

Are the effects of winter temperatures on spring budburst mediated by the bud water status or related to a whole-shoot effect? Insights in the apple tree

Juliano Dutra Schmitz · Marc Bonhomme · Hervé Cochard ·
Flavio Gilberto Herter · Gabriel Berenhauser Leite ·
Jean-Luc Regnard · Pierre-Eric Lauri

Received: 30 July 2014/Revised: 5 December 2014/Accepted: 12 December 2014/Published online: 23 December 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract

Key message In apple, the overwintering bud appears hydraulically isolated from the parent stem. Spring budburst seems more related to a whole-shoot effect than to the water status of the individual bud during winter dormancy.

Abstract The effects of winter temperatures, i.e., during dormancy, on shoot architecture are well known with budburst preferentially in the distal or the proximal part of the parent shoot in cold and mild winter conditions, respectively. However, the link with the overwintering bud water status is still scarcely documented. Our study was developed on four apple (*Malus domestica* Borkh.) cultivars covering a range of chilling requirements from low ('Condessa') to medium

('Granny Smith') and high ('Royal Gala', 'Starkrimson'), and maintained in either cold (1,428 h below 7.2 °C) or mild (99 h below 7.2 °C) fluctuating winter temperatures. Our aim was to analyze xylem conductance at the stem-to-bud junction, and relative water content and water potential of the bud itself, for buds situated in the distal third of one-year-old shoots. From dormancy to the pre-budburst stage, xylem conductance at the stem-to-bud junction increased or decreased or did not show consistent changes depending on the cultivar and the winter temperature treatment. Whatever the cultivar, there were no significant trends across dates for the effects of winter temperatures on bud water potential and relative water content. Water potential had negative values, between -4.35 and -2.24 MPa, across cultivars and winter temperature treatments without a consistent relationship with actual spring budburst frequency. These results suggested that lateral buds were hydraulically isolated from the parent stem during winter until a few days before budburst. We discussed that the temperature-related spring budburst was likely more related to a whole-shoot effect mediated by hormonal, hydraulics and/or sugar signaling, than to the individual bud water status during dormancy.

Communicated by G. Wieser.

J. D. Schmitz · F. G. Herter
Federal University of Pelotas, PPGA-FAEM, LabAgro,
C. P. 354, Pelotas 96010-900, Brazil

M. Bonhomme · H. Cochard
INRA, UMR 547 PIAF, Site de Crouël,
63100 Clermont-Ferrand, France

M. Bonhomme · H. Cochard
Clermont Université, Université Blaise Pascal, UMR 547 PIAF,
BP 10448, 63000 Clermont-Ferrand, France

G. B. Leite
Epagri, Rodovia Admar Gonzaga 1347, C. P. 502, Florianópolis,
SC 88034-901, Brazil

J.-L. Regnard
Montpellier SupAgro, UMR AGAP, 34060 Montpellier, France

P.-E. Lauri (✉)
INRA, UMR AGAP, 34098 Montpellier Cedex 5, France
e-mail: lauri@supagro.inra.fr

Keywords Apple · Axillary budburst · Relative water content · Water potential · Winter temperatures · Xylem hydraulic conductance

Introduction

Plant architecture is at any given time the expression of equilibrium between endogenous growth processes and exogenous constraints exerted by the environment (Bart-hélémy and Caraglio 2007). Dormancy is the temporary suspension of visible growth of any plant structure

containing a meristem (Lang et al. 1987) and plays a key role in budburst and branching patterns (Cook et al. 1998). Each individual bud has its own physiological state which depends both on its endogenous ability to burst and on its position within the shoot (e.g., proximal or distal part) and tree canopy (e.g., on a short or on a long shoot), and which determines its growing potential (Mauget and Rageau 1988). Two phases of dormancy have been identified during the autumn and winter: endodormancy when inhibitions rely within the bud itself, and ecodormancy when the inhibitions are imposed by unfavorable environmental factors (Lang et al. 1987). Winter temperature is the main environmental factor affecting bud dormancy (Erez 1995; Heide and Prestrud 2005) and spring budburst and branching patterns as shown in the apple (*Malus domestica*) tree (Maguylo et al. 2012; Schmitz et al. 2014). Apical dominance, which is the control exerted by the current-year shoot apex over lateral bud outgrowth, is typically the result of correlative inhibitions within the shoot during the growing season (Champagnat 1989; Cline 1997). It is a specific case of dormancy called paradormancy (Lang et al. 1987; Faust et al. 1995). Paradormancy also occurs during winter as shown by the differing budburst potential along the shoot and throughout winter (Champagnat 1983).

Acrotony is defined by the preferential growth of laterals in the distal zone of the parent annual shoot and is opposed to basitony with budburst and branching in the proximal zone (Champagnat et al. 1971; Bell 1991; Cook et al. 1998; Lauri 2007). Acrotony and basitony typically characterize the branching pattern in cold and in mild winter conditions, respectively (Cook and Jacobs 1999; Schmitz et al. 2014).

