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Germinative pretreatments to dormancy break in *Guazuma ulmifolia* Lam. seeds

Tratamentos pré-germinativos para superar a dormência de sementes de *Guazuma ulmifolia* Lam.

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ABSTRACT: *Guazuma ulmifolia* is a typical tree species of secondary forests in Brazil, recommended for restoring degraded areas. Their seeds own a mechanical layer in the tegument and slow, irregular and low germination. This work aimed to evaluate methods to dormancy break in order to increase and accelerate seed germination in this species. The fruits were harvested in Jaboticabal, São Paulo, Brazil, in October 1995 and October 1996, and stored in dry chamber. Four experiments were conducted and the seeds were extracted in the date of installation of each experiment. In the first experiment, the seeds were immersed in concentrated sulphuric acid for 0, 10, 15, 20 and 25 min. Germination tests were conducted at 30ºC and it was included one treatment in which the unscarified seeds were incubated at 20-30ºC. In the second experiment, the immersion period was increased until 100 min, in intervals of 10 min, and the seeds of all the treatments were incubated both at 30°C and 20-30ºC. Ungerminated seeds were submitted to tetrazolium test in order to verify their viability. In the third experiment, both scarified (immersion in sulphuric acid for 50 min) and unscarified seeds were imbibed in 0, 50, 100 and 150 ppm gibberellic acid (GA_3) and incubated at 30ºC. In the fourth experiment, freshly and one year stored seeds were used, scarified (immersion in sulphuric acid for 50 min) and unscarified, and incubated at 30ºC. In all the experiments, both germination percentage and speed were evaluated, as well as the viable seeds percentage in the second experiment. The results showed that to obtain better germination, seeds should be treated with concentrated sulphuric acid for 40 to 50 min and the germination tests must be conducted at constant temperature, during 28 days. The addition of gibberellic acid and the alternating temperature were not effective to stimulate seed germination. After one year dry storage, seeds retained both water impermeability and initial germinability.

KEYWORDS: *Guazuma ulmifolia*, Secondary species, Seed, Dormancy, Germination, Viability

RESUMO: *Guazuma ulmifolia* é uma espécie arbórea característica dos estádios iniciais da sucessão secundária, de ocorrência natural em toda América Latina. No Brasil é denominada principalmente de mutamba, sendo recomendada para recuperação de áreas degradadas. Suas sementes apresentam uma camada mecânica no tegumento e germinam em baixa porcentagem, lentamente e de forma irregular. O objetivo deste trabalho foi testar a aplicação de tratamentos pré-germinativos para superar a dormência das sementes dessa espécie. Os frutos foram colhidos em Jaboticabal, SP, em outubro de 1995 e outubro de 1996, e armazenados em câmara seca. Foram conduzidos quatro experimentos e as sementes foram extraídas na data da instalação de cada experimento. No primeiro experimento, as sementes foram escarificadas em ácido sulfúrico concentrado (95-98%) por 0, 10, 15, 20 e 25 min. Os testes de germinação foram conduzidos a 30ºC, tendo sido incluído um tratamento no qual as sementes não escarificadas foram incubadas a 20-30ºC. No segundo experimento, o período de imersão foi aumentado até 100 min, em intervalos de 10 min, e as sementes de todos os tratamentos foram incubadas nas temperaturas constante de 30ºC e alternada de 20-30°C. As sementes remanescentes dos testes de germinação foram submetidas ao teste de tetrazólio, para estimar a sua viabilidade. No terceiro experimento, sementes escarificadas (imersão em ácido sulfúrico por 50 min) e não escarificadas foram embebidas em ácido giberelégico (GA_3), nas concentrações de 0, 50, 100 e 150 ppm, e incubadas a 30°C. No quarto experimento foram utilizadas sementes recém-colhidas e armazenadas por um ano, escarificadas (imersão em ácido sulfúrico por 50 min) e não escarificadas, incubadas a 30°C. Foram avaliadas a porcentagem e a velocidade de germinação das sementes, em todos os experimentos, bem como a porcentagem de sementes viáveis no segundo experimento. Os resultados mostraram que as sementes de mutamba apresentam dormência causada pela impermeabilidade do tegumento à água. Para se obter melhor germinação, as sementes devem ser escarificadas com ácido sulfúrico por 40 a 50 min e os testes de germinação devem ser conduzidos em temperatura constante, com a duração de 28 dias. A adição de ácido giberélico e a alternância da temperatura não foram eficientes para estimular a germinação das sementes. Após um ano de armazenamento em câmara seca, o tegumento das sementes permaneceu impermeável à água e as sementes mantiveram sua germinabilidade inicial.

