Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

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Acta Scientiarum. Biological Sciences, vol. 35, núm. 1, enero-marzo, 2013, pp. 93-98
Universidade Estadual de Maringá
.png, Brasil

Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i1.12648

Morphological characterization of fruits, diaspores and germination of *Miconia ligustroides* (DC.) Naundim (Melastomataceae)

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ABSTRACT. *Miconia ligustroides* (DC.) Naudim, popularly known as "vassoura-preta" or "jacatirão-do-brejo" is widely distributed in Brazil. It is a recommended species for restoration of riparian areas and its fruits are attractive to wildlife. However, little is known about the morphology of its fruits and diaspores. Such morphological characterization studies are important for species identification, corroborating future studies of phenology and germination. Therefore, the fruits, diaspores and germination of *M. ligustroides* were morphologically characterized by observations through naked eye, stereoscopic microscopy and scanning electron microscopy. Analyses of chemical composition of seeds were also performed. The fruits are spherical and uniform within the analyzed sample, having a large number of tiny obtriangular yellowish-brown diaspores. The diaspores are considered ananfitropos, and have crystals in their external envelope. Concerning chemical composition, they are proteins. Germination is of phanerocotylar type and starts at 12 Days After Sowing (DAS); at 17 DAS each diaspore may produce up to three seedlings. The morphological characterization performed in this work was efficient to describe the fruits, seeds and germination of this species, thus providing the basis for future studies.

Keywords: biometric, "jacatirão-do-brejo", morphology, "vassoura-preta".

Caracterização morfológica dos frutos, diásporos e germinação de *Miconia ligustroides* (DC.) Naundim (Melastomataceae)

RESUMO. *Miconia ligustroides* (DC.) Naudim, popularmente conhecida como vassoura-preta ou jacatirão-do-brejo, é amplamente distribuída no Brasil. É uma espécie recomendada para restauração de matas ciliares, e seus frutos são atrativos à fauna. No entanto, pouco se conhece acerca da morfologia de seus frutos e diásporos, e é sabido que estudos de caracterização morfológica são importantes para a identificação de espécies, além de corroborarem estudos futuros de fenologia e germinação. Portanto, os frutos, diásporos e geminação de *M. ligustroides* foram caracterizados morfologicamente por meio de observações visuais a olho nu, em microscópio estereoscópico e por meio de Microscopia Eletrônica de Varredura. Análises da composição química das sementes também foram realizadas. Os frutos são esféricos e uniformes dentro da amostra analisada. Possuem grande número de diminutos diásporos castanho-amarelados e obtriangulares. Os diásporos são considerados ananfítropos e possuem cristais em seu envoltório externo. Quanto à composição química, são proteicos. A germinação é do tipo fanerocotiledonar e inicia-se aos 12 Dias Após Semeadura (DAS), sendo que aos 17 DAS cada diásporo pode originar até três plântulas. A caracterização morfológica realizada neste trabalho foi eficiente em descrever os frutos, sementes e germinação desta espécie, de modo a subsidiar estudos futuros.

Palavras-chave: biometria, jacatirão-do-brejo, morfologia, vassoura-preta.

Introduction

The management, conservation and restoration of tropical forests depend on the understanding of regeneration and other ecological processes that, in turn, are linked to studies for the accurate identification of plant species from their juvenile stages (OLIVEIRA, 2001). Studies addressed to the specific knowledge of seed germination morphology of a species not only contribute to its spread, but become essential for better planning and silvicultural treatment of the species, allowing the rational use of forest (MELO; VARELA, 2006).

From a purely morphological point of view, the knowledge of a plant structure is useful to characterize evolutionary and taxonomic problems. The morphology of fruits, seeds and seedlings of various species has been studied in several works like those of Antunes et al. (1998), who characterized the morphology of fruits and seeds of six plant species, including species of the genus *Miconia*; of Silva et al. (2007) who described the internal and external morphology of *Cnidosculus juercifolius* Pax and K. Hoffm (Euphorbiaceae) and of Cosmo et al. (2009) who evaluated the morphology of fruit and seed of *Vitex megapotamica* (Spreng.) Moldenke (Lamiaceae).

Besides, the morphological structures of a mature embryo and its position in the seed differ between groups of plants, and can be safely used for identification of families, genera and even species (TOLEDO; MARCOS FILHO, 1977). Generally, the most superficial characters are used in taxonomy, although internal aspects such as the presence or absence of endosperm, embryo shape and position and number of cotyledons are the most important for classification (LAWRENCE, 1973 apud FERREIRA; CUNHA, 2000).

