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ECOSYSTEMS

Morphology of pollen grains and orbicules of two threatened species of *Cedrela* P. Browne (Meliaceae A. Juss.)

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Abstract: Pollen grain morphology and the characterization of additional structures related to pollen dispersion can help to understand the strategies presented by species as well as their taxonomic circumscription. This work investigates the morphology of pollen grains and orbicules of *Cedrela fissilis* Vell. and *Cedrela odorata* L., two threatened species of the genus *Cedrela* P. Browne (Meliaceae A. Juss.) in Brazil. Observations of pollen grains and orbicules of the species were carried out pre- and post-chemical treatment of the samples, under light and scanning electron microscopy, aiming at recognizing structures, detailing morphological characterizations and taking measurements, the last of which were submitted to statistical treatment. The results demonstrate that the pollen grains of the two species are medium in size (measuring between 25 and 50µm), radially symmetrical, isopolar, prolate-spheroidal and 4,5-colporate with subcircular to subquadrangular amb and psilate ornamentation; the orbicules also have a smooth surface and measure about 7 to 10µm. Variation was observed in the size of pollen grains, which presented high coefficient of variation and in the orbicules, as well as in the number of apertures.

Key words: Cedarwood, Meliaceae, palynology, Ubisch bodies.

INTRODUCTION

The genus *Cedrela* P. Browne occurs from the Mexican coast, through to South America to northern Argentina (Cavers et al. 2003). *Cedrela* is distributed across all regions and phytogeographic domains in Brazil, where it is represented by two species: *Cedrela fissilis* Vell. and *Cedrela odorata* L. (Flores 2020). Both species are trees with compound leaves, are considered vulnerable and stand out among members of Meliaceae for the great economic potential of their wood (Carvalho 1994, MMA 2021). Morphological differentiation of the two species is based on leaflet number and presence/absence of indumentum on the abaxial face of leaf blades (Flores 2020).

Pollen grains of angiosperms constitute a fundamental dispersion unit as a species reproduction strategy, with pollination being highlighted as a specialized case of this dispersion (Faegri & van der Pijl 1979). Species of *Cedrela* can present anemochoric dispersion and entomophilous pollination, being pollinated by insects such as bees and moths (Morellato 1991, Silva et al. 2014, Steinbach & Longo 1992). The species are monoecious, with functionally distinct female and male flowers within the same inflorescence (Gouvêa et al. 2008).

The anthers have an inner tissue called the tapetum, which surrounds the sporogenous tissue and has the function of nourishing and contributing to the formation of the outermost layer of the exine of pollen grains (El-Ghazaly

1999). Secretory-type tapetum cells act by secreting substances on the surface of the pollen grain wall at the time of pollen grain ontogenesis, and this deposition is genetically determined and simultaneous on the pollen grains and the orbicules (Furness & Rudall 2001, Godwin 1968)

Orbicules are small granules coated with sporopollenin that are generally spheroidal, but can assume other shapes depending on the taxon; they are also resistant to acetolysis (El-Ghazaly 1999, Hesse 1986). Studies about the chemical composition of orbicules are scarce but include Clément & Audran (1993) with the genus *Lilium* L., who indicated that the external composition of the orbicules consists of sporopollenin and the internal composition of polyphenols and lipids. El-Ghazaly (1999) suggests that one of the functions of orbicules may be related to dispersion.

Hesse (1986) observed homologies between the genetic information for the formation of the tapetum and the sporogenic tissue that could explain similarities in the pattern of sporopollenin deposition in pollen grains and orbicules. According to Huysmans et al. (1998), the ornamentation of the exine of pollen grains and the surface of orbicules may present similarities. Studies dealing with the taxonomic value of pollen grains and orbicules have been developed by different authors for some genera (El-Ghazaly 1989, El-Ghazaly & Chaudhary 1993, Huysmans et al. 1997, Vinckier & Smets 2002a-c).

Barth et al. (1998), Carreira & Secco (1984) and Garralla & Cuadrado (1997) performed palynological studies including species of *Cedrela*. Barth et al. (1998) highlighted that it was not possible to differentiate species under light microscopy and commented on the presence of orbicules, however, without characterizing them, as with Carreira & Secco (1984) as well. Garralla & Cuadrado (1997) observed a psilate pattern of ornamentation for the genus but did not report the presence of orbicules. Other works in the areas of melissopalynology, paleopalynology and ecology have superficially characterized *Cedrela* pollen grains (Barth et al. 2009, Bauermann et al. 2008, Silva et al. 2014) without, however, commenting on its morphology in detail.

Considering the taxonomic importance of pollen grains and orbicules, and their relevance to species dispersion, the present study aimed to detail pollen grains and orbicules morphology of two endangered species of *Cedrela* using light and scanning electron microscopy.

