Sanitary analysis, transmission and pathogenicity of fungi associated with forage plant seeds in tropical regions of Brazil[1]

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ABSTRACT - Brazil is a major producer and exporter of beef in the world, 90% of the production is made in pasture and 85% of cultivated pastures in the country are *Brachiaria* sp. With a growing livestock industry in the recent years, several forage plant diseases became significant importance for causing losses in pasture productivity and quality. This study aims at quantifying the species of fungi associated with seeds and their frequency in forage plants from tropical regions of Brazil. Assays were performed considering: incidence, pathogenicity and seed-seedling transmission of fungi associated with seeds. Therefore, 28 lots of forage species seeds produced in the harvest of 2010-2011 were used. Fourteen genera of fungi associated with seeds were found, among which *Bipolaris* sp., *Phoma* sp., and *Curvularia* sp. had pathogenic potential. It was possible to note that *Bipolaris* sp., is prejudicial to forage seedlings of *Brachiaria, Panicum* and *Crotalaria*. *Bipolaris* sp. and *Curvularia* sp. have an average of seed-seedling transmission of 100% and 90%, respectively.

Index terms: *Brachiaria* sp., *Bipolaris* sp., *seed-seedling.*

Análise sanitária, transmissão e patogenicidade de fungos associados a sementes de forrageiras de regiões tropicais do Brasil

RESUMO - O Brasil é um grande produtor e exportador de carne bovina do mundo, 90% da produção é feita em pasto e 85% das pastagens cultivadas no país são do gênero *Brachiaria*. Com a intensificação da atividade pecuária nos últimos anos, várias doenças de forrageiras começaram a ter importância significativa, por causarem perdas em produtividade e qualidade de pastagens. O objetivo deste trabalho foi quantificar as espécies de fungos associados a sementes e sua frequência em plantas forrageiras oriundas de regiões tropicais do Brasil. Foram realizados ensaios abordando: incidência, patogenicidade e transmissão semente - plântula, de fungos associados a sementes. Para tanto, foram utilizados 28 lotes de sementes de espécies forrageiras produzidas na safra 2010-2011. Foram encontrados 14 gêneros de fungos associados às sementes, dentre os quais *Bipolaris* sp., *Phoma* sp., e *Curvularia* sp, com potencial patogênico. Concluiu-se que *Bipolaris* sp. é patogênico às plântulas de forrageiras de *Brachiaria*, *Crotalaria* e *Panicum. Bipolaris* sp. e *Curvularia* sp. têm taxa de transmissão média respectivamente de 100% e 90% de sementes para plântulas.

Termos para indexação: *Brachiaria* sp., *Bipolaris* sp., semente-plântula.

Introduction

Brazil is the second major producer and exporter of beef in the world, 90% of the production is made in pasture and 85% of cultivated pastures in the country are *Brachiaria* sp. (Ferraz and Felício, 2010). The country has increased its planted pasture area in approximately 341% from 1970 to 2006. Currently, it is mentioned as the largest supplier of seeds for cultivation and regeneration of pasture of the domestic market and largest exporter of tropical forage plants worldwide (Deminicis et al., 2010).

The world pressure for reduction of deforestation in Brazil and the following intensification of livestock have increased the degradation of pastures. The diseases caused

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by fungi that reduce the pasture quality and productivity are among the reasons for this degradation (Vechiato et al., 2010).

The use of low sanitary quality seed lots is frequent, seed lots with excessive vegetal residues, soil residues and/or seeds of other forage plants and weeds (Marchi et al., 2010).

In general, the seeds can host and transport microorganisms or pathogenic agents of all taxonomic groups, being disease carriers or not. From the ecologic point of view, these agents may be divided into groups of field organisms, with predominance of phytopathogenic species, and groups of storage organisms, with reduced number of species that damage the seeds in this stage. The fungi comprise the largest number of species associated with seeds, followed by bacteria, viruses and nematodes. Among the phytopathogenic fungi, most of them can be transmitted by the seeds of host plants (Lazarotto et al., 2010; Carvalho et al., 2011).

Mostly used in pasture in the Middle West and North regions, which are the largest producers of cattle in the country, grasses and legumes have been affected by fungal diseases, such as *Brachiaria brizantha* (*Pyricularia grisea*) (Verzignassi et al., 2012) and *Pythium periilum (Rhizoctonia solani*) (Duarte et al., 2007), while *Panicum maximum* and *Stylosanthes* spp. have been affected by *Bipolaris* sp., *Curvularia* sp and *Phoma* sp. (Marchi et al., 2010).

One way to avoid the occurrence of diseases is the development of species that are resistant to fungi or by treating the seeds, increasing the costs of implantation and pasture reconstitution. Any effective control alternative must investigate which fungi attack the seeds in specific regions (Silva et al., 2007; Senhor et al., 2009).

