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Morphological and anatomical studies of the seeds and seedlings of *Eucalyptus pilularis* and *E. umbra*

by

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Abstract: Morphological and anatomical aspects of seeds and seedlings of *Eucalyptus pilularis* Sm. and *E. umbra* R.T. Baker were studied in detail and found to be similar, with the exception of some anatomical features of the testa and the average length of the fertile seeds, which is significantly different for the two species.

Identification is a very practical aspect of taxonomy and many seeds of economic importance can be identified to the specific and even the varietal levels on surface features, being also possible to identify very small fragments of seeds on histological structure (Vaughan, 1968).

Eucalyptus seeds are usually so very small that identification to the specific level is difficult, if based only on morphological features. The seeds of *E. pilularis* Sm. and *E. umbra* R.T. Baker are morphologically similar, being distinguished apparently only by their size. A detailed study of these seeds and young seedlings was carried out with the purpose of finding morphological and anatomical features by which to separate these two species, and at the same time to serve as a base for possible taxonomic and ecological studies.

MATERIAL AND METHODS

The seeds of *Eucalyptus pilularis* Sm. and *E. umbra* R.T. Baker were collected from selected trees at the "Navarro de Andrade" Forest Station in Rio Claro, State of São Paulo, Brazil. These two species belong to Section Renantherae-Normales (Blakely, 1955).

The observations and drawings of the surface features of fertile and sterile seeds ("chaff") and of the young seedlings were made with a stereo microscope with camara lucida.

Determination of the mean weight of one hundred fertile seeds was done by random sampling and an analytical balance, as well as the determination of the mean percentage of fertile seeds (by weight) and the average number of fertile seeds per gram of mixed seeds. The length and width of the seeds were determined by using a calibrated eyepiece with a micrometric slide.

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Anatomic studies of the seeds were done by means of microscopical observations of cross, longitudinal and paradermal hand-made sections of soaked seeds (Corner, 1976). The sections were mounted in glycerine (10%) as semi-permanent preparations (Sass, 1951).

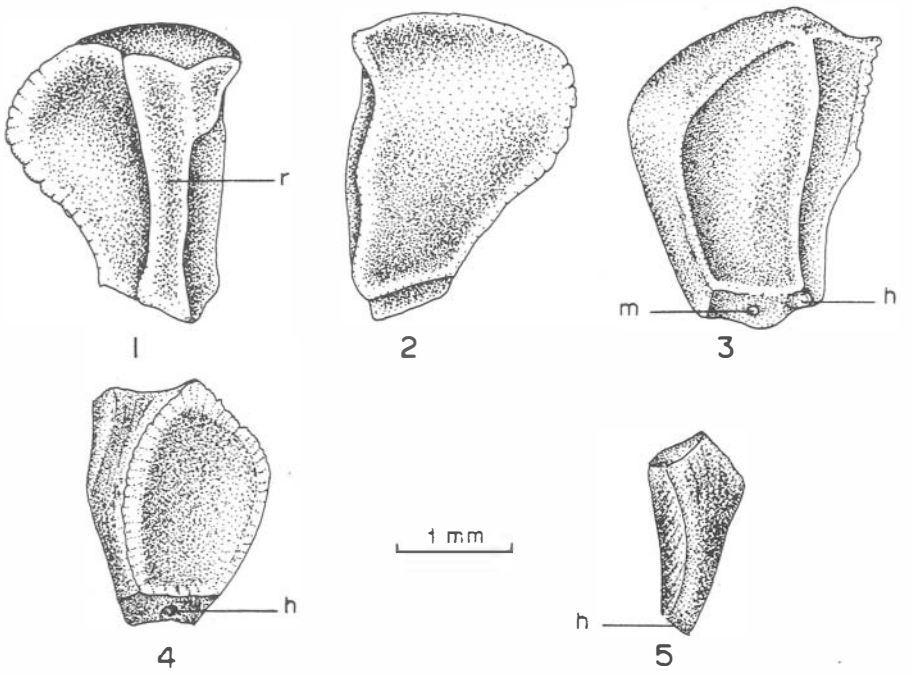
A 1% ferric chloride solution with 0,1 N hydrochloric acid was used to determine the ocurrence of phenolic compounds and tests with chloriodide of zinc were made for the identification of celulose and lignin (Jensen, 1962). Lignified walls were also localized by the phloroglucin test (Sass, 1951). The cuticle, the cutinized walls and lipid reserves became evident with Sudan IV (Johansen, 1940). Calose was localized with anilin blue and the proteinic reserves were identified by the Millon reagent (Jensen, 1962). The aleurone grains were stained by 1% alcoholic eosine solution after fixation with 20% solution of mercuric chloride in absolute alcohol (Accorsi, 1941). Liquid ammonia was used for the identification of anthocyanin in the seedlings (Johansen, 1940).

