity and rainfall) were conducive to foliar blight development.

Ten plants of each of 42 *Brachiaria* accessions (Table 1) were transplanted from a CIAT glasshouse to a 5-m single-row plot in the Caquetá site. The 42 accessions included all commercial cultivars that have been released in tropical America, accessions that had been included as parentals in developing *Brachiaria* breeding populations (J. Miles, unpublished data), as well as accessions selected at random. The 42 plots were spaced 2 m apart. One-metre-wide strips of the existing vegetation (predominantly *B. decumbens* pasture) were left between the rows of experimental materials. The 42 entries were replicated 4 times in a randomised complete block design. Plants were inoculated 1 month after transplanting by placing 5–10 sclerotia, depending on the number of tillers, on the soil surface at the base of each plant.

Plant propagation and glasshouse inoculations

Five plantlets of each of 33 *Brachiaria* accessions were separated from tillers and planted in sterile soil in cellular planting flats in the glasshouse at 19–30 °C, using a randomised complete block design in 3 replications. Two weeks after planting, each plantlet was inoculated with one sclerotium placed on the soil surface in contact

Table 1. Accessions of *Brachiaria* species evaluated for *Rhizoctonia* foliar blight in a field site at Caquetá, Colombian Amazon, and in the glasshouse.

Brachiaria species	CIAT no.	Country of origin[1]	Disease rating[2]	Assigned symbols[3]
brizantha	16827	Zimbabwe	6.25 a	F
subulifolia	16961	Zimbabwe	6.10 a	C
brizantha	16139	Ethiopia	6.10 a	J
decumbens	16499	Kenya	6.00 ab	
brizantha	6780*	Zimbabwe	5.91 abc	G
brizantha	16829	Zimbabwe	5.67 abcd	E
ruziziensis	16552	Kenya	5.63 abcd	3
brizantha	16441	Kenya	5.50 abcde	
decumbens	16498	Kenya	5.36 abcde	
decumbens	16500	Kenya	4.92 bcdef	
brizantha	16468	Kenya	4.91 bcdef	
brizantha	16335	Ethiopia	4.83 bcdefg	T
brizantha	26316	Rwanda	4.82 bcdefg	
brizantha	26124	Burundi	4.73 defg	P
brizantha	16139	Ethiopia	4.70 defg	J
decumbens	606*	Uganda	4.67 defg	6
brizantha	16126	Ethiopia	4.67 defg	D
brizantha	16472	Kenya	4.64 defg	
jubata	16531	Kenya	4.36 efgh	M
brizantha	16338	Ethiopia	4.33 efgh	B
jubata	16517	Kenya	4.33 efgh	Y
humidicola	16180	Ethiopia	4.09 fgh	H
brizantha	26646*	ND	4.00 fgh	X
brizantha	16306	Ethiopia	4.00 fgh	A
brizantha	16158	Ethiopia	3.88 fgh	
jubata	16195	Ethiopia	3.83 fgh	N
brizantha	16136	Ethiopia	3.78 fghi	U
brizantha	16457	Kenya	3.70 fghi	I
brizantha	16162	Ethiopia	3.64 ghi	L
brizantha	16122	Ethiopia	3.64 ghi	O
decumbens	16488	Kenya	3.25 hij	R
brizantha	16320	Ethiopia	3.18 hij	4
brizantha	16318	Ethiopia	3.17 hij	S
humidicola	679*	ND	2.67 ijk	5
humidicola	16887	Zimbabwe	2.36 jkl	Z
humidicola	16876	Zimbabwe	1.91 klm	V
humidicola	6369	ND	1.67 klm	7
brizantha	16312	Ethiopia	1.58 klm	K
dictyoneura	6133*	Zambia	1.55 klm	2
humidicola	16868	Zimbabwe	1.36 lm	1
humidicola	16888	Zimbabwe	1.25 lm	Q
humidicola	16886	Zimbabwe	0.82 m	W

[1] ND = Country of origin not determined.

