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Overcoming dormancy in macaw palm diaspores, a tropical species with potential for use as bio-fuel

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Summary

Methods for overcoming dormancy in macaw diaspores were evaluated to determine the type of dormancy found and the best treatments for stimulating germination. The effects of pre-germinative treatments of pyrenes and seeds were evaluated in both sand and clay soil substrates under greenhouse conditions for 12 months. The emergence of seeds, with the maintenance or removal of the opercular tegument, was also evaluated in a humid growth chamber following immersion in GA_3 solutions at concentrations of 0, 50, 100, 500, 1000, 2000 mg L^{-1}. Sowing of the pyrenes into washed sand or clay soil and the seeds into clay soil under greenhouse conditions were ineffective, irrespective of the dormancy-breaking treatment used, and are not recommended for the propagation of this species. For seed sown in sand, pre-immersion of the seeds in solutions of 100, 500 and 1000 mg L^{-1} GA_3 for 24h, immersion in water at 40°C for 24h, and the removal of the opercular tegument all favored emergence. In humid growth chambers, the removal of the opercular tegument associated with the application of GA_3 at 2000 mg L^{-1} was the most efficient treatment. We conclude that the dormancy of macaw palm diaspores is influenced by the endocarp and that the seeds show physiological dormancy.

Introduction

The macaw palm, *Acrocomia aculeata* (Jacq.) Lodd. ex. Mart., is widely distributed throughout the tropical Americas (Motta *et al.*, 2002) and there are especially dense populations in Minas Gerais State, Brazil (Lorenzi *et al.*, 2004). This palm produces large numbers of fruits and has been used for centuries by human populations as a food source and to make soap (Lorenzi, 2006). The oil content of the macaw palm fruit can exceed 16% in the pulp and 52% in the seeds (Hiane *et al.*, 2005), which makes it a potentially important agroindustrial species as a source of raw materials for bio-fuel production in dry tropical regions (Teixeira, 2005, Bandeira, 2008; Moura *et al.*, 2010).

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The large-scale use of this species would offer important economic gains both for small communities that harvest their fruits as well as for large agro-industrial projects. The establishment of commercial plantations of macaw palm is currently limited by difficulties related to its propagation, as the seeds demonstrate dormancy and germination occurs only very slowly and to very low percentages (Lorenzi *et al.*, 2004; Bandeira, 2008). As such, there is currently a risk of over-exploitation of the fruits of natural populations through over-harvesting. This situation has implications for the conservation and regeneration of *A. aculeata* and indicates the necessity of establishing efficient methodologies for promoting seed germination and seedling production.

Dormancy is defined as the lack of germination under otherwise favorable environmental conditions under which non-dormant seeds germinate. Dormancy can be classified as endogenous when related to the embryo or exogenous when related to other structures of the diaspore (Baskin and Baskin, 1998; Hartmann *et al.*, 2002). The mechanisms controlling germination and dormancy are unknown for most species of Arecaceae (Meerow, 1990; Orozco-Segovia *et al.*, 2003), although there is evidence for morphological dormancy in some species due to embryo immaturity (Baskin and Baskin, 1998; Hartmann *et al.*, 2002; Aguiar and Mendonça, 2002), physical dormancy imposed by the endocarp, and physiological dormancy (Hussey, 1958; Rees, 1962; Orozco-Segovia *et al.*, 2003; Perez, 2009).

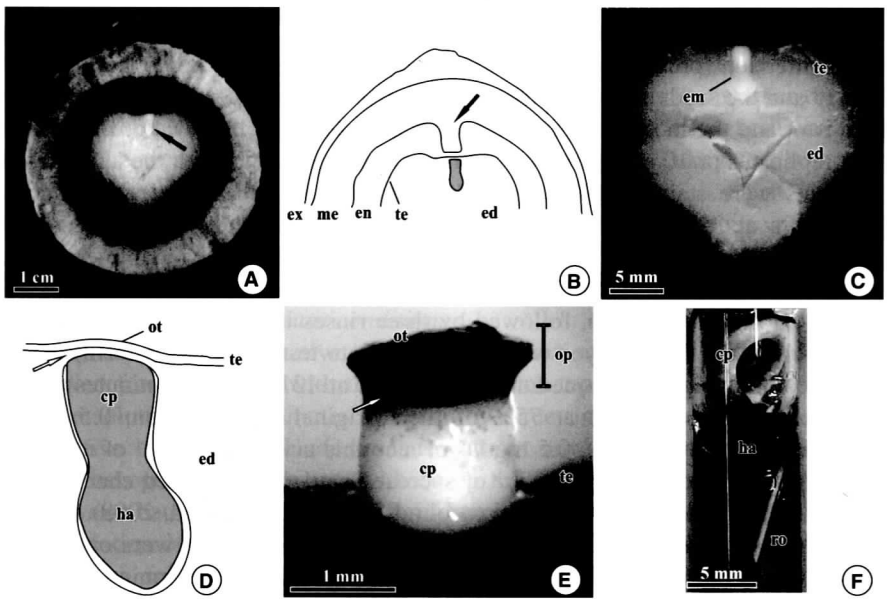
The fruits of macaw palm are globose drupes, 3-5 cm in diameter, with a woody exocarp, fibrous mesocarp which is rich in lipids, a stony endocarp, and one to three oilseeds (Lorenzi *et al.,* 2004). Under natural conditions, after abscission, the exocarp and mesocarp are usually consumed by dispersers (Lorenzi, 2006) and the pyrene (seed surrounded by the endocarp) can remain in soil for long periods. The presence of the extremely hard endocarp may be associated with limitations to germination in the species. However, this fact must be studied, considering the presence of a prominent germ pore, which is clogged by fibers of the mesocarp and a layer of endocarp at the time of abscission (figure 1A and B).

