FOLIAR ANATOMY AND HISTOCHEMISTRY IN SEVEN SPECIES OF *Eucalyptus*[1]

Leonardo David Tuffi Santos[2], Marcela Thadeo[3], Lourdes Iarema[3], Renata Maria Strozi Alves Meira[4] e Francisco Affonso Ferreira[5]

ABSTRACT – This work aimed to describe the foliar anatomy of seven species of *Eucalyptus*, emphasizing the characterization of secretory structures and the chemical nature of the compounds secreted and /or present in the leaves. Anatomical characterization and histochemical evaluation to determine the nature and localization of the secondary compounds were carried out in fully expanded leaves, according to standard methodology. Anatomical differences were verified among the species studied, especially in *E. pyrocarpa.* Sub-epidermal cavities were the only secretory structures found in the seven species studied, with higher density in *E. pellita* and lower in *E. pilularis*. The following compounds were histochemically detected: lipophilic compounds, specifically lipids of the essential or resin-oil type and sesquiterpene lactones found in the lumen of the cavities of the seven species; and hydrophilic compounds, of the phenolic compound type found in the mesophyll of all the species studied and on the epidermis of some of them. The results confirmed the complexity of the product secreted by the cavities, stressing the homogeneous histochemistry nature of these compounds among the species. However, the phenolic compounds results may be an indication of important variations in adaptations and ecological relations, since they show differences among the species.

Keywords: Anatomy, *Eucalyptus* ssp. and plant defense.

ANATOMIA E HISTOQUÍMICA FOLIAR DE SETE ESPÉCIES DE Eucalyptus

RESUMO – Objetivou-se com o presente trabalho descrever a anatomia foliar de sete espécies de ***Eucalyptus****, com ênfase na caracterização de estruturas secretoras e da natureza química dos compostos secretados e/ ou presentes no limbo foliar. A caracterização anatômica e a avaliação histoquímica para determinação da natureza e localização dos compostos secundários foram realizadas em folhas totalmente expandidas segundo metodologia usual. Houve diferenças anatômicas entre as espécies estudadas, especialmente em* ***E. pyrocarpo****. Cavidades subepidérmicas foram as únicas estruturas secretoras encontradas nas sete espécies, com maior densidade em* ***E. pellita*** *e menor em* ***E. pillularis****. Foram detectados histoquimicamente os seguintes compostos: lipofílicos, especificamente lipídios do tipo óleo essencial ou óleo-resina e lactonas sesquiterpénicas encontradas no lúmen das cavidades das sete espécies; e hidrofílicos, do tipo compostos fenólicos encontrados no mesofilo de todas as espécies estudadas e na epiderme de algumas delas. Não foram detectados alcalóides, polissacarídeos e proteínas totais. Os resultados confirmam a complexidade do produto secretado pelas cavidades, enfatizando a homogeneidade quanto à natureza destes compostos entre as espécies. Entretanto, os resultados quanto aos compostos fenólicos podem ser um indicativo de variações importantes nas adaptações e relações ecológicas, uma vez que demonstraram diferenças entre as espécies.*

Palavras-chave: Anatomia, ***Eucalyptus spp****. e defesa de plantas.*

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[2] Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais (UFMG), Montes Claros-MG. E-mail: <ltuffi@yahoo.com.br>.
[3] Programa de Pós-Graduação em Botânica da Universidade Federal de Viçosa (UFV), Viçosa-MG.
[4] Departamento de Biologia Vegetal da UFV. E-mail: <rmeira@ufv.br>.
[4] Departamento de Fitotecnia da UFV. E-mail: <faffonso@ufv.br>.

1. INTRODUCTION

Native from Australia, with a few exceptions, the eucalypt was introduced to Brazil in the second half of the 19th Century to supply wood for the production of railroad ties. Today, it is the most cultivated forest species in the world, with over 17.8 million hectares, with Brazil presenting the largest planted area according to data from the Brasil (2005).

The genus *Eucalyptus* contains around 800 species, with most being used in commercial plantations, for several applications, such as production of pulp and cellulose, vegetal charcoal, sawmill wood, essential oils, posts and stakes, civil construction wood, furniture industry wood, ornamentation wood and others.

One of the major characteristics of the genus *Eucalyptus* is the production of secondary compounds, which pleasant aroma and pharmacological properties have attracted attention along time. The genus *Eucalyptus* was histochemically characterized by the presence of phenolic compounds, oils and resins (METCALFE, 1987). The secondary compounds are involved in diverse biological activities, probably as a result of plant co-evolution with other organisms, and interaction of plants versus pathogens, herbivores, pollinators and others (CROTEAU et al., 2000). These plants produce a diversity of secondary compounds used in the production of pharmaceuticals, medicines, cosmetics, fragrances, natural pesticides, and other products. Many of such secondary compounds of economic importance, such as rubber, opium, balsam, camphor, resins and essential oils, are secreted by specialized structures (FAHN, 1979). The study of secretory structures as well as of the chemical composition of the secreted material may indicate new sources of materials to be explored by the pharmaceutical industry (FAHN, 1979).

The specific knowledge of the presence or not of secretory structures and the chemical nature of the secondary compounds secreted and/or present in *Eucalyptus* may guide their production system in relation to raw material use and management, as well as help researchers and producers in their search for materials resistant to pests and pathogens, given the protective activity of secondary compounds in plants. This work aimed to characterize the secretory structures and the chemical nature of the compounds secreted and /or present in the leaf blade of seven species of *Eucalyptus*, as well as to describe the foliar anatomy of these species.

