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Maturation of Seeds of *Caesalpinia echinata* Lam. (Brazilwood), an Endangered Leguminous Tree from the Brazilian Atlantic Forest

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ABSTRACT

The present work describes changes during the maturation process of seeds of Caesalpinia echinata *Lam. Individual flowers were tagged in the day of their anthesis and the pods were collected directly from the branches from 32 to 65 days after flowering (DAF). Results obtained suggested that physiological maturity of* C. echinata *seeds occurred ca. 60-65 DAF, immediately before shedding, when seeds had 30-40% water content.*

Key words: Harvesting, pau-brasil, pernambuco, physiological maturity, seed germination

INTRODUCTION

Caesalpinia echinata Lam. (pau-brasil, brazilwood, pernambuco) is one of the most important plant species in Brazil and has been included in the list of the Brazilian flora species at risk of extinction (Ibama, 1992), mainly due to its exploitation in the past. More recently, the idea of extremely predatory forest logging based on the brazilwood exploitation in the colonial period was not confirmed (Castro, 2002; Rocha, 2004).
Besides its current economical value for the violin bow manufacturing, this species is also an ornamental tree, commonly cultivated in streets or parks (Corrêa, 1974). It belongs to Leguminosae (Caesalpinioideae) and measures between 5 and 15m height (Lewis, 1998). The natural distribution of the species is restricted to the Atlantic Forest in the coast of Brazil, between Rio Grande do Norte and Rio de Janeiro (Aguiar and Aoki, 1983; Cunha and Lima, 1992; Rocha, 2004). Currently, the size of natural populations of this species is small (Cardoso et al., 1998; Rocha, 2004).
The flowering period of *C. echinata* growing in an experimental area in the state of São Paulo, Brazil, for about 24 years, is in August/September (at the beginning of the rainy season), the maturation of the fruits occurring in the spring and summer, as previously reported (Aguiar, 2001). The fruits are oblique, spiny, with 6-8cm length and 2-3cm width, sublunate dehiscent woody (Lewis, 1998) and contain 2-3 brownish seeds, with 1-1.5cm in diameter (Cunha and Lima, 1992). The valves of the pods twist after dehiscence, and their surfaces are pubescent with 5mm long woody spines intermixed (Lewis, 1998).
The seeds of *C. echinata* are chartaceous and exfoliate (Teixeira et al., 2004) and have been considered of short life span, not tolerating storage longer than 3 months, either under natural or

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controlled conditions (Aguiar and Barbosa, 1985). The presumably short life span had several technical implications in the past concerning seedling production (Ramalho, 1978; Aguiar and Barbosa, 1985). More recently, Barbedo et al. (2002) showed that it was possible to obtain up to 80% of germination after 18 months of storage under low temperatures, if the seeds were properly selected before storage and dried under controlled conditions. Teixeira et al. (2004) reported the presence of macrosclereides, fibres and paracytic stomata in the developing seed coat of *C. echinata*, the last feature being rarely found in legume seeds. According to these authors, the seed coat features may account for the low longevity of *C. echinata* seeds and their behaviour under storage.

Several factors may interfere in the maintenance of both seed viability and conservation, mainly its initial physiological quality. Among other factors, this quality depends on the maturation stage, being important to identify the physiological maturity to determine the best time for harvesting (Carvalho and Nakagawa, 1983). Early harvesting can result in immature and low vigour seeds. Thus, the conservation of the viability of such seeds can be harmed, either due to the incomplete development of the embryonic axis and/or to the availability of reserve compounds necessary for the germination and for the initial development of the seedlings. On the other hand, the harvesting of seeds after the point of physiological maturity can also result in accelerated seed deterioration, as the environmental conditions are often unfavorable to storage (Carvalho and Nakagawa, 1983; Mayer and Poljakoff-Mayber, 1982).

Considering the variations observed in the behaviour of seeds under storage and the little conclusive information about the best time for harvesting the seeds of *C. echinata*, the present work describes morphological and physiological changes during the maturation process of these seeds, in order to characterize their physiological maturity.

