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Short communication

Is acrotonic budburst pattern in spring a typical behavior of the low-chilling apple cultivar 'Eva' in mild winter conditions? An approach combining ex planta single-node cutting test and in planta bud water content during dormancy

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ABSTRACT

Working on the low-chilling apple cultivar 'Eva' grown in mild winter conditions of southern Brazil, we studied the influence of bud position along the one-year-old shoot on dormancy release and water content during winter, and actual budburst in spring. Firstly, during winter, the water content measured in planta increased, and the mean time to budburst measured ex planta with the single-node cutting test decreased, for buds from the distal half of shoots whereas such trends were not observed for buds in the proximal half. These two phenomena revealed a higher growth capacity of distal buds during winter compared to proximal buds. Secondly, at spring, there was an acrotonic budburst pattern similar to that of high-chilling cultivars grown under cold winter conditions. We discuss the interest of these winter bud traits as reliable markers of the adaptation of low-chilling apple cultivar to mild winter conditions.

1. Introduction

Dormancy is the temporary suspension of visible growth of any plant structure containing a meristem (Lang et al., 1987). Low temperatures, i.e., below 12 °C, typically impose growth cessation and dormancy in apple (*Malus X domestica* Borkh.) (Heide and Prestrud, 2005). Three types of dormancy have been identified: paradormancy describes correlative inhibitions among plant parts (e.g. apical dominance), and *endo*- and ecodormancy describe inhibitions within the bud itself and imposed by the environment, respectively (Lang et al., 1987).

Budburst depends both on the intrinsic capacity of a given bud to burst and on its relationships with the other buds along the parent shoot (Citadin et al., 2009). The dormancy release from fall to the end of winter, related to the satisfaction of chilling, is usually studied ex planta through the single-node cutting test (Fig. 1). This method allows studying the intrinsic growth capacity of the buds nullifying the effect of physiological correlations, and to determine the three phases of dormancy (Herter et al., 1988; Bonhomme et al., 2005). Indeed, mean time to budburst generally reaches maximal values in endodormancy and then decreases during dormancy release in ecodormancy. Therefore, the decrease in mean time to budburst indicates an increase of the intrinsic budburst capacity (Citadin et al., 2009; Bonhomme et al., 2005).

For high-chilling cultivars, winter temperatures affect budburst at the spatial level with the preferential growth of laterals in the distal zone, i.e., acrotony, and in the proximal zone, i.e., basitony, of the parent annual shoot, in cold winter and mild winter, respectively (Schmitz et al., 2014). Winter temperatures also affect the timing of budburst which is related to the position along the shoot (Petri and Leite, 2004; Schmitz et al., 2014). The resumption of bud growth after dormancy release is related to water availability which positively affects hydrolysis of stored macromolecules and enzyme activities (de Faÿ, 2000). Therefore, bud water content can be an

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Fig. 1. Single-node cutting forcing test realized in apple ex planta in a growth chamber at 25 °C and 90% air humidity (Credit: Juliano Dutra Schmitz, Pelotas, Rio Grande do Sul, Brazil, August 2013).

important indicator of the metabolic activity of the bud (Marafon et al., 2011) and is considered a good marker of bud growth potential even before the first morphological signals of budburst (Leite et al., 2006).

The apple is characterized by a high genetic variability for the length of bud dormancy under various conditions of winter temperatures (Hauagge and Cummins, 1991a,b) and for budburst frequency in spring, i.e., the proportion of buds which burst over the total number of buds on a shoot (Labuschagné et al., 2002). In Brazil, high-chilling apple cultivars, such as 'Gala' and 'Fuji', are cultivated on more than 90% of the apple crop area (Fachinello et al., 2011). However, a series of anomalies, such as desynchronized budburst and uneven distribution of growth and flowering in tree canopy, usually arise when these cultivars are grown under mild winter conditions where winter temperatures are insufficient for overcoming dormancy (Petri and Leite, 2004). There is an interest to have new cultivars which are naturally adapted to mild winter conditions with more synchronized spring budburst (Pommer and Barbosa, 2009).

The major part of studies is focused on physiological disorders of high-chilling cultivars grown under mild winter conditions (Petri and Leite, 2004). Studies of low-chilling cultivars in mild winter conditions are still scarce. Our study was carried out on the low-chilling apple cultivar 'Eva' grown under natural mild winter conditions in southern Brazil. Our hypothesis was that the position of the bud on the shoot had a significant effect on the release from dormancy and on water content during winter, prefiguring acrotonic spring budburst. The aim of this study was to analyze on winter buds in the proximal and the distal zones of shoots the release from dormancy and the water content, and to relate these evolutions to the actual spring budburst.

