Fungal endophytes from vegetative and reproductive tissues of *Eugenia uruguayensis* in Uruguay

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The main purpose of this work was to study the endophytic fungal communities of vegetative and reproductive tissues of *Eugenia uruguayensis*, a native species of Myrtaceae and to compare them with those of *Eucalyptus* spp. planted in Uruguay. Identification of fungal isolates was performed according to morphology by means of conventional mycological methods. Molecular identification was used for isolates not sporulating on culture media or those impossible to identify by morphological characters. The endophytic community was dominated by *Phomopsis* spp., *Colletotrichum gloeosporioides* and *Diaporthe phaseolorum*. Molecular analysis revealed that sterile isolates corresponded to *Xylaria acuta*, *Xylaria digitata*, *Xylaria venosula* and *Xylaria* sp. Bark and fruit tissues showed the highest number of species. The mycobiota of vegetative and reproductive organs differed, having in common only 15 % of the taxa. *Colletotrichum gloeosporioides* and *Pestalotiopsis guepinii* were dominant in fruits and *Diaporthe phaseolorum* in twigs. The highest diversity of species was found in fruits and in leaf blades and the lowest in petioles. The tissue segments infected in neotropical Myrtaceae studied in Uruguay are similar to those found in temperate trees. In Uruguay, Myrtaceae are at the southern limit of their geographical distribution reflecting adaptations to temperate climate. Therefore, it is not surprising that *E. uruguayensis* has lower rates of infection with similar diversity in endophytic communities to that of tropical trees.

Keywords: native Myrtaceae, *Xylaria, Phomopsis, Diaporthe, Colletotrichum gloeosporioides*.

In recent years fungal endophytes and some potential pathogens occurring in native Myrtaceae (Bettucci *et al*. 2004) and non-native *Eucalyptus* in South America, and particularly in Uruguay, have been studied (Bertoni & Cabral 1988; Bettucci & Saravay 1993; Bettucci & Alonso 1997; Bettucci *et al*. 1997, 1999; Lupo *et al*. 2001). Some fungal species of *Botryosphaeria* (Pérez *et al*. 2010) and *Puccinia psidii* (Alfenas & Zauza 2007) have jumped from native Myrtaceae to *Eucalyptus* plantations. Hence, it is considered to be very important to study endophytic communities as well as fungi with other strategies that are associated with different vegetative and reproductive organs from native species of Myrtaceae. Several genera of this large family of plants are distributed in tropical and subtropical forests in the

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South Eastern part of South America (Brazil, Uruguay, Northeast Argentina, South Central Paraguay), mainly between 20–35 ºS and 48–56 ºW, with a few genera restricted to the Andean highlands of the northwest (Landrum 1981).

In Uruguay a common species, *Eugenia uruguayensis*, is an evergreen tree of 3–5 m height, which grows on low hills and close to riparian vegetation, on soils of various types, except saline soils. The trees can also resist well droughts and short term floods. These trees are usually grown from seeds that germinate in about a month. It is a species of medicinal use. An infusion of digestive, diuretic and antidiarrheal properties can be prepared from the leaves; the decoction of the bark is used in gargling for angina and other infections of the throat (Legrand & Klein 1977, Barneche *et al.* 2010). The main purpose of this work was to study the composition of endophytic fungal assemblages of the native Myrtaceae *E. uruguayensis*. An additional purpose was to compare them with those of *Eucalyptus* spp. and to detect, as well, whether differences between vegetative and reproductive tissues exist.

Materials and methods

Study area

The study area named Parador Tajes is located in a riparian park of 62 ha at 34° 36′ 28″ S 56° 28′ 20″ W. It forms part of the protected wetlands of the Rio Santa Lucía in the Department of Canelones. This river flows into an old marine bay that was filled with sediments up to 2000 m depth. By its latitude Uruguay is found integrating part of the subtropical region of South America, but its climate is considered temperate, humid, rainy and without a dry season. The annual average precipitation is 1300 mm and the annual average temperature is nearly 17.5 ºC (Strahler & Strahler 1992). The riparian forest in which the study area is located constitutes a formation that occupies low zones on the margins of the Rio Santa Lucía. The plant species are distributed in stripes parallel to the course of water from hydrophilic to xerophilic species. Some species that grow on the margins with the roots in direct contact with the water or in nearby zones are *Salix humboltiana* Willd., *Cephalanthus glabratus* (Spreng.) K. Schum., *Helianthus sellowianus* Müll. Arg., *Sebastiania commersoniana* (Baill.) L.B. Smith, *Pouteria salicifolia* (Spreng.) Radlk. and *Erythrina cristagalli* L. In far away and less humid zones *Allophylus edulis* (St. Hill.) Radlk., *Myrtus communis* L., *Myrsine parvula* (Mez.) Otegui, *Scutia buxifolia* Reiss. and *Eugenia uruguayensis* Cambess., among others, are present. Finally, farthest from the river, xerophilic species appear, i.e. *Celtis tala* Gillies Planch., *Berberis laurina* Billb. and *Lithraea molleoides* (Vell.) Engler can be found. The limit with the grassland is, in general, clear (Brussa & Grela 2007).

