(DOI): 10.5935/PAeT.V8.N2.02

Brazilian Journal of Applied Technology for Agricultural Science, Guarapuava-PR, v.8, n.2, p.17-24, 2015

Cientific Paper

Light condition on the biometry and germination of dispersal units of *Cordia ecalyculata*

Conceição Aparecida Cossa[1]
Cristina Batista de Lima[2]
Maria Aparecida da Fonseca Sorace[3]
Jamile Kassem Janani[4]
Raquel Rejane Bonato Negrelle[5]

Abstract

The aim of this study was to explore the biometric characteristics of the dispersal units (fruits, pyrenes and seeds), germination and the effect of treatments for overcoming the dormancy of *Cordia ecalyculata* seeds, produced under different natural light conditions. We used ripe fruits with red coloration, collected from main trees, located in different areas regarding natural light. We determined the biometry of fruits and pyrenes. The pyrenes were exposed to different treatments for the scarifying of the seed coat. The seeds were analyzed as for imbibition, viability and germination, after being subjected to treatments for dormancy overcoming. The natural light conditions influenced the biometric parameters of the fruits, pyrenes and seeds, whose dimension varied according to the availability of solar light of the place where the mother plant was located. The germination percentage and the incidence of seeds without embryos were inversely proportional to the shading. The treatments employed did not affect satisfactorily on the overcoming of dormancy of *C. ecalyculata* seeds.

Key words: porangaba, tetrazolium, scarifying, seed viability.

Condición Luminica en la biometría y germinación de unidades de dispersión de *Cordia ecalyculata*

Resumen

El objetivo de este estudio fue investigar las características biométricas de las unidades de dispersión (frutas, pirenos y semillas), germinación y el efecto de los tratamientos para superar la latencia de las semillas de *Ecalyculata cordia* producido en diferentes situaciones de iluminación natural. Se utilizaron los frutos maduros con color rojo, recogidos de árboles matrices ubicados en diferentes zonas con relación a la iluminación natural. Se determinó la biometría de frutas y pirenos. Los pirenos fueron expuestos a diferentes tratamientos de escarificación del tegumento. Se analizaron las semillas cuanto a la imbibición, la viabilidad y la germinación, después de someterse a tratamientos para superar la latencia. La condición de iluminación natural influyó en los parámetros biométricos de las frutas, pirenos y semillas, cuyo tamaño varió de acuerdo a la disponibilidad de luz solar donde se ubicaba la planta madre. El porcentaje de germinación y la incidencia de semillas sin embrión fueron inversamente proporcionales a la sombra. Los tratamientos no tuvieron efecto satisfactorio para superar la latencia de semillas de *C. ecalyculata.*

Palabras clave: Porangaba, tetrazolio, escarificación, viabilidad de semilla.

Received at: 17/04/14 Accepted for publication at: 22/07/15

1 Dra Profa. Centro de Ciências Biológicas. Universidade Estadual do Norte do Paraná-Campus Luiz Meneghel. Bandeirantes-PR. E-mail:cossa@uenp.edu.br

2 Dra. Profa. Centro de Ciências Biológicas. Universidade Estadual do Norte do Paraná-Campus Luiz Meneghel. E-mail: crislima@uenp.edu.br

3 Dra. Profa. Centro de Ciências Biológicas. Universidade Estadual do Norte do Paraná-Campus Luiz Meneghel. E-mail:masorace@uenp.edu.br

4 Dra. Profa Universidade Estadual "Julio de Mesquita Filho" (UNESP-FCA), Botucatu-SP. E-mail:jami_janani@hotmail.com

5 Dra. Profa. Agronomia/Biologia. Universidade Federal do Paraná - UFPR. Curitiba-PR. E-mail; negrelle@ufpr.br

Condição lumínica na biometria e germinação das unidades de dispersão de *Cordia ecalyculata*