2. Materials and methods

The study was carried out in an experimental field at the Federal University of Pelotas, localized in Capão do Leão (lat. 31°S, long. 52°W, and 48 m a.s.l.), Rio Grande do Sul, Brazil. This experimental area is located in a typical mild winter region of southern Brazil (Fig. 2A). The trial was performed from fall (May in the Southern hemisphere) to spring (October) 2013 on the low-chilling apple cultivar 'Eva' (350 chilling hours (CH), i.e., hours below or equal to 7.2 °C; Hauagge and Tsuneta, 1999).

In April 2013, ca. 100 one-year-old shoots of approximately the same length (~70 cm long) and without sylleptic laterals, were randomly selected on ten 14-year-old trees trained to a central leader trained shape. Each shoot was divided in two halves, hereafter referred to as 'zone', proximal and distal, based on the total number of nodes. Bud position in one of these two zones was the main studied factor. Three analyses were done.

2.1. Dormancy release

Dormancy release was studied thanks to the ex planta single-node cutting test (Bonhomme et al., 2005;Fig. 1). Samples were taken in the field at 6 dates in 2013: 30 April, 21 May, 12 June, 01 July, 24 July and 19 August. At each date, eight shoots were randomly selected. On each shoot five seven-cm-long single-node cuttings per zone were collected, yielding to 40 single-node cuttings per date/zone. The single-node cuttings were forced in a growth chamber at 25 °C (±1 °C) and ~90% of relative humidity. The mean time to budburst (days) of each single-node cutting for a given date of sampling was recorded three times a week. Budburst was considered when the "green tip" stage, i.e., when the scales opened and the first leaves became visible, was reached. A bud was considered as totally dormant (latent) when no budburst occurred before 60 days from the entrance in the growth chamber (Zguigal et al., 2006).

2.2. Bud water content

Bud water content was analyzed in planta from the beginning of the exponential phase of chilling accumulation (Fig. 2B), i.e., on 30 June. On 01 July, date of the presumed maximum of mean time to budburst, 10 shoots were randomly chosen, and all lateral buds were excised within each zone. The same procedure was applied in the following three dates, 24 July, 19 August and 02 September, the latter date being one week before the presumed spring budburst. For each individual bud, water content was calculated as the ratio [(FM – DM)/(DM)], where FM and DM are the fresh and the dry mass (g), respectively, measured with an analytical balance. FM was determined immediately after sampling in the field, and DM was measured after drying in an oven at 70 °C until constant mass was reached.

2.3. Spring budburst in the field

Budburst occurred on 10 September 2013 onwards. Budburst rate was assessed on the remaining 10 shoots considering the position of each individual bud within either the proximal or the distal zone.

2.4. Data analyses

Data were analyzed considering the effects of factors, i.e., zone along the shoot and date of sampling, on the response variables, namely, mean time to budburst from the single-node cuttings test and bud water content, thanks to a two-way ANOVA (*F*-test). The mean time to budburst (days) was square root ($n+1$) transformed prior to analyses. The percentage of spring budburst in the field was arcsin [square root ($n+1$)] transformed prior to analysis and was analyzed thanks to a one-way ANOVA (*F*-test) considering the zone along the shoot as the only factor. When significant effects were found, means were submitted to a Duncan test to separate levels of treatments ($P<0.05$). All statistical analyses were performed using R Core Team (2013) with package 'ExpDes' (Ferreira et al., 2013).

3. Results

There was a strong amplitude of daily temperatures (up to 20 °C; Fig. 2A), and chilling accumulation amounted 454 CH (Fig. 2B)