The Melastomataceae family is considered to be a primordial group for maintaining the diversity of fruit eating species in tropical forests (GALLEETI; STOTZ, 1996), and the genus *Miconia* is noteworthy, being the largest taxon within the family, with near 1000 species (GOLDENBERG, 2004). *M. ligustroides* (DC.) Naudim, popularly known as “vassoura-preta” or “jacatirão-do-cerrado”, is widely distributed in Brazil, occurring from Ceará to Santa Catarina State. It generally occupies savannah areas along with fields, swamps and wet locations (GOLDENBERG, 2004; MARTINS et al., 1996). Its fruits are dispersed by animals, being a major wildlife attraction. It also has a large number of bromeliad associated with its stem (BONNET, QUEIROZ, 2006) and is on the list of recommended species for restoration of riparian forests according to the SMA 21/01 resolution of November 21, 2001.

Yet, little is known about the morphology of seeds, fruits and seedlings of native species in general in Brazil (ANTUNES et al., 1998), especially the genus *Miconia*. In order to expand the current knowledge of the woody savannah flora and assist in the process of identifying species, this study aimed to describe and illustrate the morphological characters of the fruits and diaspores of *M. ligustroides*, as well as to describe its germination process. Moreover, this work seeks to support parallel studies of phenology, germination and its relationship to ecological processes related to dispersal and establishment of this species.

Material and methods

Fruits of *M. ligustroides* (DC.) Naundim were collected from a population of about 30 plants in Serra do Macaia, Lavras, Minas Gerais State, Brazil. The species was identified and the exsiccates deposited in the Herbarium of Campinas State University, under the registration number 23023.

The fruits of *M. ligustroides* have uneven maturation within the same plant. We have collected fruits completely black without any visual injuries. Using 100 fruits, we measured biometric traits such as length and diameter with a caliper, and fresh weight on an analytical balance with resolution of 10^{-4} g. We have also counted the number of diaspores per fruit. The biometric data of fruits were analyzed by frequency distribution and by means of univariate statistics that included measures of position (mean, minimum and maximum values) and measures of dispersion (standard deviation and coefficient of variation, skewness and kurtosis). The remaining fruits were processed under running water, using a sieve to remove the whole pulp and obtain the diaspores. The diaspores were dried on filter paper, on bench, for 12 hours and used for the other experiments

The diaspores of *M. ligustroides* were observed under a stereomicroscope model Meiji Techno RZ, with a camera attached, to characterize the external morphological features and for the biometric determinations. For that, 100 diaspores were observed in the same microscope and photographed with the aid of the software TVR 2.5. Base to apex length and base diameter were measured with the aid of Sigma Scan-Pro 5 software and analyzed according to fruits biometric study. A detailed examination of the external surface of the diaspores was performed using a scanning electron microscope (SEM) model LEO EV040. The diaspores were fixed and prepared according to usual procedures, as described by Alves (2004).

We have also measured the moisture content by the oven method, using three replicates of 0.1 g of diaspores. The oven was set to 105 ± 3°C for 24 hours (BRASIL, 2009), and the results expressed as wet basis percentage. The 1000-seed weight (TSW) was measured as Brasil (2009), and the results expressed in grams. We calculated the variance, standard deviation and coefficient of variation (CV), with eight repetitions of 100 diaspores of the same batch.

Chemical composition analysis was performed using 2 g of diaspores crushed to powder in a refrigerated mill. The powder was defatted and subjected to chemical characterization, according to official methods of Association of Official Analytical Chemists (AOAC, 1990). We used the reductometric method of Somogyi modified by Nelson (1944) to quantify the levels of carbohydrates (total sugars, glucose, sucrose and starch), the continuous Soxhlet extractor to quantify the lipids, and the micro-Kjeldahl method to determine the level of total nitrogen, applying the factor of 6.25 to calculate the crude protein content.

The characterization of germination stages until seedlings formation was performed by sowing four replicates of 50 diaspores on a Petri dish covered with Germtest® paper. Germination occurred at Fanem 347 BOD regulated at 25°C with a photoperiod of 12 hours and 80 ± 3% RH. The methodological procedure used for the morphological characterization was based on Brasil (2009) and Aqüila (2004).