PALAVRAS-CHAVE: *Guazuma ulmifolia*, Espécie secundária, Semente, Dormência, Germinação, Viabilidade

INTRODUCTION

Guazuma ulmifolia Lam. (Sterculiaceae) is a moderate sized (8-16 m high) typical tree species of initial stages of secondary forests, heliophyte, rustic, of fast growth and native of all Latin America. In Brazil, where it is mainly called “mutamba”, it is recommended for restoring degraded areas and its wood has multiple utility (Pio Correia, 1926; Lorenzi, 1992; Barbosa e Macedo, 1993).

Seeds of the initial stages species of secondary succession (pioneer and early secondary) can remain viable for long time in the soil (Cordini, 1994). These seeds have dormancy mechanisms of relative complexity that can be interpreted as adaptation for the establishment when new gaps are formed (Vázquez-Yanes e Orozco-Segovia, 1991).

The dormancy of seeds by coat impermeable to water is often associated to high natural longevity, important for maintenance of soil seed banks (Rolston, 1978; Koller e Hadas, 1982; Kageyama e Viana, 1991). However, the delay and irregularity of germination in dormant seeds make difficult both laboratory and nursery activities (Bianchetti, 1991).

Hard seeds can be artificially softened by a variety of means that attack the integrity of the coats as acid, thermical, mechanical and manual scarification and alternating temperature (Koller e Hadas, 1982; Ramos e Zanon, 1986; Bianchetti, 1991; Todaria e Negi, 1992). Concentrated sulphuric acid has been used with considerable success on many species (Rolston, 1978) and it is reported to be the most effective treatment in tree species having hard coat (Airi et al., 1998). In some species, the action of sulphuric acid in softening hard seeds appears to result from the removal of cuticle and dissolving of the macrosclerid caps (Rolston, 1978).

In nature, scarification involves the participation of microrganisms, alternating temperature and predator animals (Carvalho e Nakagawa, 1988). So, there is a significant ecological role of seed coat in dormancy, controlling the water imbibition (Rolston, 1978). In some seeds the coat impermeability is influenced by specific environmental complexes, related to the ecological niches that the species occupy (Koller e Hadas, 1982).

However, different causes of dormancy can be associated in seed tree species (Piña-Rodrigues et al., 1990). Multiple methods to dormancy break in seeds involve two or more environmental cues in combination, and few published reports are available (Bell et al., 1993). Chemical stimulators as gibberellic acid can complement the endogen requirement for seed germination (Bewley e Black, 1982; Bell et al., 1993; Airi et al., 1998).

Lorenzi (1992) has recommended that seeds of *G. ulmifolia* be sowed soon after the harvesting without pretreatment, but emphasized that germination is normally low. Barbosa e Macedo (1993) have reported that these seeds has dormancy and require scarification and water immersion as presowing treatment. Studing the seed morphology of this species, Araújo Neto e Aguiar (1999) have detected a mechanical layer in the internal tegument.

Many indigenous tree species, including *G. ulmifolia*, are not mentioned in the Brazilian rules for seed testing (Brasil, 1992). This species also are not included in the schedule reported by Figliolia e Piña-Rodrigues (1995), which specifies the conditions for germination of seeds from 84 tree species, most native of Brazil. The objective of this work was to test methods to dormancy break in *G. ulmifolia* seeds.

