



A comparative study of the thermal ranges of three germination criteria of a tropical tree with bioeconomic interest: *Carapa surinamensis* Miq. (Meliaceae)

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Abstract

Species of the *Carapa* spp. complex, occurring in the Neotropics, Africa and India, have multiple uses, including timber, with the seed oil being used in phyto-pharmaceutical products and cosmetics. This study aimed to determine the thermal ranges of the germination process, comparing germination criteria used by seed physiologists and seed technologists, and to suggest recommendations for seed quality assessment. Germination was assessed at constant temperatures between 10 – 40 °C using three germination criteria: (1) radicle length ≥ 0.5 cm (physiological criterion); (2) epicotyl length ≥ 1 cm; and (3) epicotyl length ≥ 5 cm (criterion for seed quality tests). The base temperature was similar for the three criteria and ranged between 10 – 2 °C. The Maguire's Speed Index indicated 30 °C as most adequate. However, the upper temperature limit differed: for radicle protrusion it was above 40 °C; and for both epicotyl lengths, it was between 35 – 40 °C. Seed coat removal accelerated the germination process of these recalcitrant seeds, and is recommended for seed quality assessment, which allows completion of the germination trial in approximately one month.

Keywords: optimum temperature, pre-germination treatment, *Carapa procera* DC., seed testing, crabwood.

Estudo comparativo sobre a variação térmica em três critérios de germinação de uma árvore tropical com interesse econômico: *Carapa surinamensis* Miq. (Meliaceae)

Resumo

As espécies do complexo *Carapa* spp. ocorrem na região Neotropical, na África e na Índia, têm usos múltiplos, fornece madeira de valor comercial e o óleo extraído das sementes tem uso fitoterápico e cosmético. O objetivo deste trabalho foi determinar a faixa térmica tolerável do processo germinativo, comparando os critérios de germinação utilizados pelos fisiologistas e os tecnólogos de sementes, e sugerir recomendações para a avaliação da qualidade das sementes. A germinação foi avaliada em temperaturas constantes entre 10 e 40 °C utilizando três critérios de germinação: (1) formação da radícula $\geq 0,5$ cm (critério fisiológico); (2) alongamento de epicótilo ≥ 1 cm; e (3) alongamento de epicótilo ≥ 5 cm (critério para testes de qualidade de sementes). A temperatura de base foi semelhante para os três critérios entre 10 e 12 °C. O índice de velocidade de Maguire indicou 30 °C como a temperatura mais adequada. O limite superior de temperatura diferiu entre os critérios, sendo acima de 40 °C para protrusão da radícula e para ambos os alongamentos de epicótilo entre 35 e 40 °C. A remoção do tegumento de semente acelerou o processo de germinação dessas sementes recalcitrantes sendo recomendada para a avaliação da qualidade da semente, o que permite concluir o teste de germinação em aproximadamente um mês.

Palavras-chave: temperatura ótima, tratamento pré-germinativo, *Carapa procera* DC., avaliação de sementes, andiroba.

1. Introduction

Tropical forests harbour a high diversity of commercially important multiple-use tree species. Some of the best-known are those of *Carapa* spp. (Meliaceae). Several species of *Carapa* occur in the Neotropics as well as in tropical Africa and India (Kenfack, 2011a), and are used in similar ways. In the Amazon region they are commonly known as

andiroba, andirobinha, crabwood, roba-mahogany, among others (Ferraz et al., 2002). The tree, suitable for timber, is planted in agroforestry systems (Guarino et al., 2014). Crabwood seed oil is raw material for phyto-pharmaceutical products and cosmetics and is exported from Brazil to more than 50 international destinations (Enriquez, 2009).

The majority of the oil comes from naturally growing stands. Conservation of these populations, as well as the efficient and sustainable use of the natural resource is a priority. Thus, at the beginning of the production chain, appropriate seed handling is crucial.

Carapa surinamensis Miq. is restricted to the Guianan Shield and Central Amazonia. The species was regarded as a synonym of *Carapa procera* DC. by Pennington et al. (1981); however, based on the recent revision of the genus, the occurrence of *C. procera* is restricted to the African continent, and the Neotropical *C. procera* is now known as *C. surinamensis* (Kenfack, 2011b). Thus, earlier publications on seeds and seedlings of *C. surinamensis* collected near Manaus were published under the name *C. procera* (Fisch et al., 1995; Ferraz and Sampaio, 1996; Connor et al., 1998; Ferraz et al., 2002; Ferraz and Varela, 2003).