MATERIAL AND METHODS

The experiments were carried out during 2001 and 2002 in a homogeneous plantation (ca. 250 trees) located at the Biological Reserve and Experimental Station in Moji-Guaçu (22°15-16' S and 47°8-12' W), state of São Paulo, Brazil. Environmental data of the area are shown in Fig. 1. During the flowering period (August/September), in both years, individual flowers were tagged on the day of their anthesis (9/Sep to 14/Sep and 21/Aug to 2/Sep, in 2001 and 2002, respectively). To analyse the main phases of seed development (Kermode, 1990), the pods were collected directly from the branches at 32, 40, 48, 52 and 59 days after flowering (DAF) in 2001 and at 40, 50, 60 and 65 DAF in 2002. Each sampling period was considered as a different stage of the seed development. Additionally, seeds were collected directly from the ground, not exceeding 24 hours after shedding, and were named recently-dispersed seeds.

Fruits (four replicates of 10) were analysed for external characteristics that included size (length, width and thickness) and aspects of spines and colour. The seeds (four replicates of 10) were removed from the pods by hand and their length, width and thickness were also registered before submitting them to evaluation of their physiological quality. Water content (%, on a fresh weight basis) and the dry matter (mg seed^{-1}) for each stage of maturity were determined (four replicates of 10 seeds) after oven drying at 103ºC ± 3ºC for 24h (Ista, 1985).

Germination tests were carried out by placing four replicates of 16 seeds in 11 x 11 x 3cm plastic boxes each containing two thick germination papers, moistened previously with distilled water, in germination chambers (Marconi MA400) at 25ºC ± 1ºC, under continuous light. Germination was evaluated every two days from the sowing day, by registering the protrusion of the primary root. After 20 days, the number of seedlings with both normal radicular system and shoots was registered.

The results were analysed by applying F-test (0.05) in an entirely randomized design, with four replications, adjusted for polynomial regression equations (Steel and Torrie, 1980).

