Budbreak of pecan cultivars subject to artificial chill[1]

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ABSTRACT

Chill is a limiting factor in commercial production of temperate fruit due to their dormancy mechanism. Thus, knowledge of chill requirements of cultivars is important to reach successful production. This study aimed at evaluating responses given by different pecan cultivars subject to artificial chill. Pecan branches were collected from twelve 9-year-old cultivars – Success, Shoshoni, Farley, Elliott, Mohawk, Jackson, Desirable, Barton, Importada, Shawnee, Choctaw and Melhorada – in two orchards located in Canguçu, Rio Grande do Sul (RS) state, Brazil, in 2017 and 2018. Treatments consisted in exposing branches to 0, 250, 500, 750 and 1,000 chill hours in a cold chamber (4.0 ± 0.5 °C) and then taking them to the germination chamber (25 ± 0.5 °C and photoperiod of 16 hours of light) until the end of the evaluations. Final budbreak rate (FBR) of every cultivar and the number of days required to reach 50% of budbreak (DD50%) were evaluated. Chill required by cultivars to start budbreak varied in both years under evaluation. Both FBR and DD50 were higher in 2017 than in 2018. Due to high variation in FBR and DD50, chill requirements of pecan cultivars could not be clearly determined by the biological method.

Keywords: *Carya illinoinensis*; dormancy; biological method; nuts; cold requirements.

INTRODUCTION

The pecan [*Carya illinoinensis* (Wangenh) K. Koch] is native to temperate regions in the Northern Hemisphere, where it is commercially grown (Sparks, 2005; Walker *et al.*, 2016; Han *et al.*, 2018). However, this important fruit tree has crossed borders to be cultivated in all continents (Bilharva *et al.*, 2018). It is a large deciduous tree which can be productive for a long time. In winter, it goes through a period of vegetative dormancy, an adaptive characteristic of the species to survive low temperatures in its original region (Martins *et al.*, 2017).

Low temperatures in periods of vegetative dormancy affect plants in two ways, i. e., firstly, they contribute to stop growth and enable both cold acclimation and endodormancy induction and, secondly, they act on dormancy breaking (Petri *et al.*, 2021). The amount of chill that takes place from endodormancy induction to breaking is called chill requirement, which must be determined for every species and even every cultivar (Lang *et al.*, 1987). Thus, chill accumulation is needed to break dormancy, but many cultivation areas do not provide enough chill hours to culti-

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vars naturally. Therefore, chill accumulation has become a limiting factor in production; when it is not adequate, there is uneven budbreak, defective foliation, little ramification and many vegetative and flower buds keep dormant, a fact that leads to low yield (Grageda *et al.*, 2016; Wells, 2017b; Grageda *et al*, 2019). As a result, knowledge about chill requirements of every species and cultivar is extremely important to reach successful production.

Although pecan has adapted to several regions, its chill requirements, which may be one of the main environmental limiting factors in production, have not yet been elucidated. The literature has reported that its need for chill accumulation ranges between 50 and 1,000 hours at temperatures below 7.2 °C (Varela *et al.*, 2015; Wells, 2017a). The range is very broad and unspecific when different cultivars are considered.

From this perspective, it is essential to know chill requirements and budbreak behavior of cultivars implanted in a certain region. There are several techniques to study mechanisms involved in dormancy, such as the biological method, which is based on the evolution of time needed for budbreak of isolated buds subject to a standard temperature (Dole, 2001). This method is used for checking when endodormancy ends because the only inhibition for the bud to sprout comes from itself, since the other buds are removed and no other organ can inhibit the process (Hawerroth *et al.*, 2010).

Therefore, this study aimed at evaluating responses given by different pecan cultivars subject to artificial chill.

