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Temperature and substrate on *Plukenetia volubilis* L. seed germination

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A B S T R A C T
The objective of this work was to evaluate the effect of temperature and substrate on the germination of *P. volubilis* seeds. Seeds harvested from 25 matrix plants were submitted, in two studies, to conditions of (i) sowing in rolled paper towel at the temperatures of 10, 15, 20, 25, 30, 35, 40, and 45 °C, for the evaluation of germination, first count of germination, germination speed index and mean time for germination, and (ii) sowing in the substrates paper towel, sand, Bioplant®, Bioplant® and micron, superfine, fine, medium and coarse vermiculite. The same evaluations mentioned in the first study were conducted at the temperature of 30 ºC, as well as plant growth. The treatment replicates were distributed in a completely randomized block design and the effects of temperature were compared by polynomial regression analysis. The substrates were compared by the Scott-Knott test at 0.05 probability level. The data show that the ideal range of temperature for the germination of *P. volubilis* is between 25 and 30 °C. The temperature of 20 °C is the minimum for germination and those above 35 °C are lethal to these seeds. The most favorable substrate for *P. volubilis* seed germination is micron or fine vermiculite.

Palavras-chave:
sacha inchi
oleaginosa
Euphorbiaceae
ômega-3
planta da Amazônia

Temperatura e substrato na germinação de sementes de *Plukenetia volubilis* L.

R E S U M O
Objetivou-se, neste trabalho, avaliar o efeito de temperaturas e substratos na germinação de sementes de *P. volubilis*. Sementes colhidas em 25 plantas matrizes foram avaliadas em dois estudos: (i) semeadura em rolo de papel nas temperaturas 10, 15, 20, 25, 30, 35, 40 e 45 ºC, avaliando-se a germinação, primeira contagem, índice de velocidade de germinação e tempo médio de germinação; e (ii) semeadura nos substratos rolo de papel, entre areia, entre Basaplant®, entre Bioplant® e entre vermiculita mícron, superfina, fina, média e grossa; conduzindo-se a 30 ºC os mesmos testes descritos no primeiro estudo e também o crescimento de plântulas. Os dados foram analisados em delineamento inteiramente casualizado com análise de regressão polinomial para a avaliação das temperaturas. Para a comparação entre os substratos utilizou-se o teste de Scott Knott, a 0,05 de probabilidade. Conclui-se que a germinação de sementes de *P. volubilis* deve ser conduzida nas temperaturas de 25 a 30 ºC. A temperatura de 20 ºC é a mínima para a germinação e as acima de 35 ºC são letais para essas sementes. O substrato mais favorável à germinação de sementes de *P. volubilis* é a vermiculita mícron ou fina.

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Introduction

Plukenetia volubilis L. is a semi-perennial climbing plant from the Amazon, cultivated in Peru (Hamaker et al., 1992) and Colombia (Gutiérrez et al., 2011). The oil extracted from its seeds has been industrialized by the pharmaceutical and cosmetic sectors, which has increased the demand for seedlings for planting (Follegatti-Romero et al., 2009; Gutiérrez et al., 2011; Ruiz et al., 2013). For the production of seedlings of a certain species, it is important to identify temperatures and substrates that favor their germination and growth (Martins et al., 2012). Temperature acts on the speed of water absorption and biochemical reactions that determine the entire process of germination, and the substrate is also important, because its chemical composition, texture and structure influence the availability of water, oxygen, light and nutrients (Lima et al., 2010; Weitbrecht et al., 2011; Silva et al., 2014).

Therefore, as favorable substrates for seed germination, studies have reported sand, for *Solanum sessiliflorum* Dunal (Lopes & Pereira, 2005), and vermiculite, for *Acosmium nitens* (Vog.) Yakovlev and *Eugenia uniflora* L. (Varela et al., 2005; Sena et al., 2010), as examples of species that occur in the Amazon. For subtropical and tropical forest species, the optimal temperature for germination is usually situated between 20 and 30 °C, since these temperatures occur in their regions of origin in the period favorable to natural germination (Borges & Toorop, 2015). The temperature of 25 °C is ideal for the germination of seeds of *S. sessiliflorum*, *Copaifera langsdorffii* Desf. and *Anadenanthera colubrina* (Vellozo) Brenan (Lopes & Pereira, 2005; Brasil, 2013), while 30 °C is ideal for *A. nitens*, *Theobroma grandiflorum* (Wild ex Spreng Schum) and *Genipa americana* L. (Varela et al., 2005; Ferraz et al., 2012; Brasil, 2013), because they allow maximum germination in the shortest period of time.