Resumo

O objetivo do presente trabalho foi estudar características biométricas das unidades de dispersão (frutos, pirênios e sementes), germinação e o efeito de tratamentos para superação da dormência das sementes de *Cordia ecalyculata*, produzidos em diferentes situações de iluminação natural. Foram utilizados frutos maduros com coloração vermelha, coletados de árvores matrizes, localizadas em áreas diferenciadas no que diz respeito à iluminação natural. Foi determinada a biometria de frutos e pirênios. Os pirênios foram expostos a diferentes tratamentos para escarificação do tegumento. As sementes foram analisadas quanto à embebição, viabilidade e germinação, após serem submetidas a tratamentos para superação da dormência. A condição de iluminação natural influenciou os parâmetros biométricos dos frutos, pirênios e sementes, cujas dimensões variaram conforme a disponibilidade de luz solar do local onde se encontrava a planta mãe. O percentual de germinação e a incidência de sementes sem embrião foram inversamente proporcionais ao sombreamento. Os tratamentos empregados não surtiram efeitos satisfatórios na superação da dormência das sementes de *C. ecalyculata.*

Palavras chave: porangaba, tetrazólio, escarificação, vialibidade de sementes.

Introduction

Cordia ecalyculata Vell. (Boraginaceae) is a heliophile tree species widely distributed in Brazil, known among the population as café-de-bugre, chá-de-bugre, porangaba or louro-mole. The flowering occurs between September and February and the fruits ripen from April to August, in the State of Paraná. It has fleshy fruits with simple drupe, indehiscent, globoid shape, slightly flattened at the top and the bottom; when ripe, it turns red, with mucilaginous interior and glabrous, shiny, smooth and uniform surface (CARVALHO, 2008).

The endocarp is sclerotic with approximately 1.5 mm of thickness and constitutes the pyrene (CARVALHO, 2008), which is ligneous, bilocular, with one seed in each locule (BARROSO et al.,1999). The globoid seeds are firmly adhered to the endocarp. They are white, smooth, shiny, glabrous, reticulated with small longitudinal striae in the shape of a fingerprint (CARVALHO, 2008). The embryo is invaginated with double foliaceous cotyledons (BARROSO et al.,1999). The germination is epigeal and the seedling is phanerocotylar (PAOLI, 2010).

Substances such as the gamma-linolenic acid and the allantoin are present in leaves and new branches of *C. ecalyculata*, being scientifically proved to aid in the treatment and prevention of cardiovascular diseases and diabetes, according to the studies of LAIDLAW and HOLUB (2003); ARREBOLA et al., (2004). The allantoin has the capacity of healing wounds (SAITO and OLIVEIRA, 1986), cytotoxic activity for cancerous cells (ARISAWA, 1994) and antiviral in cases herpes simplex (HAYASHI et al., 1990).

The extractive activity, to which the trees of this species are subjected in order to supply the pharmaceutical and wood industries, has been provoking a drastic reduction of the natural its populations (DUARTE, 2006). The establishment of a rational and economical cultivation system for *C. ecalyculata* plants is indispensable for attending the increasing demands of market, reducing the extractive pressure and its negative impacts. For such, it is necessary to know some ecological and agronomical concepts about stages of the biological cycle, techniques of production and spread of seedlings, allowing its utilization in forestry programs (FERREIRA, 2000). Thereby, the aim of this study was to explore the biometric characteristics of dispersal units (fruits, pyrenes and seeds), germination and the effect of treatments for the overcoming of dormancy in *Cordia ecalyculata* seeds, produced under different natural light conditions.

Material and methods

We worked with ripe fruits (Figure 1ª), with red coloration, collected from *C. ecalyculata* plants that were located on the LUIZ MENEGUEL CAMPUS, the North Parana State University (UENP-CLM), located in Bandeirantes-PR, approximately 420 meters above sea level, in a region characterized by a subtropical climate Cfb.

Figure 1. Diaspores: ripe fruits **(A)**, pyrenes **(B)**, seeds **(C)** and seedling **(D)** of *Cordia ecalyculata.*

The botanic identification of main trees was conducted by the technicians of the Botanic Museum of Curitiba (PR), based on the material collected at the Campus, herbalized and incorporated to the collection of the Center of Environmental Education and Research of the UENP-CLM, under the register number 218.

