



# Overcoming dormancy of pecan trees in mild winter conditions

Superação da dormência de nogueira-pecã em condições de inverno ameno

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This study aimed to determine chill accumulation to overcome endodormancy of pecan trees using the biological method. Cuttings of isolated nodes in different months and their exposure to natural chill under field conditions were used. The experiment was carried out using seven pecan cultivars: Barton, Mohawk, Jackson, Success, Desirable, Farley and Melhorada (Pitol 1), planted in orchards in the municipality of Canguçu, Rio Grande do Sul, Brazil. Every 30 days from June onwards, when 50% of leaves had fallen from trees (phenological stage 95), 20 cuttings per cultivar were collected in each period until September. They were sterilized and sent to the phytotron for budbreak monitoring. Budbreak average time, time to reach 50% of budbreak and the final budbreak rate were evaluated. Average budbreak time of cultivars showed little variation along collection period. Chill requirement of pecan cultivars ranged from 51 to 369 chill hours (below 7.2 °C) to reach DD50. It was not possible to determine the chill requirement of the studied cultivars. The moment of cutting collection had little influence on average budbreak time and final budbreak rate.

Key words: bud dormancy, *Carya illinoensis*, chill accumulation.

Este trabalho teve como objetivo determinar o acúmulo de frio para superar a endodormência da nogueira-pecã através do método biológico. Foram utilizadas estacas de nós isolados em diferentes meses e sua exposição ao frio natural em condições de campo. O experimento foi realizado com sete cultivares de nogueira-pecã: Barton, Mohawk, Jackson, Success, Desirable, Farley e Melhorada (Pitol 1), implantadas em pomares no município de Canguçu, Rio Grande do Sul, Brasil. A cada 30 dias a partir do mês de junho, quando 50% das folhas caíam das árvores (estágio fenológico 95), foram coletadas 20 estacas por cultivar em cada período até setembro. As mesmas foram esterilizadas e enviadas ao fitotron para monitoramento da brotação. Foram avaliados o tempo médio de brotação, o tempo para atingir 50% de brotação e a taxa de brotação final. O tempo médio de brotação das cultivares apresentou pouca variação ao longo do período de coleta. A exigência de frio das cultivares de noz-pecã variou de 167 a 369 horas de resfriamento (abaixo de 7,2 °C) para atingir DD50. Não foi possível determinar a exigência em frio das cultivares estudadas. O momento da coleta das estacas pouco influenciou no tempo médio de brotação e na taxa final de brotação. Palavras-chave: acúmulo de frio, *Carya illinoensis*, dormência de gemas.

## 1. INTRODUCTION

Chill is one of the limiting factors to make temperate fruit trees reach commercial production since they need a dormancy period [1]. Thus, chill requirements of every species and cultivar must be known in order to plant in a suitable site for successful production. However, when chill requirements are not completed, several buds keep dormant, even though environmental conditions may be favorable to their growth [2].

Pecan trees [*Carya illinoensis* (Wangenh) Koch] have been grown commercially in all continents nowadays [3-5]. Since the species is native to North America and grown mainly in

temperate regions in the Northern Hemisphere [6, 7], cultivars have high chill requirements [8]. According to Wells (2017) [9], when it is grown in regions where chill requirements are not thoroughly met, it may show several abnormalities, such as lengthy and deficient budbreak and flowering.

Regarding pecan trees, requirements of chill hours (CH) have not been clearly determined yet; in the literature, they range from 50 to 1,000 hours at temperatures below 7.2 °C, depending on the cultivar [9-12]. To overcome endodormancy, temperatures do not need to be extremely low, but they must be regular [2].

There are techniques to study mechanisms involved in dormancy. One of them is the biological method, which is based on the evolution of time needed for budbreak of single buds subject to a standard temperature [13, 14]. This method has been used for verifying when temperate fruit trees overcome the endodormancy period, since the only form to inhibit budbreak comes from the bud itself [14-16]. Lamela et al. (2020) [17] stated that, besides this method, the rate known as DD50 enables to estimate the end of endodormancy when 50% of buds end up breaking after having been subjected to chill accumulation. This phase ends when 50% of buds (sampled on a certain date/chill accumulation) reaches the green tip stage.

Thus, this study aimed to determine the chill requirements of different cultivars of pecan trees in order to overcome endodormancy in field conditions (natural chill) of southern Brazil.

