



## **A Study of Water Dynamics and Phenology in Plant Parts of 'Eva' Apple Trees Grown under Winter Conditions in Southern Brazil**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors CFB, ESA, MBM, RMF and RRY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PCMF, FGH and RRY managed the study analyses. Authors ALSC, CFB, ESA and CSPL managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** The objective of this study was to evaluate water dynamics in different tissues of apple 'Eva' during dormancy stage under mild winter conditions of Pelotas, located in Southern Brazil.

**Study Design:** Experimental design was completely randomized, with five replications, and the experimental unit was composed by a single plant, on 3x3x4 factorial arrangement, obtained from a combination of factors: three different tissues (bud, bark, and wood), three plant positions (apical, median and basal) and four collection dates each year (during the months of August and September).

**Place and Duration of Study:** This research was carried out during two consecutive growing seasons: 2015/2016 and 2016/2017, in an experimental apple orchard located at Palma Agriculture Center, Federal University of Pelotas, Southern Brazil.

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**Methodology:** The study was conducted in an experimental orchard using young apple plants cultivar 'Eva'. Phenological data was obtained from four plants, of apical, median, and basal portions. The phenological stages adopted were 'C' (green tip), 'D' (half-inch green leafless), and 'F' (beginning of flowering). In order to determine water dynamics during dormancy stage and their fresh and dry weight, apple branches were collected and separated into three different tissues (bud, bark and wood) to evaluate water content.

**Results:** During the first growing season, there was a higher percentage of bud bursting in apical and median plant portion, while in the second season it was larger in middle and basal portion. Bark showed greater water content in most evaluations, while bud had increased water content in middle portion at the end of the dormancy stage, coinciding with the larger bud bursting.

**Conclusion:** Water content can be an indicator of overcoming endodormancy stage and bud bursting potential.

*Keywords: Malus domestica; water content; bud burst; endodormancy; plant metabolism.*

## 1. INTRODUCTION

Apple (*Malus domestica* Borkh.) is a typical fruit of temperate climate that has great economic importance, being the second fruit in production reaching 80 million tons in 2013 worldwide [1]. Brazil harvested around 1.2 million tons of apples in 2013, being the 12<sup>th</sup> biggest producer in the world. Brazilian apple production is concentrated in the southern states of Brazil (both Santa Catarina and Rio Grande do Sul states produced 96.5%) [2].

Apple production in Brazil is based mainly on clones of both 'Gala' and 'Fuji' [3], which requires medium to high chilling accumulation for dormancy release, supposed to be not well adapted to mild winter conditions of Southern Brazil [4]. Adoption of these cultivars in mild winter areas, can lead to changes in plant behavior, bringing several anomalies such as erratic bud burst and decrease in production potential [5,6]. Shift to low chilling requirement cultivars can be an alternative to reduce the possible effects of this panorama in temperate fruit crop when grown under mild winter conditions [4,7].

The breeding cultivar named IAPAR 75 'Eva' was obtained by the Agronomic Institute of Paraná (IAPAR), and it has low chilling requirement (300 to 350 hours below 7.2°C), allowing its cultivation in regions with low chilling accumulation during dormancy stage, and have high productivity and precocity [8]. This cultivar is an important alternative for apple cultivation in mild winter areas.

Temperature and water availability are important factors for temperate climate fruit species development [9]. Most of plant tissues is

composed of water and it has direct role to resume growth after dormancy, it promotes reserve substances hydrolysis and enzymes activation [10]. Thus, the water content may be regarded as an indicator of metabolic activity in buds [11]. It can also be used as a marker to determine bud bursting potential even before it occurred [12].

Temperature during dormancy stage has great effects on bud burst, where buds which were exposed to sufficient amount of chilling temperatures tends to have an acropetal bud burst, while plants submitted to a deficit in chilling accumulation tends to have a bud burst concentrated at the bottom of plant, which influences negatively in plant architecture [6,13].

Despite low chilling requirement for 'Eva' cultivar, it is necessary to know phenology in this region, and to infer growth potential of these plants when grown under mild climate situation, and analyzing water content it can be an important tool in apple crop development.

The objective of this work was to evaluate the water dynamics in different tissues of apple 'Eva' during dormancy stage under mild winter conditions of Pelotas, located in the Southern Brazil.

## 2. MATERIALS AND METHODS

This research was carried out during two consecutive growing seasons: 2015/2016 and 2016/2017, and an experimental apple orchard located at Palma Agriculture Center (CAP), Federal University of Pelotas (UFPEL), Southern Brazil, was used (latitude 31°52'00" S, longitude 52°21'24" W, 13 m above sea level). The annual average temperature and precipitation in this

region are 17.9°C and 1500 mm, respectively. Temperature data during the experiment was obtained from a weather station set at the Embrapa Clima Temperado – Estação de Terras Baixas.