MATERIAL AND METHODS

Mature fruits (dry and black) of *Guazuma ulmifolia* were harvested in Jaboticabal, SP, Brazil (21º l5' S, 48º 18' W, elevation 595m) in October 1995 and October 1996, and stored in dry chamber (40% RH) until use. Four experiments were carried out and seeds were extracted at the date of installation of each experiment. Seed moisture content was determined by the kiln at 105ºC method (Brasil, 1992).

The first experiment was installed in October 1995, soon after the harvesting. Seeds were immersed in concentrated sulphuric acid (95-98%) during 0, 10, 15, 20 and 25 min, and washed with tap water immediately after the acid treatment. Germination tests were conducted at 30ºC for 70 days and it was included a treatment in which unscarified seeds were incubated at alternating temperature of 20-30ºC.

In the subsequent experiments the germination tests period was 28 days. The second experiment was installed three months later, in which the immersion period in sulphuric acid was increased until 100 min, in intervals of 10 min. Seeds of all treatments were incubated both at 30ºC and 20-30ºC temperatures. Ungerminated seeds at the end of the tests were immersed in a 0,05% solution of tetrazolium salt for five hours at 30°C (Piña-Rodrigues e Valentini, 1995), in order to verify their viability.

The third experiment was installed in October 1996, soon after the harvesting. Both scarified (immersion in sulphuric acid for 50 min) and unscarified seeds were imbibed in 0, 50, 100 and 150 ppm gibberellic acid (GA_3) and incubated at 30ºC. This chemical was applied on the substrate, which was changed every day.

The fourth experiment was also installed in October 1996, using newly harvested and one year stored seeds. The germination tests were conducted at 30ºC and were tested scarified (immersion in sulphuric acid for 50 min) and unscarified seeds.

In each experiment were used four replicates of 50 seeds per treatment, sowed on filter paper substrate at an eight hour photoperiod. Germinated seeds (normal seedlings) were recorded each day and the germination speed was calculated according to Maguire (1962).

For all the experiments it was adopted a completely randomized experimental design and data of both second and fourth experiments were analised by the factorial scheme. In the second experiment, germination and speed data were also submitted to the polinominal regression analysis. Percentage data were transformed in arcsin $\sqrt{x/100}$ for analysis of variance, but in the tables were expressed in percentage. Means were separated using the test of Tukey.

RESULTS AND DISCUSSION

In the first experiment seeds had 8.7% moisture content and higher germination percentage of unscarified seeds was obtained at 30ºC (Table 1). In that constant temperature, acid scarification increased the germination speed and higher percentage was obtained with 25 min of immersion (Table 1 and Figure 1). For all immersion periods, a great proportion of seeds have germinated until 28 days after the sowing (Figure 1).