The drawings and diagrams of the structures were made with a camera lucida.

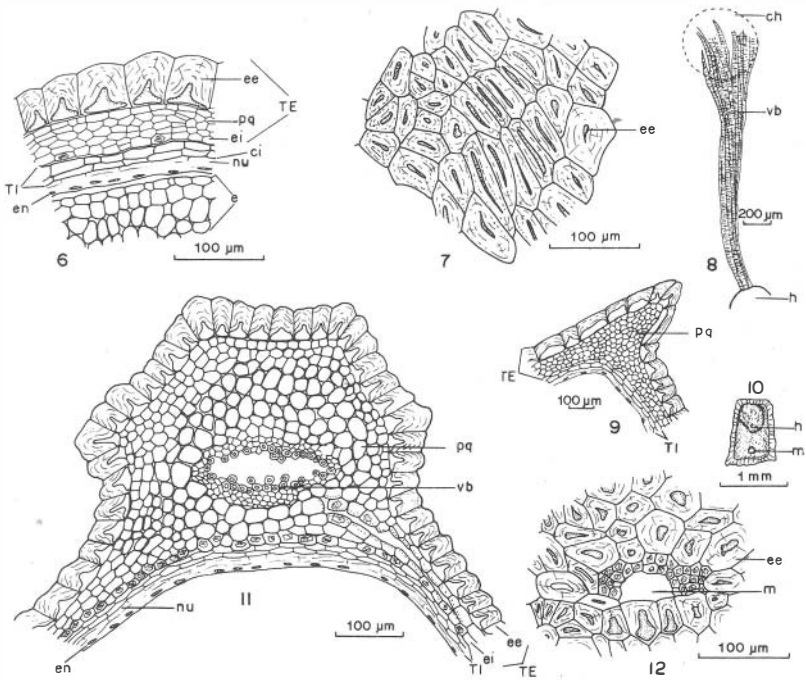
For germination studies, the seeds were placed on moist filter paper in covered Petri dishes at room temperature (mean 26 C) and light.

RESULTS

External morphology of fertile and sterile seeds: In the seeds of *Eucalyptus pilularis* and *E. umbra* there are three shapes: apparently fertile seeds, provided with embryo; and shape "A" and shape "B" embryoless sterile seeds. *E. pilularis*



Figs. 1-5. *E. pilularis* - Fertile and sterile seeds. External Morphology. Figs. 1-3 - fertile seed; Fig. 4 - shape A sterile seed; Fig.5 - shape B sterile seed.



Figs. 6-12. *E. pilularis* - Fertile seed coat anatomy. Figs. 6 and 7 - respectively, cross and paradermal sections of the fertile seed coat (antiraphe face); Fig. 8 - vascularization pattern; Fig. 9 - transversal section through the edge; Fig. 10 - basal seed face diagram showing the hilum and micropyle positions; Fig. 11 - transversal raphe section. Fig. 12 - Paradermal section at the micropylar region.

presented a mean of 6 ± 3% (by weight) of fertile seeds which represented an average of 12 ± 5 fertile seeds per gram. The mean weight of 100 fertile seeds was 155.6 ± 6.2 mg. In *E. umbra* the values were: a mean of 7± 3% (by weight) of fertile seeds which represented an average of 95± 7 fertile seeds per gram. The mean weight of 100 *E. pilularis* fertile seeds was 65.2 ± 4.1 mg.

E. pilularis fertile seeds (Figs. 1-3) are red-brown; polyhedral or trapezoidal; moderately lustrous surface with fine pitting; entire prominent edges; hilum (h) basal, circular, conspicuous by its light color; chalaza region not externally distinct; long raphe (r) as a longitudinal protuberance; antiraphe convex; exostome (m) visible as a little gap beneath the hilum; 2.20 ± 0.14 mm long x 1.53 ± 0.21 mm broad.

E. umbra: fertile seeds (Figs. 24-26) are light red-brown or light brown; polyhedral, trapezoidal or sickle-shaped; other features similar to *E. pilularis;* 1.62 ± 0.29 mm long x 1.31 ± 0.24 mm broad.