Apple (*M. domestica* Borkh.) is cultivated in regions under contrasted winter temperature conditions from the tropics to the high latitudes (Janick 1974; Palmer et al. 2003). This species is also characterized by a high genetic variability for the length of bud dormancy under various conditions of winter temperatures (Hauagge and Cummins 1991) and for budburst frequency in spring (i.e., the proportion of buds which burst in spring over the total number of buds on a shoot; Labuschagné 2002). Although progression of dormancy and consequently spring budburst frequency differ throughout winter, genotypes with high chilling requirements have a typical basitony and/or erratic budburst along the parent shoot in mild winter conditions; whereas cultivars with low chilling requirements show acrotonic budburst in the same winter temperature conditions (Cook et al. 1998; Cook and Jacobs 2000).

It has been shown in walnut (*Juglans regia* L.) that the availability of soluble sugars plays a significant role in spring budburst through the active uptake of hexoses from the xylem sap to the bud 1 month before budburst (Bonhomme et al. 2009). Based on these findings, it has been suggested that budburst potential depends on both the sink

strength of the bud itself in relation to the flux entering the bud and on the sink competition between bud, xylem parenchyma and bark (Bonhomme et al. 2009). However, this does not mean that vascular connections between stem and bud are fully developed and other pathways, apoplastic or symplasmic, could permit such transport.

As shown in Japanese Zelkova (*Zelkova serrata*; Yoda et al. 2003), stem diameter increases before budburst, and it has been shown in beech (*Fagus sylvatica*) and *M. domestica* at a more local scale that there are positive relationships between the xylem conductance at the junction of the bud to the parent stem, the number of appendages within the bud (Cochard et al. 2005) and the ability to develop a vegetative shoot or an inflorescence in spring (Lauri et al. 2008). However, to the best of our knowledge, there are no precise studies of the physiological drivers of these relationships at the stem and bud levels. Water potential (WP) is useful in understanding water movement among plant parts through the cellular membranes and xylem (Papendick and Campbell 1981). By definition, pure water (free water) has a WP equal to 0 MPa. Inside the cells as well in aqueous solutions, WP is negative (Pimenta 2008). Water flow proceeds from a compartment with less negative WP to a compartment with more negative WP and the availability of water for physiological processes decreases as the potential is more negative (Papendick and Campbell 1981). As an example, working on callus from rubberwood (*Hevea brasiliensis*; Etienne and Carron (1991) found positive correlations between values of WP close to zero, high water content and somatic embryogenesis, i.e., the ability to regenerate an embryo from a somatic cell or group of somatic cells. To the best of our knowledge, there is no research work showing a relation between bud WP and the ability of the bud to burst in spring.

Our aim was to gain more insights on the relationships between bud water status, i.e., water content and WP, xylem conductance at the junction of the bud to the parent stem, during winter and spring budburst on *M. domestica*. Our hypothesis was that a higher ability for budburst in spring would be entailed during the pre-budburst stage (ecodormancy) by an increase of the xylem conductance at the junction of the bud to the parent stem and of the relative water content of the bud itself with presumably values of bud WP close to zero. We also expected a strong effect of winter temperatures on these variations in relation to the winter temperature-dependent budburst frequency of the cultivar.

Materials and methods

Plant material and experimental setting

The trial was carried out on four *M. domestica* cultivars with different chilling requirements, as defined by the

number of hours below 7.2 °C for breaking dormancy: ‘Condessa’ (350–450), ‘Granny Smith’ (600–700), ‘Royal Gala’ (700–800), and ‘Starkrimson’ (more than 800) (Marcus Vinicius Kvitschal, EPAGRI, Brazil; unpublished data). In February 2011, twelve scions per cultivar were grafted on M.9 (Pajam[®] 2), a common rootstock in commercial orchards, at Cirad-Montpellier in southern France (lat. 43°N, long. 3°E, 44 m a.s.l.). Trees were planted in 4-liter pots filled with a potting mix including a slow-release fertilizer, and with additional nitrogen provided to the pots in April and June 2011. All trees were drip-irrigated to field capacity (ca. 0.5 L per day with drainage of excess water) during the whole trial, i.e., during the 2011-growing season through July 2012. Irrigation was maintained during the 2011–2012 winter even when winter frost occurred in outdoor conditions (see below). The pots were placed into a greenhouse during the whole growing season. Our plant material consisted of a single vertical shoot per tree directly stemming from the rootstock or at the bottom of the main cultivar shoot, were removed. On December 1, 2011, the one-year-old shoots were equally distributed into two groups, each one being submitted to one of the following treatments: (1) transferred outdoor to ensure the “cold” winter conditions (“cold” treatment), (2) put in a temperature-controlled greenhouse to ensure the “mild” winter conditions (“mild” treatment). In the mild treatment, the temperature was left to vary at 10° to 15 °C above the temperatures in the cold treatment with a minimum threshold set at 5 °C. Considering a threshold at 7.2 °C (Weinberger 1950), this setting resulted in 1,428 and 99 chilling hours in the cold and in the mild winter temperature treatments, respectively (Schmitz et al. 2014). At the end of March 2012, i.e., approximately 1 week before the budburst in outdoor conditions based on the progression of bud phenology (Fleckinger 1948), shoots from the mild treatment were transferred outside and mixed with the cold treatment shoots for budburst observations. During the winter and the spring periods, trees were completely randomized for cultivar treatment.

