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Evidence for non-occurrence of node-to-node or stem-to-bud transfer of chilling temperature signal for dormancy release

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Abbreviations: CU: Chilling unit, GDH: Growing degree hour, MTB: Mean time until bud break, PCU: Positive chilling unit.

Abstract: In the current context of global changes, phenology is expected to be one of the major processes affected by temperature increase, notably through the dynamics of endodormancy release. However, the actual impact on bud break pattern is difficult to predict due to poor knowledge about the spatial extent of chilling sensing, which likely affects bud break heterogeneity. Indeed, contrary to a widely held opinion, the strictly local perception of the chilling air signal has never been demonstrated. The present experiment addresses this issue through local chilling or heating of selected nodal groups of buds on shoots of peach tree under endodormancy. A temperature-conditioned localizable air-jet device was designed to provide the sharpest possible temperature contrast between selected treated buds and the 'not-treated' rest of the tree structure, including adjacent axis tissue. Different chilling doses were tested over two experimental seasons, and a heat treatment was applied on single nodes in a cold environment. Chilled vegetative buds did not break when the local chilling dose received was less than 50% of the requirements even though neighboring axis tissue received the standard chilling dose. The maximum rate of bud break reached 80% at chilling completion and most of the broken buds produced long shoots. We conclude that temperature sensing occurs on a very local scale with the signal not reaching neighboring untreated buds, meaning that endodormancy release should be considered a very local process. The local response permits analysis of the intra-canopy heterogeneity of bud break and the possible relationship between bud status and intra-canopy heterogeneity of bud temperature.

1. Introduction

Endodormancy is defined as a phase of suspended growth in the meristematic part of a plant structure that is controlled from within the same plant structure (Lang *et al.*, 1987). Endodormancy thus characterizes not a whole plant but individual plant structures (buds, cambial zones, seeds), and the endodormancy release enabling this meristematic part to recover full growth ability needs to be understood at this individual structure level. This allows for heterogeneity between buds in the tree structure regarding endodormancy status, which is not taken into account in standard phenological models. However, studies on temperate fruit tree species cultivated in tropical or subtropical conditions, i.e. experiencing limiting chilling conditions, have widely reported strong heterogeneity in bud break timing and spacing attributed to heterogeneity in endodormancy release (Crossa-Raynaud, 1955; Guerriero and Scalabrelli, 1982; Dennis, 1987; Bernardi, 1988; Mauget and Rageau, 1988; Lam Yam, 1990; Zguigal, 1995). This heterogeneity in endodormancy release has been shown to result in major impairment of bud break/blooming patterns, including bud-breaking rate with extended and heterogeneous bud breaking delay which in turn results in low leafing rates, the production of 'rosettes', and branching deficiency (Zguigal, 1995). In light of current global warming, such disturbances could potentially extend to new areas, starting in the south of the current temperate zone. This hypothesis is supported by recent observations: since 1988 (the start of continuous increase of yearly mean temperature in France) bloom date has advanced, compared to the 1976-1988 period, whereas endodormancy release date has tended to retreat, most visibly in southern Europe (Chmielewski *et al.,* 2004; Legave *et al.,* 2008) and Japan (Honjo, 2007; Primack *et al.,* 2009). In order to model blooming/bud-breaking date in a context of global change, an accurate endodormancy release module is needed, requiring greater knowledge of chilling signal

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sensing, transduction and response. As a step towards understanding and integrating bud break heterogeneity into relevant models, it is important to identify how locally the chilling air temperature signal is perceived and whether it is transduced between structures (buds).

Dormancy release has been investigated at more macroscopic scales, ranging from a twig up to a whole branch. Coville (1920) chilled or warmed a whole branch of blueberry plants, Timmis and Worrall (1974) chilled a whole branch of Douglas fir, Krassnosselskaya and Richter (1942) applied a warm bath on sections of poplar and ash branches, and Witkowska-Zuk (1970) applied a warm bath on a section of *Populus x berolinensis* branch. In reviews covering older works, Nooden and Weber (1978) and Saure (1985) concluded that the influence of endodormancy release factors appears confined to the buds of the treated parts; however treatments were not applied at bud scale and, consequently, did not permit identification of exactly which structure (buds, inter-node, or branch axis) hosted the actual perception zones sensing the endodormancy releasing factors (mainly temperature signal). In his review, Perry (1971) concluded that 'although there is much evidence for the concept of a control center of dormancy processes in buds and leaves, the possibility that other plant parts may be involved is not excluded'.

This issue could be partially resolved by investigations at a smaller spatial scale. Witkowska-Zuk (1970) applied warm baths to terminal buds of long shoots of *Populus x berolinensis*. Other studies used endodormancy-releasing chemicals on single buds on the shoot: Denny and Stanton (1928) applied ethylene chlorohydrin on lilac; Wang and Faust (1987) applied thidiazuron on apple. When plants or branches were subsequently exposed to mild temperatures, only the treated buds broke, prompting the conclusion that buds were effective perception zones of these endodormancy breaking factors and that only the treated bud was able to receive the signaling process borne in it.

However, with warm bath treatments it is impossible to distinguish the effects of temperature, tissue moistening and their interaction, such as the oxygen limitation that Saure (1985) highlighted as an important factor. In addition, Perry (1971) pointed out that 'all that the treatments used for breaking dormancy are severe. Often the temperature or chemical concentrations used border on being lethal. Many of the chemicals used are poisonous at relative low concentrations'. So the interpretation of such results remains questionable, even in the most convincing case of lilac bud treatment by Denny and Stanton (1928). Surprisingly, to date no experimental study at bud level has used chilling temperature, although it is undoubtedly the main natural factor driving bud endodormancy release at bud level.