E. pilularis shape A sterile seeds (Fig. 4) are red-brown; laterally flattened , trapezoidal; sickle-shaped; hilum basal; surface features similar to those of fertile seeds; 2.35 ± 0.10 mm long x 1.01 ± 0.10 mm broad.

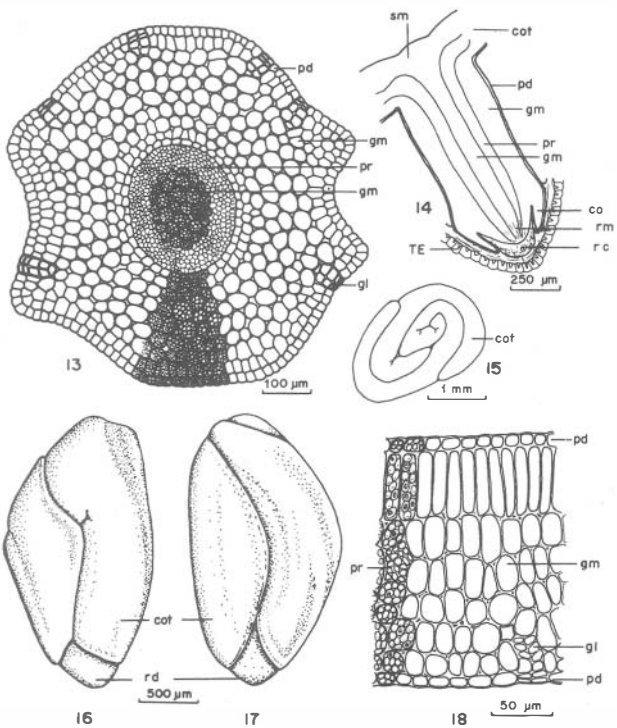
Shape B (Fig. 5) are red-brown, elongated; many conical or cylindrical; surface features similar to those of fertile seeds; 1.95 ± 0.70 mm long x 1.09 ± 0.15 mm broad.

E. umbra Shape A sterile seeds (Fig. 27) are light red-brown to brown; laterally flattened; trapezoidal; sickle-shaped; hilum basal; surface features similar to those of fertile seeds; 1.55 ± 0.16 mm long x 0.60 ± 0.08 mm broad.

In shape B (Fig. 28), color, shape and other surface features are similar to those of shape A sterile seeds; 1.09 ± 0.18 mm long x 0.82 ± 0.24 mm broad.

Internal morphology of fertile seeds: In the two species the ripe seeds are basically constituted by the seed coat and the embryo. The seed coat is formed by the two integuments (TE and TI); also it is possible to find some remnants of the nucellar tissue (nu) and a single layer of endosperm (en).

The embryo (Figs. 16,17,39 and 40) consists of the cylindrical hypocotyl-root axis and two thick cotyledons (cot), bent down along the embryo axis (Figs. 14,15,37 and 38). The root meristem (rm) and root cap (rc), are almost surrounded by the still incipient clinging disc or cupuliform organ (co). This organ is a bulge of the hypocotyl (hy) cortex (Figs. 14 and 38).



Figs. 13-18. *E. pilularis* - Embryo. Fig. 13 - middle cross section through embryo axis; Fig. 14 - Diagram of longitudinal section through embryo axis; Fig. 15 - Diagram of cross section through the cotyledons; Fig. 16 - surface view of the embryo (raphe face); Fig. 17 - surface view of the embryo (antiraphe face); Fig. 18 - cotyledon cross section.

Fertile seed coat anatomy: *Eucalyptus pilularis:* testa (TE) multilayered; exotesta (ee) as a solid layer of sclerotic cells with heavy lamellated thickenings on the outer and radial walls, the latter transversed by simple pit canals. The thickened walls are lignified and leave a very small lumen filled with amorphous dark-brown tannic material. The inner epidermis (ei) is made up of small thin-walled cells, rectangular in cross section (Fig. 6) and poligonal in paradermal section (Fig. 7). Crystals of calcium oxalate occur dispersed in these cells, being most plentiful in the raphe parenchyma, just over the hilum and at the ribs (Figs. 6 and 11).

Between the two epidermal layers there are several layers of parenchyma (tp) which frequently change in number according to their position in the seed (Fig. 6, 9 and 11). These cells have pitted walls and are filled with amorphous dark-brown tannic material.

