

SEASONAL VARIATION IN THE OCCURRENCE OF FUNGI ASSOCIATED WITH FOREST SPECIES IN A CERRADO-CAATINGA TRANSITION AREA

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ABSTRACT – Although ecotone areas occupy a significant extent in Piauí State, there is little information about these areas, especially regarding the presence of microorganisms. Thus, this study evaluated the effect of seasonality on the occurrence of fungal genera associated with forest species in an ecotone Cerrado-Caatinga in Piauí State, Brazil. The experimental area consisted of one-hectare fragment within a legal reserve, where five plots of 20m x 20m were established and the phytosociological survey was carried out. The collection of the material (healthy leaves and leaves with disease symptoms) was performed in two periods: the dry season (June and August/2017) and the rainy season (December/2017 and February/2018), totaling four collections. In the laboratory, isolation and purification of fungi were performed. The fungal identification was based on morphological characteristics and the use of identification keys. The relative abundance of fungi was calculated, and the data were submitted to multivariate cluster analysis. In total, 225 isolates were found, related to 15 fungal genera, associated with 10 forest species. The occurrence of fungi in the area was influenced by seasonal variation, with more isolates found in the dry period, as examples of important groups, whether beneficial or phytopathogenic microorganisms are: *Trichoderma* spp. and *Lasiodiplodia* spp. It was also observed that fungi are more likely to occur in forest species like *Bauhinia cheilantha* and *Pityrocarpa moniliformis*, belonging to the Fabaceae family.

Keywords: Ecotone; Mycodiversity; Seasonality.

VARIAÇÃO SAZONAL NA OCORRÊNCIA DE FUNGOS ASSOCIADOS A ESPÉCIES FLORESTAIS EM ÁREA DE TRANSIÇÃO CERRADO-CAATINGA

RESUMO – Embora as áreas de ecótonos ocupem uma extensão significativa no estado do Piauí, há pouca informação sobre essas áreas, especialmente quanto à ocorrência de microrganismos. Assim, nesse estudo avaliou-se o efeito da sazonalidade na ocorrência de gêneros de fungos associados a espécies florestais em área de transição Cerrado-Caatinga no estado do Piauí, Brasil. A área experimental consistiu em um fragmento de um hectare dentro de uma reserva legal, onde foram estabelecidas cinco parcelas de 20m x 20m e feito o levantamento fitossociológico. A coleta do material (folhas sadias e com sintomas de doenças) foi realizada em dois períodos: seco (junho e agosto/2017) e chuvoso (dezembro/2017 e fevereiro/2018), totalizando quatro coletas. No laboratório, foram feitos o isolamento e a purificação dos fungos. A identificação desses foi realizada com base nas características morfológicas, consultando à chaves de identificação. Calculou-se a abundância relativa dos fungos e os dados foram submetidos à análise multivariada de agrupamento. No total, foram encontrados 225 isolados relacionados a 15 gêneros fúngicos, associados a 10 espécies florestais. A ocorrência de fungos na área foi influenciada pela variação sazonal, com maior número de isolados encontrados no período seco, a exemplo de grupos importantes, seja de microrganismos benéficos ou fitopatogênicos como *Trichoderma* spp. e *Lasiodiplodia* spp. Foi observada também maior tendência de ocorrência dos fungos nas espécies florestais *Bauhinia cheilantha* e *Pityrocarpa moniliformis*, pertencentes à família Fabaceae.

Palavras-Chave: Ecótono; Micodiversidade; Sazonalidade.



1. INTRODUCTION

The Cerrado-Caatinga region is the third largest ecotone in Brazil, covering areas located in the states of Bahia, Minas Gerais and Piauí. Ecotones refer to areas of transition between communities, ecosystems or biomes, reflecting local and regional changes in abiotic and biotic conditions (Oliveras and Malhi, 2016).

The vegetation of the Cerrado-Caatinga ecotone comprise the floristic composition of both biomes, represented by forest species adapted to the soil and climate conditions of the region where they are inserted. Leaf senescence during the dry period is common. In general, most of the forest species that make up the Cerrado-Caatinga belong to the Fabaceae family (Silva et al., 2018).