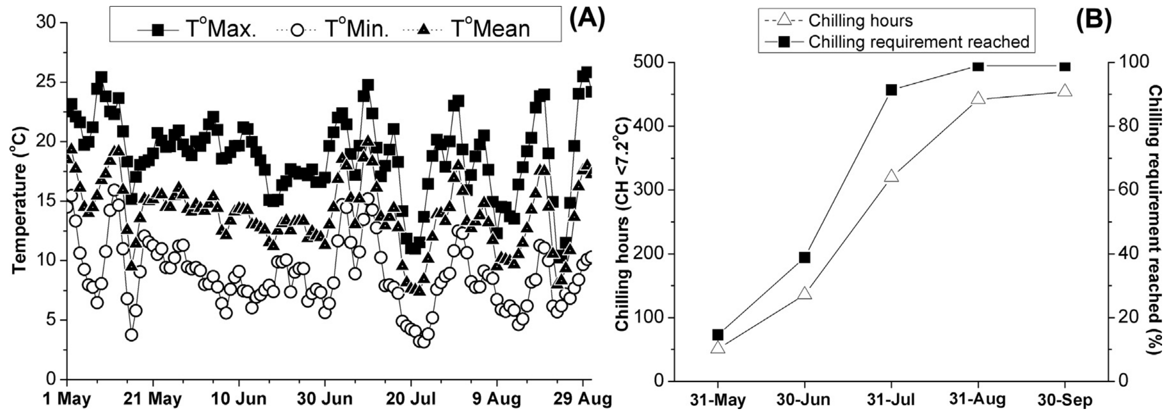

Fig. 2. Mobile average (over three days) of daily temperature (A); and chilling hours (CH) accumulation (temperature ≤7.2 °C) and percentage of chilling requirement reached (%) (B), for the low-chilling apple cultivar 'Eva' during dormancy (May to beginning of September 2013, i.e., fall-winter in southern hemisphere).

during the dormancy period (May to September 2013, i.e., from fall to the end of winter in southern hemisphere).

3.1. Mean time to budburst

The highest values of mean time to budburst were observed without difference between the two zones on 01 July (Fig. 3A), when ~40% of chilling requirement was reached (Fig. 2B). On 24 July and 19 August, buds in the distal zone had a significantly lower mean time to budburst compared to buds in the proximal zone (Fig. 3A). From 01 July to 19 August, distal buds were characterized by a higher decrease of mean time to budburst compared to buds in the proximal zone (Fig. 3A).

3.2. Bud water content

Bud water content was significantly affected by the sampling date and the zone (Fig. 3B). Within each zone, there was an increase of bud water content with time, with the highest values closer to spring budburst and with a higher amplitude for distal buds than for proximal buds (Fig. 3B). Comparing the two zones at each date, from 01 July onwards, bud water content was significantly higher in the distal zone than in the proximal zone (Fig. 3B).

3.3. Actual budburst in the field

Buds in the distal zone of the shoot had a significantly higher percentage of spring budburst than buds in the proximal zone (Fig. 4A). For some shoots this phenomenon was exacerbated with flowering on a few sub-terminal laterals (Fig. 4B).

4. Discussion

In our study endodormancy was likely reached around the 1st of July with the maximum of mean time to budburst. Our study on 'Eva' confirmed that a low-chilling cultivar had lower values of mean time to budburst, namely a maximum of 20 days on 01 July, than those observed for high-chilling cultivars, i.e., more than 30 days according to Zguigal et al. (2006). Our results also confirmed previous findings from Mauget (1984) that during endodormancy buds in the proximal zone of shoots have similar or slightly lower mean time to budburst than distal buds with a reverse trend when going through ecodormancy.

The strongest increase of water content in distal buds took place on 2 September, (Fig. 3B), approximately a week before spring budburst in the field, when 100% of chilling requirement for 'Eva' was reached (Fig. 2B). This result confirms the coupling between the increase of the xylem flux during ecodormancy and the high potential for budburst already shown in walnut (Bonhomme et al., 2009).

