An endophyte of the tropical forage grass *Brachiaria brizantha*: Isolating, identifying, and characterizing the fungus, and determining its antimycotic properties

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Abstract: *Brachiaria*, predominantly an African genus, contains species, such as *B. brizantha*, an apomictic C_4 grass, that are commercially important forage grasses in tropical America, where they now cover about 55 million hectares. From *B. brizantha* accession CIAT 6780, we isolated an endophytic fungus that may be economically significant. The fungus was identified as *Acremonium implicatum* (J. Gilman & E.V. Abott). 18S rDNA and ITS rDNA sequences were used to characterize isolates of the endophyte, and showed that they belonged to the *Acremonium* genus, being close to *A. strictum* and *A. kiliense*. Using the random amplified polymorphic DNA (RAPD) technique, involving arbitrary primers of 10 bases, we showed that the isolates were highly similar to each other. Antiserum produced from a monoconidial culture of *A. implicatum* isolated from *B. brizantha* 6780, differentiated the isolates consistently in line with the DNA data. When we compared endophyte-free with endophyte-infected *B. brizantha* CIAT 6780 plants, both artificially inoculated with the pathogenic *Drechslera* fungus, we found that the endophyte-infected plants had fewer and smaller lesions than did the endophyte-free plants. Sporulation of *Drechslera* sp. on artificially inoculated leaf sheath tissues was also much less on tissue infected with the endophyte.

Key words: endophytes, *Brachiaria*, *Acremonium*, biological control.

Résumé : *Brachiaria* est un genre principalement Africain qui contient des espèces, telles que *B. brizantha*, une herbe C_4 apomictique, représentant des herbes de fourrage commercialement importantes dans l'Amérique tropicale, où elles couvrent à ce jour environ 55 millions d'hectares. Nous avons isolé à partir de *B. brizantha* référence CIAT 6780 un champignon endophytique qui pourrait être commercialement important. Le champignon fut identifié comme étant *Acremonium implicatum* (J. Gilman & E.V. Abott). Les séquences des ADNr 18S et ADNr ITS furent utilisées afin de caractériser les isolats de l'endophyte. Les séquences démontrèrent qu'ils appartenaient au genre *Acremonium*, et étaient apparentés à *A. strictum* et à *A. kiliense*. En utilisant la technique du RAPD (ADN polymorphe amplifié au hasard), impliquant des amorces arbitraires de 10 bases, nous avons montré que les isolats étaient fort semblables entre eux. Un antisérum produit par une culture monoconidiale de *A. implicatum* isolé de *B. brizantha* 6780 a permis de différentier les isolats correctement, en harmonie avec les données d'ADN. Lorsque nous avons comparé les plants de *B. brizantha* 6780 infectés par l'endophyte avec celui dépourvu d'endophyte, les deux groupes ayant été inoculés avec le champignon pathogène *Drechslera*, nous avons découvert que les plants infectés avec l'endophyte avaient de plus petites et moins nombreuses lésions, comparativement aux plants sans endophyte. La sporulation de *Drechslera* sp. sur des tissus de gaine de feuille inoculées artificiellement était aussi diminuée de beaucoup chez les tissus infectés avec l'endophyte.

Mots clés : endophytes, *Brachiaria*, *Acremonium*, contrôle biologique.

[Traduit par la Rédaction]

Introduction

Brachiaria is predominantly an African genus, comprising about 100 species, some of which (including *B. brizantha*) have become commercially important forage grasses in tropical America. An estimated 55 million hectares are planted with *Brachiaria*, with Brazil (IBGE 1995) growing the most on an estimated 45 million hectares. Several species of *Brachiaria* are apomictic and reproduce asexually through seed (Miles and Valle 1991). This form of reproduction allows even heterozygous plant genotypes to breed true through seed. Apomictic reproduction also has many advantages for endophyte research and utilization. Almost all seeds of an endophyte-infected apomictic plant would contain not only the endophyte, but would also be genetically

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identical to each other. After treatment to eliminate the endophyte, endophyte-free seedlings can be compared with genetically identical endophyte-infected seedlings.

The popular spittlebug-resistant *B. brizantha* cv. Marandu was released in 1984 in Brazil by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). It originated from germplasm introduced to Brazil from the Zimbabwe Grasslands Research Station at Marondera (Keller-Grein et al. 1996). Spittlebugs, the most damaging pests of *Brachiaria* in tropical America, can cause complete loss of available forage. Endophytes may possibly be a component of the resistance mechanism or mechanisms employed by cv. Marandu (Rodrigues and Dias 1996).