E. umbra (Fig. 29 and 30): testa generally two-layered. The parenchyma cells occur just over the hilum, along the raphe and at the edges (Figs. 34 and 35). The other anatomic features are similar to those of *E. pilularis*.

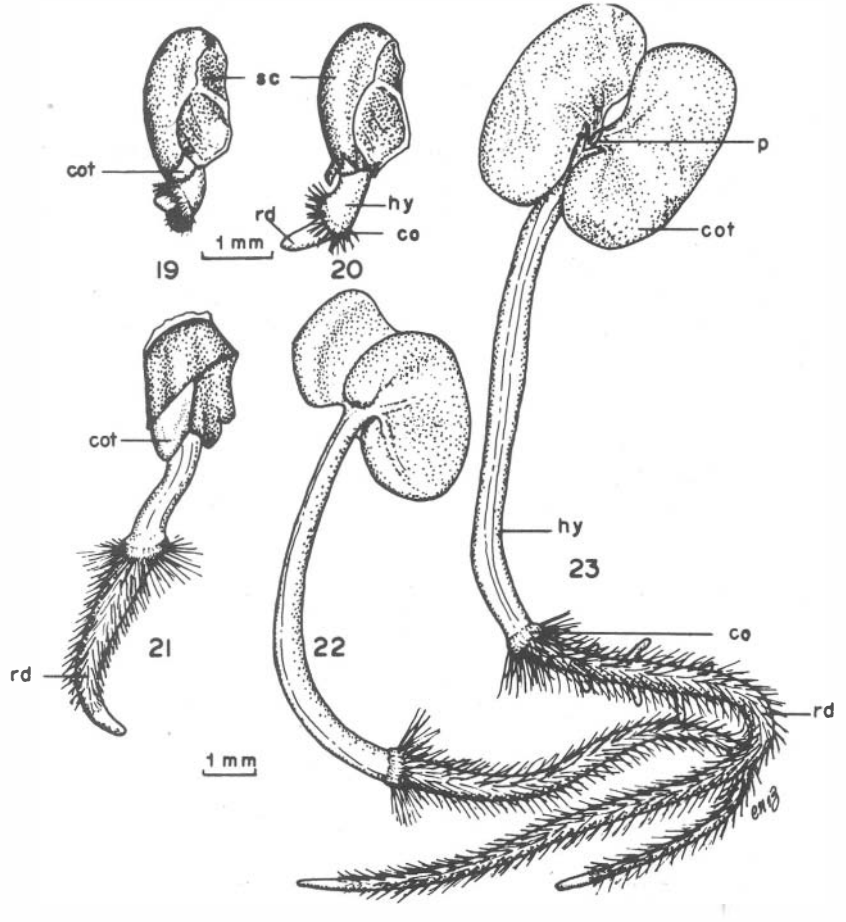
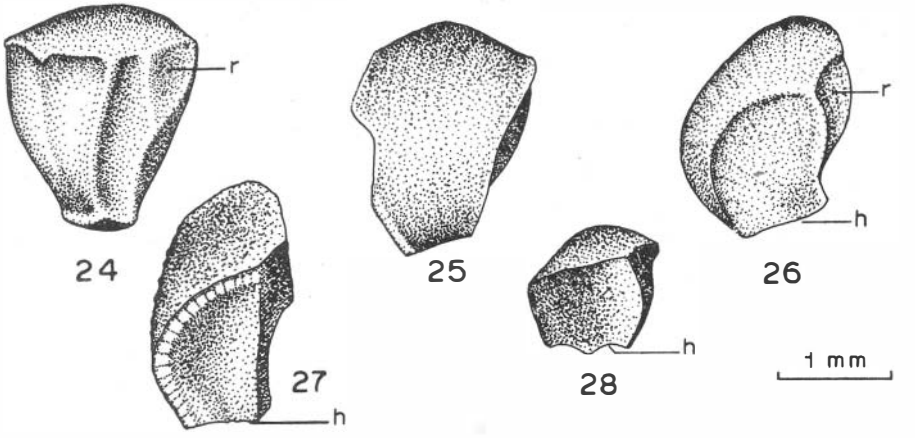


Fig. 19-23. *E. pilularis* - sequential stages of development of seedlings. Fig. 19 - seedlings at 4 days; Fig. 20 - at 5 days; Fig. 21 - at 7 days; Fig. 22 - at 10 days; Fig. 23 - at 18 days.



Figs. 24-28. *E. umbra* - Fertile and sterile seeds. External morphology. Figs. 24-26 - fertile seed. Fig. 24 - shape A sterile seed; Fig. 28 - shape B sterile seed.