Requirements related to temperature can contribute to a greater understanding of the geographic distribution of a species, the strategies for establishment (Labouriau, 1983; Larcher, 2000), and may even predict adaptive responses to climate changes. For seed quality assessment, a prerequisite for seed commercialization, the most appropriate temperature for seed germination needs to be known, as only under optimal conditions are germination rates and capacity highest. A rapid quality assessment is crucial especially for desiccation-sensitive seeds, as in the case of *C. surinamensis* (Connor et al., 1998; Sanogo et al., 2013), and no information on its germination temperatures are currently available.

The germination process, from a physiological point of view, is completed when an organ of the expanding embryo penetrates the seed coat however; for seed quality assessment, a later developmental stage is assessed. According to the International Seed Testing Association (International Seed Testing Association, 2015), the seedling must have the potential for continued development into a satisfactory plant under favourable environmental conditions. Only a few studies compare different temperatures with several germination criteria. Depending in which context the results will be needed, the physiological or technical criterion will be assessed. However, thermal requirements may be different for radicle protrusion than for more advanced stages in seedling development, as demonstrated, e.g., for a tree and a palm species from the Amazon region (Miranda and Ferraz, 1999; Bastos et al., 2017).

In light of the above, this study aims to gain an understanding of the thermal range, as well as the optimum temperature, for seed germination of *C. surinamensis*, taking into account three developmental stages to comply with needs of both physiologists and technologists, and to suggest recommendations for seed quality assessment.

2. Material and Methods

Fruits and seeds were collected at the Adolpho Ducke Forest Reserve (59° 52' 40" – 59° 52' 00" W, 03° 00' 00" – 03° 08' 00" S) and the Tropical Sylviculture Research

Station (02° 35' 55.5" S, 60° 02' 14.8" W) in plantations set up in the 1970's and 1980's, respectively. Both areas are in *terra firme* forest, located north of Manaus, Amazonas, and under the responsibility of the Brazilian National Institute for Amazonian Research (INPA). Representative specimens were collected and deposited in the INPA herbarium with accession numbers 258150 and 268723. During natural seed shedding, material was collected and weight (± 0.001 g) was determined for 100 randomly selected seeds. However, for the germination tests only average-sized seeds (± 1 standard deviation) were used. In view of the large seed size, seed moisture content was determined on 20 individual seeds for each experiment, cut into four parts and dried at 105 ± 2 °C until a stable moisture content was reached to a precision of 0.1% in repeated weighings every 24 hours.

Seeds were processed manually by removing the valves of the capsule-type fruits. Seeds with visible damage were eliminated. After submersion in water for 24 h to drown potential larvae of *Hypsipyla* sp., a lepidopteran known as a seed borer, the seeds were superficially dried above wires at room temperature (25 ± 4 °C; 45 – 60% RH) for about 2 h. Owing to the recalcitrant characteristic of the seeds, germination trials were performed immediately after processing.

Seed germination was tested in germination chambers (Eletrolab®, LMS® and Fanem®) at constant temperatures. All chambers were fitted with fluorescent white lamps (Photosynthetic Active Radiation: approximately $42 \mu\text{mol m}^{-2} \text{second}^{-1}$) and had a daily 12-hour photoperiod. Seeds were placed on medium-grain vermiculite (*Brasil Minério*®) moistened with distilled water (1 g vermiculite: 2 ml water), in plastic trays (30×22×7 cm). The trays were kept in thin transparent polyethylene bags to prevent drying.

In Experiment 1 a temperature range from 10 – 40 ± 2 °C, with intervals of 5 °C was tested. Each temperature treatment consisted of four repetitions of 20 seeds. The observation period was 140 days in the germination chambers.

Germination was assessed daily and the following morphological structures were observed: (Stage 1) radicle length ≥ 0.5 cm with positive geotropic curvature (corresponding to Labouriau (1983) (Figure 1A); (Stage 2) epicotyl length ≥ 1 cm, with visible plumule (indicating functioning of the apical meristem (Figure 1B); (Stage 3) epicotyl length ≥ 5 cm (corresponding to emergence above the substrate (Figure 1C).

Seedlings that had reached Stage 3 were transferred to the nursery in larger trays (57×25×17 cm) with the same substrate, to observe further development and expansion of the first leaves. Shoot length up to the first eophylls was measured for 50 plants for each temperature treatment (Figure 1D).

