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THE ROLE OF ENDOPHYTIC FUNGI IN *BRACHIARIA*, A TROPICAL FORAGE GRASS

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Abstract

In temperate zones, endophytic fungi are widely used as biological protection agents for forage and turf grasses. They form nonpathogenic and intercellular associations with grasses and sedges, completing their entire life cycle within the plants' aerial parts. Our surveys and studies confirmed that various endophytic fungi, including *Acremonium* spp., also inhabit native savanna grasses and introduced tropical forage grasses. We are now determining the potentially symbiotic relationships between these fungi and C₄ tropical forages, specifically between the endophyte *A. implicatum* and *Brachiaria* grasses. We treated half of a group of genetically identical clones of *Brachiaria* with fungicide to generate endophyte-free plants. So far, we have found that, under severe water stress, endophyte-infected plants of *B. arrecta* CIAT 16845 maintained better leaf expansion and produced significantly more leaf biomass than did clean plants. We also found that the endophyte protects *B. brizantha* from pathogenic fungi such as *Drechslera* sp. (causal agent of leaf spot), the grass showing fewer and smaller lesions than did endophyte-free plants. The endophyte also inhibits the growth of *Rhizoctonia solani* (causal agent of foliar blight in *Brachiaria*) and *Pyricularia oryzae* (causal agent of rice blast). It may even protect *Brachiaria* from pests such as the aphid *Rhopalosiphum maidis*. However, several years of research has shown that, in infected temperate grasses, endophytes reduce livestock productivity. Whether this is true for tropical forage grasses such as *Brachiaria* is not yet known, although what little evidence exists suggests that endophytes may cause various health disorders in livestock.

Keywords: *Acremonium implicatum*, biocontrol, *Brachiaria*, endophytes, symbiosis

Introduction

Several *Brachiaria* species are of great economic importance as pastures in tropical America, covering about 70 million hectares in Brazil alone (Miles *et al.*, 1996). The forage potential of these mostly African grasses was first recognized several years ago in tropical Australia. Of the 100 or so species, *B. brizantha* and *B. decumbens* are economically the most important, including *B. brizantha* cv. Marandu, which was released in 1984 by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) in Brazil. This cultivar, originating from germplasm introduced to Brazil from the Zimbabwe Grasslands Research Station, Marondera (Keller-Grein *et al.*, 1996), is outstanding for its ability to resist the spittlebug, the most damaging pest of *Brachiaria* in the region.

Many *Brachiaria* species are apomictic, that is, asexually reproducing through seed (Miles and Valle, 1991). This form of reproduction has great potential as a tool to allow even heterozygous plant genotypes to breed true through seed. Apomictic reproduction also has advantages for endophyte research and use. Almost all seeds of an endophyte-containing

apomictic plant would contain the endophyte and would be genetically identical to each other. After treatments to eliminate the endophyte, endophyte-free seedlings can be compared with genetically identical, but endophyte-infected, seedlings.

Endophytic fungi form nonpathogenic and intercellular symbiotic associations with many wild and cultivated grasses and sedges, completing their entire life cycle within the plants' aerial parts, and resulting in associations that, in temperate zones, have important ecological and agricultural impact (Clay, 1990; Siegel, 1993). For example, these fungi are among the most important biological plant protection agents for forage and turf grasses (Clay, 1989; Kimmons *et al.*, 1990; Gwinn and Gavin, 1992; Funk *et al.*, 1993; Rowan and Latch, 1994). Increasingly, forage and turf grass researchers want to improve known endophytes, or search for new endophytes, with desirable traits such as insect deterrence, disease resistance, drought tolerance, and persistence, thereby protecting and enhancing a grass's performance (Arechavaleta *et al.*, 1989).

Many of the most important endophytes belong to the *Acremonium* genus, including those several asexual *Acremonium* endophytes that have been newly reclassified by Glenn *et al.* (1996) to the genus *Neotyphodium* Glenn, Bacon & Hanlin. In tall fescue, symbiosis with *N. coenophialum* enhances tillering and root growth, and increases drought tolerance (Arechavaleta *et al.*, 1989) and resistance to nematodes (Kimmons *et al.*, 1990), fungal pathogens (Gwinn and Gavin, 1992), and insects (Rowan and Latch, 1994).