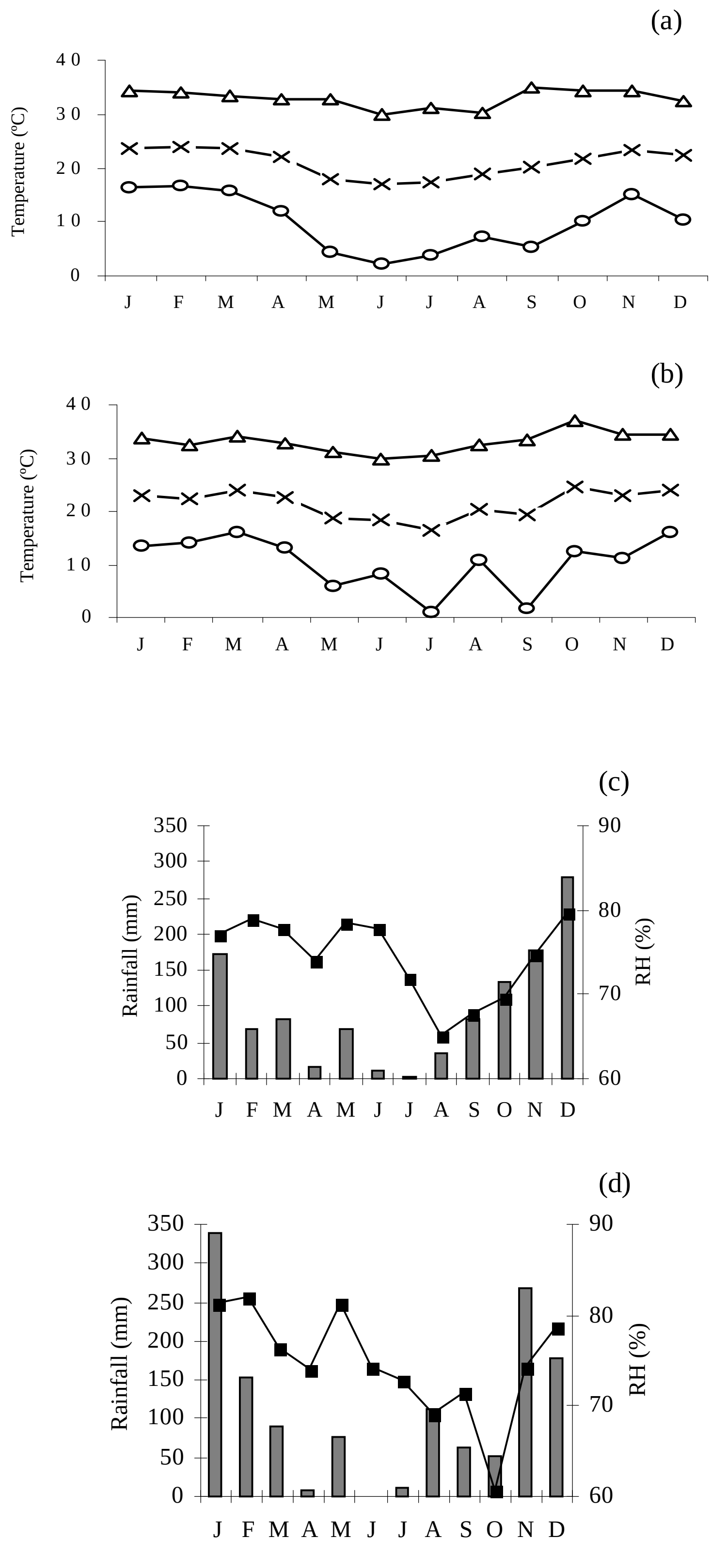

Figure 1 - Environmental data registered in Moji-Guaçu, SP, Brazil, in 2001 (a and c) and 2002 (b and d); a and b: Maximum (Δ), medium (X) and minimum (O) temperatures; c and d: rainfall (columns) and relative humidity (■).

RESULTS AND DISCUSSION

Anthesis in 2002 started ca. 15 days earlier than in 2001. This could be related to differences in rainfall distribution between these years, mainly in August (Fig. 1). The first phase of seed development, which was characterized by histodifferentiation or embryogenesis, was studied in *C. echinata* by Teixeira et al. (2004). This phase was likely to have occurred before the first period analysed in the present work (32 DAF).

The external characteristics of fruits and seeds from phase II to III are shown in Table 1 and were based on Kermode (1990) that described development/dry mass accumulation of seeds, as phase II, and final maturation/drying, as phase III. From the initial stages of development (32 DAF) until 48 DAF, fruits were green-coloured and no substantial alterations were found during the whole period of observation. Fruits started turning brown-stained at 59 and at 60 days in 2001 and 2002, respectively. The natural dehiscence of the fruits occurred a few days after 59 DAF in 2001 and after 65 DAF in 2002, when they were brownish. Thus, dehiscence in 2002 took place a few days later than in 2001 and could be associated to environmental conditions, mainly the reduction in rainfall, relative humidity and minimum temperature in 2002 (Fig. 1). These results corroborated general information of Lima et al. (2002), for which the period between flowering and shedding of *C. echinata* seeds was 60-70 days (one month from flowering to fruit and 30-40 days for ripening and seed dispersion).

The identification and characterization of the stage of maturation just before dehiscence of the fruits is important to obtain seeds of high vigour. Collecting seeds after dehiscence, when they have been subjected to uncontrolled environmental conditions, can result in seeds of low quality. Results presented in this work suggested that the colour of the fruits was a good indication of dehiscence. In both years 2001 and 2002, the change in the colour of the fruits occurred around 60 days after anthesis (Table 1), just before the dehiscence. Information concerning the association between the colour of the fruits and the stage of seed maturity, in tree species, is found in the literature, as reported for *Dalbergia cochichinensis* Pierre (Hung, 2003).

The size of the pods, including length, width and thickness, in both 2001 and 2002 increased gradually during the analyzed period (Fig. 2), being smaller in 2002, especially concerning to their lenght and width. This could be related to differences in environmental conditions, mainly the rainfall distribution and minimal temperatures in September and October, with both parameters being lower in 2002 than in 2001 (Fig. 1).

Table 1 - External characteristics of pods and seeds of *Caesalpinia echinata* during maturation in 2001 and 2002.

Phase[1]	DAF[2]	Fruit characteristics	Seed characteristics
II	32-48	light green (pale green), spines green and flexible	light green, shining and with high flexibility
II	50-60	dark green to brownish green, brownish green woody spines	dark green to brownish green, brown or purple spots and with low flexibility
III	65-sh	brownish green to brown, brown to purple spots, dark brown woody spines	brownish green to brown, brown or purple spots, non-flexible

[1]Phase I: histodifferentiation (see Teixeira et al., 2004); phase II: development/dry mass accumulation; phase III: final maturation/drying. [2] Days after flowering (sh=shedding).

