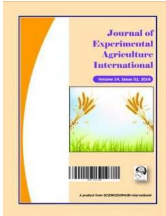

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Dormancy Studies in Apple Trees Cultivars Grown under Mild Climate

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Authors' contributions

This work was carried out in collaboration between all authors. Author IA conducted field and laboratory work and wrote the first draft of the manuscript. All other authors contributed equally in data collection, manage the study analyses and field coordination. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In order to research dormancy dynamics in apple (*Malus domestica* Borkh.) cultivars 'Eva' (low chilling requirement) and 'Mollie's Delicious' (high chilling requirement) in the year of El Niño occurrence.
Study Design: Complete randomized experimental design was arranged in bifactor scheme with four replications to each cultivar. Factor A was the conditions (Field condition and Forcing condition), and factor B comprised the sampling date (1, 2, 3, 4, 5), differing on chilling accumulation hours, which were measured by Embrapa climatic stations, cv. 'Mollie's Delicious' (89 h, 156 h, 176 h, 324 h, 367 h), and cv. 'Eva' (60h, 119h, 124h, 161h, 211h), resulting on a bifactorial 2x5 for each cultivar.
Place and Duration of Study: Experiment was developed from adult apple orchards trees at

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Arroio do Padre (31°43'S and 52°41'W) and Capão do Leão (31°56'S and 52°29'W) located near Pelotas, Southern of Brazil, between June 2014 to September 2014.
Methodology: Biological tests were performed, named Tabuenca's test (forcing conditions) and Single Bud Test (average time for budburst).
Results: During Tabuenca's evaluation period, cv. 'Mollie's Delicious' presented steady humidity until late winter, where humidity inside the bud showed a high increase, suggesting the end of endodormancy. Cultivar 'Eva', in the same test, showed steady humidity in the whole period, not indicating the end of endodormancy. In the Single Bud Test, cv. 'Eva", when subjected to forcing conditions, presented a proportional budding humidity curve with an atypical behavior and a high sprouting rate, suggesting that it has not entered into endodormancy stage.
Conclusion: The study suggests that Tabuenca's test and average time for budburst methods were not efficient to measure the end of endodormancy in cv. 'Eva' under warm winter conditions during the El Niño years. Evidence is presented that chill insufficiency is already happening in several global locations and it may causes unexpected response in crops.

Keywords: Malus domestica Borkh.; water content; endodormancy; bud weight; chilling requirement.

1. INTRODUCTION

Temperate climates are characterized by a seasonal phase where sub-optimal temperatures restrict or cease growth [1]. As a result, temperate perennial crops have developed mechanisms to survey under low temperature stress during winter [2]. Dormancy mechanisms prevent low temperature damages to flowers by protecting buds and delaying anthesis, and it can be divided into three distinct stages: paradormancy, endodormancy, and ecodormancy [3].

However, in subtropical regions, dormancy process during the winter period suffers several alterations caused by high temperature variation, which is even more problematic during El Niño years, decreasing temperate perennial plants production. The clinal phenology variation of fruit crops at warmer locations is well known: the same cultivar tends to have earlier flowering and ripening dates in warmer areas [4]. However, this trend is halted when the chilling requirements (CRs) of a cultivar are not properly satisfied in a given location, due to adaptation problems. Apple (*Malus domestica* Borkh.) orchards under this situation of inadequate chilling fulfilment present a very complex behavior, including physiological disturbs, which results in a low rate of lateral buds, irregular flower bud formation, delayed and/or reduced bud burst and, thus, low productivity [5,6]. The success of apple production in subtropical regions depends on adapted cultivars and how they maintain a high productivity in adverse conditions.

In view of phenological analyses results and the well-known effects of growing cultivars in warmer or too-warm areas, it is possible that advances or delays in phenology might be linked to the relative CRs of the species and the chilling accumulation (CA) of the area studied. If warming during winter means that the CR of the cultivar is lower than the CA of the area, then its phenology probably will still advance due to both a proper fulfilment of CR and an earlier satisfaction of the HR (in winter and/or spring). However, if warming diminishes the CA below the CR, then symptoms of inadequate CR satisfaction may appear. What is more, even a delay in CR fulfilment might imply symptoms of inadequate CA, as chilling temperatures have to be accumulated within a certain period of time to be efficient [6].

This work therefore aimed to measure the dormancy deepness of two apple cultivars with distinct chilling requirement and correlate with the water content present in reproductive spur buds.