[2] Disease severity in the field was evaluated by visual estimation based on the Horsfall-Barratt rating system, where 0 = 0% (no blight), 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–93%, 9 = 94–97%, 10 = 98–99%, and 11 = 100% (all blighted). Values followed by the same letter are not significantly different from each other (P = 0.05) according to Duncan's multiple range test.

[3] Accessions with no assigned symbols (letters or numbers) were not included in the glasshouse tests. Assigned symbols are short codes representing each *Brachiaria* accession.

* Commercial cultivars.

with the plantlet's stem. Humidity was maintained at 100% by placing the plantlet in a plastic box with one side made of cheesecloth, and immersing the whole in a tray of water. Nine of the accessions found susceptible in field tests were not included in the glasshouse tests, which were more rigorous.

Disease evaluations

Plants in the field were evaluated for disease reaction 1 month after inoculation, using the method of Horsfall and Barratt (1945). Plants in the glasshouse were evaluated 2 weeks after inoculation, following a more detailed measurement of percentage of leaves infected, percentage of leaf area infected, and number of sclerotia on infected tissues. Data were analysed, using Duncan's multiple range test, to identify accessions with low disease scores and, thus, moderate-high levels of resistance.

Results

Inoculum production

The *R. solani* CIAT 6780 in PSY broth produced mature sclerotia within 9–13 days. Sclerotia with a diameter of more than 250 μm were used for inoculation (Figure 1). These sclerotia had a 100% germination rate on acidified-water agar (pH 4.8). In the glasshouse tests, white mycelial strands were seen protruding from germinating sclerotia within 3 days.

Disease evaluation and sources of resistance

In the glasshouse, within a week of inoculation, plants showed foliar blight symptoms, beginning as oblong, ellipsoid, or circular, water-soaked spots. With time, the centre of each spot became bleached and an irregular brown border appeared around the spot (Figure 1). All inoculated plants expressed symptoms, indicating that there were no disease escapes.

Accessions differed in their reactions to foliar blight. Most *B. brizantha* accessions exhibited severe foliar blight, followed by sclerotia formation on infected tissues, thus producing inoculum for several cycles of disease (Figures 1 and 2). Only a few of the other *Brachiaria* accessions showed consistently low disease scores on a combination of criteria, indicating a moderate level of resistance in both glasshouse and field. These were *B. humidicola* CIAT 16868, 16886 and 6369; and *B. dictyoneura* CIAT 6133 (cv. Llanero) (Figures 3, 4 and 5; Table 1). Accessions that showed low-moderate levels of resistance were *B. humidicola* CIAT 679, 16876, 16887 and 16888; and *B. brizantha* CIAT 16320.

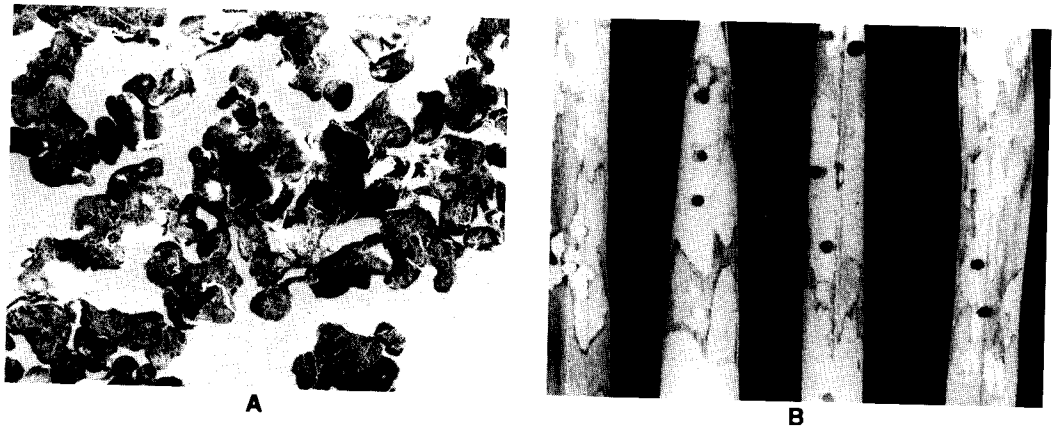

Figure 1. (A) Mature *Rhizoctonia solani* sclerotia produced in a broth of peptone, sucrose and yeast extract. (B) Typical symptoms of foliar blight disease with sclerotia loosely attached to infected leaves of *Brachiaria brizantha* CIAT 6780.

