Journal of Horticulture
ISSN: 2376-0354

Chmielewski et al., J Hortic 2017, 4:3
DOI: 10.4172/2376-0354.1000210

Research Article Open Access

Identification of Endodormancy Release for Cherries (*Prunus Avium* L.) by Abscisic Acid and Sugars

Chmielewski FM[1]*, Götz KP[1], Homann T[3], Huschek G[2], Rawel HM[3]

[1]*Faculty of Life Sciences, Institute of Agricultural and Horticultural Sciences, Humboldt-University of Berlin, Germany*
[2]*IGV-Institut für Getreideverarbeitung GmbH, Germany*
[3]*Instrumental Analysis in Nutritional Science, Institute of Nutritional Science, University of Potsdam, Germany*

Abstract

In order to develop reliable and physiologically sound models for the plant development in spring, the date of endodormancy release is always a crucial and mostly unknown model parameter. Until present, classical approaches - such as climate chamber experiments - are used to derive this unknown parameter. In these experiments, progressive plant development or significant changes in bud's fresh weight or water content are measurable markers for dormancy release. This study presents an alternative approach, which is based on four well-known metabolites. For 5 seasons (2011/12-2015/16), the content of abscisic acid (ABA) and sugars such as fructose, sucrose and glucose in sweet cherry flower buds (cultivar 'Summit') were weekly analysed between beginning of October and April. These data allow comparing the annual course of these metabolites with the date of endodormancy release, derived from a classical climate chamber experiment, published in a previous study. Results showed that ABA and sucrose are two important metabolites which can help to identify the date of endodormancy release of sweet cherries. On average, ABA content reached a plateau of 5.65 µg g^{-1} DW^{-1} during endodormancy, which was maintained for 3-6 weeks. The significant reduction of the ABA content after this period to 4.41 µg g^{-1} DW^{-1} on average during ecodormancy was nearly in agreement with the date of endodormancy release of 'Summit' on 28 November (332 DOY). The annual cycle of sucrose, which has a cryoprotective effect during winter, is well comprehensible and showed a close relationship to the annual course of minimum air temperature after leaf fall (r=-0.90). The nearly constant level of sucrose during ecodormancy (21.0 mg g^{-1} DW^{-1}, 5 yr. mean) did not only allow deriving the date of endodormancy release but can also be helpful to define the beginning of ontogenetic development.

Keywords: Endodormancy; Abscisic acid; Sucrose; *Prunus avium* L.; Flower buds; Phenological modelling

Introduction

Classical physiological parameters such as water-, carbon-, nitrogen content as well as the fresh and dry weight in flower buds were useful to identify the annual duration and timing of the dormant phase and to define the beginning of ontogenetic development for 'Summit' sweet cherries [1]. However, these parameters did not allow identifying the date of endodormancy release - a very important parameter in phenological models. For this reason, several classical experiments with plant samplings were carried out to calculate the plant specific chilling requirement [1-6]. Recently, Chuine et al. [7] suggested carrying out these experiments for a wider range of tree species and varieties in order to capture this important parameter. These classical experiments can temporarily help to identify the chilling requirement or the date of endodormancy release for a wider range of tree species, but for an improved phenological modelling a deeper knowledge of dormancy induction, maintenance and release will be necessary.

Already Perry [8] announced in his review that different plant species and different genotypes within species vary in their dormancy phenomena and defined that a dormant plant having two attributes, (i) a period of markedly reduced growth rate with few, or in some cases no, cell divisions in the terminal/lateral meristems of the plant and (ii) a winter chilling requirement. The renewed growth after dormancy release is then related to the disappearance of inhibitors from the buds and increased concentrations of growth stimulating compounds. Generally, the transitions of the metabolism from an active growth phase to a phase of quiescence, and back to the active growth are gradual and also seasonally dependent. The reason for this is that the amount of cold or the chilling requirement varies with the weather conditions of the preceding season [8,9]. Changes in day length, light quality, and temperature provide the most important environmental cues for synchronising growth with seasonality. Xin and Browse [10] summarized in a review the most notable changes, which include changes or cessation of growth, reduction of tissue water content, transient increase in ABA levels, changes in membrane lipid composition, the accumulation of compatible osmolytes such as proline, betaine, soluble sugars and antioxidants. However, cold acclimation requires many changes in cell biology and metabolism, but the knowledge of the metabolic changes that contribute to these processes is still incomplete.

Autumnal bud development is a composite of bud formation, simultaneous acclimation to dehydration and cold, and acquisition of dormancy [9]. These three processes are interconnected with each other but happen at different levels: cells throughout the plant will acclimate to dehydration and cold for survival, whereas only the youngest derivatives of the meristem participate in bud formation, and yet only those meristem parts of the bud that will resume growth in spring, become dormant. Moreover, the simultaneous activity of various closely cellular, physiological, and morphological processes confounds the dissection of the underlying developmental programs and their respective signals. Only in a few cases processes could be separated and assigned to the action of a particular signalling route [9]. Buds in fruit trees are considered to be 'utilizing sinks' but not 'storage sinks' and thus may preferentially need metabolic substrates rather than storage compounds [11]. Metabolomic studies as yet do not only allow detecting the date of endodormancy release, but also help to better understand this complex physiological process. In

***Corresponding author:** Frank-M Chmielewski, Faculty of Life Sciences, Thaer Institute of Agricultural and Horticultural Sciences, Professorship of Agricultural Climatology, Humboldt-University of Berlin, Albrecht-Thaer-Weg 5, 14195 Berlin, Germany, Tel: +49 30 2093 46395; Fax: +49 30 2093 46488; E-mail: chmielew@agrar.hu-berlin.de

Received August 24, 2017; **Accepted** September 24, 2017; **Published** September 30, 2017

Citation: Chmielewski FM, Götz KP, Homann T, Huschek G, Rawel HM (2017) Identification of Endodormancy Release for Cherries (*Prunus Avium* L.) by Abscisic Acid and Sugars. J Hortic 4: 210. doi: 10.4172/2376-0354.1000210

this study four well-known metabolites are presented which can help to identify the timing of endodormancy release in sweet cherry buds of the cultivar 'Summit'. Metabolites are organic compounds synthesized by many single enzyme-mediated chemical reactions. They are intermediates and products of plant metabolism for growth and development (primary metabolites), and e.g. for defense against herbivory (secondary metabolites). The plant hormone ABA has been shown to mediate the development of freezing tolerance, whereas several roles for sugars in protecting cells from freezing injury have been proposed Xin and Browse [10].