Individual bud traits were considered for buds in the distal third of all one-year-old shoots, in the two temperature treatments, usually characterized by the highest budburst frequency under the cold winter conditions (Maguylo et al. 2012). Destructive bud sampling was done at three consecutive times in the dormancy period during the 2011–2012 Winter: beginning of January 2012, i.e., at the end of the supposed endodormancy (Mauget and Rageau 1988), mid-February and mid-March. This latter sampling period, from March 12 to March 20, 2012, corresponded to 2–3 weeks before budburst depending on the temperature treatment.

Traits of individual buds

From beginning of January to end of March 2012, the individual bud traits were characterized in three shoots per cultivar and treatment. The following variables were considered.

WP and relative water content (RWC)—at each date, two lateral buds per shoot, the 25th and the 27th below terminal bud (Fig. 1), six per cultivar and treatment, were excised with a scalpel, immediately put into an eppendorf tube and transported to the laboratory. WP (MPa) was measured using the Wescor (HR-33T Dew Point-Microvoltmeter; www.wescor.com; Wescor 2001) apparatus, with a series of C-52 Sample Chamber, calibrated according to Montoro et al. (1995). Based on preliminary experiments, the sampled lateral buds were left for 90 min to attain equilibrium inside the C-52 Sample Chambers before reading. RWC (%) was obtained in the same buds after WP determination. RWC was calculated as the ratio $[(FM - DM)/(MFT - DM) \times 100]$, where FM is the fresh mass (mg), DM the dry (mg) mass after 72 h at 60 °C in oven (mg), and MFT the mass at full turgor (mg) obtained after immersion into pure water in dark at 4 °C for 15 h.

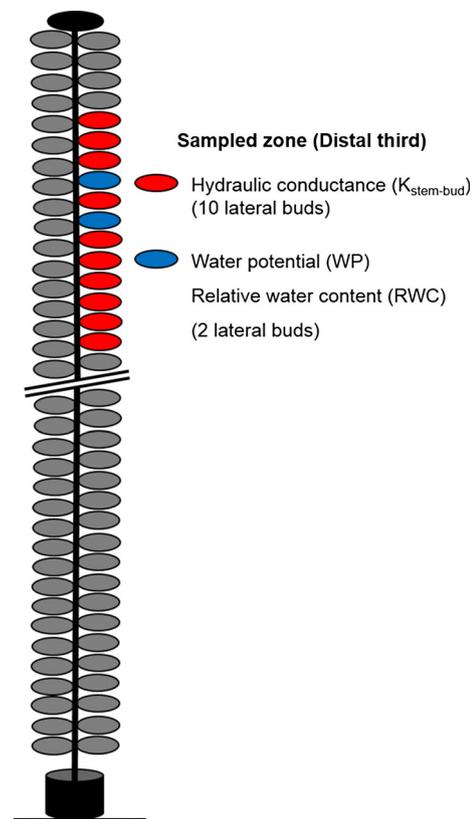


Fig. 1 Scheme of the distal third of the one-year-old shoot with positioning of sampled buds

Hydraulic conductance—the maximal hydraulic conductance of the xylem at the junction of the bud to the stem ($K_{\text{Stem-Bud}}$; $\text{mmol MPa}^{-1} \text{s}^{-1}$) was measured using the high-pressure flow meter (HPFM; Dynamax, Houston, TX, USA) apparatus, which is based on the perfusion of deionized and filtered water at high pressure (0.5–0.6 MPa) at the bottom of the 50–60 cm long distal third of the one-year-old shoot, and the measurement of the rate of water exudation at each individual lateral bud scar. Water exudation was measured by using a weighed piece of dry cotton applied for 60 s on each bud scar surface on the shoot where the lateral bud had been removed. Ten lateral buds per shoot, 30 per cultivar and treatment, were considered (Fig. 1).

Proleptic lateral growth (PLG)—in the last week of July 2012, i.e., the year following shoot growth, each node of the distal third of each remaining shoot, three shoots per cultivar, were characterized by the type of growth of the lateral bud. These lateral types entered one of the three categories, latent (L), proleptic vegetative (V) and proleptic flowering (F). PLG (%) was calculated as the ratio $[(V + F) / (V + F + L)]$.

Data analyses

The effects of the studied factors, winter temperature treatment and sampling date or cultivar, on WP, RWC, $K_{\text{Stem-Bud}}$ and PLG were analyzed through a two-way ANOVA, and when significant means were submitted to a Tukey test to separate levels of factors. Statistical analyses were done using R software version 3.0.2 (R Development Core Team 2013), with package ‘car’ with the function `lm()` for continuous variables. PLG and RWC data were transformed prior to statistical tests using the Box-Cox procedure (R Development Core Team 2013 with package ‘MASS’). Threshold for significance was set at $P < 0.05$.

Results

Effects of the winter temperature treatment on the water status of the bud

In ‘Condessa’, $K_{\text{Stem-Bud}}$ increased significantly during winter in the cold winter treatment (0.70 to $1.25 \text{ mmol MPa}^{-1} \text{ s}^{-1}$ for January and March, respectively); whereas, there were no significant changes in the mild winter treatment (Fig. 2a). There was no significant trend for the effects of the winter temperature treatment across dates on WP and RWC (Fig. 2e, i).