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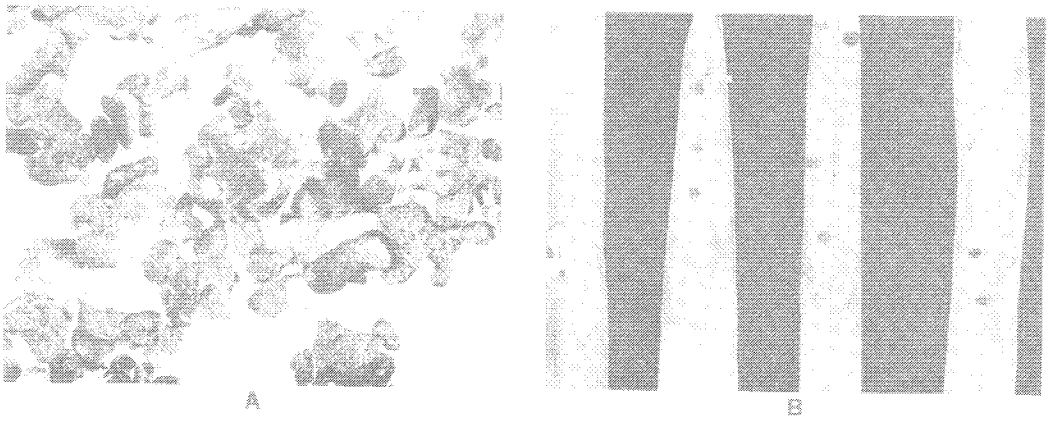

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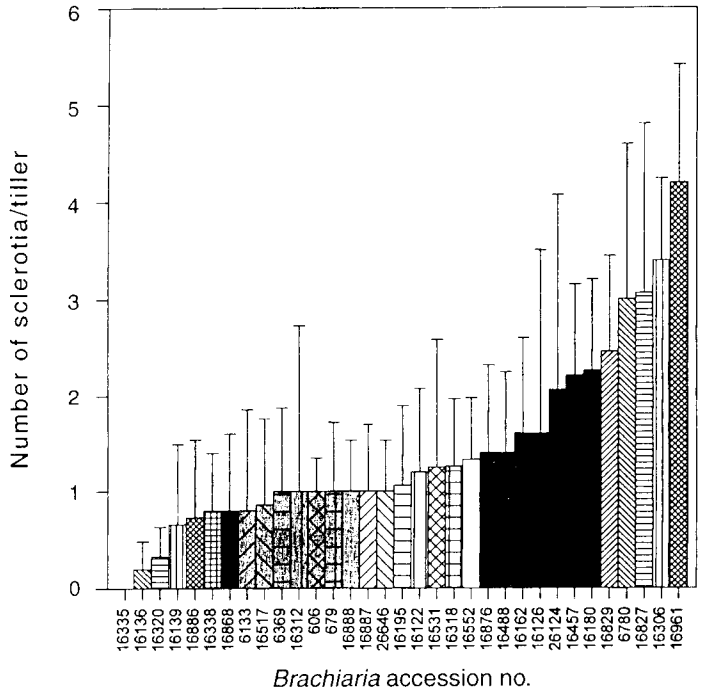

Figure 2. *Rhizoctonia solani* sclerotia production on infected tissues of *Brachiaria* accessions in glasshouse tests. Bars indicate standard deviation.