In South America, several livestock disorders have been associated with cattle grazing *Brachiaria* pastures. These include "fallen cow," which affects cows in late gestation or early lactation and grazing *B. decumbens* cv. Basilisk (signalgrass), the most widely grown pasture in humid and subhumid tropics (Lascano and Euclides 1996); and "swollen face," which occurs in horses grazing *B. humidicola* pastures in the Brazilian *Cerrados*. Deaths have been reported in Brazil, Venezuela, and Colombia. For example, in Brazil, livestock grazing signalgrass (*B. decumbens*) suffer a hepatic disorder that particularly affects young animals, causing them to lose weight and even die (Santos Filho 1996). In the State of Mato Grosso, Brazil, sheep grazing signalgrass have also suffered poisoning and death. Symptoms included swelling and dermatitis of the face, ears, and eyelids, and blindness (de Lemos et al. 1996). The exact cause or causes of these syndromes are still unknown, but endophytic fungi may possibly be involved (Lascano and Euclides 1996).

The economic importance of *Brachiaria* pastures and the associated livestock disorders were the reasons for initiating this work.

Endophytic fungi form nonpathogenic and intercellular associations with grasses and sedges, completing their entire life cycle within the plants' aerial portions. Many grasses harbor endophytic fungi (Clay 1990), which are among the most widely used biological plant protection agents for forage and turf grasses (Clay 1989; Funk et al. 1993; Gwinn and Gavin 1992; Kimmons et al. 1990; Rowan and Latch 1994). Endophyte-infected grasses also possess other properties of applied value such as growth stimulation, improved survival, and drought tolerance (Arechavaleta et al. 1989).

Considerable research has been conducted in recent years on the role of endophytes in infected temperate grasses. The greatest impact of endophyte-infected grasses is to reduce livestock productivity. Whether this is true for tropical forage grasses such as *Brachiaria* is not known because little research has so far been conducted. Nevertheless, studies have shown that various endophytic fungi inhabit native savanna grasses (Koga et al. 1995) and introduced forage grasses, including *Acremonium* endophytes in *Brachiaria* species (Kelemu and Takayama 1998), and *Acremonium*-like endophytes in *B. brizantha* cv. Marandu (Rodrigues and Dias 1996).

A leaf spot disease, caused by *Drechslera* sp., affects some *Brachiaria* species in parts of Colombia (Lenné 1990). An endophytic fungus, *Acremonium implicatum* (J. Gilman & E.V. Abbott) W. Gams, was isolated from the *B. brizantha* accession No. CIAT 6780. In this study, we report on the isolation, identification, and characterization of some isolates of *A. implicatum* from *Brachiaria* and the effect the endophyte has on the forage when the latter is infected by *Drechslera* sp. Defining the role endophytes play in the health of tropical grasses will contribute significantly to our understanding of the endophyte/C_4 grass symbiosis.

A preliminary report of parts of this work has already been published (Kelemu and Takayama 1998).

Materials and methods

Plant tissue staining, fungal isolations, and culture maintenance

Tissues (leaves or leaf sheaths) of *B. brizantha* were collected from visually symptom-free, healthy plants. Small pieces of the tissues were placed in tubes containing Carnoy's solution (6:3:1 ethyl alcohol : chloroform : 85% glacial acetic acid) for at least 24 h. They were then transferred to 70% aqueous ethyl alcohol twice, each for 24 h to remove chlorophyll. The tissues were further cut into small pieces no longer than 1.0 cm and stained with aniline blue (2:1 in 70% aqueous ethyl alcohol : 85% lactic acid) for 5–18 h, depending on the tissue's age and origin. The stained tissues were cleared by transferring them sequentially in solutions of 100% ethyl alcohol (2 × 60 min); 1:3 methyl salicylate : ethyl alcohol (60 min); 1:1 methyl salicylate : ethyl alcohol (60 min); 3:1 methyl salicylate : ethyl alcohol (60 min); and 100% methyl salicylate (60 min). The samples were then examined at ×200, using bright-field microscopy. Samples which appeared to have intercellularly growing hyphae were noted, and plants from which the samples originated were used for further fungal isolation work.

Small pieces (5 mm) of tissues were surface sterilized in 3.25% NaOCl solution for 10 min, in 70% ethanol for 1 min, and rinsed three times with sterilized distilled water. Excess moisture was removed from the samples with sterilized filter papers. They were then plated on two different media—potato-dextrose agar (PDA, Difco) and corn meal agar (CMA, Difco)—and incubated for 4 to 6 weeks at 28°C.

Cultures were effectively maintained either on PDA supplemented with 10 µg/mL tetracycline, or, for long-term storage, by lyophilization. Fungal cultures were prepared by growing the fungus on sterilized filter papers overlaid on PDA. Once the fungus fully covered them, the filter papers were carefully removed from the agar and air-dried. They were placed in envelopes and stored at –20°C. Pieces of filter paper carrying fungal mycelia can be plated on fresh agar media whenever needed for DNA extractions.