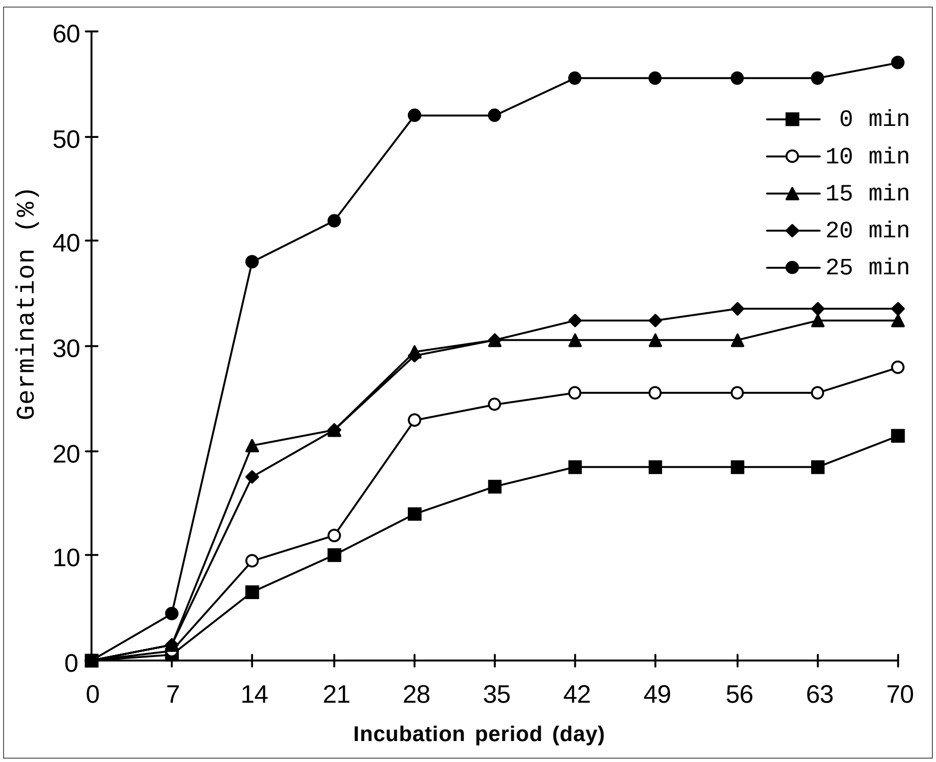

Figure 1. Cumulative germination of *Guazuma ulmifolia* seeds immersed in sulphuric acid for different periods and incubated at 30°C.

(Germinação acumulada de sementes de *Guazuma ulmifolia* imersas em ácido sulfúrico por diferentes períodos e incubadas a 30ºC).

Both germination percentage and speed were lower for unscarified seeds at constant temperature (Table 1). So, the mechanical layer identified by Araújo Neto e Aguiar (1999) in *G. ulmifolia* seed coat restricted the water absorption. Although Lorenzi (1992) have discarded the seed pretreatment for this species, immersion in sulphuric acid for 25 min favoured both germination percentage and speed. Barbosa e Macedo (1993) have recommended immersion in sulphuric acid for 15 min to reach 60% seed germination, but in this experiment, for the used seedlot, only 30% was obtained with this immersion period.

Seed moisture content was 8.8% in the second experiment. For both germination percentage and speed, the interaction immersion period x temperature regime and the results obtained for these parameters from 30 to 90 min of immersion were not significant (Table 2). However, germination was more fast at 30ºC and in this temperature, immersion period exceeding 50 min has significantly reduced the seed viability.

Bewley e Black (1982) and Bonner (1991) have reported that in the same species, there are wide variation in seed coat hardness. According to Allen e Meyer (1998), seed populations exhibit variation in dormancy levels, both among and within plants, which spreads germination across a considerable time period. Maeda e Coelho (1995) have found death of *Rubus idaeus* seeds by high temperature in its interior, caused by excessive immersion period in sulphuric acid. The authors reported that the diminution of celular membranes integrity can allow lixiviation of exudates inside the seeds, those reacted with the sulphuric acid, causing elevation of temperature. So, for longer immersion period, possibly the treatment was excessively drastic for both permeable and less impermeable coat, allowing the acid absorption and damaging the embryo of *G. ulmifolia* seeds.

Table1. Germination percentage and speed of *Guazuma ulmifolia* unscarified seeds incubated at 30ºC and 20-30ºC, and of seeds immersed for different periods in sulphuric acid and incubated at 30ºC.

(Porcentagem e velocidade de germinação de sementes de *Guazuma ulmifolia* não escarificadas, incubadas a 30ºC e 20-30ºC, e de sementes imersas em ácido sulfúrico por diferentes períodos e incubadas a 30ºC).