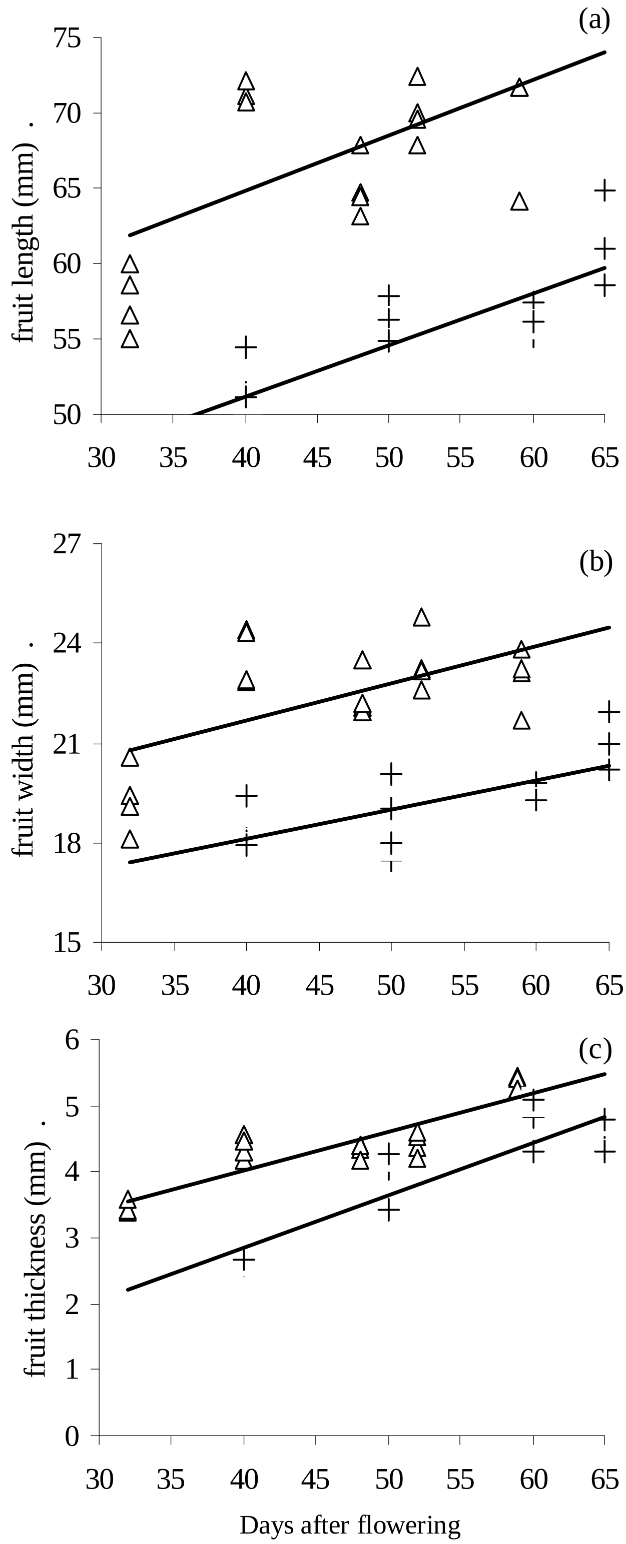

Figure 2 - Dimensions of fruits (a: length; b: width; c: thickness) of *Caesalpinia echinata* during the development and maturation in 2001 (Δ) and 2002 (+). Regressions ($p<0.05$): $Y_{(a)\Delta}=0.370X+50.025$, $r^2= 0.34$, CV=4.1%; $Y_{(a)+}=0.341X+37.504$, $r^2=0.59$, CV= 5.7%; $Y_{(b)\Delta}=0.113X+17.150$, $r^2=0.36$, CV=4.5%; $Y_{(b)+} = 0.089X+14.511$, $r^2=0.53$, CV=4.2%; $Y_{(c)\Delta}=0.058X+1.70$, $r^2=0.81$, CV=2.9%; $Y_{(c)+}=0.080X+0.344$, $r^2=0.77$, CV= 7.3%.