2. MATERIAL AND METHODOS

Eucalyptus grandis W. Hill ex Maiden, *E. pellita* F. Muell., *E. pilularis* Sm., *E. pyrocarpa* L.A.S. Johnson & Blaxell, *E. resinifera* Smith in J. White, *E. saligna* Sm. and *E. urophylla* S. T. Blake; cultivated in pots with 10 L of clayey soil fertilized with 216.6 g of N-P-K (6-30-6) and 12 g of lime in the ratio Ca: Mg = 4:1 equivalent, with 6 g/pot of N-P-K (20-5-20) applied 15 days after planting (DAP) of the seedlings, and three applications of 4 g/pot of $(NH_4)_2SO_4$ at 40, 60 and 80 DAP; were analyzed. The seedlings were produced from seeds certified arising from IPEF (Institute of Forest Research), which has collections of this species for production and distribution. The plants were maintained in unprotected environment from sun and rain, in an area owned of the Department of Plant Production, Viçosa Federal University, Brazil, from 12 December 2004 to 15 March 2005. The plants, growed at full sun, received sprinkler irrigation of water. The leaves produced by the plants are with their upper surfaces at right angles to the rays of the sun, i.e., in the horizontal position. They are about twice as long as broad and arranged in pairs, at right angles to each other. Six fully expanded leaves around third node from the shoot apex were collected from three plants per species for the histological and histochemical study. The plants were 120 days old and approximately 1.5 m high at the time of collection.

Samples from the median portion of the midrib and leaf margin were fixed in FAA_{50} and stored in ethanol 70% (JOHANSEN, 1940), dehydrated in an ethanol series and embedded in metacrylate (Historesin, Leica Instruments, Heildelberg, Alemanha) proportion 1v:1v (MEIRA and MARTINS, 2003), for the anatomical studies using light microscopy. Cross sections with 8 mm thick were obtained using an automatic advance rotative microtome (model RM 2155, Leica Microsystems Inc., Deerfield, USA) and stained with Toluidine Blue pH 4.0 (O'BRIEN and MCCULLY, 1981) for structural characterization, with Xilidine Pounceau (XP) for total proteins (O'BRIEN and MCCULLY, 1981) and PAS for neutral polysaccharides (MAIA, 1979). The slides were later mounted with synthetic resin (Permount). The other histochemical tests were applied in cuts of fresh samples sectioned transversally by using a table microtome (Model LPC, Rolemberg and Bhering, Belo Horizonte, Brazil), and the slides mounted with glycerinated gelatin. The main metabolite classes present

in the material were investigated by using histochemical tests (Table 1) like in Sant'Anna Santos et al. (2006).

Six foliar fragments from three plants per species (two fragments per plant) were dissociated using nitric acid and chromic acid, to describe the surface characters (JENSEN, 1962). The samples were stained with Astra Blue and Basic Fucsin (KRAUS et al., 1998) and the slide mounted with glycerinated gelatin. Determination of type of stomata and vascularization pattern of the midrib followed the classification of Metcalfe and Chalk (1979).

Photographic documentation of the histological material was carried out using a phototonic microscope (model AX70TRF, Olympus Optical, Tokyo, Japan) with U-Photo system.

Ten observations/plant for each epidermal face were performed in fields with area corresponding to 0.067 mm^2 to determine stomatal index, stomatal density (stomata mm^{-2}) and oil cavity density (cavities mm^{-2}), measured according to the overlying cells on the foliar epidermis. The quantitative data on the epidermal surface were obtained with the aid of the software "Image-Pro Plus". Stomatal index calculation was performed according to the formula of Cutter (1986): Stomatal Index (%) = $(N_S \times 100/(E_C + N_S))$; where N_S is the number of stomata and E_C is the number of epidermal cells.

3. RESULTS AND DISCUSSION

The seven *Eucalyptus* species presented glabrous, amphistomatal leaves (Figures 1A-B, 1E-F, 1I-J, 1M-N, 1Q-R), with anomocytic type stomata (Figs. 1C, 1G, 1K, 1O and 1S) appearing at the same level as the remaining epidermal cells (Figs. 3B, 3C and 3T). However, with lower number of stomata on the adaxial face of the epidermis, except in *E. pyrocarpa* (Fig. 1M), that had the highest stomatal index and density (Table 2). The uniseriate epidermis presented cells with shapes varying from tabular to round, except in the midrib regions in the cross sections of the leaf blade (Figures 2A, 2D, 2H and 2K) and leaf margin regions where the cells were papilose (Figures 3D, 3H, 3N, 3P, 3R, 3U and 3W). The presence of lenticels on both faces of the epidermis of *E. pyrocarpa* was common (Figure 2D), with greater intensity on the abaxial face. The lenticels was less common in the abaxial epidermis of *E. grandis*, *E. pellita*, *E. resinifera*, *E. saligna* and *E. urophylla* (Figures 2K and 2L). *E. pilularis* showed a smaller stomatal density, both on the abaxial and adaxial faces of the epidermis (Table 2), and absence of lenticels on the foliar limb epidermis.

The stomatal index, stomatal density and cavity density (Tables 2 and 3) slowed difference among the species ($p<0.05$).