Temperature	Immersion period	Germination Percentage[1]	Germination Speed
20-30ºC	0 min	7.0 *c*	0.10 *d*
30ºC	0 min	21.0 *b*	0.59 *cd*
	10 min	29.0 *b*	0.91 *bc*
	15 min	33.0 *b*	1.45 *ab*
	20 min	33.5 *b*	1.44 *ab*
	25 min	57.0 *a*	2.06 *a*
F value		22.54**	19.03**
Variation coefficient		14.70%	29.69%

(1) Means are expressed in percentage, but statistical analisys were conducted with data transformed in arcsin.

(*a,b*) Means for each column followed by the same letter are not significantly different (P>0,05).

Acid scarification has some drawbacks as (a) seeds are too easy to kill with excessive treatment time; (b) insufficient washing may leave lethal amounts of acid on the seeds; and (c) acids are dangerous for unskilled workers to handle (Bonner, 1991). However, the small size of *G. ulmifolia* seeds difficults both the manual and mechanical scarification. Barbosa e Macedo (1993) have recommended hot water soaking for 18 hours, but this method is time consumer and also has potential for seed damage.

For some species, temperature fluctuation is a factor in the softening of impermeable seed coat (Rolston, 1978). However, rather than alternating, constant temperature shortened the time for seed germination, as in the first experiment. So, fluctuation of temperature was ineffective for dormancy breaking in *G. ulmifolia*

Table 2. Germination percentage and speed of *Guazuma ulmifolia* seeds immersed in sulphuric acid for different periods and incubated at 30ºC and 20-30ºC, and viability of ungerminated seeds after incubation at 30ºC.

(Porcentagem e velocidade de germinação de sementes de *Guazuma ulmifolia* imersas em ácido sulfúrico por diferentes períodos e incubadas a 30ºC e 20-30ºC, e viabilidade das sementes não germinadas após incubação a 30ºC).

Immersion	Germinated seeds		Ungerminated seeds
period	Germinability[1]	Speed	Viability[1]
0 min	23.0 *d*	0.75 *c*	34.5 *a*
10 min	36.0 *cd*	1.59 *bc*	29.0 *a*
20 min	33.5 *cd*	1.56 *bc*	27.5 *a*
30 min	49.5 *abcd*	2.49 *ab*	27.0 *a*
40 min	58.0 *abc*	2.85 *ab*	26.0 *a*
50 min	62.1 *ab*	3.31 *a*	26.0 *a*
60 min	54.8 *abc*	2.28 *abc*	15.0 *b*
70 min	65.6 *a*	3.30 *a*	8.5 *bc*
80 min	40.8 *abcd*	2.02 *abc*	7.5 *bc*
90 min	43.8 *abcd*	2.18 *abc*	4.0 *c*
100 min	32.5 *cd*	1.74 *bc*	4.0 *c*
Mean at 30ºC	45.9 *a*	2.45 *a*	19.0
Mean at 20-30ºC	44.8 *a*	1.92 *b*	-
F for period (P)	5.3**	5.35**	27.14*
F for temperature (T)	0.09^{ns}	6.51*	-
F for interaction (PxT)	1.45^{ns}	1.41^{ns}	-
Variation coefficient	24.84%	45.89%	22.89%

(1) Means are expressed in percentage, but statistical analisys were conducted with data transformed in arcsin.

(*a,b*) Means for each column followed by the same letter are not significantly different (P>0,05).

seeds. At 30ºC, higher both germination percentage and speed were obtained about 50 min of immersion in sulphuric acid (Figure 2). Immersion for 40 min also may be used, since it promoved better germinative behaviour in relation to unscarified seeds (Table 2). For less dormant seedlots, smaller damage on viability is expected with this immersion period.

In the third experiment, seeds had 8.5% moisture content. The addition of gibberellic acid was not effective for seed germination, both in abscence and presence of sulphuric acid (Table 3). For all the gibberellic acid concentrations tested, seeds had the same germinative behaviour and acid scarification was the only effective pretreatment for germination in *G. ulmifolia* seeds.