DNA amplification and sequencing

Fresh mycelium was lifted off cellulose acetate sheets overlaid on PDA and ground in liquid nitrogen. Genomic DNA was extracted, using the Easy-DNA™ Kit Protocol #3 (Invitrogen 1997) and cleaned further, using Dneasy™ Protocol for Animal Tissues (Qiagen 1999). The internal transcribed spacer region (ITS) and partial small subunit rDNA (18S) regions were amplified from 2 µL of undiluted genomic DNA in a 100-µL reaction, using previously published protocols (White et al. 1990). Each reaction contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 12.5 pmol each dNTP, 50 pmol each primer, and 2 U *Taq* polymerase (Desai and Pfaffle 1995). PCR (30 cycles) was carried out in a GeneAmp 9600 thermocycler (Perkin Elmer Corporation, Foster City, Calif.) set to 95°C for 10 s, 56°C for 30 s, and 72°C for 2 min. Initial denaturation was conducted at 95°C for 1 min with a final extension for 10 min at 72°C. Successful PCR products were cleaned of primers and salts, using the QIAaquick PCR Purification Kit protocol (Qiagen 1997). AmpliTaq® FS cycle sequencing reactions (Perkin Elmer Corporation 1995) were prepared accord-

ing to the manufacturer's protocol, using the ITS and partial small subunit template (White et al. 1990). Reactions were analyzed on an ABI 373A Automated DNA Sequencer (Perkin Elmer Corporation, Foster City, Calif.).

Six arbitrary 10-base oligonucleotide primers from Operon Technologies [5′-AATCGGGCTG-3′ (primer code A-04), 5′-CTGTCATGCC-3′ (AK-14), 5′-TCGCAGCGAG-3′ (AK-19), 5′-ACGGGTCAGA-3′ (AJ-01), 5′-AGCACCTCGT-3′ (AJ-03), and 5′-CAGCGTTGCC-3′ (AJ-05)] were also used for PCR amplifications. The amplification products were resolved by electrophoresis in a 1.2% agarose gel (Bio-Rad), stained with ethidium bromide, and photographed under UV lighting.

Phylogenetic analysis

For Phylogenetic analysis the following sequences were obtained from GenBank: *Acremonium alabamense* (U43969), *A. alternatum* (U43970), *A. chrysogenum* (U43971), *A. furcatum* (U43972), *A. kiliense* (U43973), *A. murorum* (U43966), *A. rutilum* (U43967), *A. strictum* (U43968), *Ascosphaera apis* (M83264), *Atkinsonella hypoxylon* (U44034), *Balansia aristidae* (U44035), *B. henningsiana* (U44036), *B. obtecta* (U44037), *B. sclerotica* (U32399), *B. strangulans* (U44038), *Candida tropicalis* (M55527), *Ceratocystis fimbriata* (U32418), *Chaetomium* sp. (U44039), *Claviceps purpurea* (U44040), *Cordyceps capitata* (U44041), *Daldinia concentrica* (U32402), *Diaporthe phaseolorum* (L36985), *Echinodothis phaseolorum* (U44042), *Emericellopsis minima* (U44043), *E. terricola* (U44112), *Epichloë amarillans* (U35034), *E. baconii* (U57662), *E. festucae* (U44113), *Eurotium rubrum* (U00970), *Hypocrea pallida* (U32408), *H. polyporinus* (U32410), *Microascus trigonosporus* (L36987), *Monascus purpureus* (M83260), *Myriogenospora atramentosa* (U44114), *Nectria cinnabarina* (U32412), *N. viridescens* (U44116), *Neocosmospora vasinfecta* (U44117), *Neotyphodium coenophialum* (U45942), *N. uncinatum* (U45943), *Saccharomyces cerevisiae* (M27607), *Taphrina deformans* (U00971), and *Xylaria hypoxylon* (U20378).

The GCG programs Gap, Pileup, and the SeqLab interface for the Wisconsin Package v. 9.1 (Genetics Computer Group (GCG), Madison, Wis.) were used to analyze sequences, generate alignments, and make manual adjustments. The PAUP v. 4.0b2 for Macintosh (Swofford 1999) was used for phylogenetic analysis. Heuristic searches were performed, using maximum parsimony as previously described (Reddy et al. 1998), starting trees via stepwise addition (100 replicates) with other options in default mode. Bootstrapping, using the same criteria with 500 replicates, was performed to determine the confidence levels of the inferred phylogenies.

Antiserum

Antiserum was produced by BioWorld (Ohio), using a monoconidial culture of *A. implicatum* isolate 6780 in rabbits. The antiserum was purified (McLaughlin et al. 1981; Voller et al. 1976) and conjugated with alkaline phosphatase, using standard procedures (Lister and Rochow 1979).