Unlike annual cultures, the forage plant seeds are cropped by soil sweeping and in this operation they are frequently contaminated with impurities, such as clod, pieces of plants, fungi and insects (Quadros et al., 2012). The seed health is an important factor for the establishment and maintenance of good quality tropical pastures (Marchi et al., 2010). According to Vechiato et al. (2010), in order to obtain healthy seeds, there must be the creation of a seed certification program, yet it has been difficult to create it due to the absence of researches on the seed pathology field, providing information about field health, seeds tolerance to pathogenic elements and products and techniques that are efficient for the seeds treatment.

Besides the lack of scientific information, there are no regulatory measures to satisfy the Brazilian need. The Normative Instruction n. 9 of the Ministry of Agriculture Livestock and Supply, June 2, 2005, approves rules for the production, commercialization and use of seeds; however, it does not include the health regulations. This issue is included in other rules for seeds of other vegetal species, but not for forage plants (Brasil, 2005; Brasil, 2013).

Despite the great demand for information on fungi control by forage plant seed producers and exporters, the subject has not been largely studied (Lasca et al., 2004).

With the purpose of increasing this knowledge, a health analysis was carried out aiming at quantifying the species of fungi associated with seeds and their frequency in forage plants from tropical regions of Brazil.

Material and Methods

The present study was conducted at the Phytopathology Laboratory and in greenhouses at Universidade Federal do Tocantins, campus Gurupi, from July, 2012 to March, 2013. Assays were made approaching: I – Incidence, II – Pathogenicity and III – Seed-seedling transmission, of fungi associated with seeds. Therefore, 28 lots of tropical forage species seeds, produced in the harvest of 2010-2011 in different cities of the states of Bahia, Goiás, São Paulo and Tocantins, were used as shown in Table 1.

Assay I – Incidence

For this assay, the blotter test method was used, following the description in the Rules for Seed Testing - RAS (Brasil, 2009a). Seeds from each lot were submitted to the following treatments: with disinfestation of tegument (CDT) and without disinfestation of tegument (SDT). The disinfestation was made by immersion of the seeds in alcohol solution (70%) for 30 seconds, followed by immersion in sodium hypochlorite (2%) for two minutes and, finally, in sterilized distilled water. The seeds that were not disinfested were washed in sterile water during one minute.

The experimental design was completely randomized (DIC), with ten replications, each on a Petri plate with 40 seeds of each forage plant cultivar/species. The assay was conducted in incubation chamber at 24 ± 2 °C of temperature and photoperiod of 12 hours during seven days (Sá et al., 2011). At the end of this period, the fungi growth was evaluated. After that, with the help of a stereomicroscope, the fungi were directly identified by the observation of structures and comparison with the specialized literature (Barnett and Hunter, 1972), or after the monosporic isolation (Camera et al., 2013) after the identification of the fungi and their incidence on each forage plant species/cultivar.

"Potentially pathogenic fungi" are those capable of causing diseases in tropical forage plants, or that, although not evident, present potential to it. The "secondary or storage fungi" are those that present lower economic importance for tropical forage plants or that may cause deterioration of seeds from these species during their storage period (Marchi et al., 2010).

Table 1. Origin of forage plant seeds produced in tropical regions of Brazil.

Lot	Forage species – cultivar	Geographic Origin
1	*B. brizantha* Marandu	Aparecida do Rio Doce – GO
3	*B. brizantha* Marandu	Quirinópolis – GO
7	*B. decumbens* Basilisk	Quirinópolis – GO
9	*B. ruziziensis*	Quirinópolis – GO
15	*B. brizantha*	Quirinópolis – GO
17	*B. humidicola*	Quirinópolis – GO
14	*P. maximum* Massai	Quirinópolis – GO
12	*Sthilosanthes* sp. Campo Grande	Quirinópolis – GO
8	*B. humidicola*	Gurupi – TO
25	*B. brizantha* MG5	Paraíso do Tocantins – TO
24	*B. brizantha* Piatã	Guaraí – TO
27	*P. maximum* Mombaça	Colinas do Tocantins – TO
18	*B. brizantha* Piatã	Araguaína – TO
19	*B. brizantha* MG5	Araguaína – TO
21	*B. brizantha* Marandu	Araguaína – TO
20	*B. humidicola*	Araguaína – TO
23	*P. maximum* Mombaça	Araguaína – TO
22	*C. juncea*	Araguaína – TO
28	*Sthilosanthes* sp. Campo Grande	Araguaína – TO
26	*C. juncea*	Araguatins – TO
16	*B. brizantha*	Barreiras – BA
10	*B. ruziziensis*	São Desidério – BA
4	*B. brizantha* Marandu	São Desidério – BA
5	*B. brizantha* Marandu	São Desidério – BA
2	*B. brizantha* Marandu	Cosmorama – SP
6	*B. decumbens* Basilisk	Presidente Prudente – SP
11	*P. maximum* Massai	Cosmorama – SP
13	*P. maximum* Massai	General Salgado – SP