The aim of this study was to show whether temporal changes of these four metabolites during autumn and winter allow defining the date of endodormancy release of 'Summit' sweet cherries.

Material and Methods

Experimental site

The study was conducted for 5 seasons (2011/12-2015/16) at the experimental sweet cherry orchard at Berlin-Dahlem (52.47 °N, 13.30 °E, h = 51 m a.s.l.), established in 2000. The orchard (980 m^2) comprises 80 cherry trees (cultivars 'Summit', 'Regina' and 'Karina') growing in 8 rows with 10 trees each. Trees are grafted on Gisela-5 rootstocks and pruning was performed on demand. All investigations in this study are focused on the cultivar 'Summit', of origin in British Columbia. The soil type and long-term climatic conditions are given in [1].

Bud sampling

In order to analyse ABA and sugar content, weekly 3 flower-bud cluster per tree were taken, randomly over 4 trees (replications) during the cold season from beginning of October until April. After beginning of bud development sampling was conducted development-related at the stages 'swollen bud' (SB), 'side green' (SG), 'green tip' (GT), 'tight cluster' (TC), and 'open cluster' (OC). After freeze-drying and milling of the buds [1], the content of abscisic acid (ABA) and sugars (fructose, sucrose, glucose) were analysed during 5 seasons.

Analytic work

Extraction method: Freeze dried samples (50 mg) were extracted with 1.5 ml 60% methanol using an ultrasonification treatment (Sonorex RR 100, Bandelin electronic GmbH & Co. KG, Berlin, Germany) for 3 minutes followed by incubation at 4°C overnight (AEG Santo 60240 DT 28, Electrolux Hausgeräte GmbH, Nürnberg, Germany). The extracts were centrifuged at 9300 x g for 10 minutes at 4°C, the extraction repeated, and the supernatants pooled together and stored at -20°C until needed.

Identification of ABA: The extraction of the phytohormone was performed as described above. The determination was adapted using the high-performance liquid chromatography and tandem mass spectrometry [12-14]. Chromatography was carried out on a Dionex Ultimate 3000 HPLC system (Thermo Fisher GmbH, Idstein, Germany) using a Phenomenex Luna C18 column (Phenomenex Inc., Aschaffenburg, Germany; 150 × 3 mm; 3 µm) at 40°C , connected to a C18 pre-column containing the same material with a flow rate of 0.32 ml/minutes. The eluents were A (distilled water with 0.1% formic acid) and B (methanol with 0.1% formic acid). The gradient was applied under the following conditions: 50% eluent B, 0-1 minutes; 50-75% eluent B, 1-12 minutes; 75% eluent B, 12-13 minutes; 75-50% eluent B, 13-13.1 minutes; 50% eluent B, 13.1-17 minutes. Run time was 18 minutes and the injection volume was 10 µl. Concentrations of 0.01-10 µg ml^{-1} of selected phytohormones in a mixture (abscisic acid, gibberellic acid, 6-benzyl adenine, indole-3-acetic acid, benzoic acid, acid, indole-3-butyric acid, naphthalene-acetic acid, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) were used for external calibration. Negative ion mass spectra of the samples were recorded in the MRM modus using a 4000 Q Trap® mass spectrometer (AB Sciex Germany GmbH, Darmstadt, Germany). The ion source temperature was set at 600°C and a dwell time of 120 ms for negative ionization were used.

Identification of sugars: The detection of sugars was performed by analysing the samples with a Shimadzu HPLC system (Shimadzu Europa GmbH, Duisburg, Germany) equipped with an evaporative light-scattering detection (Shimadzu ELSD-LT II at 40°C, Gain=3). An X-bridge Amide (particle size 3.5 µm, 150 mm of length and 4.6 mm of internal diameter; Waters GmbH, Eschborn, Germany) column with 12% carbon load was used. The extracts were centrifuged at 16000 x g for 2 minutes and 50 µl of the supernatant were used for HPLC analysis. Acetonitrile (80%) and 0.1% ammonia in water (20%) was used as eluent and a gradient with changing flow rate under isocratic conditions was applied: 0-30 minutes, 1-1.5 ml/minutes; 30-32 minutes, 1.5-1 ml/minutes; 32-35 minutes, 1 ml/minutes. External calibration was made using solutions of fructose, glucose and sucrose at 0.2-2 mg ml^{-1} each (dissolved in 60% methanol).

Statistical analysis

For all sampling dates, mean values and standard deviations of the ABA- and sugar contents were calculated for 4 replications. Significance of temporal changes of the metabolites was tested with the Tukey-HSD test ($p \leq 0.05$). In order to quantify the relationship between mean minimum temperature during endodormancy and mean sucrose content during ecodormancy the Pearson correlation coefficient was calculated.

Results

Timing of para-, endo-, ecodormancy and ontogenetic development

The annual timing of para-, endo-, ecodormancy and ontogenetic development for 'Summit' in Figures 1-3 was indicated by different colours in the figure's background, derived from Chmielewski and Götz [1]. The beginning of endodormancy was equated with the date of complete leaf fall (LF, BBCH 97). Its end (t_1) was derived from classical experimental studies with cherry twigs, which were cut off weekly from November until December and placed in 500 ml plastic flasks, filled with water, to observe the beginning of blossom in a climate chamber with 12 h light, temperatures of ~20/~15°C (day/night) and 70% relative humidity. When twigs started to bloom under controlled conditions (BBCH 60), it was the indication that the chilling requirement at sampling in the orchard was fulfilled. Thus, it was possible to time t_1. Beginning of ontogenetic development (t_1*) was defined as the date with an ongoing increase of bud's water content, which was related to continuously rising air temperatures above the freezing point [1].

