Rodriguésia 73: e00052021. 2022
http://rodriguesia.jbrj.gov.br
DOI: http://dx.doi.org/10.1590/2175-7860202273040

Original Paper

Ascomycota in the litter of *Inga edulis* and *Lafoensia pacari* in an Atlantic Forest remnant in southeastern Bahia state, Brazil

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Abstract

The Ascomycota population in the litter of *Inga edulis* and *Lafoensia pacari* trees was identified and its richness was evaluated. The collections were carried out from October 2018 to July 2019. Twenty fallen leaves were collected in different stages of decomposition. The leaf samples were carefully washed in running water and incubated in humid chambers. The fungal structures were mounted in PVLG resin and observed under a light microscope. The identification was done by consulting the specific literature. Distribution studies included richness, frequency, constancy, and similarity of the fungal populations. The total richness was 48 species and 36 genera corresponding to 58.33% in *I. edulis* and 60.41% in *L. pacari.* Most taxa had sporadic frequency and accidental constancy. There was low similarity between plant species. Ascomycota populations are well represented in *Inga edulis* and *Lafoensia pacari* litter. Richness, frequency, constancy, and similarity of these populations varied little in function of the collection date, climate and host plant. Lower richness observed in the second collection may reflect the effect of reduced humidity observed in that time of the year.

Key words: decomposing fungi, Fabaceae, Fungi, Lythraceae.

Resumo

A população de Ascomycota na serapilheira das árvores *Inga edulis* e *Lafoensia pacari* foi identificada e avaliada a sua riqueza. As coletas foram realizadas no período de outubro de 2018 a julho de 2019. Foram coletadas 20 folhas caídas em diferentes estágios de decomposição. As amostras de folhas foram cuidadosamente lavadas em água corrente e incubadas em câmaras úmidas. As estruturas do fungo foram montadas em resina PVLG e observadas ao microscópio de luz. A identificação foi realizada consultando a literatura específica. A riqueza total foi de 48 espécies e 36 gêneros correspondendo a 58,33% em *I. edulis* e 60,41% em *L. pacari.* A maioria dos táxons possui freqüência esporádica e constância acidental. Houve baixa similaridade entre as espécies de plantas. *Inga edulis* e *L. pacari* possuem grandes populações de Ascomycota na separilheira delas. A riqueza, freqüência, constância e similaridade dessas populações variaram pouco em função da época de coleta, clima e planta hospedeira. A baixa riqueza observada na segunda coleta pode refletir o efeito da menor umidade observada naquela época do ano.

Palavras-chave: fungos em decomposição, Fabaceae, Fungi, Lythraceae.

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Introduction

The Atlantic Forest is the most biodiverse tropical forest in the world, with an estimated 20,000 plant species and 2,420 vertebrates, including endemic and endangered species (Rezende *et al*. 2018). Being considered as a worldwide hotspot (Mittermeier *et al*. 2011). Unfortunately, its diversity has been threatened by anthropogenic actions since the discovery of Brazil, with the extraction of brazilwood (*Caesalpinia echinata* Lam.) (Sanquetta 2008). This biome includes countless species of living organisms some of which yet unknown to science (Paglia & Pinto 2010).

The fragments of the Atlantic Forest have plant and soil heterogeneity and diverse microclimatic characteristics generating a large amount of organic matter very rich in microorganisms (Marques *et al* 2007; Santos *et al.* 2011). The organic matter accumulated on the soil surface consists mainly of dead plant material (leaves, wood, roots, branches, fruits and flowers) and functions as a reservoir of biodiversity organisms (Penna-firme & Oliveira 2017; Santa-Izabel & Gusmão 2018). In addition, forest productivity can be estimated by the rate of litter decomposition responsible for nutrient cycling (Penna-Firme & Oliveira 2017).

Litter fungi along with other organisms guarantee the cycling of nutrients, essential for the nutrient balance in forest ecosystems (Hättenschwiller *et al.* 2011). Fungi exhibit great morphological and physiological adaptations to this type of environment and their activity depend on many ecological factors including temperature and humidity at ground level (Marques *et al*. 2008).