In this context and due to the lack of information on the optimal temperature and the adequate substrate for the germination of this species, this study aimed to evaluate the effect of temperatures and substrates on the germination of *P. volubilis* seeds.

Material and Methods

P. volubilis seeds were obtained from ripe fruits, with dark brown color, harvested in the crown of 25 matrix trees in Manaus, AM (3° 8' S; 59° 52' W). After drying the fruits in the shade, the seeds were extracted, placed in Kraft paper bag and taken to the laboratory. The seeds had water content of 8%, determined at 105 ± 3 °C for 24 h (Brasil, 2009). The germination potential of the seeds was evaluated in two studies:

The first study evaluated germination temperatures of 10, 20, 25, 30, 35, 40 and 45 °C in regime of 8 h of light and 16 h of darkness. For this, four replicates of 20 seeds were sown in each treatment, in rolls of paper towel previously moistened with distilled water, at the proportion of 2.5 times the dry weight of the substrate. Germinated seedlings were considered as those with hypocotyl-radicle axis longer than 6 cm and epicotyl present between the cotyledons at 60 days after sowing. The results were expressed in percentage of germination (Brasil, 2009). Simultaneously to the germination test, the speed of the process was evaluated through the following variables:

First count of germination – performed 9 days after sowing, through the count of the normal seedlings present in the test. The counting date was established based on the criterion of 50% of seed germination in most treatments (Brasil, 2009; Ferraz et al., 2012).

Germination speed index – determined through counts every two days and applying the equation established by Maguire (1962).

Mean time for germination – calculated using the formula proposed by Labouriau & Valadares (1976), with results expressed in days.

The second study evaluated the following substrates: roll of paper towel, between sand, between Basaplant® (Pine bark, vermiculite, peat, charcoal, nitrogen, phosphorus, potassium and micronutrients), between Bioplant Prata® (Pine bark, aged manure, sawdust, coconut fiber, vermiculite, rice hull, ash, agricultural gypsum, calcium carbonate, magnesium, magnesium thermophosphate, nitrogen, phosphorus, potassium and micronutrients) (Lopes et al., 2013; Bioplant, 2015) and between micron (particles smaller than 0.46 mm), superfine (particles smaller than 0.65 mm), fine (particles smaller than 1.29 mm), medium (particles smaller than 2.60 mm) and coarse (particles smaller than 5.20 mm) vermiculites (Ugarte et al., 2005).

The tests were installed with seven replicates of 20 seeds and conducted at the most favorable temperature, identified in the previous step. The substrate paper was moistened with 2.5 times its dry weight in water. The sand, commercial substrates and vermiculite were moistened with 60% of their water retention capacity, according to the recommendations for sand of the Rules for Seed Analysis (Brasil, 2009). Germination in sand, Basaplant®, Bioplant® and vermiculite was conducted in transparent plastic boxes (18.0 x 13.5 x 6.5 cm) containing 2.0 cm of substrate, with sowing performed at the depth of 1.0 cm.

The effect of the substrates on the germination performance of the seeds was evaluated by the previously described tests of germination, first count of germination and germination speed index. At the end of the test, the percentages of hard and dead seeds were also calculated.

The normal seedlings produced were measured using a ruler graduated in centimeter for the determination of shoot length (from the base to the insertion of the first leaf) and root length (from the apex of the main root to the base). The weight of shoot and root dry matter of the seedlings was determined after drying the normal seedlings in Kraft paper bags in a forced-air oven at 65 °C for 48 h (Nakagawa, 1999).