Eliminating endophytes with Folicur

Endophyte-free clones were generated, using the fungicide Folicur® (common name: tebuconazole; chemical name: (RS)-1-p- = chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol). Twenty plantlets were propagated from the original mother plant already infected with the endophyte. Half of these plantlets were soaked in a solution of 0.1 mL/L of Folicur (250 g a.i./L) for 6 h (Bacon and White 1996) to eliminate the endophyte, and the other half were left untreated. Both treated and untreated tillers were individually planted in small pots. Leaf sheath and leaf blades were examined 4 to 6 weeks later for the presence of endophytic mycelium. These genetically identical plants were then challenged

Fig. 1. Scanning electron microscope photographs of *Acremonium implicatum*. (A) Attached conidium within *Brachiaria brizantha* leaf sheath tissue. (B) Hyphae of *A. implicatum* in *B. brizantha*. (C) Attached conidium from a pure culture isolated from leaf sheath tissue of *Brachiaria*.

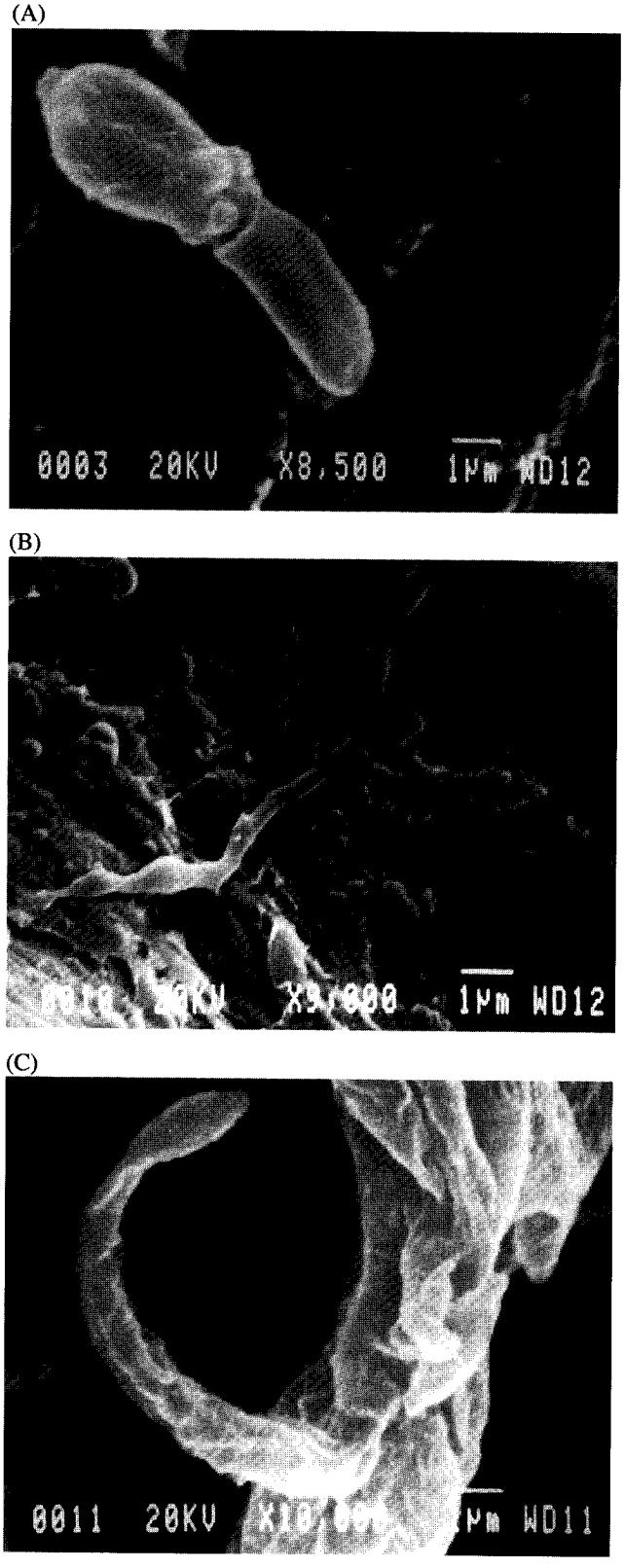
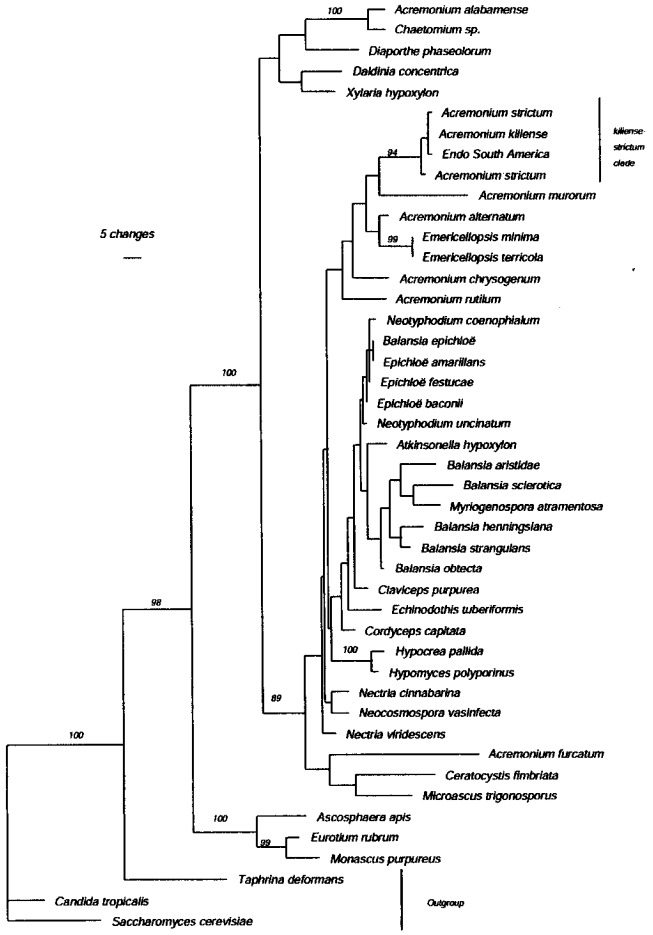