The temperature signal was also investigated by authors working on vernalization signal, but the treatment was not applied strictly to the bud; for example Metzger (1988) chilled the upper part of the twig. Furthermore, the process investigated is not exactly the same because, in the vernalization process, leaves are involved (Crosthwaite and Jenkins, 1993).

Thus, contrary to a widely held opinion, a strictly local perception of the chilling air signal has never been demonstrated.

The present experiment addresses this issue through local application of different temperatures to either buds or neighboring non-bud areas in an attempt to answer the following questions: 1) which structures are able to perceive the chilling signal inducing dormancy release - bud only, non-bud area only, or both; 2) is the chilling signal applied to one bud able to break dormancy in other buds on the twig; 3) is the signal applied to non-bud area able to break dormancy in buds; 4) is non-bud chilling required in addition to bud chilling for dormancy release?

2. Materials and Methods

The experiment was conducted on 'Redhaven' peach trees (three years old in 2003) grown outside in 200-l containers filled with a peat-local soil mixture. Thermal conditioning was applied locally to parts of one-year-old shoots at bud scale, with two contrasting levels: either (i) target buds were submitted to chilling temperature while the rest of the tree structure, including shoot tissues adjacent to these buds, remained at non-chilling temperature (T>15°C in a greenhouse, experiments 1 and 2); or (ii) a symmetrical treatment, with target buds kept at non-chilling temperature while the rest of the tree structure remained at chilling temperature in a cold chamber (experiment 3). As the focus of the study was on endodormancy release in relation to outgrowth and branching pattern, the term 'target buds' refers to vegetative buds only; the one or two floral buds that could be on either side of the single vegetative bud on each node, sharing the same thermal conditions, were not taken into account.

Conditioning device

Local thermal conditioning was achieved by a thermostated air-jet (Fig. 1). Low-pressurized air was circulated in plastic pipes from a thermostated water bath and delivered through nozzles (modified pipette tip cones) directly blowing onto node buds. The neighboring shoot axis structures were kept isolated from the air jet by a plastic deflector set up at the base of each air-chilled or air-warmed bud group.

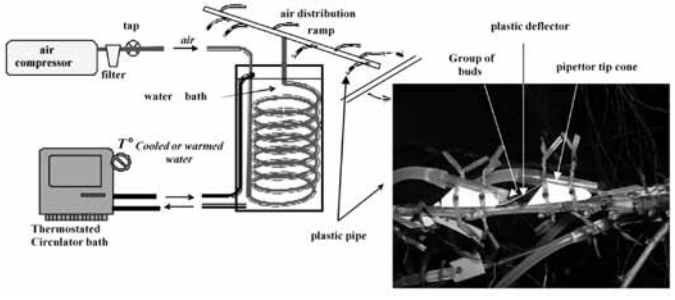

Fig. 1 - Schematic diagram and picture of the device developed for selectively chilling or heating buds (for the warming treatment, warm water replaced cooled water in the water bath).

Bud temperature was monitored by thermocouples inserted between the basal scales of one floral bud (Fig. 2), and internode temperature corresponded to ambient temperature. Thermocouples inserted into the bark of the shoot axis 3-5 mm away from the bud point connection made it possible to verify that adjacent axis tissues (AAT), very close to the buds but protected by the deflector (Fig. 1), were not significantly affected by the cooled air jet. This device allowed efficient local, differential thermal control of buds and the non-bud area (Fig. 3). Thermocouple data were recorded on a Delta T logger (Delta-T-Device-SIIS, Cambridge, UK) every 10 min. Ambient temperature, corresponding to the inter-node temperature, was recorded (time lapse: 10 min) through a thermistor (Hobo Temp Pro 64K, Prosensor).

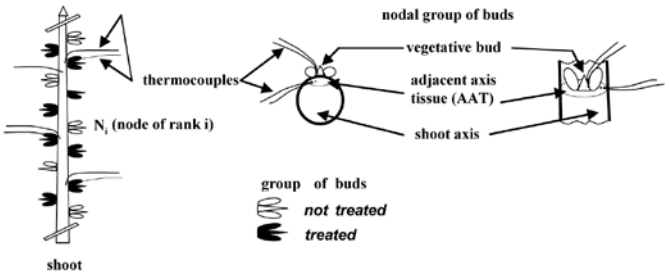

Fig. 2 - Schematic diagram of the experimental design for the treatment of bud groups and for collecting the temperature data for treated bud groups and the corresponding adjacent axis tissues.

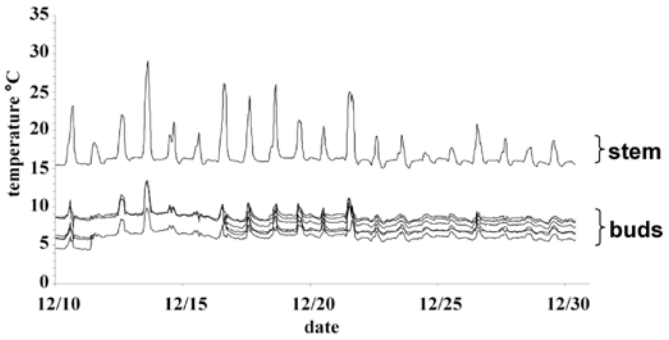

Fig. 3 - Temperature applied at stem and bud level in experiment 1 (partial recording as example).

Selective chilling of buds on cold-deprived trees under endodormancy (experiments 1 and 2)

Trees were cold-deprived from the time of deepest endodormancy onwards by transferring them to a greenhouse kept at a temperature that was high enough to prevent any chilling effect (heating threshold: 15°C) and moderate enough to prevent heat shocks (cooling threshold: 25°C).