Weather conditions in the seasons 2011/12 - 2015/16

With leaf fall (LF) of cherry trees, in the end of October or mid of November (mean 05/11, Table 1), air temperature continuously decreased during the dormancy phases. Mean air temperature reduced from 5.8°C during the relatively short endodormancy phase (LF-t_1: 22.8 d) to 2.2°C during the long period of ecodormancy (t_1-t_1*: 86.2 d). Beginning of ontogenetic development was contrasting between the season 2012/13 (t_1*= 85 day of year (DOY), 26 March) and 2013/14

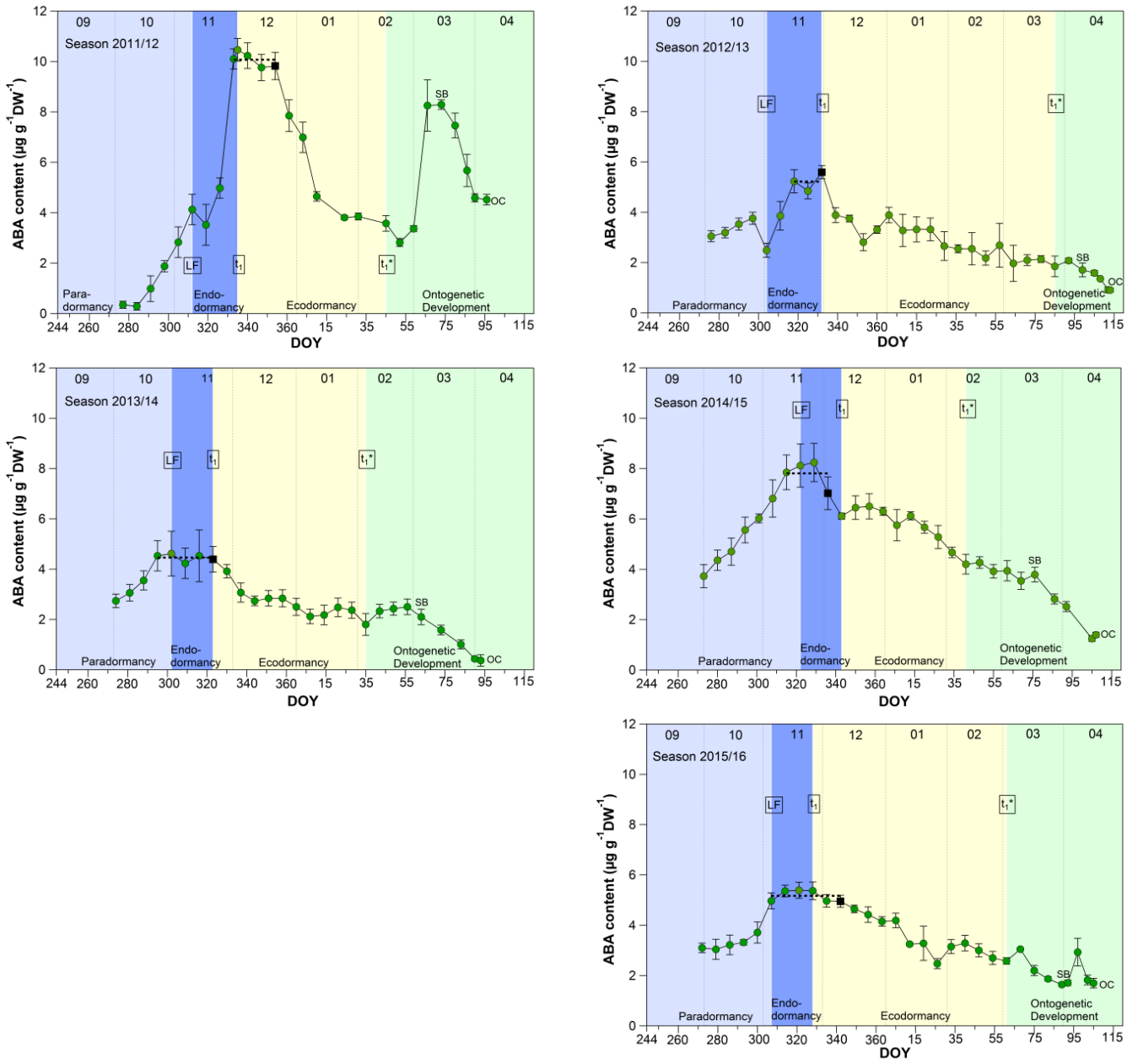

Figure 1: Weekly and development orientated changes of ABA content, 2011/12 - 2015/16. Black squares indicate timing of t_1 according to ABA content. LF: leaf fall, SB: swollen bud, OC: open cluster, t_1: end of endodormancy and t_1*: beginning of ontogenetic development, according to the classic approach, DOY: day of year.

(t_1*= 35 DOY, 4 February, Figure 1). Between LF and SB mean air temperature in 2013/14 was always higher compared to 2012/13, leading to an earlier beginning of ontogenetic development and finally to an early start of bud swelling (2013/14: SB = 63 DOY, 4 March, Figure 1). Between SB and SG air temperature further increased in all seasons and reached on average 7.7°C. In the following 16 days from SG until OC, air temperature was strongly influenced by the annual weather variability. For this reason no homogeneous rise in air temperature was observed.

Variability of ABA content in cherry buds

Figure 1 shows weekly changes in ABA content of 'Summit' sweet cherry buds. It can be seen that in all seasons the ABA content increased from the first sampling date (S1: beginning of October) to maximum values in November or beginning of December. This plateau of maximum ABA contents (on average 5.65 µg g^{-1} DW^{-1}, Table 2) were held for 3 (2012/13) to 6 (2015/16) weeks and marks approximately the endodormancy phase. Subsequently, ABA content steadily decreased until open cluster (OC) in April. Only in the season 2011/12 a second peak during ontogenetic development occurred, which cannot be explained and was never repeated in the following seasons. Additionally, 2011/12 was the season with the lowest ABA content at the first sampling date (0.35 µg g^{-1} DW^{-1}) and the highest mean ABA content during endodormancy (10.1 µg g^{-1} DW^{-1}, Table 2).