In ‘Granny Smith’, $K_{\text{Stem-Bud}}$ was significantly higher in the mild winter treatment in January ($1.16 \text{ mmol MPa}^{-1} \text{ s}^{-1}$) and February ($0.90 \text{ mmol MPa}^{-1} \text{ s}^{-1}$) compared to

March ($0.33 \text{ mmol MPa}^{-1} \text{ s}^{-1}$; Fig. 2b). There was no significant trend for the effects of the winter temperature treatments across dates on WP and RWC (Fig. 2f, j).

In ‘Royal Gala’, $K_{\text{Stem-Bud}}$ significantly decreased in the mild winter treatment from January ($0.61 \text{ mmol MPa}^{-1} \text{ s}^{-1}$) to February and March (0.32 and $0.25 \text{ mmol MPa}^{-1} \text{ s}^{-1}$, respectively); whereas, it significantly increased in the cold winter treatment from January ($0.30 \text{ mmol MPa}^{-1} \text{ s}^{-1}$) to February ($0.80 \text{ mmol MPa}^{-1} \text{ s}^{-1}$) with intermediate values in March (Fig. 2c). There was no significant trend for the effects of the winter temperature treatment across dates on WP and RWC (Fig. 2g, l).

In ‘Starkrimson’ there was no significant increase of $K_{\text{Stem-Bud}}$ across dates whatever the treatment (Fig. 2d). In cold winter, WP increased significantly from February (-4.09 MPa) to March (-2.38 MPa) (Fig. 2h). RWC was significantly higher in cold winter in March (56.71 %) compared to January and February (39.27 and 35.68 %, respectively) (Fig. 2m).

Relation between PLG and water status at the pre-budburst stage (mid-March)

There were contrasted budburst patterns depending on the winter temperature treatment with a typical acrotony in the cold winter treatment and basitony in the mild winter treatment (Fig. 3, the example of ‘Starkrimson’). Focusing on the distal third of one-year-old shoots, all cultivars had the highest PLG in the cold winter treatment and the lowest in the mild winter treatment (Table 1). However, considering the cold winter treatment, it is worth noticing that the low-chilling ‘Condessa’ had the lowest value; whereas the other three cultivars with higher chilling requirements (‘Royal Gala’, ‘Starkrimson’ and ‘Granny Smith’) had higher values (Table 1). There was a reverse trend in the mild winter treatment with, although not significantly different, the highest mean value of PLG for ‘Condessa’ compared to the other three cultivars (Table 1). It should also be noticed that apart from the differences in the mean PLG values, the mild winter treatment was also characterized by a high variability of PLG, with coefficients of variation varying from 69 to 108 %, compared to a range of 4–31 % for shoots in the cold treatment (data not shown, Table 1). Considering the two contrasted cultivars with regards to chilling requirements, ‘Condessa’ and ‘Starkrimson’, before budburst, ‘Condessa’ has a higher $K_{\text{Stem-Bud}}$ ($1.25 \text{ mmol MPa}^{-1} \text{ s}^{-1}$) in cold winter than ‘Starkrimson’ ($0.77 \text{ mmol MPa}^{-1} \text{ s}^{-1}$), and there were no significant differences between the two cultivars in mild winter (0.49 and $0.32 \text{ mmol MPa}^{-1} \text{ s}^{-1}$, for ‘Condessa’ and ‘Starkrimson’, respectively) (Table 1). For these two cultivars and for ‘Granny Smith’, $K_{\text{Stem-Bud}}$ was significantly higher in the cold winter compared to the mild

