

# Temperature on *Annona cherimola* Mill. x *Annona squamosa* L. seed germination

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## ABSTRACT

In the production of atemoya rootstocks (*Annona cherimola* Mill. x *Annona squamosa* L.), the seed propagation is hindered by dormancy resulting in low germination, similar to temperature that affects the percentage, speed, and uniformity of germination. This study aimed to evaluate the effect of constant and alternating temperature on the seed germination of three atemoya genotypes: 'Gefner', 'Thompson' and 'PR-1' at 25°C and 20-30°C (8-16h) using germination paper in absence of light, with four samples of 25 seeds per treatment. The evaluations occurred each seven days up to 35 days, which were assessed: germinated seeds, seedlings, dormant seeds and germination speed index. The experimental design was completely randomized in a factorial arrangement of 3x2, using four replications. The water content, fresh and dry matter of 100 seeds were analyzed in a completely randomized scheme with four replications. The alternating temperature 20-30°C provided favorable condition and higher germination percentage for atemoya seeds, achieving 0.638 for germination speed index and 37% for percentage of germination at 35 days. 'Gefner' and 'Thompson' cultivars presented higher percentage of germination than 'PR-1'.

**Key words:** *Annonaceae*, atemoya, seed propagation, seed quality, dormancy, fruit science.

## INTRODUCTION

During the multiplication of atemoya (*Annona cherimola* Mill. x *Annona squamosa* L.), the fruit plant is propagated asexually through grafted rootstock obtained via seed. Among the recommended species to be used as rootstocks is the atemoya itself, whenever the interest is to produce a compatible rootstock. In this way, the uniformity and a viable germination percentage are necessary for the commercial production of rootstocks (Santos et al., 2015), which does not occur with atemoya seeds (Stenzel et al., 2003) because of its dormancy (Smet et al., 1999; Gimenez et al., 2014; Ferreira et al., 2019).

According to Rizzini (1973), embryonic dormancy occurs in several genera and species of the Annonaceae family due to embryo immaturity, which results in a slow and irregular germination. The morpho-physiological dormancy of *Annona crassiflora* seeds was reported by Silva et al. (2007), who proposed low temperature and/or temperature fluctuations preceding the rainy season to break seed dormancy. However, Smet et al. (1999) and Bispo and Ferreira (2018) suggest that dormancy may be caused by the presence of inhibiting substances as reported by Assis et al. (2018) and Vargas et al. (2018) for *Passiflora* spp., or even by resistance and tegument impermeability.

Like dormancy, temperature also affects all the biochemical and physiological processes, interfering in the total, speed and uniformity of seed germination. Thus, germination will only take place within determined temperature limits, which includes temperature or a temperature range, where the process occurs with maximum efficiency (Carvalho and Nakagawa 2012). Although, seeds of many species germinate at constant temperatures (Lima et al., 2011; Carvalho et al., 2018a), others show a favorable germination reaction under alternating temperatures, probably an adaptation to temperature fluctuations during the daytime/nighttime periods, which occur in the nature (Copeland and McDonald 1995; Carvalho and Nakagawa 2012).

In some species such as *Sebastiania commersoniana* (Santos and Aguiar 2000), *Cnidoculus phyllacanthus* (Silva and Aguiar 2004), *Passiflora alata* (Osipi and Nakagawa 2005), *Caesalpinia pyramidalis* (Lima et al., 2011), among others, alternating temperatures seem to act, predominantly, at the germination levels and

uniformity. However, in some of these species and others, the effects on induced dormancy breakage are well defined, which some of them seem to have a fixed demand for an alternation in temperature (Bewley et al., 2013).