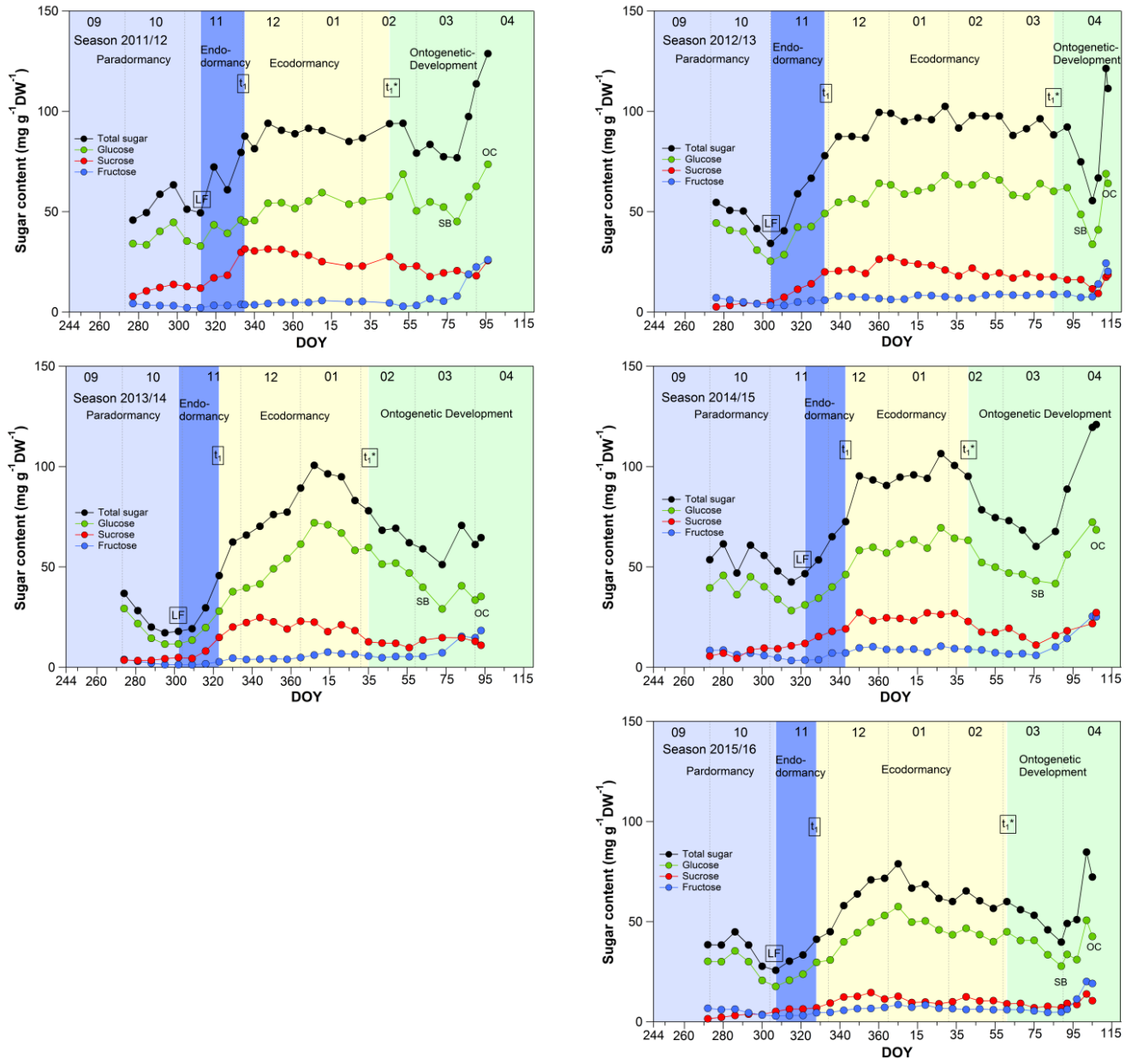

Figure 2: Weekly and development orientated changes of sugar content (glucose, sucrose, fructose, total sugar), 2011/12 - 2015/16. LF: leaf fall, SB: swollen bud, OC: open cluster, t_1: end of endodormancy and t_1*: beginning of ontogenetic development according to the classic approach, DOY: day of year.

The initial mean ABA content in the other 4 seasons was much higher (3.15 µg g^{-1} DW^{-1}).

Despite this fact, in all seasons the highest mean ABA content (marginal exception 2011/12) was reached during the endodormancy phase (Table 2: LF-t_1). In this period also the absolutely highest ABA content was observed (Figure 1). In the seasons 2012/13 and 2013/14 the ABA content significantly reduced after t_1, derived from the classic approach [1]. However, in the other 3 seasons the experimentally found date for t_1 was not exactly in agreement with a significant reduction in the ABA content (Table 3). In 2011/12, it significantly reduced 4 and in 2015/16, 3 weeks after t_1 and in 2014/15 it already declined 1 week before t_1. Assuming that the timing of t_1 is not an absolutely fixed date, it can be conclude that the significant reduction in ABA content after a period of constantly high values (3-6 weeks) was the first hint for endodormancy release. Our previous classical experiments yielded a mean chilling requirement for 'Summit' from 1 September until t_1 of about 40 chill portions (classic approach, Table 3). Using the significant reduction of ABA content after reaching the plateau ($p<0.05$), the chilling requirement for 'Summit' would be about 44 CP (metabolomic approach, Table 3), which nearly confirms our previously derived value for the chilling requirement (C*). The highest differences in C* between the classic and metabolomic approach occurred in the season 2011/12 and 2015/16, where C* reached 55.1 and 52.4 CP, respectively (Table 3).