Whether the traits described for endophyte-temperate grass associations hold true for tropical forage grasses such as *Brachiaria* is not yet known. Recent surveys and studies have confirmed that various endophytic fungi, including *Acremonium* endophytes, do inhabit native savanna grasses (Koga *et al.*, 1995) and introduced tropical forage grasses (Kelemu and Takayama, 1998; Kelemu *et al.*, 2000). For example, *A. implicatum* (J. Gilman & E. V. Abbott) W. Gams was isolated from *B. brizantha* accession CIAT 6780, *B. arrecta* accession CIAT 16845, and *B. decumbens* accession CIAT 606. Also found are "Acremonium-like" endophytes in *B. brizantha* cv. Marandu (Rodrigues and Dias Filho, 1996), and *Balansia* endophytes in the savanna grass *Andropogon bicornis* L.

***Balansia* endophytes**

The savannas of the Colombian Eastern Plains cover an area of about 5.5 million hectares, with at least 153 documented plant species that belong to 93 genera and 34 families, with *An. bicornis* L. and *Panicum campestre* Nees among the predominant native species. A survey (Koga *et al.*, 1995) was conducted in November 1994, in Carimagua, Colombia (4°37' N, 71°26' E; 150 to 175 m in altitude; mean annual rainfall, 2100 mm; mean annual temperature, 26°C). On the young, unrolling leaves of *An. bicornis*, dark brown to black ascomata on abaxial surfaces were observed (Figure 1). Perithecia were ovoid and ostioles were short necked. Filamentous asci contained ascospores that were about the same length as the asci.

Gray to black circlets of ascomata were also observed on *P. campestre* stems just below the nodes (Figure 2), characterizing the fungus as *Balansia henningsiana* (A. Möller) Diehl. We also found *B. subnodosa* Atk. on *An. bicornis* (Koga *et al.*, 1995). Scanning electron microscopy of transverse sections of infected tissues of both plants showed that the hyphae of these fungi developed in the intercellular spaces of the host leaf blades.

These findings suggest that the two *Balansia* species are endophytic, associating primarily with warm-season grasses. As far as we know, this is the first report of finding *Balansia* spp. on *P. campestre* and *An. bicornis* in the Colombian savannas.

Further research is needed to ascertain the relationship between these endophytes and the savanna grasses. Because these grasses comprise a major component of the extensive cattle-raising industry of the Eastern Plains, the potential effect of these endophytes on livestock productivity also needs to be examined.

***Acremonium* endophytes**

Rodrigues and Dias Filho (1996) documented a preliminary survey report on the isolation of “*Acremonium*-like” fungi from leaf sheaths of *B. brizantha* cv. Marandu grown in *Brachiaria* pastures in the State of Pará in the Brazilian Amazonia. As far as we know, no detailed work on the identification or characterization of these fungi has yet been reported.

We took several isolates of an endophytic fungus from *B. brizantha* cv. Marandu, *B. decumbens* accession CIAT 606, and *B. arrecta* accession CIAT 16845, identifying them as *A. implicatum* (J. Gilman & E. V. Abott), according to morphological criteria (Figures 3 and 4). Molecular analysis, using 18S rDNA and ITS rDNA sequences, showed that the isolates belonged to the genus *Acremonium*, and were close to *A. strictum* and *A. kiliense* (Kelemu *et al.*, 2000). *Acremonium implicatum* has also been isolated from seeds (Dwivedi and Shukla, 1990) and soil (Singh, 1991).

Disease and insect tolerance

In temperate zones, endophyte-plant associations are mutually beneficial by nature (Bacon *et al.*, 1986; Siegel *et al.*, 1987; Schardl and Phillips, 1997). The endophyte receives nutrients and completes its life cycle within the plant and, in return, the host plant benefits by receiving protection from fungal and other pathogens (Christensen, 1996) and pests, including aphids (Siegel *et al.*, 1991; Clement *et al.*, 1997), beetles (Kirfman *et al.*, 1986; Siegel *et al.*, 1987), crickets (Ahmad *et al.*, 1985), and nematodes (West *et al.*, 1988; Kimmons *et al.*, 1990). *In vitro* studies confirmed the antagonism existing between endophytic fungi and grass pathogens (White and Cole, 1986; Siegel and Latch, 1991; Christensen, 1996).