Assay II – Pathogenicity

For this assay, the used samples were 16 isolated *Bipolaris* sp. and *Curvularia* sp. obtained from eight lots of seeds: Lot 15–*B. brizantha*, Lot 18–*B. brizantha* Piatã, Lot 2-*B. brizantha* Marandu, Lot 19-*B. brizantha* MG5, Lot 7-*B. decumbens* Basilisk, Lot 17-*B. humidicola*, Lot 10-*B. ruziziensis* and Lot 11-*P. maximum* Massai. One isolated monosporic of each fungus was obtained from all lots (Camera et al., 2013). They had their pathogenicity evaluated by means inoculating the shoot part of seedlings of the 28 forage plant lots used in this study.

The experimental design was completely randomized, with 16 treatments (fungal isolated) and 10 replication. Each replication was composed by two cells (100 x 100 mm) of tray containing 10 forage plant seedlings in each cell. The seedlings were obtained by sowing disinfested seeds following the same methodology described for the incidence assay, in plastic trays containing sterilized commercial substrate. The germination evaluation of each lot was made in accordance with RAS (Brasil, 2009a).

The inoculate suspension resultant from each fungal isolated was applied five days after the seedlings emergence, in the concentration of 2 x 10^6 spores/mL (Macedo and Barreto, 2007). In order to quantify the spores, a Neubauer chamber was used, and the suspensions were prepared in fungi communities cultivated in PDA media and incubated for eight days, under temperature of 25 ºC and photoperiod of 12 hours (Sá et al., 2011). After the inoculation, the seedlings were maintained in dark humid chamber, for 72 hours and were later transferred to a greenhouse for 20 days. The symptoms were evaluated five, 10, 15 and 20 days after the inoculation. Compared to Anjos et al. (2004), the leaves with symptoms were isolated, in PDA media, after the last symptoms evaluation, 20 days after the inoculation in order to confirm Koch's principles. To satisfy the seedlings' need for nutrients, they were fertilized with urea (5 g/L) 20 days after the emergence.

With the purpose of verifying if there is influence of spots in the *Brachiaria* seeds tegument during their germination, another assay was made. Thus, seeds from nineteen lots of *Brachiaria,* without superficial disinfestation of tegument, were separated into two groups: with spots in the tegument (CMT) and without spots in the tegument (SMT). The sowing was made on plastic trays containing sterilized sand. For each lot, 400 seeds were used, being 200 CMT and 200 SMT. The germination evaluation of each lot was made in accordance with RAS (Brasil, 2009a).

Assay III – Seed-seedling transmission

For the seed-seedling transmission assay, 100 seeds in four replications, totalizing 400 seeds from each lot of forage plant seeds, were sowed without disinfestations of tegument, on two plastic trays/lots, containing autoclaved sand (subsoil, 40cm). After the sowing, the trays were transferred to a greenhouse and irrigated with sterilized water on a daily basis. The seedlings evaluation was made 5, 10, 15 and 20 days after the emergence by observation of the characteristic symptoms. For confirmation of Koch's principles, leaf fragments that presented symptoms were isolated in PDA media. The seedlings emergence and the incidence of unhealthy seeds were evaluated and, then, the percentage of seedlings with leaf spots in relation with seedlings without leaf spots was evaluated.

The statistical procedures were held with Sisvar software. The multiple comparisons between the averages were made by Scott-knott and/or Tukey's test at 5% of probability.

Results and Discussion

Transportation of fungi associated with forage plant seeds

As seen in Table 2, fourteen genres of fungi were detected in this assay. In the treatment without disinfestation of tegument (SDT), the saprophytic fungus *Fusarium* sp., was more frequent (89.3%), followed by the potentially pathogenic fungi *Curvularia* sp. (75.1%), *Bipolaris* sp. (67.9%), *Phoma* sp. (67.9%) and *Sclerotium* sp. (28.6%). However, in the treatment with disinfestation of tegument (CDT) these fungi presented frequency of 39.3%, 46.4%, 39.3%; 39.3% and 28.6% respectively. A relevant difference between the treatments SDT and CDT in the incidence of fungi *Bipolaris* sp., *Fusarium* sp., *Penicillium* sp., *Phoma* sp., *Rhizophus* sp. was observed (Table 2). The difference shows that fungus inoculum was adhered to the seed tegument, yet the frequency and incidence of fungi in the treatment CDT, even in lower percentage, indicate that these fungi are present on the surface, as well as on the tegument tissue of tropical forage plant seeds.