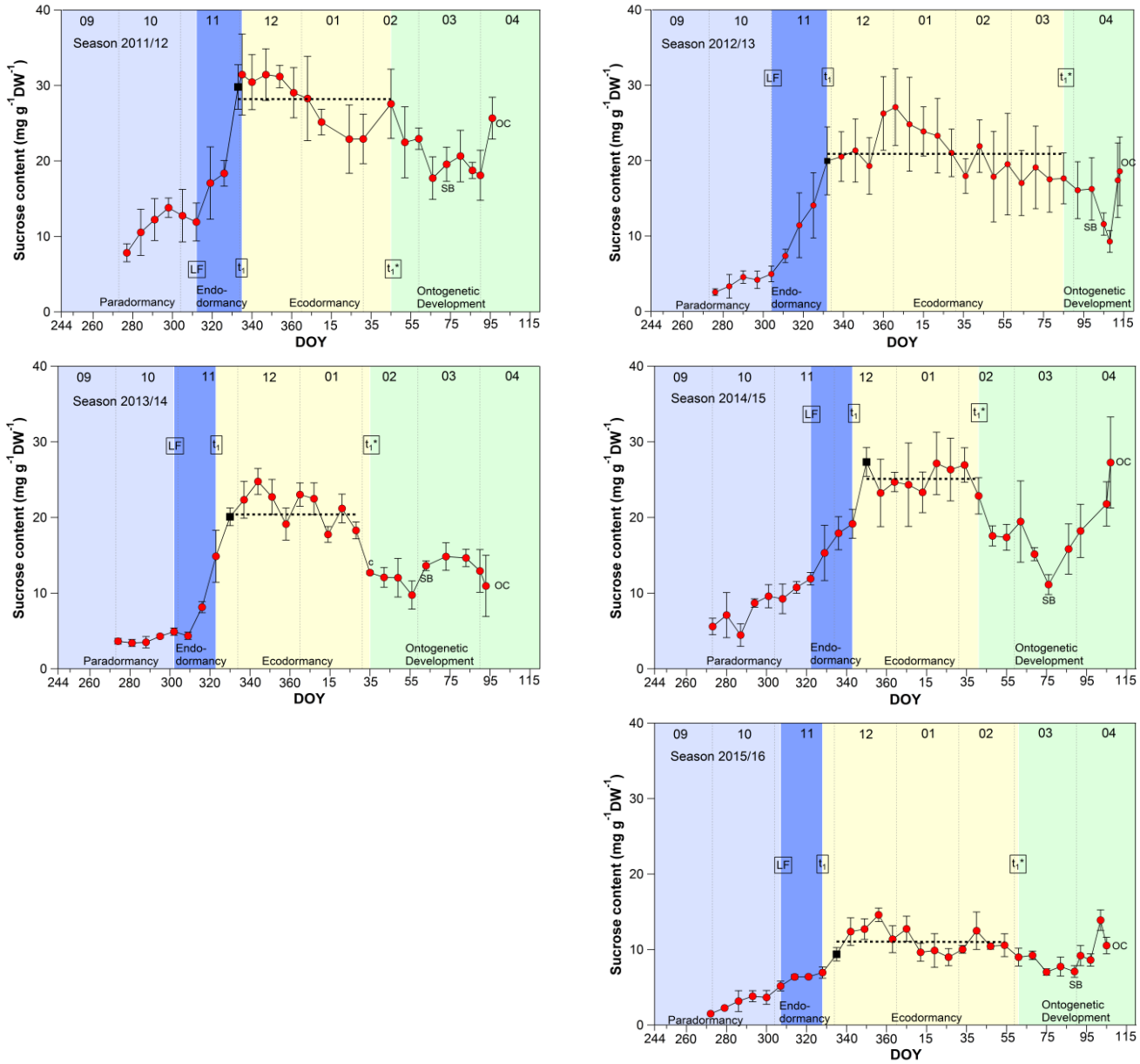

Figure 3: Weekly and development orientated changes of sucrose content, 2011/12 - 2015/16. Black squares indicate timing of t_1 according to SUC content. LF: leaf fall, SB: swollen bud, OC: open cluster, t_1: end of endodormancy and t_1*: beginning of ontogenetic development according to the classic approach, DOY: day of year.

Phase	Av. timing of phases	Av. duration / s (d)	T 2011/12	T 2012/13	T 2013/14	T 2014/15	T 2015/16	x	s
LF - t_1	05/11 - 28/11	22.8 / 3.0	4.0	5.8	7.0	2.6	9.4	**5.8**	2.6
t_1 - t_1*	28/11 - 22/02	86.2 / 21.8	1.3	0.4	2.8	2.8	3.7	**2.2**	1.3
t_1* - SB	22/02 - 21/03	26.6 / 7.7	4.8	1.2	5.7	4.1	4.7	**4.1**	1.7
SB - SG	21/03 - 28/03	7.2 / 2.9	8.4	8.8	7.2	6.7	7.4	**7.7**	0.9
SG - GT	28/03 - 03/04	6.0 / 2.5	10.3	16.0	9.5	6.6	12.8	**11.0**	3.5
GT - TC	03/04 - 10/04	6.6 / 3.8	10.0	13.0	7.4	8.3	9.2	**9.6**	2.1
TC - OC	10/04 - 13/04	3.0 / 1.9	5.9	12.9	10.7	13.5	11.4	**10.9**	3.0

Table 1: Average timing, duration and air temperature (T in °C) in the phenophases: endodormancy [leaf fall (LF) - end of endodormancy (t_1)], ecodormancy [t_1 - beginning of ontogenetic development (t_1*)], and during ontogenetic development after t_1* (SB: swollen bud, SG: side green, GT: green tip, TC: tight cluster, OC: open cluster), 2011/12 - 2015/16, x: mean air temperature, s: standard deviation.

Variability of sugar content in cherry buds

The mean total sugar content of 69.1 mg g^{-1} DW^{-1} between first sampling and OC (S1-OC) consisted of 11% fructose (7.3 mg g^{-1} DW^{-1}), 22% sucrose (15.5 mg g^{-1} DW^{-1}) and 67% glucose (46.3 mg g^{-1} DW^{-1}, Table 4). During this period, mean total sugar content in the buds reaches a minimum around leaf fall (Figure 2). Starting from LF, values raised during endodormancy (LF-t_1) and partially in the beginning of ecodormancy (t_1-t_1*), in order to reach a maximum during the latter phase (Table 4). Subsequently, total sugar content decreased until SB. After SB, values raised again until the stages TC or OC (Figure 2).

The seasonal variability of the total sugar content was mainly driven by changes of glucose, because it usually supplies the energy for metabolic processes in the buds. In contrast to this, fructose showed relatively constant values during both dormant phases and started to rise after SB with always significant higher values at the stage GT. Thus, the highest sucrose, glucose and total sugar content was found during ecodormancy (t_1-t_1*) and for fructose during the ontogenetic development (t_1*-OC, Table 4).

Sucrose was the only sugar component which strongly rose during endodormancy phase and stayed nearly constant during ecodormancy phase (Figure 3). With the beginning of ontogenetic development, it decreased nearly until SB and subsequently rose again until TC or OC. In all years the maximum sucrose content was observed during ecodormancy phase, ranging between 11.1 mg g^{-1} DW^{-1} (2015/16) and 27.4 mg g^{-1} DW^{-1} (2011/12, Table 5). It stepwise increased from the end of paradormancy (5.9 mg g^{-1} DW^{-1}) to endodormancy (12.4 mg g^{-1} DW^{-1}) until ecodormancy (21.0 mg g^{-1} DW^{-1}).