Sampling

Twigs and leaves

Eugenia uruguayensis is sparsely distributed in the study area since it is located at the southern limit of its geographical distribution. From five ran-

domly selected trees ten asymptomatic branches with leaves, two from each tree, were collected. Trees were marked at the start of the sampling. All the material was taken to the laboratory in paper bags, stored at 5 °C and processed within 24 h. From the middle part of each branch one asymptomatic twig (2–5 mm in diameter) was selected. From each of these 10 twigs, ten segments, of approximately 2–5 mm in diameter and 5 mm in length, were cut and the bark was stripped off the xylem. From the same twigs 100 asymptomatic leaves (10 from each twig) were collected and one disc of 4 mm in diam. of each blade was dissected. Petioles were also examined, one segment from each leaf (100) since they are very small. Segments from each tissue were pooled and surface-sterilized by sequential immersion in 80 % ethanol for 1 min and in sodium hypochlorite (0.4 g active Cl/100 ml) for 2 min, washed with sterile distilled water and then dried on sterile filter paper.

Flowers, fruits and seeds

From the same five trees, ten branches with flowers, two from each tree, were collected in spring (October) and also from these trees five months later (March), ten branches with fruits were cut off. All the material was taken to the laboratory in paper bags, stored at 5 °C and processed within 24 h.

Flowers and fruits were taken from their branches and surface sterilization was performed as for leaves and twigs. After flowers were separated from the peduncles, they were sectioned longitudinally at the middle. Each part was plated in sets of ten segments per plate, 100 from the half of each flower and 100 segments from peduncles, were plated out as described above. Fruits without seeds, and previously separated from peduncle, were also sectioned longitudinally in the middle. Each part was plated in sets of ten per plate, 200 from fruits and 100 from peduncles. From seeds, tegument was stripped from cotyledons and plated separately in sets of ten segments per plate, 100 from tegument and 100 from cotyledons.

To test the effectiveness of surface sterilization, imprints were performed of all segments on culture medium.

Isolation and identification

The total segments from all tissues (400 from vegetative and 700 from reproductive) were plated in sets of ten segments per plate containing MEA 2 % (Difco), pH 4,5 and incubated at 24 °C for six weeks or more depending on the growth rate of fungi. Each colony that emerged from segments was transferred to fresh medium (MEA 2% Difco, PDA Difco and oatmeal agar) for identification.

Morphological identification was performed by means of conventional mycological methods following description of the cultural and micromorphological characteristics of each isolate (Ellis 1971, 1976; Sutton 1980; Dennis 1981; Gams 1983). For not sporulating isolates or those that were not possible to identify by their morphology, molecular identification was used to place them in a putative taxonomic position. DNA was obtained from

fresh aerial mycelia by extraction with cetyltrimethylammonium bromide (CTAB), followed by organic extraction and isopropanol precipitation of the DNA using the method described by Lee & Taylor (1990). The 5.8 S gene and flanking internal transcribed spacers (ITS1 and ITS2) regions of rDNA were amplified using the fungal specific primer set (ITS4 and ITS5) (White *et al.*1990). When necessary, large subunit (LSU) of RNA was also amplified to provide taxonomic information. The primers used to amplify LSU sequences were LROR and LR7 (Vilgalys & Hester 1990). PCR protocol described by Rehner & Uecker (1994) was performed. Representative isolates were subjected to similarity searches against those deposited in Gene Bank using BLAST (Basic Local Alignment Search Tool). In cases in which multiple matches were equally probable it was recorded the lowest taxonomic level shared by the disparate matches. As Genbank lacks sequence data for most fungi at species level, some isolates were named only by the corresponding genus. Sterile isolates that did not match with any genus, remained as sterile mycelia.

Data analysis

The relative frequency of isolation was calculated as the number of segments colonized by a given fungus, divided by the total number of segments, expressed as percentage. The abundance distribution and species accumulation curves, derived from each tissue, were performed to evaluate to what extent fungal community was revealed by the sampling. Moreover, the curves of relative abundance were compared to lognormal theoretical model using the Kolmogorov-Smirnov test (Krebs 1989). Endophytic fungal diversity was measured for each tissue, organ and tree host by means of Shannon diversity index with the package MVSP for Windows version 3.21 (Kovach Computing, Anglesey, UK).