Results and discussion

The fruits of *M. ligustroides* are tiny, bacoid type, bacaceous globous, vinaceous, with a non-juicy pulp (APPROBATO; GODOY, 2006). Fruit maturation is quite uneven within the same plant and also in trees of a given population. At the beginning of development, fruits are smaller and have a bright green color. As they develop, the following colors occur: light green with pink spots, light blue, dark blue, purple and black. The flowering and fructification of other Melastomataceae species such as *Miconia cinnamomifolia* (PEREIRA, MANTOVANI, 2001) and *Tibouchina mutabili* (SIMÃO et al., 2007) are also irregular, with fruits at different stages of development in sampled trees.

According to Simão et al. (2007), fruits are drains that compete with vegetative organs for nutrients, water and other compounds. Likewise, the phytohormones produced by fruits and seeds, for their development, could have an inhibitory effect on growth and development of neighboring units (LEE, 1990). It is suggested that the first fruits and seeds developed have a greater availability of resources that accelerate their development. Fruits that develop later cannot find resources in enough quantities to ensure complete development, occurring abortion of seeds, as well as senescence and early abscission of fruits (SIMÃO et al., 2007).

Fruits of *M. ligustroides* had measures of length, diameter, fresh weight and seed number rather uniform within the analyzed sample. The average fruit length was 0.53 ± 0.40-0.70 cm (Figure 1A). The average diameter was 1.09 ± 0.90-1.30 cm, with 53% of fruits having a 1.13 cm diameter (Figure 1B). The average fresh weight was 0.11 ± 0.06-0.70 g, with 55% of fruits having 0.13 g and 42% 0.06 g (Figure 1C). Moreover, the average number of diaspores was 20.93 ± 9-70, with 51% of fruits having 22.05 diaspores and 39% 15.77 (Figure 1D).

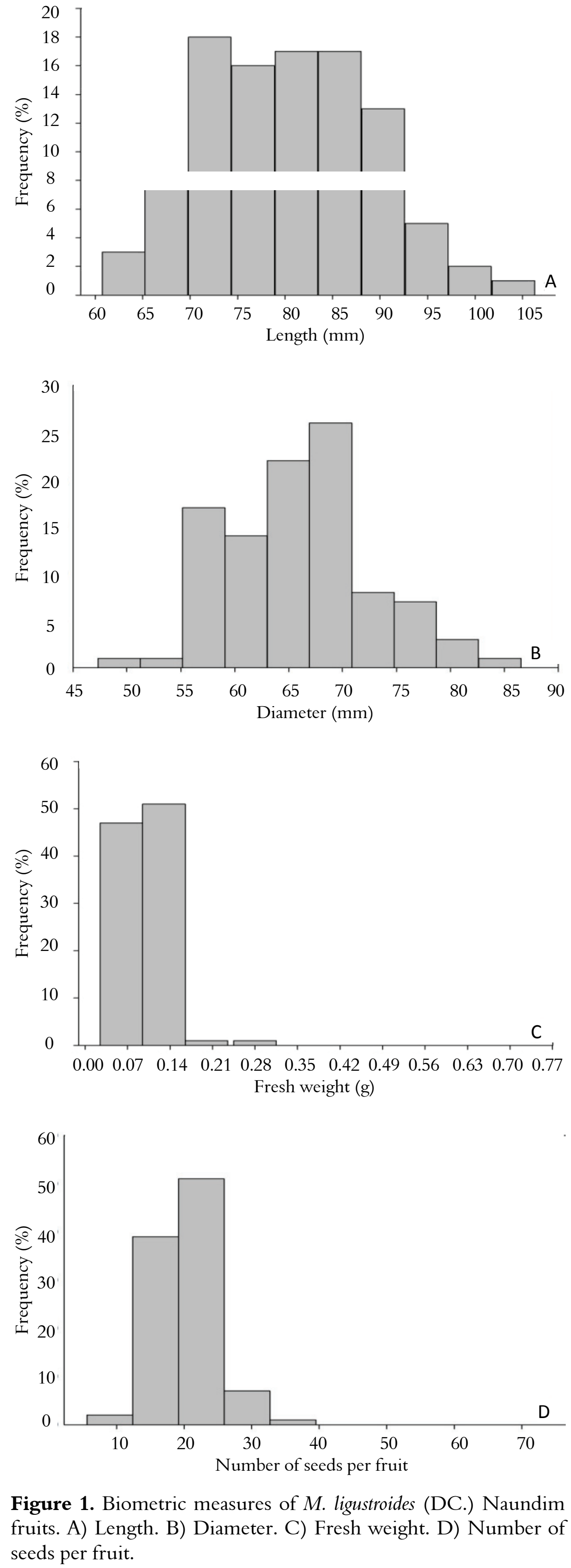

Figure 1. Biometric measures of *M. ligustroides* (DC.) Naundim fruits. A) Length. B) Diameter. C) Fresh weight. D) Number of seeds per fruit.

