



Seed characters and their usefulness in the separation of Asteraceae species

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ABSTRACT. Studies on seed structure of Asteraceae have received little attention of botanists. Seed structure in Asteraceae is analyzed to evaluate the usefulness of characters in the separation of species through the analysis of nine weedy species. Seeds originate from anatropous, unitegmic and tenuinucellate ovules. The partial collapse of the developing seed coat is a common characteristic in the Asteraceae species studied. The testa consists of crushed and thin-walled cells in almost all of the species studied herein, except for *Elephantopus mollis* and *Parthenium hysterophorus*, which showed exotesta cells with U-shaped thickening. The analysis revealed high uniformity in seed characters.

Keywords: ovule, exotesta, endosperm, embryo.

Caracteres de sementes e sua utilidade na separação de espécies de Asteraceae

RESUMO. Estudos da estrutura da semente de Asteraceae receberam pouca atenção de botânicos. A estrutura da semente de Asteraceae é examinada com a finalidade de avaliar a utilidade dos caracteres na separação de espécies, mediante a análise de nove espécies de plantas daninhas. As sementes são originárias de óvulos anátropos, unitegumentado e tenuinucelados. O colapso parcial do revestimento da semente em desenvolvimento é uma característica comum nas espécies estudadas de Asteraceae. A testa é composta por células colapsadas e de paredes finas em quase todas as espécies estudadas, com exceção de *Elephantopus mollis* e *Parthenium hysterophorus*, que demonstraram células da exotesta com espessamento em U. A análise revelou uma grande quantidade de uniformidade dos caracteres estudados das sementes.

Palavras-chave: óvulo, exotesta, endosperma, embrião.

Introduction

While most botanists are satisfied with external features of the achene and a few researches have analyzed the development of the embryo and ovule, little attention has been given to the seed-coat of Asteraceae (Compositae) (CORNER, 1976). However, this author complements that the seed-coat not completely deteriorate in the achene but has retained an exxotestal palisade (Cynareae), a variety in the thickening of the exotestal cells, in the retention of the mesophyll and in the vascular supply.

Pallone and Souza (2014) showed that the seeds of *Crepis japonica* (L.) Benth., *Porophyllum ruderale* (Jacq.) Cass. and *Tridax procumbens* L. consist of endothelium in the developing seeds, and the seed-coat does not completely deteriorate in the cypsela of the three species, mainly in *C. japonica*, which has exotestal seed composed of thick-walled cells.

Besides the intrinsically interesting features of the cypsela (achene) structure, we sought to

contribute to a better understanding of the seed characters of nine Asteraceae species, which occur with relative frequency as weed plants in Brazil. An attempt is made to answer the question concerning the usefulness of the seed characters in the separation of these species of Asteraceae.

Material and methods

Developing flowers and cypselas of nine Asteraceae species: *Cosmos sulphureus* Cav., *Eclipta alba* Hassak., *Elephantopus mollis* Kunth, *Emilia sonchifolia* (L.) DC., *Erechtites valerianifolius* (Link ex Spreng.) DC, *Galinsoga quadriradiata* Ruiz & Pav., *Parthenium hysterophorus* L., *Praxelis dematidea* (Griseb.) R. M. King & H. Rob. and *Sigiesbeckia orientalis* L. (all listed in Table 1) were collected in the region of Maringá, Paraná State, Brazil. Voucher material was deposited in the UEM Herbarium (HUEM).

Table 1. Species from Asteraceae collected at Maringá, Paraná State, Brazil.

Species	Habit	Accession number
<i>Cosmos sulphureus</i> Cav.	Shrubby	18589 HUEM
<i>Eclipta alba</i> (L.) Hassk.	Herb	18587 HUEM
<i>Elephantopus mollis</i> Kunth	Herb	18588 HUEM
<i>Emilia sonchifolia</i> (L.) DC.	Herb	18586 HUEM
<i>Erechtites valerianifolius</i> (Link ex Spreng.) DC.	Herb	21182 HUEM
<i>Galinsoga quadriradiata</i> Ruiz & Pav.	Herb	18590 HUEM
<i>Parthenium hysterophorus</i> L.	Herb	20828 HUEM
<i>Praxelis dematidea</i> (Griseb) R. M. King & H. Rob.	Herb	21181 HUEM
<i>Sigesbeckia orientalis</i> L.	Herb	20836 HUEM