On the other hand, in *Anigozanthus manglesii* seeds, both scarification and addition of gibberellic acid were required to dormancy break (Sukhivibul, 1991, *apud* Bell et al., 1993). Among dormant seeds, germination of both those with coat-imposed dormancy and those with embryo dormancy are promoted by gibberellic acid (Bewley e Black, 1982). This stimulator favours the release of an enzyme

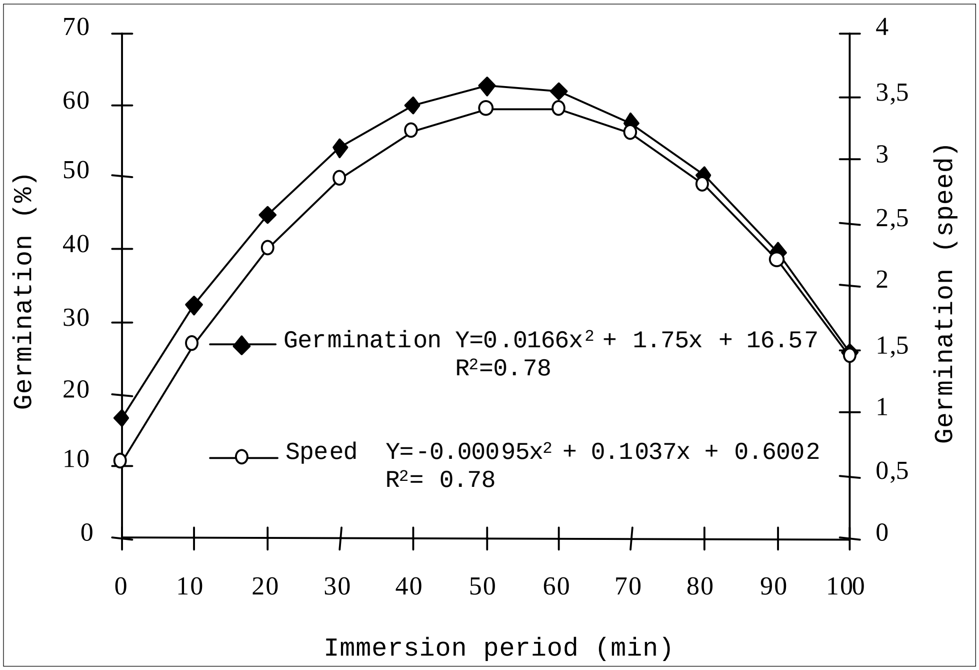

Figure 2. Germination percentage and speed of *Guazuma ulmifolia* seeds immersed in sulphuric acid for different periods and incubated at 30ºC.

(Percentagem e velocidade de germinação de sementes de *Guazuma ulmifolia* imersas em ácido sulfúrico por diferentes períodos e incubadas a 30ºC).

which weakens the tensile strength of the seed coat and is reported to be responsible for mobilization of nutrients from endosperm to the embryo (Airi et al., 1998).

In the fourth experiment the stored and those newly harvested seeds had 8.5 and 10.5% moisture content, respectively. Seeds harvested in 1995 and dry stored for one year had a similar behaviour than those newly harvested in 1996 (Table 4). More that 20% of unscarified seeds newly harvested in 1995 have germinated at 30ºC (Table 1), but very low germination of unscarified seeds harvested in 1996 was obtained (Table 4). This difference can be result of wide variation in seed coat hardness within a species due to crop year, among several other factors, as have been reported by Bewley e Black (1982) and Bonner (1991).

G. ulmifolia seed moisture content varied from 8.5 to 8.8% during storage in dry chamber (experiment 1 to 4) and have retained water impermeability, since the unscarified seeds almost have not germinated (Table 4). Water impermeability is not an absolute condition, but it can be reversed by dry storage; the results obtained in this work are consistent, as coat impermeability is essentially irreversible during storage at lower than 10% seed moistures (Rolston, 1978). Maximum germination percentage in Table 1 was similar to the one showed in Table 4; hence, initial germinability was also retained after one year dry storage.