It was used young trees of apple 'Eva' grafted on 'Marubakaido' rootstock and 'M.9' interstock. The orchard was established in September 2012, with trees density of 2522 plants ha<sup>-1</sup> (0.9 m between plants and 5.0 m between lines), and was conducted in tall-spindle system.

Phenology data was obtained from four plants, of apical, median and basal portions, using the phenology scale proposed by Flekinger [14]. Evaluations during 2015/2016 season were carried out from August 18<sup>th</sup> (with 122 accumulated chilling hours below 7.2°C) to September 15<sup>th</sup> 2015, whereas during 2016/2017 season, the evaluations were performed from August 19<sup>th</sup> (180 accumulated chilling hours below 7.2°C) to September 14<sup>th</sup> 2016. The phenological stages adopted were C (green tip), D (half-inch green leafless), and F (flowering).

In order to determine the water dynamics in apple shoots, branches were collected from three portions of each plant (apical, median and basal). These branches were sectioned in three portions of 1.5 cm containing a single bud and then separated into three different tissues (bud, bark, and wood), before their fresh weight (FW) determination. Then, this material was packed in paper packets and taken to the oven during 48 hours for drying and subsequent determination of tissue dry weight (DW). Mass measurements were performed with a precision analytical balance of 0.1 mg. Water content (WC) in apple tissues was determined according to the formula:

$$WC = (FW - DW) / DW$$

Experimental design was completely randomized, with five replications, and the experimental unit was composed by a single plant, on 3x3x4 factorial arrangement, obtained from a combination of factors: three different tissues (bud, bark, and wood), three plant positions (apical, median and basal) and four collection dates each year (during the months of August and September). The results of the experiment were submitted to analysis of variance and when statistical significance was reached, the means of the treatments were submitted to the Tukey test (P = 0.05).

### 3. RESULTS AND DISCUSSION

According to Table 1, water content (WC) during 2015/2016 season had no significant interaction in each analyzed factor (plant tissue, plant portion and sampling date) and combinations of sampling date x plant portion and sampling date x plant tissue. During 2016/2017 season, water content showed similar interaction, but there was no interaction in the combination dates collections x plant tissues. The water content in bark tissue was superior to other plant tissues in most evaluations.

**Table 1. Analysis of variance, summary for water content of single-node 'Eva' apple shoots in different plant portions (apical, median, and basal portion) during 2015/2016 and 2016/2017 growing seasons**

Source of variation	DF	Mean square	
		2015/2016	2016/2017
Tissue (T)	2	0.94*	1.98*
Plant portion (P)	2	0.27*	3.69*
Sampling Dates (C)	3	1.09*	0.72*
T x P	4	0.02 <sup>ns</sup>	0.05 <sup>ns</sup>
P x C	6	0.31*	1.20*
C x T	6	0.29*	0.13 <sup>ns</sup>
T x P x C	12	0.05 <sup>ns</sup>	0.10 <sup>ns</sup>
Mean		1.10	1.38
CV (%)		17.19	22.09

*ns* – not significant. \*significant differences ( $p \leq 0.05$ ).  
DF = Degrees of freedom

According to Fig. 1, during 2015/2016 season, bud burst occurred mainly in apical and median plant portions, with 30.00% and 24.78% of buds, respectively, reached the phenological stage "D". In basal portion; however, only 12.30% of buds have reached the phenological stage "D". The percentages of flower buds in the upper and median shoots portions were 13.7% and 11.9%, respectively. In the basal part, only 3.7% of the buds differentiated in flower. The bud bursting started approximately on August 15<sup>th</sup> and the full bloom occurred on September 1<sup>st</sup>. In the 2016/2017 growing season, bud bursting was concentrated in the middle portion of the plant, with 47.5% of buds reaching stage "D", followed by basal and apical portions, with 39.7% and 34.2% of buds reaching stage "D". Despite the higher percentage of bud bursting, number of mixed buds at stage "F" was lower than 2015/2016 season, where only 7.3%, 4.4% and