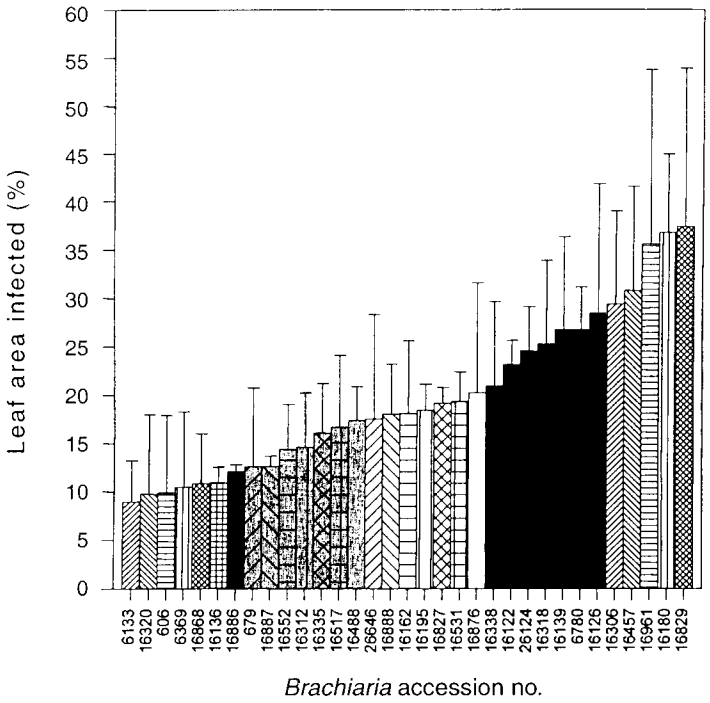

Figure 3. Percentage of leaf area infected with foliar blight in *Brachiaria* accessions in glasshouse tests. Bars indicate standard deviation.

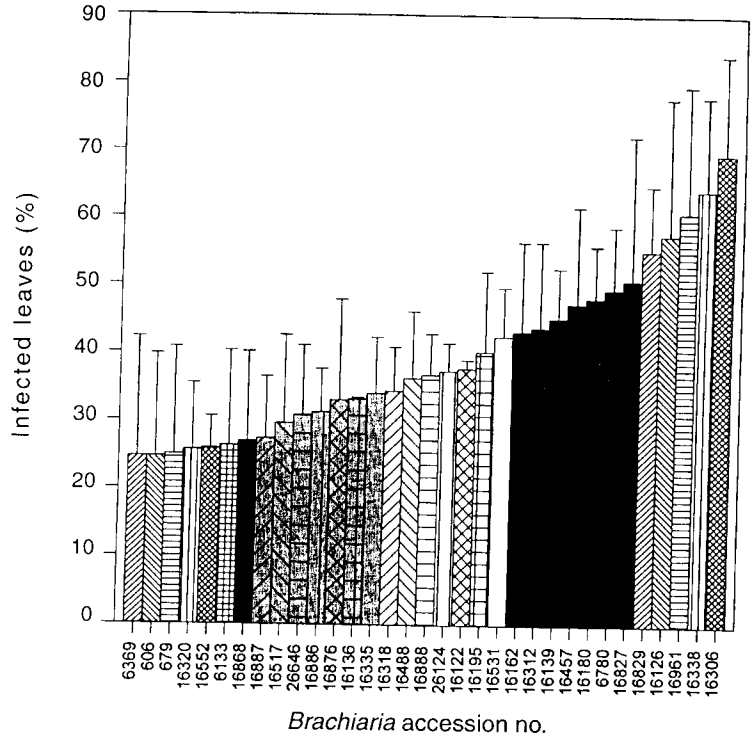

Figure 4. Percentage of leaves infected with foliar blight in *Brachiaria* accessions in glasshouse tests. Bars indicate standard deviation.