Experiment 1. In 2003, six trees were transferred from outside into the greenhouse on October 14. Due to technical constraints, two 80-cm-long shoots were selected on one of the six trees for the cooling treatment. On each shoot (*Sx*), eight bud groups at *Nx* node position from the terminal (one vegetative bud with one or in most cases two floral buds) were chilled under the cooled air-jet from November 18 onwards. On December 23, a thermocouple was inserted under the bark of the shoot axis at mid-internode between nodes N14 (not chilled) and N15 (chilled) of shoot S2. The data (not shown) revealed that during the cooling period, mid-internode temperature was very close to the ambient temperature as recorded with the thermistor. This enabled us to map the temperature dynamics of each shoot section (buds, internodes) of the treated zone.

The buds of nodes N6 and N9 on shoot S1 fell at the beginning of December due to mechanical injury while setting up the air device. They were replaced by two new groups, i.e. nodes N5 and N7 on S1. On December 30, the cooling systems on all buds were stopped. We noticed damage on the buds at node N5, which were consequently discarded from the analyses. Consequently, this treatment actually had two levels: a 'short chilling' treatment (buds of node N7 on S1) and a 'long chilling' treatment (the other buds).

Experiment 2. In order to investigate the impact of insufficient chilling on dormancy release and bud breaking patterns, a second experiment, similar to experiment 1, was conducted with low and medium chilling doses. In 2004, six trees were transferred from outside into the greenhouse on October 10. Four one-year-old shoots on one of the six trees and six bud groups were selected on each shoot. Air jet cooling started on 11 February 2005 (Fig. 2). It was terminated on March 15 for shoots S1 and S4 ('short chilling' treatment) and on April 6 for shoots S2 and S3 ('long chilling' treatment'). On March 15, when switching off the nozzles, we noticed damage on the buds at node N12 on S1; the corresponding data were discarded.

Calculating chilling and heating doses. The chilling doses received by different parts of the treated shoots were calculated from the thermocouple (cooled buds) or thermistor data (other parts of the shoot) by applying the classical Utah model (CU) from Richardson *et al.* (1974), the Positive Utah Chill Units (PCU) model (Linsley-Noakes *et al.,* 1994) and the Dynamic model (Fishman *et al*., 1987 a, b). The PCU model was chosen because it appears more relevant in warm country (with October 1 as start date and hourly temperature data) and the Dynamic model because it is, described as the most accurate model for walnut by Luedelling *et al*. (2009 a, b; 2011).

For each experiment, we calculated the heat unit doses between the end of the bud cooling phase and the mean date of bud break. We used the classical Growing Degree Hours (GDH) model, with 4.5°C as base temperature (Richardson *et al.,* 1975).

Selective warming of buds on chilled trees under endodormancy (experiment 3)

Our objective was to condition targeted buds at temperatures unable to release endodormancy (>15°C) while the rest of the tree structure, including shoot tissues adjacent to these buds, remained at chilling temperatures. Two trees were cold-deprived by transferring them on 15 September 2008 to a greenhouse maintained at a temperature that was high enough to prevent any chilling effect on the trees (heating threshold: 15°C). On 17 October 2008, they were transferred into a cold chamber set around 8°C with

a short photoperiod (8 h) for 52 days, which corresponded to the target accumulation of ca. 1000 CU calculated with the PCU or Utah models as chilling requirements were estimated as 870 CU by Richardson *et al*. (1974, 1975) and 1180 CU by Werner *et al.* (1988) for Redhaven peach trees. The corresponding value for the Dynamic model was initially set as 75 'portions' for this variety (Erez *et al.,* 1988) but later revised to 45 'portions' (Erez, personal communication) and optimized by Bonhomme *et al.* (2010) at 48 'portions'. During this period, the same bud conditioning device was used to provide warm air (>15°C) to selected buds on six twigs of two trees. Finally, the trees were transferred on December 8 into a greenhouse where the temperature was kept above 15°C until bud break.

Biological observations

Dormancy assessment. To ascertain if vegetative buds were endodormant at the start of the chilling or warming treatments, we characterized dormancy status using the "single node cuttings" forcing test at 25°C (Rageau, 1978; Dennis, 2003) on buds from non-treated shoots. Mean time to bud break (MTB) was then calculated. At the end of both treatments, MTB was calculated to verify that endodormancy had not been released in experiments 1 and 2 for non-chilled buds but had been released in experiment 3 for non-warmed buds.

Bud phenology and shoot growth. Each of the buds of treated shoots was checked twice a week after the end of the cooling or warming treatment (i.e. non-chilling); the buds, like the other parts of the trees, were kept under the forcing temperatures (>15°C) of the greenhouse. We watched for bud break in vegetative buds, at "green tip" stage, #09 in BBCH scale (Meier, 2001).

After the cooling treatment, shoot length was measured several months after bud break, in late May and June in experiments 1 and 2, respectively, when growth had almost completely stopped, in order to check for alterations in shoot growth. The same control checks were done in June for experiment 3.

Statistical analysis

Bud breaking rates obtained for the different levels of chilling doses were compared with Fisher's exact test.

The relationship between bud break delay (DB) and chilling doses received, and between the length of shoots (L) and chilling doses were analyzed by linear correlation.

The effect of year (experiment) on DB and L was tested using the non-parametric Kruskal-Wallis test.

All the analyses were performed using the R software package (R Development Core Team 2011; R Foundation, http://www.r-project.org/).

