

Seed Germination of *Chresta sphaerocephala* DC. and *Lessingianthus bardanoides* (Less.) H. Rob. (Asteraceae) from *Cerrado*

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ABSTRACT

This work aimed to study the effects of different temperature conditions on the germination of *L. bardanoides* and *C. sphaerocephala* seeds, compare the germination rates of these two species and estimate the occurrence of embryoless seeds. The results indicated that the best temperatures for the germination of *L. bardanoides* seeds were 20, 25 and 20-30°C and for *C. sphaerocephala*, 20-35°C and 40.32 $\mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance; *L. bardanoides* had a higher germination rate since the number of seeds with embryos higher compared with *C. sphaerocephala*.

Key words: Gemmiferous root; savanna, vegetative propagation; xylopodium

INTRODUCTION

The *Cerrado* biome comprises a 1.5 million km² area within Brazil, with a diversified vegetation ranging from *campos limpos* (grassland) open country forms to *cerradão* (dry forests), relatively dense forest formations. Between the two extremes, intermediate forms are found, in which *campos sujos* (shrubland), *campos cerrados* (brushland) or *Cerrado sensu stricto* (Coutinho, 2002) may be observed. In the São Paulo state territory, *Cerrado* occurs especially in the mid-northern region (Toledo Filho et al., 1984), and small *cerradão* spots may occur in the mid-western region. The eastern state comprises more open physiognomies such as *campos cerrados* and *campos sujos*, especially preserved in the São Carlos region (Durigan et al., 2004). The destruction in the *Cerrado* today is intense and,

according to Almeida et al. (2005), the flora of this biome is not fully known yet, in that in each survey new species are added to this list of biome's angiosperm. The family Asteraceae is an important part of its flora in the herbaceous and subshrubby layers. This family includes the plants with diverse habits such as weeds, subshrubs, creepers or exceptionally trees, with most genera constituted by small-sized plants, several of them may be used as food, in pharmaceutical products, in beverage preparation, as rubber producers, or insecticides (Joly, 1998).

In the state of São Paulo, Almeida et al. (2005) performed a survey on Asteraceae species found in eight localities of *Cerrado sensu stricto* and emphasized the need for further studies on the herbaceous-subshrubby flora in these fragments, in which many were sole species and exclusive, considering the conservation of these areas as

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fundamental. Among the different Asteraceae tribes listed in this study, Vernoniae was the one with the highest number of species; Appezzato-da-Glória et al. (2008) reported the presence of different thickened underground organs in two frequent species in the state of São Paulo *Cerrado* inside this tribe. In *Lessingianthus bardanoides* (Less.) H. Rob., the thickened underground organ is represented by a xylopodium and in *Chresta sphaerocephala* DC., by a diffused underground system of radicular structure that produces aerial stems (Appezzato-da-Glória et al., 2008). This underground system is an important vegetative reproduction device in the *Cerrado* flora. As to the xylopodium, according to Appezzato-da-Glória et al. (2008), it is characterized by woody consistency, self-grafting among stem branches, and budding ability. The formation may occur due to environmental influences, or may be genetically determined, and the anatomical structure may have a root, mixed or stem nature. Furthermore, the xylopodium cannot propagate vegetatively and the only means of the reproduction for *L. bardanoides* is through sexual propagation, whose efficiency not only assures the maintenance of the species but also places it as one of the most recurrent in the São Paulo state *Cerrado*.

The reproductive efficiency of a species can be estimated through seed germination. There are many studies involving *Cerrado* Asteraceae germination (Achutti, 1978; Ruggiero and Zaidan, 1997; Sasaki et al., 1999; Gomes and Fernandes, 2002; Souza et al., 2003; Rosal, 2004; Velten and Garcia, 2005; Bertasso-Borges and Coleman, 2005). Nevertheless, only Sasaki et al. (1999) and Velten and Garcia (2005) related the germination ratio to vegetative production.

A common phenomenon in the family Asteraceae and directly related to germination rate is the significant amount of seed production, although many of them are embryoless (Sasaki et al., 1999), which has been confirmed by others (Achutti, 1978; Sajo and Menezes, 1986; Maluf and Wizenier, 1998; Sasaki et al., 1999; Bertasso-Borges and Coleman, 2005; Velten and Garcia, 2005; Tonetti et al., 2006); this although common, has not been fully elucidated yet, and since it affects the seed germination rates, it must always be considered in germination trials of the family Asteraceae.