To test for these responses in *Brachiaria*, half of a group of genetically identical clones of the grass were treated with the fungicide Folicur® (tebuconazole) to generate endophyte-free plants. The entire group of clones was challenged with *Drechslera* sp., a fungus causing leaf spot in various grasses, and with the aphid *Rhopalosiphum maidis*. Endophyte-infected plants manifested enhanced resistance to *Drechslera* sp. with fewer and smaller leaf spot lesions and significantly reduced fungal sporulation (Kelemu, *et al.*, 2000). *In vitro* studies showed that *A. implicatum* produced diffusible substances that inhibited the growth of *Drechslera* sp. and other grass pathogens.

The aphid preferred to feed on endophyte-free *B. arrecta* plants than on endophyte-infected plants. Populations were higher on endophyte-free than on endophyte-infected plants, whether the insect was given a choice between endophyte-free and endophyte-infected plants, or was confined to either individual endophyte-free or endophyte-infected plants. The aphid *R. maidis* has been reported to be affected by endophyte-infected grasses in the temperate zone (Johnson *et al.*, 1985; Buckley *et al.*, 1991).

Drought tolerance

Increased fitness and enhanced drought tolerance have been reported in endophyte/grass associations (Arechavaleta *et al.*, 1989; Hill *et al.*, 1990; West *et al.*, 1993). Our preliminary results showed that, under moderate levels of water stress, the presence of *A. implicatum* had no significant effect on shoot growth characteristics of *B. arrecta* accession CIAT 16845. But, under severe water stress, endophyte-infected plants maintained better leaf expansion and produced significantly more leaf biomass than did the clean plants.

Livestock productivity

Cattle have been observed to suffer health disorders when grazing *Brachiaria* pastures. One syndrome, known as “fallen cow”, affects cows in early lactation when they graze *B. decumbens* cv. Basilisk (signalgrass), the most widely grown pasture in the humid and subhumid tropics (Lascano and Euclides, 1996). Another syndrome, known as “swollen face”, occurs in horses grazing *B. humidicola* pastures in the Brazilian *Cerrados*. Other neurological disorders in sheep grazing *B. decumbens* (Salam Abdullah *et al.*, 1989) and toxicity in cattle grazing *B. decumbens* (Noordin *et al.*, 1989) have been reported. The exact cause(s) of these syndromes are still unknown, although these and other observations may suggested a role for endophytic fungi in these tropical grasses. This hypothesis is supported by findings from research, conducted in recent years, that endophytes in infected temperate grass forages reduce livestock productivity (Fletcher and Harvey, 1981; Ball *et al.*, 1993).

Conclusions

By defining the role of endophytes in tropical grasses, we will contribute significantly to the understanding of the endophyte/C₄ grass symbiosis. Identification and characterization of new species and strains of endophytes from a variety of exotic tropical grasses may also provide a valuable source of microbial germplasm for biological applications. Interest in endophytes increased as their role in cattle toxicosis and insect and pathogen deterrence and other beneficial traits became known. It is not unrealistic to expect that a great diversity of plant hosts and corresponding endophytes may occur in the tropics. Modern molecular technologies may then let us exploit the desirable qualities of the endophyte/grass symbiosis while eliminating toxicity to livestock. Because of their intrinsic and unique relationships with their hosts, endophytes may be used as safe delivery systems of useful foreign cloned genes to their hosts, enabling the foreign genes to be maternally inherited through the seed.

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Figure 1 - Dark brown to black ascomata of *Balansia subnodosa* Atk. on abaxial surfaces of young unrolling leaves of the Colombian savanna grass *Andropogon bicornis* L.