3. Results

Chilling doses received by the different parts of the shoots

Accumulated chilling doses (in CU, PCU and portions) received by the different parts of the shoots were computed according to experiment and chilling treatment duration (Table 1).

Table 1 - Chilling amounts (computed with PCU and CU and Dynanic models) received by different organs and tissues: all internodes and at cooled and non-cooled node buds according to experiments 1 and 2 on shoots (Si) and nodes (Nj)

Buds at cooled nodes					
			Endodormancy release model		
Shoot	Node	Class of chilling amounts	Positive CU (PCU)	Utah (CU)	Dynamic (portions)
Experiment 1					
"Short chilling" treatment					
S1	N5	**medium**	**622**	**537**	**24**
S1	N7	*medium*	*620*	*540*	*20*
"Long chilling" treatment					
S1	N11	*high*	*1060*	*970*	*37*
S1	N12	**high**	**1078**	**993**	**38**
S1	N13	*high*	*1060*	*970*	*37*
S1	N14	*high*	*1060*	*970*	*37*
S1	N15	*high*	*1060*	*970*	*37*
S1	N17	**high**	**991**	**906**	**37**
S2	N6	**high**	**1061**	**976**	**38**
S2	N8	*high*	*1060*	*970*	*37*
S2	N9	*high*	*1060*	*970*	*37*
S2	N11	**high**	**1083**	**998**	**37**
S2	N13	*high*	*1060*	*970*	*37*
S2	N15	*high*	*1060*	*970*	*37*
S2	N16	*high*	*1060*	*970*	*37*
S2	N19	**high**	**1079**	**993**	**37**
Chilling amounts at all internodes and non-cooled-node buds		**very low**	**97**	**0**	**3**
Experiment 2					
"Short chilling" treatment					
S1	N8	**low**	**469**	**334**	**30**
S1	N10	*low*	*500*	*370*	*30*
S1	N15	**medium**	**629**	**499**	**32**
S1	N17	**low**	**531**	**400**	**31**
S1	N20	**medium**	**652**	**523**	**32**
S4	N9	**low**	**451**	**313**	**30**
S4	N12	*low*	*440*	*300*	*30*
S4	N17	**low**	**378**	**231**	**28**
S4	N21	**low**	**528**	**386**	**30**
S4	N24	*low*	*440*	*300*	*30*
S4	N29	**low**	**399**	**249**	**29**
"Long chilling" treatment					
S2	N10	**low**	**558**	**410**	**29**
S2	N11	*medium*	*750*	*610*	*29*
S2	N13	**medium**	**674**	**534**	**29**
S2	N17	**medium**	**711**	**571**	**29**
S2	N20	**medium**	**862**	**726**	**30**
S2	N23	*medium*	*750*	*610*	*29*
S3	N9	**medium**	**761**	**626**	**30**
S3	N11	**medium**	**773**	**639**	**30**
S3	N16	**medium**	**887**	**982**	**30**
S3	N19	**high**	**1025**	**756**	**31**
S3	N24	**medium**	**806**	**667**	**30**
S3	N29	*medium*	*810*	*730*	*30*
Chilling amounts at all internodes and non-cooled node buds		**very low**	**129**	**0**	**11**

For non-thermocoupled buds, chilling amounts were estimated as means (rounded to the nearest 10 PCU or CU) of all experimental values from the same class of chilling on the same shoot in the same experiment and indicated in italics

Based on the recorded thermistor data, some chilling units were accumulated during experiments 1 and 2 (97 and 129 units, respectively, which is very low compared to the chilling requirements as mentioned above) at the inter-node tissues as well as at buds and AAT at non-cooled nodes. About half of this chilling occurred before transfer to the greenhouse, the rest occurred before the cooling treatment.

At the cooled nodes, chilling doses were computed, either from actual data for the thermocoupled buds or non thermocoupled buds, as means of all experimental values of the same chilling class from the same experiment (Table 1). Four classes of chilling dose were defined: 'very low' (<150 PCU), 'low' (350-550 PCU), 'medium' (600-900 PCU) and 'high' (>950 PCU). Internodes and non-cooled buds received very low doses (Table 1), mainly before the transfer into the greenhouse.

In experiment 3, the accumulated chilling dose of non-warmed buds reached 973 PCU (Table 2). As the temperature was around 8°C, almost the same value was obtained with the classical Utah model, thus meeting the chilling requirements of the Redhaven peach bud (≈900 CU). For warmed buds, the PCU dose was very low (32 PCU maximum, i.e. only 3% of chilling requirements, 1 portion i.e. around 2% of chilling requirements for Dynamic model) for the coldest bud, and zero for the warmest bud. The classical Utah model yielded zero CU for all warmed buds.

Endodormancy status

After trees were transferred to the greenhouse, the MTB values of non-chilled vegetative buds decreased over the first two to three months and leveled down to ca. 22.5±0.5 days in experiment 1, and 21.5±0.5 days in experiment 2 (data not shown). At periods marking the start of bud cooling, MTB was 31.5±0.5 d in experiment 1 and 21.5±0.5 d in experiment 2, thus indicating that vegetative buds were actually endodormant [MTB threshold between endodormancy and ecodormancy for vegetative 'Redhaven' peach tree buds is cited as 12 days by Bonhomme *et al.* (2000)].

In experiment 3, at the beginning of the treatment, the MTB value of the non-chilled vegetative buds was 32±10 d (data not shown), thus indicating that the vegetative buds were actually endodormant and close to the maximum of endodormancy. When trees were transferred from the cold chamber to the greenhouse for bud break forcing, the MTB value was 12±1.5 d, indicating endodormancy release for these chilled buds. It was impossible to determine MTB of the warmed buds due to insufficient replications. Nevertheless, bud break did not occur, which clearly indicated that endodormancy was not released.