The statistical design was completely randomized. For the evaluation of temperatures, the means were fitted to polynomial regression models, while the means of the substrates were compared by the Scott-Knott at 0.05 probability level. Before the analyses, the percent data were transformed to arc sine $((x+1)/100)^{1/2}$ (Banzatto & Kronka, 2006). The means presented in the tables are from the original data.

Results and Discussion

There was significant effect of temperature on all variables related to the germination of *P. volubilis* seeds

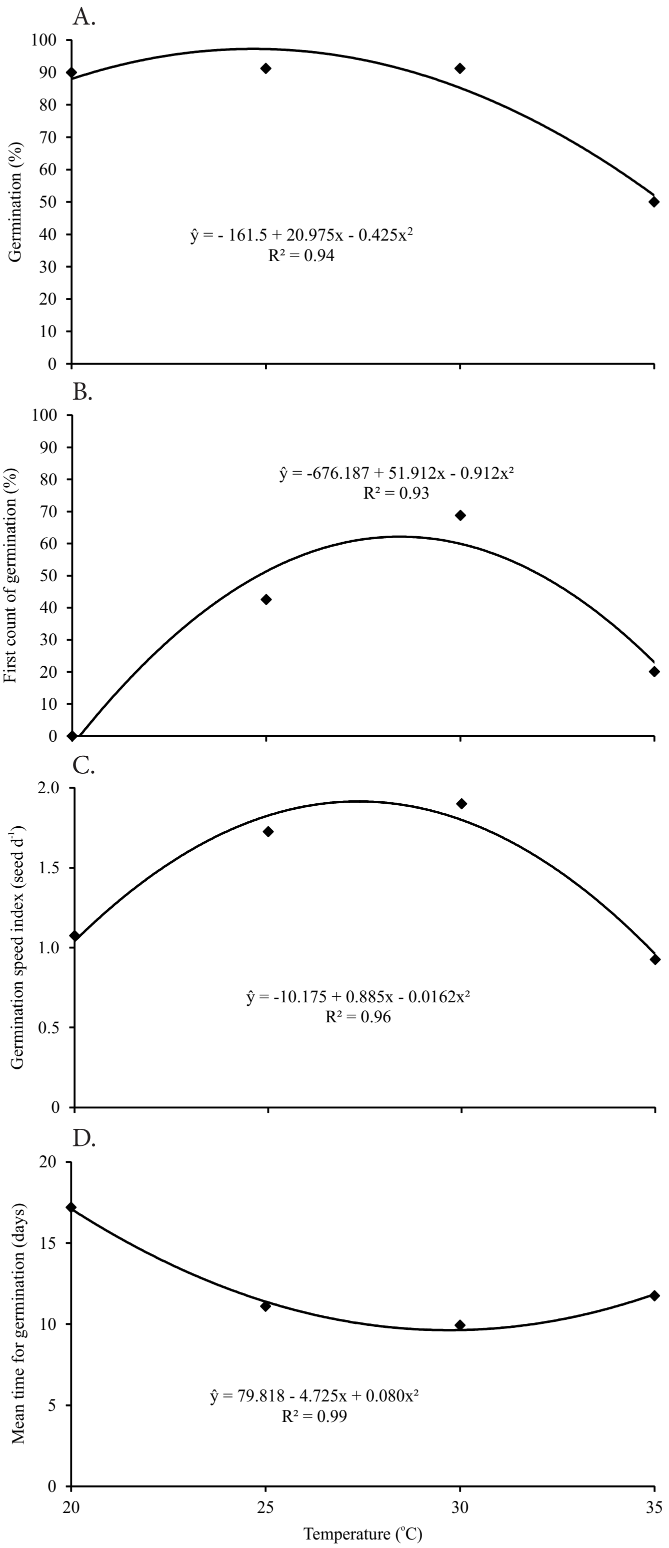

Figure 1. Germination (A), first count of germination (B), germination speed index (C) and mean time for germination (D) of *Plukenetia volubilis* L. seeds germinated at different temperatures

(Figure 1). Germination occurred between 20 and 35 °C, with maximum of 97% at 25 °C (Figure 1A). The temperature of 25 °C has also been recommended for the conduction of germination tests of other Euphorbiaceae plants, such as *T. grandiflorum*, *Croton floribundus* Spreng and *Croton urucurana* Baill, *J. curcas* and *Joannesia princeps* Vellozo (Ferraz et al., 2012; Brasil, 2013).