Research related to the effect of ecotones on biodiversity suggests that these transition areas have high species richness, besides phenotypic and genetic diversity (Kark, 2013). Although it occupies a significant area in Piauí, there is very little information about Cerrado-Caatinga ecotone, especially regarding the diversity of microorganisms.

The study of microbial diversity, particularly of fungi, is of paramount importance due to the various roles they perform as beneficial or noxious microorganisms. The most recent estimate of fungal diversity in the world is 2.2 to 3.8 million species (Hawksworth and Lücking, 2017) and, by that time, only approximately 144,000 had been described (Kew Gardens, 2018), mainly in tropical regions.

Some locations in the Northeast of Brazil, such as the Southwestern region of Piauí, present a climate with well-defined seasons: a dry season, which corresponds to the months from May to September and a rainy season from October to April (Reis et al., 2017). In ecosystems that commonly present environmental stress, such as long periods of drought, diversity and microbial activity are influenced (Schimel et al., 1999). Thus, work related to temporal variation in the microorganisms occurrence are needed.

Soltis (2017) highlights that the available climate data enable the comparison and evaluation of how climate change can influence the genetic diversity both spatially and temporally. By linking data from collections in different landscape and seasonal variations, it is possible to explain how young and old lineages have diversified through space and time (James et al., 2018).

Thus, it is worth emphasizing the importance of generating reliable information on fungal diversity, to compose databases, confirming the influence of both spatial and temporal variation. In this context, this work evaluated the effect of seasonality on the occurrence of fungal genera associated with forest species in the Cerrado-Caatinga transition area in Piauí State, Brazil.

2. MATERIAL AND METHODS

2.1 Location and description of the study area

The present study was carried out in a Cerrado-Caatinga transition area, situated at the geographical coordinates 8°51'7.48"S and 44°11'39.95"W, located in the municipality of Cristino Castro, Southwest region of Piauí State, Brazil (Figure 1).

The climate of the region, according to the Köppen's (1948) classification, is Aw, hot and semi-humid. The mean annual temperature is 27 °C, with mean annual rainfall of 900 mm and rainy season from October to April.

2.2 Characterization of the experimental area and experimental design

The experimental area consists of a one-hectare fragment within a legal reserve (Figure 1). Five 20 x 20m plots, with a distance of 20m between the plots were arbitrarily marked in the area and the phytosociological survey for the identification of forest species was carried out.

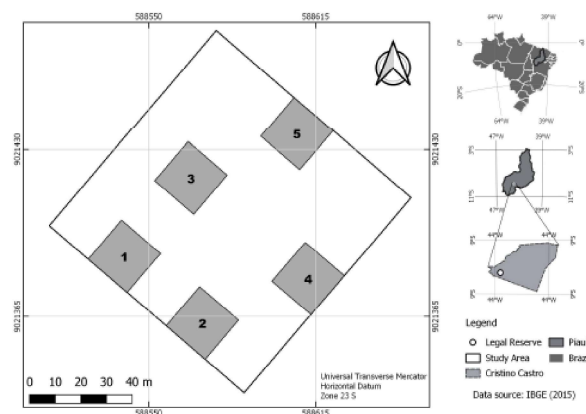


Figure 1 – Representative map of the experimental area in Cristino Castro municipality, Piauí State, Brazil.

Figura 1 – Mapa representativo da área experimental, no município de Cristino Castro, Piauí, Brasil.

2.3 Collection and isolation of material

The collections were performed in two periods, the dry period (June and August/2017) and the rainy period (December/2017 and February/2018), totaling four collections. Throughout the period, weather data on temperature, humidity and precipitation were collected from a CONRAD Funk-Wetterstation Professional USB weather station.

Within each plot, five trees of each species present in the area were selected, and leaves at different stages of development, with and without necrotic spots, possibly caused by pathogens, were collected from the middle section of the canopy. The leaves were packed in plastic bags identified with the forest species and the plot from which they were collected, stored in thermal boxes, and forwarded to the Laboratory of Phytopathology of the Federal University of Piauí, Campus Professora Cinobelina Elvas (9°05'03.7"S and 44°19'36.4"W), Bom Jesus, Piauí.