Flower and fruit samples were fixed in glutaraldehyde 24 hours (1% in 0.1 M phosphate buffer, pH 7.2). These were dehydrated and embedded in historesin Leica according to the method of Guerriets (1991). Sections were serially cut from the embedded samples with a rotary microtome to the thickness 8-12 μm . Sections were stained with 0.05% Toluidine Blue, pH 4.7 (O'BRIEN et al., 1964). Light microscope photographs were taken with a Leica EZ4D digital camera, and subsequently processed using the software Leica Application Suite 1.8. The following stains and reagents were used for specific color tests: Iodine-Potassium Iodide Test, for starch; Sudan IV, for lipids; and Ferric Chloride for phenolic substances (JOHANSEN, 1940; RUZIN, 1999).

Results

Developing seed

Seeds originate from anatropous, unitegmic and tenuinucellate ovules (Figure 1A). Ovules are sessile, but *Sigesbeckia orientalis* has a short funicle (Figure 1B). The vascular supply of the ovules has postchalazal course (Figure 1C). The integument consists of outer epidermis with narrow and tangentially elongated cells and (Figure 1C) in the micropyle region the cells are short and quadrilateral. The multiseriate parenchyma mesophyll is made up of polygonal and large cells, eventually elongated attached to the outer epidermis (Figure 1C). The integument inner epidermis shows cuboid or slightly prismatic cells (Figure 1C). The hypostase is well-defined in the chalaza by the cells more densely stained (Figure 1C). More developed ovules undergo longitudinal elongation (Figure 1D).

Young seeds also show a hypostase and the inner epidermis cells become specially differentiated in endothelium, in which the cells have pronounced radial elongation and more intense staining (Figure 1E).

Ovary general view with a single ovule in longitudinal section. B. Ovule with short funicle in

longitudinal section. C. Detail of chalazal and micropylar region of the ovule in longitudinal section. D. Longitudinal section of the ovary and more developed ovule. E. Cross section of the young seed showing the mesotesta and endotesta (endothelium) (black asterisk). Outer ovule epidermis (white arrow-head), inner ovule epidermis (black arrow-head), procambial strand with postchalazal branches (white arrow), hypostase (white asterisk), mesophyll (double arrow), ovary (ov) and ovule (ou).

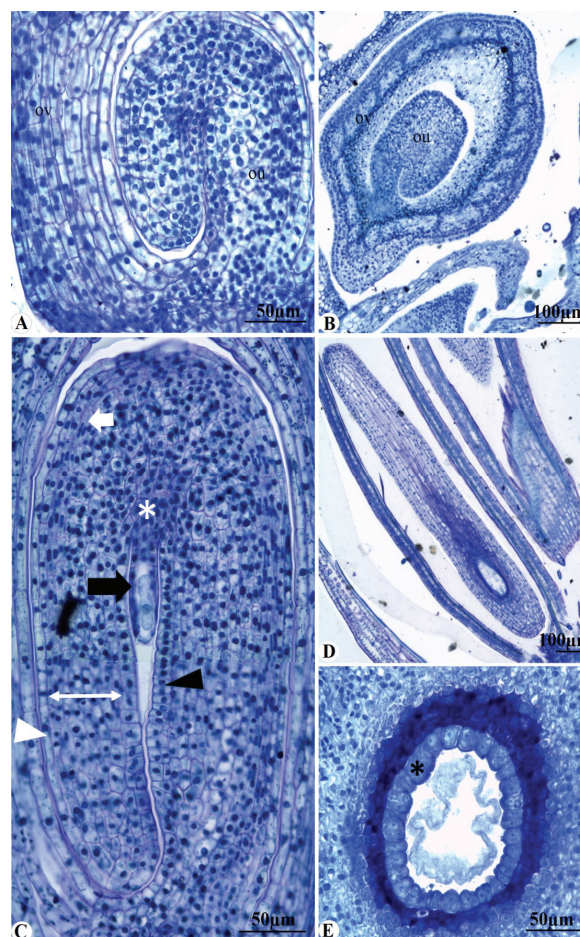


Figure 1: Structure of ovule and young seed. A C - *Emilia sonchifolia*, B - *Sigesbeckia orientalis*, D - *Erechtites valerianifolia*, E- *Cosmos sulphureus*. A.