Table 1 – Methodologies used to detect the main classes of metabolites and result of histochemical tests on the leaves of the *Eucalyptus* species. Abbreviations: + (positive reaction) / - (negative reaction) / sps. (species)
Quadro 1 – *Metodologias utilizadas para a detecção das principais classes de metabólitos e resultado dos testes histoquímicos nas folhas das espécies de Eucalyptus. Abreviaturas: + (reação positiva) / - (reação negativa) / sps. (espécies)*

	Metabolite Group	Test applied	Result		
			Cavitie	Mesophyl	Epiderm
Lipids	Total lipids	Sudan III	+	-	Only the cuticle
	Acid lipids	Nile blue A	+	-	-
Fatty acids	Copper acetate and rubianic acid	-	-	-	
Terpenoids	Essential oils and resin oils	Nadi reagent	+	-	Some granules
	Sesquiterpene Lactones	Sulfuric acid	+	-	-
Phenolic Compounds	General phenolic compounds	Potassium dichromate	-	+	5 sps.+ 2 sps. -
	Tannins	Vanillin-hydrochloric acid	-	3 sps.+ 4 sps.-	1 sps. + 6 sps. -
	Lignin	Phloroglucinol	-	-	-
Proteins		Xilidine Pounceau	-	-	-
Alkaloids		Wagner reagent	-	-	-
Polysaccharides	Starch	Lugol	-	+	-
	General Polysaccharides	PAS	-	+ starch	-
	Pectins	Ruthenium Red	-	-	-

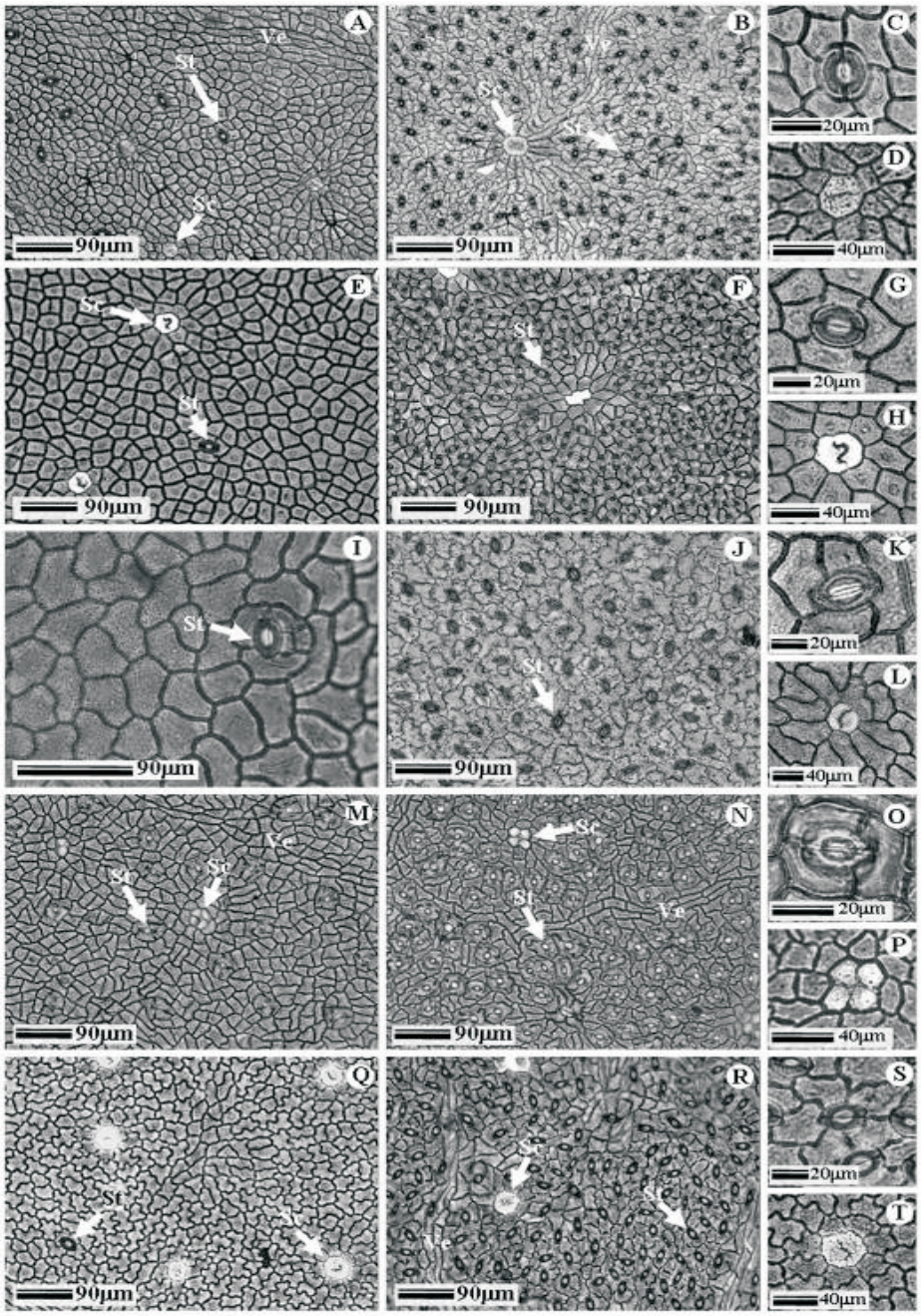

Figure 1 – Frontal view of the epidermis of the leaf blade of the species of *Eucalyptus*. (A-D) *E. grandis*; (E-H) *E. pellita*; (I-L) *E. pilularis*; (M-P) *E. pyrocarpa*; (Q-T) *E. resinifera*; (A, E, I, M and Q) adaxial face; (B, F, J, N and R) abaxial face; (C, G, K, O and S) stomata detail; (D, H, L, P* and T) detail of the secretory cavity; *secretory cavity presenting four overlying cells. Sc = secretory cavity; St = stomata, Ve = vein.