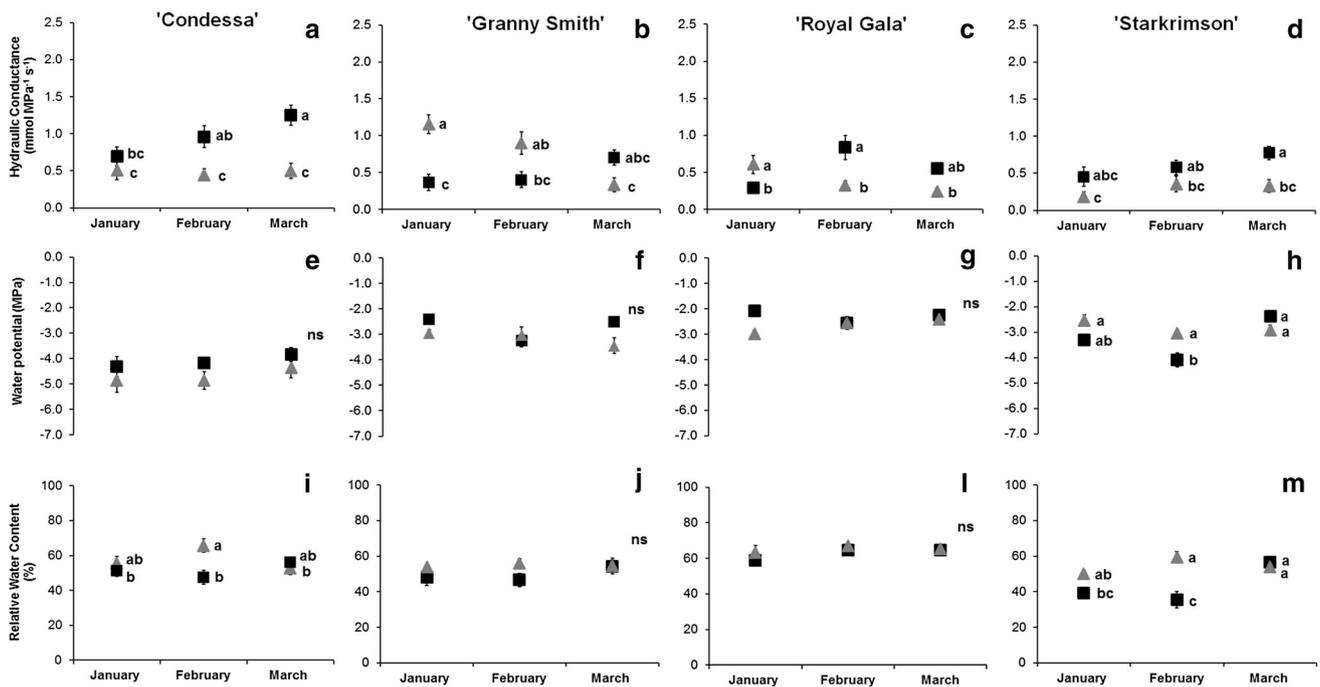


Fig. 2 The effect of winter temperature treatment (*black square*, cold winter; *gray triangle*, mild winter) during dormancy on hydraulic conductance at the stem-to-bud junction ($K_{Stem-Bud}$; $\text{mmol MPa}^{-1}\text{s}^{-1}$, $n = 30$ per cultivar \times date \times temperature treatment), and bud water potential (WP; MPa) and relative water content (RWC; %)($n = 6$ per cultivar \times date \times temperature treatment) for ‘Condessa’ (a, e, i),

‘Granny Smith’ (b, f, j), ‘Royal Gala’ (c, g, l), and ‘Starkrimson’ (d, h, m) from beginning of January to mid-March. Symbols are mean \pm se. Within each variable and cultivar, mean values with the *same letter* are not significantly different ($P < 0.05$); *ns* no significant difference (Tukey multiple means comparison test following an ANOVA with temperature treatment and sampling date as factors)



Fig. 3 Proleptic lateral growth on one-year-old shoots of ‘Starkrimson’ grown under two winter temperature treatments. **a** Acrotonic budburst on plants submitted to ‘cold’ winter temperatures (1,428 h below 7.2 °C), **b** basitonic budburst on plants submitted to ‘mild’ winter temperatures (99 h below 7.2 °C). (Credit, Juliano Dutra Schmitz, Montpellier, France, June 2012)

treatment (Table 1). There was no effect of the temperature treatment on $K_{Stem-Bud}$ for ‘Royal Gala’. On the other hand, WP had very negative values whatever the cultivar and the winter temperature treatment. This latter varied between -4.35 MPa for ‘Condessa’ in the mild treatment to -2.24 MPa for ‘Royal Gala’ in the cold treatment without consistent relationship with PLG (Table 1). There was no significant effect of the winter temperature treatment and the cultivar on RWC which varied between 52.86 % for ‘Condessa’ in the mild treatment to 65.35 % for ‘Royal Gala’ in the mild treatment despite strong differences in PLG (Table 1).

Discussion

Our study included two contrasted winter temperature treatments, one of which was a mild winter condition with 99 chilling hours below 7.2 °C. This experimental setting entailed a strong effect on the budburst pattern of the one-year-old *M. domestica* shoot with a typical acrotony in the cold winter temperature treatment and a basitony in the mild winter temperature treatment (Fig. 3), confirming previous studies (Cook and Jacobs 1999).

Working on *M. domestica* buds located in the upper third of one-year-old shoots our hypothesis was that, under

Table 1 Hydraulic conductance ($K_{\text{stem-bud}}$; $\text{mmol MPa}^{-1}\text{s}^{-1}$), water potential (WP; MPa) and relative water content (RWC; %) before budburst (mid-March 2012), and proleptic lateral growth (PLG; %) on one-year-old wood (July 2012) in the distal third of parent shoots

Temperature treatments	Cultivars	$K_{\text{stem-bud}}$ ($\text{mmol MPa}^{-1}\text{s}^{-1}$)	WP (MPa)	RWC (%)	PLG (%)
Cold winter	CO	1.25 ± 0.13 a	−3.83 ± 0.27 cd	56.04 ± 1.52	38.30 ± 12.0 b
	GR	0.70 ± 0.10 b	−2.49 ± 0.12 abc	54.43 ± 4.42	90.00 ± 5.00 a
	RG	0.55 ± 0.06 bc	−2.24 ± 0.12 a	64.52 ± 2.87	75.00 ± 2.90 a
	ST	0.77 ± 0.08 b	−2.37 ± 0.13 ab	56.71 ± 1.58	83.30 ± 11.70 a
Mild winter	CO	0.49 ± 0.10 bc	−4.35 ± 0.39 d	52.86 ± 3.62	10.80 ± 7.40 c
	GR	0.33 ± 0.09 c	−3.45 ± 0.31 bcd	54.70 ± 3.07	2.50 ± 2.50 c
	RG	0.24 ± 0.04 c	−2.41 ± 0.17 ab	65.35 ± 2.36	0.83 ± 0.83 c
	ST	0.32 ± 0.08 c	−2.91 ± 0.20 abc	53.94 ± 2.47	0.83 ± 0.90 c
Factor		<i>F</i> , <i>P</i>	<i>F</i> , <i>P</i>	<i>F</i> , <i>P</i>	<i>F</i> , <i>P</i>
Cult (<i>df</i> = 3)		10.55, 1.15×10^{-6} ***	5.54, 0.02 **	2.07, 0.11 ^{ns}	3.07, 4.37×10^{-2} *
Temp (<i>df</i> = 1)		44.42, 1.02×10^{-10} ***	1.87, 0.18 ^{ns}	0.69, 0.41 ^{ns}	13.02, 1.10×10^{-3} **
Cult*Temp (<i>df</i> = 3)		3.01, 0.03 *	0.70, 0.55 ^{ns}	0.28, 0.83 ^{ns}	6.17, 2.30×10^{-3} **