Internally to the inner epidermis, there are mesotesta cells, which are more stained than the other cells (Figure 1E) and later are crushed (Figure 2A). The partial collapse of the developing seed coat is a common feature in the studied species of Asteraceae. Subepidermal mesotesta, inner epidermis and central cells of the chalazal region undergo collapse in the nine species (Figure 2B). As consequence, the embryonic cavity increases in size to some extent (Figure 2B).

Outer epidermis of the developing seed coat of *Elephantopus mollis* (Figure 2C) and *Parthenium hysterophorus* is strikingly different from the other species herein investigated, due to the thickened cell walls.

Endosperm formation in the analyzed nine species is of the cellular type (Figure 2D), in which ontogenetic seed studies are needed to precisely evaluate the endosperm type. Abortive seeds were found in *Emilia sonchifolia* (Figure 2E and F), *Cosmos sulphureus* and *Praxelis clematidea*.

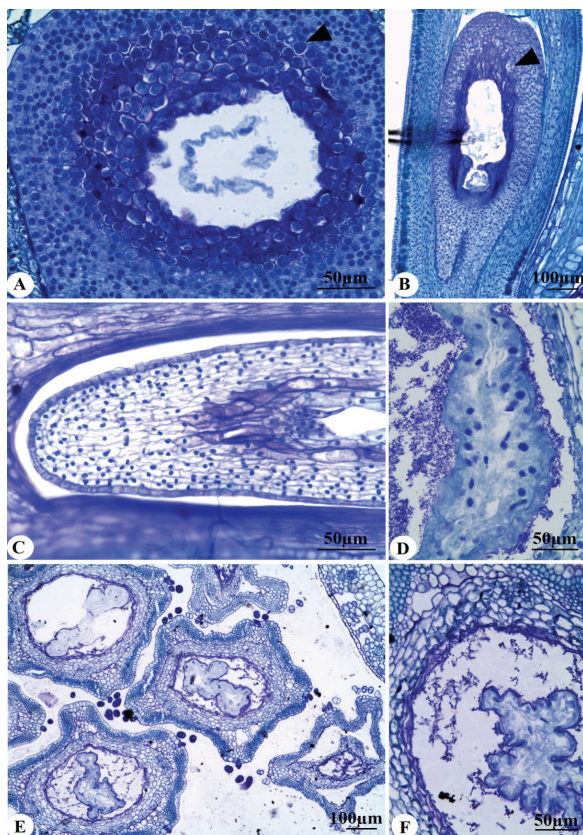


Figure 2. Structure of the young seed. A, B - *Cosmos sulphureus*. C - *Elephantopus mollis*. D - *Parthenium hysterophorus*. E, F - *Emilia sonchifolia*. A - Cross section showing the collapse of mesotesta cells (arrow-head). B - Seed in longitudinal section showing endotesta and part of mesotesta already collapsed (arrow-head). C - Longitudinal section with exotesta cells of thicker walls. D - Cross section showing cellular endosperm (asterisk). E - F - Cross section showing the seed abortion.

Ripe seed

The testa is formed of crushed and thin-walled cells in almost all of the species studied here, but the cellular collapse is more pronounced in seeds of *Eclipta alba*, *Emilia sonchifolia* (Figure 3A) and *Sigiesbeckia orientalis*, except for the located cells in the raphe region. On the other hand, seeds of *Elephantopus mollis* and *Parthenium hysterophorus* (Figure 3B) showed exotesta cells with U-shaped thickening.

The endosperm persists in the ripe seed, and it is composed of one or two layers of relatively thick-walled cells (Figure 3A, B, C and E).

All nine species have seed vasculature consisting of a single collateral vascular bundle that terminates blindly in the proximities of the micropyle. The embryo of the nine Asteraceae species is erect, and consists of two cotyledons with homogeneous mesophyll (Figure 3C), plumule with leaf primordia (Figure 3D), and hypocotyl-radicle axis relatively long (Figure 3E).