Fig. 2. Phylogentic relationship of *Acremonium implicatum* isolated from *Brachiaria brizantha* to other endophytic fungi, deduced from sequences of the partial small subunit rDNA (18S rDNA). Numbers along branches indicate branch lengths.



with *Drechslera* sp., a causal agent for one of the leaf spot diseases.

Inoculation and disease evaluation

Drechslera conidial suspensions were prepared with sterilized distilled water, adjusted to a concentration of 9.0×10^4 condia/mL, and sprayed onto detached plant tissue until wet. Or, pieces of filter papers, soaked with conidial suspension, were clipped onto leaf blades of potted plants. Plants were kept in humidity chambers for 48 h, then transferred to the greenhouse until symptoms were expressed. Parameters of disease lesions were transferred onto overhead transparencies and scanned. The length and area of lesions were then measured, using the WinRHIZO image analyzer software, v. 3.6 D (Regent Instruments, Canada).

In vitro inhibition tests

An agar disk of *A. implicatum* was placed in the middle of a sterilized filter paper that overlaid PDA. This was incubated at 28°C until about one fourth of the filter paper was covered with mycelia and (or) conidia. The filter paper carrying *Acremonium* was then carefully removed. An agar disk of *Drechslera* sp. was placed in the center of each plate from which the filter paper carrying *Acremonium* sp. was removed. Control plates contained agar disks of *Drechslera* sp. in the absence of *Acremonium* or its products. When *Drechslera* mycelia covered the control plates, measurements were taken. Plates were scanned and areas of *Drechslera* growth recorded, using the WinRHIZO image analyzer, v. 3.6 D (Regent Instruments, Canada).

A second method was used to determine the presence of antifungal activity of *A. implicatum* when grown in vitro. PDA containing petri plates were inoculated with a colony of the endophyte on one side of each plate and incubated for 2 weeks. Two weeks later, an agar disk of *Drechslera* sp. (4 mm diameter) removed from the actively growing edge was placed on the opposite side of each plate. The plates were further incubated and when *Drechslera* mycelia covered the control plates, measurements were taken as described above. All tests were replicated three times.

Results and discussion

Isolation, identification, and characterization of the endophyte

The appearance of fungal colonies on PDA or CMA frequently took as long as 5 weeks. One colony type was consistently isolated. The colony was white, cottony, and slow-growing. Colony characteristics on CMA are similar to those on PDA. The fungus sporulated on both CMA and PDA. This isolate produced individual conidiogenous cells on vegetative hyphae. The conidiogenous cells had conidia from their apex by a replacement wall-building apex system with percurrent proliferation, and the conidia adhered in weak unbranched chains with the youngest at the base. The vegetative hyphae were hyaline, smooth, and 1.5–2 μm wide.

The fungus was identified as *A. implicatum* (J. Gilman & E.V. Abbott) W. Gams (Figs. 1A–1C), previously known as *Paecilomyces terricola* (Miller et al.) Onions and Barron and as *A. terricola* (Miller et al.) W. Gams. The International Mycological Institute, U.K., confirmed the identity of the isolates as being *A. implicatum*. The same fungus was also isolated from *B. decumbens* accession No. CIAT 606 and *B. arrecta* accession No. CIAT 16845. It has been reportedly isolated from soils and a wide range of plant substrates (Bilgrami and Prasad 1998).