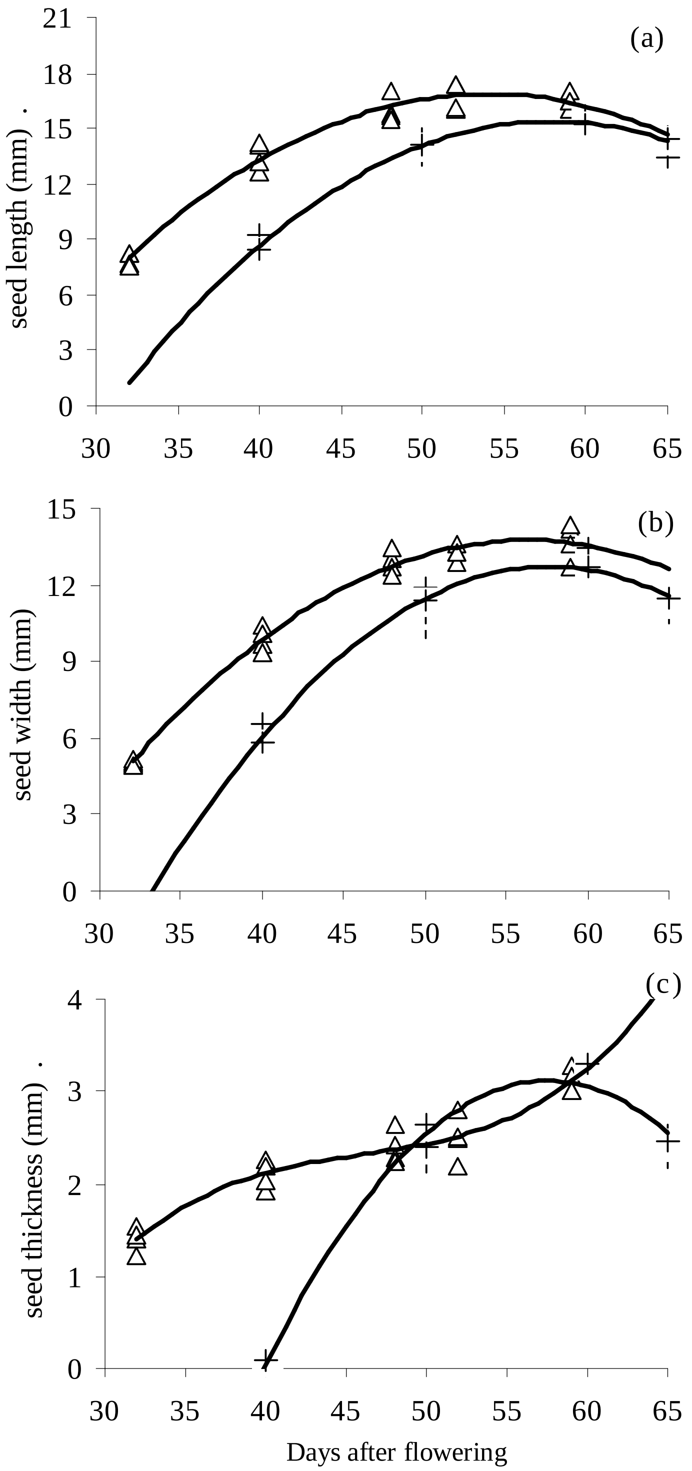

Figure 3 - Dimensions of *Caesalpinia echinata* seeds (a: length; b: width; c: thickness) during the development and maturation in 2001 (Δ) and 2002 (+). $Y_{(a)\Delta} = -0.018X^2+1.968X-36.266$, $r^2 = 0.97$, CV = 4.4%; $Y_{(a)+} = -0.021X^2+2.451X-55.538$, $r^2 = 0.96$, CV = 3.1%; $Y_{(b)\Delta} = -0.0147X^2+1.658X-32.915$, $r^2 = 0.98$, CV = 4.3%; $Y_{(b)+} = -0.021X^2+2.451X-55.538$, $r^2 = 0.98$, CV = 4.5%; $Y_{(c)\Delta} = 0.0002X^3-0.028X^2+1.310X-18.547$, $r^2 = 0.93$, CV = 7.6%; $Y_{(c)+} = -0.01X^2+1.147X-29.877$, $r^2 = 0.98$, CV = 5.2%.

Even so, in both years of analysis, the length and the width of the fruits reached values reported by Lewis (1998) for *C. echinata*, which were 6-8cm and 2-3cm, respectively. The colour of the seeds followed the same pattern of changes observed in fruits, remaining greenish during most of the period of fruit development, and becoming brownish at 59-60 DAF (Table 1). At the end of the maturation period, some seeds presented purple spots on the surface.

The size of the seeds presented similar pattern of development found in fruits. The width and the length reached maximal values at ca. 55 DAF (Fig. 3, a and b) and thickness increased up to 59 DAF (Fig. 3c). Differently from the fruits, the size of the seeds was very similar concerning both years of analysis, indicating a lower influence of environmental conditions. The influence of temperature on the development of seeds has been shown for several species. For example, the temperate woody perennial *Aesculus hippocastanum* growing under warmer conditions presented seeds larger than those under cooler temperatures (Daws et al., 2004).