In order to enhance the knowledge on the herbaceous-subshrubby flora of the family Asteraceae in the São Paulo state *Cerrado*, thus

contributing for its preservation, the aims of this work were: (i) to study the effects of different temperature conditions on the germination of *L. bardanoides* and *C. sphaerocephala* seeds; (ii) to compare the germination rates of both the species, and (iii) to estimate the occurrence of embryoless seeds for each species.

MATERIAL AND METHODS

Seed collection and storage

According to Marzinek et al. (2008) in Asteraceae, the fruit type is cypsela. Here, this term was considered as synonymous of seed. The seeds of *Lessingianthus bardanoides* (Less.) H. Rob. (Fig. 1 a, b) and *Chresta sphaerocephala* DC. (Fig. 1 c, d) were collected from the natural *Cerrado* area populations in the state of São Paulo, Brazil, at *Estação Ecológica de Itirapina* (22°13'S and 47°54'W). The time of collection varied between the two species (June for *L. bardanoides* and November for *C. sphaerocephala*).

The criterium of collecting was capitula in which the seeds easily removed by hand were chosen and soon after the collection, they were left to dry at room temperature (18-22°C) for three days and then transferred to a refrigerated chamber (20°C) with 45-50% relative humidity. The storage period varied as the experiments were set up at different times. For the *L. bardanoides* experiment, the seeds were stored for 17 days and for the *C. sphaerocephala* the seed storage lasted 62 days.

Experimental set-up, seed germination and seedling development monitoring

To set up the experiments, the capitula were removed from the refrigerated chamber, the seeds were removed by hand, homogenized in a tray and randomly selected when placed for germination. Withered and preyed seeds were rejected and it was not possible to differentiate those with embryo-containing seeds from those without through aspect or by pressure.

For the *L. bardanoides* experiment, 25 seeds were placed onto four sheets of filter paper soaked in 23.5mL distilled water in previously sterilized (with absolute ethanol) plastic boxes for germination, with five replications per treatment with 125 seeds in each temperature. The boxes were then distributed in six germination chambers (Marconi, model MA 402) at 20, 25, and 30°C with eight-hour photoperiod and 15–35, 20–30,

20–35°C with eight-hour light in the top temperature. In all the germination chambers, the light was supplied by fluorescent light bulbs with an irradiance of $40.32 \mu\text{mol m}^{-2}\text{s}^{-1}$. The *C. sphaerocephala* germination experiment was set up following the model of the experiment of *L. bardanoides*, although with 10 replications per treatment, with 250 seeds for each temperature.

The germination process was monitored weekly, from three to four days and this periodicity was defined by the low number of germinated seeds daily. When necessary, the filter paper was moistened to ensure the seeds have enough water supply throughout the test. This process was followed during 80 days for *L. bardanoides* and 115 days for *C. sphaerocephala*. In each observation, the emission of the primary root (Fig. 2 a) was first recorded and then the rise of the other seedling structures. For the germination, the seedlings having two cotyledons, hypocotyl and primary root were considered as normal ones.

With the results of the germination tests, the germination speed was calculated by the germination speed index (GSI), according to the formula proposed by Maguire (1962) and the mean germination time (t), according to Labouriau (1983).

Analysis of nongerminated seeds

In both the experiments, following the last evaluation, all the seeds were kept under the same conditions for an additional 30-day period to verify whether there would be germinating seeds or not. At the end of this period, the experiments were stopped and all the seeds, which did not germinate, were sectioned longitudinally to verify the presence or absence of the embryo. The seeds in which a normal-appearing embryo was observed were submitted to tetrazolium test (Brasil, 1992; Velten and Garcia, 2005) to determine their viability. In this test, immediately following the longitudinal section, the seeds were placed into a small plastic cup containing 1% tetrazolium. This container was covered with aluminum sheet to prevent the evaporation of the solution and kept at 30°C for 24 hours. The seeds with red-colored embryo were considered viable.

Statistical analysis

For the statistical analysis a completely randomized design was used and was performed by using the Sistema de Análise Estatística para Microcomputadores (SANEST) (Zonta & Machado, 1984). For the variance analysis, the data of germination percentage were transformed in $\text{arc sen } \sqrt{x/100}$ and the Tukey test was followed at 5 and 1% probability for multiple comparison of means to detect the differences among the temperature treatments for each species.