## 2. MATERIAL AND METHODS

The plant material used in the experiment was collected in two commercial orchards in Canguçu, state of Rio Grande do Sul (RS), Brazil. The first orchard (31°28"S; 52°56"W) had trees of cultivars Barton, Mohawk, Jackson, Success, Desirable and Farley, eight years old at 9 m x 6 m spacing. The second orchard (31°28"S; 52°41"W) had trees of cultivar Melhorada (Pitol 1), nine years old at 10 m x 10 m spacing.

The experimental design used was completely randomized in plots subdivided in time, considering two factors in the analysis. The plot factor was the treatment with seven levels (cultivars) and the subplot factor with four levels (months). Five replicates composed of four stakes each were used. Branches of pecan cultivars were collected every 30 days from June to September 2019. The dates were June 11<sup>th</sup>, July 17<sup>th</sup>, August 14<sup>th</sup> and September 24<sup>th</sup>, a total of four collections. At the beginning of branch collection, 50% of leaves had fallen from the trees. It corresponded to the phenological stage 95 of the pecan scale proposed by De Marco et al. (2021) [18].

Twenty branches of one year old and 20 cm in length were collected from every cultivar, in each collection period. A cultivar sample was composed of four trees. The Meteorologic Station Canguçu-A811, INMET (2019) [19], provided the data of number of natural chill hours (CH) in the orchard (Table 1).

*Table 1: Number of chill hours ( $\leq 7.2$  °C) in Canguçu, RS, Brazil in 2019.*

Period	Chill hours	Accumulated chill hours
Up to 11/06	51	51
12/06 to 17/07	116	167
18/07 to 14/08	66	233
15/08 to 24/09	136	369
Year Accumulation	369	-

The Köppen-Geiger climate classification showed that climate in the experimental location is Cfa: humid subtropical with hot and humid summers and mild winters [20].

After having been collected, the plant material was taken to the Embrapa Clima Temperado, in Pelotas, RS, Brazil. Branches were kept moist, wrapped in moist paper and stored in plastic

bags, from their collection to the moment they were prepared to be placed in the Biochemical Oxygen Demand (BOD) analyzer. Branches were cut to measure 10 cm in length; only the median portion was used. After the cut, a single 2 cm bud was kept below the upper cut, so as to eliminate the other buds. To decrease branch dehydration, and consequently, bud dehydration, the upper extremity of the branch was wrapped in plastic film. Then, branches were placed on trays with moist vermiculite and kept in growth chambers at 25 °C and a 16-hour photoperiod for 30 days, conditions considered optimal for overcoming dormancy in temperate fruit tree, as proposed by the literature [21].

Evaluations were carried out every two days; beginning of budbreak was considered the moment in which buds had green tips, in agreement with stage 07 of the phenological scale proposed by De Marco et al. (2021) [18]. Based on these data, the following were calculated: mean time to budbreak (MTB) of every cultivar, which represents the average number of days between the installation of the experiment and the detection of the green tip stage; time needed to reach 50% of budbreak (DD50), which was estimated by the methodology proposed by Lamela et al. (2020) [17]; and the final budbreak rate (FBR), which represents the percentage of cuttings with buds that exhibited green tips.

To meet the assumptions of analysis of variance (ANOVA), Shapiro-Wilk's and Hartley's tests and graphical analysis of residuals were performed. Subsequently, two approaches analyzed the data: the means of the MTB and FBR variables were analyzed using the Tukey's test at 5% error probability. In a second approach, regression analysis and sigmoid regression were performed.

For the DD50, regression analysis it was made using the 3-parameter model. The analysis of the 3-parameter sigmoid regression was carried out with cumulative data on budbreak (%) as the response variable ( $y$ ) and time in the growth chamber as the predictive variable ( $x$ ):

$$y = a/(1+e^{-(x-DD50)/b})$$

where:  $y$  is the budbreak percentage for each cultivar at each evaluation period;  $x$  is time expressed as days;  $a$  represents the difference between maximum and minimum points of the variable;  $b$  is the curve inclination; and DD50 = time needed to reach 50% of budbreak.

The regression is logistic with sigmoid curve and actual values of sprouting and values estimated by the model were analyzed by the mean square error (RMSE) by the equation:

$$RMSE = \sqrt{1/n \sum_{i=1}^n (P_i - O_i)^2}$$

where:  $P_i$  represents the predicted cumulative percentage of sprouting;  $O_i$  is the effective cumulative percentage of sprouting; and  $n$  is the number of observations.