MATERIALS AND METHODS

Material examined

The material analyzed in the present study is deposited in Herbário Leopoldo Krieger of Universidade Federal de Juiz de Fora (CESJ, acronym according to Thiers (2022)). About ten specimens of both species tested were examined, many of which had already been in fruit, or even had only the vegetative part. We selected the materials in the best condition for the palynological study:

Cedrela fissilis Vell. – BRASIL. Minas Gerais: Barroso. Mata do Baú, 02/X/2003, L. C. S. Assis 239 and M. K. Ladeira (CESJ); Descoberto. Reserva Biológica da Represa do Grama, 21/X/2000, L. D. Meireles, S. M. Verardo, F. Magalhães, P. O. Costa, P. C. Zampa &, L. F. Fazza (CESJ 31435); 25/X/2004, A. Valente & V. R. Almeida 377 (CESJ).

Cedrela odorata L. – BRASIL. Minas Gerais: Iguatama: Fazenda Faroeste, margem esquerda do Rio Miguel, 16/XI/2002, P.H.A Melo, L.M. Versieux 214 (CESJ).

Methods for light microscopy

Pollen grains were taken from anthers of flowers taken at random and prepared following the method of Wodehouse (1935) and acetolysis of

Erdtman (1960). Measurements were taken of acetolyzed pollen grains using a micrometric eyepiece. Measurements of polar and equatorial diameters in equatorial view, apocolpium side and width and length of endoapertures and colpi were obtained from 25 pollen grains selected at random from three slides (Salgado-Labouriau 1973). Measurements of the exine were taken from 10 pollen grains while those of largest diameter were taken from 25 orbicules taken at random from three acetolyzed slides. The terminology follows Punt et al. (2007).

Analysis of flowers

Aiming to observe possible sexual variations in the examined materials, six flowers of the two studied species were analyzed in a Petri dish under a stereoscopic microscope with the aid of tweezers and needle, to determine sex differentiation. The method of Wodehouse (1935) was subsequently employed to separately evaluate pollen grains in the anthers of male and female flowers. The sample was covered with a coverslip and sealed with paraffin for observation under a light microscope.

Methods for scanning electron microscopy

Analysis under scanning electron microscopy used pollen grains that were separated from anthers, with the aid of tweezers and needle, and spread on double-sided tape previously adhered to a specific numbered support (Melhem et al. 2003). The samples were then metallized with a thin layer of gold (20nm) and taken for observation.

Descriptive statistcs

Statistical treatment was performed and presented as: size range (Xmin – Xmax), arithmetic mean (X), standard deviation (S), standard error (Sx), 95% confidence interval (CI) and coefficient of variation (CV%).

RESULTS

Description of pollen grains

Cedrela pollen grains (Figs. 1a-h) comprise isopolar monads of medium size that are prolate-spheroidal and 4,5-colporate with radial symmetry, subcircular to subquadrangular amb, psilate ornamentation and sexine thicker than nexine.

The pollen grains of the studied species are of medium size, with those of *C. odorata* being larger than those of *C. fissilis* (Table I). The acetolyzed slides reveal evident size discrepancy between the pollen grains of the functionally male and mixed female anthers, mainly for *C. fissilis* (Fig. 1a). The coefficient of variation was high for both species (Table I). The amb of *Cedrela odorata* is subcircular while that of *C. fissilis* is subquadrangular (Fig. 1c). The pollen grains of both taxa are prolate-spheroidal in shape (Figs. 1b, 1d, 1h). *Cedrela odorata* is 4-colporate, less often (about 10%), 5-colporate while *C. fissilis* is 4-colporate (Fig. 1c). The colpi of both species are narrow with a circular to lalongate (Figs. 1b, 1d) (Table I) and an evident margin. Scanning electron microscopy revealed a granular aspect to the colpus membrane (Figs. 1d, 1h). The ornamentation of the pollen grains of both species is psilate (Figs. 1d, 1h). The sexine is the thickest stratum of the exine in both taxa (Table I).

Orbicules

Orbicules were observed in the direct, acetolyzed preparations (Figs. 1a, 1e) and under scanning electron microscopy (Figs. 1f-g). They were adhered to the exine in some pollen grains (Figs. 1f-h) or isolated (Fig. 1e) and are individualized or grouped (Figs. 1e-h) and possess a spheroidal shape and psilate walls (Figs. 1e, 1f-h). Their appearance, when observed under light microscopy, resembled oil droplets (Figs. 1a, 1e). The orbicules of *C. fissilis* were smaller than