Isolated macaw palm embryos germinate rapidly and in high percentages when cultivated *in vitro* (Tabai, 1992; Bandeira, 2008), indicating that the degree of maturity of the embryo is not a limiting factor for germination and that physiological dormancy, if present, is not deep (Baskin and Baskin, 2004). These observations suggest that relatively simple methods could be employed to overcome dormancy, as has been observed in a number of other species that demonstrate non-deep physiological dormancy (Bewley and Black, 1994; Baskin and Baskin, 1998; Hartmann *et al.*, 2002; Finch-Savage and Leubner-Metzger, 2006).

Baskin and Baskin (1998) observed that diaspore responses to different treatments could help define the type of dormancy involved. Various methods have been utilized to break dormancy in Arecaceae seeds (Meerow, 1990; Hartmann *et al.*, 2002). Nagao *et al.* (1980) observed positive effects of GA treatments on the germination of *Archontophoenix alexandrae* (F.Muell.) H.Wendl. Drude and *Ptychosperma macarthurii* H.Wendl. seeds, observations also made in *Euterpe edulis* Mart. by Roberto and Habermann (2010). Studies with *Elaeis guineensis* Jacq. (Hussey, 1958), *Rhapidophyllum hystrix* (Frazer ex Thouin) H.Wendl. & Drude (Carpenter *et al.*, 1993), and *Phoenix dactylifera* L. (Al-Wasel and

Warrag, 1998) demonstrated positive effects resulting from the removal of the operculum. In some species a combination of a number of different treatments were necessary to promote germination (Hussey, 1958; Nagao *et al.*, 1980; Orozco-Segovia *et al.*, 2003). Rigorous control of environmental conditions is also required due to the susceptibility of these seeds to deterioration by pathogen attack (Meerow, 1990; Orozco-Segovia *et al.*, 2003).

Studies directed towards overcoming dormancy in macaw palm diaspores may increase our knowledge of the biology of seeds and of the control of germination in palms. Such studies have previously been restricted to a relatively small number of species (Orozco-Segovia *et al.*, 2003; Panza *et al.*, 2004). This work would also contribute to the development of technologies that can promote the use of this important oil-producing palm (Clement *et al.*, 2005). Treatments applied to pyrenes and seeds should be able to clarify the influence of the hard endocarp on dormancy and the physiological nature of dormancy. Within this perspective, the present work evaluated the efficiency of different methods of stimulating germination in macaw palm seeds and addressed the following questions: i) does the endocarp impose some type of resistance to seed germination?; ii) do these seeds show physiological dormancy?; and iii) which treatments favour the interruption of dormancy and the subsequent germination of macaw palm seeds?



cp: cotyledon petiole; ed: endosperm; em: embryo; en: endocarp; ex: exocarp; ha: haustorium; me: mesocarp; op: operculum; ot: opercular tegument; ro: root; te: tegument

Figure 1. (A) Transverse section of an *Acrocomia aculeata* fruit collected on the day of abscission showing the embryo (arrow); (B) structures of the fruit indicating the germination pore filled by tissue of the mesocarp (arrow) and the embryo (highlighted in gray); (C) transverse section of an *Acrocomia aculeata* seed showing the embryo; (D) localization of the opercular tegument and the micropylar endosperm (arrow) and regions of the embryo (highlighted in gray); (E) germinated seed showing the operculum components: opercular tegument and micropylar endosperm (arrow); (F) seedlings developed *in vitro* after 12 days of embryo culture.

Materials and methods

Collections, storage and preliminary evaluations

Fruits were collected from 20 individuals of *A. aculeata* (Jacq.) Lodd. ex. Mart. from a native population in the municipality of Montes Claros, Minas Gerais State, Brazil, (16°42'34"S; 43°52'48"W) immediately after their natural abscission. Two collection periods were established: fruits collected in November and December 2008 were stored in the shade until February 2009; fruits collected in February and March were stored until April 2009. Samples were collected at different dates in order to get the number of fruits needed for the experiments, since fruit abscission is gradual. The storage period was necessary for to allow some dehydration of the seeds, allowing release of the endocarp and enabling seed extraction (figure 1A and B). After the storage period, fruits collected in November and December were mixed and used for experiments in the greenhouse and fruits collected in February and March were mixed and used for laboratory experiments.

After the initial storage periods, assessment of water content, the tetrazolium test and *in vitro* embryo culture were performed separately for fruits of the two collection periods in order to characterize the lots, determine the viability of embryos and the possible occurrence of embryo dormancy.