2. MATERIALS AND METHODS

The experiment was developed from adult apple orchards trees of a private property at Arroio do Padre (31°43'S and 52°41'W) and Capão do Leão (31°56'S and 52°29'W), both places located near Pelotas, Southern Brazil. This region is characterized by having a humid subtropical climate. As plant material it was used apple cultivar of low CR 'Eva' ('Anna' x 'Gala') and high CR 'Mollie's Delicious' from NJ 14 ('Golden Delicious' x 'Edgewood') x NJ 4 ('Red Gravenstein' x 'Close') breeding, both cultivars grafted on 'Marubakaido' rootstock.

Complete randomized experimental design was arranged in bifactor scheme with four replications to each cultivar. Factor A was the condition (Field condition and Forcing condition), and factor B comprised the sampling date (1, 2, 3, 4, 5), differing on CA hours below 7.2ºC, which were measured by Embrapa climatic stations, cv. ‘Mollie’s Delicious’ (89 h, 156 h, 176 h, 324 h, 367 h), and cv. ‘Eva’ (60 h, 119 h, 124 h, 161 h, 211 h), resulting on a bifactorial 2x5 for each cultivar.

For one-bud cutting test, long annual shoots bearing lateral vegetative buds were regularly collected in orchard from June 2014 to September 2014, made 40 cuttings and placed inside a forcing room at the laboratory (25ºC±1; 16 hours of light) with water supplies. For each collection date, observations were made three times by week for each bud in forcing room [7].

At the end of the observation period, the percentage of budburst and the average time for budburst (ATB value) were observed for each cutting sample. Regarding the Tabuenca’s test [8], spurs bearing terminal flower buds were sampled regularly from June 2014 to September 2014. At each sampling date, shoots were separated in two sets: the first one was used to estimate humidity in orchard condition; the second set was placed inside the forcing room, shoots were stored in containers with water supplies. One week later, primordia were also extracted from forcing buds and weighted. Time-course changes in water content of flower primordia were graphically elaborated before and after forcing.

Data were analyzed for normality by Shapiro Wilk’s test; for homoscedasticity by Hartley’s test; and for residues independence by graph analysis. Data were later submitted to variance analysis by test F ($p \leq 0.05$). When statistical significance was extant, the effects of different conditions were compared by test t ($p \leq 0.05$). Whenever treatment interaction was presented, confidence intervals at 95% were plotted on the graph and the difference was significant when there was no overlaying between the vertical bars. The effects of collect date (CA) were evaluated by linear regression.

3. RESULTS AND DISCUSSION

Water content (%) of ‘Eva’ cultivar, with 4.4% coefficient of variation (CV), presented no statistical significance between treatment factors conditions ($F = 2.86$; $p = 0.1011$), and collection ($F = 2.03$; $p = 0.1155$) and neither to the interaction between the treatment evaluated ($F = 0.16$; $p = 0.9581$). Cultivar ‘Mollies Delicious’ presented statistical significance between the treatment factors condition and collection date, for the variable evaluated ($p < 0,05$).

Cultivar ‘Eva’ presented an atypical behavior during the period evaluated, according to Campoy and co-workers [6], this behavior may be due to the high temperature variation not allowing plant reaches the endodormancy stage. Physiological development of cv. ‘Eva’ could have been affected by the lack of low temperatures associated to high temperature fluctuations commonly observed during El Niño years, which implicates in a shallow dormancy throughout the rest of the cycle. As observed in ATB (Table 1), even during winter the present cultivar has shown an elevated rate of bud burst during the forcing conditions, which indicates an unexpected plant behavior. Moreover, the fact that cv. ‘Eva’ requires a low chilling rate may have modified the normality of dormancy procedure, putting this cultivar in the ecodormancy stage (quiescence condition) throughout the winter.

Table 1. Bud bursting (%) observation of cv. ‘Eva’ under forcing conditions (25ºC±1; 16 hours of light)

Collection	Bud burst (%)		
	2 D.A.C	**4 D.A.C**	**7 D.A.C**
1 (60 c.a)	12.5%	60.0%	67.5%
2 (119 c.a)	10.0%	55.0%	62.5%
3 (124 c.a)	0%	65.0%	67.5%
4 (161 c.a)	12.5%	50.0%	60.0%
5 (211 c.a)	10.0%	65.0%	72.5%

**D.A.C: Days after collection.*
**c.a: Hours of chilling accumulation*

In addition, Tabuenca’s test, which correlates bud humidity with dormancy stage, seems not to be appropriated to measure the development of a low CR cultivar during an atypical year, facing that there were no statistical differences in bud humidity during the collect dates. The consequence of the El Niño during dormancy stage resulted in different conclusion obtained by Yaacoubi and co-workers [9], who confirmed the ability of Tabuenca’s test to discriminate low- (‘Eva’) and high-chill cultivars (‘Gala’ and ‘Fuji’).