At the end of the germination test, the following variables were calculated for each germination criterion: total germination (*TG*), mean germination time (*MGT*), and Maguire's Germination Speed Index (*GSI*): the sum

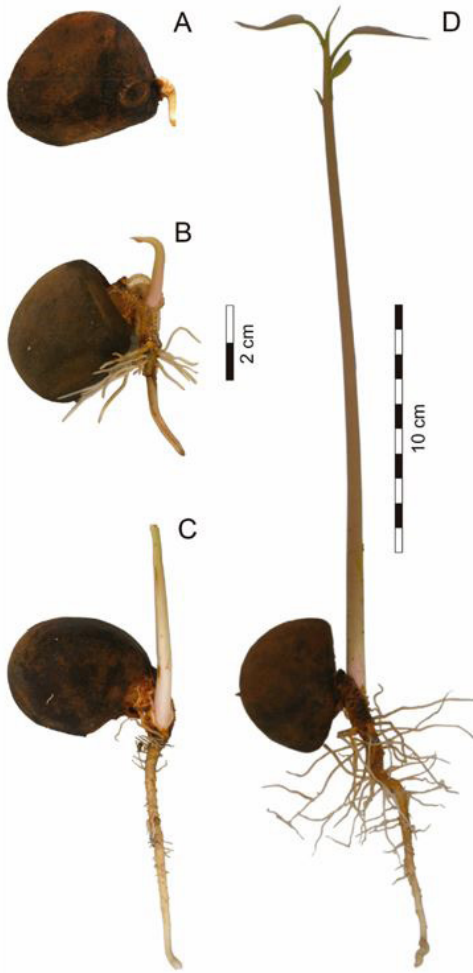


Figure 1. Stages of development after radicle protrusion of *Carapa surinamensis*. Stage 1 (A) Radicle protrusion ≥ 0.5 cm. Stage 2 (B) Epicotyl length ≥ 1 cm. Stage 3(C) Epicotyl length ≥ 5 cm. (D) Seedling expanding the first pair of eophylls.

of germinated seeds (G) on first, second and final count divided by the number of days (D) after sowing at first, second and final count: $GSI = G1/D1 + G2/D2... + G60/D60$ (Maguire, 1962). The lower temperature limit (Base Temperature: T_b) was estimated by the reciprocal of time for 50% of total germination against temperature ($1/T50$) and calculating the x-intercept through linear regression analysis (Roché et al., 1997).

The experimental design was entirely random. Residual normality was checked by the Shapiro-Wilk's test and the homogeneity of variances by the Levene's test, with 0.01% significance; once assumptions were achieved, treatments were compared using analysis of variance (ANOVA) and the means were compared with the Tukey test (5%).

In Experiment 2 only the optimal temperature (30 °C) was tested and used to evaluate seed coat removal and

determine first and final count according to Seed Testing Rules (Brasil, 2009; International Seed Testing Association, 2015). The above-mentioned three germination criteria (Figure 1) were assessed during 60 days. Seeds were dried above a fan in an air-conditioned room (25 ± 2 °C; 50% RH) for approximately two hours before manual seed coat removal with a metal spatula, avoiding any damage to the embryo. The experimental design was entirely random and each treatment consisted of four repetitions of 20 seeds each. Mean and standard deviations were evaluated for first and final count.

3. Results

Carapa surinamensis seeds presented a wide variation in size, and minimum and maximum individual seed weights ranged between 2.7 g – 36.2 g. Average weight was 14.5 g (SD: ± 7.9) and only these seeds were used in the germination trials. Seed moisture content was 48.8% (Experiment 1) and 46.6% (Experiment 2).

In Experiment 1 where germination was assessed in a temperature range from 10 – 40 °C, the lower temperature limit was detected. The seeds did not tolerate 10 °C, as no germination was assessed and the cutting test after 140 days revealed deterioration (Table 1). The lower temperature limit, T_b , for radicle protrusion, was estimated as 9.9 °C ($y = 0.023x - 0.0227$, $R^2 = 0.988$); T_b for epicotyl length ≥ 1 cm as 11.6 °C ($y = 0.0153x - 0.0177$, $R^2 = 0.937$); and for epicotyl length ≥ 5 cm as 12.3 °C ($y = 0.0141x - 0.0174$, $R^2 = 0.932$) (Figure 2). Thus the base temperature for radicle protrusion was slightly lower (1.7 – 2.4 °C) than for more advanced developmental Stages.