In the laboratory, the leaves were cut into 0.5 cm discs. The symptom-free leaf discs were washed with sterile distilled water and placed in the PDA culture medium (Potato-Dextrose-Agar). For symptomatic leaves, foliar fragments were disinfested in sequential solutions of 70% alcohol, 2% sodium hypochlorite and immersed in sterile water, dried on filter paper, transferred to Petri dishes containing PDA and kept at 25 °C.

2.4 Obtaining of pure cultures and fungal identification

After cultivation, macroscopic screening of the isolates was carried out, in order to separate the fungi based on phenotypic characteristics (pigmentation, surface, border and texture of the colonies). Fragments of each of the fungal colonies were transferred individually to Petri dishes containing PDA. For the identification of filamentous fungi, semi-permanent slides were prepared with lactophenol + cotton blue and PVLG (Polyvinyl-Lacto-Glycerol) for microscopic observation and measurement of the major vegetative and reproductive structures of each of the isolates obtained. Dicotomic keys to genera were followed and generic descriptions available in the specific literature were compared (Cole and Kendrick, 1981; Barnett and Hunter, 1998; Seifert et al., 2011).

2.5 Pathogenicity tests

Pathogenicity tests were performed with one isolate of the potentially pathogenic fungal genera with the highest occurrence. For this purpose, leaves without damage or symptoms were collected from the trees in the study area. In the laboratory, the leaves were initially washed with SDW (sterile distilled water) and blotted dry with filter paper. A small needle wound was done on the abaxial and adaxial faces of the detached leaves and a 5 mm diameter disk taken from cultures of the pathogen in PDA was placed on the lesion. The control consisted of depositing a filter paper disk soaked in SDW over injured leaves. The leaves were kept in Petri dishes (150 x 30 mm), with a SDW-soaked cotton pad, at 25 °C and photoperiod of 12 hours lighting, for seven days, when the presence of lesions was evaluated. The experimental design was completely randomized, with three replications.

2.6 Statistical analysis

The relative abundance (π_i) of the fungus was calculated from the expression: $\pi_i = n_i/N$ (May, 1975), in which: n_i = number of isolates belonging to the genus i ; N = total number of isolates in each period (dry or rainy). The relative abundance data of fungi was subjected to multivariate grouping analysis, using the Jaccard index as a measure of similarity and Ward's binding method (Ward Junior, 1963), using R statistical analysis software.

3. RESULTS

The phytosociological survey identified 10 forest species in all experimental plots, five belonging to Fabaceae family (*Bauhinia cheilantha* (Bong.) Steud., *Pityrocarpa moniliformis* (Benth.) Luckow & Jobson, *Cenostigma macrophyllum* Tul., *Mimosa tenuiflora* (Willd.) Poir., and *Diptychandra aurantiaca* (Tul.) H. C. Lima et al.); two to Combretaceae family (*Combretum glaucocarpum* Mart. and *Combretum laxum* Jacq.); one to Myrtaceae (*Myrcia tomentosa* (Aubl.) DC.); one to Moraceae (*Brosimum gaudichaudii* Trécul.); and one to Boraginaceae (*Cordia toqueve* Aubl.).

Observing the average values of air temperature and relative humidity, as well as the monthly precipitation accumulated during the interval of the data collection (Figure 2), it can be noted that the definition of the dry and rainy periods is evident. It was found that in the

months of June and August (dry period), respectively, the average temperature was 29.3 °C and 30.2 °C, and average humidity was 41.2% and 40.9%, without occurrence of precipitation. In the months of December and February (rainy period), temperatures of 30.1 °C and 28.9 °C were observed and humidity of 55.1% and 50.2%, with accumulated monthly precipitation of 200 mm and 103 mm, respectively.

The temperature oscillation between June 2017 and February 2018 was from 28.6 °C (Jul/17) to 32.2 °C (Oct/17), while the average humidity was between 30.3% (Sept - Oct/17) and 55.1% (Dec/17). The rains occurred between November/17 and February/18, with a maximum of 265 mm in January/18. The decline of the rainy season was observed in Feb/18.