Vegetative bud response

Vegetative bud behavior resulting from chilling dose in experiments 1 and 2. Table 3 presents the vegetative bud responses to the chilling doses received: break, abscission, or no detected event. Neither very low (<115 PCU) nor low (mean: 469 PCU) doses resulted in bud break. Consequently the bud break rate obtained with medium chilling dose is significantly higher ($p<0.0001$) than very low and

Table 2 - Chilling amounts (computed with PCU and CU and Dynamic models) received by different organs and tissues: all internodes and at non-cooled node buds according to experiment 3; at warmed nodes, shoots (Si) and nodes (Nj)

Buds at cooled nodes					
			Endodormancy release model		
Shoot	Node	Class of chilling amounts	Positive CU (PCU)	Utah (CU)	Dynamic (portions)
Experiment 3					
"Warming" treatment					
S1	N16	**very low**	**0**	**0**	**0**
S1	N17	**very low**	**18**	**0**	**0**
S1	N21	**very low**	**0**	**0**	**0**
S1	N24	*very low*	*6*	*0*	*0*
S2	N16	**very low**	**30**	**0**	**1**
S2	N21	*very low*	*27*	*0*	*1*
S2	N27	**very low**	**24**	**0**	**1**
S3	N16	**very low**	**0**	**0**	**0**
S3	N21	*very low*	*0*	*0*	*0*
S3	N24	**very low**	**0**	**0**	**0**
S3	N28	*very low*	*0*	*0*	*0*
S3	N29	**very low**	**0**	**0**	**0**
S4	N16	**very low**	**0**	**0**	**0**
S4	N18	*very low*	*0*	*0*	*0*
S4	N20	**very low**	**0**	**0**	**0**
S5	N13	**very low**	**0**	**0**	**0**
S5	N20	**very low**	**0**	**0**	**0**
S5	N24	*very low*	*0*	*0*	*0*
S6	N14	**very low**	**3**	**0**	**0**
S6	N18	*very low*	*14*	*0*	*0*
S6	N23	**very low**	**32**	**0**	**1**
S6	N24	*very low*	*14*	*0*	*0*
S6	N28	**very low**	**6**	**0**	**0**
Chilling amounts at all internodes and non-warmed node buds		**high**	**973**	**941**	**41**

For non-thermocoupled buds, chilling amounts were estimated as means (rounded to the nearest 10 PCU or CU) of all experimental values from the same class of chilling on the same shoot in the same experiment and indicated in italics.

low rates. Almost no buds fell after very low doses, but 20% of buds fell after low doses. Medium and high doses (mean: 745 and 1057 PCU, respectively) both resulted in about 10% bud fall and in 69 and 80% bud break, respectively, values which were not statistically different.

On the shoots with medium and high doses, none of the non-cooled vegetative buds broke (Fig. 4a, 4b), either on the equipped tree or on the other five trees kept under the greenhouse.

Figure 5a shows that within each experiment, the individual delay to bud break (DB), i.e. the time between the end of cool air application and bud break, was not dependent on the PCU received by the bud (for experiment 1, $R^2 = 0.22$, $p = 0.12$; for experiment 2, $R^2 = 0.10$, $p =$

0.41; and if both experiments 1 and 2 were analyzed as one single dataset, $R^2 = 0.08$, $p = 0.21$). There was a clear experiment effect on the mean DB ($p < 0.001$): 35.5 d in experiment 1 and 28.0 d in experiment 2. This difference could be mainly explained based on mean temperature under the greenhouse after the end of bud cooling, which was higher in experiment 2 (19.1°C) than experiment 1 (16.2°C). In addition, Figure 5b shows that the length of a growth unit sprouted from a broken bud was not dependent on the cumulated PCU that had been received by that bud (for experiment 1, $R^2 = 0.05$, $p = 0.51$; for experiment 2, $R^2 = 0.08$, $p = 0.47$; and if both experiments 1 and 2 were analyzed as one single dataset, $R^2 = 0.008$, $p = 0.79$). The

Table 3 - Vegetative bud responses to the received chilling doses

Buds at cooled nodes					
			Endodormancy release model		
Shoot	Node	Class of chilling amounts	Positive CU (PCU)	Utah (CU)	Dynamic (portions)
Experiment 3					
"Warming" treatment					
S1	N16	**very low**	**0**	**0**	**0**
S1	N17	**very low**	**18**	**0**	**0**
S1	N21	**very low**	**0**	**0**	**0**
S1	N24	*very low*	*6*	*0*	*0*
S2	N16	**very low**	**30**	**0**	**1**
S2	N21	*very low*	*27*	*0*	*1*
S2	N27	**very low**	**24**	**0**	**1**
S3	N16	**very low**	**0**	**0**	**0**
S3	N21	*very low*	*0*	*0*	*0*
S3	N24	**very low**	**0**	**0**	**0**
S3	N28	*very low*	*0*	*0*	*0*
S3	N29	**very low**	**0**	**0**	**0**
S4	N16	**very low**	**0**	**0**	**0**
S4	N18	*very low*	*0*	*0*	*0*
S4	N20	**very low**	**0**	**0**	**0**
S5	N13	**very low**	**0**	**0**	**0**
S5	N20	**very low**	**0**	**0**	**0**
S5	N24	*very low*	*0*	*0*	*0*
S6	N14	**very low**	**3**	**0**	**0**
S6	N18	*very low*	*14*	*0*	*0*
S6	N23	**very low**	**32**	**0**	**1**
S6	N24	*very low*	*14*	*0*	*0*
S6	N28	**very low**	**6**	**0**	**0**
Chilling amounts at all internodes and non-warmed node buds		**high**	**973**	**941**	**41**

For very low chilling level of the non-treated shoots in experiments 1 and 2, the number (Nb) of non-broken buds was not exactly determined (>1000) but corresponded to all the buds (100%) of the six trees placed under the greenhouse.
Significantly different rates, as assessed by Fisher's exact test, are indicated by different letters.