The distribution of taxa in vegetative and reproductive organs was examined by multivariate ordination using correspondence analysis (StatSoft 1998). This analysis was applied on the log of the relative frequency higher or equal to 2 % of each species occurring in more than one tissue. Species with lower frequency, but isolated from two different tissues, were also included (Howard & Robinson 1995).

Results

A total of 1100 segments, 400 from vegetative organs and 700 from reproductive structures, were colonized by 53 taxa. From all twigs and leaves 291 isolates belonging to 30 taxa were obtained, with the number of taxa ranging from 6 to13 in twigs and from 6 to 12 in leaves (Tab.1). The endophytic community was dominated by *Phomopsis* spp., *Colletotrichum gloeosporioides* (Penz.) Sacc. and *Diaporthe phaseolorum* (Cooke & Ellis) Sacc.

The morphological identification of isolates corresponding to *Phomopsis* was difficult in culture. The analysis of phylogenetic sequences of the ITS region revealed that some isolates corresponded to *Diaporthe phaseolorum*

Tab. 1. Endophytic fungi from vegetative and reproductive tissues of *Eugenia uruguayensis*. Frequency of colonization (%).

Fungi	Code	Fl	Fr	Se	Bl	Pe	Ba	Xy
Alternaria alternata Keissl.			4,7					
Bartalinia robillarioides Tassi							2,0	
Beltrania rhombica Penz.						7,0		
Bionectria ochroleuca (Schwein.) Schroers & Samuels			0,3					
**Botryosphaeria dothidea* (Moug.) Ces. & De Not.			1,0					
**Botryosphaeria obtusa* (Schwein.) Shoemaker			0,3					
**Botryosphaeria ribis* Grossenb. & Duggar			0,7					
**Botryosphaeria stevensii* Shoemaker	Bos	0,5	1,0					
Cladosporium cladosporioides (Fresen.) de Vries	Cla		1,3	1,5	1,0			1,0
Cladosporium sphaerospermum Penz.	Cls							1,0
Colletotrichum acutatum Simmonds			2,3					
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	Cog	11,5	10,0		18,0		16,0	
Colletotrichum phormii (Henn.) Farr & Rossman		1,0						
Coniochaeta velutina (Fuckel) Cooke						2,0		
Conoplea fusca Pers.							1,0	
**Daldinia* sp.							8,0	
**Diaporthe phaseolorum* (Cooke & Ellis) Sacc.	*Dia*		3,0			1,0	46,0	
Diplodia pinea (Desm.) Kickx.		3,0						
Epicoccum purpurascens Ehrenb.	*Epi*		1,0	2,0				
Eupenicillium brefeldianum (B. O. Dodge) Stolk & D. B. Scott					2,0			
*Fungal endophyte (Ascomycete)			0,7					
Fusarium graminearum Schwabe			0,7					
Fusarium lateritium Nees			1,3					
Fusarium oxysporum Sm. & Swingle			1,0					
Gliocladium catenulatum Gilman & Abbott		1,0						
Glomerella cingulata (Stoneman) Spauld & Schrenk	*Glo*		0,3		4,0			
Kuskia oryzae Huds.			0,7					
Lecanicillium lecanii (Zimm.) Zare & Gams				0,5				
Lentinus tigrinus (Bull.) Fr.					1,0			

Fungi	Code	Fl	Fr	Se	Bl	Pe	Ba	Xy
**Lophiostoma* sp. 1 Ces. & De Not.							1,0	
**Lophiostoma* sp. 2 Ces. & De Not.			0,3					
Metarhizium anisopliae (Metschn.) Sorokīn								1,0
**Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & Phillips	Neo	2,0	4,3		3,0	1,0		
Nigrospora sacchari (Speg.) Mason	Nis		0,3		3,0			
Nigrospora sphaerica (Sacc.) Mason	Nip		3,0			2,0	1,0	
**Paraconiothyrium fungicola* Verkley & Wicklow							2,0	
Pestalotiopsis guepinii (Desm.) Steyaert	Pes		14,3				1,0	
Phaeoacremonium sp. W. Gams, Crous & M. J. Wingf.	Pha		0,7	0,5				3,0
*"*Phomopsis micheliae* C. Q. Chang, Z. D. Jiang & P. K. Chi, illeg."					12,0			
Phomopsis sp.	Pho	22,0	28,0	2,5	20,0	86,0	25,0	
Scytalidium sp.			0,3					
Sordaria fimicola (Roberge ex Desm.) Ces & De Not.	Sor		1,3	1,0				
Trichoderma atroviride Bissett	Tra	6,5	1,0	5,0				
Trichoderma harzianum Rifai			0,7					
Trichoderma saturnisporum Hammill					1,0			
**Xylaria acuta* Peck							2,0	
**Xylaria digitata* (L.) Grev.					2,0			
**Xylaria* sp.					7,0			
**Xylaria venosula* Speg.			0,7					
Sterile mycelium (233)							3,0	
Sterile mycelium (235)							1,0	
Sterile mycelium (244)								2,0
Sterile mycelium (245)								1,0
Total isolates 668		**95**	**256**	**26**	**74**	**99**	**109**	**9**
Total taxa		**8**	**29**	**7**	**12**	**6**	**13**	**6**
Total segments		**300**	**200**	**200**	**100**	**100**	**100**	**100**

Bl = blade; Pe = petiole; Ba = bark; Xy = xylem; Fl = flower; Fr = fruit; Se = seed.
*: species identified by molecular methods.