Figura 1 – *Vista frontal da epiderme foliar de espécies de Eucalyptus. (A-D) E. grandis; (E-H) E. pellita; (I-L) E. pilularis; (M-P) E. pyrocarpa; (Q-T) E. resinifera; (A, E, I, M e Q) face adaxial; (B, F, J, N e R) face abaxial; (C, G, K, O e S) detalhe dos estômatos; (D, H, L, P* e T) detalhe das cavidades secretoras; * cavidade secretora apresentando quatro células de cobertura. Sc=cavidades secretoras; St=estômato; Ve= nervuras.*

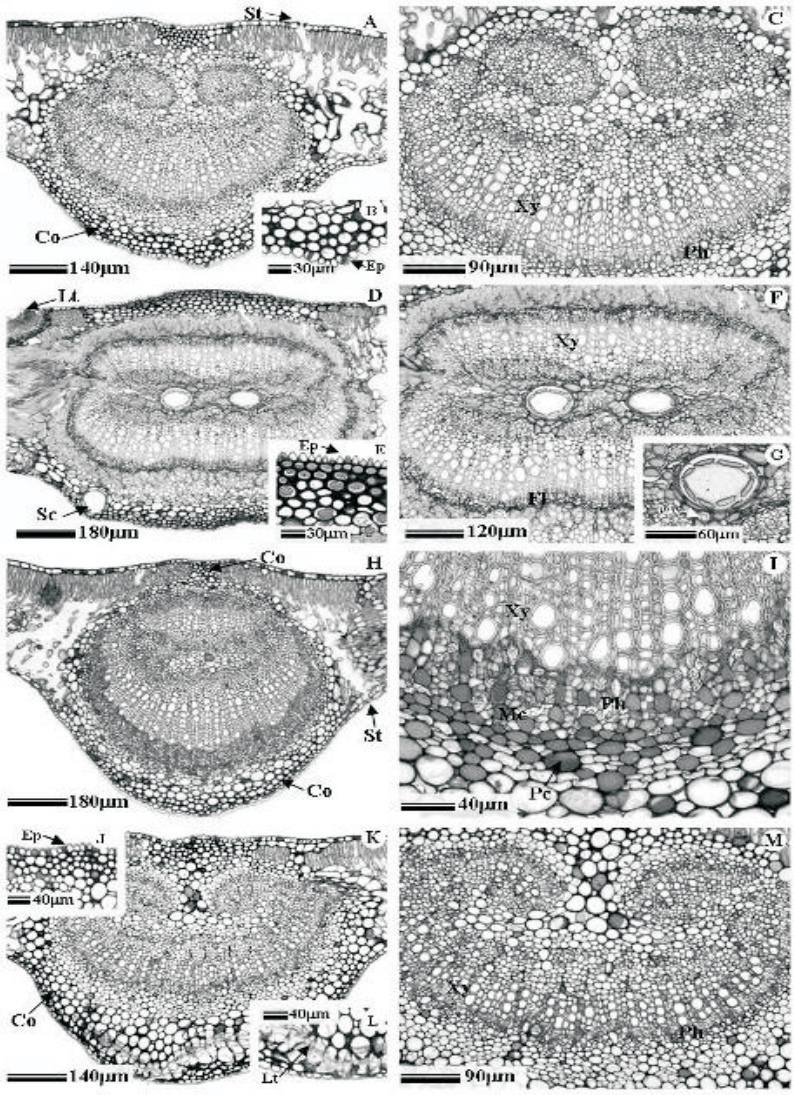

Figure 2 – Transverse sections of the midrib (Mi) of the leaf of *Eucalyptus* species. (A-C) *E. grandis*; (A) overall Mi aspect; (B) abaxial face detail; (C) detail of the vascular bundle in the shape of flat arc; (D-G) *E. pyrocarpa*; (D) overall Mi aspect with the presence of lenticel; (E) adaxial face detail; (F) detail of the siphonostele type of vascular bundle; (G) detail of the secretory cavity; (H-I) *E. resinifera*; (H) midrib with the pattern arc with invaginated ends and dorsal trace type; (I) detail of Mi presenting innumerous monocrystalls; (J-M) *E. urophylla*; (J) detail of the adaxial face; (K) overall Mi aspect; (L) detail of the abaxial face with beginning of lenticel formation; (M) detail of the vascular bundle in the flat arc shaped. Co = collenchyma; Ep = epidermis; Lt = lenticel; Mc = monocrystals; Pc = phenolic compounds; Ph = phloem; Sc = secretory cavity; St = stomata; Xy = xylem.

Figura 2 – *Seção transversal da região mediana da folha (Mi) de espécies de Eucalyptus. (A-C) E. grandis; (A) vista geral da Mi; (B) detalhe da face abaxial; (C) detalhe do feixe vascular em forma de arco fletido; (D-G) E. pyrocarpa; (D) vista geral da Mi com presença de lenticela; (E) detalhe da face adaxial; (F) detalhe da região vascular do tipo sifonostelo; (G) detalhe da cavidade secretora; (H-I) E. resinifera; (H) região da nervura central com feixes vasculares com invaginações do tipo traço dorsal; (I) detalhe da Mi com inúmeros monoscristais; (J-M) E. urophylla; (J) detalhe da face adaxial; (K) vista geral da Mi; (L) detalhe da face abaxial com lenticelas em formação; (M) detalhe do feixe vascular do tipo arco fletido. Co = colênquima; Ep = epiderme; Lt = lenticela; Mc = monocristais; Pc = compostos fenólicos; Ph = floema; Sc = cavidade secretora; St = estômato; Xy = xilema.*