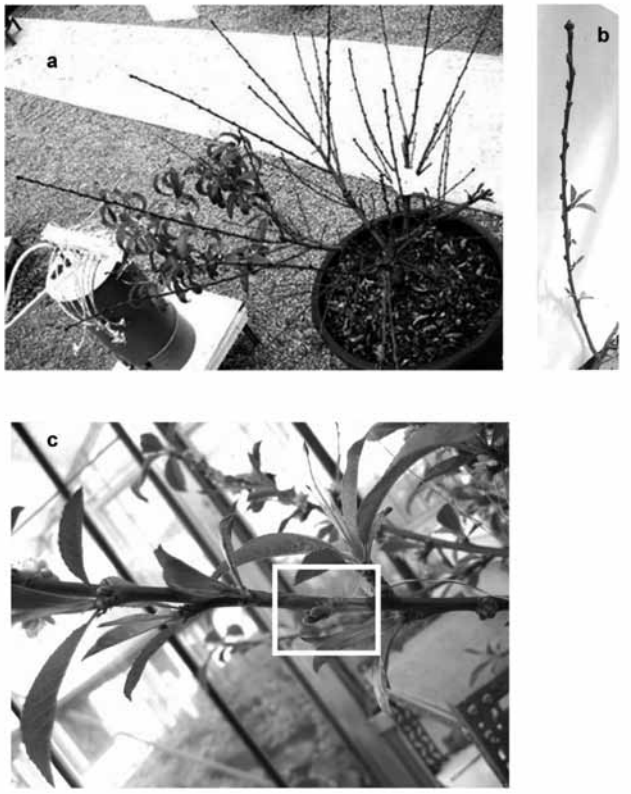

Fig. 4 - Bud break in the localized-chilled experiment (a and b) and localized-warmed experiment (c).

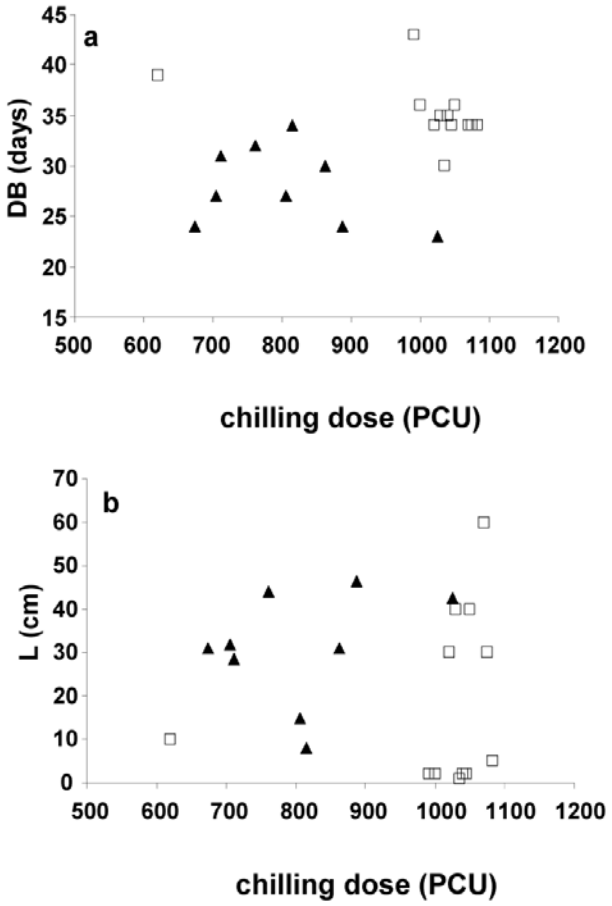

Fig. 5 - Responses of vegetative buds according to chilling dose. a: individual delay to bud break (DB); b: individual final length (L) of shoots in experiments 1 (□) and 2 (▲).

experiment effect on sprouted shoot length was not significant ($p = 0.06$).

It can be concluded that selective chilling of a nodal group of buds was effective in releasing the endodormancy of its vegetative bud. No endodormancy release of buds of any other node was observed, which shows that the signal is not transmitted.

Vegetative bud behavior resulting from the warming treatment in experiment 3. With a few exceptions at the stem bases, almost all buds chilled in the cold chamber broke, corresponding to the classical bud break pattern observed in temperate climates (82% of bud break on the control twigs). On the treated tree, not a single warmed vegetative bud broke, indicating that the chilling signal received at the stem was not transmitted to non-chilled buds (Fig. 4c).

For non-warmed trees, bud break was observed on 9 January 2009 for terminal buds and 16 January 2009 for axillary buds, corresponding to 8419 and 10479 GDH (with the 4.5°C base) after chilling, respectively, i.e. around 10% higher than was calculated for axillary buds in experiments 1 and 2.

Mean temperature in the greenhouse over the 39-day period between the end of chilling and axillary bud break was 15.8°C.

4. Discussion and Conclusions

Vegetative bud behavior resulting from chilling dose in experiments 1 and 2

Contrasting with medium and high chilling doses, the low chilling dose treatment allowed some events to take place in the buds during further forcing, that ultimately led to abscission of a significant percentage of these buds.