The fruit collecting occurred in main trees located in the following natural light conditions: a) full sunlight; b) partial lateral shading; c) partial superior shading; d) totally shaded. The fruits collected (Figure 1-A) were taken to the laboratory of seed analysis of the institution for the checking of:

Fruit biometry – we conducted the measuring of length (mm), diameter (mm) and fresh mass (g). On the determinations of length and diameter, we used a digital caliper (resolution: 001"/0,1 mm), and for the obtaining of fresh mass we used an analytical scale of precision (0.0001). We calculated the averages and determined the variation amplitude for each treatment, within each caliper.

Pyrene biometry – the fruits were pulped by manual friction in sieve under current water (BINOTTO, 2004) for the removal of the mucilage and obtaining of the pyrenes (Figure 1-B), which were placed over newspaper, inside plastic trays, and maintained during 15 days, under room condition of the laboratory, with averages of 24ºC temperature and 67 % relative humidity. After this period, they were subjected to the measuring of length (mm), diameter (mm) and determination of the fresh mass (g) with the same equipments described on the fruit biometry.

Imbibition curve (BRASIL, 2009) – established through the method of seed submerged in water (MSSA). The seeds were conditioned in plastic containers, containing 200 mL of distilled water, maintained under the temperature of 25 ± 3 ºC. The weighing was conducted in an analytical scale of precision (0.0001), at the first 12 hours, spaced in one hour and, after, in intervals of 12 hours, until the total of 156 hours.

Viability – the scarifying of the pyrenes for obtaining the seeds (Figure 1C) was conducted through five treatments: 1 – sulfuric acid for 15 minutes; 2 – sulfuric acid for 30 minutes; sulfuric acid for 60 minutes; 4 – sulfuric acid for 90 minutes; 5 – abrasion with pruning shears. After, the seeds were immersed in distilled water for 15 minutes (in order to soften and facilitate the removal, with tweezers, of the thin membrane that involves the seed), and placed in tetrazolium solution at 1%, 30ºC, during 24 hours. After this period, the seeds were removed from the solution, washed in distilled water, dried in paper filters and analyzed internal and externally, regarding the viability, through a stereomicroscope

Category	Class	Seed description
Viable	1	Light pink color, uniform and with all tissues with normal and firm aspect.
	2	Dark pink color, uniform and with cotyledons presenting symptoms of damages inferior to 50%.
Impracticable	3	Intense red color, uniform and with cotyledons presenting symptoms of damages superior to 50%, indicating a sharp deterioration process.
	4	Without embryo.
	5	Totally white, indicating death of vital tissues.

with magnification of 20 to 100 times the original size. We opted for not conducting the cutting in the seeds, because the foliaceous embryos could easily fragment in contact with the slide, in the moment of the cutting (MENDONÇA et al., 2001).

The seeds were divided into two categories and five classes of viability, defined based on the uniformity of color and vital areas (axles of embryo and cotyledon), adapting to the proposed by DELOUCHE et al. (1976).

Germination – conducted with seeds obtained after eight treatments of scarifying of the pyrenes: 1 – sulfuric acid for 15 minutes; 2 – sulfuric acid for 30 minutes; 3 – sulfuric acid for 60 minutes; 4 – sulfuric acid for 90 minutes; 5 – sulfuric acid for 15 minutes + solution of potassium nitrate (KNO_3) at 0.2%; 6 – pruning shears; 7 – pyrenes worn with pruning shears + experimental solution composed by 0.1 g L^{-1} of IBA, 0.05 g L^{-1} of gibberellic acid, 0.2 g L^{-1} of kinetin and 3 g L^{-1} of thiamine; 8 – pyrenes places to germinate without previous preparation (control).