Season	S1-LF	LF - t_1	t_1 - t_1*	t_1*-OC	$ABA_{plateau}$
2011/12	1.26^a / 0.11	6.64^c / 0.22	6.73^c / 0.33	5.90^b / 0.20	10.1 / 0.29
2012/13	3.38^b / 0.16	4.78^c / 0.19	3.21^b / 0.27	3.08^a / 0.34	5.2 / 0.37
2013/14	3.47^c / 0.26	4.03^d / 0.67	2.43^b / 0.19	2.38^a / 0.13	4.6 / 0.15
2014/15	5.57^b / 0.31	7.61^c / 0.32	5.87^b / 0.13	5.66^a / 0.11	7.8 / 0.55
2015/16	3.33^b / 0.24	5.21^c / 0.20	3.83^b / 0.11	3.65^a / 0.11	5.2 / 0.23
x	**3.40**	**5.65**	**4.41**	**4.13**	**-**
s	1.52	1.45	1.82	1.57	-

Table 2: ABA content (µg g^{-1} DW^{-1}) of 'Summit' sweet cherry buds during the end of paradormancy (S1-LF), endo- (LF-t_1), ecodormancy (t_1-t_1*) and ontogenetic development (t_1*-OC), 2011/12 - 2015/16, given are means/standard deviations, different letters indicate significant changes (Tukey HSD, n=4, p≤0.05), $ABA_{plateau}$ gives the highest mean ABA content in each season (see dashed line in Fig. 1), S1: first sampling date, OC: open cluster, x: mean, s: standard deviation.

Season	Classic approach		Metabolomic approach			
	t_1 in DOY	C*(t_1) in CP	t_1 (ABA) in DOY	C*(t_1, ABA) in CP	t_1 (SUC) in DOY	C*(t_1, SUC) in CP
2011/12	335	40.7	354	55.1	335	40.7
2012/13	332	41.0	332	41.0	332	41.0
2013/14	323	38.0	323	38.0	330	42.8
2014/15	343	38.9	336	32.1	350	44.3
2015/16	328	40.7	342	52.4	335	46.8
x (date)	**332.2** (28/11)	**39.9**	**337.4** (03/12)	**43.7**	**336.4** (02/12)	**43.1**
s	7.5	1.3	11.6	9.7	7.9	2.5

Table 3: Dates of endodormancy release (t_1) and chilling requirement (C*) of 'Summit' sweet cherries, 2011/12 - 2015/16 according to experimental studies with twigs (classic approach) and derived from changes in abscisic acid (ABA) and sucrose (SUC) content (metabolomic approach), x: mean, s: standard deviation, CP: chill portion, DOY: day of year.

Component	S1-LF	LF - t_1	t_1 - t_1*	t_1*-OC	S1-OC
Fructose	4.6 / 1.61	3.7 / 1.42	6.8 / 0.33	11.7 / 2.16	7.3 / 1.35
Sucrose	5.9 / 3.63	12.4 / 5.76	21.0 / 6.28	15.1 / 4.51	15.5 / 4.77
Glucose	32.7 / 8.50	31.3 / 9.91	55.7 / 6.72	48.4 / 8.55	46.3 / 6.71
Total sugar	43.3 / 11.6	47.4/ 16.0	83.4 / 12.6	75.2 / 14.4	69.1 / 11.59

Table 4: Mean sugar content (mg g^{-1} DW^{-1}) of 'Summit' sweet cherry buds in the seasons 2011/12 - 2015/16, during para- (S1-LF) endo- (LF-t_1), ecodormancy (t_1-t_1*), ontogenetic development (t_1*-OC) and for the whole period (S1-OC), given are means/standard deviations, S1: first sampling date, OC: open cluster

Season	S1-LF	LF - t_1	t_1 - t_1*	t_1*-OC	SUC_{max}
2011/12	11.4^a / 2.08	20.2^b / 3.85	27.4^c / 3.48	20.6^b / 2.19	31.5
2012/13	3.7^a / 0.72	11.6^b / 2.90	21.1^c / 4.06	14.9^b / 2.88	27.1
2013/14	3.7^a / 0.18	8.1^b / 1.17	20.4^d / 0.45	12.6^c / 0.47	24.8
2014/15	7.9^a / 0.84	16.1^b / 1.14	25.1^d / 2.62	18.2^c / 2.27	27.3
2015/16	3.0^a / 0.51	6.2^b / 0.40	11.1^d / 0.98	9.2^c / 0.49	14.6
x	**5.9**	**12.4**	**21.0**	**15.1**	**-**
s	3.63	5.76	6.28	4.51	-

Table 5: Sucrose content (mg g^{-1} DW^{-1}) of 'Summit' sweet cherry buds during end of paradormancy (S1-LF), endo- (LF-t_1), ecodormancy (t_1-t_1*) and ontogenetic development (t_1*-OC), 2011/12 - 2015/16, given are means/standard deviations, different letters indicate significant changes (Tukey HSD, n=4, p≤0.05), SUC_{max} gives the absolutely highest sucrose content during ecodormancy in each season, S1: first sampling date, OC: open cluster, x: mean, s: standard deviation

Interestingly, the high sucrose content during ecodormancy in the seasons 2011/12 (27.4 mg g^{-1} DW^{-1}) and 2014/15 (25.1 mg g^{-1} DW^{-1}) corresponded with high ABA contents in the same seasons (6.73 µg g^{-1} DW^{-1} and 5.87 µg g^{-1} DW^{-1}).

The stable level of sucrose content during ecodormancy phase (dashed line Figure 3) allowed us to use this metabolite to identify the generally unknown dates of t_1 and t_1*. If one assumes, that the first sampling date with a constantly high sucrose content during ecodormancy marks the timing of t_1, the mean date of endodormancy release for 'Summit' would be on 2 December, if C* reaches 43 CP (Table 3). This result was very close to the value which was previously derived from our classic approach. On the other hand, the beginning of ontogenetic development (t_1*) would be the first date when the stable sucrose content during ecodormancy starts to reduce, significantly (Figure 3). This always happened around t_1*, previously derived by the classic approach.

Environmental drivers for the sucrose content

If one assumes that sucrose is a cryoprotective compound which protects buds to freeze during winter, the maximum sucrose content during ecodormancy is plausible and matches the coldest winter period (Table 1). For this reason the relationship between mean air temperature and sucrose content was further investigated (Figure 4). The strongest linear correlation coefficient ($r=-0.90$, $n=20$, $p<0.05$) was found between the sucrose content during ecodormancy and the average minimum temperature during endodormancy, where the strongest increase of sucrose took place (Figure 3). The correlation coefficient between sucrose content during ecodormancy and mean air temperature ($r=-0.86$) or maximum temperature during endodormancy ($r=-0.78$) was lower, which highlights the relevance of sucrose as a cryoprotective compound.