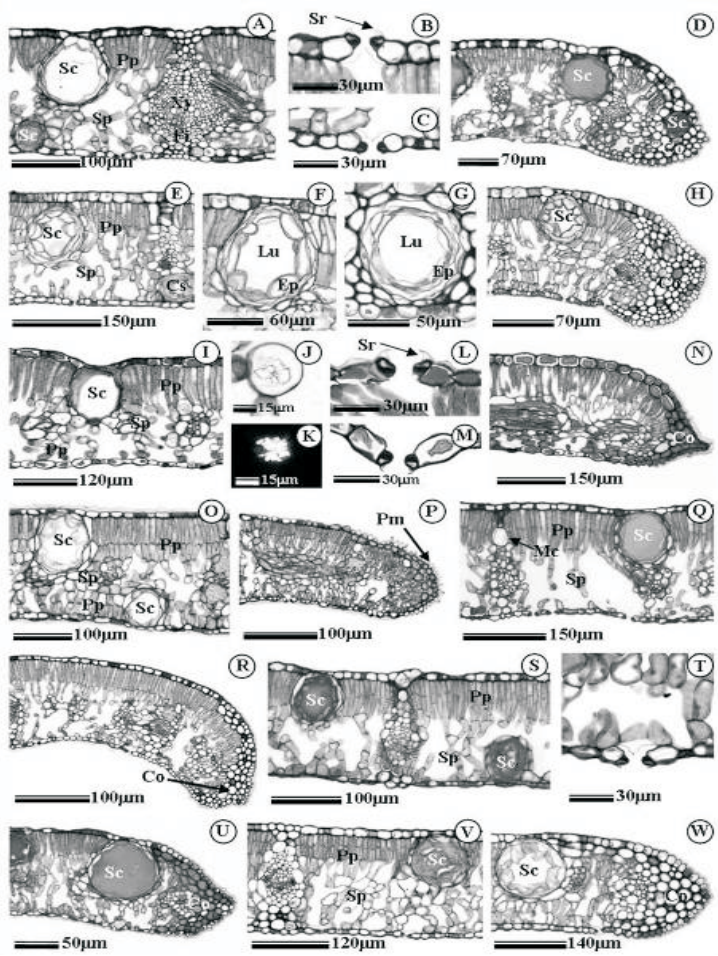

Figure 3 – Transverse sections of the leaf blade of the *Eucalyptus* species (A-D) *E. grandis*; (A) leaf blade with secretory cavities (Sc) at the epidermis abaxial and adaxial; (B-C) detail of the stomata on the adaxial and abaxial faces, respectively; (D) leaf margin; (E-H) *E. pellita*; (E) leaf blade; (F-G) detail of the secretory cavity; (H) leaf margin; (I-N) *E. pilularis*; (I) leaf blade; (J-K) detail of the idioblast containing calcium oxalate crystal in the form of druse; (K) visualized under polarized light; (L-M) detail of stomata on the adaxial and abaxial faces, respectively; (N) leaf margin; (O-P) *E. pyrocarpa*; (O) leaf blade with two layers of palisade parenchyma (Pp) on the adaxial surface; (P) leaf margin with the papillose epidermis; (Q-R) *E. resinifera*; (Q) limb with the presence of monocrystals associated to second order vein; (R) leaf margin; (S-U) *E. saligna*; (S) leaf blade; (T) detail of the stomata on the abaxial face of the leaf; (U) leaf margin; (V-W) *E. urophylla*; (V) leaf blade; (W) leaf margin. Co = collenchyma; Ep = epidermis; Fi = fibers; Lu = lumen; Mc = monocrystals; Pm = papillose leaf margin; Pp = palisade parenchyma; Sc = secretory cavity; Sp = spongy parenchyma; Sr = stomatal ledge; Xy = xylem.

Figura 3 – *Secções transversais do limbo foliar das espécies de Eucalyptus. (A-D) E. grandis; (A) limbo evidenciando as cavidades secretoras (Cs) voltadas para a epiderme das faces abaxial e adaxial; (B-C) detalhe dos estômatos nas faces adaxial e abaxial, respectivamente; (D) bordo; (E-H) E. pellita; (E) limbo; (F-G) detalhe da cavidade secretora; (H) bordo; (I-N) E. pillularis; (I) limbo; (J-K) detalhe do idioblasto contendo cristal de oxalato de cálcio na forma de drusa; (K) visualizado em luz polarizada; (L-M) detalhe dos estômatos nas faces adaxial e abaxial, respectivamente; (N) bordo; (O-P) E. pyrocarpo; (O) limbo destacando as duas camadas de parênquima paliçádico (Pp) na superfície adaxial; (P) bordo evidenciando a epiderme papilosa; (Q-R) E. resinifera; (Q) limbo destacando a presença de monocristais associados à nervura de segunda ordem; (R) bordo; (S-U) E. saligna; (S) limbo; (T) detalhe do estômato na face abaxial da folha; (U) bordo; (V-W) E. urophylla; (V) limbo; (W) bordo. Co = colênquima; Cr = cristas estomáticas; Cs = cavidade secretora; Ep = epiderme; Fi = fibras; Lu = lume; Mc = monocristais; Pm = borda papilosa; Pp = parênquima paliçádico; Sc = cavidade secretora; Sp = parênquima esponjoso; Sr = cristas estomáticas; Xy = xilema.*