RESULTS

Germination tests, analysis of embryo presence and tetrazolium test

The results of the germination tests are shown in Table 1. After performing the longitudinal section of all the seeds that failed to germinate, except for the ones killed by the fungus attack, the number of seeds without embryo, malformed embryo and nongerminated viable seeds for each temperature, are shown in Table 2. Out of 750 *L. bardanoides* seeds placed for germination, 124 seeds germinated, and out of 1,500 *C. sphaerocephala* seeds, 44 germinated. In *L. bardanoides*, 58% did not have an embryo, while in *C. sphaerocephala*, this was 85%. In addition to the absence of embryo and occurrence of dead seeds, another cause for the germination not to occur was the presence of a malformed embryo (Table 2). In *L. bardanoides*, three nongerminated seeds had a normal looking embryo and when submitted to the tetrazolium test, revealed a red color (Fig. 2 d), thus characterizing the seeds as alive, probably viable.

Morphology of *Lessingianthus bardanoides* seedlings

L. bardanoides seeds germinated normal (Fig. 2 c) and abnormal (Fig. 2 b) seedlings. The morphological differences, between them can be viewed in Table 3.

Table 1 - Total number of seeds (n), temperature, germination rate, calculation of the germination speed index (GSI) and mean germination time (t) for different temperatures and 40.32 $\mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance. Same letters in the same columns do not differ significantly at 5 and 1% probability.

Species	n	Temperature (°C)	Germination (%)	GSI	t (days)
<i>L. bardanoides</i>	125	20	27.2 (a)	0.138 (a)	46 (a)
	125	25	30.4 (a)	0.214 (a)	36 (a)
	125	30	11.2 (b)	0.068 (b)	39 (a)
	125	15-35	7.20 (b)	0.019 (b)	78 (b)
	125	20-30	18.4 (a.b)	0.094 (a.b)	43 (a)
	125	20-35	4.80 (b)	0.018 (b)	51 (a)
Total	750				
<i>C. sphaerocephala</i>	250	20	4.8 (a)	0.066 (a)	19 (a)
	250	25	2.0 (b)	0.029 (b)	20 (a)
	250	30	2.4 (b)	0.042 (a.b)	14 (a)
	250	15-35	2.0 (b)	0.039 (a.b)	41 (b)
	250	20-30	2.4 (b)	0.042 (a.b)	14 (a)
	250	20-35	4.0 (a)	0.060 (a)	16 (a)
Total	1,500				

Table 2 - Number of seeds with embryo, no embryo, dead seeds, malformed embryo, nongerminated viable seeds and germinated seeds ignoring the dead seeds, seeds with malformed embryos and embryoless for different temperatures and 40.32 $\mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance.

Species	Temperature (°C)	With embryo	No embryo	Dead seed	Malformed embryo	Viable (alive)	Germinated (n ^a)	Germinated (%)
<i>L. bardanoides</i>	20	34	84	5	2	0	34	100
	25	38	66	21	0	0	38	100
	30	14	71	40	0	0	14	100
	15-35	11	91	20	3	2	9	82
	20-30	23	62	38	2	0	23	100
	20-35	7	62	56	0	1	6	86
Total		127	436	180	7	3	124	16.5
<i>C. sphaerocephala</i>	20	12	203	33	2	0	12	100
	25	5	223	21	1	0	5	100
	30	6	216	24	4	0	6	100
	15-35	5	208	36	1	0	5	100
	20-30	6	214	29	1	0	6	100
	20-35	10	221	19	0	0	10	100
Total		44	1,285	162	9	0	44	3

Table 3 - Morphological differences of *L. bardanoides* seedlings.

Morphology	N seedlings (n)	(%)	Cotyledons	Hypocotyl	Primary root	Adventitious root
abnormal	13	10.5	2	swollen	degenerated	hypocotyl-derived
normal	111	89.5	2	not swollen	present	absent