MATERIAL AND METHODS

The experiment was carried out in two orchards located in Canguçu, RS, Brazil, in 2017 and 2018, when pecan branches of cultivars Success, Shoshoni, Farley, Elliott, Mohawk, Jackson, Desirable, Barton, Importada, Shawnee, Choctaw (31°28"S 52°56"W) and Melhorada (31°28"S 52°41"W) were collected. Plants had been implanted in 2009 and were nine years old when the material was collected. Spacing is 9 x 6 m and 10 x 10 m. The orchards do not have any irrigation system.

Branches were about 30 cm long when they were collected in June 2017 and May 2018. Before collection, there was natural chill accumulation of 34 hours below 7.2 ºC in the first year while there were 18 hours in the second year. Data were provided by the Meteorological Station in Canguçu-A811, INMET. When branches were collected, 50% of leaves had already fallen. It corresponded to Phenological Stage 97 in the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) (Han *et al.*, 2018).

After their collection, branches were taken to the Cascata Experimental Station, which belongs to the Embrapa Clima Temperado, in Pelotas, RS, to carry out the treatments. Branches were soaked in water with sodium hypochlorite (1:1000 v/v) for five minutes. They were then wrapped in moist newspaper and placed in plastic bags (non-toxic polyethylene) in a cold chamber (4.0 ± 0.5 °C) to simulate chill hour accumulation.

Treatments consisted in exposing branches to 0, 250, 500, 750 and 1,000 chill hours. After the number of chill hours of every treatment was reached, the biological test was carried out to evaluate dormancy of lateral buds in 10 cm long branches. Only intermediate parts of branches were used, i. e., the bud that was 2 cm below the highest cut was kept while the other buds were eliminated. In order to mitigate branch and bud dehydration, the highest part of the branch was protected with paraffin (Figure 1).

Figure 1: Detail of the branch protected with paraffin and its bud in the phenological stage named beginning of bud break (green tip).

Branches were placed on trays with moist vermiculite and kept in a Biochemical Oxygen Demand (BOD) germination chamber at 25 ± 0.5 °C and a 16-hour photoperiod of light up to the end of evaluations of every treatment/collection. In the treatment with no chill hours (zero), branches were not placed in the cold chamber.

Dormancy intensity was evaluated by the biological test of single node cutting. Evaluations were carried out every two days; the beginning of budbreak was considered the moment in which buds had green tips (Figure 1), i. e., Stage 07 in the BBCH scale (Han *et al.*, 2018). Calculations of final budbreak rate (FBR) – which represents the percentage of cuttings with buds that had green tips – and the number of days required to reach 50% of budbreak (DD50%) were based on these data. Calculations were carried out by equations proposed by Lamela *et al.* (2020).

The experiment had a completely randomized design with four replicates; every replicate was composed of five cuttings. Two approaches were used for analyzing data. Firstly, a 12 x 5 bifactorial scheme (cultivars x artificial chill hours) was used for finding the FBR. When the effect was significant, the Tukey's test was carried out at 5% error probability by the SISVAR program (Ferreira, 2011). Afterwards, a 3-parameter logistic regression was applied. The response variable (y) was cumulative data on budbreak (%) while the predictor variable (x) was time in the growth chamber: $y = a/(1 + exp(-(x - x0)/b))$, where y is budbreak percentage, x is time expressed as days, a represents the difference between maximum and minimum points of the variable, b is the curve inclination and $x0 = DD50$. This analysis led to equations of logistic regression adjusted by the mean squared error and by the Akaike Information Criterion (AIC).

RESULTS AND DISCUSSION

The analysis of variance showed significant interaction ($p \leq 0.05$) between pecan cultivars x treatments of chill hours in both years under evaluation (Table 1), i. e., different responses were given by cultivars to treatments applied in both years.