Discussion

Role of ABA and sugars during dormancy

The increase of ABA content and the transient plateau/peak is consistent with findings for poplar, which report an upregulation of critical enzymes (NCED3, ABA1, and ABA2) for ABA biosynthesis at 3 to 4 weeks of short days [9]. In grape [15], bud dormancy is also induced by the short day photoperiod and both the ABA content and the expression of ABA biosynthesis genes (VvNCED1 and VvNCED2) increased during the induction and maintenance of endodormancy. ABA inhibits respiration in non-dormant buds and down-regulates the expression of respiration-related genes such as VvaNAD, VvCYTC and VvCOX6, thus providing a molecular mechanism by which ABA induces bud endodormancy. Because the short day photoperiod induces bud endodormancy in grapevines, the up-regulation of the ABA biosynthesis genes VvNCED1 and VvNCED2 by the short day photoperiod is consistent with the putative role of ABA in the induction and maintenance of endodormancy. The elevated ABA content during ecodormancy could be also the reason for preventing growth and development of small bud tissues during early stages of development, as assumed for seed germination related to embryogenesis [16].

Sugars are providers of carbon and energy and fulfill an important role in coordination with hormonal signalling pathways and controlling various plant physiological processes [17]. Distinct glucose, sucrose, and fructose signalling pathways can be discerned. These signalling pathways might be strongly influenced by the activities of sucrose splitting enzymes (vacuolar, cell wall and neutral invertases, sucrose synthase) since they have strong impact on sucrose to hexose ratios, which are an important parameter in plant responses to the environment, especially under stress. Next to growth and stress responses, it can be expected that sugar signalling is of great importance in flowering time control. This major developmental transition directly affects yield and its exact timing is essential for plant fitness. Recent findings suggest that both subcellular compartmentation and route of sucrolysis are important for plant development, growth, and yield. Enzymes of sucrose metabolism (invertase, hexokinase, fructokinase, sucrose synthase, sucrose-6-phosphate synthase) are not produced from single genes, but from paralog families in plant genomes [18]. It is important to note that it is the differential localisation of both the sugars themselves as well as the sugar metabolizing enzymes, that ultimately led to sugar signalling, and the authors conclude that a combination of subcellular complexity and gene duplication/sub-functionalization gave rise to sugar signalling as a regulatory mechanism in plant cells. Since carbon metabolism occurs simultaneously in different organelles, different sensors may be required under specific conditions or circumstances. Different sucrolytic enzymes might be therefore important for channelling carbon into different metabolic routes, and they further postulate that sugar signals are specific for each paralog/pathway. It was shown by Bonhomme et al. [19] that floral peach buds - that have come out of endodormancy - have a high capacity to synthesize ATP, which is involved in many metabolic pathways. This indicates an important role of soluble carbohydrates in providing carbon and energy required for the biosynthetic and respiratory metabolism.

Sucrose, as well as glucose and fructose accumulations during winter in plants have often been reported and appear to be related to their properties of cryoprotection and osmotic regulation in the control of bud development [20-22]. Our study clearly confirmed the cryoprotective role of sucrose during ecodormancy in sweet cherry buds. Additionally, it was shown that the annual sucrose content in cherry buds strongly depends on minimum temperatures during endodormancy, where the sucrose content strongly increased. Spring brings warmer and longer days, and the carbohydrate content generally decreases together with bud burst. This supports remobilization of stored resources for the plant to support leaf and stem production, independently of an external supply of nutrients during the active period of growth at flushing time [20]. However, reports that have been described the relationship between bud growth and carbon metabolism in fruit trees are scarce [11]. In addition to the modification in sugar levels, the onset of bud development is linked with variations in almost all metabolic pathways. ABA, auxin and cytokines have been described as major plant growth regulators, involved. Autumn accumulation of soluble sugars (mainly sucrose) is considered a determinant factor for cold tolerance or winter survival and low(er) concentration(s) of accumulated carbohydrates are associated with poor winter survival and reduced spring regrowth. Ito et al. [11] postulate that higher activities of sugar catabolizing enzymes should enhance the capacity of buds to attract assimilates, thereby stimulates bud growth.

The increase of the total sugar content, as well as glucose and sucrose indicate that cherry buds importing/synthesise these carbohydrates until LF/t_1. Accumulation of sugars in buds of other species during autumn and early winter followed similar patterns [22]. The high and stable sucrose content was likely a perfect indicator for the description of the ecodormancy phase, which allows to derive the timing of t_1 and t_1*.

The decline in total, glucose and sucrose content after t_1* mark the resumption of bud metabolic activity. This pre-growth activation has also been observed in other species and was correlated with increased

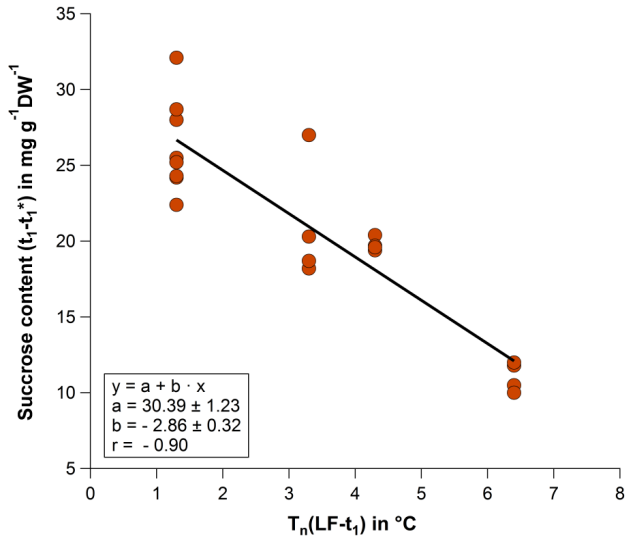

Figure 4: Relationship between mean minimum temperature (T_n) during endodormancy (LF and t_1) and sucrose content during ecodormancy (t_1-t_1*) for all 4 replications (trees) in 5 years (2011/12 - 2015/16).

sugar metabolism [23]. The reactivation of sugar metabolism in buds precedes the start of flower morphogenesis, which has high energy requirements. The drop of sucrose and glucose content approximately at t_1*, but comparatively the significant increase in fructose content at GT, indicates a rapid onward metabolism of hexoses. Maurel et al. [23] demonstrated in *Prunus persica* that hexoses, or at least glucose, may play a key role in triggering the onset of bud break in peach trees. This conclusion is consistent with many reports describing the ability of soluble carbohydrates (glucose and sucrose) to regulate a variety of physiological processes, including induction of flowering. Nevertheless, we agree with Xin and Browse [10] that further investigations are necessary to fill the gap of knowledge of the precise role and the cross-talk of sucrose and hexoses during cold acclimatisation, especially in fruit trees during winter rest and plant development in spring. For deeper and comprehensive understanding the complex of metabolism and to identify the most relevant components during the different phases of dormancy integrative, multidisciplinary approaches using tools from transcriptomics in conjunction with metabolomics and biochemical analysis, including different compartments, from plant tissue to plant organ, are necessary.