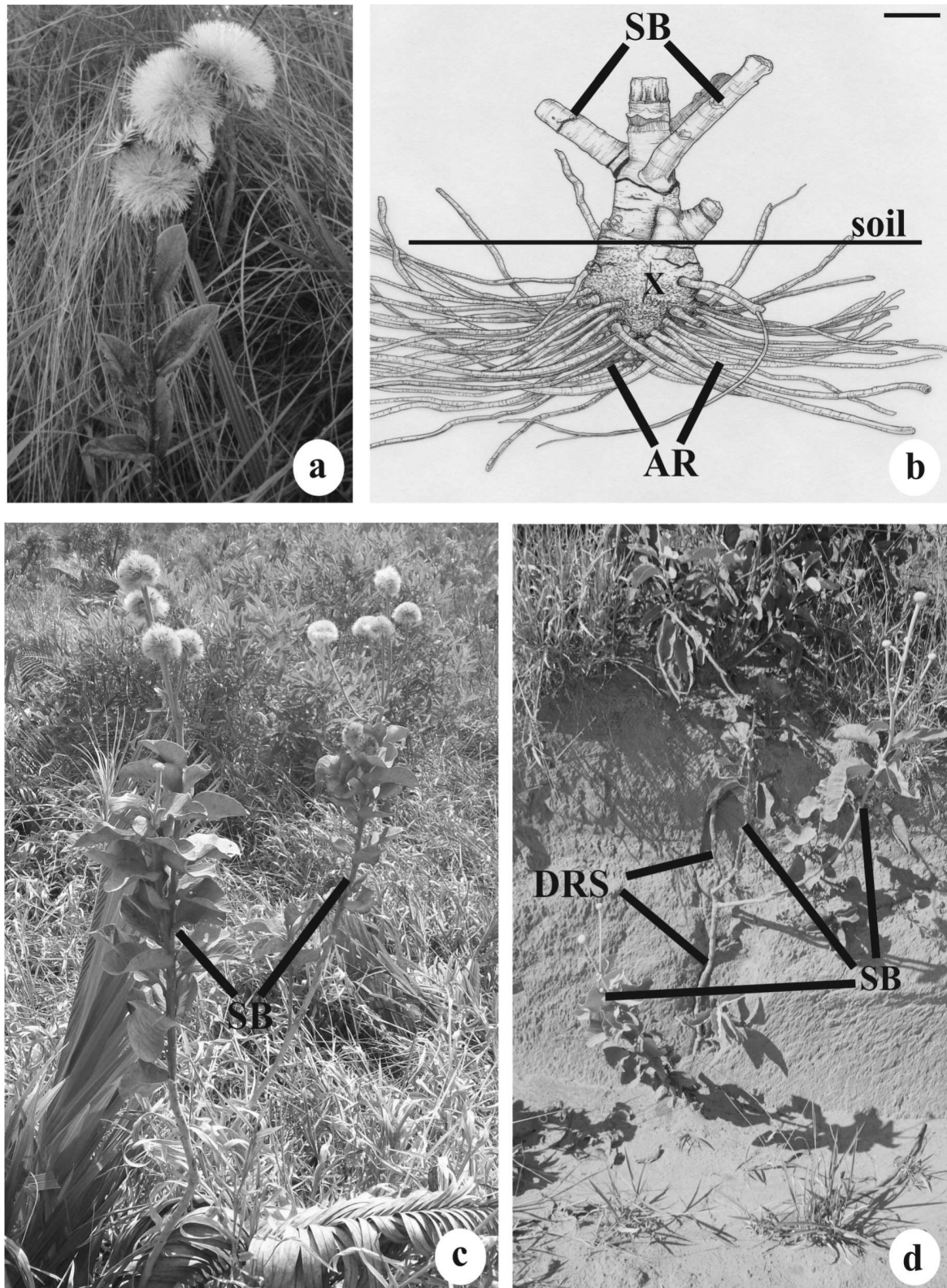


Figure 1 - *Lessingianthus bardanoides* (Less.) H. Rob (a-b). General view of the plant in field (a). Xylopodium with emission of stem branches and adventitious roots (b). *Chresta sphaerocephala* DC. (c-d). General view of the plant in field (c). Diffuse root system with emission of stem branches (d). AR = adventitious roots; SB = stem branches; DRS = diffuse root system; X = xylopodium. Bar: 1 cm (b).