To identify multicollinearity, variance inflation factors (VIFs) were calculated using the formula [22]:

$$VIF = \frac{1}{1 - R^2}$$

### 3. RESULTS AND DISCUSSION

Tables 2 and 4 showed significant interaction ( $p \leq 0.05$ ) between pecan cultivars and collection periods, from 13.0 to 22.2. Concerning Success, Jackson and Melhorada, the period of cutting collection did not affect MTB significantly. However, in the case of Farley, the lowest MTB was found when collection took place in September (369 CH), while, in the case of Mohawk, it occurred in July (167 CH). The lowest MTB of cultivars Desirable and Barton varied among periods of cutting collection (Table 2). According to Mauget and Rageau (1988) [23] and Hawerth (2010) [21], decrease in MTB is associated with the end of endodormancy. Stabilized values correspond to the ecodormancy phase. Thus, even though differences in MTB of cultivars

were not so evident in the evaluation periods, there was a little decrease in MTB from July (167 CH) on, by comparison with the previous period (June: 51 CH), with the exception of Barton, which in July had a slight increase.

Table 2. Mean time to budbreak (MTB) (days) of pecan cultivars in different periods of cutting collection in Canguçu, RS, Brazil, in 2019.

Cultivar	MTB (days)				Ac*
	June	July	August	September	
Farley	15.0±1.1 bA	14.4±2.3 bA	14.8±2.3 bcA	13.2±0.5 bB	14.4
Success	15.0±1.2 bA	14.0±1.1 bA	13.6±0.6 cA	15.2±2.7 abA	14.5
Jackson	17.0±0.9 bA	14.0±1.2 bA	14.8±0.9 bcA	15.2±2.1 abA	15.3
Mohawk	22.2±1.8 aA	16.0±1.5 bB	18.4±1.9 aA	17.6±0.9 aB	18.6
Desirable	18.0±0.7 abA	16.0±1.6 abB	19.6±1.7 aA	16.8±2.2 abAB	17.6
Melhorada	15.0±0.7 bA	14.0±1.2 bA	13.0±0.0 cA	16.0±4.1 abA	14.5
Barton	15.0±0.0 bAB	17.0±1.0 aA	13.0±1.3 cB	16.4±0.9 abAB	15.4
Ma**	16.7	15.1	15.3	15.8	

Averages followed by a certain lowercase letter in a column and an uppercase one in a line do not differ by the Tukey's test at 5% significance. \*Ac= Average of cultivar; \*\*Ma= Monthly average. ± Mean standard error. General average: 15.86. Coefficient of variation of the plot (%): 11.51; Coefficient of variation of the subplot (%): 14.84.

MTB of cultivars showed little variation in all collection periods; therefore, cultivars obtained similar responses (Table 2). According to Fowler et al. (2018) [24], the highest MTB is characterized as the deepest endodormancy period of cultivars. Thus, cultivars under study showed similar characteristics, that is, the MTB varied between the evaluation periods.

There was an oscillation in MTB both in dates of collection and in the cultivars under study. Fowler et al. (2018) [24] stated that differences in MTB occur due to chill requirements of every cultivar and fluctuations are observed as a function of temperature variations over the period. Furthermore, Petri et al. (2021) [1] stated that it is very difficult to determine chill requirements of a species or cultivar under field conditions, since environmental conditions can incite plants to generate different responses.

Figure 1 shows that temperatures were not constant and that the amplitude in all months of the experiment was 29.64°C, on average. Thus, that high temperatures are common in the winter period and have a negative effect on chill accumulation. In this context, it is important to highlight that temperatures above 20 °C cancel out accumulated chill, which may interrupt biochemical reactions that take place inside plants at the beginning of sprouting [1, 25, 26]. In addition, the same authors also report that this effect is stronger as a function of its duration and intensity. While this test was conducted, there were 20 days at average temperatures above 20 °C and 67 days whose maximum temperatures exceeded 20 °C, the one reported by the authors. This could be one of the factors that may have influenced results of this trial.