Table 1: Final Budbreak Rate (%) of pecan cultivars subject to different artificial chill temperatures in 2017 and 2018

	Chill hours in 2017					M.cv
Cultivar	**0**	**250**	**500**	**750**	**1000**	
Success	75 eC*	95 bB	100 aA	100 aA	95 bB	93
Shoshoni	90 cC	95 bB	100 aA	90 bC	95 bB	94
Farley	75 eC	85 cA	80 cB	80 cB	80 eB	80
Elliott	100 aA	95 bB	100 aA	100 aA	100 aA	99
Mohawk	60 gE	70 eC	65 eD	80 cB	85 dA	72
Jackson	95 bB	100 aA	100 aA	100 aA	100 aA	99
Desirable	95 bB	100 aA	90 bC	100 aA	100 aA	97
Barton	80 dC	85 cB	75 dD	100 aA	85 dB	85
Melhorada	90 cB	75 dC	100 aA	100 aA	100 aA	93
Importada	95 bB	95 bB	100 aA	100 aA	90 cB	96
Shawnee	90 cB	95 bB	100 aA	100 aA	95 bB	96
Choctaw	70 fC	85 cB	65 fD	90 bA	90 cA	80
M.t	85	90	90	95	93	

	Chill hours in 2018					M. cv
Cultivar	**0**	**250**	**500**	**750**	**1000**	
Success	60 dC	60 eC	95 aA	95 bA	85 cB	79
Shoshoni	55 eC	50 fD	80 cA	60 gB	55 hC	60
Farley	65 cD	70 cC	75 dB	75 eB	90 bA	75
Elliott	60 dC	45 gD	90 bA	85 dB	80 dC	72
Mohawk	55 eC	50 fD	55 fC	60 gB	65 gA	57
Jackson	50 fC	80 aB	90 bA	90 cA	90 bA	80
Desirable	65 cB	65 dB	60 eC	60 gC	70 fA	64
Barton	55 eE	45 gF	70 dC	65 fD	75 eA	62
Melhorada	80 bB	75 bC	90 bA	85 dB	95 aA	85
Importada	45 gC	50 fC	80 cB	100 aA	75 eB	70
Shawnee	85 aC	75 bD	90 bB	95 bA	90 bB	87
Choctaw	45 gD	45 gD	50 fC	85 dB	95 aA	64
M.t	60	59	77	80	80	

where M.t = mean of treatment; M.cv = mean of cultivar. *Means followed by the same lowercase letter in a column and uppercase letter on a line do not differ by the Tukey's test at 5% significance.

FBR of cultivars (Table 1) that were not subject to any chill hour (zero) exhibited the lowest values, in general. However, 'Jackson' reached maximum FBR between 250 and 500 chill hours while cultivars Success, Shoshoni, Melhorada, Importada and Shawnee exhibited maximum FBR between 500 and 750 chill hours in 2017 and 2018. 'Mohawk', 'Barton' and 'Choctaw' were the most demanding cultivars since they required between 750 and 1,000 chill hours. In the case of 'Farley', 'Elliott' and 'Desirable', there was high variation in FBR in both years under study.

Thus, their chill requirements could not be precisely determined. Grageda *et al.* (2016) stated that studies have reported that 'Desirable' requires 500 chill hours while other studies showed that 'Success' and 'Desirable' require from 300 to 400 chill hours.

Therefore, studies of chill requirements of pecan cultivars are not conclusive. As a result, further studies are needed so as to elucidate chill requirements of cultivars. Even though plants may be in the same phenological stage (about 50% of fallen leaves) when material is collected on the field, cultivars may be in different dormancy phases.

In this scenario, cultivars may not have been in deep dormancy, i. e., when they were exposed to the growth chamber, budbreak took place. Besides, this result suggests that cultivars require high chill accumulation to start deep dormancy, by comparison with the other cultivars whose lowest FBR were found in the treatment with no chill hours (zero). Lang *et al.* (1987) reported that low temperatures act on dormancy in two ways. Firstly, they contribute to growth paralyzation and enable cold acclimation and endodormancy induction. Afterwards, they act to reverse this situation.