This low variability in fresh weight and number of seeds is probably due to uniformity of fruits in terms of length and diameter. Another feature of fruits is that they are spherical (Figure 2A), smooth, and when pressed, loose their pulp very easily.

Approbato and Godoy (2006), dealing with the same species, found smaller fruit dimensions (0.26-0.38 cm in length and 0.29-0.43 cm in diameter). This difference is probably due to environmental conditions, *i.e.* different soil and climate, since the fruits collected by these authors were harvested in 'campo sujo', a grassland with scattered trees and shrubs, and the data collected in this work were obtained from grassland region.

The diaspores of *M. ligustroides* are small, devoid of associated structures, and yellow-brown in most of their surface. The surface is very irregular, with two concave faces, one always continuous with the base (Figure 2B) and the other very rough (Figure 2C) with a color ranging from purple to black. In most of the diaspores, the remaining faces are convex. The diaspores are pyramidal, with a concave base and different number of faces (Figures D and E). Electronmicrographs show that the entire surface is striated, and the darkest face is even rougher and wavy (Figure E and F), having thicker and more porous surface than the others. The diaspores are amphitropous, since the hilum and micropyle are close to each other (Figure G and I). Both are on the darker face of the diaspore, near the pyramid apex (Figure E and I). We observed the presence of crystals in the outer envelope of some diaspores (Figure E and H).

The diaspores of *M. ligustroides* have a very small size, measuring 0.8 mm in length and 0.66 mm in base diameter. Approbato and Godoy (2006) found higher values for seed length and diameter in this same species (0.9-1.2 mm and 0.7-1.1 mm, respectively). These values are higher probably due to larger fruit sizes found by the same author. Seed moisture content is 16.25% and 1000-seed weight (TSW) 0.0397 g. Therefore, extrapolating this result (0.0397 g 1000^{-1} diaspores), there are 25.190 diaspores per gram.

Since the diaspores have proteins as the predominant chemical reserve (Table 1), they can be qualified as protein. There is an enormous variation in seed composition, but the substances stored in large quantities are carbohydrates, lipids and proteins. The first two serve as carbon and energy source for seed germination and seedling development, while the proteins function is to store nitrogen and sulfur, which are essential for protein synthesis, nucleic acids and secondary compounds during seedling growth (BUCKERIDGE et al., 2004). High protein content can contribute to reduce storage potential due to the high affinity of this substance with water (MARCOS FILHO, 2005).