A number of researchers studied the fungal population in the Atlantic Forest litter seeking for the relations of litter fungi to plant species, time of the year and geographical location (Magalhães *et al.* 2011; Costa & Gusmão 2015; Santos *et al.* 2017; Santa Izabel & Gusmão 2018; Grandi & Silva 2003; Paulus *et al*. 2006; Morath *et al.* 2012).

The tree species *Inga edulis* Mart. (Fabaceae) and *Lafoensia pacari* A. St.-Hil. (Lythraceae) are widely distributed in tropical and subtropical forests (Dias *et al.* 2010) and both are important for restoring riparian forests and degraded areas (Barbosa *et al.* 2017).

The mycobiota associated with litter of these plants had not been studied and the main objective of this research was to explore the Ascomycota fungi associated with them and study their population distribution.

Materials and Methods

Study area

Collections and analyses were carried out at the Executive Committee of the Cocoa Farming Plan - CEPLAC, of the Ministry of Agriculture - MAPA. The area is located at km 22 of the Jorge Amado highway, municipality of Ilhéus, Bahia, Brazil (latitude: 14º47'20"S; longitude: 39º02'58"W). This locality is inserted in the central corridor of the Atlantic Forest, formed by the dense ombrophilous forest, belonging to the neotropical zone (Veloso *et al.* 1991). The climate in the region, according to the Köppen classification, is of the Af type, hot and humid tropical forest without a dry season, with an average rainfall of 1,300 mm distributed throughout the year, an average temperature of 23 °C and relative air humidity 80%. The soil of the experimental area was classified as Nutrosolo Háplico Eutroferrico as Santana *et al.* (2002).

Collection

The authors collected litter from two individuals of *Inga edulis* (14°45'25"S and 39°14'23"W) and two of *Lafoensia pacari* (14°45'26"S and 39°14'23"W) located in the experimental area H' of CEPEC/CEPLAC from October 2018 to July 2019. They used 50 × 50 cm (0.25 m²) square frames to mark areas under the trees to pick 20 leaves of each plant species at different stages of decomposition. The samples were transported to the Fungal Diversity Laboratory of CEPLAC for processing. On July 26, 2018, the climate conditions were: average temperature 23.7 °C, 97 mm of rain in the month, humidity of 85%; on March 12, 2019, were: average temperature 25.5 °C, rain 125 mm/ in the month, humidity 82% and on July 5, 2019, were: average temperature 21.6 °C, rain 109 mm/ in the month, humidity of 86%.

Samples processing

The samples were cleansed gently in plastic sieves for one hour in tap water. After cleaning the leaves were placed in moist chambers made of plastic containers which were opened daily for fifteen minutes for air renewal, according to Castañeda-Ruiz *et al*. (2006). Stereoscopic observation of the samples started 48 h after incubation during 30 days.

Fungal characterization

Fungal structures (somatic, reproductive and resistance structures) were mounted in permanent

mounting medium (PVLG resin: polyvinyl alcohol + lactoglycerol) (Morton *et al*. 1993), between microscope slides and coverslips. The preparations were examined in a light microscope provided with a photographic camera and a micrometric ocular. The structures were interpreted, measured and photographed. Species identification was achieved through specific literature consultation.

Fungal population estimation

The variation of Ascomycota population was based on estimation of richness, frequency, constancy, and similarity of the species found.

Richness was defined as the total number of species found in the collection sites and in each plant species (Brower *et al*. 1998).

The calculation of frequency of occurrence was based on the following formula:

$$F = n \times 100/N$$

Where: n = number of samples in which a species was found; N = total sample in each plant species (2 plant species × 3 collections = 6).

Frequencies were classified according to the classes proposed by Dajoz (1983), adapted as: Sporadic, when found only in one collection; Uncommon, when found in two collections; Frequent, when found in three collections, but in only one species; and Very Frequent, when found in three collections and in both plant species.