Tab. 2. Species of endophytic fungi from vegetative and reproductive tissues of *Eugenia uruguayensis*. Identified by molecular analysis (ITS and LSU).

Taxa	Sequence	Nearest match	Query coverage	Max identity
Bionectria ochroleuca (Schwein.) Schroers & Samuels	ITS - LSU	*Bionectria ochroleuca* (AY686634.1, LSU)	100% (LSU)	99% (LSU)
Botryosphaeria dothidea (Moug.) Ces. & De Not.	ITS - LSU	*Botryosphaeria dothidea* (JQ936677.1, LSU)	100% (LSU)	99% (LSU)
Botryosphaeria ribis Grossenb. & Duggar	ITS - LSU	*Botryosphaeria ribis* (DQ246263.1, LSU)	100% (LSU)	99% (LSU)
Botryosphaeria stevensii Shoemaker	ITS - LSU	*Botryosphaeria stevensii* (DQ377864.1, LSU)	99% (LSU)	99% (LSU)
Colletotrichum phormii (Henn.) Farr & Rossman	ITS - LSU	*Colletotrichum phormii* (DQ286147.1, LSU)	100% (LSU)	100% (LSU)
Coniochaeta velutina (Fuckel) Cooke	ITS - LSU	*Coniochaeta velutina* (FJ167402.1, LSU)	100% (LSU)	100% (LSU)
Conoplea fusca Pers.	ITS - LSU	*Conoplea fusca* (EU552114.1, LSU)	100% (LSU)	99% (LSU)
Daldinia sp.	ITS - LSU	*Daldinia childiae* (EF562505.1, LSU)	85% (LSU)	94% (LSU)
Diaporthe phaseolorum (Cooke & Ellis) Sacc.	ITS	*Diaporthe phaseolorum* (HQ328006.1)	100%	99%
Diplodia pinea (Desm.) Kickx.	ITS - LSU	*Diplodia pinea* (EU754157.1, LSU)	100% (LSU)	99% (LSU)
Fungal endophyte (Ascomycete)	ITS - LSU	*Fungal endophyte* (EF420054.1, LSU)	100% (LSU)	98% (LSU)
Fusarium oxysporum Sm. & Swingle	ITS - LSU	*Fusarium oxysporum* (HM210091.1, LSU)	100% (LSU)	98% (LSU)
Lecanicillium lecanii (Zimm.) Zare & Gams	ITS - LSU	*Lecanicillium lecanii* (EF026005.1, LSU)	100% (LSU)	100% (LSU)
Lentinus tigrinus (Bull.) Fr.	ITS - LSU	*Lentinus tigrinus* (AY615974.1, LSU)	100% (LSU)	100% (LSU)
Lophiostoma sp. 1	ITS - LSU	*Lophiostoma hysterioides* (AB619019.1, LSU)	98% (LSU)	99% (LSU)
Lophiostoma sp. 2	ITS	*Lophiostoma cynaroidis* (EU552138.1)	89%	100%
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & Phillips	ITS	*Neofusicoccum parvum* (IQ647911.1)	100%	100%
Paraconiothyrium fungicola Verkley & Wicklow	ITS	*Paraconiothyrium fungicola* (AY642532.1)	95%	98%
"*Phomopsis micheliae* C. Q. Chang, Z. D. Jiang & P. K. Chi"	ITS	"*Phomopsis micheliae*"(AY620820.1)	100%	98%
Phomopsis sp.	ITS	*Phomopsis* sp. (EF687936.1)	100%	99%
Xylaria acuta Peck	ITS - LSU	*Xylaria acuta* (AY544676.1, LSU)	100% (LSU)	98% (LSU)
Xylaria digitata (L.) Grev.	ITS	*Xylaria digitata* (GU322456.1)	100%	99%
Xylaria sp.	ITS	*Xylaria venosula* (EF026149.1)	100%	99%
Xylaria venosula Speg.	ITS	*Xylaria venosula* (EF026149.1)	100%	99%