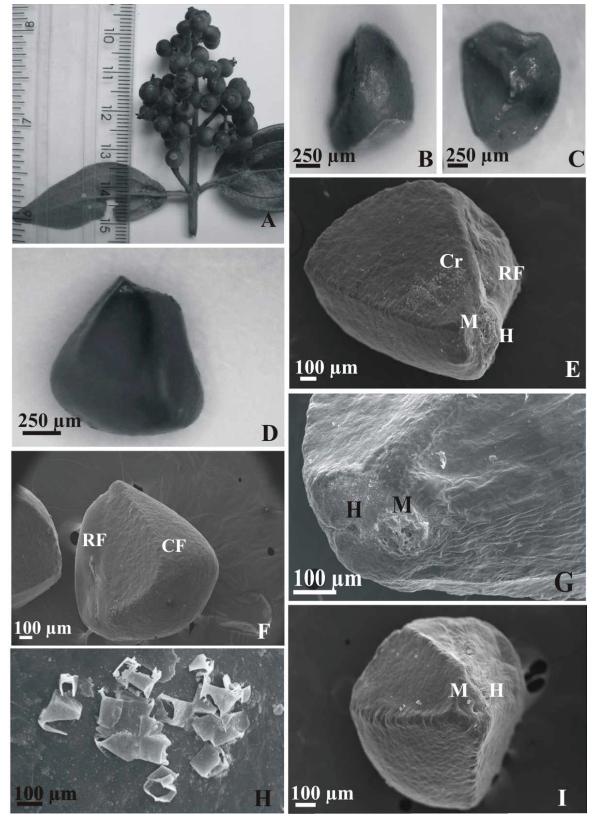

Figure 2. Characterization of sexual propagation structure of *M ligustroides*(DC.) Naundim. A) Mature inflorescence. B) Face continual to basis. C) Rougher side. D) Pyramidal appearance. E) Electronmicrograph showing RF, the region of M and H, and Cr found in outer envelope. F) Electronmicrograph of the general characterization of diaspore showing RF and CF. G) Detail of the M and H region. H) External Cr found in outer envelope. I) Region where M and H can be viewed at diaspore apex . Legend: RF: rough face, M: micropyle, H: hilum, Cr: crystals, CF: continuous face.

Table 1. Mean values on a dry basis and standard deviation of compounds present in *M. ligustroides* diasporas.

Chemical compound		% (g 0.01 g^{-1})	Standard Deviation
	Glucose	1.15	0.04
Carbohydrate	Sucrose	0.34	0.03
	Total sugars	1.51	0.03
	Starch	6.69	0.75
Crude Protein		13.70	0.09
Lipids		0.58	0.02

As the diaspore initiates water uptake, its rough face, which has a characteristic dark color, becomes less and less rough and begins to swell. At 12 Days After Sowing (DAS) this face is completely swollen and micropylar pole is starting to break, probably due to water absorption by diaspore and embryonic development (Figure 3A). Radicle protrusion becomes visible under a magnifying glass at 14 DAS (Figure 3B), almost completely surrounded by the outer envelope (Figure 3C). Hypocotyl hook is formed at 16 DAS, with cotyledons still attached to the diaspore outer envelope (Figure 3D). These come off completely at 17 DAS (Figure 3E), forming a seedling (Figure 3F).

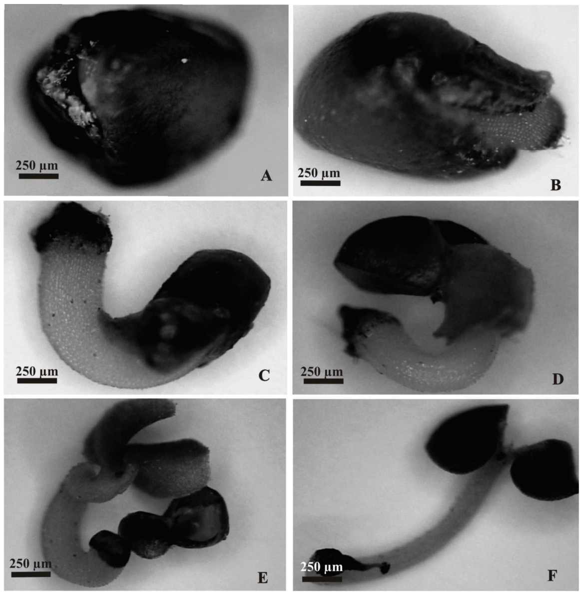

Figure 3. Stereomicrographs characterizing the germination of *M. ligustroides* (DC.) Naundim until seedling formation. A) Opening of micropylar pole at 12 DAS. B) Radicle protrusion at 15 DAS. C) Formation of hypocotyls hook, at 16 DAS, the cotyledons being still attached to the outer envelope. D) At 17 DAS, formation of seedling. E) Seedling detachment of outer envelope. F) Seedling.

The cotyledons are photosynthetic and germination is epigeal and phanerocotylar, since the cotyledons rise of the substrate and free themselves completely from the outer envelope, forming the seedling. In a small percentage of cases, the cotyledons emerge from the diaspores prior to the roots.

We observed polyembryony in a large number of diaspores, causing each diaspore to originate up to three seedlings (Figure 4). In case that two seedlings grew, one was of normal size (about 1 cm) while the other was of minor size (about 0.7 cm).

However, when three seedlings grew, all had the same size, about 0.7 cm. Polyembryony is the presence of more than one embryo in a single seed (JOHRI, 1984). According to Maheshwari (1950), supernumerary embryos of sporophytic nature can originate from nucellus or integuments cells, by zygote or pro-embryo cleavage and differentiation of suspensor cells. Supernumerary embryos of gametophytic nature originate from cells of the embryo sac different from egg cell. Another possibility is the development of more than one embryo sac in the same egg.