The presence of fungi on vegetal tissues was also reported by Yago et al., (2011) who studied sorghum and foxtail millet seeds and identified *Curvularia* sp. on the seeds' endosperm and *Alternaria* and *Fusarium* on the seeds' endosperm and embryo.

Two fungi with greatest incidence in the treatment SDT were *Fusarium* sp. (saprophytic) and *Phoma* sp. (pathogenic). For genre *Fusarium* sp., the largest incidence was in *Brachiaria humidicola* while *Phoma* sp. had greater incidence on *B. brizantha* Marandu, *B. brizantha* MG5, *P. maximum* Mombaça, *B. ruziziensis*, *B. decumbens* Basilisk. The other fungi has low incidence (<10%) (Table 3 e Table 4). The fungi incidence diverged among the lots when the treatment was SDT (Table 4), but not when treatment was CDT (Table 5).

Table 2. Frequency and incidence of fungi after treatment in forage plant seeds produced in tropical regions of Brazil.

Fungus	Frequency (%)		Incidence (%)	
	SDT*	CDT**	SDT*	CDT**
Aspergillus niger	3.6a	3.6a	0.5a	0.03a
Aspergillus sp.	64.3a	21.4a	4.21a	0.5a
Bipolaris sp.	67.9a	39.3a	4.35a	0.53b
Botrytis sp.	17.9a	10.7a	0.42a	0.07a
Chaetomium sp.	21.4a	17.9a	0.92a	0.35a
Curvularia sp.	75.14a	46.4a	3.71a	0.57a
Fusarium sp.	89.3a	39.3a	21.82a	0.42b
Helminthosporium sp.	3.6a	0a	0.35a	0a
Penicillium sp.	71.4a	10.7a	6.21a	0.21b
Phoma sp.	67.9a	39.3a	13.89a	0.71b
Pithomyces sp.	7.1a	3.6a	0.5a	0.03a
Rhizophus sp.	46.4a	10.7a	6.53a	0.1b
Sclerotium sp.	28.6a	28.6a	0.89a	0.67a
Trichoderma sp.	14.3a	3.6a	0.25a	0.03a

*SDT – Without Disinfestation of the Tegument.
**CDT – With Disinfestation of the Tegument.
Measures followed by the same letter in the line do not differ inwardly by Tukey's test ($p<0,05$). Analysis made between both treatments: SDT and CDT.

The incidence of fungi in seeds can happen in the field, during the storage or after the harvest, and it interferes negatively with their physiological potential (Gama et al., 2012). Saprophytic fungi, such as *Fusarium* sp., which are storage fungi, are capable of affecting the seeds viability, the seedlings emerged and may even kill the seedlings (Vechiato et al., 2010). The presence of storage fungi associated with the seeds is related with the harvest and post-harvest methods, as well as with the relative air humidity during storage (Lacerda et al., 2003).

The presence of fungi *Aspergillus* sp., *Rhizopus* sp., *Bipolaris* sp., *Fusarium* sp. and *Phoma* sp. had already been reported in seeds of *P. maximum* Massai, Mombaça and Tanzania and *Stylosanthes* Campo Grande (*S. capitata* e *S. macrocephala*), even under extremely clean physical conditions (Marchi et al., 2010). The fungi *Phoma* sp. and *Fusarium* sp. were also found in *Brachiaria* seeds (Lasca et al., 2004).

It is important to note that genre *Rhizopus* sp., *Penicillium* sp. and *Aspergillus* sp. harmed the seeds physiological quality, reducing their germination capacity (Vechiato et al., 2010), therefore, even though their incidence was not large in this study, they may represent future damages to cultivation.

Table 3. Incidence average of fungi in tropical forage plant seeds, without disinfestation of tegument (lots average).

