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Dynamic of blueberry buds dormancy in a region of low chill occurrence

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Abstract

Several cultivars of blueberry were introduced in the colder regions in Brazil, but presented varied vegetative and reproductive growth, due to the heterogeneity of adaptation to the regional climate. The objective of this research was to determine the dormancy dynamics of blueberry buds grown in a region of low chill occurrence. Four methods of bud dormancy evaluation were used: biological test of single node cuttings, dormancy index, tetrazolium test and Tabuenca test. The dormancy of floral and vegetative buds of the cultivars Bluebelle, Climax, Delite and Powderblue of the Rabbiteye group was studied in collections every two weeks from April 26 to July 21. In the evaluated period there were only 76 h of temperatures below 7.2° C. The biological test and the dormancy index were viable methods for the dynamic evaluation of buds dormancy. The tetrazolium test was efficient to predict the exit of the endodormancy of floral and vegetative buds, but the Tabuenca test was efficient to predict the exit of floral buds dormancy only for the cultivars of less intense dormancy. The cultivars Bluebelle and Powderblue presented better adaptation to the conditions of low cold occurrence, with well defined installation and overcoming of the dormancy. The cv. Delite is more susceptible to budding heterogeneity and the cv. Climax is the least indicated to the region.

Keywords: dormancy, plant physiology, *Vaccnium ashei*

Introduction

Blueberries were introduced in Southern Brazil in the 1980s, but cultivars with greater production potential were introduced only after 2010, with low chill requirements and early production, which could be grown in warmer regions of the country (Cantuarias-Aviles et al., 2014). Cultivars of *Rabbiteye* group are more cultivated in Southern Brazil, such as Bluebelle, Climax, Delite and Powderblue (Fischer et al., 2014), because they are more adapted and more productive (Pasa et al., 2014; Medeiros et al., 2017). The knowledge about the dormancy dynamics of blueberry buds and the artificial cold effects on the plant is not consolidated yet. Thus, basic studies of their physiology become relevant to support decision making in the implantation and maintenance of orchards, such as the breakdown of dormancy with chemicals (Picolotto et al., 2014).

The activity of buds during dormancy can be very variable and difficult to determine, but their knowledge is fundamental to conclude whether dormancy has been naturally overcome or producer interference is required to stimulate a new growth cycle. Among the tests to evaluate the dormancy dynamics, we highlight the biological method of single node cuttings (Carvalho & Biasi, 2019) that allows the quantification of the bud dormancy as a function of its average time for bud burst under controlled conditions. Other methods support the interpretation of the biological test as the dormancy index created for apple buds (Carvalho & Silva, 2010) and extended for application in other fruit plants by Carvalho and Biasi (2012).

The test of Tabuenca (1964) is also used to determine the dormancy exit of floral buds by the fresh and dry mass variation of the buds during the resting period and after growth induction under controlled

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conditions, but also requires a week to its accomplishment. To give an alternative evaluation of dormancy in shorter time, Carvalho et al. (2010) studied the application of the tetrazolium test in the evaluation of fruit buds dormancy.

It is necessary for the producer to determine the entry and exit of dormancy of the floral and vegetative buds of the plant and evaluate the adaptation of a cultivar to a region climate, avoiding losses in production due to the uniform bud burst of the plant.

The objective of this study was to determine the dormancy dynamics of blueberry buds grown in a low cold region.

Material and Methods

The dormancy of floral and vegetative buds was studied for the cultivars Bluebelle, Climax, Delite and Powderblue. The branches with healthy and intact buds were collected in the orchard of Experimental Station of Canguiri of UFPR in the Municipality of Pinhais, PR, Brazil, in latitude 25°23'30" South, longitude 49°07'30" West and altitude of 920 m. The number of temperatures below 7.2 °C hours in the region was 76 h. The orchard is located in a low chill occurrence region and with frequent high temperatures in the fall and winter.

The branches with a diameter of 3.5 to 4.5 mm were collected at intervals of two weeks from April 26 to July 21, 2017. Biological and biochemical tests were performed to determine the dormancy intensity and to relate the feasibility of applying the tests in the buds dormancy evaluation.

The biological test of single node cuttings was carried out with cuttings of 7 cm in length, keeping only the upper bud and eliminating the others. Each plot was formed by a group of ten cuttings conditioned in vermiculite moistened in growth room with temperature of 25 °C and photoperiod of 16 h. The evaluations were done three times a week until the maximum period of 40 days identifying the stages of green tip (GT) and open bud (OB). Based on these data were calculated:

- Mean time of budburst (MTB): The mean number of days between the start of the experiment and the day that GT stage was observed for each bud.