To calculate constancy, the following formula was used:

$$C = p.100/P$$

Where: p = number of collections in which a fungal species was found; P = total number of collections (three collections).

Constancy was classified as: Accidental, when less than or equal to 33%, that is, present in only one collection in this study; Accessory, when greater than 50% and less than or equal to 66%, present in 2 collections; and Constant, when greater than 66%, present in three collections. The methodology was adapted from Cavalcanti & Mobim (2004).

Similarity was obtained from the Sorensen Index (Muller-Dombois 1981), using the following formula:

$$S = 2c.100/a + b$$

Where: c = number of fungi common to both plant species; a + b = number of fungi present on the trees.

The three collections were compared according to the following formula:

$$S = 3d.100/a + b + c$$

Where: d = number of fungi common to the three collections; a + b + c = number of fungi present in the collections.

The analyses were conducted in the PAST 3:01.

Results and Discussion

A total of 48 fungal taxa were identified inserted in 37 genera. *Inga edulis* litter yieldd 28 taxa included in 18 genera and *Lafoensia pacari* 29 taxa in 26 genera. All taxa identified in the present study are reported as decomposers of plant substrates of various plant species and are recorded in inventories on tropical regions (Santa Izabel & Gusmão 2018; Monteiro *et al.* 2019) and temperate regions (Hernández-Restrepo *et al*. 2017) in various regions of the world. Many studies that have investigated saprobic fungi have revealed new genera, species and records, especially when tropical areas were investigated (Heredia-Abarca *et al.* 2018; Cantillo *et al*. 2019; Pem *et al.* 2019; Barbosa *et al.* 2020; Hyde *et al.* 2020).

Fungal species with frequency of occurrence appeared in all collections (Tab. 1). The genus *Thozetella* was prevalent in *I. edulis* litter and was represented by eight species.

Taxa richness was 58.33% for the leaf litter of *I. edulis*, and 60.41% for the leaf litter of *L. pacari* (Fig. 1a), taxa richness was highest for *L. pacari* (39.58%) in the first collection and for *I. edulis* (37.50%) in the third collection. Lowest richness was found for both plants in the second collection (29.08 % for *I. edulis* and 25 % for *L. pacari*) (Fig. 1b).

Regarding taxa richness, Marques *et al*. (2008) studying a dense forest area and a sparse forestry area in the Atlantic Forest of Bahia observed higher richness in the dense area. Other surveys also showed variations in richness depending on the studied area and the amount of collections made (Magalhães *et al.* 2011; Costa & Gusmão 2015; Santos *et al.* 2017; Santa Izabel Gusmão 2018). Polishook *et al.* (1996) studied fungal richness in mixed leaf litter in forest ecosystems, and concluded that fungi prefer certain substrates. This fact was also observed by Hyde & Alias (2000), who reported that different plant parts (leaves, petioles, barks, among others) harbor different fungi.

Sporadic taxa showed up in *L. pacari* (58.62%) and in *I. edulis* (57.14%) (Tab. 2). Uncommon taxa surged more in *L. pacari* (24.13%) than in *I. edulis* (17.85%). *Inga edulis*

Table 1 – Frequency of occurrence of fungi found in *Inga edulis* and *Lafoensia pacari* trees in an Atlantic Forest remnant in Ilhéus city, Bahia State, Brazil.