In general, it may be stated that the highest mean FBR was reached when chill accumulation was 750 hours, in both years under study. However, some cultivars reached maximum FBR when chill accumulation was equal to or below 750 hours. Thus, FBR was found to be higher in 2017 than in 2018, regardless of the treatment. The difference between both years may result from chill accumulated on the field when branches were collected, i. e., 34 and 18 chill hours below 7.2 ºC in 2017 and 2018, respectively. Besides, it may be due to the drought that occurred in the 2017/2018 summer (from November 2017 to March 2018) (SEMA-RS, 2018), which affected plant development and, consequently, reserve accumulation for the next year.

In this circumstance, it is clear that FBR is complex and may be influenced not only by chill hour accumulation but also by other factors. Therefore, further studies should correlate winter chill hours and the other climate variables of previous cycles to better elucidate differences in budbreak throughout developmental cycles.

In the literature, some studies have shown very broad ranges of chill requirements of pecan trees, from 50 to 1,000 hours (Varela *et al.*, 2015; Wells, 2017a). The experiment reported by this paper corroborates the studies, since some cultivars exhibited 100% budbreak when they were exposed to 250 (or fewer), to 500 or to 750 chill hours. Melke (2015) assures that pecan buds can sprout when they are exposed to 100 chill hours (or fewer), but it may lead to uneven sprouting and subsequent pollination problems. Thus, studies of whole plants and follow-up of plants on the field are needed to evaluate effects of chill hours on vegetative and reproductive development of the crop in order to get conclusive data.

The number of days required to reach 50% of budbreak (DD50%) varied much, depending on treatments and years under study (Tables 2 and 3). Based on regression equations adjusted for 2017 (Table 2), both 'Melhorada' and 'Choctaw' reached DD50 in the treatment with no chill hours (zero). Cultivars Success, Farley, Elliott, Mohawk, Jackson and Shawnee reached 50% of budbreak (DD50) faster when they were exposed to 250 chill hours. 'Barton' required 500 chill hours, 'Shoshoni' and 'Desirable' needed 750 chill hours and 'Importada' needed 1,000 chill hours to reach DD50.

Table 3 shows that there was a change in time required to reach DD50 in 2018. Five out of 12 cultivars under study – Elliott, Desirable, Barton, Melhorada and Importada – reached 50% of budbreak faster when they were exposed to 750 chill hours while cultivars Success, Shoshoni, Farley, Mohawk, Jackson, Shawnee and Choctaw required 1,000 chill hours. Therefore, DD50 was found to exhibit a wide range in 2017 and in 2018. In the latter, cultivars required more chill hours to reach this parameter. According to Lamela *et al.* (2020), DD50 is a simple parameter used for estimating the end of endodormancy. It means that this phase ends when the percentage (50% of budbreak) is reached. In this case, both FBR (Table 1) and DD50 were reached at the lowest chill accumulation in 2017, by comparison with 2018.

Another observed factor was the number of days required to reach DD50. Cultivars required from 5 to 11 days to reach 50% budbreak in 2017 (Table 2) while they needed from 10 to 15 days in 2018 (Table 3). Thus, buds that were in the growth chamber required more days to overcome dormancy, by comparison with the previous year.

Table 2: Regression equations adjusted for the number of days required to reach 50% of budbreak (DD50) in treatments under investigation in 2017