Conclusions

In this study the annual variability of ABA and sucrose, glucose and fructose in 5 seasons was presented. Already these metabolites allowed a deeper understanding of dormancy regulation in sweet cherry buds. Following highlights can be summarised:

During endodormancy, ABA content in cherry buds reaches a maximum (plateau), which was maintained for 3-6 weeks. The significant reduction of the ABA content after this period was nearly in agreement with the date of endodormancy release of 'Summit' trees.

Minimum temperature during endodormancy regulated the increase of sucrose up to a maximum value, which nearly stayed constant during ecodormancy. This highlights the cryoprotective role of sucrose during the period with the lowest temperatures. The constant level of sucrose during ecodormancy allowed to derive the timing of t_1 and t_1* for cherry trees.

These results show, the use of selected metabolites allow identifying the unknown phenological phases during winter rest of trees, which are important to improve classical phenological model approaches.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) in the project "Progress in Phenological Modelling on the Basis of Metabolomic Approaches" by the grants (CH 228/5-1, RA 1008/9-1). We are very thankful to Mrs. Susanne Moryson and Mr. Stefan Heider who supported our experimental works at Humboldt-University.

References

1. Chmielewski FM, Götz KP (2017) Identification and timing of dormant and ontogenetic phase for sweet cherries in Northeast Germany for modelling purposes. J Hortic 4: 205.
2. Alburquerque N, García-Montiel F, Carrillo A, Burgos L, (2008) Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. Environmental and Experimental Botany 64: 162-170.
3. Basler D, Körner C (2012) Photoperiod sensitivity of bud burst in 14 temperate forest tree species. Agricultural and Forest Meteorology 165: 73-81.
4. Laube J, Sparks TH, Estrella N, Höfler J, Ankerst DP, et al. (2014) Chilling outweighs photoperiod in preventing precocious spring development. Global Change Biology 20: 170-182.
5. Götz KP, Chmielewski FM, Homann T, Huschek G, Matzneller P, et al. (2014) Seasonal changes of physiological parameters in sweet cherry (*Prunus avium* L.) buds. Scientia Horticulturae 172: 183-190.
6. Chmielewski FM, Götz KP (2016) Performance of models for the beginning of sweet cherry blossom under current and changed climate conditions. Agricultural and Forest Meteorology 218-219: 85-91.
7. Chuine I, Bonhomme M, Legave JM, Garcia De Cortazar Atauri I, Charrier G, et al. (2016) Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. Global Change Biology 22: 3444-3460.
8. Perry TO (1971) Dormancy of trees in winter. Science 171: 29-36.
9. Ruttink T, Arend M, Morreel K, Strome V, Rombauts S, et al. (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. The Plant Cell 19: 2370-2390.
10. Xin Z, Browse J (2000) Cold comfort farm: the acclimation of plants to freezing temperatures. Plant, Cell and Environment 23: 893-902.
11. Ito A, Hayama H, Kashimura Y (2002) Sugar metabolism in buds during flower bud formation: a comparison of two Japanese pear [Pyrus pyrifolia (Burm.) Nak.] cultivars possessing different flowering habits. Scientia Horticulturae 96: 163-175.
12. Durgbanshi A, Arbona V, Pozo O, Miersch O, Sancho JV, et al. (2005) Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. Journal of Agricultural and Food Chemistry 53: 8437-8442.
13. Hou S, Zhu J, Ding M, Ly G (2008) Simulataneous determination of gibberellic acid by solid-phase extraction and liquid chromatography-electrospray tandem mass spectrometry. Talanta 76: 798-802.
14. Ma Z, Ge L, Lee ASY, Yong JWH, Tan SN, et al. (2008) Simultaneous analysis of different classes of phytohormones in coconut (*Cocos nucifera* L.) water using high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry after solid-phase extraction. Analytica Chimica Acta 610: 274-281.
15. Parada F, Noriega X, Dantas D, Bressan-Smith R (2016) Differences in respiration between dormant and non-dormant buds suggest the involvement of ABA in the development of endodormancy in grapevines. Journal of Plant Physiology 201: 71-78.
16. Zeevaart JAD, Creelman RA (1988) Metobolism and physiology of abscisic acid. Annual review of plant physiology and plant molecular 39: 439-473.
17. Bolouri Moghaddam MR, Van den Ende W (2013) Sugars, the clock and transition to flowering. Frontiers in Plant Science 4: 1-6.
18. Thiessen A, Padillia-Chacon D (2013) Subcellular compartmentation of sugar signalling: links among carbon cellular status, route of sucrolysis, sink-source allocation, and metabolic partitioning. Frontiers in Plant Science 3: 1-6.

19. Bonhomme M, Rageau R, Gendraud M (2000) Influence of temperature on the dynamics of ATP, ADP and non-adenylic triphosphate nucleotides in vegetative and floral peach buds during dormancy. Tree Physiology 20: 615-621.

20. Jouve L, Jacques D, Douglas GC, Hoffmann L, Hausmann JF (2007) Biochemical characterization of early and late bud flushing in common ash (*Fraxinus excelsior* L.). Plant Science 172: 962-969.

21. Lipavská H, Svobodá H, Albrechtová J (2000) Annual dynamics of the content of non-structural saccharides in the context of structural development of vegetative buds of Norway spruce. Journal of Plant Physiology 157: 365-373.

22. Richardson AC, Walton EF, Meekings JS, Boldingh HL (2010) Carbohydrate changes in kiwifruit buds during the onset and release from dormancy. Scientia Horticulturae 124: 463-468.

23. Maurel K, Leite GB, Bonhomme M, Guilliot A, Rageau R, et al. (2004) Trophic control of bud break in peach (*Prunus persica*) trees: a possible role of hexoses. Tree Physiology 24: 579-588.