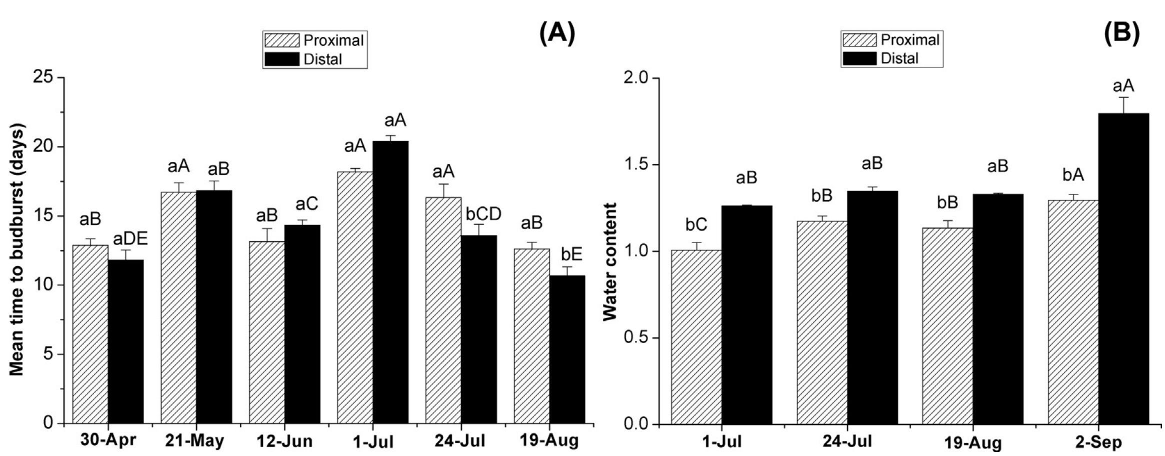

Fig. 3. Characteristics of the lateral buds of the low-chilling apple cultivar 'Eva' depending on their position (zone: proximal, distal) along the one-year-old shoot. Mean time to budburst (days; mean ± SE, n = 40) analyzed with the single-node cutting test carried out on buds ex planta, from 30 April to 19 August, 2013 (A), and water content (mean ± SE; all buds sampled on 10 shoots) measured in planta from 01 July to 02 September, 2013 (B). For each date, different lower case letters indicate significant differences between proximal and distal zones (Duncan test at $P < 0.05$). For each zone along the shoot, different higher case letters indicate significant differences between dates (Duncan test at $P < 0.05$).

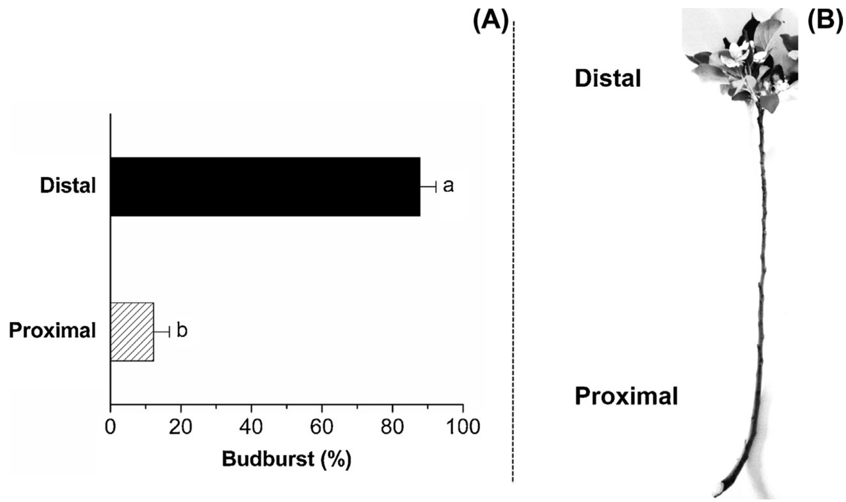

Fig. 4. Spring budburst frequency (%; mean ± SE, $n = 10$) in the proximal and distal zones along the one-year-old shoot (A), and acrotonic budburst pattern as it appears in the orchard (B), for the low-chilling apple cultivar 'Eva' on 09 October, 2013 (Credit: Juliano Dutra Schmitz, Pelotas, Rio Grande do Sul, Brazil). For budburst frequency, different letters indicate significant differences (Duncan test at $P < 0.05$).

However, this fast hydration of buds was late compared to what has been observed in walnut, cv. 'Franquette', grown in cold winter where water content increased a month before budburst (Citadin et al., 2009; Bonhomme et al., 2009).

Our results suggested that the release from endodormancy, i.e., ecodormancy, was related to both a decrease in the mean time to budburst observed here with the ex planta single-node cuttings (Fig. 3A) and an increase of bud water content measured in planta (Fig. 3B), leading to the actual acrotonic spring budburst pattern on shoots in the orchard (Fig. 4A and B). Therefore, the low-chilling cultivar 'Eva' grown under mild chilling conditions typical of southern Brazil, presented a typical acrotonic budburst pattern similar to what is usually described for high-chilling cultivars grown under cold winter conditions (Cook et al., 1998). This acrotonic budburst is responsible for the subsequent branching acrotony related to the fulfillment of chilling requirements with a high frequency and length of proleptic branches in the distal zone (Cook et al., 1998; Cook and Jacobs, 1999).

5. Conclusions

The low-chilling apple cultivar 'Eva' grown in mild winter conditions presents an acrotonic pattern of spring budburst that is mainly established after chilling requirements are fulfilled approximately one week before budburst. This behavior, typical of that of a high-chilling cultivar grown in cold winter conditions, suggests a pivotal role of the dynamics of bud water content during winter to determine spring budburst. To the best of our knowledge this is the first time an acrotonic behavior has been evidenced on a low-chilling cultivar under mild winter conditions. Nevertheless, this result has to be used carefully since our study was conducted in only one year. Other studies should be developed to analyze a possible year-to-year variations in these spatial and temporal patterns.

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