Seeds of *Mimosa bimucronata* stored for one year germinated substantially more than freshly seeds. During the storage, coat permeability has raised, decreasing the number of dormant seeds and making easy the imbibition (Ferreira et al., 1992). Unscarified seeds of *Senna macranthera*, both freshly and one year stored had low germination (1%),

Table 3. Germination percentage and speed of *Guazuma ulmifolia* seeds scarified and unscarified, submitted to different concentrations of gibberellic acid and incubated at 30ºC.

(Percentagem e velocidade de germinação de sementes de *Guazuma ulmifolia* escarificadas e não escarificadas, submetidas a diferentes concentrações de ácido giberélico e incubadas a 30ºC).

Treatment	Germination	
	Percentage[1]	Speed
Control	0.0 *b*	0.00 *c*
GA_3 (50 ppm)	3.5 *b*	0.24 *c*
GA_3 (100 ppm)	1.5 *b*	0.10 *c*
GA_3 (150 ppm)	8.8 *b*	0.14 *c*
H_2SO_4	69.0 *a*	7.56 *a*
H_2SO_4 + GA_3 (50 ppm)	55.0 *a*	4.81 *b*
H_2SO_4 + GA_3 (100 ppm)	57.0 *a*	5.25 *ab*
H_2SO_4 + GA_3 (150 ppm)	71.0 *a*	6.46 *ab*
F value	83.37**	42.36**
Variation coefficient	18.64%	32.62%

(1) Means are expressed in percentage, but statistical analisys were conducted with data transformed in arcsin.

(*a*,*b*) Means for each column followed by the same letter are not significantly different (P>0,05).

whereas those two year stored presented higher germination (22%), showing wastage of coat. Two year stored seeds were more sensible to sulphuric acid action than those one year stored and these were more sensible than those freshly seeds (Santarém e Aquila, 1995).

Table 4. Germination percentage and speed of *Guazuma ulmifolia* seeds harvested in 1995 and 1996, unscarified and scarified, and incubated at 30ºC. Seeds harvested in 1995 were dry stored for one year.

(Percentagem e velocidade de germinação de sementes de *Guazuma ulmifolia* colhidas em 1995 e 1996, não escarificadas e escarificadas, e incubadas a 30ºC. As sementes colhidas em 1995 permaneceram armazenadas em câmara seca por um ano).

Treatment	Germination	
	Percentage[1]	Speed
Scarified seeds	57.0 *a*	4.51 *a*
Unscarified seeds	3.6 *b*	0.15 *b*
F value for scarification (S)	443.54**	75.54**
F value for seed age (A)	0.42^{ns}	3.58^{ns}
F value for interaction (SxA)	2.49^{ns}	2.70^{ns}
Variation coefficient	12.20%	60.93%

(1) Means are expressed in percentage, but statistical analisys were conducted with data transformed in arcsin.

(*a,b*) Means for each column followed by the same letter are not significantly different (P>0,05).

The maintenance of both coat impermeability and seed germinability observed in this work suggest that *G. ulmifolia* contribute to the soil seed bank composition. Due to the uncontrolled natural conditions in the field, it is important to study, in posterior research, both coat impermeability and seed longevity in the soil.

CONCLUSIONS

The results obtained in this study allow the following conclusions:

✓ Seeds of *Guazuma ulmifolia* have dormancy imposed by water impermeable coat;

✓ To obtain better germination, seeds must be treated with concentrated sulphuric acid for 40 to 50 min;

✓ The germination tests must be conducted at constant temperature (30ºC) for 28 days;

✓ The addition of gibberellic acid and alternating temperature were ineffective for seed dormancy break;

✓ After one year dry storage, both seed coat impermeability and initial germinability were retained.

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