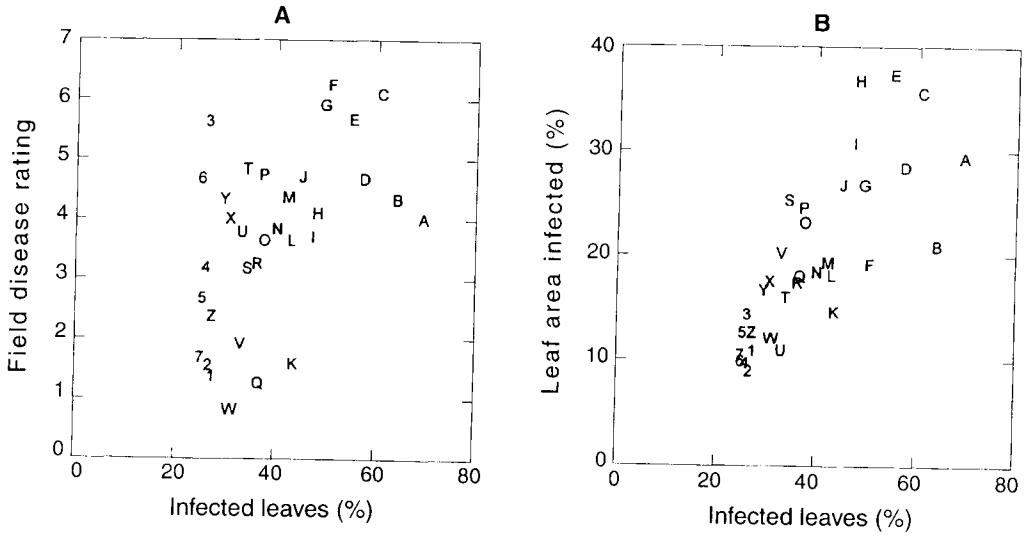

Figure 5. Correlations of: (A) field ratings of foliar blight and percentage of infected leaves in the glasshouse (correlation coefficient, r = 0.49; P = 0.004); (B) percentage of infected leaf area in the glasshouse and percentage of leaves infected with foliar blight (correlation coefficient, r = 0.77; P <0.001). Letters and numbers represent *Brachiaria* accessions — see Table 1. Disease ratings in the field were conducted by visual estimation based on the Horsfall-Barratt system, where 0 = 0% (no blight), 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–93%, 9 = 94–97%, 10 = 98–99%, 11 = 100% (all blighted). In the glasshouse, all infected leaves were counted to determine the percentage of leaves infected with foliar blight.

Discussion

Control of foliar blight through cultural practices such as crop rotation and destruction of infected plant debris is not feasible for perennial pasture plants such as *Brachiaria* grasses. Thus, the use of resistant plant material remains the cheapest and cleanest method of plant disease control. To reliably identify sources of resistance, a reproducible and uniform method of inoculation is crucial. The inoculation method described in this study is rigorous and allowed no disease escapes. Virulent inoculum and optimal environmental conditions, such as high relative humidity for foliar blight, are basic to successful inoculation. McCoy and Kraft (1984) showed that virulence of sclerotia of *R. solani* is directly affected by sucrose and peptone concentrations in the medium. In our initial inoculation method experiments, we found that sclerotia produced on potato-dextrose agar were less virulent on *Brachiaria* plants than those produced in PSY broth (data not shown).

Highly susceptible accessions usually supported the formation of a large number of fungal sclerotia under high humidity. These sclerotia dislodged and accumulated in the soil, thus providing sources of inoculum for subsequent disease cycles. On some resistant accessions, sclerotia were not produced, even though the plants were not completely free of disease, thus reducing the production of primary inoculum. Lee and Rush (1983) found that growing rice cultivars resistant to sheath blight, which is also caused by *R. solani*, can reduce sclerotia production.

Disease severity was usually higher in the glasshouse than in the field, probably because of the high humidity maintained or reduced microbial competition for *R. solani* in the steam-sterilised soil, or a combination of these factors.

Although no data are available on the effect of foliar blight on feed quality of *Brachiaria*, sheath blight in rice is known to affect the quality of the grain (Marchetti 1983).

We selected resistant accessions on the following bases: (i) reduced numbers of fungal sclerotia formations on infected plant tissues; (ii) degree of the disease's progress upward from the inoculation point at the soil line; (iii) percentage of diseased leaves; and (iv) percentage of leaf area infected. Accessions with moderate levels of disease resistance have coarse and spiky leaf textures, and highly susceptible accessions have smoother leaves, leading us to conclude that resistance to foliar blight in *Brachiaria* spp. may be correlated with plant morphology and texture.

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