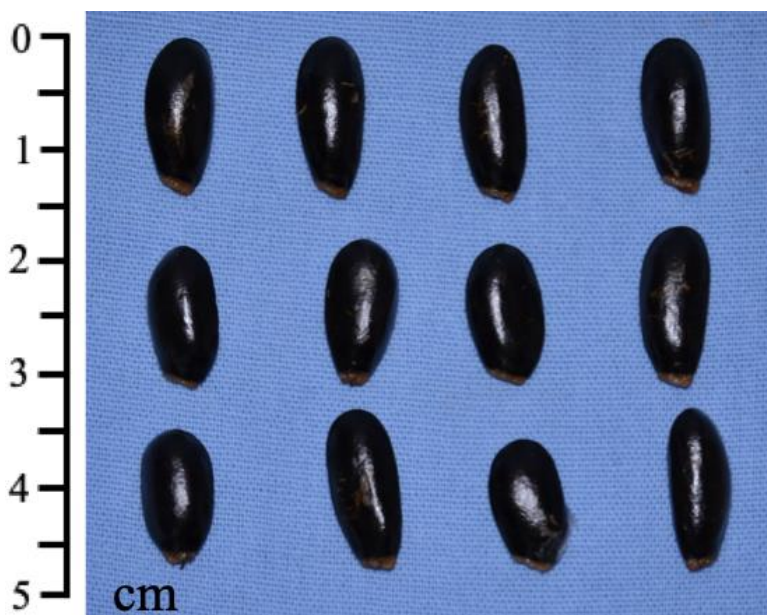
Some hypotheses try to explain the reasons for this seed reaction to alternating temperatures such as dormancy breakage and hormonal imbalance, but none is fully satisfactory (Carvalho and Nakagawa 2012). There are also species that germinate under both constant and alternating temperatures (Scalon et al., 2009; Lima et al., 2011).

In fruit plants, the commercial use of sexual propagation occurs in restricted situations, including rootstock production and genetic development. Consequently, seed technology is not well developed in the fruit propagation, and investigations in this area with fruit plant species are scarce, lacking basic information regarding the ideal conditions for germination. It is also important to emphasize that the irregularity and low germination percentage are among the existing problems regarding the use of atemoya seeds for rootstock production. Thus, the objective of this work was to evaluate the effect of constant and alternating temperatures on seed germination of three atemoya cultivars.

## MATERIALS AND METHODS

Trials were performed in the department of Plant Production of the Universidade Estadual do Norte do Paraná – *Campus* Luiz Meneghel, Brazil, using fruits from three atemoya genotypes: ‘Gefner’, ‘PR-1’ and ‘Thompson’, from a commercial orchard in the city of Assaí, Paraná, Brazil.

Seeds were manually extracted from the fruits, washed in running water, dried over a paper towel for three days (Figure 1), and packed in kraft paper for five days. Soon after, a germination trial at the constant temperature of 25°C and alternating temperatures from 20-30°C (8h for 20°C and 16h for 30°C) were conducted, in the absence of light, in a B.O.D chamber.



**Figure 1.** Seeds of atemoya cv. ‘Gefner’ extracted from ripe fruits.

The germination trial was performed on a germitest paper, with four samples of 25 seeds per treatment, previously immersed for 3 minutes in a metalaxyl-M+fluodioxonil (Maxin xl<sup>®</sup>) at 0.2% and carboxin+tirame (Vitavax-Thiram 200 SC<sup>®</sup>) at 0.2% fungicide suspension, in the proportion of 1:1.

Evaluations were carried out every seven days up to 35 days after the trial installation. There was considered as germinated seed those with radicle length equal or greater than two millimeters, a seedling with cotyledon roots and leaves, regardless the presence of anomaly and dormant seeds (non-germinated).

After the evaluations, the germination speed index (GSI) was calculated by the formula proposed by Maguire (1962). The experimental design was complete randomized, in a 3x2 factorial scheme (three cultivars x alternating temperatures of 20-30°C and constant temperature of 25°C), with four replications. Characteristics such as, water content, fresh and dry matter of 100 seeds were analyzed at random, with four replications, considering the cultivars as treatments. All data were computer processed by the R

software version 3.6.0 (<http://www.r-project.org>) using the ExpDes package (Ferreira et al., 2014) for analysis of variance, the means were compared by Tukey's test at 0.05 level.