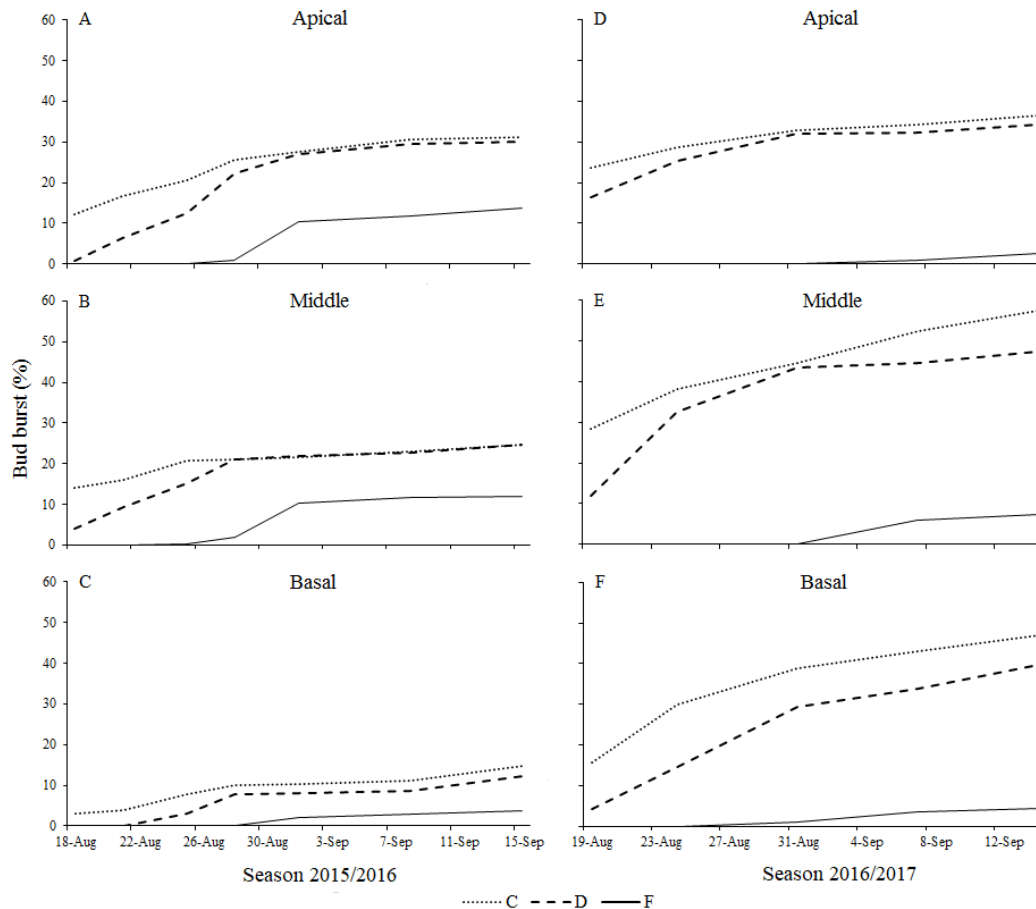
2.4% of buds in median, basal and apical portions, respectively, had flowered.

Petri and Leite [15] observed low axillary bud break in apple grown in Southern Brazil, which induced a larger growth of apical bud with greater intensity of apical dominance. Such condition demands more pruning due to high vegetative vigor.

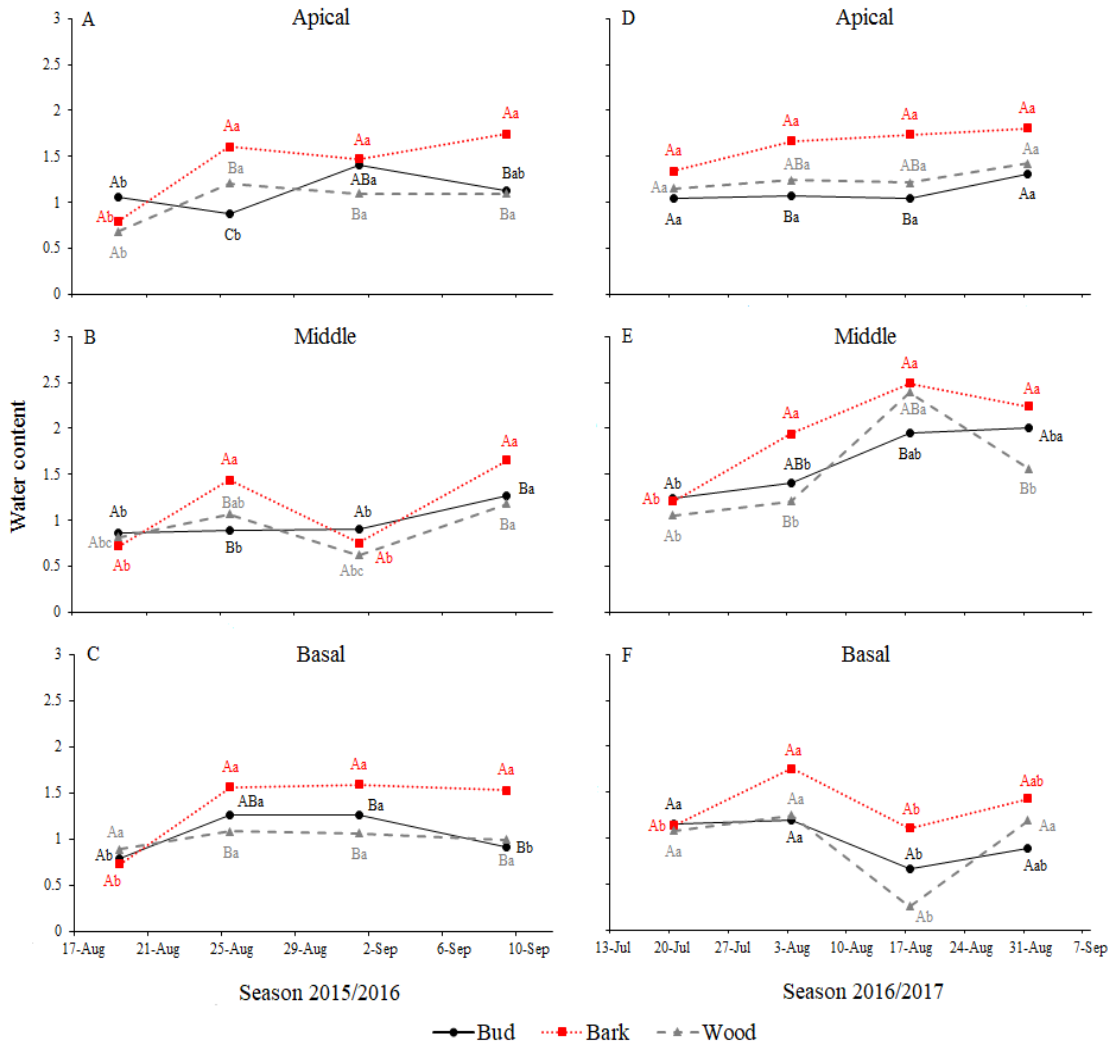
According to Fig. 2, during 2015/2016 season, WC of both bark and wood tissues from apical portion had an increase in the second sampling date, and maintained until the last, while WC in buds increased in the third sampling and had a small reduction in the last sampling. In the middle plant portion, WC bud increased only in the last sampling, while the WC of both bark and wood increased in the second sampling, reduced in the