It was found that there was greater occurrence of fungi in the dry season (131 isolates) than in the rainy season (94 isolates). Sterile mycelium fungi had a significant occurrence in both dry (31) and rainy (19) periods, corresponding to 22.2% of the total isolates obtained in all collections (225). These isolates did not form reproductive structures under the conditions in which they were grown.

The genus with greatest abundance in the dry period was *Pestalotiopsis* Steyaert with 15.27% and the genera with smallest abundance were *Aureobasidium* Viala & Boyer, *Chaetomium* Kunze, *Phoma* (Fr.) Sacc. and *Phomopsis* (Sacc.) Bubák, all with 0.76%. In the rainy period, *Pestalotiopsis* (48.94%) was the most abundant and *Cladosporium* Link, *Colletotrichum*

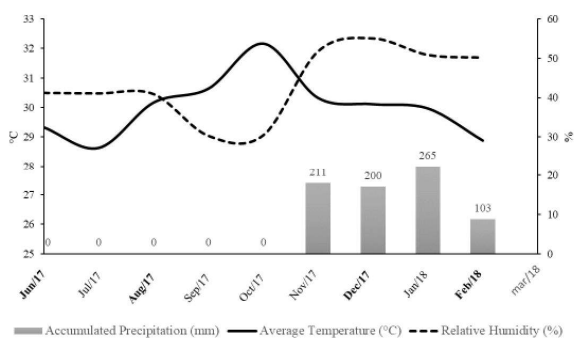


Figure 2 – Monthly averages of temperature and relative humidity of the air and monthly rainfall accumulated during the collection period in Cristino Castro municipality, Piauí State, Brazil.

Figura 2 – Médias mensais de temperatura e umidade relativa do ar e precipitação mensal acumulada durante o período de coleta no município de Cristino Castro, Piauí, Brasil.

Corda, *Mucor* P. Micheli, *Phomopsis* and *Trichoderma* Pers., all with 1.06%, the least abundant. The isolates of the genera *Chaetomium*, *Phoma*, *Curvularia* Boedijn and *Lasiodiplodia* Ellis & Everh. were only found in the dry period and 18 of the 19 isolates of *Trichoderma* spp. were found during this period (Figure 3).

In total, 15 genera of fungi were found belonging to the phylum Ascomycota, except *Mucor* (Zygomycota). These genera were associated with 10 forest species found in the studied fragment (Table 1). More fungal isolates have been found in *B. cheilantha* (41) and *P. moniliformis* (39), both from Fabaceae family. In number of fungal genera *C. glaucocarpum*, stood out with 11 genera followed by *B. cheilantha* with 10 genera, *M. tomentosa* with nine genera, *C. macrophyllum*, with eight genera and *C. toqueve* with seven genera. The forest species *C. laxum* had the highest number of fungal genera (6) but yielded only seven isolates. Therefore, two arboreal species of the Combretum genus stand out in relation to variety of fungal taxa.

Of 68 isolates *Pestalotiopsis* 18 were associated with *B. cheilantha* and 25 with *P. moniliformis*. *Pestalotiopsis* was found in eight of the forest species studied, with the exception of *B. gaudichaudii* and *D. aurantiaca*, of which fewer isolates of other fungi were obtained (Table 1). It was found that *Pestalotiopsis* sp. was pathogenic to four of the total hosts from which they were isolated (*B. cheilantha*, *C. macrophyllum*, *C. glaucocarpum* and *C. toqueve*), showing lesions on the leaves four days after inoculation.

Lasiodiplodia sp. was pathogenic to all the hosts with which it was associated (*B. cheilantha*, *C. macrophyllum*, *C. glaucocarpum*, *C. toqueve*, *M.*

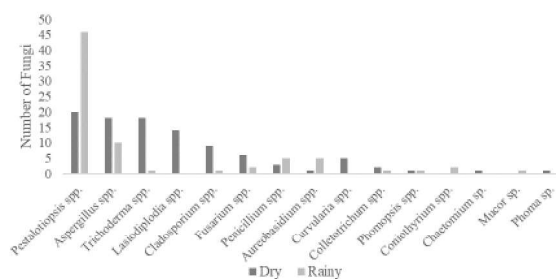


Figure 3 – Number of fungal isolates from leaves of forest species during dry and rainy periods, in a Cerrado-Caatinga transition area, in Piauí State, Brazil.