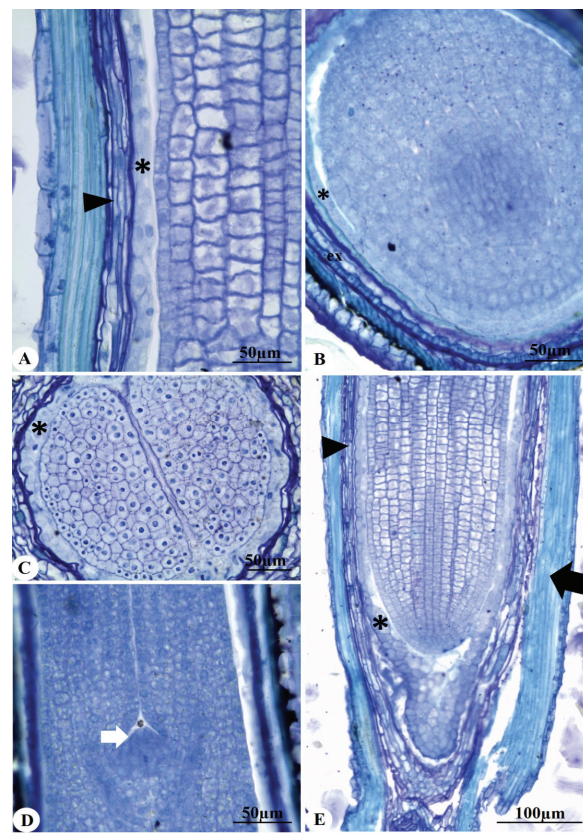


Figure 3. Structure of the ripe seed. A, C, E - *Emilia sonchifolia*. D - *Erechthites valerianaeifolia*. B - *Parthenium hysterophorus*. A - Longitudinal section of the ripe seed showing the collapsed testa (black arrow-head). B - Cross section of the fruit with seed showing endosperm (asterisk) around the embryo. C - Cross section of the fruit showing the cotyledons with homogeneous mesophyll. D - Embryo in longitudinal section showing the plumule (white arrow-head). E - Fruit in cross section showing the hypocotyl-root axis, endosperm (asterisk), collapsed testa (black arrow-head) and pericarp (black arrow).

Discussion

Ovules and young seeds of the analyzed species maintain the structural pattern observed in Asteraceae (Compositae) (CORNER, 1976). The endothelium is normally restricted to tenuinucellate and generally unitegmic ovules and it is registered in Asteranae (DAHLGREN, 1991) and (CORNER, 1976; BOUMAN, 1984). In several Asteraceae

species, the endothelium was reported, for instance, in *Vernonia platensis* (Spreng.) Less. (GALASTRI; OLIVEIRA, 2010), *C. japonica*, *P. ruderae* and *T. procumbens* (PALLONE; SOUZA, 2014), and it is apparently involved with several processes (WERKER, 1997). There seems to be no doubt that the endothelium is a nutritive layer, whose main role is to work as an intermediate for the transport of nutrients from the integument to the embryo sac (MAHESHWARI, 1971). In a literature review on endothelium, Werker (1997) suggests several functions for this tissue, such as transference of nutrients, temporary accumulation of nutrients, ability to act in metabolizing dissolved products in its own cells, secretion of digestive enzymes, and also act as a limiting barrier, preventing the embryo and endosperm from excessive growth. The presence of endothelium may be considered a derived character (VON TEICHMAN; VAN WIK, 1991).

The collapse of the integument and the increase in the cavity, observed in the nine species, were also evidenced in other species, as *Vernonia cinerea* Less. (PANDEY; SINGH, 1980), *Schlechtendalia luzulifolia* Less. (MELLO et al., 2009), *Vernonia platensis* (Spreng.) Less. (GALASTRI; OLIVEIRA, 2010), *C. japonica*, *P. ruderae* and *T. procumbens* L. (PALLONE; SOUZA, 2014).

The endosperm is of the cellular type, however Dahlgren (1991), mentioning Wunderlich (1959), argued that the endosperm formation in Asteraceae is surprisingly variable, suggesting that both the cellular and the nuclear types are associated with most Asteraceous tribes, and are also reported for many genera, which is extremely unusual in eudicots. Other authors, such as Singh (1964) and Corner (1976), also indicate both the cellular and nuclear types of endosperm for the family.