Taxon	*Inga edulis*	*Lafoensia pacari*
	FO (%)	FO (%)
Actinocymbe sp.	1.0	0.0
Beltrania rhombica Penz.	0.3	0.0
Beltraniella portoricensis (F. Stevens) Piroz. & S.D. Patil	0.3	0.3
Castanediella ramosa (Matsush.)	0.0	0.5
Ceratocladium sp.	0.0	0.3
Chaetopsina fulva Rambelli	0.0	1.0
Cladosporium aff. *cladosporioides* (Fresen.) G.A. de Vries	0.0	0.3
Cladosporium sp.	0.5	0.3
Clonostachys aff. *rosea* (Preuss)	0.3	0.3
Clonostachys aff. *compactiuscula* (Sacc.) D. Hawksw. & W. Gams	0.3	0.0
Coccomyces leptosporus Speg.	1.0	0.0
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	0.0	1.0
Colletotrichum sp. (sexual morph)	0.3	0.0
Cryptophiale hamulata K.D. Hyde & McKenzie	0.0	0.3
Graphium sp.	0.5	0.0
Hansfordia pulvinata (Berk. & M.A. Curtis) S. Hughes 1958	0.0	0.3
Idriella lunata P.E. Nelson & S. Wilh.	0.3	0.0
Lauriomyces acerosus Somrith., Suetrong & E.B.G	0.0	0.5
Menispora britannica (M.B. Ellis) P.M. Kirk	1.0	0.3
Menisporopsis profusa Piroz. & Hodges	0.5	0.0
Menisporopsis theobromae S. Hughes	0.3	0.0
Metulocladosporiella sp.	0.0	0.3
Muyocopron corrientinum Speg	0.0	0.3
Nectria sp.	0.0	0.3
Neopestalotiopsis pernambucana Silvério, M.A.Q. Cavalc. & J.L. Bezerra	0.0	0.3
Ophioceras sp.	1.0	0.0
Ophiostoma sp.	0.0	0.3
Parasympodiella laxa (Subram. & Vittal) Ponnappa	0.0	0.5
Penicillium spp.	0.0	1.0
Periconia byssoides Pers.	0.3	0.0
Pestalotiopsis sp.	1.0	1.0
Pyrenochaeta sp.	0.0	1.0

Taxon	*Inga edulis*	*Lafoensia pacari*
	FO (%)	FO (%)
Sporidesmium tropicale M.B. Ellis	0.0	0.3
Sympodiella sp**.**	0.0	0.3
Thozetella aff. *submersa* F.R. Barbosa & Gusmão	0.3	0.0
Thozetella buxifolia Allegr. Cazau, Cabello & Aramb	1.0	0.0
Thozetella cristata Piroz. & Hodges	1.0	0.3
Thozetella falcata B.C. Paulus, Gadek & K.D. Hyde	0.5	0.0
Thozetella havanensis R.F. Castañeda	1.0	0.0
Thozetella queenslandica B.C. Paulus, Gadek & K.D. Hyde	0.3	0.0
Thozetella radicata (E.F. Morris) Piroz. & Hodges	0.3	0.0
Thozetella sp.	0.3	0.3
Triposporium sp.	0.0	0.5
Vermiculariopsiella cf. *cubensis* (R.F. Castañeda)	0.3	0.0
Vermiculariopsiella pediculata (J.L. Cunn.)	0.3	0.5
Volutella sp.	0.0	0.0
Volutellonectria consors (Ellis & Everh.)	0.3	0.0
Zygosporium sp.	0.5	0.5
Total = 48		

presented 21.42% frequent taxa and *L. pacari* 17.24 %. The species composition of frequent taxa was different in the two plants: *Menispora britannica*, *Ophioceras* sp., *Thozetella buxifolia*, *T. cristata* and *T. queenslandica* were frequent taxa in *I. edulis*, while *Chaeptopsina fulva*, *Penicillium* spp., *Pyrenochaeta* sp., and *Sympodiella* sp. were frequent in *L. pacari*. Only one taxon (*Pestalotiopsis* sp.) was very common in all collections and on both trees, accounting for 3.57% and 3.44% richness in *Inga edulis* and *Lafoensia pacari*, respectively.

As for taxa constancy in the trees, accidental fungi collected one time in one collection predominated (Fig. 2). In *I. edulis*, 57.14% of the taxa were accidental, 17.85% accessory, and 25% constant. In *L. pacari*, 58.62% of the taxa were accidental, 24.13% accessory, and 17.24% constant. Santana *et al*. (2017) also observed a greater occurrence of accidental species (61.54%) in the Atlantic Forest fragment.