At temperatures above 30 °C, there was a tendency of reduction in germination, intensified at 35 °C (Figure 1A). The sharp decrease in the germination percentage of the seeds from 35 °C on must have been caused by damages to their structure, because high temperatures inhibit embryo development, cause enzymatic alterations and reduce the amount of free amino acids of the synthesis of RNA, modifying the speed of metabolic reactions (Weitbrecht et al., 2011; Borges & Toorop, 2015).

At temperatures lower than 20 °C, between 10 and 15 °C, it was possible to observe that the seeds absorbed water, because they increased in size; however, germination did not complete and there was no production of root, even after 60 days of test. Therefore, 15 °C can be classified as the minimum temperature, the value below which germination does not occur (Carvalho & Nakagawa, 2012).

The absence of germination was also observed in seeds that germinated at temperatures above 35 °C (between 40 and 45 °C); however, at the latter ones, *P. volubilis* seeds showed evident signs of deterioration and death, such as: darkened tegument, fungi proliferation and release of exudates in the substrate. Hence, for the seeds of this species, the temperature of 40 °C can be classified as maximum temperature, the value above which germination does not occur (Carvalho & Nakagawa, 2012).

In the evaluation of germination speed through the tests of first count, speed index and mean time for germination, the best performances of the seeds (62% of germination, speed index of 1.91 and time of 10 days for germination) were observed at 29, 27 and 30 °C, respectively (Figure 1B, C and D). Therefore, the temperatures most favorable to germination speed and that allowed the formation of seedlings in shorter time were higher than those observed to obtain maximum germination percentage, as reported for the seeds of *J. curcas* (Pascuali et al., 2012). This phenomenon can be explained by the acceleration of the metabolic reactions of the seeds due to the high temperatures, associated with the denaturation of proteins and alteration of cell membranes, so that the number of seeds that are able to complete germination rapidly decreases (Kapoor et al., 2011).

Considering the data of final germination percentage and the speed of this process, it is inferred that temperatures between 25 and 30 °C can be considered as optimal for the conduction of the test of seed germination and production of *P. volubilis* seedlings (Figure 1), because they allowed maximum germination in the shortest time.

As to the effect of the substrates on *P. volubilis* germination, micron and fine vermiculites allowed maximum seed germination, with values of 92 and 95%, respectively. These percentages of germination were significantly higher than those of the seeds in the other substrates tested (Table 1).

It should be pointed out that the vermiculite with these two particle sizes led to favorable results to the seeds, which were similar with respect to the germination speed index and the percentages of hard and dead seeds; however, the fine vermiculite allowed a faster germination compared with micron vermiculite, according to the test of first count, with values of 64 and 54%, respectively.

Germination speed can be influenced by the texture of the substrate and, depending on the species, the seeds may

Table 1. Mean values of germination percentage (G), first count of germination (FCG), germination speed index (GSI), hard seeds (H) and dead seeds (D) of *Plukenetia volubilis* L. from different substrates

Substrates	G	FCG	GSI	H	D
	(%)			(%)	
Paper	71 c	67 a	1.44 b	0 a	29 b
Sand	87 b	67 a	1.52 a	9 b	4 a
Bioplant®	79 b	44 b	1.31 b	13 b	8 a
Basaplant®	56 d	2 e	0.69 d	39 c	5 a
Micron vermiculite	92 a	54 b	1.56 a	5 a	3 a
Superfine vermiculite	82 b	13 d	1.14 c	13 b	5 a
Fine vermiculite	95 a	64 a	1.60 a	1 a	4 a
Medium vermiculite	87 b	59 a	1.39 b	2 a	11 a
Coarse vermiculite	56 d	27 c	1.00 c	32 c	12 a
F treatment	24.8**	39.2**	21.5**	25.0**	4.9**
C.V. (%)	9.9	23.3	13.2	32.0	48.1