- Budburst rate (BR): percentage of buds that reached the GT stage;

- Vigorous Bud Rate (VBR): percentage of buds that reached the GT stage and progressed to the OB stage;

- Velocity of budburst (VB): budburst occurrence as a function of time for sprouting (buds day^{-1})

Carvalho and Silva (2010) have determined a dormancy index (DI) for apple buds that can be adapted to other fruit species, by assigning in the general formula values calculated for the constants *k* and *w*. The general formula described is:

$$ID = MTB \times (k \times BR + w \times VB + VBR)^{-1}$$

The constants *k* and *w* are calculated by means of the relationship between the correlation coefficients of the MTB, BR, VBR and VB variables obtained in the biological test as follows:

$$|k| = |r_{(BR/VB)}| \times |r_{(BR/VBR)}| / |r_{(BR/VB)}| - |r_{(BR/VBR)}|$$
$$|w| = |r_{(VB/BR)}| \times |r_{(VB/VBR)}| / |r_{(VB/BR)}| - |r_{(VB/VBR)}|$$

being $|r_{(x/y)}|$ the modulus of the correlation coefficient between the variables "x" and "y".

After identifying the constants of the formula, the reference values for each dormancy intensity were determined and the specific dormancy intensity interpretation groups were set up for floral and vegetative buds of the blueberry tree in classes of absent, weak, moderate, intense and deep dormancy.

The biochemical method of bud dormancy analysis proposed by Carvalho et al. (2010) was adopted. The buds were detached from the branches and sectioned longitudinally to expose their internal tissues. Samples with 300 mg of floral buds and 50 mg of vegetative buds were maintained in 5 mL of 1% (w / v) 2.3.5 triphenyl tetrazolium chloride solution in open flasks kept in a growth chamber at 25° C for two hours to stain the living tissues. Then the colored buds were removed from this solution and kept in another solution of 6 mL absolute ethyl alcohol (AS) at room temperature for one hour to extract the red color of the buds. The color intensity obtained in the ethyl alcohol solution was measured by absorbance spectrophotometry at 560 nm. At each collection date samples of equal mass (MF) were taken for oven drying at 65° C under forced ventilation until constant mass (MS) for determination of moisture by means of equation:

$$U\ (\%) = [(MF - MS) \times MF^{-1}] \times 100$$

The absorbance values were corrected according to the humidity of the sample, resulting in absorbance per 100 mg of dry mass of buds. The method assumes that the higher the dormancy intensity, the lower the staining of the buds being evaluated, due to the lower cellular metabolic activity in the dormant bud.

The test of Tabuenca (1964) was carried out in

each collection with terminal floral buds of each cultivar. Four replicates with five cuttings of 10 cm long containing terminal floral buds were kept in a growth chamber at 25° C and photoperiod of 16 hours for seven days, with their bases immersed in deionized water to simulate favorable conditions for bud burst. At the end of this period, the terminal floral buds were detached and subjected to oven drying at 60° C until constant mass to determine the dry and fresh masses to calculate the moisture of the buds.

The experimental design was a randomized complete block design in split plots. The main plots were the four cultivars and the subplots the seven collection dates, with four replications. Floral and vegetative buds were analyzed separately. The homogeneity of the variances was tested by the Bartlett test and the means with significant differences by the F test in the analysis of variance were submitted to the Tukey test ($p \leq 0.05$).

Results and Discussion

The MTB variation of floral buds was distinct for each cultivar, indicating important physiological characteristics of entrance and exit of dormancy during autumn and winter. The cv. Bluebelle showed the lowest values of MTB in the beginning of June, but after this date it had similar values to the other cultivars. The cv. Powderblue presented higher MTB up to the beginning of June, but was reduced significantly at the end of June, indicating the reduction of the dormancy intensity and the sprouting arrangement of the bud. This early arrangement of buds to sprout in mild winter climates may be a problem because of the frequent occurrence of high temperatures, which stimulate sprouting, followed by low temperatures, even frost, which can damage the new open flowers. In this sense the cv. Powderblue, due to presenting dormancy at the beginning of the period, would be less subject to these thermal fluctuations. The cultivars Climax and Delite presented higher MTB until the end of June, followed by a significant reduction in the month of July (Table 1).