Forage plant	Incidence of Fungi (%)														Total
	An	As	Bi	Bo	Ch	Cu	Fu	He	Pe	Ph	Pi	Rh	Sc	Tr	
B. brizantha	0.0a	5.5a	3.0a	0.0a	2.0a	5.5a	19.0c	0.0a	6.5a	6.0b	6.0a	14.5a	3.5a	1.0a	72.5
B. brizantha Marandu	2.3a	2.3a	6.3a	0.2a	3.5a	5.2a	26.5b	0.0a	2.2a	31.8a	0.0a	7.5a	1.8a	0.0a	89.7
B. brizantha MG5	0.0a	3.0a	13.5a	0.0a	0.0a	13.5a	13.5c	0.0a	17.0a	24.5a	0.0a	2.5a	2.0a	0.0a	89.5
B. brizantha Piatã	0.0a	9.0a	4.0a	0.0a	0.0a	0.5a	34.0b	0.0a	12.5a	0.0b	0.0a	0.0a	1.5a	0.0a	61.5
B. decumbens Basilisk	0.0a	0.5a	3.0a	4.0a	0.0a	3.0a	26.0b	0.0a	0.5a	18.0a	0.0a	0.0a	0.0a	2.0a	57.0
B. humidicola	0.0a	0.0a	5.0a	0.0a	0.0a	4.0a	55.0a	0.0a	0.0a	1.0b	0.0a	4.7a	0.0a	0.0a	69.7
B. ruziziensis	0.0a	1.0a	9.5a	1.5a	0.0a	1.5a	14.5c	0.0a	2.5a	18.5a	1.0a	23.5a	0.0a	0.5a	74.0
C. juncea	0.0a	9.5a	0.0a	0.0a	0.0a	0.0a	5.0c	0.0a	27.0a	0.0b	0.0a	0.0a	0.0a	0.0a	41.5
P. maximum Mombaça	0.0a	2.5a	0.0a	0.0a	0.0a	3.0a	8.5c	0.5a	1.5a	22.5a	0.0a	8.5a	0.0a	0.0a	47.0
P. maximum Massai	0.0a	8.0a	1.0a	0.0a	0.3a	2.3a	15.3c	0.0a	5.0a	5.3b	0.0a	8.7a	0.0a	0.0a	46.0
Sthilosanthes sp.	0.0a	8.8a	0.0a	0.0a	0.0a	0.0a	0.0c	0.0a	5.3a	0.0b	0.0a	0.0a	0.0a	0.0a	0.0
Média	0.2	4.6	4.1	0.5	0.5	3.5	19.8	0.0	7.3	11.6	0.6	6.3	0.8	0.3	58.9

An: *Aspergillus niger;* As: *Aspergillus* sp.; Bi: *Bipolaris* sp.; Bo: *Botrytis* sp.; Ch: *Chaetomium* sp.; Cu: *Curvularia* sp.; Fu: *Fusarium* sp.; He: *Helminthosporium* sp.; Pe: *Penicillium* sp.; Ph: *Phoma* sp.; Pi: *Pithomyces* sp.; Rh: *Rhizophus* sp.; Sc: *Sclerotium* sp.; Tr: *Trichoderma* sp.
Measures followed by the same letter in the column do not differ inwardly by the Scott-knott's test (p<0.05).

Table 4. Incidence of fungi in tropical forage plant seeds, without disinfestation of tegument.

Forage plant	Lot	Incidence of Fungi (%)														Total
		An	As	Bi	Bo	Ch	Cu	Fu	He	Pe	Ph	Pi	Rh	Sc	Tr	
B. brizantha	16	0a	5a	3a	0a	2a	3a	21a	0a	5a	8a	0a	29a	5a	0a	81
	15	0a	6a	3a	0a	2a	8a	17a	0a	8a	4a	12a	0b	2a	2a	64
B. brizantha Marandu	1	0a	0a	4a	0a	0a	6a	23a	0a	3a	66a	0a	13a	2a	0a	117
	21	14a	3a	4a	0a	0a	3a	48a	0a	1a	15a	0a	26a	0a	0a	114
	2	0a	2a	10a	0a	0a	11a	33a	0a	5a	45a	0a	4a	0a	0a	110
	3	0a	3a	4a	0a	3a	7a	22a	0a	1a	33a	0a	2a	5a	0a	80
	4	0a	6a	3a	0a	8a	0a	11a	0a	2a	7a	0a	0a	3a	0a	40
	5	0a	0a	13a	1a	10a	4a	22a	0a	1a	25a	0a	0a	1a	0a	77
B. brizantha MG5	19	0a	0b	16a	0a	0a	5b	22a	0a	0b	11b	0a	5a	4a	0a	63
	25	0a	6a	11b	0a	0a	22a	5b	0a	34a	38a	0a	0b	0b	0a	116
B. brizantha Piatã	18	0a	1a	2a	0a	0a	1a	65a	0a	0a	0a	0a	0a	3a	0a	72
	24	0a	17a	6a	0a	0a	0a	3a	0a	25a	0a	0a	0a	0a	0a	51
B. decumbens Basilisk	6	0a	0a	2a	5a	0a	3a	24a	0a	0a	19a	0a	0a	0a	1a	54
	7	0a	1a	4a	3a	0a	3a	28a	0a	1a	17a	0a	0a	0a	3a	60
B. humidicola	20	0a	0a	0a	0a	0a	1a	93a	0a	0a	0a	0a	3a	0a	0a	97
	8	0a	0a	4a	0a	0a	9a	23a	0a	0a	3a	0a	0a	0a	0a	39
	17	0a	0a	11a	0a	0a	2a	49a	0a	0a	0a	0a	11a	0a	0a	73
B. ruziziensis	9	0a	2a	4a	2a	0a	2a	14a	0a	2a	4a	2a	42a	0a	0a	74
	10	0a	0a	15a	1a	0a	1a	15a	0a	3a	33a	0a	5b	0a	1a	74
C. juncea	22	0a	17a	0a	0a	0a	0a	0b	0a	48a	0a	0a	0a	0a	0a	65
	26	0a	2b	0a	0a	0a	0a	10a	0a	6b	0a	0a	0a	0a	0a	18
P. maximum Mombaça	23	0a	0a	0a	0a	0a	1a	15a	1a	0a	16a	0a	17a	0a	0a	50
	27	0a	5a	0a	0a	0a	5a	2a	0a	3a	29a	0a	0a	0a	0a	44
P. maximum Massai	11	0a	5a	3a	0a	1a	5a	20a	0a	1a	11a	0a	0a	0a	0a	46
	13	0a	12a	0a	0a	0a	2a	19a	0a	5a	5a	0a	2a	0a	0a	45
	14	0a	7a	0a	0a	0a	0a	7a	0a	9a	0a	0a	24a	0a	0a	47
Sthilosanthes sp.	12	0a	0b	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0
	28	0a	17.5a	0a	0a	0a	0a	0a	0a	10.5a	0a	0a	0a	0a	0a	0