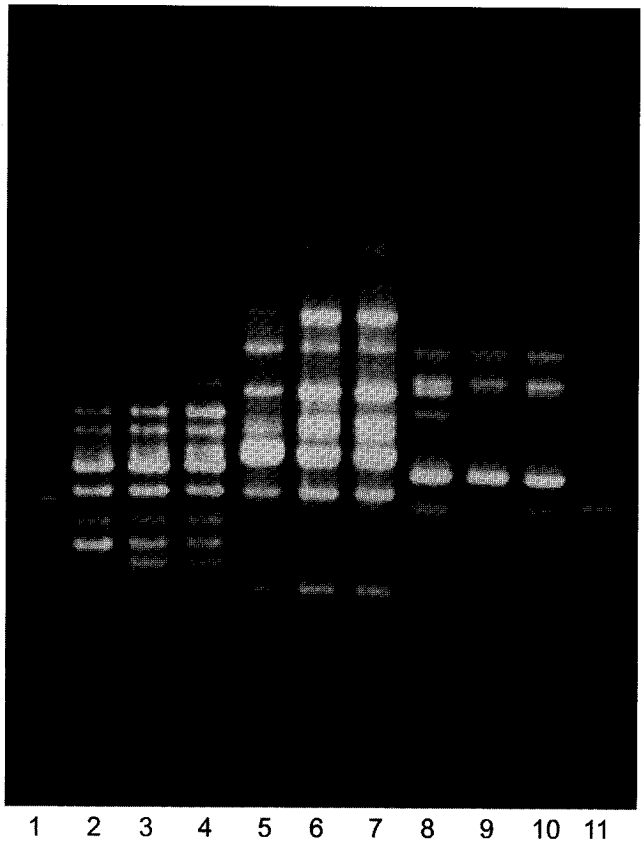
We used 18S rDNA (primers NS1 and NS6) and ITS rDNA (primers ITS4 and ITS5) sequences (White et al. 1990) to characterize two isolates of endophytic fungi, which were isolated from *B. brizantha* accession No. CIAT 6780. We found that they clearly belonged to the *Acremonium* genus, being close to *A. strictum* and *A. kiliense* (Fig. 2). The ITS rDNA sequence results showed that the isolates from *B. brizantha* are very similar to each other (data not shown). The random amplified polymorphic DNA (RAPD) technique, involving arbitrary primers of 10 bases, also showed that the isolates were highly similar to each other (Fig. 3). Results from the antiserum, produced from a monoconidial culture of an isolate of *A. implicatum* were consistent with the DNA data (Table 1).

The cultures have been submitted to the American Type Culture Collection.

In vitro inhibition of *Drechslera* sp. by *Acremonium implicatum*

The two in vitro assays indicated that the *Acremonium* fungus has a strong antibiotic effect, inhibiting growth in *Drechslera* spp., an important pathogen of a wide range of grasses (Fig. 4). In the first method where the fungal patho-

Fig. 3. Random amplified polymorphic DNA profiles of three monoconidial isolates of *Acremonium implicatum* from (A) *Brachiaria decumbens* accession No. CIAT 606 (lanes 2, 5, and 8); (B) *B. brizantha* accession No. CIAT 6780 (lanes 3, 6, and 9); and (C) *B. arrecta* accession No. CIAT 16845 (lanes 4, 7, and 10). Lanes 1 and 11 are repetitions of a 100-bp marker. Arbitrary primers A-04 (5′-AATCGGGCTG-3′) in lanes 2, 3, and 4; AK-14 (5′-CTGTCATGCC-3′) in lanes 5, 6, and 7; and AK-19 (5′-TCGCAGCGAG-3′) in lanes 8, 9, and 10 were used.



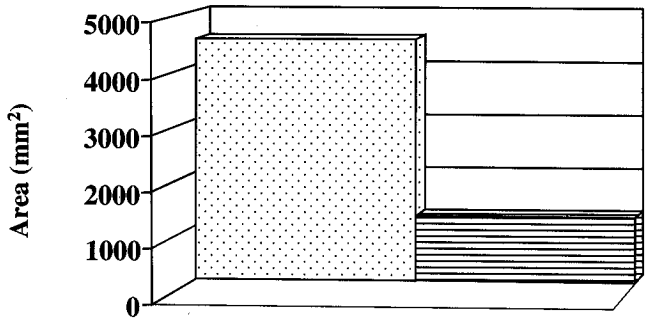
gen was placed directly on agar with diffusible substance(s) from the endophyte, very little or no growth was recorded (data not shown). In the second, growth of *Drechslera* ceased when the colony margin approached some distance from the endophyte, indicating the release of some diffusible substance(s) by the endophyte into the agar medium (Fig. 4). Isolates of *Acremonium* spp., and *Epichloë typhina* grown in culture have been reported to have antifungal activity (Christensen et al. 1991; Christensen and Latch 1991; Siegel and Latch 1991). White and Cole (1985) reported the capacity of *A. coenophialum* (= *Neotyphodium coenophialum*), an endophyte of *Festuca arundinacea*, to inhibit some soil fungi in vitro. Application of some dominant phylloplane fungal spores on barley leaves, either collectively or individually, inhibited disease lesion development by *Drechslera graminea*, but no significant effect was observed in vitro (Rai and Singh 1980). Using a dual-culture in vitro test, Christensen (1996) demonstrated inhibition of plant pathogens *Drechslera erythrospila* and *Rhizoctonia zeae* by strains of *Acremonium* spp., and *Epichloë festucae*. Our results are compatible with those reported by Christensen (1996).