For determination of water content, four replicates of 10 seeds were extracted from the fruits with the aid of a bench-top vice. During removal of the seeds they were checked for fungal infection (presence of mycelium) and insect attack.The seeds fresh weight was determined and the seeds were dehydrated in an oven at 105°C for 24 hours after removal from the fruit and the moisture content was calculated (Brasil, 2009). For the tetrazolium test, five replicates of 10 embryos were extracted from the seeds with the aid of a scalpel. The embryos were stained in a 0.5% solution of 2,3,5-triphenyl tetrazolium chloride for four hours at 35°C. After staining, embryos were evaluated according to criteria established by Ribeiro *et al.* (2010) to determine the percentage of viable embryos. For the *in vitro* embryo culture, five replicates of 10 embryos were disinfected in a 0.5% solution of chlorine for 10 min, followed by three rinses in sterile distilled water. Using an air flow chamber, the embryos were inoculated into test tubes (12 × 1 cm) containing 2 mL of the following culture medium (autoclaved at 121°C for 20 minutes): MS salts (Murashige and Skoog, 1962) at 75% of their original concentration, 0.5 mg L^{-1} of thiamin, 1 mg L^{-1} of pyridoxine, 0.5 mg L^{-1} of nicotinic acid, 100 mg L^{-1} of myo-inositol, 0.5 g L^{-1} of hydrolyzed casein, 30 g L^{-1} of sucrose, 3 g L^{-1} of activated charcoal, 6 g L^{-1} of agar; with the pH adjusted to 5.7 (protocol adapted from Bandeira, 2008). The culture was done in an incubator at 30°C in the dark for 30 days. Cultures were observed daily and after the cultivation period, the germination percentage was determined, considering that germinated embryos showed elongation of the cotyledonary petiole (length greater than twice the average length of the embryos measured before inoculation).

Treatments of the pyrenes under greenhouse conditions

The exocarps of the fruits were broken using wooden rollers and the pyrenes were subjected to 15 pre-germination treatments: manual removal of the pulp by hand with a knife (control); mechanical removal of the pulp using motor-driven wire brushes

(mechanical de-pulping); scarification of the germination pore with a knife to extract the mesocarp fibrous tissues that fill the germination pore (figure 1A and B) (scarification); fermentation of the mesocarp by immersing the fruits in water for seven days (fermentation); immersion of the pyrenes in boiling water for 2 seconds (100°C/2s); and for 5 seconds (100°C/5s); warming pyrenes that were buried under 5 cm of dried leaves by burning the leaves for 3 minutes (fire/3 min); immersion of the pyrenes in water in a water bath at 40°C for 48 hours (40°C/48h) and for 72 hours (40°C/72h); storage of the pyrenes in humid sand in a cold chamber at 8°C for 15 days (stratification 15d) and for 30 days (stratification 30d); immersion of the pyrenes in water at 40°C for 48 h followed by storage in humid sand in a cold chamber at 8°C for 15 days (40°C/48h – stratification 15d); immersion of the pyrenes for 72 h in solutions of gibberellic acid (GA_3; Sigma Chemical Co., St. Louis, Missouri, USA) at concentrations of 500 mg L^{-1} (GA/500), 1000 mg L^{-1} (GA/1000), and 5000 mg L^{-1} (GA/5000). In all of the treatments (except the control) the fruit were mechanically de-pulped to obtain the pyrenes. In the treatments involving GA, the germination pore was scarified beforehand.

The treated and control pyrenes were sown in a greenhouse (8 cm deep) in two different types of substrate (washed sand or clay soil) and watered daily. The experiment was established in a randomized design, with 15 (dormancy breaking treatments) × 2 (substrates) factors. Five replicates of 20 pyrenes were used for each treatment. The effects of the different treatments on seed germination were determined by weekly evaluations of the percentage emergence of seedlings for 12 months. The emergence velocity index (EVI) was calculated by the formula $\sum E_n N_n^{-1}$, where E represents the number of seedlings emerged in each week and N the number of weeks after sowing (Borghetti and Ferreira, 2004). The data were submitted to variance analysis and the Tukey test was used for comparing averages (Sas Institute, 1990). The values of the emergence percentages were arcsine converted $[(x/100)^{0.5}]$ for comparisons to normalize heterogeneous variation across the data.

Seed treatments under greenhouse conditions

The effects of nine pre-germinative treatments of intact seeds were evaluated. The seeds were removed from the fruits with the aid of a bench-top vice and then disinfected in a 6% chlorine solution for 10 minutes followed by three rinses in running water. The nine pre-germinative treatments included: seeds without any additional treatment (control); removal of the opercular tegument (without op te); immersion in water at 40°C for 12 hours in a water bath (40°C/12h); storage in humid sand in a cold chamber at 8°C for 30 days (stratification 30d); immersion in water at 40°C for 12 hours followed by storage in humid sand in a cold chamber at 8°C for 15 days (40°C/12h stratification 15d); immersion in water at 70°C for 10 seconds (70°C/10s); immersion in solutions of GA for 24 hours at concentrations of 100 mg L^{-1} (GA/100), 500 mg L^{-1} (GA/500), and 1000 mg L^{-1} (GA/1000). The opercular tegument was removed with a scalpel while viewing under a stereomicroscope; the micropylar endosperm and embryo remained fully intact (figure 1C and D). The immersion temperature was reduced from the 100°C applied to pyrenes to 70°C for seed to avoid damaging the embryo in the absence of the endocarp and was based in the descriptions by Loomis (1958) of practical treatments applied in *Acrocomia* diaspores.