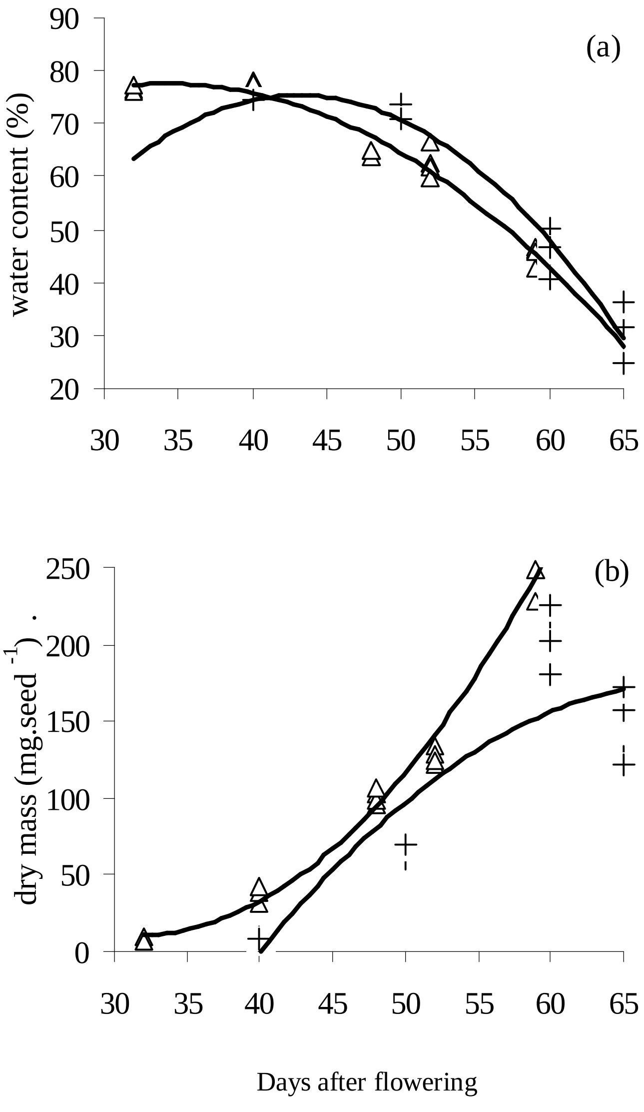

Figure 4 - Water content (a) and dry matter (b) of *Caesalpinia echinata* seeds during the development and maturation in 2001 (Δ) and 2002 (+). $Y_{(a)\Delta}$ = -0.052X^2+3.530X+17.418, r^2 = 0.96, CV = 2.3%; $Y_{(a)+}$ = - 0.096X^2+8.243X-102.32, r^2=0.97; CV=14.0%; $Y_{(b)\Delta}$ = 0.313X^2-19.811X+324.340, r^2=0.98, CV=8.0%; $Y_{(b)+}$ = - 0.194X^2+27.277X-780.790, r^2=0.78, CV=15.0%.