Table 1. Absorbance values of 60-min DAS-ELISA reactions of isolates of *Acremonium implicatum*, using antiserum produced against a monoconidial culture of *A. implicatum* from *Brachiaria brizantha* accession No. CIAT 6780.

Treatment	Absorbance at 405 nm
Blank control	0.197
Control nutrient broth	0.147
A. implicatum[a]	1.505
A. implicatum[b]	1.567
A. implicatum[c]	1.670
Hyalodendron sp.[d]	0.100

[a]Isolated from *B. brizantha* CIAT 6780.
[b]Isolated from *B. arrecta* CIAT 16845.
[c]Isolated from *B. decumbens* CIAT 606.
[d]Isolated from *B. brizantha* CIAT 26110.

Fig. 4. Mycelial growth area measurements of *Drechslera* sp. in the presence (▤) or absence (▨) of *Acremonium implicatum* on potato-dextrose-agar plates.



Disease reaction

Results indicate that *A. implicatum* protects *B. brizantha* CIAT 6780 from infections by *Drechslera* sp. Endophyte-infected plants had fewer and smaller lesions than endophyte-free plants (Figs. 5 and 6). Resistance to pathogens associated with endophyte infection of temperate grasses has been documented (Gwinn and Gavin 1992; Kimmons et al. 1990; West et al. 1988), but no similar reports existed before now in the literature on tropical grasses.

Fungal sporulation on *Brachiaria* tissue

One dramatic finding was the difference between fungal sporulation on endophyte-infected and that on endophyte-free leaf sheaths of *B. brizantha*. Abundant sporulation of *Drechslera* sp. was observed on artificially inoculated pieces of leaf sheaths of endophyte-free plants on moist filter paper kept in petri dishes. In contrast, endophyte-infected leaf sheaths showed little sporulation and hyphal development (Fig. 7).

Clay (1994) stated, "It is noteworthy that the seed-borne *Acremonium* (Link) Fr. endophytes are known only from the cool-season [*grass*] subfamily Pooideae with the C_3 photosynthetic mechanism." To the best of our knowledge, this is the first report on the (1) presence of an *Acremonium* endophyte in a C_4 grass, that is, *Brachiaria* sp.; and (2) the ef-

Fig. 5. Endophyte-infected (left) and endophyte-free (right) tissues of *Brachiaria brizantha* both challenged with the pathogen *Drechslera* sp.