The selective chilling of a nodal group of buds was effective in releasing endodormancy of its vegetative bud, and did not result in endodormancy release of buds of any other node.

Nevertheless, some other aspects of the results were unexpected. Medium chilling doses (620-887 PCU) were almost as effective as high doses (991-1083 PCU): the percentage of broken buds was only slightly lower (Table 3) and the shoots borne from the broken buds showed normal growth. The chilling requirement for Redhaven peach trees is cited at 870 CU (Utah model) by Richardson *et al.* (1975), 1180 CU by Werner *et al.* (1988) and 45 portions in the dynamic model (Erez, personal communication), optimized to 48 portions (Bonhomme *et al*., 2010) from one-node cutting test results. This points out the three critical elements in the models: (1) the determination of the starting date for chilling accumulation, (2) taking into account (or not) the negative temperatures and (3) the reality and intensity of the reverse effect of mild temperatures and the duration during which the reversion is possible (a short period in Dynamic model, without limits in Utah model, 24 h in PCU).

The computed heat requirement for bud break (around 9000 GDH) was much higher than the value given by Richardson *et al.* (1975) for 'Redhaven', i.e. 4922 GDH (for the same 4.5°C baseline) for floral buds (full bloom) with 870 CU of prior chilling, and closer to that found by Scalabrelli and Couvillon (1986), i.e. 8000 GDH (same base) for vegetative bud break with 900 CU of prior chilling. The heat requirements would, surprisingly, have been quite similar for buds under medium chilling doses (8900 GDH) and buds under high chilling doses (9200 GDH). This suggests that chilling requirements may have been overestimated and/or that these buds were in deeper endodormancy in experiment 1 than in experiment 2 when chilling started.

Although some shoots had not totally stopped growing when the shoot lengths were recorded, Figure 5b shows that the length of a growth unit borne from a broken bud was not dependent on the cumulated chilling dose received by this bud.

Vegetative bud behavior resulting from warming treatment in experiment 3

Excluding some buds at the base of the twigs, almost all the buds chilled in the cold chamber broke, thus reproducing the classical bud break pattern observed in temperate climates. Not a single warmed vegetative bud broke, indicating that the chilling signal received at the stem was not transmitted to the non-chilled buds.

Normal bud break occurred at a chilling mean temperature of around 9.3°C, showing once again that the Weinberger model is not accurate, even under temperate climates. In this range of chilling temperatures, the Utah and PCU models gave the same cumulated chilling (990 CU), close to the chilling requirement cited by Richardson *et al.* (1974).

The heat requirement for bud break estimated in this experiment was a little higher than that found by Scalabrelli and Couvillon (1986), i.e. 8000 GDH (same base) for vegetative bud break with 900 CU of prior chilling, and also than the amounts deduced from experiments 1 and 2. The small difference (2-3 days) could probably be attributed to the uncertainty in determining bud break date or starting date (i.e. endodormancy release date).

All these results indicate that the temperature for endodormancy release has to be applied at the level of the structure, where the cause of growth blockage is located, i.e. the bud itself, according to the definition of endodormancy given by Lang *et al.* (1987).

Accuracy of the chilling and heat requirements

These requirements are very approximate determinations that are not fully suitable for modeling the impacts of global changes. Figures on chilling requirements for endodormancy release are often confounded by the chilling received during the period between growth arrests or leaf fall and bud break. Heat requirements given in the literature are also approximations, as the thermal threshold permitting growth is generally unknown. Moreover, heat and chilling action could combine at mild temperature

(around 10°C), and determination of the start date for heat action remains problematic.

Further, the delay to bud break (or bloom) and the heat requirements calculated may also be dependent on factors other than the sole bud response to temperature, such as bud water and nutrient uptake rate and, consequently, the temperature of the roots as revealed in Young *et al.* (1987).

Thus, the questions about chilling and heat requirements will probably have to be revisited before chilling and heat requirement figures can be used to model climate change impacts.

Temperature signal transfer

From the literature, the most clear response is that the chilling (temperature) signal is not transferred from one terminal bud of a branch to the terminal bud of another branch.

Regarding vernalization process studies, it is clear that a signal is transferred from the leaves of sugar beet to the terminal meristem (Crosthwaite and Jenkins, 1993) but this signal could be a thermic signal or a secondary biochemical signal. This signal could also be originated in roots (Metzger *et al.,* 1992). Some grafting approaches (Metzger, 1988) seem to show that the vernalized condition is not transferred to other buds but it is not exactly the same process (transfer of a status and not simultaneous transfer of the signal received by one bud to another bud). Moreover, vernalization seems to be correlated with the presence of dividing cells and the dormancy release process occurs on buds with their cell cycle blocked in G1 phase (Cottignies, 1987).

Therefore, even if vernalization and dormancy release seem to be very similar (Metzger, 1996), the literature does not make it possible to conclude about the absence of transfer for the chilling signal from the close part of the twig to the bud or from one bud to another bud located on the next internode or the complementarity of both tissues.

Synthesis: interpretation in terms of chilling signal transfer

Candidates for the chilling receptive zone are the different nodal bud groups and the axis; candidates for the meristematic targets are the vegetative buds of different nodal groups (only two groups represented). With regard to possible paths, we considered 1) 'univocal' paths, i.e. endodormancy release response can only result from the signal coming from one sensing zone; 2) 'parallel' paths, i.e. each of the signals borne in either a bud group or an axis results in effective endodormancy release in a given bud; 3) 'cooperative' paths, i.e. effective endodormancy release in a given bud needs signals from both bud and axis; 4) 'short' paths, i.e. the signal reaching a bud comes from its bud group, and 5) 'long' paths, i.e. the signal reaching a bud comes from another group.