Cultivar	Hours	a	b	DD50	R²
		2017			
Success	0	95.0355	0.6922	11.5323	0.97
	250	95.1888	2.1096	8.3088	0.92
	500	98.6031	1.6898	10.4175	0.97
	750	101.3627	2.1620	8.3953	0.94
	1000	70.0023	0.5179	11.6728	0.90
Shoshoni	0	88.8417	2.2095	10.5303	0.87
	250	94.0317	2.5305	8.9378	0.90
	500	99.3548	1.6198	10.6795	0.97
	750	88.7722	1.6012	7.2985	0.94
	1000	90.1594	0.8288	12.004	0.93
Farley	0	74.1207	2.6262	14.5190	0.84
	250	79.9312	2.5730	11.3323	0.91
	500	82.0320	2.0604	13.8808	0.92
	750	80.0851	2.0045	12.2982	0.91
	1000	81.1581	1.3571	13.2397	0.92
Elliott	0	97.5548	2.2612	6.8554	0.93
	250	87.3926	1.1831	5.3710	0.88
	500	98.6127	1.3148	5.9250	0.96
	750	98.2807	1.0566	5.6023	0.95
	1000	100.2373	2.6700	9.2583	0.93
Mohawk	0	62.5884	2.0823	15.9514	0.93
	250	69.7365	2.2065	9.2386	0.88
	500	65.0063	0.5434	11.6535	0.93
	750	79.3147	2.9740	11.5370	0.88
	1000	82.9645	1.1671	12.2605	0.93
Jackson	0	92.0912	2.5627	9.0711	0.86
	250	99.7284	1.5069	6.0201	0.94
	500	100.8808	2.1376	8.8030	0.93
	750	99.2767	2.3425	10.1523	0.95
	1000	100.9162	2.362	9.638	0.92
Desirable	0	96.3941	2.7078	8.9625	0.91
	250	98.9877	2.6651	10.1416	0.94
	500	91.7234	2.4165	9.1079	0.90
	750	100.0382	1.2967	8.3562	0.98
	1000	100.1808	1.6434	8.3969	0.95

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Barton	0	82.9470	3.3454	9.8735	0.84
	250	86.6008	3.1587	9.1398	0.86
	500	71.9938	1.1309	6.1458	0.83
	750	99.0746	0.9859	7.7024	0.98
	1000	84.4158	1.1247	7.8531	0.95
Melhorada	0	87.6850	1.7146	6.4619	0.90
	250	73.1874	2.5727	7.2712	0.77
	500	96.8796	0.9360	6.5685	0.96
	750	94.1667	0.1454	6.9820	0.95
	1000	99.6262	2.0646	8.1035	0.94
Importada	0	96.2375	2.5933	9.0749	0.92
	250	96.2919	2.7002	8.7454	0.92
	500	100.0221	1.6776	8.6960	0.95
	750	99.8242	2.2680	9.4412	0.94
	1000	85.8191	2.3781	8.0202	0.88
Shawnee	0	95.5619	2.1707	8.6773	0.92
	250	94.1670	0.4590	5.9779	0.96
	500	97.5000	0.1355	6.8369	0.98
	750	99.2146	0.7675	6.3312	0.95
	1000	95.0429	0.7422	6.5998	0.94
Choctaw	0	69.0114	1.6086	6.1927	0.87
	250	84.7173	1.3339	6.4487	0.92
	500	58.9045	1.2774	7.0191	0.81
	750	88.9454	1.2969	6.3983	0.92
	1000	89.3510	1.9180	6.8476	0.92

p value < 0.0001.

It should be highlighted that, besides conditions found when plant material was collected and other data on climate in both years under investigation, other factors, such as alternate bearing, may have contributed to results. Thompson *et al.* (2019) stated that alternate bearing is a great challenge for pecan growers and the industry since it refers to the tendency to have an irregular crop load from year to year. The production of a heavy crop one year may be followed by a light one the next. It is common in fruit trees, but especially severe in pecan ones. According to the authors, in the "on" year, too many fruit are set while the subsequent year is "off" (few fruit). The intensity of this mechanism in cultivars depends on environmental conditions found in a certain region throughout the most sensitive phenological phases and on crop management. Thus, an "on" year, with good yield and, consequently, more waste of energy, by comparison with an "off" year, may influence budbreak and plant vigor. This factor helps to explain the difference found between 2017 and 2018, i. e., the former was "on" while the latter was "off".

Besides the difference found between 2017 and 2018, which may have been influenced by "on" and "off" years, other environmental variables and factors, such as management, may have significantly contributed to budbreak. Thus, studies of different techniques and longer periods of evaluation are needed to better understand dormancy mechanisms of different pecan cultivars. In addition, further studies that correlate other environmental variables are relevant to better elucidate results.