Table 2 – Stomatal index and stomatal density of seven species of *Eucalyptus*
Quadro 2 – *Índice estomático e densidade estomática de sete espécies de Eucalyptus*

Specie	Stomatal index (%)		Stomatal density (stomata mm^{-2})	
	Adaxial	Abaxial	Adaxial	Abaxial
Eucalyptus grandis	1.03 bc	16.05 bc	32.46 ab	566 b
Eucalyptus pellita	0.28 c	17.28 b	12.31 b	569 b
Eucalyptus pillularis	1.21 bc	16.62 bc	11.90 b	280 d
Eucalyptus pyrocarpo	*4.72 a*	*14.67 c*	*106.13 a*	*436 c*
Eucalyptus resinifera	2.05 b	20.01 a	35.13 ab	560 b
Eucalyptus saligna	0.35 c	15.55 bc	22.19 ab	707 a
Eucalyptus urophylla	1.52 bc	15.59 bc	72.60 ab	608 b
CV (%)	7.51	6.49	39.97	21.09

Means followed by same letter in the column do not differ by the Tukey Test at 5% probability.

E. grandis (Fig. 2A), *E. pellita*, *E. pilularis* and *E. urophylla* (Figs. 2K and 2M) presented the midrib vascularization pattern in flat arc. *E. resinifera* (Fig. 2H) and *E. saligna* presented the pattern arc with invaginated ends and dorsal trace type and *E. pyrocarpa* (Fig. 2D) the siphonostele type. In the midrib the sub-epidermal collenchyma appeared as two caps turned towards the two leaf faces (Figures 2A-B, 2D-E, 2H and 2J-L). The vascular bundles was bicollateral and delimited by 3 to 5 layers of fibers in *E. pyrocarpa* (Figure 2F) and in the remaining species by achlorophyll parenchyma and dispersed fibers (Figures 2C, 2I and 2M). The presence of monocrystals and druses in the seven species was common in the vascular bundle region, both in the midrib (Figures 2G and 2I) and mesophyll (Figures 3I-K and 3Q).

The mesophyll was isobilateral in *E. pyrocarpa* and *E. pilularis*. *E. pyrocarpa* had a palisade parenchyma with two cell layers on the adaxial surface (Figure 3O); and in *E. pilularis* only a palisade parenchyma layer on the adaxial surface was observed to be clearly defined, while the abaxial surface parenchyma presents smaller, elongate and irregular cells, with the number of layers varying from one to two (Figure 3E). The mesophyll of the remaining species was dorsiventral with a prominent spongy parenchyma (Figures 3A, 3E, 3Q, 3S, 3V). The anatomical characterization was in agreement with the description for the genus *Eucalyptus* (METCALFE and CHALK, 1979), regarding the occurrence of isobilateral mesophyll. The record of a dorsiventral mesophyll in some of the species studied was common for juvenile leaves, isobilateral for adults (JOHNSON, 1926).

The isobilateral mesophyll in *E. pyrocarpa* and *E. pilularis*, as well as the palisade parenchyma with two cell layers in *E. pyrocarpa* can be considered an adaptive strategy to support high light intensities, since such characteristics are common in sun leaves (DOLEY, 1978; GUTSCHICK, 1999). Leaves with thicker palisade parenchyma present higher light extinction coefficient, with the photosynthetic rates expected to be higher in these leaves being (BOLHAR-NORDENKAMPF and DRAXLER, 1993).

The presence of abaxial palisade parenchyma with smaller, elongate, and irregular cells, as observed in *E. pilularis*, had been reported for *E. globulus* (JOHNSON, 1926), and named pseudopalisade. Information on the selective advantage of such characteristics had not been found in the literature.

Cavities were dispersed in the mesophyll and midrib of the *Eucalyptus* species, predominantly located in the sub-epidermal region of the two faces of the leaf (Figures 3A, 3O and 3S). *E. pyrocarpa* was the only species showed variation in the number of overlying cells, displaying up to four cells (Figure 1P), with two cells being observed in the remaining species (Figures 1D, 1H, 1L and 1T). *E. pellita* showed the greatest cavity density, both on the abaxial and adaxial faces of the epidermis (Figure 3E), while *E. pilularis* showed cavities only on the adaxial face of the epidermis (Figure 3I) and with lower density among the species (Table 3).

Stomatal density on the adaxial surface was lower than on the abaxial, and cavity density in both surfaces was prominently lower than stomata density in all the species studied. Similar results were obtained for *Eucalyptus camaldulensis* (JAMES and BELL, 1995). Stomatal density varies according to plant age and is directly influenced by environmental conditions (CAO, 2000; JUSTO et al., 2005). However, such characteristic

was positively related to CO_2 assimilation (ABRAMS et al., 1994; JUSTO et al., 2005), due to the positive relation between stomatal density and gas exchanges (ARAUS et al., 1986) and greater stomatal conductance (BOARDMAN, 1977). On the other hand, stomatal index was a relatively constant for each between species (CUTTER, 1986), witch greater taxonomic relevance.

The natural openings such as stomata and lenticels were considered indirect routes pathogen penetration in eucalypt plants, typically occurring for bacteria, virus, viroid and some species of fungi such as *Coniella fragariae* (Oudem.) B. Sutton and *Cryptosporiopsis eucalypti* Sankaran & B. Sutton (ALFENAS et al., 2004). Thus, species such as *E. saligna*, *E. grandis* and *E. urophylla*, presenting higher stomata density, would be most inclined to be infected by these pathogens, while *E. pilularis*, with a lower stomatal density among the species, would have an advantage.