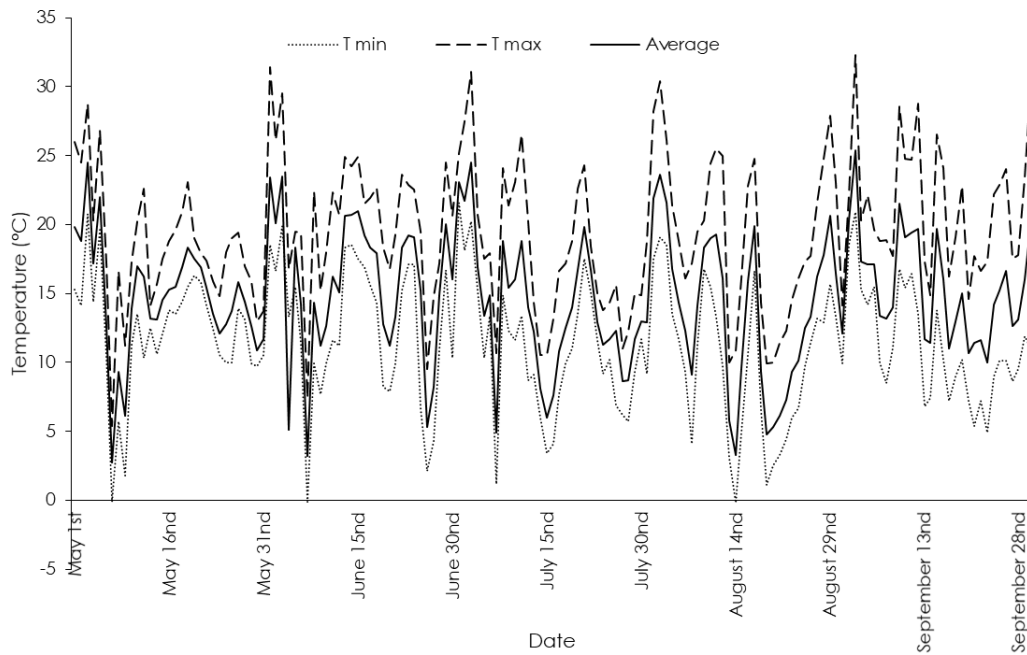


Figure 1. Minimum, maximum and average daily temperature in the 4-month experiment in Canguçu, RS, Brazil, 2019.

DD50 is a parameter that has been used for establishing the moment in which cultivars overcome endodormancy [17]. Cultivars were found to need from 12 to 17 days to end endodormancy (Table 3). However, to make it happen, CH accumulation differed between cultivars. Cultivars Jackson, Mohawk and Farley took about 16 days to reach DD50, but, in the case of Jackson, it took place in the collection carried out in July (167 CH), while, in the cases of Mohawk and Farley, it happened in the last collection (September, 369 CH).

Regarding DD50 (Table 3) of all seven cultivars under evaluation, both Jackson and Desirable reached 50% of budbreak faster in July, while Success, Melhorada and Barton reached DD50 faster in the collection carried out in August (233 CH). Finally, both Farley and Mohawk reached DD50 faster in the last collection period. Thus, the results are in agreement with Rovani and Wollmann (2018) [27], different pecan cultivars have distinct chill requirements.

Final budbreak rate (FBR) varied between periods of cutting collection and pecan cultivars (Table 4). In the first collection period (June, 51 CH), the cultivar Mohawk reached the lowest FBR, 75% of budbreak. The other cultivars did not exhibit any significant difference. In the second collection period (July, 167 CH), Mohawk also exhibited the lowest FBR. In the third collection period (August, 233 CH), there was no significant difference in FBR of cultivars. In the last collection period, (September, 369 CH), the lowest FBR were found. It was the only period of cutting collection in which no cultivars exhibited 100% of budbreak. It was not expected since this period had the highest number of CH in the whole period under study. However, when material was collected in September, budbreak had already started. As a result, when branches were cut, budbreak stopped and only the buds that had not started the process yet could break in the growth chamber. Therefore, the biological method with the use of branches collected in the last month should not be used anymore. Hawerth et al. (2010) [21] stated that one of the main problems that limit the use of cuttings is related to time limitation due to their short lifespan. Thus, using the biological method to evaluate buds in this developmental stage may not be adequate. It shows that the moment of branch collection is an important parameter which must be taken into consideration [28].

Table 3: Regression equations adjusted to time expressed as days to reach 50% of budbreak (DD50) according to collection dates.