In *E. pilularis,* from the hilum an amphicribal vascular bundle (vb) with helically thickened tracheids extends in the raphe parenchyma right up to the chalaza where it spreads out (Figs. 8 and 11). In *E. umbra* the vascular bundle gives rise to some branchlets before reaching the chalaza region (Figs. 31, 32 and 35). The chalaza (ch) is a parenchymatous tissue made up of thin-walled suberized cells filled with a dense reddish-brown tannic material.

In *E. pilularis* the inner integument (TI) is formed by two epidermal layers of suberized tabular cells (Fig. 6). In *E. umbra* the two epidermal layers of the inner integument are distinct only along the raphe. At the inner limit there is a rather evident cuticle which forms short rib-like projections of cutine between the nucellus epidermal cells. The formation of this cuticle is supressed in the chalazal region.

Remnants of the nucellar tissue (nu) with empty and largely obliterated cells are present, especially in the chalaza region, where the celular structure is evident.

The endosperm (en) is almost completely absorbed, remaining only a layer of cells filled with proteins and oil droplets (Figs. 6, 9, 11, 29, 34).

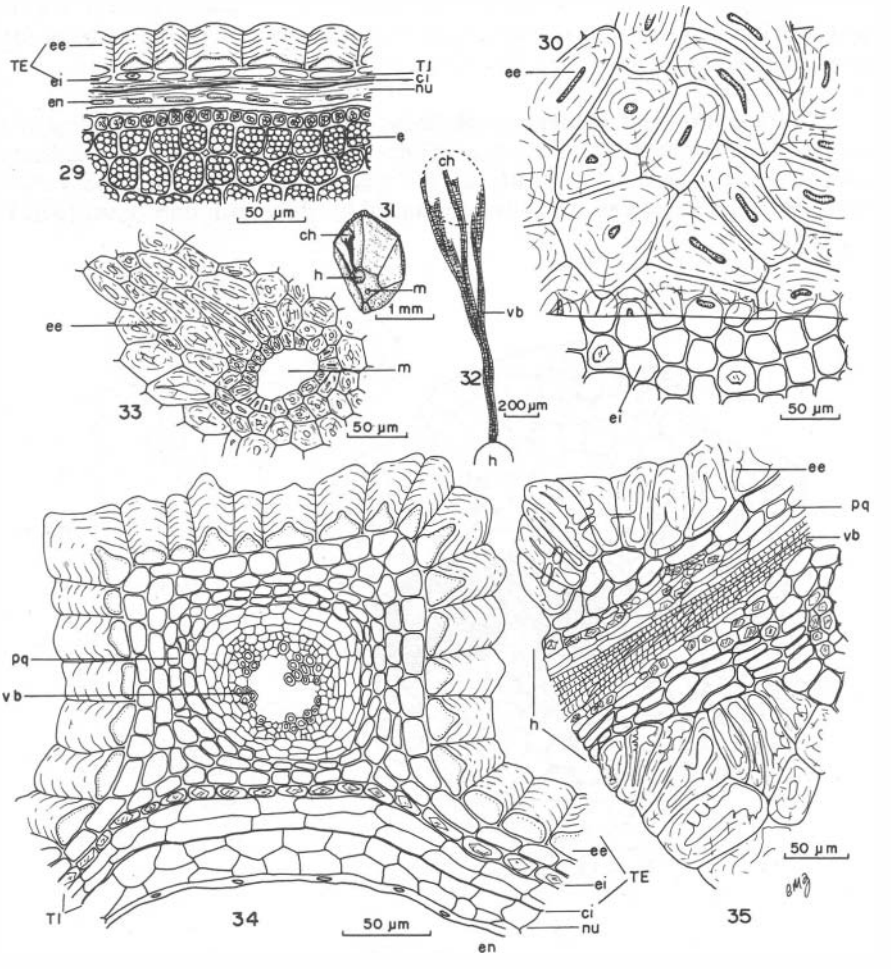
In both species, the hilum (h) is a scar of thin-walled non-suberized cells surrounded by a rim of sclereids (Fig. 35).

In *E. pilularis* the micropylar gap (m) is bordered at its upper and lateral edges by small thin-walled suberized cells, each containing a calcium oxalate crystal, and at its lower edge, by sclereids (Figs. 10 and 12).

In *E. umbra* surrounding the micropylar gap there is a complete rim of small sclereids (Fig. 33).