Table 1. Mean time of budburst (MTB) of floral buds of four blueberry cultivars in Pinhais, PR, Brazil.

Dates	Cultivars							
	Bluebelle		Climax		Delite		Powderblue	
	MTB (days) of floral buds							
April 26	9.2	Ab	9.4	Bab	15.3	ABCa	11.5	ABab
May 12	5.9	Ab	6.7	Bb	21.7	Aa	16.7	Aa
May 26	8.0	Ab	16.3	Aa	10.3	CDEb	17.3	Aa
June 09	4.7	Ab	12.6	ABa	17.8	ABa	17.2	Aa
June 23	7.2	Aa	9.7	ABa	11.5	BCDa	9.0	Ba
July 07	6.6	Aa	7.7	Ba	8.0	DEa	5.1	Ba
July 21	3.5	Aa	7.2	Ba	4.6	Ea	5.9	Ba
CV(cultivars)	22.2%		CV(dates)		27.4%			

* Values that are followed by different letters, uppercase in the same column and lowercase in the same line, are significantly different using Tukey's test ($P \leq 0.05$). CV=coefficient of variation.

The dormancy dynamics of vegetative buds was similar to floral buds dormancy, but with greater intensity. The cv. Bluebelle showed lower dormancy intensity of vegetative buds in the beginning of June in relation to the other cultivars, which was naturally surpassed only at the end of July, characterized by a significant drop in MTB. The vegetative buds of the cv. Powderblue and Delite presented dormancy from April to the end of June and the beginning of July respectively. For cv. Clímax the significant increase of MTB from the beggining to the end of May indicated the stablishment of the dormancy that remained until the end of June (Table 2). Although according to Strik (2007), cultivars of the *Rabbiteye* group have a cold requirement of 300 to 400 h of temperatures below 7.2° C, the dormancy of the cv. Bluebelle, Delite and Powderblue had already been overcome even with a winter of only 76 h cold. These characteristics indicate the possibility of growing the plant in a protected environment without occurrence of characteristic cold of autumn and winter (Picolotto et al., 2014).

The analysis of the DI that considers the MTB, BR, VBR and VB variables obtained in the biological test, confirms the dormancy dynamics of the floral and vegetative buds observed by MTB analysis alone and indicates in a practical way the intensity of dormancy and disposition to sprouting (Table 3). The reference DIs for the dormancy analysis were established as follows for floral buds: Absent dormancy: $DI \leq 2.0$; Weak dormancy: $2.0 < DI \leq 3.0$; Moderate dormancy: $3.0 < DI \leq 4.4$; Severe dormancy: $4.4 < DI \leq 6.0$; Deep dormancy: $DI > 6.0$. For the vegetative buds, the reference DIs developed were: Absent dormancy: $DI \leq 4.0$; Weak dormancy: $4.0 < DI \leq 6.0$; Moderate dormancy: $6.0 < DI \leq 8.4$; Severe dormancy: $8.4 < DI \leq 12.0$; Deep dormancy: $DI > 12.0$.

By the end of June all flower buds were no longer dormant and consequently were able to sprout as soon as

environmental conditions were favorable, which naturally occurred at the end of July. The floral buds of the cv. Bluebelle showed only weak dormancy at the beginning of the period, while for the others the dormancy evolved from April and lasted until the beginning of June, in a heterogeneous way for the cv. Climax and Delite and homogeneously for the cv. Powderblue (Table 3). The homogeneous dormancy dynamics of the buds of the cv. Powderblue shows the good adaptation of this cultivar to the region, without risk of very early blooms. Moreover, this cultivar presents high levels of starch in its branches in the winter (Oliveira et al., 2012), which is favorable to the internal metabolism of the plant and constitutes a source of reserves for a good budding in the next cycle.

The analysis of the DI of the vegetative buds emphasizes the higher dormancy intensity of these buds in relation to the floral ones, with important differences of their dynamics during autumn and winter. The cv. Bluebelle presented dormancy less intense than the others, but this one was totally surpassed at the end of July, even with the low occurrence of cold in the region. The cv. Powderblue presented deep and constant dormancy from late May to early June and was fully overcome in the beginning of July. Deep dormancy early in the fall and winter period is interesting to avoid unwanted early sprouting. The vegetative buds of cv. Delite presented deep dormancy that was partially overcome at the end of July, while the buds of cv. Climax showed deep dormancy that was not overcome by the natural cold of the region. These two cultivars would require the artificial overcoming of dormancy to guarantee full sprouting necessary for the production cycle quality of that year and the following year (Table 3). However, the application of sprouting inducers in the cultivar Climax did not have an effect on sprouting, besides reducing the production by phytotoxic effect (Coletti et al., 2011). As for the cv. Powderblue, with more homogeneous dormancy dynamics, the application of sprouting inducers in protected environment was favorable to sprouting and fruit production (Picolotto et al., 2014).