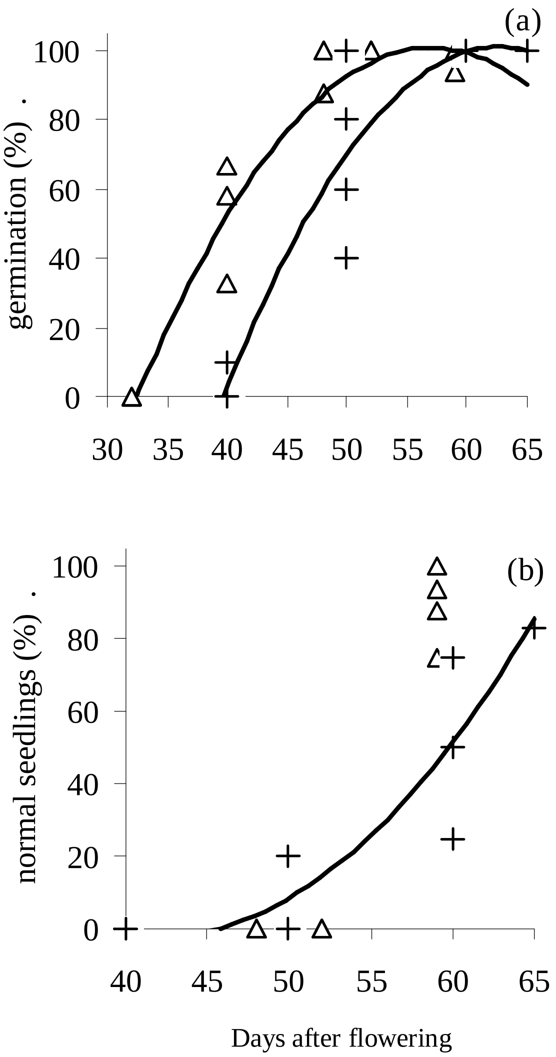

Figure 5 - Germination (a) and normal seedling growth (b) of *Caesalpinia echinata* during the development and maturation in 2001 (Δ) and 2002 (+). $Y_{(a)\Delta}$=-0.168X^2+19.123X-444.23, r^2=0.91, CV=18.6%; $Y_{(a)+}$=-0.191X^2+23.972X-650.700, r^2=0.92, CV=19.3%; $Y_{(b)+}$=0.169X^2-14.314X+300.690, r^2=0.90, CV=36.0%.

The physiological features analysed during maturation of *C. echinata* seeds followed the classical pattern described for orthodox seeds (Kermode, 1990).

The water content of seeds in both years of analysis (Fig. 4a) decreased gradually from the beginning to the end of the maturation process. In 2001, the water content of the seeds was 76.8% (equivalent to 3.35g of water.g^{-1} of dry mass) at 32 DAF, decreasing to 45.4% (0.82 g of water.g^{-1} of dry mass) at 59 DAF (Fig. 4a). The water content of recently-dispersed seeds (not exceeding 24 hours after shedding) was 13.7% (0.16 g of water.g^{-1} of dry mass), a typical value for orthodox seeds (Roberts, 1973). Results suggest that as the seeds undergo maturing drying, there was a period of fresh weight loss as estimated by a decline in the values of the seed water content. In 2002, 40 DAF seeds presented water content of 72.2% (2.85 g of water.g^{-1} of dry mass), a value that was reduced to 30.6% (0.45 g of water.g^{-1} of dry mass) at 65 DAF (Fig. 4a).

It was interesting to note that the values of water content in seeds from both years were very close at 59-60 DAF (0.83 and 0.84g.g^{-1}, corresponding to 45.0% and 45.8%, Fig. 4a). The regression analysis performed allowed to estimate the water content for seeds just before shedding, in both years, being ca. 30%. Thus, it could be assumed that the natural dehiscence of *C. echinata* pods, in Moji-Guaçu, took place just after the seeds reached 30% of water content on wet basis ($0.43g.g^{-1}$).

Based on the results showed in this work, the colour of the fruits and both dimensions and water content of the seeds could be used to indicate the beginning of the dehiscence phase. This information might improve current technical recommendation for harvesting seeds of *C. echinata*, a practical management previously based exclusively on the natural dehiscence (Lorenzi, 1992). At the same time water content decreased, seed dry matter increased up to 59 DAF in 2001, reaching $250.3mg.seed^{-1}$, and up to 65 DAF in 2002, reaching $170.9mg.seed^{-1}$ (Fig. 4b). The wide variation found in seed dry matter between both years could be related to differences in environmental conditions, mainly rainfall and temperature (Fig. 1), as discussed above. Indeed, Ellis et al. (2000) showed that ending irrigation early in rapid-cycling brassica plants resulted in earlier mass maturity, altered the time course of maturation drying and reduced final seed weight. Similar changes seemed to have occurred in *C. echinata* seeds in 2002, the lower rainfall and lower temperature probably reducing the final dry mass of the seeds.

The regression analysis performed to seed dry matter revealed an increase in both years 2001 and 2002, especially in the former. According to several authors, seeds can reach the physiological maturity when maximum values of dry matter are achieved (Carvalho and Nakagawa, 1983). In 2001 recently-dispersed seeds of *C. echinata* (collected up to 24 hours after dehiscence) presented dry matter content ($295.5mg.seed^{-1}$, data not shown) higher than those found at 60 DAF (pre-dehiscence phase, Fig. 4b), indicating that seed dry matter increased gradually up to the dehiscence of the fruits.