The seeds and pyrenes (control) were placed to germinate in rolls of paper filters, previously moistened in distilled water, at the proportion of 2.5 times the weight of the dry paper (BRASIL, 2009). In the treatments for verifying the effect of substances that promote germination (5, 6 and 7), the paper filter was firstly moistened in these solutions and, subsequently, in distilled water. The evaluations were conducted daily, during ninety days, registering the numbers of germinated seeds, from the emission of cotyledons (Figure 1D).

Table 1. Maximum and minimum values and averages of diameter, length and fresh mass of fruits and pyrenes collected from trees of C. ecalyculata, found under different natural light conditions, at the Campus of UENP-CLM (Bandeirantes-PR).

Description	Maximum	Average	Minimum	CV (%)
Fruits				
Diameter (mm)				
Full sunlight	12.54	10.45a*	9.11	10.9
Partial lateral shading	10.99	8.45b	6.88	
Partial superior shading	10.87	7.91b	6.38	
Totally shaded	9.87	6.96c	5.47	
Length (mm)				
Full sunlight	14.42	11.43a	7.84	9.6
Partial lateral shading	11.95	9.25b	7.14	
Partial superior shading	11.13	8.96b	6.75	
Totally shaded	10.80	7.82c	6.36	
Fresh mass (g)				
Full sunlight	0.85	0.53a	0.34	26.0
Partial lateral shading	0.57	0.27b	0.14	
Partial superior shading	0.57	0.24b	0.10	
Totally shaded	0.28	0.10c	0.07	
Pyrenes				
Diameter (mm)				
Full sunlight	11.81	9.08a	8.61	7.2
Partial lateral shading	9.81	7.80b	5.67	
Partial superior shading	9.18	7.48b	5.20	
Totally shaded	7.94	5.99c	4.98	
Length (mm)				
Full sunlight	9.32	8.36a	6.97	10.9
Partial lateral shading	8.81	6.83b	5.25	
Partial superior shading	7.72	5.95c	4.71	
Totally shaded	6.79	5.66c	4.63	
Fresh mass (g)				
Full sunlight	0.34	0.24a	0.14	36.4
Partial lateral shading	0.26	0.11b	0.05	
Partial superior shading	0.25	0.13b	0.05	
Totally shaded	0.19	0.09c	0.01	

**Averages followed by the same lower case letter, in the column, within each characteristic, do not differ statistically by the Tukey test at 5% probability; CV = variance coefficient.*

The statistical design was entirely randomized with four replicates of fifty seeds for each evaluation and treatment. The original data obtained in these tests were subjected to variance analysis and the averages compared by the Tukey test at 5% probability.

Results and discussion

The fruits presented significant biometric differences in relation to the natural light condition under which they were placed (Table 1). The biggest diameters (mm), lengths (mm) and fresh masses (g) were found in the fruits collected from plants that were located in full sunlight, decreasing the dimensions with the increase of shade. The same tendency was observed in the pyrenes, whose fresh mass varied between 0.34 to 0.01 grams (Table 1 and Figure 2). According to LORENZI and MATOS (2002), one kilo of pyrenes of C. ecalyculata contains 5,400 units, therefore, each of it has, approximately, 0.19 grams, this being the only reference available for pyrene weight of café-de-bugre. SMITH (1970) attributed to fruits of plants of C. ecalyculata 12 mm diameter and PAOLI (2010) 15 mm for fruits collected in Rio Claro/SP. Therefore, the results of this study indicate the variation in the biometry pattern presented by the diaspores of this species, probably in function of an interaction between genotype and environment.

The imbibition behavior of seeds of *C. ecalyculata* showed itself similar in the different areas of collecting, progressing according to the three-phase pattern. Thereby, we opted for presenting only one representative graphic (Figure 3) for the studied light conditions. Phase I, characterized by the fast transference of water to the seed, due to the sharp difference between the water potentials, occurred linearly throughout the first 24 hours. Phase II was evidenced between 48 and 96 hours and, the

Figure 2. Pyrenes of C. *ecalyculata*, produced under different light conditions: **(A)** full sunlight, **(B)** partial lateral shading, **(C)** partial superior shading and **(D)** totally shaded.