Seed abortion was observed in fruits of *Emilia sonchifolia*, *Cosmos sulphureus* and *Praxelis clematidea*. Chican and Palser (1982) speculated that the proliferation of the endothelium, failure in embryo formation, and failure in endosperm development seem to be either directly or indirectly involved in abortion of seeds at early stages of development of *Cichorium intybus* L. The occurrence of self-incompatibility, which is common in Asteraceae (HEENAN et al., 2005–não consta ne referência), seems to be a cause of seed abortion. Evidently, all these possible factors in seedless cypselas should be investigated in the species here studied.

The chief protective function for the embryo with unspecialized seed coat (thin-walled and collapsed cells) seems to be exercised by the pericarp wall of the cypselas (achene). In this case, the pericarp wall may to substitute the seed coat which is rather obliterated during the development. The

transfer of protecting function of the embryo was already enhanced for Júlio and Oliveira (2008). It is probable that in *E. mollis* and *P. hysterothorus*, which have exotesta with secondary thickening, the seed coat furnishes additional protection to the embryo. Among the Asteraceae, *Wedelia calendulacea* Rich. (PANDEY; SINGH, 1994) and *C. japonica* (PALLONE; SOUZA, 2014) also have exotesta with thick-walled cells. Pandey and Singh (1994) referred to fibrous layers in the seed coat of species belong to the tribes Eupatorieae and Heliantheae.

One or two layers of endosperm of the nine species of Asteraceae persist in the mature seed with cell walls relatively thick. The occurrence of endosperm in mature seeds is also known in *Helichrysum bracteatum* Andr. and *Vicoa indica* DC (PANDEY; SINGH, 1983), and *P. ruderae* and *T. procumbens* (PALLONE; SOUZA, 2014). It is likely that the persistent endosperm protects the embryo, mainly in the seeds which do not have a specialized seed coat, if the suggestions of Souza and Paoli (2009), who consider the protective function of the endosperm for Bignoniaceae seeds, are taken into consideration.

According to Corner (1976) the vascular supply of Asteraceae (Compositae) could help to solve infrafamilial relationships, and the author proposed three vascular supply states for the families, which may be useful for separation of genus. The seeds of the species analyzed in this study belong to the type I (CORNER, 1976), which is characterized by the “vascular supply as a single bundle extending round the seed from the funicle more or less to the micropyle”.

The Spatulate embryo, according to Martin (1946) classification, is defined as “[...] embryo erect; cotyledons variable, thin to thick and slightly expanded to broad; stalk not invested by cotyledons or only slightly so [...]”.

The possible relevance of the seed characters in the separation of the nine species of Asteraceae, as suggested by Corner (1976), was not satisfactorily evidenced (as shown in the Table 2). On the contrary, the analysis revealed high uniformity in these characters. Although the number of analyzed species is insufficient, it is important to note that the exotesta with thick-walled cells seems to be relevant in the species of the two genera of the subfamily Cichorioideae (*Elephantopus* and *Crepis*) (PALLONE; SOUZA, 2014). This same exotesta character is exhibited in the tribes Asteroideae, Heliantheae (*Wedelia*) (PANDEY; SINGH, 1994) and Senecioneae (*Emilia*), but it was not verified in the genera Heliantheae (*Cosmos*, *Eclipta*, *Galinsoga*, *Parthenium* and *Sigesbeckia*) studied here.

Table 2. Significant features in seed characterization of the nine species of Asteraceae.

Species	Presence of funicle	Seed coat (exotesta)
<i>Cosmos sulphureus</i>	Sessile	Non - specialized
<i>Eclipta alba</i>	Sessile	Non - specialized
<i>Emilia sonchifolia</i>	Sessile	Secondary thickening
<i>Elephantopus mollis</i>	Sessile	Secondary thickening
<i>Erechtites valerianifolius</i>	Sessile	Non - specialized
<i>Galinsoga quadriradiata</i>	Sessile	Non - specialized
<i>Parthenium hysterophorus</i>	Sessile	Non - specialized
<i>Praxelis clematidea</i>	Sessile	Non - specialized
<i>Sigesbeckia orientalis</i>	Short funicle	Non - specialized