Seeds began to be able to germinate around 30-35 DAF in 2001 and 40 DAF in 2002. Germinability increased from these points to 55-60 DAF in 2001 and to 60-65 DAF in 2002 (Fig. 5a). Normal seedlings have developed from seeds at 50 DAF in 2002, reaching maximum values at the end of the maturation period, that was 65 DAF (Fig. 5b). In 2001 the percentage of normal seedlings from seeds collected at 59 DAF was higher than that observed in 2002 at the same stage of development (Fig. 5b). The influence of water availability to development of seeds was shown by Ellis et al. (2000). They found that in rapid-cycling brassica plants, the reduction of irrigation diminished the time course of seed maturation, increasing the maximal quality of the seeds. The normal development of *C. echinata* seeds could be affected by the irregular distribution of rainfall recorded in 2002.

Corroborating previous observations of Barbedo et al. (2002), recently-dispersed seeds presented lower germinability (77% and 91% of germination in 2001 and 2002, respectively, data not shown) and vigour (59% of normal seedlings, in both years, data not shown) than seeds collected before shedding (Fig. 5). It is important to emphasize the fact that in both years, seeds at 40 DAF, presenting less than a third part of the amount of the final dry matter content (Fig. 4b), were capable to germinate (40% in 2001 and 2% in 2002, Fig. 5a). However, although seeds of *C. echinata* acquired the germination capacity very early in the maturation process, they were able to develop normal seedlings only very close to dehiscence.

According to Teixeira et al. (2004) the endosperm of *C. echinata* seeds is completely consumed during embryogenesis, thus characterizing an exendospermic seed. This process occurred probably during the histodifferentiation phase of the embryo that preceded the first stage of seed maturation (32 DAF) analysed in the present work. Therefore, the capacity to produce normal seedlings, not found in the early stages of seed maturation, was more related to the reserve deposition, in the expansion phase (Kermode, 1990), than to the histodifferentiation of the embryo. The analysis of the reserve compounds in cotyledons and embryo axis during maturation could contribute significantly to understand the process of seedling development from seeds at different stages of maturation.

One of the major factors influencing vigour and viability is physiological maturity of the seeds at harvest (Śliwińska, 2000) and this process is markedly affected by environmental factors, mainly temperature and water availability. Experiments with the leguminous white lupin and yellow lupin seeds indicated that the response of maturing seeds to environmental changes in temperature was associated to changes in the content and composition of soluble carbohydrates stored in those seeds (Górecki et al., 2001).

Results obtained for seed dimensions, water and dry matter contents, germination and seedling development suggested that physiological maturity of *Caesalpinia echinata* seeds occurred ca. 60-65 DAF, immediately before shedding. This short period between maturity and shedding is critical for obtaining lots of seeds with high physiological quality, affecting further the germinability and storability of the seeds (Barbedo et al., 2002). Conversely, harvesting premature seeds led to lower seed quality although they germinated (Fig. 5a). Therefore, the precise characterization of the physiological maturity stage is crucial to define the best time for harvesting *C. echinata* seeds. In Moji-Guaçu, SP, Brazil, this time was reached when seeds had 30-40% water content, ca. 60-65 days after flowering.

Data reported in this article increase the information about an endangered species of the Atlantic Forest and contribute to the protection of this environment, particularly concerning the Leguminosae flora, that is one of the richest and most important families of the tropics (Lewis, 1987 and 1998).

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RESUMO

Sementes de *Caesalpinia echinata* Lam. têm sido consideradas como de curta longevidade. Contudo, quando lotes são submetidos à seleção prévia ao armazenamento, é possível conservar sua viabilidade por até 18 meses. Considerando a falta de informações conclusivas quanto à melhor época de colheita dessas sementes, o presente trabalho descreve as modificações que ocorrem durante o processo de maturação das sementes. Flores foram etiquetadas no dia de sua antese e os frutos foram colhidos diretamente dos ramos dos 32 aos 65 dias após a antese (DAA). Sementes dispersas naturalmente por período não superior a 24 horas também foram coletadas, sendo designadas sementes recém-dispersas. As características externas e as dimensões (comprimento, largura e espessura) de frutos e sementes foram registradas. A avaliação da qualidade fisiológica das sementes foi baseada no teor de água, no conteúdo de matéria seca e na germinação. Os resultados sugerem que a maturidade fisiológica das sementes de *C. echinata* ocorreu por volta de 60-65 DAA, imediatamente antes da deiscência, quando as sementes tinham 30-40% de água.

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