According to Maheshwari (1950), apomixis can be defined as the replacement of sexual reproduction by an asexual process. Apomixis is usually accompanied by formation of more than one embryo in the same egg (FAHN, 1990). Sporophytic apomixis appears to be facultative in most cases (RICHARDS, 1997) and can occur concurrently with sexual reproduction. Goldenberg and Shepherd, (1998) confirm that apomictic species have a greater ability to colonize extreme environments, showing wider distribution than non-apomictic species. According to Goldenberg and Shepherd (1998) some species of Melastomataceae have nearly complete male sterility, apparently always associated with apomixis. Relatively low pollen fertility levels, such as those found in *M. ligustroides* and *M stenostachya* are usually accompanied by apomixis (GOLDENBERG; SHEPHERD, 1998) and may be responsible for polyembryony in *M. ligustroides.*

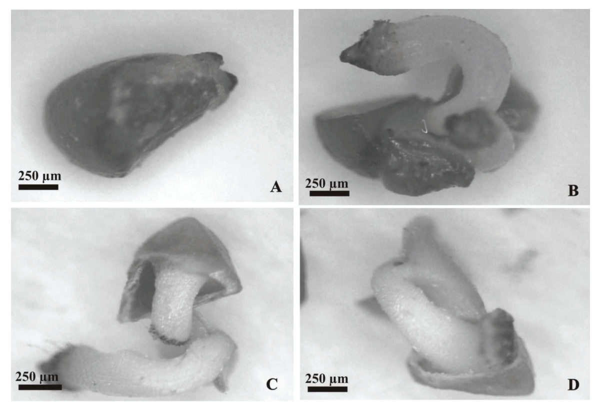

Figure 4. Development of polyembryonic seedling in the germination of *M. ligustroides* (DC.) Naundim. A) Presence of two roots in the same seed. B) Formation of hypocotyl hook in one of the seedlings. C) Formation of a seedling and root development of another within the same seed. D) Development of second seedling.

Conclusion

The fruits of *M. ligustroides* have uneven ripening, but vary little in size, diameter, and number of diaspores per fruit. The diaspores are considered ananfitropos and protein in chemical composition. Germination is epigeal and phanerocotyledonal. Due to polyembryony each diaspore may originate one to three seedlings. The morphology of the fruit, seed and seedling of M. *ligustroides* described here was sufficiently reliable to identify and characterize the germination and early development stages of this species, supporting future studies such as the possible occurrence of seed dormancy.

Acknowledgements

CAPES, CNPq and Fapemig for financial support.

References

ALVES, E. **Curso introdutório de microscopia eletrônica de varredura**. Lavras: UFLA, 2004.

ANTUNES, N. B.; RIBEIRO, J. F.; SALOMÃO, A. N. Caracterização de frutos e sementes de seis espécies vegetais em matas de galeria do distrito federal. **Revista Brasileira de Sementes**, v. 20, n. 1, p. 112-119, 1998.

AOAC-Association of Official Analytical Chemists. **Official methods of analysis**. Washington, D.C.: AOAC, 1990.

APPROBATO, A. U.; GODOY, S. A. P. Levantamento de diásporos em áreas de Cerrado no Município de Luiz Antônio, SP. **Hoehnea**, v. 33, n. 3, p. 385-401, 2006.

AQÜILA, M. E. A. Tipos de diásporos e suas origens. In: FERREIRA, A. G.; BORGHETTI, F (Ed.). **Germinação**: do básico ao aplicado. Porto Alegre: Artmed, 2004. p. 69-92.

BONNET, A.; QUEIROZ, M. H. Estratificação vertical de bromélias epifíticas em diferentes estádios sucessionais da Floresta Ombrófila Densa, Ilha de Santa Catarina, Santa Catarina, Brasil. **Revista Brasileira de Botânica**, v. 29, n. 2, p. 217-228, 2006.

BRASIL. **Regras para análise de semente**. Secretaria de Defesa Agropecuária. Brasília: Mapa/ACS, 2009.

BUCKERIDGE, M. S.; SANTOS, H. P.; TINÉ, M. A. S.; AIDAR, M. P. M. Mobilização de reservas. In: FERREIRA, A. G.; BORGHETTI, F. (Ed.). **Germinação**: do básico ao aplicado. Porto Alegre: Artmed, 2004. p. 163-185.

COSMO, N. L.; GOGOSZ, A. M.; NOGUEIRA, A. C.; BONA, C.; KUNIYOSHI, Y. S. Morfologia do fruto, da semente e morfo-anatomia da plântula de *Vitex megapotamica* (Spreng.) Moldenke (Lamiaceae). **Acta Botanica Brasílica**, v. 23, n. 2, p. 389-397, 2009.