During the collections, the cultivar ‘Mollie’s Delicious’ showed a typical behavior during the

ATB observations [10,11,12,13]. This cultivar presented no response to the increased temperature and photoperiod, which indicates endodormancy stage inside the bud (Table 2). According to Chen and co-workers [14], this stage of dormancy is closely related to changes in abscisic acid (ABA), which is responsible for affect dormancy progression through its action on dehydrins or membrane permeability. Plant tissues in endodormancy stage have a diminished ability to convert biochemical and energetic compounds needed for normal development, thus, metabolic processes are blocked [15,16].

Table 2. Bud bursting (%) observation of cv. 'Mollie's Delicious' under forcing conditions (25ºC±1; 16 hours of light)

Collection	Bud burst (%)		
	2 D.A.C	**4 D.A.C**	**7 D.A.C**
1 (89 c.a)	0%	0%	0%
2 (156 c.a)	0%	0%	0%
3 (176 c.a)	0%	0%	0%
4 (324 c.a)	0%	0%	0%
5 (367 c.a)	2.5%	52.5%	60.0%

**D.A.C: Days after collection.*
**c.a: Hours of chilling accumulation*

The difference between field and forcing conditions are exposed in Table 3. Values obtained indicates that there is no distinction between bud humidity extracted directly from the field and those exposed to the forcing conditions during all collection dates, except for the collection "3" which had demonstrated statistical difference. Buds that were submitted to high temperature and photoperiod presented a higher water content (%) than buds that were evaluated directly from the field, this could be an attempt to overcome endodormancy through the ambient stimulus.

Bud water content variation during the collection is demonstrated on Fig. 1. There was no statistical significance in bud humidity between the collections "1" to "4", which corroborates to the previous results indicating the endodormancy stage. The collection "5" was characterized by an increasing in bud water content, indicating an enhanced metabolic activity inside the bud. This behavior of apple trees during dormancy has been pointed as the transition from endo- to ecodormancy stage [15]. These values were similar to those found previously (77%) by Yaacoubi and co-workers [9] and Malagi and co-workers [16], who considered as a biological marker of effective establishment of the ecodormancy stage.

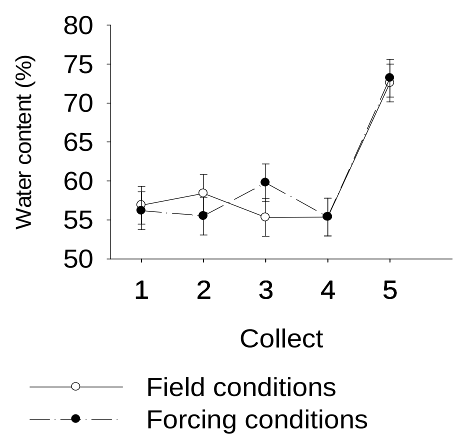

Fig. 1. Bud water content on 'Mollie's Delicious' cultivar during 5 collections date under field and forcing conditions. Collects 1, 2, 3, 4, 5 correspond to chilling accumulation (89 h, 156 h, 176 h, 324 h, 367 h). Vertical bars present confidence intervals (95%)

During the experimental year of 2014, Southern Brazil suffered effects of El Niño, resulting in above-average temperatures and severe drought. Climate changes might result in deficient dormancy release (late phenology) or reduce ecodormancy stage (early phenology) [6], affecting the suitability for some cultivars to yield

Table 3. Bud water content (%) on 'Mollie's Delicious' cultivar under different conditions during collection date

Condition	Water content (%)				
	1	**2**	**3**	**4**	**5**
Field conditions	56.88[a]	58.38[a]	55.31[b]	55.38[a]	72.56[a]
Forcing conditions	56.16[a]	55.49[a]	59.77[a]	55.36[a]	73.19[a]

**Different letters within columns indicate statistical differences between means (Fisher P ≤ 0.05)* in several locations of mild climate [17]. Similar consequences could be observed in apple trees grown under mild climates during El Niño year occurrence.

4. CONCLUSION

The presented results point out different responses between cultivars, low CR could have been the factor that determined cv. 'Eva' presented shallow dormancy, whereas high CR cv. 'Mollie's Delicious' presented a deep dormancy, characterized by a typical low metabolism even during El Niño year. Several authors have also demonstrated that there is good evidence of climate warming reducing winter CA. Impacts on perennial fruit trees due insufficient winter chilling are clearly pointed. Evidence is presented that chill insufficiency is already happening in several global locations and it may causes unexpected response in crops. Developing our understanding of these processes is vital and critically important.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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