Sowing was performed under basically the same conditions as in the previous experiment, with the seeds being sown into 4 cm-deep furrows and then covered with sand or clay soil. Four replicates of 25 seeds were used in each treatment. The evaluations and data analyses were undertaken as described above. The experiments with the pyrenes and the seeds were performed at the same time, and the maximum and minimum temperatures were noted to determine the average temperatures and thermal ranges.

To evaluate the effect of the endocarp on water absorption by seeds, isolated seeds and pyrenes were placed in sand under the same conditions of the experiment described above. After 30, 60, 90 and 120 days, four replicates of ten of each type of diaspore were taken and water content of seeds, both when isolated from the pyrenes and taken from within the pyrenes (after breaking the endocarp), was determined by oven method as described previously. The data were compared by F test at 5% probability (SAS Institute, 1990).

Seed treatments under laboratory conditions

The effects of GA_3 on germination were evaluated by immersing macaw palm seeds, both with and without removal of the opercular tegument, in solutions with various concentrations of this growth regulator under controlled laboratory conditions. The seeds were removed from the fruits, inspected for possible damage, and subsequently disinfected with a 6% solution of chlorine for 10 minutes followed by three rinses in running water. The seeds were hydrated (Rees, 1962; Ferreira and Gentil, 2006) by maintaining them in trays with vermiculite for ten days in a humid growth chamber (relative humidity 95 ± 5%) at 30 ± 2°C; the final water content of the seeds was 21.3%. The opercular tegument was then removed from half of the seeds as under seed treatments under greenhouse conditions. The seeds were transferred to plastic beakers (200 mL) and covered with 100 mL of GA solutions at the following concentrations: 0, 50, 100, 500, 1000, 2000 mg L^{-1}. After immersion for 24 hours, the seeds were placed in polystyrene trays and once again incubated in the humid growth chamber under vermiculite substrate. Four weeks later, the seeds were removed from the substrate and the GA treatment was repeated. The seeds were removed from the growth chamber on a weekly basis for 18 weeks (when stabilization of germination occurred by one week in all treatments) and examined for germination and deterioration. The emergence of the cotyledonary petiole was considered indicative of germination (figure 1 D) (Bewley and Black, 1994; Meerow, 1990) and seeds were considered deteriorated when they showed necrotic areas and / or the presence of fungal mycelium. Any germinated or deteriorated seeds were removed from the trays. At the end of the experiment, embryos from undamaged seeds that did not germinate were tested using tetrazolium staining, as previously described. The experiment was established in a randomized design, with 6 (GA concentrations) × 2 (removal or maintenance of opercular integument) factors. Five replicates of 20 seeds per treatment were used, and the percentages of germinating seeds and their germination velocity were calculated as described earlier.

The germination percentage was calculated after four weeks (before the second application of GA), eight and eighteen weeks. The GVI was calculated after eighteen weeks using the same formula for EVI described previously, but considering the seeds

that had germinated and not the emergence. The percentage of deteriorated seeds, and the percentage of seeds that did not germinate but were shown to be viable by tetrazolium test, were also calculated. Data analysis was performed as described for previous experiments, including the arcsine transformation.

Results

Treatments of the pyrenes and seeds under greenhouse conditions

The batch of seeds collected in November and December showed no detectable fungal or insect attack and had a water content of 15.3% after the storage period. The tetrazolium test indicated that 96% of embryos were viable and 92% of embryos cultured *in vitro* germinated. The germination started after two days of inoculation and occurred uniformly; after ten days of inoculation most of the seedlings had developed roots (figure 1E).

Seedling emergence from pyrenes was observed after 36 weeks, but percentages remained below 7% in all of the treatments using a sand substrate; only the 40°C/48h treatment was superior to the control in relation to the emergence percentage and EVI (figure 2A). Emergence in clay soil was effectively zero, since only one seedling emerged, and this result was significantly lower than observed for sand. In the experiments with seeds, seedling emergence in the sand substrate began in the eighth week after sowing (figure 2B); no emergence occurred when the clay soil substrate was utilized. The removal of the opercular tegument significantly accelerated emergence between the eighth and 13th week compared with control, but then stabilized between the 13th and 33rd week before increasing again after the 33rd week. The treatments with GA, immersion in water at 40°C, and removal of the opercular tegument resulted in emergence percentages that were significantly greater than those seen with the controls. In relation to the EVI (data not presented), removal of the opercular tegument was the only treatment that gave results statistically different from the control and was also superior to other treatments.