third and was the highest in the last sampling. In the basal plant portion, WC bark increased in the second sampling, and remained until the end. WC of wood remained unchanged in all evaluations, and WC of buds had an increase in the second sampling, with a reduction in the last sampling.

During 2016/2017 season, WC of bud, bark and wood from apical and basal plants portions did not show significant change during the different samplings, but WC of buds reduced in the third sampling. In the middle plant portion, WC in bark increased in the second collection, and has remained until the end, while the WC bud showed an increase only in the last collection and WC wood peaked in the third collect, but the other three collects did not show differences.



**Fig. 1. Phenological stages C (green tip), D (half-inch green leafless), and F (flowering) of apple 'Eva' in different plant portions (apical, middle, and basal portion) during 2005/2016 season [A, B, C] and 2016/2017 season [D, E, F]**



**Fig. 2. Water content of single-node apple ‘Eva’ shoots in different plant portions (apical, middle, and basal portion) during 2015/2016 season [A, B, C] and 2016/2017 season [D, E, F]**

Water state of buds is associated with tissues metabolism and consequent potential for bud bursting [12]. Thus, if we compare WC of different plant portions with their phenology, it is possible to see that during 2015/2016 season both apical and median portions had the highest bud bursting, which coincided with an increase in bud WC from the third sampling date (bud bursting time).

During 2016/2017 season, middle portion showed the highest levels of bud bursting, coinciding with an increase in WC in bud from the third sampling. The increase of WC in buds coinciding with the larger bud bursting indicating that hydration of these tissues has an important

role on increasing metabolic activity to overcome dormancy. Cronjé et al. [16] presented results that corroborate with this work, where apple tree buds during dormancy period had 41% humidity, and increased until 59% at the beginning of bud bursting.

According to Marafon et al. [11], climate conditions where chilling requirement for dormancy release are not fully satisfied, may not provide buds rehydration, thus preventing bud bursting. This fact might be related to results obtained in basal plants portion during 2015/2016 growing season, where most demand of chilling accumulation in this plant portion prevented resumption of its metabolism, resulting

in decrease of bud bursting rate. Another point, proposed by Yaacoubi et al. [17], is that low chill cultivars can confer capacities to tolerate shallow dormancy, with a transition from endo- to ecodormancy without heterogeneous rehydration, and can tolerate high temperatures during ecodormancy.

#### 4. CONCLUSION

Highest bud burst percentage, which was lower than 60% during 2016/2017 season, coincided with an increase in water content of apple shoots. Increase in water content of apple tissue is an important factor for dormancy release and consequent growth resumption. Great increase in water content of apple tissue indicated a physiological indicator of metabolic activity and seems to be a marker of endodormancy overcoming.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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