Anatomy of the embryo: In *E. pilularis* the hypocotyl-root axis is about 1.2 mm long and 0.4 mm in diameter and in *E. umbra* it is about 0.8 mm long and 0.3 mm in diameter.

In both species the embryo axis is covered by the protoderm (pd, Figs. 13 and 16). Inside to the protoderm there are 7 or 8 layers of ground meristem (gm), precursor of cortical ground tissue, of round thin-walled cells. Below this tissue there is a cylinder of narrow procambial cells (pr), 6 or 7 layers thick in *E. pilularis* and 4 or 5 layers in *E. umbra.*



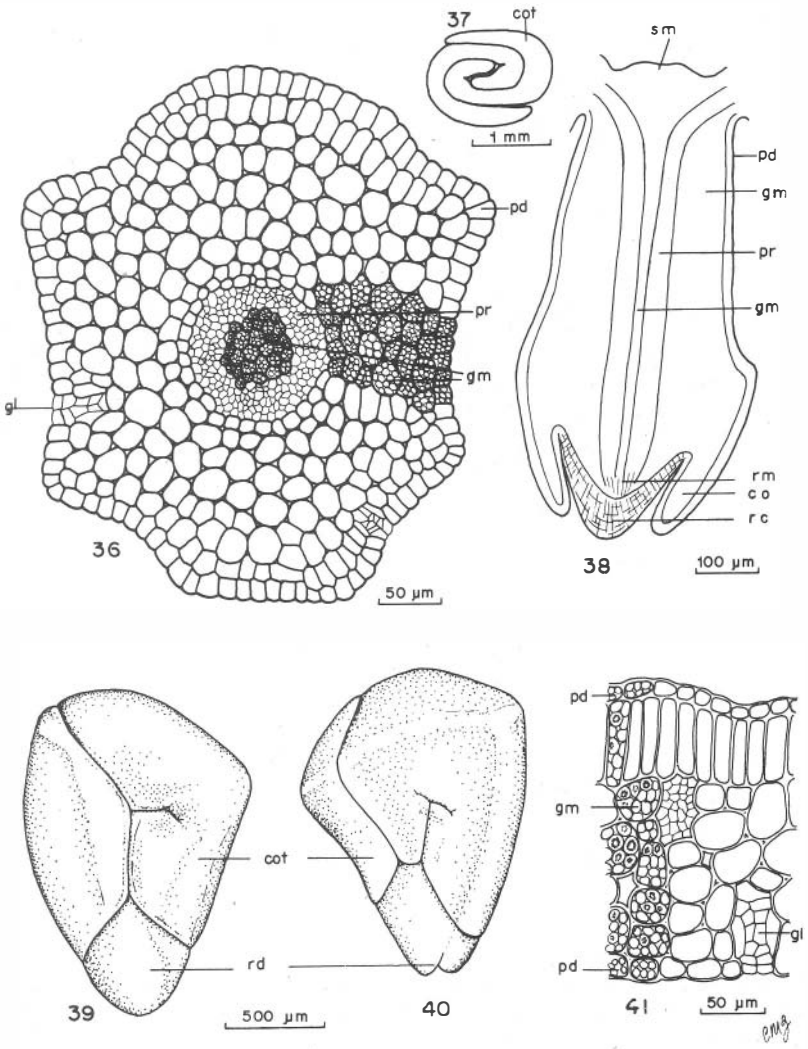
Figs. 29-35. *E. umbra* - Fertile seed coat anatomy. Figs. 29 and 30 - respectively cross and paradermal sections of the fertile seed coat (antiraphe face); Fig. 31 - Seed diagram showing the hilum, chalaza and micropyle positions; Fig. 32 - vascularization pattern; Fig. 33 - paradermal section at the micropylar region. Fig. 34 - raphe transversal section; Fig. 35 - longitudinal section through the hilum region.

Arising from the protoderm of the axis, as well as from that of the cotyledons, and deeper in the ground meristem there are small oil glands (gl) scattered along the embryo axis, and in both faces of the cotyledons, mainly at the abaxial surface.

The cotyledons (Figs. 18 and 41) are covered by thin-walled protodermal cells, rectangular in cross section and irregular in paradermal section. The ground meristem below the protoderm, on the adaxial face consists of a row of palisade cells, and on the abaxial side there are 5 layers of round cells which present small intercelular spaces.

Most of the embryo tissues except procambial and glandular cells, are filled with aleuron grains and oil droplets. Each one of the aleuron grains contains one or more small druses of calcium oxalate (Figs. 18 and 41).