An: *Aspergillus niger;* As: *Aspergillus* sp.; Bi: *Bipolaris* sp.; Bo: *Botrytis* sp.; Ch: *Chaetomium* sp.; Cu: *Curvularia* sp.; Fu: *Fusarium* sp.; He: *Helminthosporium* sp.; Pe: *Penicillium* sp.; Ph: *Phoma* sp.; Pi: *Pithomyces* sp.; Rh: *Rhizophus* sp.; Sc: *Sclerotium* sp.; Tr: *Trichoderma* sp.
Measures followed by the same letter in the column, among forage plants, do not differ inwardly by Tukey's test (p<0.05).

Table 5. Incidence of fungi in tropical forage plant seeds, with disinfestation of tegument.

Forage	Lot	Incidence of Fungi (%)														Total
		An	As	Bi	Bo	Ch	Cu	Fu	He	Pe	Ph	Pi	Rh	Sc	Tr	
B. brizantha	16	0a	0a	1a	0a	1a	0a	1a	0a	0a	2a	0a	0a	3a	0a	8
	15	0a	0a	0a	0a	1a	1a	1a	0a	2a	1a	1a	1a	1a	0a	9
B. brizantha Marandu	1	0a	0a	0a	0a	0a	0a	1a	0a	0a	0a	0a	0a	5a	1a	7
	21	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0
	2	0a	5a	1a	0a	0a	2a	0a	0a	0a	1a	0a	0a	1a	0a	10
	3	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0
	4	1a	1a	2a	1a	0a	1a	0a	0a	0a	2a	0a	1a	0a	0a	9
	5	0a	0a	0a	0a	1a	2a	1a	0a	0a	1a	0a	0a	3a	0a	8
B. brizantha MG5	19	0a	1a	0a	0a	3a	0a	0a	0a	2a	3a	0a	0a	1a	0a	14
	25	0a	1a	1a	0a	4a	0a	0a	0a	0a	0a	0a	0a	1a	0a	7
B. brizantha Piatã	18	0a	0a	1a	0a	0a	1a	1a	0a	0a	3a	0a	0a	4a	0a	10
	24	0a	0a	3a	0a	0a	0a	0a	0a	0a	1a	0a	0a	0b	0a	4
B. decumbens Basilisk	6	0a	3a	1a	1a	0a	1a	0a	0a	0a	4a	0a	0a	0a	0a	10
	7	0a	0a	0a	0a	0a	0a	1a	0a	0a	1a	0a	0a	0a	0a	2
B. humidicola	20	0a	0a	0a	0a	0a	0a	1a	0a	0a	0a	0a	0a	0a	0a	1
	8	0a	0a	0a	0a	0a	1a	0a	0a	0a	0a	0a	0a	0a	0a	1
	17	0a	0a	0a	0a	0a	1a	0a	0a	0a	0a	0a	1a	0a	0a	2
B. ruziziensis	9	0a	0a	0a	0a	0a	1a	0a	0a	0a	0a	0a	0a	0a	0a	1
	10	0a	0a	0a	0a	0a	0b	0a	0a	0a	0a	0a	0a	0a	0a	0
C. juncea	22	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0
	26	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0
P. maximum Mombaça	23	0a	0a	0a	0a	0a	0a	1a	0a	0a	0a	0a	0a	0a	0a	1
	27	0a	0a	2a	0a	0a	2a	2a	0a	0a	1a	0a	0a	0a	0a	7
P. maximum Massai	11	0a	0a	1a	0a	0a	1a	1a	0a	0a	0a	0a	0a	0a	0a	3
	13	0a	3a	1a	0a	0a	1a	1a	0a	2a	0a	0a	0a	0a	0a	8
	14	0a	0a	1a	0a	0a	1a	0a	0a	0a	0a	0a	0a	0a	0a	2
Sthilosanthes sp.	12	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0
	28	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0