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References

- BOUMAN, F. The ovule. In: JOHRI, B. M. **Embryology of angiosperms**. Berlin: Springer-Verlag, 1984. p. 123-157
- CORNER, E. J. H. **The seeds of dicotyledons**. Cambridge: University Press, 1976.
- CHICAN, M. A.; PALSER, B. F. Development of normal and seedless achenes in *Cicorium intibus* (Compositae). **American Journal of Botany**, v. 69, n. 06, p. 885-895, 1982.
- DAHLGREN, G. Steps toward a natural system of the dicotyledons: embryological characters. **Aliso**, v. 13, n. 1, p. 107-165, 1991.
- GALASTRI, N. A.; OLIVEIRA, D. M. T. Morfoanatomia e ontogênese do fruto e semente de *Vernonia platensis* (Spreng.) Less. Asteraceae. **Acta Botanica Brasilica**, v. 24, n. 1, p. 73-83, 2010.
- GUERRITS, P. O. The application of glycol methacrylate in histotechnology; some fundamental principles. Netherlands: University Groningen, 1991
- HEENAN, P. B.; SMISSEN, R. D.; DAWSON, M. I. Self-incompatibility in the threatened shrub *Oleria adenocarpa* (Asteraceae). **New Zealand Journal of Botany**, v. 43, n. 4, p. 831-841, 2005.
- JOHANSEN, D. A. **Plant microtechnique**. New York: McGraw-Hill Book Company, 1940.
- JÚLIO, P. G. S.; OLIVERIA, M. T. O.. Morfoanatomia comparada e ontogênese do pericarpo de *Bidens gardneri* Baker e *B. pilosa* L. (Asteraceae). **Revista Brasileira de Botânica**, v. 32, n. 1, p. 109-116, 2008.
- MAHESHWARI, P. **An introduction to the embryology of angiosperms**. New Delhi: McGraw-Hill publishing company, 1971.
- MARTIN, A. C. The comparative internal morphology seeds. **The American Midland Naturalist**, v. 36, n. 3, p. 513-660, 1946.
- MELLO, M. C.; FIOR, C. S.; OLIVEIRA, J. M. S. Anatomia do fruto de *Schlechtendalia luzulifolia* Less. (Barnadesioideae, Asteraceae Bercht. & J. Presl). **Iheringia: série botânica**, v. 64, n. 1, p. 77-80, 2009.
- O'BRIEN, T. P.; FEDER, N.; MCCULLY, M. E. Polychromatic staining of plant cell walls by toluidine blue. **O Protoplasma**, v. 59, n. 2, p. 368-373, 1964.
- PALLONE, S. F.; SOUZA, L. A. Pappus and cipsela ontogeny in Asteraceae: structure considerations of the tribal category. **Revista Mexicana de Biodiversidad**, v. 85, n. 1, p. 62-77, 2014.
- PANDEY, A. K.; SINGH, R. P. Development and structure of seeds and fruits in tribe Vernoniae: some *Vernonia* and *Elephantopus* species. **Flora**, v. 169, n. 1, p. 443-452, 1980.
- PANDEY, A. K.; SINGH, R. P. Development and structure of seeds and fruits in Compositae: tribe Eupatorie. **Journal of the Indian Botanical Society**, v. 62, n. 1, p. 276-28, 1983.
- PANDEY, A. K.; SINGH, R. P. Development and structure of seed and fruit in Eupatorieae and Heliantheae (Compositae). **Proceedings of the National Academy of Sciences of India**, v. 64, n. 1, p. 115-126, 1994.
- RUZIN, S. E. **Plant microtechnique and microscopy**. Oxford University Press, New York, 1999.
- SINGH, D. A further contribution to the endosperm of Curcubitaceae. **Proceedings of the National Academy of Sciences of India**, v. 60, n. B, p. 399-411, 1964.
- SOUZA, L. A.; PAOLI, A. A. S. Estrutura da semente. In: Souza, L. A. **Sementes e plântulas**: germinação, estrutura e adaptação. Ponta Grossa: Todapalavra Editora, 2009.
- VON TEICHMAN, I.; VAN WYK, A. E. Trends in the evolution of dicotyledonous seeds based on character associations, with special reference to pachychalazy and recalcitrance. **Botanical Journal of the Linnean Society**, v. 105, n. 3, p. 211-237, 1991.
- WERKER, E. **Seed anatomy**. Berlin: Gebrüder Borntraeger, 1997.
- WUNDERLICH, R. Zur frage der phylogenie der endospermtype bei den Angiospermen. **Osterreichische Botanische Zeitschrift**, v. 106, n. xx, p. 203-293, 1959.

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