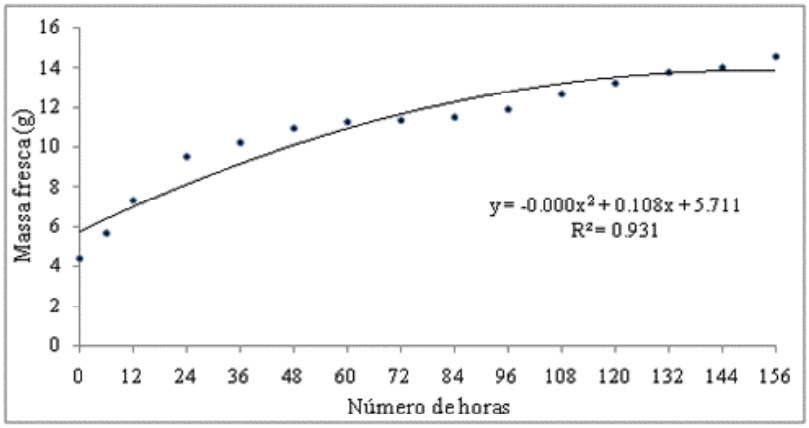

Figure edited in portuguese by the author

(vertical: Fresh mass (g) | horizontal: Number of hours)

Figure 3. Fresh mass **(g)** determined in seeds of C. *ecalyculata*, during the period of imbibition.

beginning of Phase III, with the growth of the embryo with the gain of mass, was observed between 84 and 96 hours.

For most species, the imbibition curve is established in two hours (CARVALHO and NAKAGAWA, 2012), however, species whose dispersal units present stony involucres, similar to the *C. ecalyculata*, can demonstrate higher time for imbibition, because the nature and composition of the integument, as well as the temperature and dormancy (CASTRO et al., 2004), influence on the speed of absorption and quantity of soaked water. The external integument of seeds of *C. trichotoma* hampered the absorption of the tetrazolium solution, causing MENDONÇA et al. (2001) to suggest the removal of the referred integument, after the preconditioning of the seeds.

The use of sulfuric acid caused irreversible damages to the seeds, regardless of the exposure time and the light condition. Such seeds presented dark red color and signs of damages. Thereby, the results presented in Table 2 are referent to the seeds whose integument was worn with pruning shears.

The tetrazolium test allowed us to distinguish viability levels for seeds of *C. ecalyculata*. The average percentages were distributed in the categories and classes of pre established viability (Figure 4), with predominance of unviable seeds under the different conditions of light (Table 2). However, the highest expression of infeasibility was registered in seeds from the location that was totally shaded (93.5). The incidence of seeds with increased deterioration process (class 3) and absence of embryo (class 4) were the main causes of the low viability.

The low viability of native forest seeds can be attributed to biotic and/or abiotic factors, from the ones who contribute for the adequate nutrition of the plant, production and pollination of its flowers, to the ones that interfere on the fertilization of the eggs (AGUIAR et al., 1993). MACHADO and CÍCERO (2003), emphasize that the lack of uniformity in the flowering, a common event among forest species, can be associated to the production of empty seeds and/or with malformed embryos.

In the treatments where we used sulfuric acid, as well as in the employment of the pyrenes without previous preparation, no seed germinated. The germination was evidenced only in the procedures with wear of the integument with pruning shear, allied or not to the use of experimental solution (Table 2), therefore, it is probable that the stony involucres of these seeds is one of the inhibiting causes of the germination, related to the water impermeability (hardness). Accordingly AMORIM (1996) used a vise to extract the seeds from the pyrenes, due to the difficulty of scarifying by conventional chemical and mechanical methods. The methodology of mechanical wear with pruning shear was of difficult execution, due to the size of the seeds, being necessary to employ with caution, in order to avoid cuts and/or wounds to whomever is performing the procedure.