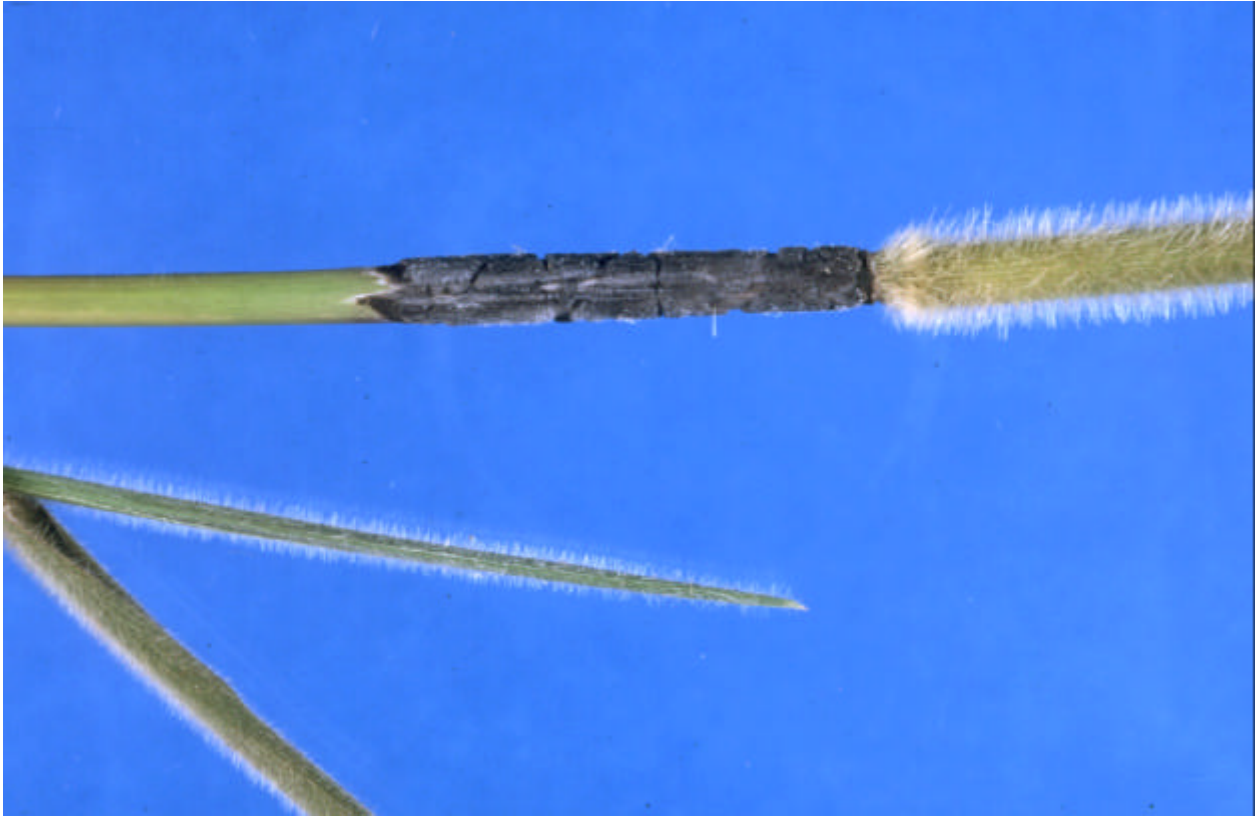


Figure 2 - Gray to black circlets of ascomata of *Balansia henningsiana* (A. Möller) Diehl on the stems of a Colombian native savanna grass, *Panicum campestre*, just below the nodes.