The beginning of seedling emergence from pyrenes and the increase in emergence observed after the 33rd week in the greenhouse experiments coincided with an elevation of the natural environmental temperature (figure 2A, B and C). Between the 13th and 33rd weeks, when the emergence had not occurred from pyrenes and was less than 5% from seeds, with the exception of where the opercular tegument had been removed, the average minimum temperature was 17.7°C. After this period the average minimum temperature increased ca. 5°C with a decrease in the daily thermal amplitude (from 13.9°C to 10.42°C).

The water content of isolated seeds (21.4%) was significantly higher than in seeds that remained within pyrenes (18.5%) after 30 days of sowing. At 60, 90 and 120 days there was no difference between treatments, with the average water contents ranging between 21.7% and 22.9%.

Seed treatments under laboratory conditions

For the seed lot used in this experiment, the average water content was 14.5% after the storage period, the tetrazolium test indicated that all embryos were viable and 97% of embryos germinated *in vitro*. No fungal or insect attack on seeds was detected.

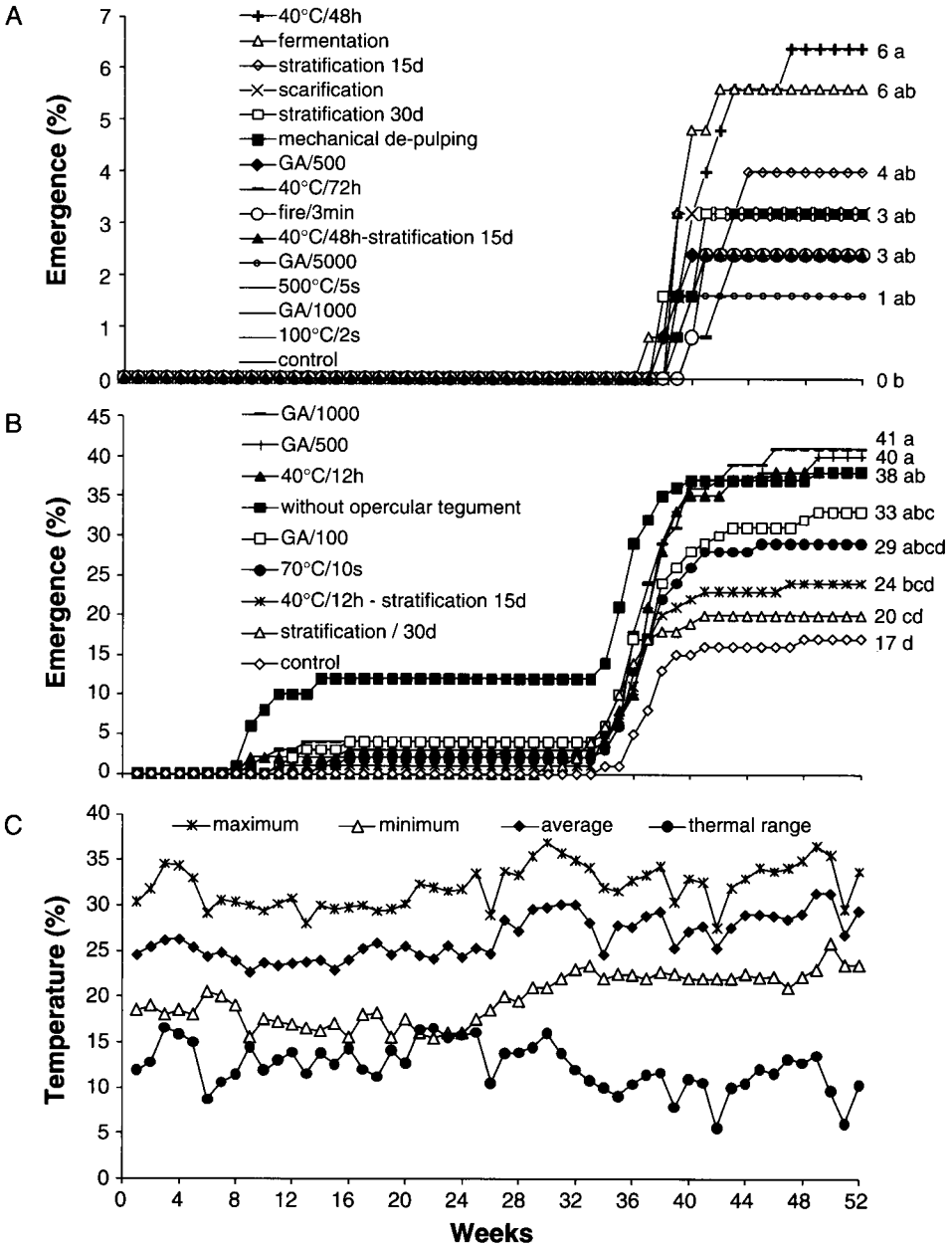


Figure 2. Emergence from diaspores of *Acrocomia aculeata* in the greenhouse as a function of various treatments designed to overcome dormancy. Seedling emergence (percentage) from pyrenes sown into sand substrate (A); seedling emergence (percentage) grown from seeds sown into sand substrate (B) and weekly averages of the maximum, minimum, and average temperatures, as well as the variations between the maximum and minimum temperatures during the course of germination experiments (C). Different letters indicate significant differences between treatments, using the Tukey test at a 5% level of probability.