For the upper temperature limit, differences among the Stages of development were noted. At the maximum temperature tested (40 °C) there was 12.5% radicle protrusion, whereas no development of the aerial parts was noted. Thus the upper temperature limit for shoot development was between 35 °C – 40 °C (Table 1).

Radicle protrusion was high between 15 – 35 °C (TG 76.2 – 87.5%), and was reduced to TG 12.5% at 40 °C, which indicates the latter to be in the supra-optimal temperature range. Mean germination time (MGT) for radicle protrusion decreased with increasing temperature, from 15 °C (42.1 d) to 40 °C (6.6 d) (Table 2). Considering as optimum when the highest germination percentage is achieved in the least amount of time, the optimum temperature range for radicle protrusion was between 25 – 35 °C (12.3 – 15.8 days). For the more advanced Stages of development, the optimum temperature range was smaller and ranged between 25 – 30 °C (Table 1).

The GSI index was calculated for the three Stages of development of *C. surinamensis* (Figure 3) and a positive relation with increasing temperature up to 30 °C was obtained, followed by a decrease at higher temperatures. Despite differences between the absolute values, the pattern for the three Stages of development was similar and a

Table 1. Total Germination (%) for the three developmental Stages of *Carapa surinamensis* between 10 – 40 °C. †= death of seed verified by the cutting test).

Temperature °C	Total Germination (%)		
	Radicle (≥0.5 cm) Stage 1	Epicotyl (≥1 cm) Stage 2	Epicotyl (≥5 cm) Stage 3
10	†0.0 c	†0.0 c	†0.0 c
15	77.5 ± 13.2 a	71.2 ± 18.8 ab	66.2 ± 17.0 a
20	85.0 ± 12.9 a	72.5 ± 19.3 ab	71.2 ± 20.9 a
25	80.0 ± 15.8 a	80.0 ± 15.8 a	77.5 ± 13.2 a
30	87.5 ± 2.8 a	87.5 ± 2.8 a	87.5 ± 2.8 a
35	76.2 ± 6.2 a	47.5 ± 19.3 b	32.5 ± 18.4 b
40	12.5 ± 12.5 b	†0.0 c	†0.0 c
	CV= 17.8%	CV= 27.2%	CV = 27.9%
	LSD= 24.5	LSD =32.1	LSD =30.7
	W= 0.480	W= 0.434	W= 0.210
	F= 0.153	F= 0.247	F= 0.456

Means followed by different letters in the columns differ by Tukey test at 0.05 of significance; W; F: statistics using Shapiro-Wilk test and Levene, respectively. Values in bold indicate residues with normal distribution and homogeneous variances to 0.01 significance level. CV= coefficient of variation. LSD = least significant difference

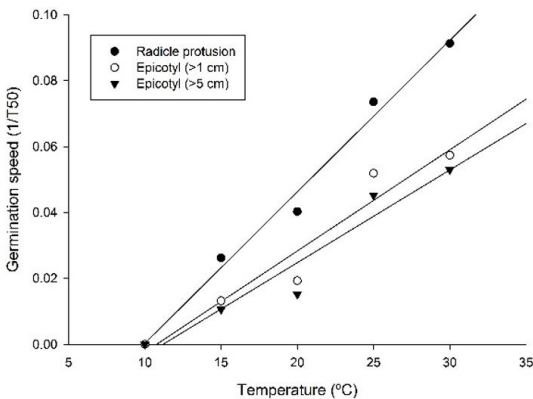


Figure 2. Germination speed (1/T50) 140 days after sowing of *Carapa surinamensis* seeds using three germination criteria (Stages of development) under constant temperatures.

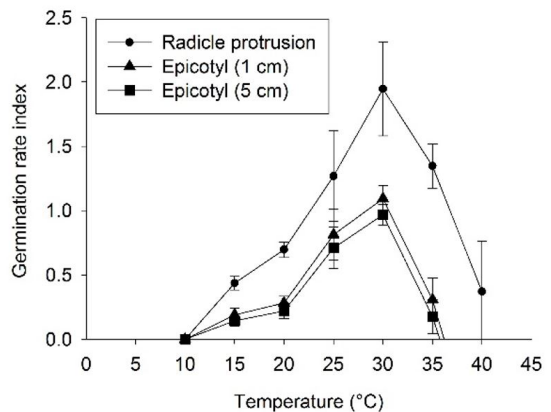


Figure 3. Germination speed index (GSI) of *Carapa surinamensis* for three Stages of development, at constant temperatures.

temperature of 30 °C can be indicated by this variable as the optimal germination temperature of *C. surinamensis*.