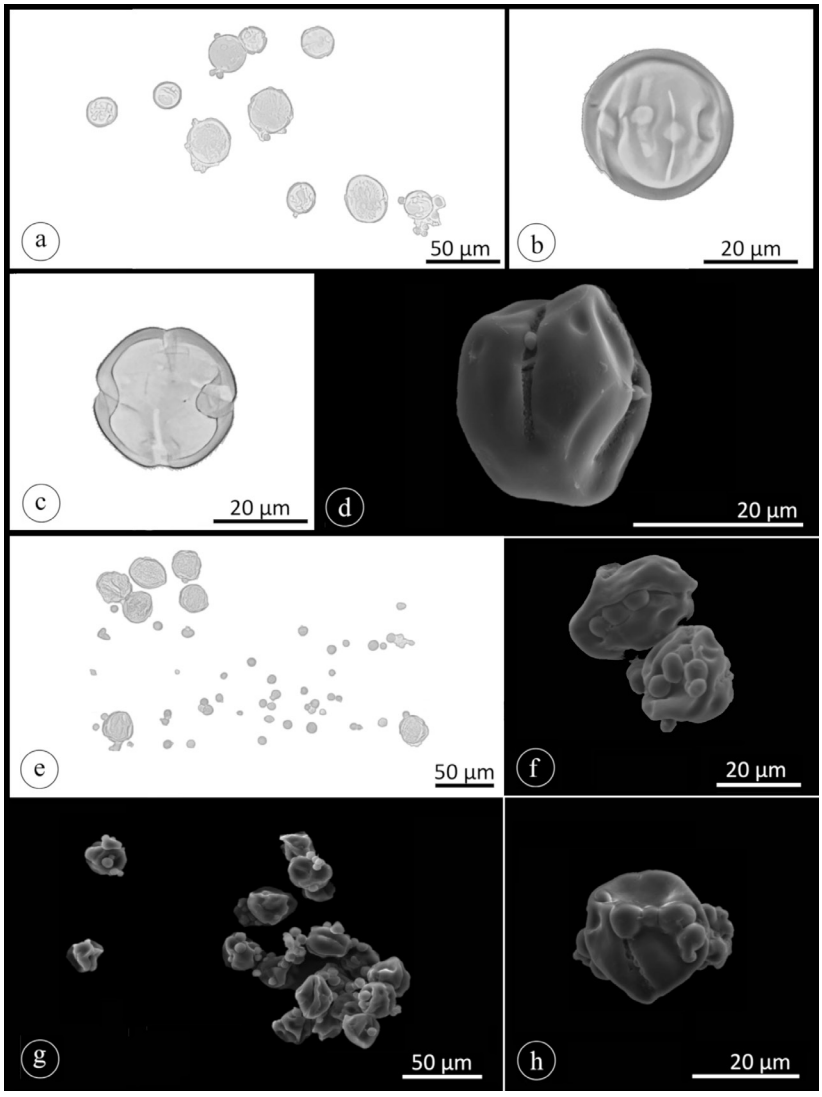

Figure 1. Light photomicrographs (LP) and scanning electromicrographs (SE) of pollen grains of *Cedrela* P. Browne. a-d. *Cedrela fissilis* Vell. a. General view (LP). b. Equatorial view (LP). c. Polar view (LP). d. Equatorial view (SE). e-h. *Cedrela odorata* L. e. General view (LP). f. Detail of grouped orbicules (SE). g. General view (SE). h. Subequatorial view (SE).

those of *C. odorata* (Table I), measuring 7.16µm and 10.34µm, respectively.

Analysis of flowers

The pollen grains of female flowers of both species are larger than those of male flowers and with deformations and little cellular content, while those of male flowers are intact and with much cellular content.

DISCUSSION

The pollen morphology results found that it is possible to distinguish the two species, even with light microscopy. Barth et al. (1998) highlighted that it was not possible to separate species within some genera of Meliaceae, such as *Cedrela*, using light microscopy. However, the present study observed the pollen grains and orbicules of *Cedrela odorata* to be larger than those of *Cedrela fissilis*.

The number of colporus-type apertures varied between the studied species, from four for *C. fissilis* to five for *C. odorata*. Garralla & Cuadrado (1997) investigated Meliaceae from Argentina and found up to five apertures for both species, a number not found for *C. fissilis* in the present study. Barth et al. (1998) found

Table I. Morphometric data for pollen grains and orbicules of species of *Cedrela* P. Browne (measurements in micrometers).