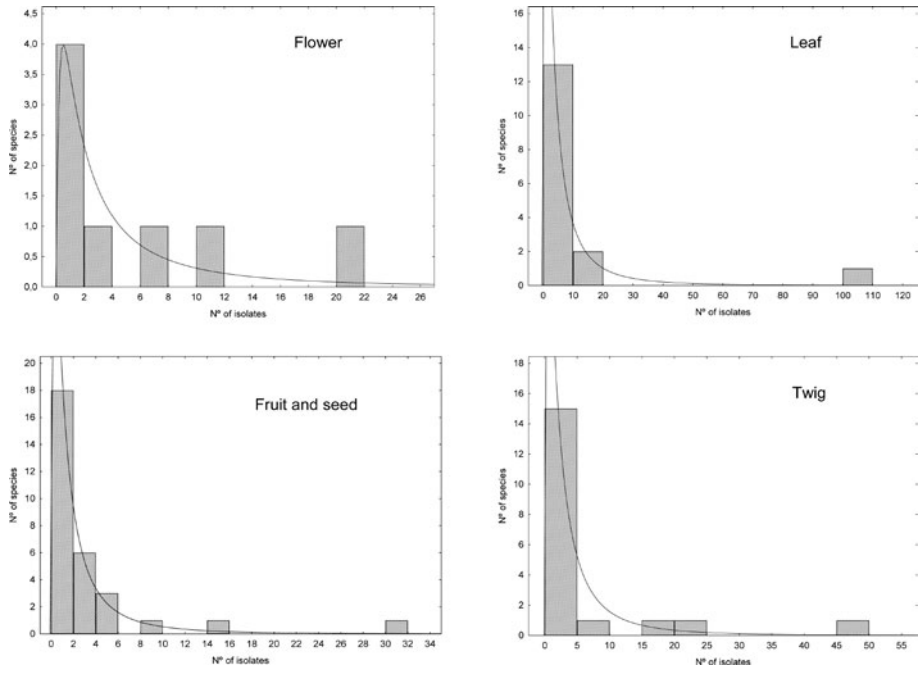

Fig. 1. Lognormal distribution of species abundances from each tissue. Few species were isolated with high frequency and several were rare. The lognormal distribution expected (line) did not differ significantly (P> 0.05) from observed data (Kolmogorov-Smirnov). Boxes represent the number of species within each range of isolates.

and to "*Phomopsis micheliae* (C. Q. Chang, Z. D. Jiang & P. K. Chi), illeg.". The remainder of the isolates were named *Phomopsis* spp. This genus is the anamorph of *Diaporthe* though some authors use *Diaporthe* to name species of *Phomopsis* and others consistently use the anamorph due to the abundance of *Phomopsis* species. When identifying according to ITS sequences we used both names *Diaporthe* or *Phomopsis*, depending on how the isolates were identified in GenBank. Identification of the isolates corresponding to Xylariaceae according to culture morphology was difficult since they remained sterile in culture; the analysis of phylogenetic sequences of ITS and LSU revealed that most isolates corresponded to the genus *Xylaria* and some of them to *Xylaria acuta* Peck, *Xylaria digitata* (L.) Grev., *Xylaria venulosa* Speg. and *Xylaria* sp. (Tab. 2).

From flowers, fruits and seeds 377 isolates belonging to 33 taxa were obtained, with the number of taxa ranging from 7 to 29 according to the organs (Tab. 1). The endophyte community was dominated by *Colletotrichum gloeosporioides, Diaporthe phaseolorum*, *Phomopsis* spp., *Trichoderma atroviride* Bissett and *Pestalotiopsis guepinii* (Desm.) Steyaert. *Phomopsis* spp. were more abundant in leaves, *C. gloeosporioides* and *P. guepinii* in fruits

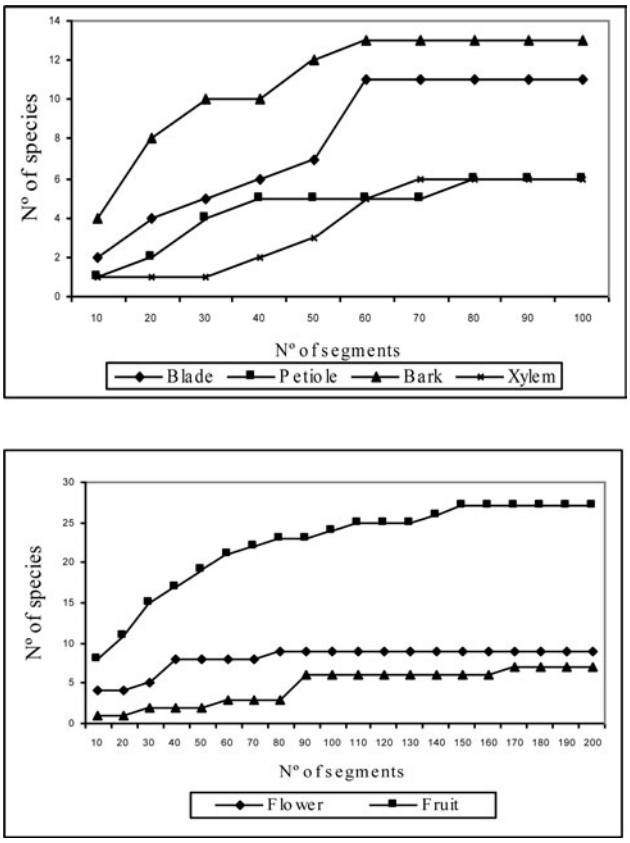

Fig. 2. Species accumulation curves showing the number of species found with each ten additional segments plated out.