The average germination percentage did not

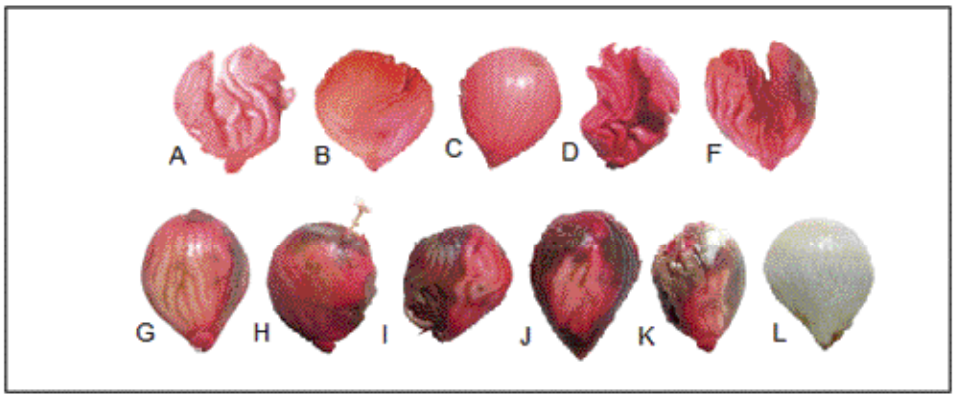

Figure 4. Classification of seeds of *Cordia ecalyculata* according to the tetrazolium test: viable class 1 (A, B and C), class 2 (D and E).Infeasible class 3 (F to J), class 5 (K).

surpass 19%, having a decrease of germinated seeds with the increase of shading. The low germination percentage of *C. ecalyculata* seeds seems to be inherent to the species, coinciding with the reports presented by LORENZI and MATOS (2002), being considered low and irregular, inferior to 50% to AMORIM (1996) and in a maximum of 25.5% in the study of SUGANUMA et al. (2008). The causes of low viability and germination, as well as efficient techniques for removing the involucres that protect the seeds, are worthy of being investigated, aiming to improve the capacity of production of seedlings of this species.

Conclusions

The natural light condition influenced the biometric parameters of the fruits, pyrenes and seeds, whose dimensions varied according to the availability of solar light of the place where the mother plant was located. The germination percentage and the incidence of seeds without embryo were inversely proportional to the shading. The treatments employed did not affect satisfactorily on overcoming the dormancy of *C. ecalyculata* seeds.

References

AGUIAR, I. B.; PIÑA-RODRIGUES, F. C. M.; FIGLIOLIA, M. B. Sementes florestais tropicais. Brasília: ABRATES, 1993. 350p.

AMORIM, I. L. Morfologia de frutos, sementes, germinação, plântulas e mudas de espécies florestais da região de Lavras - MG. 1996. 127f. Dissertação (Mestrado em Engenharia Florestal) - Departamento de Silvicultura, Universidade Federal de Lavras, Lavras.

ARISAWA, M. Cell growth inhibition of KB cells by plant extracts. Natural Medicines I, Sendai, v. 48, n. 4, p. 338-47, 1994.

ARREBOLA, M. R. B.; PETERLIN, M. F.; BASTOS, D. H. M.; RODRIGUES, R. F. O.; CARVALHO, P. O. Estudo dos componentes lipídicos das sementes de três espécies do gênero Cordia L. (Boraginaceae). Revista Brasileira de Farmacognosia, v. 14, n. 1, p. 57-65, 2004.

BARROSO, G. M.; MORIM, M. P.; PEIXOTO, A. L.; ICHASO, C. L. F. Frutos e sementes: morfologia aplicada à sistemática de dicotiledôneas. Viçosa: UFV, 1999. 443p.

BINOTTO, A. F. Beneficiamento de sementes florestais. In: HOPPE, J.M. (Org.). Produção de sementes e mudas florestais. Santa Maria: [s.n.], 2004. p.71-80 (Caderno Didático n. 1, 2. ed.).

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasília: Mapa/ACS, 2009. 399p.

CARVALHO, P. E. R. Espécies Arbóreas Brasileiras. v. 3. Brasília: Embrapa, 2008. 593p.