Morphometric data		*Cedrela fissilis*	*Cederela odorata*
Equatorial diameter	Xmin - Xmax	25.60 – 46.08	28.16 – 46.08
	X±sx	32.35 ± 1.27	36.55 ± 0.90
	S	6.35	4.52
	CI (95%)	29.73 - 34.67	34.69 - 38.41
	CV (%)	19.62	12.36
Polar diameter	Xmin - Xmax	25.60 - 46.08	33.28 - 46.08
	X±sx	32.46 ± 1.31	39.62 ± 0.72
	S	6.55	3.62
	CI (95%)	29.76 - 35.17	38.13 - 41.11
	CV (%)	20.17	9.13
Orbicules	Xmin - Xmax	5.12 – 10.24	7.68 – 12.80
	X±sx	7.16 ± 0.36	10.34 ± 0.34
	S	1.81	1.72
	CI (95%)	6.42 – 7.90	9.63 – 11.05
	CV (%)	26.27	16.63
Colpi	Length	18.43	24.67
	Width	7.98	5.52
Endoaperture	Length	3.63	5.22
	Width	4.24	5.73
General data	P/E	1.00	1.08
	E(PV)	32.76	35.07
	A	9.72	9.98
	Sexine	1.53	1.48
	Nexine	0.56	0.56

Xmin-Xmax: minimum and maximum values of pollen grains size diameter, X±Sx: mean and standard error, S: standard deviation, CV%: coefficient of variation, CI 95%: confidence interval, P: polar axis, E: equatorial axis, E(PV): equatorial axis in polar view, A: apocolpium side.

a pattern of four colporate for the genus and Carreira & Secco (1984) described the pollen grain of *C. odorata* as 4-colporate, which differs from the findings of the present study.

Psilate ornamentation, described by Garralla & Cuadrado (1997) as standard for *Cedrela*, was consensual among most of the works listed here, like the medium size of pollen grains, the presence of a margin, referred to by Carreira & Secco (1984) as "invaginated vertices", and the prolate-spheroidal shape. The exception being Garralla & Cuadrado (1997) who found small to medium pollen grains with an oblate-spheroidal shape. Granules in the colpus membrane were also observed by Garralla & Cuadrado (1997) for both species.

The present study found the medium size of the orbicules to help separate the species. Palynological studies that included a characterization of orbicules for species of

different families discussed their taxonomic relevance, however, such was not found in the reviewed literature for the genus *Cedrela*. For other taxonomic groups, El-Ghazaly (1989) investigated pollen grains and orbicules morphology of nine species of the genus *Euphorbia* and concluded that the orbicules, despite showing differences between species, did not corroborate the taxonomic groups suggested by the author. El-Ghazaly & Chaudhary (1993) studied the taxonomic application of orbicules to sixty species of the same genus and emphasized that they can help with taxonomic issues.

Vinckier & Smets (2002a-c) investigated the diversity, morphology and ultrastructure of orbicules of the order Gentianales and of two of its families: Loganiaceae and Apocynaceae. In conclusion, the authors highlighted the common presence of orbicules in the order and their importance in differentiating genera and tribes. Huysmans et al. (1997) studied 14 genera of Cinchonoideae (Rubiaceae) and reported similarities between the sexine ornamentation (microspines) of pollen grains and orbicules with a thorny surface.

El-Ghazaly (1999) suggested that one of the functions of orbicules may be associated with the dispersion of pollen grains. The presence of orbicules in the two studied species, which are pollinated by insects such as moths and bees (Morellato 1991, Silva et al. 2014, Steinbach & Longo 1992), can corroborate this inference, since these structures have the potential to adhere to the pollen grain and increase the contact surface between the body of the pollinator and the pollen grain itself.

Following the floral development of the genera *Toona* (Endl.) M. Roem. and *Cedrela*, Gouvêa et al. (2008) observed that female flowers of *Cedrela* had pollen grains with deformations and devoid of content, while male flowers contained well-formed microspores with robust cytoplasm. The present work found the same pattern using slides prepared according to Wodehouse (1935) with an anther of different sexes, that is, the material with an anther of the functionally female flower presented larger pollen grains with some deformations, however, both sexes exhibited cellular content.

In both functionally male and female flowers, the pollen grains were observed to differ in size, which justifies the high coefficient of variation. Barth et al. (1998) found variation in the maximum and minimum size of pollen grains for species of *Cedrela*, similar to that found in the present work, but they did not differentiate functional male or female flowers, nor did they perform statistical analyses.

In this study is presented a detailed characterization of the pollen morphology of *Cedrela fissilis* Vell. and *Cedrela odorata* L. and the orbicules. It was possible to observe that the pollen grains of the two species overlapped in size in terms of amplitude, this characteristic being insufficient to separate the taxa. However, we highlight a high coefficient of variation in both species and that the species *C. odorata* showed higher averages in all analyzed parameters and pollen grains ranging from 4 to 5 apertures.

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Andrea Pereira Luizi-Ponzo: Coordinator of the project in which this work is inserted, study design, obtaining the botanical material, supervision of the methodology employed, participation in obtaining the results and in the discussion. Fernanda da Costa Fernandes: Participation in the study design, preparation of botanical material for observation, measurement, statistical treatment, participation in the elaboration of results and discussion.