Figura 3 – Número de isolados de fungos obtidos em folhas de espécies florestais, nos períodos seco e chuvoso, em área de transição Cerrado-Caatinga, no Piauí, Brasil.

Table 1 – Number of isolates of fungal genera by forest species in a Cerrado-Caatinga transition area in Piauí State, Brazil.
Tabela 1 – Número de isolados de gêneros fúngicos, por espécies florestais, em área de transição Cerrado-Caatinga, no Piauí, Brasil.

Fungal Genera	Bauhinia cheilantha Fabaceae	Brosimum gaudichaudii Moraceae	Cenostigma macrophyllum Fabaceae	Combretum glaucocarpum Combretaceae	Combretum laxum Combretaceae	Cordia toqueve Boraginaceae	Diphychandra aurantiaca Fabaceae	Mimosa tenuiflora Fabaceae	Myrcia tomentosa Myrtaceae	Ptyrocarpa moniliformis Fabaceae
<i>Aspergillus</i>	8	2	3	1	1	3	-	1	2	6
<i>Aureobasidium</i>	1	1	1	-	-	-	-	1	1	-
<i>Chaetomium</i>	-	-	-	-	-	-	-	1	-	-
<i>Cladosporium</i>	2	-	-	-	-	1	-	-	2	4
<i>Colletotrichum</i>	1	-	-	1	-	-	-	-	-	1
<i>Coniothyrium</i>	-	-	-	1	-	-	-	-	-	-
<i>Curvularia</i>	1	-	-	-	-	1	-	1	1	1
<i>Fusarium</i>	3	-	1	2	1	1	-	1	-	-
<i>Lasiodiplodia</i>	3	-	1	2	-	3	-	-	4	1
<i>Mucor</i>	-	-	-	-	-	-	-	-	1	-
<i>Penicillium</i>	1	1	1	1	1	-	-	-	2	-
<i>Pestalotiopsis</i>	18	-	5	3	2	3	-	8	4	25
<i>Phoma</i>	-	-	1	-	-	-	-	-	-	-
<i>Phomopsis</i>	-	-	-	2	-	-	-	-	-	-
<i>Trichoderma</i>	3	-	4	1	1	1	1	4	3	1
Total	41	4	17	16	7	13	1	17	20	39

Note: - non occurrence of fungi.
 Nota: Não ocorrência de fungos.

tomentosa and *P. moniliformis*) showing leaf lesions from the third day after inoculation. The pathogens were reisolated and the cultures showed the same characteristics of the original isolate.

Of the 19 *Trichoderma* isolates six were present in leaves with symptoms associated with *Lasiodiplodia*, *Pestalotiopsis*, *Curvularia* and *Phoma*. It is worth mentioning that for *B. cheilantha*, *C. macrophyllum* and *M. tomentosa* hosts, *Trichoderma* isolates were found both in symptomatic and asymptomatic leaves. It is also worth noting the significant presence of other fungi with biocontrol potential, such as *Aureobasidium* Viala & Boyer and *Coniothyrium* Corda genera.

From the grouping analysis (Figure 4) relating the abundance of the fungal genera to forest species hosts four groups were formed in the dry period and three in the rainy period.

There was no fungal association with *D. aurantiaca* in the rainy season. In this period, *B. gaudichaudii* species was grouped with *M. tomentosa*, *C. macrophyllum* and *M. tenuiflora* in relation to fungal abundance. The same species in the dry season grouped together with *D. aurantiaca* just because they hosted a smaller number of fungi.

More fungi were observed in Fabaceae as it was the most represented family in the study area. It was observed a trend of more fungal abundance in *B. cheilantha*, *P. moniliformis*, *C. macrophyllum* and *M. tenuiflora* both in dry and rainy seasons.