Data are mean ± se. In each column, mean values with the same letter are not significantly different across cultivars and temperature treatments ($P < 0.05$, Tukey multiple means comparison test)

CO ‘Condessa’, GR ‘Granny Smith’, RG ‘Royal Gala’, ST ‘Starkrimson’, Cult cultivar, Temp temperature

***, **, * Significant at $P < 0.001$, 0.01, 0.05, and ^{ns}, not significant at $P < 0.05$

the cold winter treatment with presumably higher budburst, both xylem conductance at the junction between bud and stem and relative water content would increase, and water potential would become closer to zero, from dormancy (January; possibly endodormancy not completely released combined with ecodormancy and paradormancy), to the release of dormancy (mid-March). Our results did not support this hypothesis except for xylem conductance in ‘Condessa’ (Fig. 2; Table 1) which, however, had a slightly lower budburst in cold winter compared to the other three cultivars (Table 1). It is known that the freeze–thaw cycles occurring in winter may increase vulnerability to embolism entailing a decrease of the stem xylem conductance (Sperry and Sullivan 1992). We were unable to detect an effect of winter temperatures on the xylem conductance at the junction between stem and bud with, depending on the case, higher values in the cold (e.g., ‘Condessa’ in February and March) or in the mild (‘Granny Smith’ in January and February) treatment (Fig. 2). Similarly, water potential and relative water content did not show any specific trend along the same period (Fig. 2). The low negative values of WP before budburst (from −4.35 to −2.24 MPa; Table 1) without consistent changes during dormancy (beginning of January) through ecodormancy (Fig. 2) strongly suggested that buds were hydraulically isolated from the parent stem. Indeed, although stem WP was not investigated in our study previous findings in *M. domestica* showed that, even during water restriction in

of four apple cultivars grown under contrasted winter temperature treatments, cold winter and mild winter, with 1,428 and 99 h below 7.2 °C, respectively

horticultural conditions, i.e., compatible with shoot and fruit growth, midday stem water potential generally does not decrease below −1.5 MPa (Naor 2006). Our hypothesis of a stem-to-bud hydraulic barrier during winter is supported by findings in alpine dwarf shrubs in which structural features of the tissues at the base of the pedicel impede or prevent ice progression from the frozen stem into the pedicel of the reproductive shoot (Kuprian et al. 2014). Previous results on poplar (*Populus nigra*) trees submitted to a drastic water stress also showed that hydraulic connections between the bud and parenchyma cells around the pith allow bud desiccation to be delayed (Barigah et al. 2013). More generally, Lintunen et al. (2013) state that the susceptibility of freezing and cavitation are linked together in the xylem of plants suggesting similarities of adaptation of plants to cold and drought stress.

Our study evidenced that changes of the bud water status during winter was not sufficient in itself to explain budburst potential in the following spring, even at ca. 2 weeks before budburst (mid-March sampling). A first explanation could be that the bud water status would evolve only a few days prior to budburst which depends not only on the plant material but also on the climatic conditions during this period leading to the quasi-impossibility to determine precisely, in our experiment, when to sample. However, our results strongly suggested that the growing potential of the bud depends both on an endogenous growing ability

(Lauri and Térouanne 1998) in relation to the number of appendages in the bud positively related to $K_{\text{stem-bud}}$ (Lauri et al. 2008) and on a whole-shoot effect. This latter effect is likely related to highly dynamic competitions among buds before budburst through a temporal (e.g., the first bud to burst has a higher growth potential, also called primigenic dominance) and/or a positional effect along the stem (Maguylo et al. 2012), and between buds and the parent shoot (Champagnat 1983). However, interactions between the bud endogenous potential and the parent stem are still not fully understood, and large buds with functional vascular connections may be fully dormant (Brewer et al. 2009). It has been shown that branching on the one-year-old shoot is usually composed of consecutive zones each one characterized by a homogeneous composition of laterals of different types (Lauri and Térouanne 1998; Napoli et al. 1999). The genetic variability of these branching patterns has been shown in the apple (Costes and Guédon 2002). The fact that buds were likely hydraulically isolated from the parent stem strongly suggested that the effects of winter temperatures are not mediated by the individual bud water status, at least until the very last days before budburst, contrary to what we initially expected. In the dominant branching model, this whole-shoot effect is under the control of three long-range hormonal signals involving auxin transported basipetally, strigolactones synthesized in roots and cytokinin synthesized in shoots and roots (Leyser 2010). Although the modes of actions of these hormones are still in debate (Ferguson and Beveridge 2009; Beveridge et al. 2009; Müller and Leyser 2011), it has been shown that this signaling and the resulting budburst patterns are under the influence of the environment, e.g. light (Leyser 2009; Kebrom et al. 2010). How the dramatic effects on shoot architecture of winter temperatures as shown here can be explained by these hormonal controls at the stem-to-node continuum scale needs more studies. Recent discoveries also put forward the possible role of sugar signals, instead of auxin, to initiate lateral budburst with the hypothesis of competitions for sugars, especially sucrose, among buds (Mason et al. 2014; Van den Ende 2014). Whatever the underlying signaling entailing the inhibition or the development of the lateral bud, further understanding of the effects on whole-shoot architecture of winter temperatures need more studies.