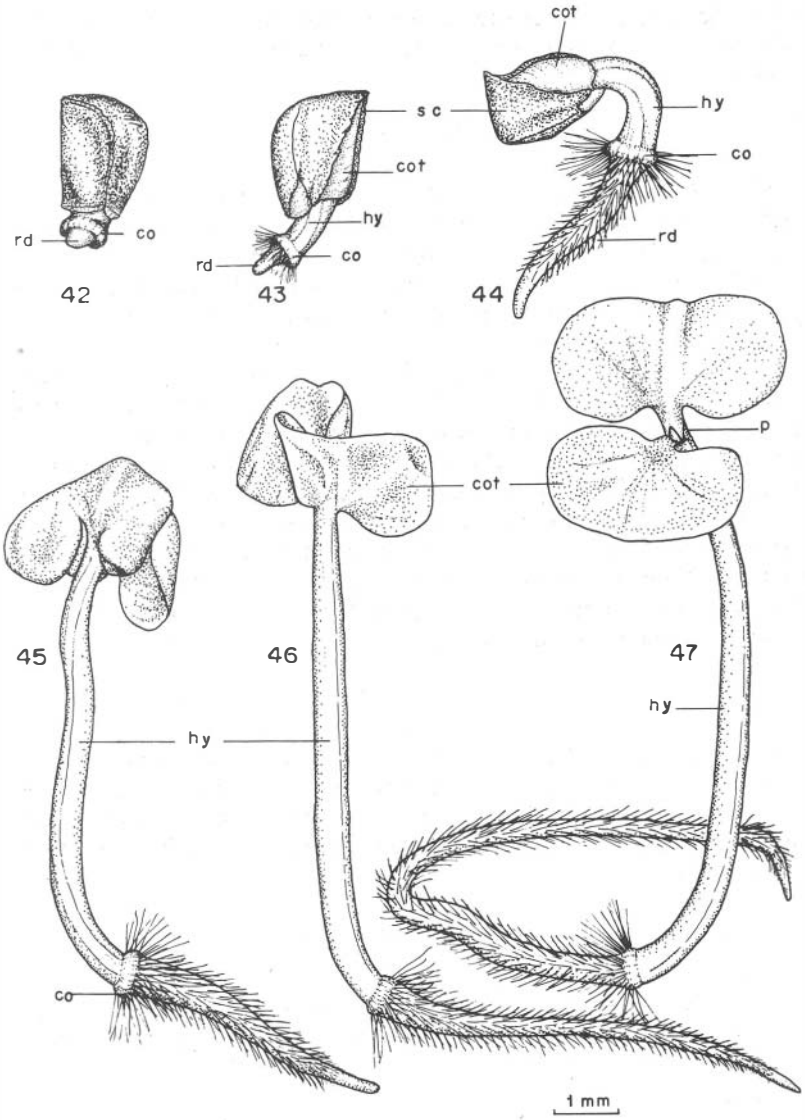
Germination and seedling morphology: Germination and morphology of the seedlings in the two species are similar. Three days after sowing (in petri dishes) the rupture of the seed coat occurs at the micropylar region and the radicular primordium (rd) emerges, partially surrounded by the cupuliform organ (co). This



Figs. 36-41. *E. umbra.* Embryo. Fig. 36 - middle cross section through the embryo axis; Fig. 37 - diagram of cross section through the cotyledons; Fig. 38 - Diagram of longitudinal section through the embryo axis. Fig. 39 - surface view of the embryo (raphe face); Fig. 40 - surface view of the embryo (antiraphe face); Fig. 41 - cotyledon cross section.

organ, that is very narrow (0,3 mm in *E. pilularis* and 0,2 mm in *E. umbra*), soon develops long absorbing hairs (Figs. 20 and 43) and remains functional for about 30 days when the hairs begin to dry.

The radicle and the hypocotyl (hy) grow simultaneously and after 7 or 8 days the seedling is fixed to the substratum (Figs. 21 and 44) when the seed coat (sc) is released. The hypocotyl is pink, due to the presence of anthocyanins in the epidermal cells.



Figs. 42-47. *E. umbra* - sequential stages of seedling development. Fig. 42 - seedling at 4 days; Fig. 43 - at 6 days; Fig. 44 - at 8 days; Fig. 45 - at 10 days; Fig. 46 - at 11 days; Fig. 47 - at 18 days.