C. surinamensis can be classified as seedling type B-2-2-2-2, with hypogeal germination, based on the International Seed Testing Association (ISTA), where epicotyl elongation is evaluated and the primary root may be replaced by secondary roots (International Seed Testing Association, 2013). Developmental Stage 3 meets this technological germination criterion. According to International and National Rules for Seed Testing (Brasil, 2009; International Seed Testing Association, 2015), the time after sowing for a first and final count is included in the recommendations. This study considered 80% of the germinable seeds adequate for a first count

and a final count when all seeds had germinated. With seed coat intact, the first count could be done 51.0 days after sowing and the final count after 55 days (Table 3). Seed coat removal reduced these times considerably to 24.8 days (first count) and 30.5 days (final count). Germination assessment with the physiological criterion could be finished in 23.8 days. Seed coat removal did not negatively affect further development and no pathogens on the exposed seed reserves were observed. All the seedlings of Stage 3, transferred from the controlled conditions to the nursery, continued normal development and shoot length up to the first photosynthetically active leaves varied between 19.0 and 55.5 cm, with an average of 34.5 cm (Figure 1).

Table 2. Mean germination time (d) for the three developmental Stages of *Carapa surinamensis* between 10 – 40 °C. (NA= not assessed).

Temperature °C	Mean Germination Time (days)		
	Radicle (≥0.5 cm) Stage 1	Epicotyl (≥1 cm) Stage 2	Epicotyl (≥5 cm) Stage 3
10	N.A	N.A	N.A
15	42.1 ± 2.1 c	79.9 ± 8.0 d	96.5 ± 8.5 d
20	35.0 ± 5.9 c	57.9 ± 9.4 c	69.9 ± 10.4c
25	15.8 ± 1.5 b	22.7 ± 1.5 a	23.9 ± 2.2 a
30	12.3 ± 1.2 ab	18.5 ± 1.6 a	20.5 ± 1.8 a
35	15.8 ± 2.9 b	37.6 ± 8.6 b	44.4 ± 9.1 b
40	06.6 ± 4.5 a	N.A	N.A
	CV = 17.7%	CV = 18.7%	CV = 17.1%
	LSD = 7.4	LSD = 13.3	LSD = 14.4
	W = 0.112	W = 0.775	W = 0.522
	F = 0.127	F = 0.019	F = 0.049

Means followed by different letters in the columns differ by Tukey test at 0.05 of significance; W; F: statistics using Shapiro-Wilk test and Levene, respectively. Values in bold indicate residues with normal distribution and homogeneous variances to 0.01 significance level. CV= coefficient of variation. LSD = least significant difference

Table 3. Time required (mean ± standard deviation) to reach 80% germination (first count) and to conclude the germination test (final count) at the optimum temperature indicated for the three development Stages of *Carapa surinamensis*, sown with and without the seed coat.

Processing	Development Stage	Evaluation time (days)	
		First count	Final count
With seed coat	1 Radicle (≥0.5 cm)	34.5 ± 2.5	44.3 ± 1.0
	2 Epicotyl (≥1 cm)	38.8 ± 3.3	51.3 ± 1.3
	3 Epicotyl (≥5 cm)	51.0 ± 12.1	55.0 ± 0.0
Without seed coat	1 Radicle (≥0.5 cm)	15.8 ± 5.5	23.8 ± 5.9
	2 Epicotyl (≥1 cm)	21.3 ± 3.0	30.3 ± 6.3
	3 Epicotyl (≥5 cm)	24.8 ± 3.3	30.5 ± 7.5

4. Discussion

The size of the seeds of *C. surinamensis* agrees with earlier reports on seeds collected near Manaus, which had been designated at that time as *C. procera*, with 16 g (1 – 40 g) (Ferraz et al., 2002), and those of *C. procera*, from mountainous regions in Africa, with 15.4 g (5.9 – 37.4 g) (Sanogo et al., 2013). Both have high variation in seed size. Seed moisture content was also similar to earlier studies of this species from the Amazon with 51% (Ferraz et al., 2002); however, lower than the African *C. procera* with 69% (Sanogo et al., 2013). The present study supports the descriptions of Kenfack (2011a) that both species have similar seed size, and that seeds of the Neotropical *C. surinamensis* cannot be distinguished from the African *C. procera* solely by seed size.