An: *Aspergillus niger;* As: *Aspergillus* sp.*;* Bi: *Bipolaris* sp.*;* Bo*: Botrytis* sp.*;* Ch: *Chaetomium* sp.*;* Cu: *Curvularia* sp.*;* Fu: *Fusarium* sp.*;* He: *Helminthosporium* sp.*;* Pe: *Penicillium* sp.*;* Ph: *Phoma* sp.*;* Pi: *Pithomyces* sp.*;* Rh: *Rhizophus* sp.*;* Sc: *Sclerotium* sp.*;* Tr: *Trichoderma* sp.
Measures followed by the same letter in the column, among forage plants, do not differ inwardly by Tukey's test ($p<0.05$).

Pathogenicity

On the first assay, from a total of 28 lots tested, sixteen present seedlings emergence lower than 50% (Table 6), for lot 9 *B. ruziziensis*, the emergence percentage was zero. One of the causes may be the presence of fungi *Fusarium* sp. and *Rhizopus* sp. (Table 3), which may affect the viability of forage plant seeds (Marchi et al., 2010).

During the evaluations, after the seedling inoculations, leaf spots were detected in seedlings of three forage species: *B. brizantha*, *C. juncea* and *P. maximum*. The leaf spots presented characteristics of damages caused by *Bipolaris* sp. In general, other studies report the susceptibility of these grasses to attacks of fungi from genre *Bipolaris, Exserohilum* and *Curvularia*, which cause spots in leaves and stalks, besides drying the leaves and killing the seedlings (Macedo and Barreto, 2007; Martinez et al., 2010; Kleczewski et al., 2012; Braz et al., 2013; Kumar et al., 2013), however, some authors realized that this result may vary in accordance with the genotype used (Braz et al., 2013).

Yago et al. (2011) observed that there was an increase in the seedling death percentage for sorghum and foxtail millet seeds inoculated with *Curvularia lunata* and increase in the severity index for infected seedlings 10 days after the inoculation.

With re-isolation in culture media, there was presence of conidia of fungus *Bipolaris* sp*.,* showing a crossed pathogenicity, since the inoculum applied was removed from other seed lots (Table 7).

As seen in Table 8, the lots of *Brachiaria* that presented spots on the tegument had lower emergence percentage, staying under 50% in this treatment. Although not quantified in the transport assay, one explanation for this low germination percentage is that the *Brachiaria* seeds with dark (spotted) teguments had a larger incidence of potentially pathogenic fungi, which are capable of affecting the forage plant seeds germination, such as *Bipolaris* sp., *Curvularia* sp., *Fusarium* sp., *Phoma* sp. and *Rhizophus* sp.

Table 6. Emergence (%) of tropical forage plant seedlings sowed in commercial sterilized substrate, with disinfestation of tegument.

Forage plant	Lot	Emergence (%)
B. brizantha	16	16
	15	68
B. brizantha Marandu	18	36
	24	28
B. brizantha MG5	1	72
	21	12
	2	4
	3	8
	4	8
	5	28
B. brizantha Piatã	19	84
	25	68
B. decumbens Basilisk	6	84
	7	48
B. humidicola	20	4
	8	40
	17	16
B. ruziziensis	9	0
	10	8
C. juncea	22	16
	26	28
P. maximum Mombaça	11	80
	13	100
	14	80
P. maximum Massai	23	76
	27	28
Sthilosanthes sp.	12	100
	28	94

Table 7. Inoculation of eight *Bipolaris* sp. isolated in forage seedlings.

Forage Species	Lot*	Lot of origin of the isolated/Pathogenicity**							
		2	7	10	11	15	17	18	19
B. brizantha	16	-	-	-	-	-	+	+	+
	15	+	-	-	-	+	-	-	-
B. brizantha Piatã	18	+	-	+	-	-	-	-	+
	24	-	+	+	+	-	+	+	-
C. juncea	22	+	-	-	-	-	-	+	-
	26	+	-	-	-	-	-	-	-
P. maximum Massai	11	-	+	+	+	-	-	-	-
	13	+	-	+	-	+	-	-	-
	14	-	-	-	+	-	-	-	-
P. maximum Mombaça	23	+	+	-	-	-	-	-	-
	27	+	-	-	-	-	-	-	-

*Lot 2-*B. brizantha* Marandu. Lot 7-*B. decumbens* Basilisk. Lot 10-*B. ruziziensis*. Lot 11-*P. maximum* Massai. Lot 15-*B. brizantha*. Lot 17-*B. humidicola*. Lot 18-*B. brizantha* Piatã and Lot 19-*B. brizantha* MG5.
** + isolated pathogenic species – non pathogenic isolated species.