**Significant by F test at 0.01 probability level. Means followed by the same letter in the column do not differ by the Scott-Knott test at 0.05 probability level

Table 2. Mean values of length and dry matter of shoots and roots of *Plukenetia volubilis* L. seedlings from different substrates

Substrates	Length (cm)		Dry matter (g)	
	Shoots	Roots	Shoots	Roots
Paper	4.4 e	12.3 b	0.139 d	0.082 c
Sand	10.8 c	8.3 d	0.297 b	0.091 c
Bioplant®	22.4 a	14.9 a	0.383 a	0.166 a
Basaplant®	8.7 d	8.3 d	0.272 c	0.119 b
Micron vermiculite	16.8 b	11.2 c	0.371 a	0.139 b
Superfine vermiculite	8.7 d	9.0 d	0.261 c	0.134 b
Fine vermiculite	16.5 b	9.6 d	0.327 b	0.136 b
Medium vermiculite	10.9 c	13.3 b	0.305 b	0.146 b
Coarse vermiculite	11.4 c	10.6 c	0.323 b	0.126 b
F treatment	84.9**	14.5**	18.7**	9.2**
C.V (%)	12.60	13.97	14.83	18.20

**Significant by F test at 0.01 probability level. Means followed by the same letter do not differ by the Scott-Knott test at 0.05 probability level

exhibit better germination performance in vermiculite with certain granulometry. This phenomenon was observed for seeds of *Archontophoenix alexandrae* H. Wendl & Drude in micron vermiculite (Martins et al., 2011), *E. uniflora* (Sena et al., 2010) and *Adenanthera pavonina* L. (Alves et al., 2015) in fine vermiculite.

Basaplant® and coarse vermiculite were the least adequate substrates for the germination of *P. volublis* seeds, because they led to the lowest percentages of germination and highest percentages of hard seeds. A probable reason for this phenomenon would be a lower water retention capacity of these substrates in comparison to the others, as reported by Alves et al. (2015) for seeds of *A. pavonina*. Another factor that may have influenced water absorption by the seed is the area of contact between the seed coat and the substrate (Carvalho & Nakagawa, 2012), considering that substrates with larger particle sizes have smaller surface of contact with the seeds.

The other substrates, paper, sand, Bioplant® and superfine and medium vermiculite, led to intermediate values of germination, between 71 and 87%, and the seeds that did not germinate in the roll of paper were dead (Table 1). Therefore, it can be claimed that the substrate paper caused the death of the seeds, as observed for the seeds of other species of the Amazon, such as *S. sessiliflorum* (Lopes & Pereira, 2005) and *A. nitens* (Varela et al., 2005).

The substrate roll of paper is the most used for the standard test of germination according to the Rules for Seed Analysis (Brasil, 2009), due to the ease of standardization, practicality in the utilization and disposal and for occupying less space in the storage.

The utilization of Bioplant® as substrate allowed maximum growth of *P. volubilis* seedlings, observed through the length and dry matter of shoots and roots, in comparison to the others, although it did not differ regarding shoot dry matter from seedlings produced in micron vermiculite (Table 2).

Bioplant® has in its composition calcium carbonate, magnesium, magnesium thermophosphate, ashes, aged manure, coconut fiber, rice hull and sawdust; these components are not found in the other substrates (Lopes et al., 2013; Bioplant, 2015). Therefore, from seed germination and root growth on, the absorption of the nutrients present in the substrate and their mobilization to the structures of the seedlings began, promoting their development, as observed for *Peltophorum dubium* (Spreng.) Taub. by Dutra et al. (2012).

Although Bioplant® favored the growth of the seedlings, it was not one of the most favorable to seed germination; hence, a strategy that could be used for the production of *P. volubilis* seedlings would be the planting of seeds in micron or fine vermiculite and later transplanting the pre-germinated seeds to the substrate Bioplant®.

Conclusions

1. The germination of *P. volubilis* seeds must be conducted at temperatures from 25 to 30 °C.

2. The temperature of 20 °C is the minimum for germination and those above 35 °C are lethal to the seeds.

3. The substrate most favorable to the germination of *P. volubilis* seeds is micron or fine vermiculite.

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