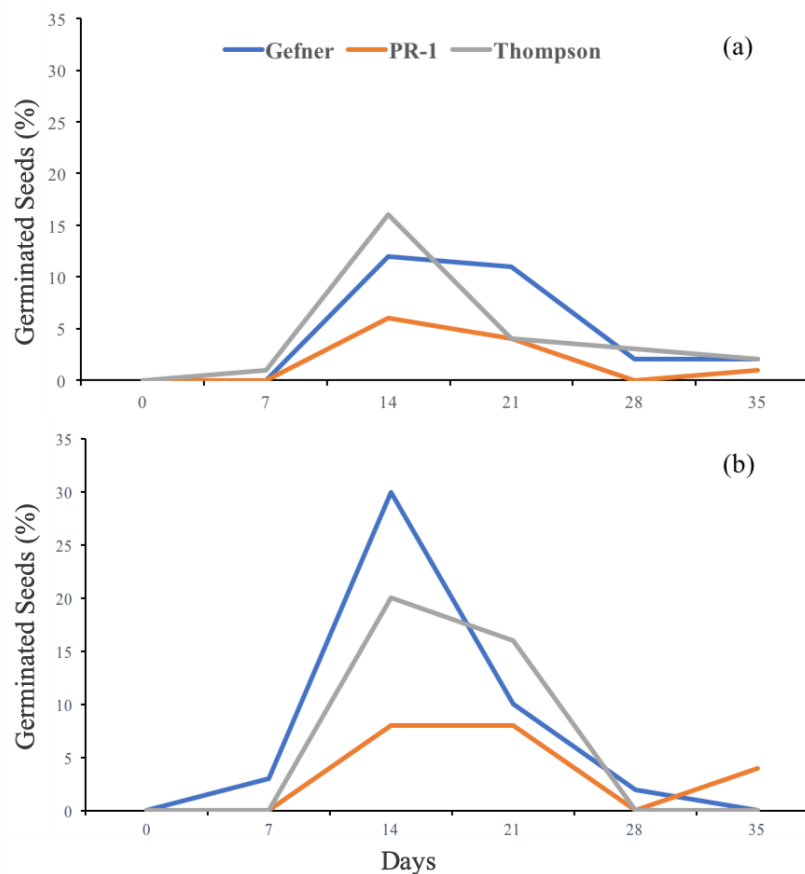
## RESULTS AND DISCUSSION

Values obtained for water content, fresh and dry matter of 100 seeds indicate that the cultivars showed similarities for these characteristics (Table 1). In regards to the tested temperatures, seeds germination period for the three cultivars occurred mainly from day 7 to day 28, concentrated between the 7th and the 14th day (Figure 2). These results corroborate those found by Stenzel et al. (2003) and Carvalho et al. (2018b), who obtained, for the same genotypes evaluated, greater percentiles of germination between days 7 and 14.

**Table 1.** Water content (%), fresh and dry matter of 100 seeds (g) from the 'Gefner', 'PR-1' and 'Thompson' atemoya cultivars. Bandeirantes, PR.

Cultivars	Fresh matter	Dry matter	Water content
'Gefner'	37.29 <sup>ns</sup>	31.37 <sup>ns</sup>	1.48 <sup>ns</sup>
'PR-1'	35.05	28.88	1.54
'Thompson'	35.05	28.87	1.54
Coefficient of Variaton (%)	6.9	6.6	7.7
DMS	4.94	3.96	0.29

ns – non-significant by the Tukey test ( $p \leq 0.05$ ).



**Figure 2** – Percentage of atemoya seeds germinated at the constant temperature of 25°C (a) and alternating temperature between 20-30°C (b), in the period from zero to 35 days.

Results from the germination test (Tables 2 and 3) indicate a significant interaction between temperature and cultivars only for the percentiles of seeds germinated at 28 and 35 days. However, this did not occur at days 7, 14 and 21, for the same variable, both for seedlings and dormant seeds at the end of the test.

**Table 2.** Percentages of germinated seeds (GS), seedlings (S) and dormant seeds (DS) for ‘Gefner’, ‘PR-1’ and ‘Thompson’ atemoya cultivars, under alternating (20-30°C) and constant (25°C) temperatures. Bandeirantes, PR.

Treatments	7 days GS	14 days GS	21 days GS	35 days	
				S	DS
‘Gefner’	2 ab*	23 a*	33 a*	34 a*	66 b*
‘PR-1’	0 b**	10 b**	14 b**	17 b**	83 a
‘Thompson’	4 a	22 a	32 a	35 a	65 b
20-30°C	3 A	25 A	34 A	33 A	63 B
25°C	0 B	12 B	18 B	25 B	79 A
MSD <sub>Treatment</sub>	3.9	10.2	12.8	9.5	13.4
MSD <sub>Temperature</sub>	2.6	6.9	8.6	6.4	9.0
Coefficient of Variation (%)	15.9	21.7	22.0	20.5	13.8

\*Means followed by the same letter in the column do not differ among them by the Tukey test ( $p \leq 0.05$ ). \*\*Data transformed in  $\arcsin \sqrt{x/100}$ .

**Table 3.** Percentages of germinated seeds (GS) from the ‘Gefner’, ‘PR-1’ and ‘Thompson’ atemoya cultivars, under alternating (20-30°C) and constant (25°C) temperatures. Bandeirantes, PR.