CARVALHO, N.M.; NAKAGAWA, J. Sementes: ciência, tecnologia e produção. 5.ed. Jaboticabal: FUNEP, 2012. 590p.

CASTRO, R. D.; BRADFORD, K. J.; HILHORST, H. W. M. Embebição e reativação do metabolismo. In: FERREIRA, A. G.; BORGHETTI, F. (Orgs.). Germinação: do básico ao aplicado. Porto Alegre: Artmed, 2004. p.149-162.

DELOUCHE, J. C.; STILL, T. W.; RASPET, M.; LIENHARD, M. O teste de tetrazólio para viabilidade da semente. Brasília: AGIPLAN, 1976. 103p.

DUARTE, M. C. T. Atividade antimicrobiana de plantas medicinais e aromáticas utilizadas no Brasil. Revista Multiciência, n.7, 2006. Disponível em: <http://www.multiciencia.unicamp.br/artigos_07/a_05_7.pdf>. Access in: august, 2012.

FERREIRA, C. A. C. Recuperação de áreas degradadas. Informe Agropecuário, v. 21, n. 202, p.127-30, 2000.

HAYASHI, K.; HAYASHI, T.; MORITA, N.; NIWAYAMA, S. Antiviral activity of an extract of *Cordia salicifolia*

on herpes simplex virus type 1. Planta Medica, v. 56, n. 5, p. 439-43, 1990.

LAIDLAW, M.; HOLUB, B.J. Effects of supplementation with fish oil–derived n-3 fatty acids and γ-linolenic acid on circulating plasma lipids and fatty acid profiles in women. American Journal of Clinical Nutrition, v. 77, n. 1, p. 37-42, 2003. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/12499320>. Access in: august, 2012.

LORENZI, H.; MATOS, A. F. J. Plantas medicinais: nativas e exóticas. Nova Odessa: Instituto Plantarum, 2002. 512p.

MACHADO, C. F.; CICERO, S. M. Aroeira–branca [Lithraea molleoides (Vell.) Engel.–Anacardiaceae] seed quality evaluation by the x- Ray test. Scientia Agricola, v. 60, n. 2, p. 393-397, 2003. DOI: 10.1590/S0103-90162003000200026.

MENDONÇA, E. A. F.; RAMOS, N. P.; PAULA, R. C. Viabilidade de sementes de Cordia trichotoma (Vellozo) Arrabida ex Steudel (louro-pardo) pelo teste de tetrazólio. Revista Brasileira de Sementes, v. 23, n. 2, p. 64-71, 2001. Disponível em: <http://www.abrates.org.br/revista/artigos/2001/v23n2/artigo09.pdf>. Access in: august, 2012.

PAOLI, A. A. S. Caracterização morfológica do diásporo e plântulas de Cordia ecalyculata Vell. e de Cordia abyssinica R. BR. (Boraginaceae). Naturalia, v. 33, p. 20-33, 2010. Disponível em: <http://132.248.9.34/hevila/NaturaliaRioclaro/2010/vol33/3.pdf>. Access in: august, 2012.

SAITO, M. L.; OLIVEIRA, F. Morfodiagnose e identificacao cromatográfica em camada delgada de chá de bugre - Cordia ecalyculata Vell. Revista Brasileira de Farmacognosia, v. 67, n. 3, p. 1-16, 1986.

SMITH, L. B. Boragináceas. In: Reitz, P. R. (Ed.). Flora Ilustrada Catarinense. Itajaí: Herbário Barbosa Rodrigues, 1970. 85p.

SUGANUMA, M. S.; BARBOSA, C. E. A.; CAVALHEIRO, A. L.; TOREZAN, J. M. D. Enriquecimento artificial da diversidade de espécies em reflorestamentos: análise preliminar de dois métodos, transferência de serapilheira e semeadura direta. Acta Scientiarum Biological Sciences, v. 30, n. 2, p. 151-158, 2008. DOI: 10.4025/actascibiolsci.v30i2.3629.