and *D. phaseolorum* in twigs. Of the 53 taxa recorded, only 15 were isolated from fruits (Tab. 1).

Endophytic communities in all tissues fit a lognormal distribution (P>0.05) (Fig. 1). The cumulative species abundance (Fig. 2) shows the number of species found for each ten additional segments plated out. The point at which the asymptote was achieved varied for each tissue segment of vegetative organs, from 60 in blades, 80 in petioles, 60 in bark and 70 in xylem and from reproductive organs, 80 in flowers, 150 in fruits and 90 in seeds. The percent of segments colonized ranged from 9 % in xylem and 13 % in seeds to 85.3 % in fruits and 97 % in bark (Fig. 3). The fungal diversity in *E. uruguayensis* taking all analyzed tissues into consideration was 2.396. Diversity in reproductive tissues (2.379) was higher than that found in vegetative tissues (2.07). The diversity in blades was higher than that in petioles and that in the bark was lower than that in the xylem. Diversity was higher in twigs (1.976) than in leaves (1.55). Diversity in fruits (2.411) was higher than in

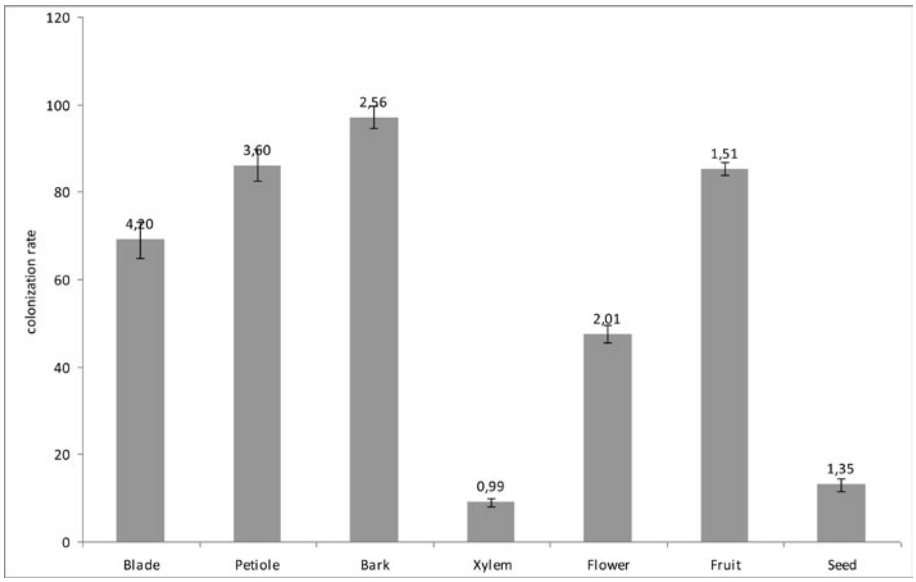

Fig. 3. Percentage of segments colonized from tissues of *Eugenia uruguayensis*.

flowers (1.49) and in seeds (1.67). The highest species diversity was found in fruits (2.411) and in leaf blades (2.003) and the lowest in leaf petioles (0.56) (Tab. 3).

Tab. 3. Measures of diversity for endophytic communities in tissues and plant organs of *Eugenia uruguayensis*.

	Eugenia uruguayensis	Blade	Petiole	Leaf	Bark	Xylem	Twig	Vegetative tissues	Flower	Fruit	Seed	Reproductive tissues
H'	2.396	2.003	0.56	1.55	1.709	1.834	1.976	2.07	1.49	2.411	1.67	2.379
J	0.603	0.806	0.313	0.559	0.666	0.943	0.671	0.609	0.717	0.716	0.858	0.681
S	53	12	6	16	13	7	19	30	8	29	7	33

H´= Shannon´n diversity index; J = evenness; S = total number of species in the community.