4. DISCUSSION

The occurrence of many fungi is directly related to seasonal variation. In general, temperature and humidity of the atmosphere are the main factors influencing formation, germination and longevity of fungal spores (Smilanick and Mansour, 2007). Non-sporulating fungi have been isolated in large numbers in studies carried out in tropical regions, which may occur due to poor adaptation to the artificial conditions they are subjected to (Bezerra et al., 2012; Bezerra et al., 2013).

It was noted that *Pestalotiopsis* spp. had the largest occurrence in both dry and rainy periods. In the last period, specifically in February, the frequency of isolates of this genus was 60%. Tejesvi et al. (2005) also found higher occurrence of *Pestalotiopsis* species in the rainy period. This may be due to the antimycotic effects of species of this genus (Strobel et al., 2002), which may

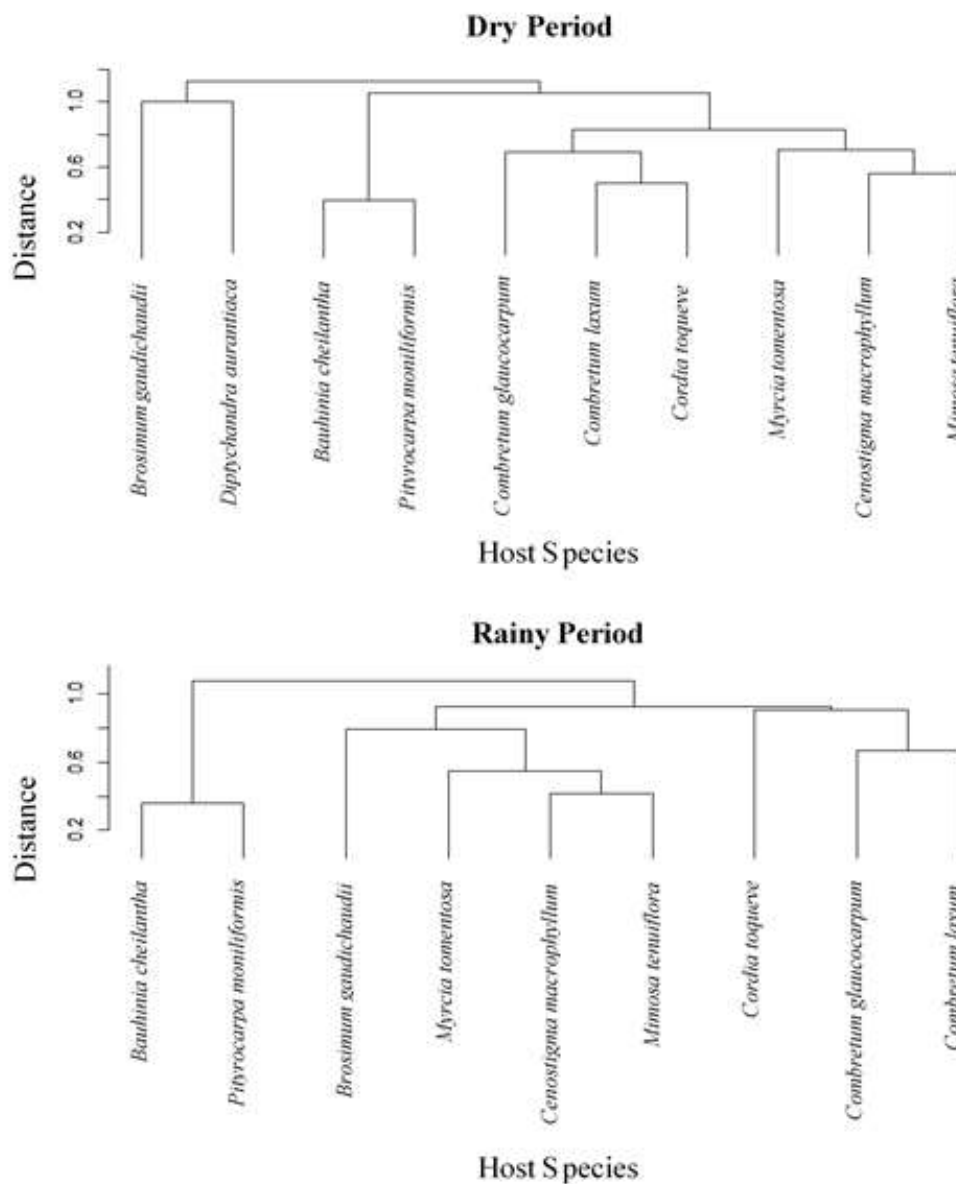


Figure 4 – Grouping analysis based on the relative fungal abundance per host forest species, in dry and rainy periods, in a Cerrado-Caatinga ecotone area, in Piauí State, Brazil.

Figura 4 – Análise de agrupamento baseada na abundância relativa de fungos por espécie florestal hospedeira, nos períodos seco e chuvoso, no ecótono Cerrado-Caatinga, no Piauí, Brasil.

have exerted suppressive action on the growth of other endophytes in this season.

Pestalotiopsis sp. was pathogenic to four of their hosts. This genus comprises a complex of 338 catalogued species (Index Fungorum, 2019) initially classified according to the host where they were first found (Maharachikumbura et al., 2014). Species

of *Pestalotiopsis* do not cause serious problems in forest plantations, as in *Eucalyptus* spp. Despite of its abundance in the environment, they need an initial injury to cause infection (Alfenas et al., 2009).

Some fungi were found only in the dry period, such as *Lasiodiplodia* sp. Such findings indicate the effect of seasonality on the occurrence of these genera. It is

worth noting that, in this period, the forest species in the area of Cerrado-Caatinga were in the process of foliar senescence. Sadaka and Ponge (2003) report that during the process of leaf senescence there is generally an increase in the wealth of colonizing fungal species and also an increase in the internal colonization of foliar tissue. Such evidence may be related to the fact that, when the plants are under biotic or abiotic stress, and during leaf senescence, there is an accumulation of sugars, such as glucose, fructose and sucrose (Wingler and Roitsch, 2008). Thus, it is deduced that the process of foliage senescence can modify the ecological niche of some species, enabling greater frequency of fungi adapted to saprophytic life (Ghizelini et al., 2006).