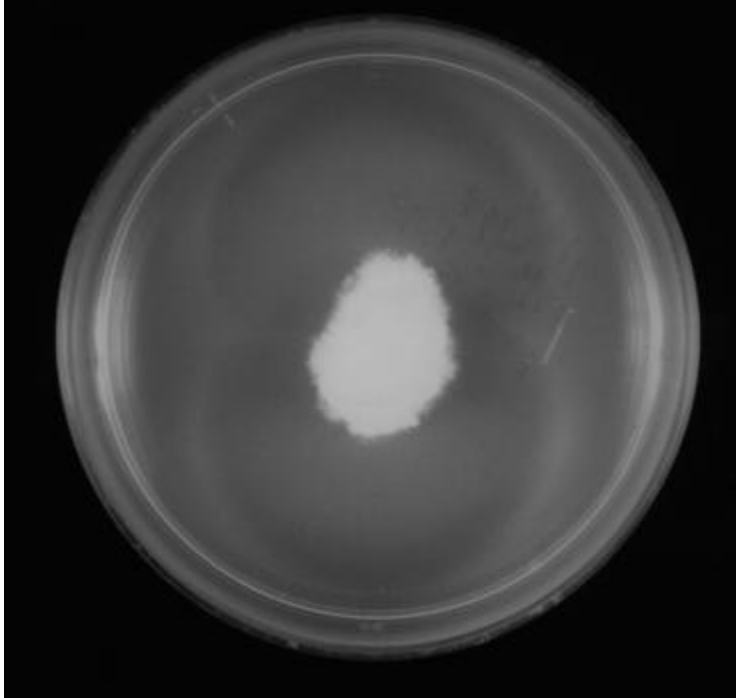


Figure 3 - A pure culture of the endophyte *Acremonium implicatum* was isolated from the grass *Brachiaria arrecta* CIAT 16845 after being incubated for 27 days at 28°C on potato-dextrose agar.

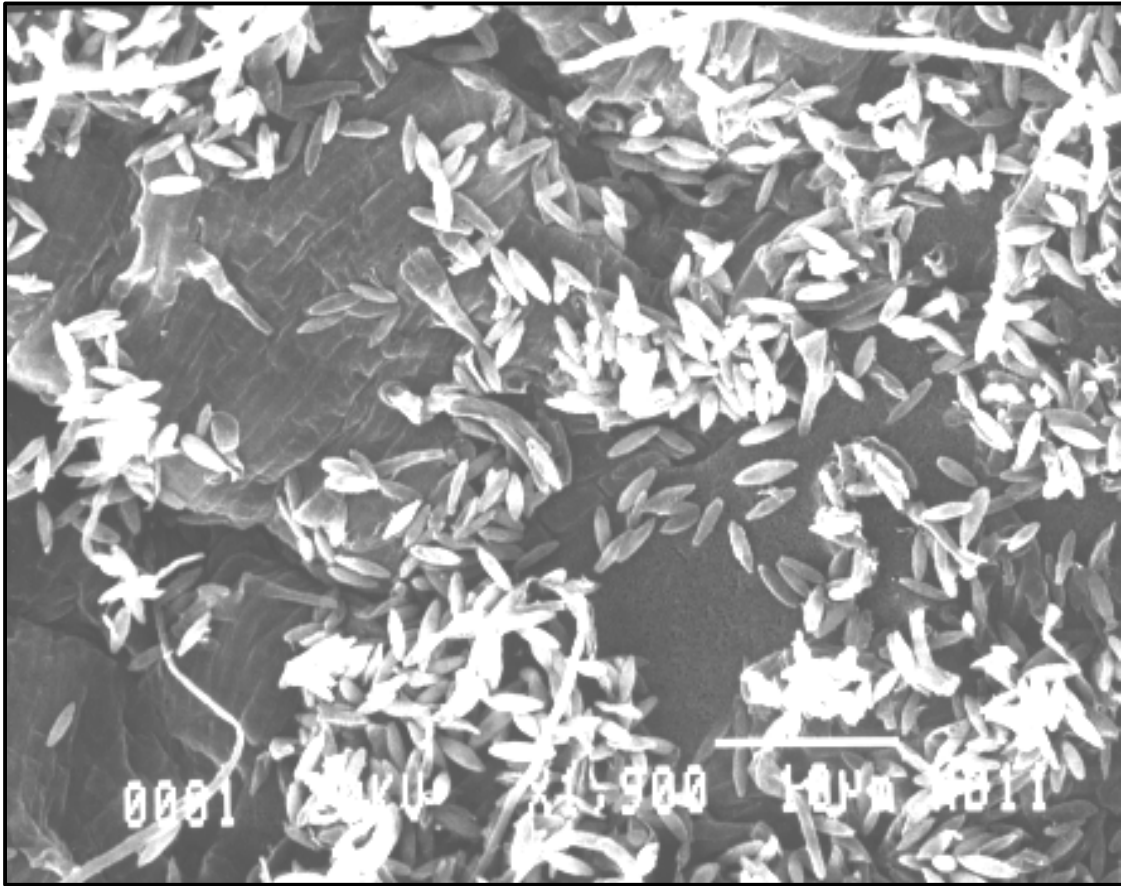


Figure 4 - Conidia of the endophyte *Acremonium implicatum*, isolated from the tropical grass *Brachiaria arrecta* CIAT 16845, as seen under a scanning electron microscope.