FAHN, A. **Plant anatomy**. New York: Pergamon Press, 1990.

FERREIRA, R. A.; CUNHA, M. C. L. Aspectos morfológicos de sementes, plântulas e desenvolvimento da muda de craibeira (*Tabebuia caraiba* (Mart.) Bur.) - Bignoniaceae e pereiro (*Aspidosperma pyrifolium* Mart.) - Apocynaceae. **Revista Brasileira de Sementes**, v. 22, n. 1, p. 134-143, 2000.

GALLEETI, M.; STOTZ, D. *Miconia hypoleuca* (Melastomataceae) como espécie chave para aves frugívoras no Sudeste do Brasil. **Revista Brasileira de Biologia**, v. 56, n. 2, p. 435-439, 1996.

GOLDENBERG, R. O gênero *Miconia* (Melastomataceae) no Estado do Paraná. **Acta Botanica Brasilica**, v. 18, n. 4, p. 927-947, 2004.

GOLDENBERG, R.; SHEPHERD, G. J. Studies on the reprodutive biology of Melastomataceae in "cerrado" vegetation. **Plant Systematics and Evolution**, v. 211, n. 1-2, p. 13-29, 1998.

JOHRI, B. M. **Embryology of Angiosperms**. Berlin: Springer Verlag, 1984.

LEE, T. D. Patterns of fruit and seed production. In: LOVETT DOUST, J.; LOVETT DOUST, L. (Ed.). **Plant reproductive ecology - Patterns and strategies**. Oxford: Oxford University Press, 1990. p. 179-209.

MAHESHWARI, P. **An Introduction to the Embryology of Angiosperms.** New Dehli: Tata McGraw-Hill, 1950.

MARCOS FILHO, J. **Fisiologia de sementes de plantas cultivadas**. Piracicaba: Fealq, 2005.

MARTINS, A. B.; SEMIR, J.; GOLDENBERG, R.; MARTINS, E. O gênero *Miconia* Ruiz and Pav. no Estado de São Paulo. **Acta Botanica Brasilica**, v. 10, n. 2, p. 267-316, 1996.

MELO, M. F. F.; VARELA, V. P. Aspectos morfológicos de frutos, sementes, germinação e plântulas de duas espécies florestais da amazônia. I. *Dinizia excelsa* Ducke (angelimpedra). II *Cedrelinga catenaeformis* Ducke (cedrorana) - leguminosae: mimosoideae. **Revista Brasileira de Sementes**, v. 28, n. 1, p. 54-62, 2006.

NELSON, N. A. Photometric adaptation of Somogyi method for determination of glucose. **Journal of Biological Chemistry**, v. 153, p. 136-175, 1944.

OLIVEIRA, E. C. Morfologia comparada de plântulas e plantas jovens de leguminosas arbóreas nativas: espécies de Phaseoleae, Sophoreae, Swartizieae e Tephrosieae. **Revista Brasileira de Botânica**, v. 24, n. 1, p. 85-97, 2001.

PEREIRA, T. S.; MANTOVANI, W. Maturação e dispersão de *Miconia cinnamomifolia* (DC.) NAUD. Na reserva biológica de poço das antas, município de Silva Jardim, RJ, Brasil. **Acta Botanica Brasilica**, v. 15, n. 3, p. 335-348, 2001.

RICHARDS, A. J. **Plant breeding system**. London: George Allen and Unwin, 1997.

SIMÃO, E.; NAKAMURA, A. T.; TAKAKI, M. Época de colheita e capacidade germinativa de sementes de *Tibouchina mutabilis* (Vell.) Cogn. (Melastomataceae). **Biota Neotrópica**, v. 7, n. 1, p. 67-73, 2007.

SILVA, L. M. M.; AGUIAR, I. B.; TERTULIANO, S. S. X. Morfologia de frutos, sementes e plântulas de *Cnidosculus juercifolius* Pax & K. Hoffm (Euphorbiaceae). **Revista de Biologia e Ciências da Terra**, v. 7, n. 2, p. 83-92, 2007.

TOLEDO, E. F.; MARCOS FILHO, J. **Manual de sementes**: tecnologia da produção. Piracicaba: Agronômica Ceres, 1977.

Received on February 28, 2011.
Accepted on June 9, 2011.