Table 3: Regression equations adjusted for the number of days required to reach 50% of budbreak (DD50) in treatments under investigation in 2018

Cultivar	Hours	a	b	DD50	R^2
		2018			
Success	0	50.0309	0.6314	19.7480	0.78
	250	109.5031	4.9917	30.3409	0.81
	500	94.2495	1.4919	16.71	0.94
	750	95.9037	1.4434	16.7429	0.93
	1000	84.1001	0.7119	13.1359	0.81
Shoshoni	0	51.6885	2.5742	24.0681	0.73
	250	52.6968	2.1181	23.8726	0.80
	500	71.1464	1.4299	15.8501	0.84
	750	57.6516	0.6892	16.4583	0.76
	1000	53.0713	0.6953	12.8312	0.78
Farley	0	67.5619	2.6938	22.8657	0.90
	250	72.8507	2.5679	16.9662	0.83
	500	72.0888	2.2513	15.0728	0.88
	750	75.0337	0.8977	14.6243	0.84
	1000	88.7528	1.5354	12.7646	0.92
Elliott	0	57.4931	1.3665	19.0127	0.76
	250	46.7259	1.7591	14.7729	0.84
	500	88.7626	2.3212	16.9989	0.90
	750	84.2158	0.8117	13.2768	0.91
	1000	81.3970	3.5385	14.7402	0.81
Mohawk	0	63.3833	3.1722	25.1821	0.80
	250	51.4213	2.3774	17.9754	0.83
	500	52.4149	1.6734	16.5600	0.65
	750	60.0441	0.9661	14.6738	0.74
	1000	60.5832	1.0648	13.0389	0.9
Jackson	0	59.4977	4.1036	24.3084	0.80
	250	82.2562	1.8862	19.1909	0.89
	500	90.7704	1.2409	17.1916	0.96
	750	90.0001	0.2388	14.0533	0.91
	1000	91.0347	1.5703	12.7836	0.95
Desirable	0	66.3731	2.0170	21.3208	0.85
	250	64.7719	1.8603	18.1035	0.88
	500	56.9327	1.0749	18.6309	0.92
	750	58.8328	0.7396	15.7350	0.73
	1000	58.8021	0.7423	15.7300	0.72

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Continuation

Barton	0	81.5904	4.6391	26.3289	0.86
	250	57.1393	4.6613	24.3171	0.78
	500	78.9660	3.7751	21.8035	0.90
	750	64.8519	1.6867	14.1287	0.80
	1000	74.9238	2.0114	15.6182	0.83
Melhorada	0	80.0887	2.6555	21.4386	0.90
	250	74.5213	1.4176	17.4588	0.85
	500	89.7921	1.5329	16.3801	0.90
	750	81.0577	1.1768	13.2852	0.95
	1000	94.2106	1.8816	14.8683	0.96
Importada	0	38.5014	1.7115	20.6510	0.83
	250	52.9788	2.4511	24.2071	0.78
	500	77.5115	2.0881	17.5160	0.91
	750	100.00	0.2361	13.7406	0.99
	1000	75.4040	1.7910	14.6358	0.95
Shawnee	0	78.7175	1.6388	19.2711	0.85
	250	77.1619	2.2758	19.3433	0.81
	500	87.7493	1.4646	17.7720	0.91
	750	94.6513	0.9545	13.7028	0.97
	1000	88.5012	1.9479	10.2203	0.89
Choctaw	0	47.7895	2.9040	21.4517	0.76
	250	43.3757	1.3329	16.6213	0.58
	500	49.9406	1.4026	17.7674	0.70
	750	85.0410	1.5334	17.8532	0.94
	1000	95.0075	2.4247	12.7122	0.96

p value < 0.0001.

CONCLUSION

The final lowest sprouting rate of cultivars took place in the absence of artificial cold while the highest one was reached after 750 chill hours.

Chill requirements that made cultivars reach budbreak varied in both years under evaluation. Final Budbreak Rate (FBR) and DD50 were higher in 2017 than in 2018.

Due to high variation in FBR and DD50, chill requirements of pecan cultivars could not be clearly studied by the biological method.

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