Seed germination was first observed in the week after immersion in GA_3, with a pronounced increase in germination after the re-application of GA_3 during the fourth week (figure 3). In the more effective treatments, germination tended to stabilize after eight weeks, while in the less effective treatments and in the control, stabilization occurred after 17 weeks.

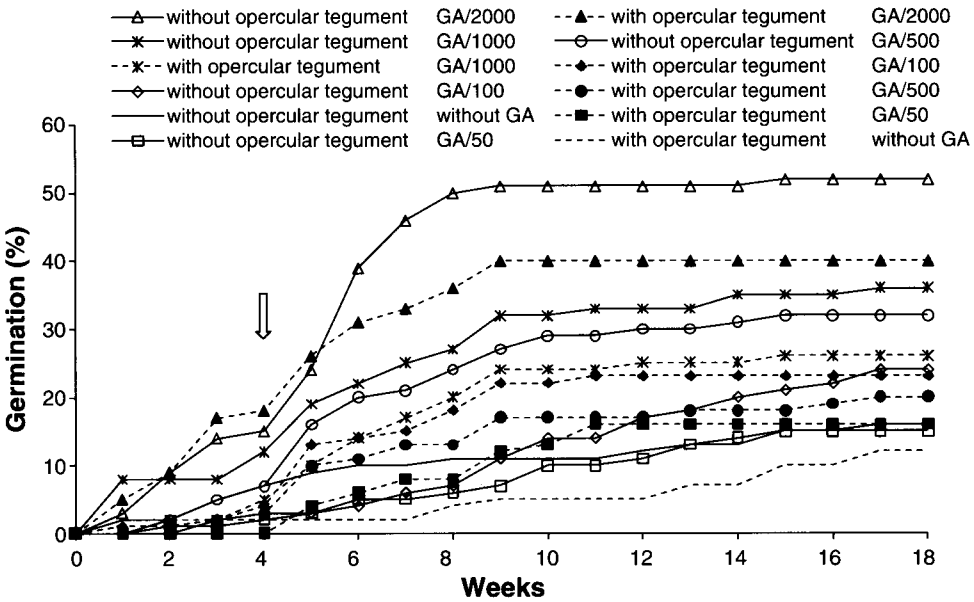


Figure 3. Changes in germination percentage over eighteen weeks in moist chamber at 30°C, of *Acrocomia aculeata* seeds following immersion in solutions with different concentrations of GA_3 either with or without the opercular tegument. The arrow indicates the time of the second application of GA_3.

The responses of seeds to levels of GA_3 changed during the experiment. Four weeks after the first application of GA_3, the evaluation of seed germination indicated that only the dose of 2000 mg L^{-1} showed divergent results from the control both in seeds with or without the opercular tegument (table 1). This same dose was superior to other treatments in seeds with the opercular integument. There was no significant effect of removal of opercular tegument within each dose of GA_3. After eight weeks (four weeks after the second application of GA_3) doses of 1000 and 2000 mg L^{-1} resulted in germinations significantly higher than the control in seeds with the opercular tegument. In seeds without the opercular tegument, the doses of 500 mg L^{-1} and above gave higher germinations than control. The removal of the opercular tegument combined with immersion in GA_3 at a concentration of 2000 mg L^{-1} was the most efficient treatment, reaching 50% germination and in this treatment the removal of opercular tegument was particularly effective. The evaluation after eighteen weeks showed the same trend as in the eighth week, with the exception of increased germination at lower doses. Thus the dose of 100 mg L^{-1} resulted in a statistically higher germination to the control in seeds with opercular integument.

Table 1. Germination percentages (G) after four, eight and eighteen weeks and germination velocity index (GVI) after eighteen weeks in moist chamber at 30°C, following immersion of *Acrocomia aculeata* seeds with or without opercular tegument in solutions with different concentrations of GA [(1)].

GA (mg L^{-1})	weeks							
	4		8		18			
	G		G		G		GVI	
	with	without	with	without	with	without	with	without
0	2bA	3bA	4cA	7dA	13dA	17deA	0.4bA	0.6bA
50	0bA	3bA	8cA	7dA	16cdA	15eA	0.6bA	0.6bA
100	4bA	7abA	18abcA	11cdA	24bA	25cdA	0.7bA	0.9bA
500	3bA	7abA	13bcA	24bcA	20bB	32bcA	0.7bA	1.3bA
1000	5bA	12abA	20abA	27bA	27bB	36bA	1.0bB	2.5aA
2000	18aA	15aA	36aB	50aA	40aB	53aA	2.7aA	2.8aA

Within each week, separately, for germination and GVI, the lowercase letters in each column and the uppercase letters in each row, when are the same indicates no significant differences assessed by Tukey test at 5% probability.

The removal of the opercular tegument increased the GVI only at GA_3 concentration of 1000 mg L^{-1} (table 1). In seeds with intact opercular teguments, only GA_3 concentrations of 2000 mg L^{-1} showed GVI increases in relation to the other concentrations examined. The GVI was greatest among seeds without their opercular tegument at concentrations of 1000 and 2000 mg L^{-1} s.