The simple correspondence analysis showed that the three coordinate axes explained 73.14 % of the total inertia, indicating a good fit of the data to the model (Fig. 4). The differences among tissues and organs in this ordination represented changes in species composition and abundance. The first axis that contributed with 33.26 % to the total inertia separated bark and fruits from the remaining tissues and organs and particularly the seeds and xylem. Moreover, this axis evidenced differences between bark and blade. The species which contributed most to the inertia in this axis was *C. gloeosporiodes* (11.73 %). *Diaporthe phaseolorum* (26.10 %) characterized to bark while *P. guepini* (7.37) to fruit, *T. atroviride* (11.04 %) to seeds and

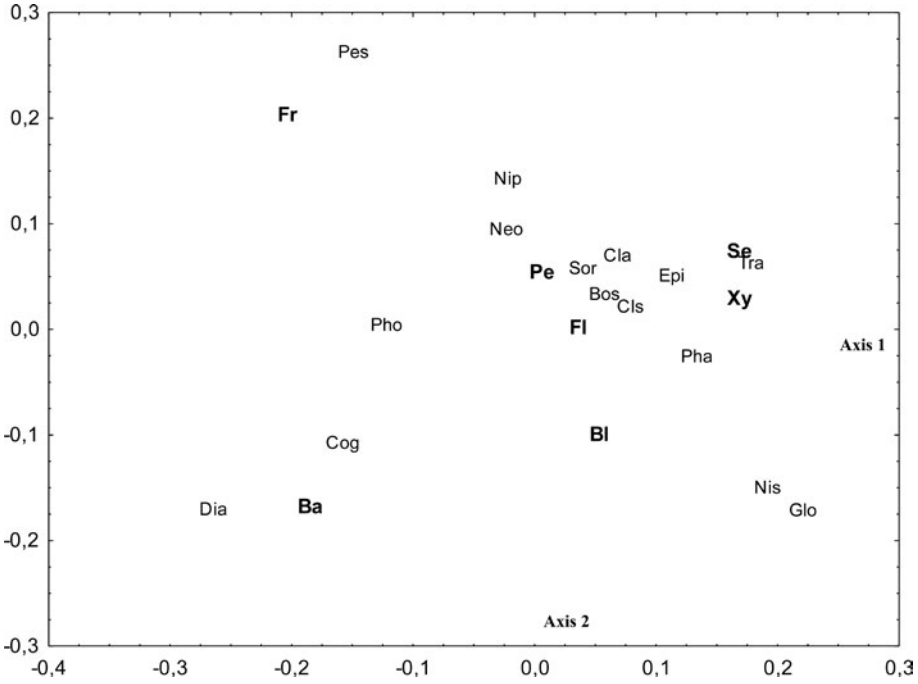

Fig. 4. Simple correspondence analysis. Ordination of tissues according to the endophytic composition on the two first axes. Total inertia explained by the first two axes was 55.84 %. Variables are the relative frequencies of isolation of species with frequency equal or higher than 2 % and those with lower frequency, but isolated from two host species. Bl = blade; Pe = petiole; Ba = bark; Xy = xylem; Fl = flower; Fr = fruit; Se = seed. Symbols for the species are indicated in Tab. 1.

Phaeoacremonium sp. (5.07%) to xylem. The blade was characterized by *Glomerella cingulata* (Stoneman) Spauld & Schrenk (11.01 %) and by *Nigrospora sacchari* (Speg.) Mason (10.45 %). The second axis that contributed with 22.58 % to the total inertia allowed separating fruit from bark. *Pestalotiopsis guepinii* (31.44 %), *D. phaseolorum* (17.7 %), *G. cingulata* (12.8 %) and *Nigrospora sacchari* (10.6 %) were the species that contributed more (72.5 %) to the construction of this axis. The third axis (17.32 %), not represented here, allowed to separate bark from xylem. *Diaporthe phaseolorum* (34.2 %), *Phomopsis* spp. (29.5%) and *C. gloeosporiodes* (10 %) were the species that contributed more to the total inertia of this axis. A set of species were related at the same time with flower and petiole tissues.

Discussion

The richness of fungal endophytes was low, 53 taxa in total, as was the number of tissue-specific fungal species. Several species isolated from *E. uruguayensis* were found in *Vitis*, *Pinus* and *Eucalyptus* reflecting that

spores from fructifications developed on debris of native plants can colonize introduced species. Endophyte composition from vegetative and reproductive organs, have in common only 15 % of the taxa. Differences in species diversity (H) and in equitability (J) were related with the abundance of *Phomopsis* spp. and *Diaporthe phaseolorum* in petiole, blade and bark tissues; although in flowers and fruits, species of *Phomopsis* were also abundant. The dominant species could be related to community structure and be responsible for affecting host physiology. A dominant species is considered a good colonizer in a host with high levels of ecological specialization under selection pressure (Yuan *et al*. 2010). Moreover, the distribution of the isolation frequency of some species could reflect tissue preference as in temperate trees (Carroll 1995). The diversity in twigs was, at least, similar to that of low and mid branches of *Guarea guidonia* (L.) Sleumer, from Puerto Rico (Gamboa & Bayman 2001).