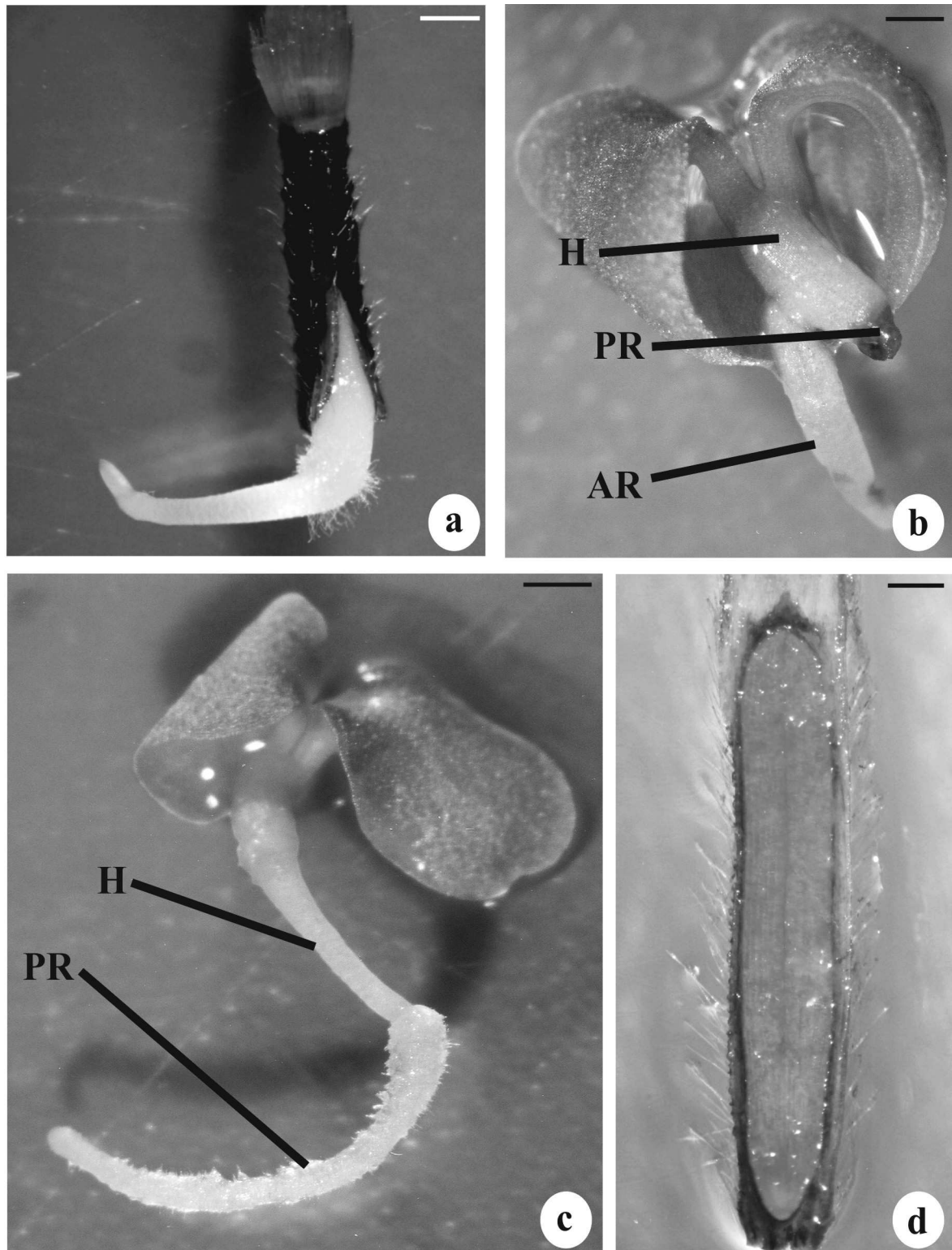


Figure 2 - *Lessingianthus bardanoides* (Less.) H. Rob. Emission of the primary root (a). Abnormal seedling (b). Normal seedling (c). Longitudinally sectioned fruit showing red-colored embryo after tetrazolium test (d). H = hypocotyl; AR = adventitious root; PR = primary root. Bars: 870 μm (a); 700 μm (b); 900 μm (c); 300 μm (d).

DISCUSSION

L. bardanoides seeds had the highest germination rates and the highest GSI values at 20, 25, and 20-30°C. For *C. sphaerocephala*, the best germination rates occurred at 20 and 20-35°C and the highest GSI values were obtained at all the temperatures, except for 25°C. Comparing these results with those of other studies (Ruggiero and Zaidan, 1997; Ferreira et al., 2001; Gomes and Fernandes, 2002; Velten and Garcia, 2005; Garcia et al., 2006; Tonetti et al., 2006), in which embryoless-seeds were not previously separated, within the tribe Vernonieae, the various species were observed to show distinct temperature ranges for the germination, and that variation also occurred among the germination rates in the family Asteraceae.