Table 8. Percentage of *Brachiaria* spp. seedlings emergence sowed without disinfestation of tegument in sterilized sand, with separation of seeds with spots in the tegument (CMT) and seeds without spots in the tegument (SMT).

Forage plant	Lot	Emergence (%)	
		CMT	SMT
B. brizantha	16	16	24
	15	4	55
B. brizantha Piatã	18	2	27
	24	16	28
B. brizantha Marandu	1	16	53
	21	0	44
	2	2	6
	3	4	4
	4	10	32
	5	12	8
B. brizantha MG5	19	23	60
	25	0	44
B. decumbens Basilisk	6	12	80
	7	9	9
B. humidicola	20	20	18
	8	16	3
	17	0	12
B. ruziziensis	9	0	0
	10	1	3
Mean		8.57b	26.84a

Measures followed by the same letter in the column do not differ inwardly by Tukey's test ($p<0.05$).

Dias and Toledo (1993) noted that the increase in the incidence of fungi *Curvularia* and *Phoma* in seeds of *B. decumbens* corresponded to decrease in the seeds germination percentage, while Lasca et al. (2004) saw that these fungi, when present on the seeds of forage species, affected the emergence of seedlings and provoked their death.

Seed-seedling transmission

The seed-seedling transmission was confirmed to *Bipolaris* sp. and *Curvularia* sp. as shown in Table 9. The incidence of *Bipolaris* sp. and *Curvularia* sp. in the seeds varied from 0 to 16% and from 0 to 22% respectively, as seen in Table 4. However, the absence or the reduced incidence in some lots has not reflected a lower transmission of these fungi that are pathogenic to the forage plant seedlings evaluated (Table 9).

Fungus *Curvularia* was identified by Lasca et al. (2004) being transmitted by *Brachiaria* seeds and causing leaf spots in seedlings of this forage plant.

Medina et al. (2009) observed lack of effects resultant from the incidence of *Curvularia lunata* (0.5 to 1.5%) and *Phoma* spp. (0.5 to 4.0%) in the *X. triticosecale* Wittmack seed germination and in the transmission of these pathogenic elements to the seedlings. These authors believe, however, that infected seeds play an important role in the epidemiology of diseases caused by these pathogenic elements, due to the introduction of inoculum sources in agriculture since the early stages of the plants.

Table 9. Incidence of *Bipolaris* sp. and *Curvularia* sp. transmitted via seed-seedling.

Forage Species	Lot	Emergence (%)	Incidence (%)	
			Bipolaris sp.	*Curvularia* sp.
B. brizantha	16	20	7	3
	15	23	13	4
B. brizantha Marandu	18	30	0	0
	24	22	0	0
B. brizantha MG5	1	90	0	0
	21	22	31	0
	2	9	30	0
	3	8	0	0
	4	10	0	0
	5	33	0	0
B. brizantha Piatã	19	92	57	0
	25	50	0	30
B. decumbens Basilisk	6	77	0	0
	7	50	22	0
B. humidicola	20	9	0	15
	8	42	0	0
	17	19	0	0
B. ruziziensis	9	0	0	0
	10	16	15	0
C. juncea	22	16	0	39
	26	32	0	2
P. maximum Massai	11	65	0	0
	13	100	0	24
	14	85	0	7
P. maximum Mombaça	23	64	0	0
	27	37	0	0
Sthilosanthes sp.	12	100	0	0
	28	98	0	0

Medeiros et al. (2012) observed that the high incidence of fungi in *Caesalpinia pulcherrima* seeds increased the transmission of the pathogenic elements to seedlings of this species.

In some seed lots there was no seed-seedling transmission; the seeds germinated, produced seedlings with no symptoms of diseases. Yet, these seedlings may produce low quality seeds with tegument tissue infested by the identified pathogenic elements, being, thus, transported, because the transmission can be influenced by several factors.

Some factors that influence the transmission of a pathogenic element by the seed are: cultivated species (varietal resistance), environment conditions (environment and soil humidity, temperature, wind, rain and light), inoculums (viability, location in the seed, type), culture practices (soil type, pH, plant population, sowing depth and planting season, fertilization), inoculums survival, soil and seed microflora, among others (Barba et al., 2002).

Conclusions

The fungus *Bipolaris* sp. is prejudicial to forage plant seedling of the genres *Brachiaria*, *Crotalaria* and *Panicum*.

Bipolaris sp. and *Curvularia* sp. are transmitted from seeds to seedlings.

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