The phenolic compounds in the parenchymatic and epidermal tissues varied among the species regarding, however, no marked differences were verified in the chemical composition of the material secreted by the cavities (Table1).

The reaction to Sudan III showed lipophylic substances in the lumen of the cavities (Figs. 4C-F) of the seven species. Complementary tests detected that such lipids have an acid nature, as they were stained by Nile blue, and violet to blue when submitted to the Nadi reagent, with the secreted substance thus constituting a resin-oil (Fig. 4G). The reaction showed the presence of oil, what might be due to variations in the functional stage of the epithelial cells of the cavities. The presence of essential oil drops on the epidermis of the eucalypt species was also detected. The presence of sesquiterpene lactones was also noted in the substance secreted at the cavities lumen. Tests applied to detect phenolic compounds showed the presence of such substances in the mesophyll, especially in the palisade parenchyma region (Figures 4H, 4I and 4L), of all eucalyptus species, and in the epidermis of the abaxial and adaxial faces of *E. grandis* (Figure 4H), *E. pellita, E. pilularis* (Figure 4I), *E. resinifera* and *E. urophylla*. The positive result for the test using vanillin-hydrochloric acid confirmed the tanniniferous nature of these phenols in the mesophyll of *E. pilularis* (Figure 4J), *E. pyrocarpa* (Figure 4K) and *E. saligna* (Figure 4M) and in the epidermis of *E. pilularis* (Figure 4J).

Lipids of the essential oil or resin-oil type, sesquiterpene lactones and phenolic compounds were detected by the histochemical tests (Table 1). Alkaloids, polysaccharides (Figures 4N-O) and total proteins were not detected.

The secretion of secondary compounds is generally associated to specialized structures (FAHN, 1979; ROSHCHINA and ROSHCHINA, 1993). Sub epidermal cavities were the only secretory structures found in the seven species studied, with the most abundant being found in *E. pellita* and less frequent in *E. pilularis*. The difference among these structures densities may be an indicative of the amount of secondary compounds produced by each species, representing relevant information for extraction of resin-oil and sesquiterpene lactones, compounds presents in the cavities lumen. However, further quantitative chemical studies must be carried out to measure the productive capacity of such compounds by each species.

Some characteristics reported in the species studied may be dealt with plant defense. The presence of calcium oxalate crystals adjacent to the vascular bundles may be related to the ecology of the plant, conferring umpalatability to phytophagous insects (MAUSETH, 1988). The essential oil found in the cavities of the seven eucalyptus species studied may be related to the strategy of decreasing excessive loss of water by acting as a thermal isolating agent (CRAVEIRO and MACHADO, 1986), besides acting in defense of the plant against phytophagous or pathogen attack (RODRIGUEZ et al., 1984). Likewise, steroids of the sesquitepene lactone type possess important biological activities, acting as antimicrobial and antihelminthic agents as well as in hepatotoxic and phytotoxic actions (CROTEAU et al., 2000).

Table 3 – Density of the cavities seen through the guard cells on the abaxial and adaxial epidermis of seven species of *Eucalyptus*

Quadro 3 – *Densidade de cavidades vistas por células de cobertura na epiderme abaxial e adaxial de sete espécies de Eucalyptus*

Specie	Cavities mm^{-2}	
	Abaxial	Adaxial
E. grandis	0.29 b	0.36 ab
E. pellita	0.40 a	0.42 a
E. pillularis	-	0.09 c
E. pyrocarpo	0.22 cd	0.30 b
E. resinifera	0.24 c	0.33 ab
E. saligna	0.19 d	0.38 ab
E. urophylla	0.31 b	0.36 ab
CV (%)	12.03	32.11

Means followed by the same letter in the column do not differ by the Tukey test at 5% probability.