Cultivar	GS at 28 days		GS at 35 days	
	20-30°C	25°C	20-30°C	25°C
‘Gefner’	42 aA *	30 aB*	42 aA*	31 aA*
‘PR-1’	27 bA**	16 aA**	28 bA**	17 bB**
‘Thompson’	41 abA	29 aB	41 abA	30 aA
MSD <sub>Line</sub>		11.8		10.9
MSD <sub>Column</sub>		14.4		13.3
Coefficient of Variation (%)		25.9		23.2

\*Means followed by the same letter, lowercase in the column and uppercase in the line, do not differ significantly among them by the Tukey test ( $p \leq 0.05$ ). \*\*Data transformed in  $\arcsin \sqrt{x/100}$

The ‘Gefner’ and ‘Thompson’ atemoyas showed greater germination capacity, with similar behavior in relation to the percentiles for seedlings and dormant seeds obtained at the end of the experiment (Table 2). On the other hand, the ‘PR-1’ atemoya resulted in lower percentiles of germinated seeds compared to the others genotypes during the evaluation at day 21. These values are similar to those found by Carvalho et al. (2018b) for the evaluation of the same atemoya genotypes submitted to treatments with phyto regulators.

Atemoya ‘PR-1’ stood out for showing greater percentile of dormant seeds, being also surpassed by other two cultivars in the germination speed index, a variable parameter which showed non-significant interaction between cultivars and the evaluated temperatures (Table 4). Stenzel et al. (2003) did not find difference regarding germination rate (at 25°C) for the ‘Gefner’ and ‘PR-1’ atemoyas from their control, in a study of dormancy breakage using scarification and phyto regulators for ‘Gefner’, ‘PR-1’, PR-3 atemoyas and *A. squamosa* (fruta-do-conde) seeds.

Concerning temperatures tested up to 21 days from the germination trial, regardless of the cultivars, the alternating temperature between 20-30°C was more efficient than constant temperature at 25°C, presenting superior percentiles of germinated seeds and seedlings, and lower percentiles of dormant seeds.

Costa et al. (2011) investigating the effect of temperature on the germination of *A. emarginata* (araticum-de-terra-fria) seeds observed that the alternating temperature of 20-30°C was more efficient for germination (total percentile and speed index) than constant temperature of 20°C and 30°C during the analyzed period, corroborating with the results from this experiment.

**Table 4.** Germination speed index (GSI), of ‘Gefner’, ‘PR-1’ e ‘Thompson’ atemoya seeds, in alternating temperatures (20-30°C) and constant temperature (25°C). Bandeirantes, PR.

Treatment	GSI
‘Gefner’	0.579 a*
‘PR-1’	0.249 b
‘Thompson’	0.604 a
20-30°C	0.638 A
25°C	0.316 B
MSD <sub>Treatment</sub>	0.234
MSD <sub>Temperature</sub>	0.157
Coefficient of Variation (%)	23.17

\* Means followed by the same letter in the column do not differ significantly among them by the Tukey test ( $p \leq 0.05$ ).

After 28 days under alternating temperatures, the ‘Gefner’ and ‘Thompson’ atemoyas had already stabilized the percentiles of germinated seeds, as shown by the equal values obtained at 35 days (Table 3). However, under constant temperature, at 28 days, stabilization had not occurred for the referred cultivars. After 35 days, the ‘PR-1’ atemoya had not reached germination stability (Figure 2) and, only from this point onwards, showed differences regarding germinated seeds percentile between the temperatures tested. This was probably the result of the slower and uneven germination presented by the ‘PR-1’ atemoya, with greater percentile of dormant seeds and late stabilization.

As in this study, others authors reported better seed germination for alternating temperature treatments between 20-30°C when compared to constant temperatures, for many species (Santos and Aguiar 2000; Silva and Aguiar 2004; Osipi and Nakagawa 2005; Lima et al., 2011; Costa et al., 2011).

The best performance of the atemoya seed germination at 20-30°C observed in this study can be explained by the interference of this alternating temperature on the inhibitor/promotor balance, which promoted greater amount of germination according to Copeland and McDonald (1995). Results from *A. cherimola* x *A. squamosa* (Cruz et al., 2016), as well as from *A. squamosa* (Zucareli et al., 2007) and *A. emarginata* (Costa et al., 2011) seeds, corroborate those found in the present investigation. In general, regardless of the cultivars, the alternating temperature presented higher germination speed index than that obtained for the constant temperature at 25°C.

## CONCLUSIONS

The alternating temperature between 20-30°C promotes an adequate condition for the germination of the atemoya seeds, providing greater speed and percentage of germination for the tested genotypes. ‘Gefner’ and ‘Thompson’ show better germination capacity than ‘PR-1’.

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