Author contribution statement JD Schmitz collected the data, made most of the analyses and interpretation of results, and wrote a first draft of the manuscript. M Bonhomme and H Cochard contributed to the interpretation of results. FG Herter, GB Leite and JL Regnard defined the main lines of the experiment. PE Lauri managed the study, and contributed to the analyses and interpretation of results, and to the writing of the manuscript.

Acknowledgments This work was partly funded by the French-Brazil bilateral CAPES-COFECUB Research Program 2009–2013,

n° Sv 686/10. [CAPES (Brazil, Coordination for the Improvement of Higher Level Personnel); COFECUB (French Committee for the Evaluation of Academic and Scientific Cooperation with Brazil)], and by the CNPq (National Council for Scientific and Technological Development) for grant support in Brazil. The authors thank Sébastien Martinez and Gilbert Garcia for taking care of the trees and help in measurements, Dr Thierry Améglio for helpful suggestions, and two anonymous reviewers for helpful comments on a first version of the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Barigah TS, Bonhomme M, Lopez D, Traore A, Douris M, Venisse JS, Cochard H, Badel É (2013) Modulation of bud survival in *Populus nigra* sprouts in response to water stress-induced embolism. *Tree Physiol* 33:261–274. doi:10.1093/treephys/tpt002
- Barthélémy D, Caraglio Y (2007) Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Ann Bot* 99:375–407. doi:10.1093/aob/mcl260
- Bell AD (1991) Plant form, an illustrated guide to flowering plant morphology. Oxford University Press, Oxford
- Beveridge CA, Dun EA, Rameau C (2009) Pea has its tendrils in branching discoveries spanning a century from auxin to strigolactones. *Plant Physiol* 151:985–990. doi:10.1104/pp.109.143909
- Bonhomme M, Peuch M, Améglio T, Rageau R, Guillot A, Decourteix M, Alves G, Sakr S, Lacoite A (2009) Carbohydrate uptake from xylem vessels and its distribution among stem tissues and buds in walnut (*Juglans regia* L.). *Tree Physiol* 30:89–102. doi:10.1093/treephys/tpp103
- Brewer PB, Dun EA, Ferguson BJ et al (2009) Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and *Arabidopsis*. *Plant Physiol* 150:482–493. doi:10.1104/pp.108.134783
- Champagnat P (1983) Bud dormancy, correlation between organs, and morphogenesis. *Russ J Plant Physiol (Fiziol Rastenii)* 30:587–601
- Champagnat P (1989) Rest and activity in vegetative buds of trees. *Ann des Sci For* 46:9–26
- Champagnat P, Barnola P, Lavarenne S (1971) Premières recherches sur le déterminisme de l'acrotonie des végétaux ligneux. *Ann des Sci For* 28:5–22. doi:10.1051/forest/19710101
- Cline M (1997) Concepts and terminology of apical dominance. *Am J Bot* 84:1064–1069
- Cochard H, Coste S, Chanson B, Guelh JM, Nicolini É (2005) Hydraulic architecture correlates with bud organogenesis and primary shoot growth in beech (*Fagus sylvatica*). *Tree Physiol* 25:1545–1552
- Cook NC, Jacobs G (1999) Suboptimal winter chilling impedes development of acrotony in apple shoots. *HortScience* 34:1213–1216
- Cook NC, Jacobs G (2000) Progression of apple (*Malus × domestica* Borkh.) bud dormancy in two mild winter climates. *J Hort Sci Biotech* 75:233–236
- Cook N, Rabe E, Keulemans J, Jacobs G (1998) The expression of acrotony in deciduous fruit trees: a study of the apple rootstock M.9. *J Amer Soc Hort Sci* 123:30–34
- Costes E, Guédon Y (2002) Modelling branching patterns on 1-year-old trunks of six apple cultivars. *Ann Bot* 89:513–524. doi:10.1093/aob/mcf078