Table 2. Mean time of budburst (MTB) of vegetative buds of four blueberry cultivars in Pinhais, PR, Brazil.

Dates	Cultivars							
	Bluebelle		Climax		Delite		Powderblue	
	MTB (days) of vegetative buds							
April 26	15.1	ABa	15.1	Ca	20.3	ABa	17.8	Aba
May 12	18.9	Aab	15.1	Cb	24.4	Aa	17.5	ABab
May 26	21.2	Ab	30.1	Aa	26.9	Aab	21.1	Ab
June 09	14.8	ABb	23.7	ABa	22.2	Aa	22.5	Aa
June 23	19.1	Ab	25.5	ABab	26.0	Aa	21.8	Aab
July 07	21.2	Aa	21.6	BCa	21.1	ABa	12.5	BCb
July 21	11.4	Bb	22.5	Ba	14.8	Bb	10.2	Cb
CV(cultivars)	5.9%		CV(dates)	15.5%				

* Values that are followed by different letters, uppercase in the same column and lowercase in the same line, are significantly different using Tukey's test ($P \leq 0.05$). CV=coefficient of variation.

Table 3. Dormancy Index (DI) of floral and vegetative buds of four blueberry cultivars in Pinhais, PR, Brazil.

Dates	Cultivars							
	Bluebelle		Climax		Delite		Powderblue	
	Floral buds							
April 26	2.1	Weak	2.9	Weak	6.1	Deep	4.6	Moderate
May 12	0.9	Absent	1.1	Absent	9.5	Deep	13.4	Deep
May 26	1.3	Absent	5.2	Severe	2.2	Weak	7.7	Deep
June 09	0.5	Absent	3.4	Moderate	6.9	Deep	7.7	Deep
June 23	1.3	Absent	2.0	Absent	3.9	Moderate	2.5	Weak
July 07	0.8	Absent	1.1	Absent	1.2	Absent	0.7	Absent
July 21	0.3	Absent	1.0	Absent	0.5	Absent	0.7	Absent
	Vegetative buds							
April 26	5.8	Weak	12.9	Deep	42.2	Deep	9.3	Severe
May 12	12.1	Deep	6.6	Moderate	18.9	Deep	10.8	Severe
May 26	8.9	Severe	42.6	Deep	18.1	Deep	14.4	Deep
June 09	4.8	Weak	14.8	Deep	18.8	Deep	15.2	Deep
June 23	5.9	Weak	21.3	Deep	13.9	Deep	11.1	Severe
July 07	9.2	Severe	12.7	Deep	7.9	Moderate	3.5	Absent
July 21	2.5	Absent	16.5	Deep	4.7	Weak	2.2	Absent

The tetrazolium test analyzes the activity of the internal buds tissues and proves that the floral or vegetative buds, even with established dormancy, present significant variations in their metabolism (Tables 4 and 5). The high

metabolic activity in the resting period indicates that other factors are involved in the bud burst capacity, such as the availability and transport of organic compounds and minerals at short distances (Carvalho & Zanette, 2004) or the balance of substances of cellular metabolism such as reactive oxygen species (ROS) (Beauvieux et al., 2018) or reactive nitrogen species (RNS) (Sudawan et al., 2016). When dormancy is completely overcome the existing metabolic activity translates into intense cell division and synthesis and necessary compounds transport for tissue growth and stretching. This variation of metabolic activity during the autumn and winter period occurred for floral and vegetative buds, but in both cases, the dormancy exit only took place after the month of June, except for the cv. Climax, that although there was an increase of the bud metabolic activity, was not completely dormant with the biological test and the DI, indicating a greater complexity of natural dormancy overcoming.

The control of the entry and exit of bud dormancy is complex and may also be under the effect of antioxidant enzymes whose activity decreases during exposure to low temperatures occurring during the winter. The reduction of the enzymatic activity causes the increase of the levels of hydrogen peroxide in the buds, which initiates a process of signal transduction, which result is the sprouting of the buds when the climatic conditions are favorable (Sudawan et al., 2016).