Santos *et al.* (2017) did a very accurate study of the litter fungi in three tree species in a large reserve of the Atlantic Forest in the municipality of Una, Bahia and they found that 43.6% of the taxa were constant.

Our results showing the predominance of sporadic taxa fits those reported by many authors in previous studies on litter fungi of the Atlantic Forest (Barbosa *et al.* 2009; Magalhães *et al*. 2011; Santos *et al.* 2017). *Pestalotiopsis* sp. was the only very frequent species detected probably because it is an extremely effective saprobe and endophyte (Strobel & Daisy 2003; Jeewon *et al*. 2004; Devarajan & Suryanarayanan 2006; Kruschewisky *et al.* 2014). The predominance of accidental taxa was reported by Magalhães *et al.* (2011) studying three plant species in three areas in southern Bahia. Marques *et al.* (2008) and Barbosa *et al.* (2009) published similar results in other fragments of the Atlantic Forest in Bahia. The authors concluded that the rapid succession of litter fungi during colonization in a humid chamber may cause the overlook of some species. This fact was noted also in the present study. Accidental fungi species seems to be highly influenced by temperature and

Table 2 – Distribution of the taxa obtained in the plant species *Inga edulis* and *Lafoensia pacari* by frequency class.

Frequency	*Inga edulis*	(%)	*Lafoensia pacari*	(%)
Sporadic	16	57.14	17	58.62
Uncommon	5	17.85	7	24.13
Frequent	6	21.42	4	17.24
Very frequente	1	3.57	1	3.44
Richness/plant	28	100	29	100

humidity, as well as, by litter decomposition stage. Constant species appears to be less influenced by these conditions and are perhaps more resistant to climate changes (Lima & Sousa 2014; Costa & Gusmão 2017).

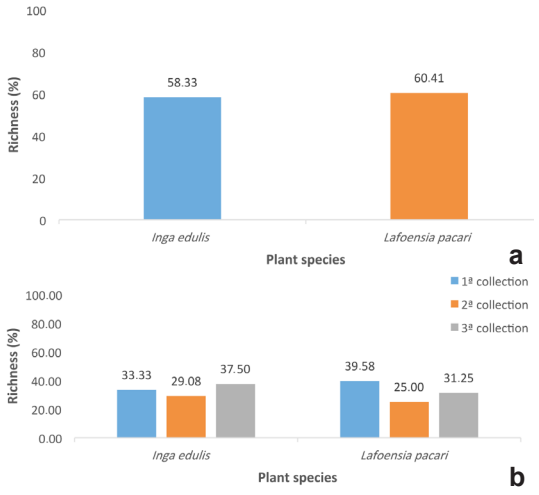

Figure 1 – a-b. Fungal richness of each plant species – a. *Inga edulis* and *Lafoensia pacari*; b. between collections 1, 2, and 3.

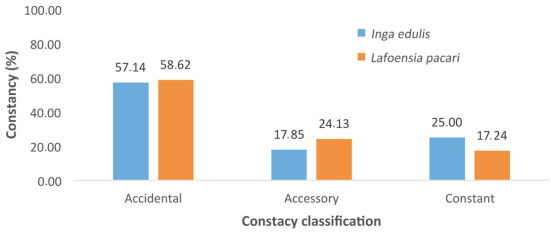

Figure 2 – Taxa constancy in the litter of plant species *Inga edulis* and *Lafoensia pacari*.

Fungal similarity between plant species was 31.57% (Tab. 3), that is, of the 48 taxa identified in the two trees, only the following nine fungi were common: *Beltraniella portoricensis*, *Cladosporium* sp., *Clonostachys* aff. *rosea*, *Menispora britannica*, *Pestalotiospsis* sp., *Thozetella cristata*, *Thozetella* sp., *Vermiculariopsiella pediculata*, and *Zygosporium* sp. Comparing the three collections a low similarity (26.82%) was found but by pairing them a similarity above 50% was obtained.