Lasiodiplodia sp. was pathogenic to all its hosts. Species of this genus may infect a wide variety of host plants or survive as saprobes or endophytes in leaves and other living tissues (Sakalidis et al., 2011; Phillips et al., 2013). This evidence, combined with the information of the period of greatest occurrence of this fungus is important to support preventive control measures of diseases caused by species of *Lasiodiplodia* which may be potentially pathogenic to the plantations of the region where the study area is inserted.

The occurrence of genera considered as biological control agents, such as *Trichoderma*, *Aureobasidium* and *Coniothyrium* may show that the environment is in balance. *Trichoderma* isolates were found in symptomatic and asymptomatic leaves, indicating a possible antagonistic relationship of this genus with the associated fungi. These isolates may have a potential as inductors of resistance in local forest species. Consolo et al. (2012) reported that biocontrol by *Trichoderma* spp. occurs indirectly through competition for space and nutrients, antibiotic production, induction of plant growth and its defense mechanisms, or directly by parasitism.

The clusters results show that the degree of similarity between species varies with season. This variation is probably due to factors related to the host plant in its different phenological phases, and to the fungi themselves, which may present different survival strategies in each season (Miranda et al., 2010).

Some species of the Fabaceae family were grouped, in both dry and rainy seasons. This is due to the fact that these species are associated with the greater abundance of fungi in common, which suggests that there is a

preference of some fungi for species in Fabaceae family, such as *Pestalotiopsis* spp. Such knowledge is of great value to support programs for diseases management in Fabaceae local crops.

5. CONCLUSIONS

The occurrence of fungi in the Cerrado-Caatinga ecotone area were influenced by seasonal variation. There was greater tendency for fungi to occur in forest species belonging to Fabaceae family and to a lesser extent to Combretaceae family which presented a significant number of fungi. Mycological studies in areas of Cerrado-Caatinga transition are important to evaluate fungal diversity in different periods of the year.

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