The percentage of deterioration of the seeds was not influenced by treatments, with an average of 16%. In undamaged seeds that did not germinate, the tetrazolium test showed that 92% of embryos remained viable, independent of the treatment.

Discussion

The rapid germination of macaw palm embryos when cultured *in vitro*, as already evidenced by Bandeira (2008), indicates lack of embryo dormancy (Baskin and Baskin, 2004) and suggests that there are no limitations to germination related with anatomical immaturity, which would need time to overcome (Baskin and Baskin, 1998). Furthermore, the results of preliminary evaluations indicated that the brief period of storage before the experiments began did not affect significantly their viability and germinative capacity.

The low emergence percentages observed in experiments involving pyrenes were consistent with the reported dormancy of macaw palm diaspores under natural conditions (Tabai, 1992; Teixeira, 2005). The inability of the pre-germinative treatments to alleviate dormancy in the pyrenes (in contrast to the seeds) indicated that the endocarp imposes limitations on the germination of seeds still within the pyrenes - a situation that would

severely restrict the utilization of pyrenes in the propagation of macaw palm trees. Various studies have demonstrated the influence of the hard endocarp on palm seed dormancy and its relationship to limitations of water and gas fluxes (Meerow, 1990; Orozco-Segovia *et al.*, 2003; Ferreira and Gentil, 2006) and to the presence of inhibitory substances (Khan, 1982; Fernandes *et al.*, 2007). However, as seeds still within the endocarp had the same water content 60 days after sowing as did isolated seeds, the influence of the endocarp could not be directly related to the restriction of water absorption to the seeds.

Earlier studies reported elevated oxygen demands during palm seed germination (Hussey, 1958; Pech y Aké *et al.*, 2004) and their susceptibility to predation (Orozco-Segovia *et al.*, 2003). As such, the very low germination observed in clay soils in the present study may be related to gas diffusion restrictions in that substrate (especially in light of the compaction of the superficial soil layer) and the greater insect presence observed, especially ants, which could be involved in predation. Specific research will be needed to confirm these possibilities. Seed germination was promoted under greenhouse conditions in experiments in which the operculum was removed and GA was applied - corroborating results reported for other palm species (Hussey, 1958; Nagao *et al.*, 1980; Carpenter *et al.*, 1993; Al-Wasel and Warrag, 1998; Roberto and Habermann, 2010).

The temperature data presented here suggests that low temperatures limit germination, and/or periods with the lowest temperatures act as stratification treatments that can promote germination when temperatures rise. The climate record shows that the observed temperatures during the experiment are close to those that occur in the area where the fruits were collected, where in winter daily thermal amplitudes greater than 10°C are observed for about three months. According to Baskin and Baskin (1998), many effective treatments to overcome dormancy simulate the natural conditions under which species occur and include stratification at low or warm temperatures or storage of seeds at high or alternating temperatures. These treatments has proven effective in overcoming physiological dormancy, the type of dormancy that occurs in Arecaceae species of tropical or subtropical climate such as *Elaeis guineensis* (Hussey, 1958; Rees, 1962) and *Prichard remote* (Perez *et al.*, 2008) respectively.

The inefficiency of stratification for 30 days at 8°C, compared to GA treatment and removal of the opercular tegument (figure 2B), indicates that the treatment period may have been insufficient or that the use of constant temperature is not adequate. The rise in temperature began around week 25 and thermal amplitude decreased significantly from week 30, but germination response was not observed until week 33. These observations suggest that the germination process in *A. aculeata* is slow and either requires a period between the perception of the environmental signal and germination, or that there is a limit related to the temperature that was reached only close to week 33, thus allowing germination. Whereas the germination of the control increased after 33 weeks, but was inferior to many treatments, it is possible to conclude about the heterogeneity of response in the population of seed whose complexity of dormancy requires a combination of treatments.

Gibberellins can stimulate elongation of the embryonic axis as well as weaken seed tissues and mobilize metabolic reserves (Bewley and Black, 1994; Baskin and Baskin, 1998; Finch-Savage and Leubner-Metzger, 2006), although these effects can vary greatly

among different species. Pech y Aké *et al.* (2007) observed a promotional effect of GA on embryo elongation in *Cocos nucifera* L. (Arecaceae) cultivated *in vitro*, and suggested that this response to exogenous gibberellin was related to embryo immaturity.

The response to doses of GA during germination in a humid growth chamber changed during the evaluations, with only higher doses being effective in the first evaluations and increased effectiveness of lower doses at the end of the experiment. These facts indicate that there is heterogeneity among the seeds in relation to the demand pattern of GA_3, as only a proportion of the seeds was stimulated to germinate with an application of 2000 mg L^{-1} GA_3. In the remaining seed, germination was stimulated only by repeating the application of GA_3. Alternatively internal changes may have occurred during the maintenance period in the moist chamber.

The initiation of germination observed in the first week after treating seeds with GA is indicative of an absence of morphological dormancy. This condition would require longer periods (normally 30 days) of embryo growth under favorable conditions before germination could be initiated (Baskin and Baskin, 2004). We have also completed morpho-anatomical studies of macaw palm in which embryos were measured at different stages of development and the ability of the embryo axis to elongate at each stage determined (unpublished data). These studies indicated that the elongation of the embryonic axis is quite rapid and is not restricted by the degree of differentiation of the embryo, occurring concomitantly with the elongation of the cotyledon petiole.