Cultivar	Period	a	b	DD50	R <sup>2</sup>	p value
Farley	June	106.05	4.31	22.68	0.73	<0.0001
	July	92.02	4.30	22.77	0.65	<0.0001
	August	79.47	4.02	17.40	0.63	<0.0001
	September	63.01	4.14	16.74	0.68	<0.0001
Success	June	83.88	3.75	18.55	0.71	<0.0001
	July	99.48	3.01	16.21	0.89	<0.0001
	August	78.97	1.00	14.39	0.77	<0.0001
	September	47.06	4.04	26.17	0.62	<0.0002
Jackson	June *	100.64	2.09	18.70	0.98	<0.0001
	July *	82.08	3.00	16.65	0.98	<0.0001
	August *	137.41	4.20	25.28	0.91	<0.0001
	September	92.20	5.52	21.12	0.71	<0.0002
Mohawk	June	72.01	2.61	20.84	0.73	<0.0001
	July	59.58	3.24	19.57	0.56	<0.0002
	August	138.35	5.22	27.27	0.76	<0.0001
	September	55.48	3.85	16.09	0.86	<0.0001
Desirable	June	94.23	0.64	17.96	0.88	<0.0001
	July	93.20	3.39	17.10	0.79	<0.0001
	August	427.76	5.94	27.17	0.83	<0.0001
	September	46.13	4.24	17.49	0.66	<0.0001
Melhorada	June	100.12	3.37	13.18	0.79	<0.0001
	July	99.70	3.87	18.95	0.89	<0.0001
	August *	96.74	0.95	12.57	0.95	<0.0001
	September*	80.02	0.74	15.98	0.99	<0.0001
Barton	June *	100.11	2.45	13.60	0.93	<0.0001
	July	97.85	3.07	19.84	0.89	<0.0001
	August	96.41	2.09	13.46	0.83	<0.0001
	September	57.99	3.98	20.62	0.58	<0.0002

\*VIF (variance inflation factors) values greater than 10, indicating the presence of severe multicollinearity [27].

In the last period of cutting collection (September, 69 CH), FBR of cultivars Farley, Jackson and Melhorada did not differ from those found in previous collections, although most cultivars had decreased their FBR by comparison with those found in the August collection (233 CH) (Table 4). Wells (2017) [29] stated that pecan trees should be exposed to, at least, 100 CH below 7.2 °C. Thus, this study showed that FBR over 50% could be reached by all cultivars and evaluation periods. However, in general, the third period of cutting collection (August: 233 CH) reached the best budbreak percentages, whose average was 96%.

Most researchers who study or work with pecan trees highlighted that the crop needs accumulation of more than 400 CH to overcome endodormancy [30]. However, other authors stated that it may be grown in regions where there are between 50 and 100 to 550 CH [9-11]. In the literature, CH range from 50 to 550; as shown by this study, pecan budbreak is reached at different numbers of CH. Further studies of this topic are needed to clarify it, such as tests on whole plants instead of cuttings with isolated nodes and empirical methods (climatic models) that

relate phenological behavior of plants, regarding the level of budbreak, with climatic data from a particular region where the study is being conducted.

Table 4: Final budbreak rate (%) of pecan cultivars in different periods of cutting collection in Canguçu, RS, Brazil, in 2019.

Cultivar	Final budbreak rate (%)				Ac*
	June	July	August	September	
Farley	85 ±5.4 aA	65±9.8 bcA	80±7.2 aA	70±3.6 abA	75
Success	85±5.4 aA	100±0.0 aA	100±0.0 aA	50±8.9 bB	84
Jackson	100±0.0 aA	80±7.2 bA	100±0.0 aA	85±8.1 aA	91
Mohawk	75±4.5 aAB	55±11.6 cB	90±5.8 aA	55±7.2 bB	69
Desirable	100±0.0 aA	90±5.4 abA	100±0.0 aA	55±7.2 bB	86
Melhorada	100±0.0 aA	95±3.6 aA	100±0.0 aA	85±5.4 aA	95
Barton	100±0.0 aA	95±3.6 aA	100±0.0 aA	65±5.4 abB	90
Ma**	92	83	96	66	

Averages followed by a certain lowercase letter in a column and an uppercase one in a line do not differ by the Tukey's test at 5% significance. \*Ac= Average of cultivar; \*\*Ma= Monthly average. ± Mean standard error. General average: 84.28. Coefficient of variation of the plot (%): 20.82; Coefficient of variation of the subplot (%): 17.19.

#### 4. CONCLUSIONS

Chill requirements of pecan cultivars ranged from 51 to 369 chill hours to reach DD50 (50% of sprouted buds). The month of collection had little influence on average budbreak time and final budbreak rate of pecan cultivars. Branches collected in September in Brazil should no longer be used for dormancy studies of pecan cultivars, as pecans plants are beginning their budding period and may interfere with the results.

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