This can be translated into different hypotheses:

Hypothesis 1: a local application of chilling or warming induces a local response for dormancy release. As chilled buds broke and warmed buds did not, the present results are consistent with this hypothesis.

Hypothesis 1': the chilling (or warming) signal can be transmitted to another bud. As on a twig in warm conditions only chilled buds broke, this hypothesis has to be rejected.

Hypothesis 2: a chilling signal applied to a stem could reach the bud and permit bud break. As warmed buds on stems placed in a cold environment did not break, this hypothesis has to be rejected.

Hypothesis 3: chilling applied to buds does permit bud break even if parallel warming on the stem is applied. As chilled buds broke on a twig in warm conditions, the present results are consistent with this hypothesis. The reciprocal hypothesis - chilling applied to a twig does permit bud break even if parallel warming on the bud is applied - is rejected consequent to the rejection of hypothesis 2.

Hypothesis 3': the chilling signal applied to a bud could be transmitted to other buds, permitting bud break, even if a parallel warm signal is applied to the stem. As only chilled buds broke on a twig in warm conditions, this hypothesis has to be rejected.

Hypothesis 4: chilling applied to buds needs additional chilling on the stem to permit bud break. As chilled buds broke on a twig in warm conditions, this hypothesis has to be rejected.

Hypothesis 4': chilling applied to buds could be transmitted to other buds if additional chilling is applied to the stem. Since in a cold environment (i.e. not-warmed buds and twig are chilled), warmed buds did not break, this hypothesis has to be rejected.

Figure 6 summarizes the different hypotheses tested and the possible "chilling receptive zone → vegetative meristematic target" pathways for endodormancy release signal.

In conclusion, our results validated hypotheses 1 and 3; the other cases are to be rejected. Thus, chilling clearly has to be applied to the given bud to be effective for its subsequent endodormancy release. Chilling the stem without chilling the bud is not effective for endodormancy release. Chilling on the bud does not need additional chilling on the stem.

Consequently, the physiological processes involved in chilling-temperature response for endodormancy release have to be investigated at bud level. This is not necessarily (and probably not actually) the case for primordial growth in buds during the ecodormancy phase, as water and nutrient uptake fluxes from roots are needed.

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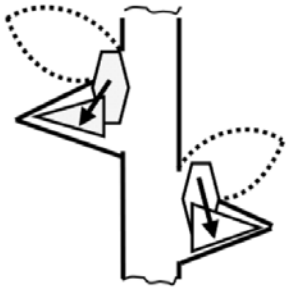
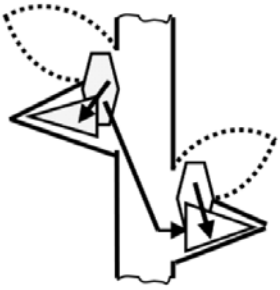
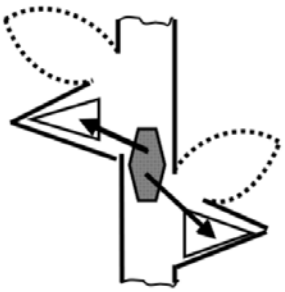


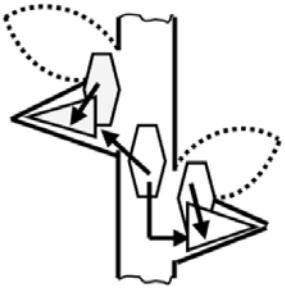
Hyp 1: chilling or warming application on group of buds, local response.
Result : Hyp 1 valid



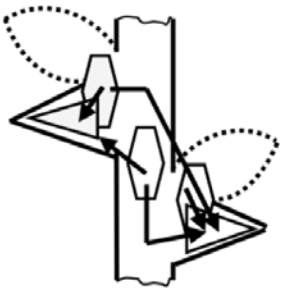
Hyp 1': chilling application on buds, possible transmission of signal to others buds
Result : Hyp 1' not valid



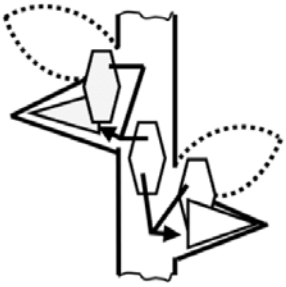
Hyp 2: chilling application on stem, possible transmission of signal to buds.
Result : Hyp 2 not valid



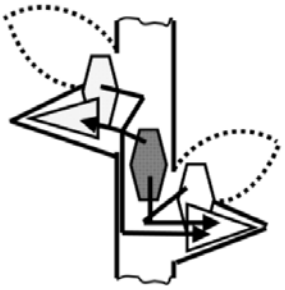
Hyp 3: response of chilled buds did not need parallel chilling signal from stem.
Result : Hyp 3 valid



Hyp 3': the chilling signal could be transmitted to other buds even if parallel warm temperature signal is applied on stem.
Result : Hyp 3' not valid



Hyp 4: response of chilled buds need additional chilling signal from stem.
Result : Hyp 4 not valid



Hyp 4': the chilling signal could be transmitted to other buds if additional chilling signal is applied on stem.
Result : Hyp 4' not valid

'perception zone' with effective chilling dose in experiments

'perception zone' of warm temperature in experiments

Bud break observed i.e. endodormancy released

No bud break observed i.e. endodormancy not released

Fig. 6 - Schema of the different hypotheses tested for "chilling perception" → "meristematic response" spatial paths in the endodormancy release response to chilling; possible and rejected cases for the vegetative buds of peach trees, based on our experimental findings.

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