- Erez A (1995) Means to compensate for insufficient chilling to improve bloom and leafing. *Acta Hort* 395:81–91
- Etienne H, Carron MP (1991) Water status of callus from *Hevea brasiliensis* during induction of somatic embryogenesis. *Physiol Plant* 82:213–218
- Faust M, Liu D, Wang SY, Stutte GW (1995) Involvement of apical dominance in winter dormancy of apple buds. *Acta Hort* 395:47–56
- Ferguson BJ, Beveridge CA (2009) Roles for auxin, cytokinin, and strigolactone in regulating shoot branching. *Plant Physiol* 149:1929–1944. doi:10.1104/pp.109.135475
- Fleckinger J (1948) Les stades végétatifs des arbres fruitiers en rapport avec les traitements. *Pomol Fr Suppl* 81–93
- Hauagge R, Cummins J (1991) Phenotypic variation of length of bud dormancy in apple cultivars and related *Malus* species. *J Am Soc Hortic Sci* 116:100–106
- Heide OM, Prestrud AK (2005) Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. *Tree Physiol* 25:109–114
- Janick J (1974) The apple in Java. *HortScience* 9:13–15
- Kebrom TH, Brutnell TP, Finlayson SA (2010) Suppression of sorghum axillary bud outgrowth by shade, phyB and defoliation signalling pathways. *Plant Cell Environ* 33:48–58. doi:10.1111/j.1365-3040.2009.02050.x
- Kuprian E, Briceño VF, Wagner J, Neuner G (2014) Ice barriers promote supercooling and prevent frost injury in reproductive buds, flowers and fruits of alpine dwarf shrubs throughout the summer. *Environ Exper Bot* 106:4–12
- Labuschagné I (2002) Genetic variation in chilling requirement in apple progeny. *J Am Soc Hortic Sci* 127:663–672
- Lang G, Early J, Martin G, Darnell R (1987) Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. *HortScience* 22:371–377
- Lauri PÉ (2007) Differentiation and growth traits associated with acrotony in the apple tree (*Malus × domestica*, Rosaceae). *Am J Bot* 94:1273–1281
- Lauri PÉ, Térouanne É (1998) The influence of shoot growth on the pattern of axillary development on the long shoots of young apple trees (*Malus domestica* Borkh.). *Int J Plant Sci* 159:283–296
- Lauri PÉ, Bourdel G, Trottier C, Cochard H (2008) Apple shoot architecture: evidence for strong variability of bud size and composition and hydraulics within a branching zone. *New Phytol* 178:798–807. doi:10.1111/j.1469-8137.2008.02416.x
- Leyser O (2009) The control of shoot branching: an example of plant information processing. *Plant Cell Environ* 32:694–703. doi:10.1111/j.1365-3040.2009.01930.x
- Leyser O (2010) The power of auxin in plants. *Plant Physiol* 154:501–505. doi:10.1104/pp.110.161323
- Lintunen A, Hölttä T, Kulmala M (2013) Anatomical regulation of ice nucleation and cavitation helps trees to survive freezing and drought stress. *Sci Rep* 3:2031. doi:10.1038/srep02031
- Maguylo K, Cook NC, Theron KI (2012) Environment and position of first bud to break on apple shoots affects lateral outgrowth. *Trees* 26:663–675. doi:10.1007/s00468-011-0634-y
- Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. *Proc Natl Acad Sci USA* 111:6092–6097
- Mauget J, Rageau R (1988) Bud dormancy and adaptation of apple tree to mild winter climates. *Acta Hort* 232:101–108
- Montoro P, Etienne H, Carron M (1995) Effect of calcium on callus friability and somatic embryogenesis in *Hevea brasiliensis* Müll. Arg.: relations with callus mineral nutrition, nitrogen metabolism and water. *J Exp Bot* 46:255–261
- Müller D, Leyser O (2011) Auxin, cytokinin and the control of shoot branching. *Ann Bot* 107:1203–1212. doi:10.1093/aob/mcr069
- Naor A (2006) Irrigation scheduling and evaluation of tree water status in deciduous orchards. *Hortic Rev* 32:111–165
- Napoli C, Beveridge C, Snowden KC (1999) Reevaluating concepts of apical dominance and the control of axillary bud outgrowth. *Curr Top Dev Biol* 4444:127–169
- Palmer JW, Privé JP, Tustin DS (2003) Temperature. In: Webster AD, Wertheim SJ (eds) *Tromp J. Fundamentals of temperate zone tree fruit production* Backhuys, Leiden, pp 217–236
- Papendick RI, Campbell GS (1981) Theory and measurement of water potential. In: Parr JF, Gardner WR, Elliot LF (eds) *Water potential relations in soil microbiology*. Soil Science Society of America, Madison, pp 1–22
- Pimenta JA (2008) Relações hídricas. In: Kerbauy GB (ed) *Fisiologia Vegetal*, 2nd edn. Guanabara, Rio de Janeiro, pp 1–32
- R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>. Accessed July 2014
- Schmitz JD, Guédon Y, Herter FG, Leite GB, Lauri PÉ (2014) Exploring bud dormancy completion with a combined architectural and phenological analysis: the case of apple trees in contrasting winter temperature conditions. *Am J Bot* 101:398–407. doi:10.3732/ajb.1300335
- Sperry JS, Sullivan JE (1992) Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. *Plant Physiol* 100:605–613
- Van den Ende W (2014) Sugars take a central position in plant growth, development and stress responses. A focus on apical dominance. *Front Plant Sci* 5:1–3. doi:10.3389/fpls.2014.00313
- Weinberger J (1950) Chilling requirements of peach varieties. *Proc Soc Hortic Sci* 56:122–128
- Wescor (2001) HR-33T Dew point microvoltmeter instruction/service manual. © 2001 Wescor, Inc
- Yoda K, Wagatsuma H, Suzuki M, Suzuki H (2003) Stem diameter changes before bud opening in *Zelkova serrata* saplings. *J Plant Res* 116:13–18. doi:10.1007/s10265-002-0065-3