The Tabuenca test was efficient to detect the moisture variations of the bud at the end of the dormancy of the floral buds only of those cultivars which dormancy was totally overcome, such as Bluebelle and Powderblue. For cultivars with more intense dormancy and greater requirement to environmental conditions, the variations were not perceptible (Table 6).

Table 4. Test of tetrazolium in floral buds of four blueberry cultivars in Pinhais, PR, Brazil.

Dates	Cultivars							
	Bluebelle		Climax		Delite		Powderblue	
	Absorbance (560 nm) of floral buds							
April 26	0.106	Aa	0.103	Aa	0.063	Ab	0.087	BCab
May 12	0.092	Aba	0.088	Aba	0.075	Aa	0.089	BCa
May 26	0.104	Aa	0.101	Aa	0.093	Aa	0.124	Aba
June 09	0.088	ABab	0.080	ABCb	0.064	Ab	0.119	ABa
June 23	0.073	ABab	0.067	ABCb	0.084	Aab	0.106	ABCa
July 07	0.058	Ba	0.039	Ca	0.048	Aa	0.071	Ca
July 21	0.090	ABb	0.053	BCc	0.080	Abc	0.135	Aa
CV(cultivars)	32.7%		CV(dates)	18.4%				

* Values that are followed by different letters, uppercase in the same column and lowercase in the same line, are significantly different using Tukey's test (P ≤ 0.05). CV=coefficient of variation.

Table 5. Test of tetrazolium in vegetative buds of four blueberry cultivars in Pinhais, PR, Brazil.

Dates	Cultivars							
	Bluebelle		Climax		Delite		Powderblue	
	Absorbance (560 nm) of vegetative buds							
April 26	0.173	BCa	0.140	BCa	0.138	BCa	0.133	CDa
May 12	0.173	BCa	0.121	CDa	0.139	BCa	0.115	CDa
May 26	0.288	Aa	0.254	Aa	0.288	Aa	0.313	Aa
June 09	0.145	BCa	0.118	CDa	0.133	BCa	0.176	BCDa
June 23	0.205	Ba	0.206	Aba	0.171	Ba	0.215	Ba
July 07	0.121	Ca	0.059	Da	0.072	Ca	0.097	Da
July 21	0.145	BCa	0.140	BCa	0.167	Ba	0.187	BCa
CV(cultivars)	17.1%		CV(dates)	19.5%				

* Values that are followed by different letters, uppercase in the same column and lowercase in the same line, are significantly different using Tukey's test (P ≤ 0.05). CV=coefficient of variation.

Table 6. Humidity (%) of floral buds in the Tabuenca test of four blueberry cultivars in Pinhais, PR, Brazil.

Dates	Cultivars							
	Bluebelle		Climax		Delite		Powderblue	
April 26	61.1	Bab	61.9	Ca	62.9	Aa	58.2	Cb
May 12	62.5	Ba	63.5	BCa	63.2	Aa	60.8	BCa
May 26	62.4	Ba	65.1	BCa	62.9	Aa	63.6	Ba
June 09	63.6	Ba	62.5	BCa	60.7	Aa	61.5	BCa
June 23	62.3	Ba	64.3	BCa	60.7	Aa	62.1	BCa
July 07	62.0	Bbc	70.4	Aa	61.0	Ac	64.8	Bb
July 21	72.0	Aa	66.3	ABbc	62.7	Ac	69.2	Aab
CV(cultivars)	2.8%		CV(dates)	2.6%				

* Values that are followed by different letters, uppercase in the same column and lowercase in the same line, are significantly different using Tukey's test (P ≤ 0.05). CV=coefficient of variation.

Conclusions

It is concluded that there are important differences in the adaptation of blueberry tree cultivars in relation to the establishment and dormancy overcoming of floral and vegetative buds in the studied region. The biological test and the dormancy index are viable methods for the evaluation of dormancy of floral and vegetative buds. The tetrazolium test was efficient to predict the dormancy exit of the buds of the cultivars more adapted to the region and the Tabuenca test was efficient to predict the exit of the dormancy of floral buds only for the cultivars of light dormancy. The cultivars Bluebelle and Powderblue presented better adaptation to the conditions of low cold occurrence, with well defined installation and overcoming of the dormancy. The cv. Delite, due to the heterogeneous behavior of floral and vegetative buds dormancy, although its dormancy has been overcome, is more susceptible to budding heterogeneity, perhaps related to the diameter of the branches. The cv. Climax is the least indicated to the region and it needs the management of dormancy overcoming to express all its budding potential.

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