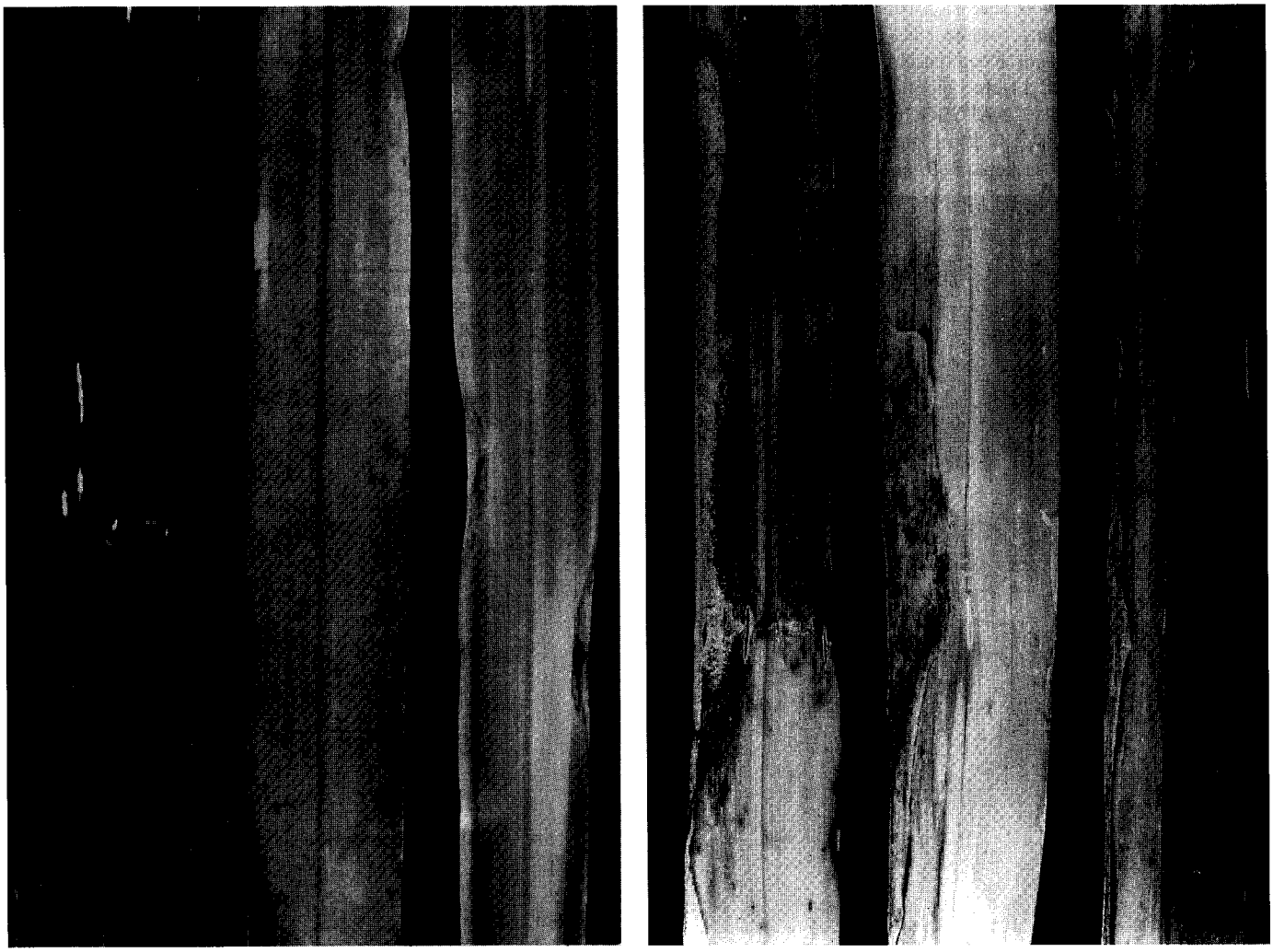
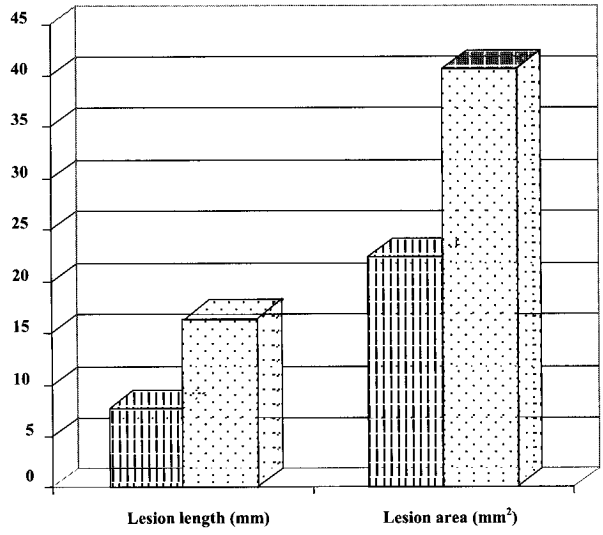


Fig. 6. Lesion sizes measured in endophyte-infected (▥) and endophyte-free (▨) plants of *Brachiaria brizantha* inoculated with *Drechslera* sp.



fect of this endophyte on a pathogen of *Brachiaria.* Rodrigues and Dias (1996) reported the finding of *Acremonium*-like fungi in *B. brizantha* cv. Marandu in the State of Pará in the Brazilian Amazonia. However, they had not conclusively established the existence of species of *Acremonium* in *Brachiaria.*

Practical implications

Several disorders have been reported in livestock grazing *Brachiaria* pastures in South America. The cause or causes of these disorders are still unknown, and the possible involvement of endophytic fungi needs to be checked out.

The economic importance of *Brachiaria* is well recognized in tropical America (Miles et al. 1996). A significant constraint to *Brachiaria* production is damage by spittlebugs (Homoptera: Cercopidae), which can cause complete forage loss (Valério et al. 1996). Some *Brachiara* species can resist spittlebugs, although the mechanism of resistance is poorly understood. According to Lapointe et al. (1992), the forage contains a toxin or antifeedant that deters insect feeding during nymphal stadia, leading to death by starvation. Whether this phenomenon involves endophytes remains to be seen.

Fig. 7. Endophyte-infected and endophyte-free leaf sheaths of *Brachiaria brizantha* accession No. CIAT 6780 inoculated with the pathogen *Drechslera* sp. Tissues on the left are infected with the endophyte *Acremonium implicatum*.

Work on the role of this endophytic fungus in other aspects of disease and pest control, and in drought resistance and other traits is in progress.

Acknowledgements

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