In relationship with the mean germination time, although there was a significant difference between the species, for both at 15-35°C, the germination was slowest and no difference was observed at temperatures. The mean time evaluates the germination process and, as proposed by Ferreira et al. (2001), the species can be classified as rapid-germination ($t < 5$ days), intermediate germination ($5 < t < 10$ days), and slow germination ($t > 10$ days). According to such classification, both *L. bardanoides* and *C. sphaerocephala* was slow germination species. Other species of the family Asteraceae which also have slow germination are *Trixis praestans* (Vell.) Cabrera, *Senecio heterotrichus* DC., *S. oxyphyllus* DC. and *S. selloi* DC, while other species of the tribe Vernonieae such as *Elephantopus mollis* H.B. and K., *Vernonia nudiflora* (Less) (Ferreira et al., 2001), *Eremanthus elaeagnus* (Mart. ex DC.), *E. glomeratus* Less. and *E. incanus* (Less) Less (Velten and Garcia, 2005) have rapid germination. Regarding the total germination of the two species, the germination rate of *L. bardanoides* (16.5%) was 5.5 times higher than that of *C. sphaerocephala* (3%). This seemed plausible when these germination rates were associated with the types of underground systems of each species. The underground organ of *L. bardanoides* was made up of one xylopodium which did not have vegetative propagation capacity, therefore, the sexual reproduction would be ensured by the higher seed germination rate. *C. sphaerocephala*, which has a low germination rate it can be propagated vegetatively through its diffused underground system of radicular structure

(Appezato-da-Glória et al., 2008). Sprouting from root buds could represent an effective process of spatial reoccupation and is different from the sucker shoots, because these have the potential to emerge at variable distances from the main trunk, but develop their own adventitious root system, and become independent (Rodrigues et al., 2004; Hayashi and Appezato-da-Glória, 2009).

In germination trials, Sasaki et al. (1999) achieved a germination rate of 11% for *Vernonia herbacea* (Vell) Rusby, lower than the value of 16.5% achieved in this work for *L. bardanoides*. According to Hayashi and Appezato-da-Glória (2005), *V. herbacea* is one of the *Cerrado* species whose underground system is a rhizophore with vegetative propagation capacity. Velten and Garcia (2005) also found low values in the germination tests for *Eremanthus elaeagnus* (Mart. Ex DC.) and attributed this to the occurrence of asexual reproduction in the species through the underground system. Moreover, in the clonal species, the production of viable seeds tends to be always low (Bell et al., 1993), which could explain the high number of embryoless seeds in *C. sphaerocephala* (85%) in relation to *L. bardanoides* (58%) in this study. Sajo and Menezes (1986) reported that in three species of *Vernonia* Scrb. most seeds in each capitulum did not contain embryo. This was due to the rhizophores that constituted vegetative propagation units of these plants by developing new aerial branches from the parts close to the soil surface. In *Vernonia herbacea* (Vell.) Rusby, Sasaki et al. (1999) reported 85% embryoless seeds. Velten and Garcia (2005) observed 50 to 90% seeds as embryoless in *Eremanthus* species.

Overall, as previously stated, in Asteraceae species a higher number of embryoless seeds occurred, thus affecting the germination rate negatively. Table 2 showed a large number of embryoless seeds. At the germination trial, ignoring the dead seeds, the seeds with malformed embryos and the embryoless seeds, the germination rate was high and almost equivalent to the total number of the seeds. Table 2 showed that for *C. sphaerocephala* at all the temperatures, the germination rate was 100%, and for *L. bardanoides* only at two temperatures, the germination rate was not 100% due to occurrence of nongerminated viable seeds. Achutti (1978) found only six embryo-bearing seeds (0.25%), out of 2,300 analyzed in *Piptocarpha rotundifolia* (Less.) Baker. Bertasso-Borges and Coleman (2005) reported that out of

3,632 seeds of *Eupatorium laevigatum* Lam., 28.2% had an embryo. In *Eremanthus erythropappus* (DC.), Tonetti et al. (2006) found approximately 15% embryo-bearing seeds.