The reduced level of dormancy-break observed with the isolated removal of the opercular tegument (without GA_3) may reflect the restrictive effect of the micropylar endosperm (which was not removed in order to avoid any damage to the embryo). Moura *et al.* (2010) noted that the rigidity of the endosperm in macaw palm is due to the thick walls of its component cells. Gong *et al.* (2005) reported that the rigid endosperm of *P. dactylifera* seeds, which is rich in mannans, likewise restricted embryonic elongation. Additionally, Nagao *et al.* (1980) noted that scarification of the tegument favored penetration of the GA_3 solutions into *A. alexandrae* and *P. macarthurii* (Arecaceae) seeds, which could explain the synergistic effects of the removal of the opercular tegument and the highest concentrations of GA_3 that were observed in the present study. Baskin and Baskin (2004) considered the restrictive mechanical effects of the tissues adjacent to the embryo as an important component of the physiological dormancy, since the non-occurrence of germination is related to the inability of the embryo to grow. In the present study increases in seed germination percentages were only observed after stimulating embryo growth with GA_3, independent of the presence or absence of the opercular tegument. At doses of $GA_3 \geq 500$ mg L^{-1} the embryo acquired a growth capacity capable of overcoming the restrictive effects of the tegument. This resulted in divergence of the responses between treatments with or without the opercular tegument. Studies with different species have shown that the effect of GA_3 on germination may be related to the weakening of the tissues surrounding the embryo and or the stimulation of embryonic elongation (Bewley and Black, 1994; Kucera *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006). Pech y Aké *et al.* (2007) observed positive effects of exogenous GA_3 on the germination of isolated embryos of *Cocos nucifera* L. (Arecaceae) and suggested this fact reflected a hormonal disequilibrium (GA/ABA).

The observed increase in germination after the second immersion in GA_3 indicated that 24 hours was too short a time for the seed / embryo to absorb enough of the plant hormone to induce germination, or that sensitivity to GA_3 increased with time under those experimental conditions (30°C and high humidity). The amount of GA_3 necessary to overcome physiological dormancy is dependent on the degree of restriction of embryonic elongation imposed by the structural characteristics of the endosperm and the tegument, which will vary as a function of the environmental and temporal conditions (Debeaujon and Koornneef, 2000; Finch-Savage and Leubner-Metzger, 2006). Work with several species has demonstrated that seed incubation at high temperatures (as performed in the present study) can overcome physiological dormancy and promote increased sensitivity to GA_3 (Baskin and Baskin, 1998; Finch-Savage and Leubner-Metzger, 2006). On the other hand, Jimenez *et al.* (2008), working with *E. guineensis* showed that dry-heat treatment applied to to the seeds (40°C for 50 days) caused a significant reduction in the level of ABA in the embryo and endosperm, which was related to break dormancy in the species. In *A. aculeata*, although not evaluated in this study, it is possible that a reduced level of ABA also may have occurred, which would favour an increase in the GA_3/ABA ratio that was able to induce germination (Finch-Savage and Leubner-Metzger, 2006). Although germination stabilized after the eighth week, 92% of embryos remained viable in seeds that did not germinate, which suggests that heat treatment was not sufficient to reduce the level of ABA or increase sensitivity to GA_3 in all the seeds.

The percentage of deterioration was relatively high (16%) throughout the experiment. This indicates that the disinfection process needs to be improved. The susceptibility to degradation by microorganisms, common in palm seeds (Meerow, 1990, Orozco-Segovia *et al.,* 2003), is increased due to injuries caused by the process of extraction of seeds from fruits. As the endocarp of *A. aculeata* is extremely hard, seed extraction is only possible by using a bench-top vice to break the structure of the fruit (Bandeira, 2008; Moura *et al.*, 2010). After the process, the seeds that are visibly damaged are discarded. However, seeds with imperceptible damage are commonly used and eventually show a peculiar pattern of deterioration, with necrotic areas coinciding with cracks in the endosperm. The development of more efficient technologies for extraction of the seeds will surely contribute to the advancement in the propagation of the species.

The present study demonstrated that the dormancy of macaw palm diaspores under natural conditions is related to the influence of the endocarp and physiological dormancy and there is a component related to mechanical restriction of the embryo by adjacent tissues. Pyrenes sown into clay soil substrates demonstrated very low emergence percentages, and this technique is not efficient for propagating this species. Under greenhouse conditions, and using sand as a planting substrate, pre-immersion in a GA solution $\geq$ 100 mg L^{-1} for 24h as well as immersion in water at 40°C for 12h were simple and efficient procedures that could be used for large-scale production of *Acrocomia aculeata* seedlings. The removal of the opercular tegument associated with pre-immersion of the seeds for 24h in a solution of 2000 mg L^{-1} GA_3 and repetition of this GA_3 treatment after 4 weeks in a humid growth chamber resulted in greater than 50% germinability in eight weeks, and is considered the best treatment now available to stimulate germination in macaw palm seeds.

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