On the other hand, the rarely isolated species could be truly rare taxa, with an obligatory endophytic lifestyle or could become dominants on adjacent plants (Joshee *et al*. 2009). One interesting species, *Metarhizium anisopliae*, isolated here only once from xylem, but commonly found in soils is an effective entomopathogen used in biocontrol of ants (*Acromyrmex* spp.) in *Eucalyptus* spp. plantations located in the southeast of Uruguay (Lupo *et al*.2008).

Pestalotiopsis guepinii was present in fruit at the highest frequency and few isolates were recovered from twig bark. This is a common endophyte in temperate (Bills & Polishook 1992, Barengo *et al*. 2000) and tropical (Bayman *et al*. 1998) tree species and in *Eucalyptus* spp. (Bettucci *et al*. 1997, 1998). Some isolates of *P. guepinii* have been reported to produce taxol, an important anti-cancer metabolite (Strobel *et al*. 1997). Some strains of *Bartalinia robillarioides* are also important producers of taxol (Gangadevi & Muthumary 2008).

Colletotrichum gloeosporioides present in fruits is a frequent decomposer of fleshy indehiscent fruits. *Phomopsis* spp, were present in nearly all tissues of *E. uruguayensis* except in xylem. "*Phomopsis micheliae"* was present only in leaf blades and *D. phaseolorum* was present with the highest frequency in twig bark. *Diaporthe/Phomopsis* constitute a fungal complex of high genetic diversity that includes pathogens of great economic importance, producers of secondary metabolites (Ting *et al*. 2009, Li *et al*. 2010, Ahmed *et al*. 2011) and finally numerous are endophytic (Udayanga 2011). Species prevalent as endophytes of many hosts in both temperate and tropical regions are especially common in the sapwood of angiosperms (Boddy & Griffith 1989, Rossman *et al*. 2007, Botella & Diez 2011, González & Tello 2011). In *Eucalyptus globulus* and *E. grandis* planted in Uruguay *Phomopsis arnoldiae* was found being one of the two most important species (Bettucci *et al*. 1999). In *E. uruguayensis* twigs the xylem was the unique tissue in which *Phomopsis* was not found.

Species of *Xylaria* have been isolated as endophytes of almost all tropical plants and to a minor extent of temperate trees (Dreyfuss & Petrini 1984,

Petrini & Petrini 1985, Boddy & Griffith 1989, Bills & Polishook 1992, Rodrigues *et al*. 1993, Bayman *et al*. 1998, Takeda *et al*. 2003) but the species found here were not the same recorded from others native Myrtaceae in Uruguay (Bettucci *et al*. 2004).

Wood rotting Basidiomycetes are frequent colonizers of forest leaf litter. *Lentinus tigrinus*, although with low frequency in *Eugenia uruguayensis* leaf blades, had not previously been found in native Myrtaceae, but was present in sapwood, heartwood and bark as latent colonizers of *Eucalyptus* spp. stems (Simeto *et al*. 2005) and as basidiomata surrounding *Eucalyptus* stumps (Alonso *et al*. 2012) and on a Salix trunk (Bettucci & Silva 1992). Probably, the large amount of spores produced by *L. tigrinus* found a good niche in leaf blade of this native Myrtaceae. This wood rotting fungus is an active producer of extracellular oxidative enzymes. It has been used for lignin wood degradation of *Eucalyptus globulus* and *E. grandis* (Bettucci *et al*. 1998) and for bleaching of *Eucalyptus* spp. paste paper (Speranza 2003).

From seven species present in seeds all except one were also present in the fruit tissues suggesting that seed infection came from there. This fact was also observed in young seeds inside the capsules of *E. globulus* (Lupo *et al*. 2001). In relation with *Botryosphaeria dothidea* and *Colletotrichum gloeosporioides* there is no specific evidence to assume that they could have adopted *Eucalyptus* spp. as a host considering that they colonize also several other hosts and have a wide world distribution (Fisher *et al*. 1993, Smith *et al*. 1996, Bettucci *et a1*. 1999).

In general, the proportion of twig segments infected in tropical trees (Arnold *et al*. 2001, Cannon & Simons 2002, Arnold & Herre 2003) exceed that recorded in neotropical Myrtaceae that we have studied (Bettucci *et al*. 2004) and also in *E. uruguayensis*, being similar to that found in temperate trees. In Uruguay, Myrtaceae are at the southern limit of their geographical distribution presenting morphological characteristics that reflect adaptations to temperate climate (Legrand & Klein 1977, Landrum 1981). Therefore, it is not surprising that *E. uruguayensis* has lower rates of infection with similar diversity in endophytic communities to that of tropical trees.

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