Low similarity of fungal population was expected since the host plants belonged to different families. Studies show that the similarity index is higher among plants of the same genus or species than between different taxa (Maia 1983; Polishook *et al*. 1996). Similar results were found by Polishook *et al*. (1996) when studying the leaf litter of *Guarea guidonea* Sleumer and *Manilkara bidentata* Chev. The authors obtained fungal similarity of 32 % and 26 %, respectively in two different areas. Magalhães *et al.* (2011) obtained a slightly higher similarity when comparing three plant species (39.6%). According to Mueller-Dombois & Ellenberg (1974), two communities are considered similar when the Sorensen index is higher than 50%. In Pernambuco, Assunção (2010) also found similarity between the areas where she collected banana endophytic fungi. Analyzing the leaf litter of *Caesalpinia echinata* in two areas with and without impact of air pollution in São Paulo, Silva (2007) obtained a similarity index of 53.3%.

Of the taxa detected, 48 were asexual morphs and only the following six were teleomorphic: *Actinocymbe* sp., *Colletotrichum* sp., *Muyocopron corrientinum*, *Nectria* sp., *Ophioceras* sp., *Ophiostoma* sp. Many of the asexual (conidial) species were recorded in other studies (Cruz *et al.* 2007; Marques *et al.* 2008; Barbosa *et al.* 2009; Magalhães *et al.* 2011; Santos *et al.* 2017; Monteiro *et al.* 2019). The scarcity of teleomorphs of litter Ascomycota detected now in *I. edulis* and *L. pacari*

Table 3 – Fungal similarity between the plant species *Inga edulis* and *Lafoensia pacari*, and between collections 1, 2, and 3.

Plant species and collection	Similarity (%)
Inga edulis and *Lafoensia pacari*	31.57
Collection 1 and collection 2	61.11
Collection 1 and collection 3	55.93
Collection 2 and collection 3	64.70
Collection 1, collection 2, and collection 3	26.82

occurs with other types of litter (Parungao *et al*. 2002; Duong *et al.* 2008; Santana *et al.* 2017). These authors reported a vast majority of asexual morphs of Ascomycota in litter.

Most species encountered in this work were typical litter fungi such as: *Beltrania rhombica*, *Beltraniella portoricensis*, *Castanediella ramose*, *Ceratocladium* sp., *Volutella* sp., and *Volutellonectria consors.* Other species correspond to epifoliar pathogens or parasites of canopy leaves, e.g., *Actinocymbe* sp., *Hansfordia pulvinata*, *Colletotrichum gloeosporioides* and others. *Actinocymbe* is an ascomycete belonging in the Chaetothyriales which has never been reported as a litter fungi since it is an epifoliar fungus. *Hansfordia pulvinata* is a mycoparasite which can be used as a biocontrol agent of several plant patogenic ascomycetes. The finding of epifoliar fungi in the litter of the studied plants implies in alterations in the fungus metabolism (Pugh *et al.* 1972). Many leaf pathogenic ascomycetes complete their life cycle on the fallen leaves of their hosts (Bowen *et al*. 2011).

Inga edulis and *Lafoensia pacari* have high populations of Ascomycota in their litter. Richness, frequency, constancy, and similarity of these populations varied little in function of epoch of collection, climate and host plant. Low richness observed in the second collection may reflect the effect of lower humidity observed in that time of the year.

Acknowledgements

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting the scholarship to the first (88882.451313/2019-01) and second (88882.451314/2019-01) authors. A research grant from the Conselho Nacional de Pesquisas - CNPq (309340/2017-9) to the last author is also acknowledged. We are also grateful to the Executive Committee of the Cacao Crop Plan (CEPLAC), for providing the Laboratory and materials to conduct the fungal research.

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Area Editor: Dr. Anibal de Carvalho Junior
Received in February 04, 2021. Accepted in May 03, 2021.