Many authors have discussed about the causes leading to low production of seeds with embryo in the family Asteraceae. Recently Marzinek (2008) suggested that one of the possible causes of the phenomenon was related to the abortion of ovules and seeds due to scarce maternal resources, since many flowers occurred in one capitulum. According to Lloyd (1980), however, the canceling of the maternal investment can not be considered simply as a loss of reproductive potential, instead it represented an adaptational response to the boundaries imposed by the available resources. Nevertheless, a possible cause of abortion in Asteraceae seeds was apomixis, a quite common occurrence in the family (Werpachowski et al., 2004). In capitula with cross-pollination, the development of abnormal ovules involves the proliferation of the endothelium, a failure in the formation of the embryo and failure in the endosperm development, which according to Cichan and Palser (1982) can cause the abortion of seeds in the early development stages in *Cichorium intybus* L.

Self-incompatibility is frequent in family Asteraceae (Richards, 1986). Velten and Garcia (2005) suggested that this can be one of the possible causes of the reduced production of viable seeds in *Eremanthus*. Ferrer and Good-Avila (2007) reported that 63% of 572 Asteraceae species were with self-incompatibility.

As to the ecologic aspects, many Asteraceae species host insect larvae which feed on their seeds (Klinkhamer et al., 1988), and according to Fenner et al. (2002), these seeds are more available while they are attached to the capitulum and represent a source of proteins and minerals to be exploited. For Louda and Potvin (1995), in addition to the direct consumption to developing flowers and seeds in Asteraceae, which can reduce the seed production and result in lesser number of seedlings and adult individuals, the insects feed on the inflorescence in the early development stages, causing injuries to the phloem and disrupting the nutrient supply to future seeds, thus decreasing the number of viable seeds per capitulum. Louda and Potvin (1995) also reported that the capitula of *Cirsium canescens* Nutt. located in the upper part, matured before those located in the lower portion and recorded that when the herbivory interference

was experimentally reduced, a higher number of viable seeds was achieved, in view of their higher contribution from the lower part of the plant. Considering these results, two considerations were established: (1) this type of herbivory could decrease the number of viable seeds in a plant; (2) the difference between the maturation time of inflorescences within the same individual could affect significantly the seed collection, thus increasing the proportion of embryoless seeds.

The results obtained in the germination tests of *L. bardanoides* indicated the presence of three seeds that, although being viable following the tetrazolium test, failed to germinate. Among the possible causes that prevented these seeds from germinating, dormancy could be one of them. However, dormancy is inherent to the species and according to Rizzini (1997), it would achieve the germination thoroughly, which does not justify the absence of embryonal development in only three seeds. There were delays in the germination which caused fungal infestation. The presence of these microorganisms could have interfered with the germination process and prevented the three seeds from germinating.

The germination trials of *L. bardanoides* showed the development of normal and abnormal seedlings. According to Brasil (1992), only the seedlings with normal development could be considered. However, the seedlings with abnormal development, according to the work mentioned above, had morphological characters such as swollen hypocotyl and emission of adventitious roots and primary root degeneration, quite similar to the xylopodium of that species found in the field. The morphological characters shown by these seedlings gave rise to a cauline xylopodium and the primary root is degenerated. Rizzini and Heringer (1961) reported that the xylopodium in *Mimosa multipinna* Benth. was formed through the thickening of the hypocotyl and the upper primary root. Other authors found through the anatomical studies (Paviani, 1977; Appezzato-da-Glória and Estelita, 2000; Hayashi and Appezzato-da-Glória, 2007) that in *Brasilia sickii* G.M. Barroso (Asteraceae), *Mandevilla illustris* (Vell.) Woodson, *Mandevilla velutina* (Mart. ex Stadelm.) Woodson (Apocynaceae) and *Vernonia grandiflora* Less. (Asteraceae), the xylopodium had a cauline and root structure and considered the xylopodium a morphological unit.

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RESUMO

Este trabalho teve como objetivo estudar os efeitos de diferentes condições de temperatura na germinação de sementes de *L. bardanoides* e *C. sphaerocephala*, comparar as taxas de germinação dessas duas espécies e estimar a ocorrência de sementes sem embrião. Os resultados indicaram que as melhores temperaturas para a germinação das sementes de *L. bardanoides* foram 20, 25 e 20-30°C e, para *C. sphaerocephala*, 20 e 20-35°C e 40.32 $\mu\text{mol m}^{-2}\text{s}^{-1}$ de irradiância; *L. bardanoides* apresentou maior taxa germinativa, já que o número de frutos contendo semente com embrião é maior, comparado com *C. sphaerocephala*.

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