The Maguire's Germination Speed Index (*GSI*) takes into account germinability, speed and uniformity of germination and is a tool usually employed for the evaluation of vigour (Maguire, 1962). Due to the importance of these variables in determining optimum temperature, this index was calculated for *C. surinamensis* and revealed a temperature

of 30 °C as optimal for all three developmental Stages. In a worldwide revision on the optimum temperature for tree seed germination, the most adequate conditions for radicle protrusion for the majority of species (43% of 99 species) ranged between 26 – 30 °C (Dürr et al., 2015). In a survey of Brazilian forest species, 25 °C was appropriate for most species (80% of 272) (Brancalion et al., 2010), as well as in a survey restricted to tropical and subtropical trees (Ferraz and Calvi, 2011). However, taking into account solely tree seeds from Central Amazonia, the optimum temperature for radicle protrusion was higher than 30 °C for most species (87% of 30) for example, *Aniba roseodora* Ducke (Lauraceae), *Dinizia excelsa* Ducke (Leguminosae) and *Clarisia racemosa* Ruiz & Pav (Moraceae) (Ferraz and Varela, 2003).

According to the Seed Testing Rules, the germination test should be completed within two months (Brasil, 2009), and particularly for recalcitrant seeds, an earlier result is desirable. For the germination type of *C. surinamensis*, developmental Stage 3 meets the ISTA recommendations (International Seed Testing Association, 2013). Without seed coat or by cutting the seeds, Sanogo et al. (2013) obtained

a significant reduction in *TG* for *C. procera*. In this study, the slight drying of the seeds before cutting facilitated the loosening of the embryo from the tegument. The same *TG* was obtained for seeds with and without seed coat, and seed quality can be assessed within 30 days.

A temperature tolerance of 15 °C is known for tropical recalcitrant seeds from some studies (Kioko et al., 2006; Pritchard et al., 2014). Curious is the high germination percentage of *C. surinamensis* achieved at this temperature, near the lower limit, as over a ten-year survey in a forest clearing near Manaus, this temperature was only recorded once, for a few hours (Ribeiro, 1976).

In the vast literature on seed germination temperature (Brancalion et al., 2010; Ferraz and Calvi, 2011; Dürr et al., 2015; Ribeiro and Costa, 2015; Andrade and Cardoso, 2016), only radicle protrusion has been assessed, and few studies evaluate more than one Stage of development. In this study the three developmental Stages of *C. surinamensis* differed in their thermal tolerance primarily involving the upper temperature limit. In other species the protrusion of the first organ through the seed coat had a lower as well as a higher temperature limit compared to further developmental Stages, such as in *Clitoria fairchildiana* R. A. Howard (Silva and Cesarino, 2014), *Oenocarpus bataua* Mart. (Bastos et al., 2017) *Parkia pendula* (Willd.) Walp. (Rosseto et al., 2009), *Qualea grandiflora* Mart. (Bilio et al., 2013) and *Theobroma grandiflorum* (Willd. ex Spreng.) K.Schum. (Ferraz et al., 2012).

Temperature limits of a given species are generally related to its geographical distribution and serve to match germination timing to favourable conditions for subsequent seedling growth and development (Bewley et al., 2013). Some of the above-mentioned tree species have wide geographical distribution in tropical Central and/or South America (e.g., *P. pendula*, *Maquira sclerophylla* (Ducke), *Q. grandiflora* and *C. fairchildiana*), while others are restricted to the central Amazon region (*C. racemosa* and *T. grandiflorum*) (Tropicos, 2017).

Plant development after root protrusion may alter its temperature tolerance as the plant may experience different environmental conditions in its microenvironment, caused by growth in height and depth, or even by climatic changes during the seasons. Furthermore, the onset of metabolic events related to the mobilization of seed reserves, providing nutrients to support early seedling growth, may have other temperature thresholds than the protrusion of the first organ. This study showed that the thermal tolerance of radicle protrusion is not always equal to normal seedling development. Radicle protrusion as a germination criterion assessed in physiological studies may not always be suitable to predict successful establishment under natural conditions.

Seeds of *C. surinamensis* display the typical requirement of an Amazon forest species, and this study shows that a temperature of 30 °C can be considered optimal. Thermal conditions in the forest understorey reveal, in fact, a constant temperature of almost 26 °C (Oliveira et al., 2008). Thus this species, with its recalcitrant seeds, which do not persist

in natural seed banks, may still have a small thermal buffer in view of the potential increase in temperatures due to climate change scenarios.

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