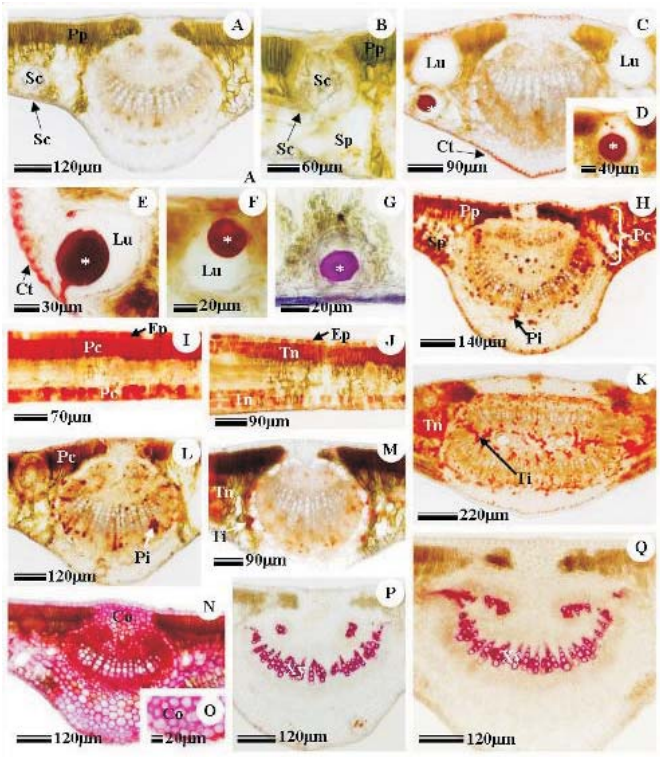

Figure 4 – Histochemical characterization of the foliar lamina of *Eucalyptus*. (A) Midrib of *E. saligna* in fresh samples not submitted to reagents (white), cavity with secretion (Ss) in the mesophyll; (B) Detail of cavity (white) of *E saligna*, secretion (Ss) of translucent aspect and part of the mesophyll; (C-F) Sections submitted to Sudan III, positive reaction for lipids (*) on the secretion of the cavities of *E. saligna* (D = detail), *E. resinifera* and *E. urophylla*, respectively; (G) Positive reaction for resin-oil in the substance secreted (*) from *E. resinifera*, for the Nadi reagent; (H-L) Sections of the midribs of *E. grandis* and *E. saligna*, respectively, submitted to potassium dichromate. Note positive reaction for phenolic compounds (Pc) only on the palisade parenchyma cells and on the idioblasts (Id) of the vascular bundle for *E. saligna*, and in all the mesophyll and in the idioblasts for *E. grandis*. (I-J) Mesophyll of *E. pilularis* submitted to potassium dichromate and vanillin-hydrochloric acid tests, respectively. Observe the presence of tanniniferous substances in the parenchyma and epidermis. (K and M) Reaction to vanillin-hydrochloric acid in the midribs of *E. pyrocarpa* and *E. saligna*, respectively, phenolic compounds of the tannin type. (N-O) Section of midrib of *E. pilularis* submitted to Ruthenium red (N) highlighting collenchyma turned towards the midrib abaxial surface (O). (P-Q) Positive reaction to phloroglucinol in the midrib xylem of *E. urophylla* and *E. pellita*, respectively. Co = collenchyma; Ct = cuticle; Ep = epidermis; Lu = lumen; Pc = phenolic compounds; Pi = phenolic idioblast; Pp = palisade parenchyma; Sc = secretory cavity; Sp = spongy parenchyma; Ss = substance secreted; Ti = tanniniferous idioblast; Tn = tannin, Xy = xylem.

Figura 4 – *Caracterização histoquímica da lâmina foliar de Eucalyptus. (A) Nervura mediana de E. saligna em amostras frescas não submetidas a reagentes (branco), evidenciando cavidade com secreção (Sc) no mesofilo; (B) Detalhe da cavidade (branco) de E saligna, evidenciando a secreção (Sc) de aspecto translúcido e parte do mesofilo; (C-F) Cortes submetidos ao sudan III, reação positiva para lipidios (*) na secreção das cavidades de E. saligna (D = detalhe), E. resinifera e E.urophilla, respectivamente; (G) Reação positiva para óleo-resina no secretado (*) da cavidade de E. resinifera, ao reagente de nadi. (H-L) Secções das nervuras medianas de E. grandis e E. saligna, respectivamente, submetidas ao dicromato de potássio, notar reação positiva para compostos fenólicos (Cf) nas células do parênquima palicádico e nos idioblastos (If) do feixe vascular para E. saligna, e em todo o mesofilo e nos idioblastos para E. grandis. (I-J) Mesofilo de E. pilularis submetido ao teste com dicromato de potássio e vanilina clorídrica, respectivamente. Observar a presença de substâncias taníferas no parênquima e na epiderme. (K e M) Reação à vanilina clorídrica nas nervuras medianas de E. pyrocarpo e E. saligna, respectivamente, evidenciando compostos fenólicos do tipo tanino. (N-O) Corte da nervura mediana de E. pilularis submetido ao vermelho de rutênio (N) destacando o colênquima voltado para a superfície abaxial da nervura mediana (O). (P-Q) Reação positiva ao floroglucinol no xilema da nervura mediana de E. urophylla e E. pellita, respectivamente. Co = colênquima; Ct = cutícula; Ep = epiderme; Lu = lume; Pc = compostos fenólicos; Pi = idioblasto fenólico; Pp = parênquima paliçádico; Sc = cavidade secretora; Sp = parênquima lacunoso; Ss = secretado; Ti = idioblasto taninífero; Tn = tanino, Xy = xilema.*

The phenolic compounds found on different tissues of the *Eucalyptus* species way have restringing effect to certain visitors, such insects, with tannin being known for its action against herbivores and pathogens (SANT'ANNA SANTOS et al., 2006), as well as protection of the cellular structures against the excess of ultraviolet radiation and maintenance of protoplast integrity under water stress situations (TAIZ and ZEIGER, 2004). Based strictly on these characteristics, *E. grandis*, *E. pellita*, *E. pilularis*, *E. resinifera* and *E. urophylla*, would be more adapted to high radiation and pest attack conditions, since they present phenolic compounds in the epidermis. The production of terpenes and phenolic compounds and their relation with growth in *Eucalyptus polybractea* plants had a positive correlation between plant growth and the production of total secondary compounds (terpenes + phenolic compounds) was suggest that the plants producing higher quantities of total secondary compounds possess greater adaptative advantages in relation to environment variations, and, consequently, greater growth (KING et al., 2004).

Some volatile and water-soluble toxins of the tissues of *E. camaldulensis* can inhibit seed germination and plantlet development (MORAL and MULLER, 1970). Many of these toxins were identified as terpenes and phenolic compounds, which had possible involvement with allellophatic properties.

The complexity of the product secreted by the cavities, highlighting the homogenous histochemical nature of these compounds in eucalyptus species, was confirmed. However, the phenolic compounds may be indicative of important variations in resistance against herbivores and pathogens, since they showed differences among the eucalyptus species.

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