After about 12 days the cotyledons (cot) unfold (Figs. 22, 23 and 45-47). They are bilobed, dark-green adaxially and reddish-purple abaxially, due to anthocyanins. The epicotyl (p) initiates its growth in about 20 days in *E. pilularis* and in about 25 days in *E. umbra.*

Comparison of seed length averages: As additional information to the morphological comparisons, the variation of the fertile seed length was studied in greater detail. The Kruskal-Walles technic (Sokal & Rohlf, 1969) was used to verify whether the existing difference in the average length of the fertile seeds was significant or not. According to the test, *E. pilularis* seeds differ significantly in length from those of *E. umbra.*

DISCUSSION

The present results suggest that these two species have many common morphological and anatomical features but they can be distinguished mainly by the average length of fertile seeds and by the seed coat anatomy.

In *E. pilularis*, but not in *E. umbra,* the testa is multilayered in all its extent and according to Corner (1976) "multiplicative seeds occur in families acknowledged to be primitive".

Another important observation is that in other previously studied species (Beltrati, 1977a; 1977b; 1978a; 1978b; 1979; 1980) but not in *E. pilularis* and in *E. umbra,* the exotesta is unlignified and the endotesta is composed of closely packed cells, each containing a heavy cellulosic thickening of its inner periclinal wall and one or more calcium oxalate crystals each. According to Gauba & Pryor (1958) the discontinuous presence or complete absence of calcium oxalate crystals in generally unmodified cells of the inner epidermis of the testa among the Renantherae-Normales is likely to have taxonomic significance since in the Angiosperms the ocurrence of a "crystal epithelium" is widely distributed, especially in the more primitive families.

RESUMEN

Se estudiaron en detalle aspectos morfológicos y anatómicos de semillas y plántulas jóvenes de *Eucalyptus pilularis* Sm. y *E. umbra* R.T. Baker. Se encontró que eran similares a excepción de algunos aspectos anatómicos de la testa y del promedio de longitud de las semillas fértiles, que es significativamente diferente en estas dos especies.

LITERATURE CITED

Accorsi, W.R.
1941. Contribution to the comparative anatomical study of *Eucalyptus tereticornis* Smith and *Eucalyptus citriodora* Hooker. Thesis. Esc. Sup. Agr. "Luiz de Queiroz". Piracicaba, Brasil.

Beltrati, C.M.
1977a. Comparacão morfológica entre sementes procedentes do Brasil e da Austrália, de *Eucalyptus alba* Reinw. Rev. Brasil. Biol., 37: 463-471.

Beltrati, C.M.
1977b. *Eucalyptus grandis* (Hill) Maiden: morfologia das sementes e de sua germinação. Phyton, 35: 93-101.

Beltrati, C.M.
1978a. Morphological and anatomical studies of the seeds and seedlings of *Eucalyptus citriodora* and *E. maculata*. Rev. Biol. Trop., 26: 213-225.

Beltrati, C.M.
1978b. Morfologia e anatomia das sementes e plântulas de *Eucalyptus maidenii*. Turrialba, 28: 209-214.

Beltrati, C.M.
1979. Morfologia e anatomia das sementes de *Eucalyptus punctata* DC. Anais da Sociedade Botânica do Brasil, XXX Congresso Nacional de Botânica: 17-21.

Beltrati, C.M.
1980. Morfologia e anatomia das sementes e plântulas de *Eucalyptus saligna* Sm. Rev. Brasil. Biol., 40: 441-446.

Blakely, W. F.
1955. A key to the eucalyptus (2d ed.). Comm. Austr. For. Timber Bur. Canberra, Australia.

Corner, E.J.H.
1976. The Seeds of dicotyledons. Vol. 1. Cambridge University Press. Cambridge.

Gauba, E., & L.D. Pryor
1958. Seed coat anatomy and taxonomy in *Eucalyptus*. I. Proc. Linn. Soc. N.S.W., 83: 20-32.

Jensen, W.A.
1962. Botanical histochemistry. Principles and pratice. Freeman, San Francisco.

Johansen, D.A.
1940. Plant microtechnique. McGraw-Hill. New York.

Sass, J.E.
1951. Botanical microtechnique (2d ed.). Ames, Iowa State College Press.

Sokal, R.R., & F.J. Rohlf
1969. Biometry. Freeman. San Francisco.

Vaughan, J.G.
1968. Seed anatomy and Taxonomy. Proc. Linn. Soc. Lond., 79: 251-255.