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Identification and Timing of Dormant and Ontogenetic Phase for Sweet Cherries in Northeast Germany for Modelling Purposes

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Abstract

Some important stages of plant development, such as dormancy release and beginning of ontogenetic development, cannot be easily observed so that semi-mechanistic phenological models of spring phases mostly does not accurately reflect the control of these physiological stages. For this reason, over 6 years we studied weekly changes of Fresh Weight (FW), Dry Weight (DW), Water (WC), Nitrogen (NC) and Carbon Content (CC) in sweet cherry buds (*Prunus avium* L., cultivar 'Summit'), from October to mid of April (stage 'open cluster', BBCH 56). Together with an experimental study to determine the date of endodormancy release, we were able to define the annual variability and average duration in the timing of para- endo-, and ecodormancy phase for sweet cherries, cultivar 'Summit'. The secession of growth in autumn starts when all leafs has fallen down (BBCH 97) and marks the transition from para-to endodormancy. During endodormancy all investigated parameters reached a constant level which also did not change during ecodormancy. Our results showed that ecodormancy can be a relatively long phase in temperate climates, which must be adequately handled in phenological models. Beginning of ontogenetic development was clearly related to steadily rising water contents in cherry buds, which stayed stable during endo- and ecodormancy. Thus, this study highlights the importance of bud's water content to define dormancy phases as well as beginning of ontogenetic development. The latter one was induced by continuously rising air temperatures above the freezing point. Physiologically, beginning of ontogenetic development is a flexible date which occurred on average 26 days before bud swelling was observed.

Keywords: *Prunus avium* L.; 'Summit'; Dormancy; Ontogenetic development; Physiological parameters; Phenological modelling

Introduction

After leaf fall in autumn, deciduous trees in mid- and high latitudes enter into a long period of rest during winter, until growth and development restarts in spring. According to Lang et al. [1] dormancy is divided into 3 phases: para-, endo- and ecodormancy (for further definitions [2]). During endo- and ecodormancy, no visible changes on the tree occur, which complicates the detection and subdivision of these 'hidden' phases. Thus, dormancy is frequently a black box in semi-mechanistic phenology models [3]. The first visible sign of biological activity after winter rest is bud swelling (SB). In phenology, bud break, beginning of leaf unfolding/flowering are usually the first observed and registered stages, which can be used for modelling purposes [4]. This makes phenological modelling to a challenge, because in semi-mechanistic models nearly all model parameters must be parameterised to these observations. There is usually no precise information of the date of endodormancy release (t_1) or the beginning of ontogenetic development (t_1*), later one must start some weeks before bud swelling is observed, in order to initiate this process.

It is common that endodormancy release is described by chilling and the ontogenetic development by forcing models (2-phase models), which are well presented in Chuine et al. [3]. There are different assumptions to link both phases by sequential [5-7], parallel [6-9] or alternating [10,11] approaches. Sequential models assume that plants cannot response to forcing temperatures until the chilling requirement is fulfilled. Theoretically, these models can be easily applied if the chilling requirement of the plant is known. In parallel models chilling and forcing accumulation may act simultaneously and in the alternating model, chilling units can compensate for forcing units, expressed by a negative exponential relationship in the model.

Most phenological models are driven only by air temperature, some of them consider photoperiodic constrains [12-14] or other parameters, such as radiation, precipitation [15] or air humidity [16].

In recent decades many efforts have been made to determine the chilling requirement of trees (C*). The use of 2-phase semi-mechanistic models to derive reliable values for C* or t_1 is limited, because the parameter estimates strongly depend on the equations which are used to calculate the chilling and forcing requirement. Additionally, the linkage between the chilling and forcing phase influences the results. Without any knowledge of the real date for t_1, the optimised model parameters cannot be validated. For this reason, these models cannot be used to calculate the timing of phenological events for distinctly warmer climate conditions [17]. Modelling studies [18-21] and some observations [22,23] showed that rising temperatures could lead to a later date of endodormancy release, which is modified by the climatic region and the species specific chilling requirement [24].

A further modelling approach to estimate t_1 or C* was introduced by Luedeling and Gassner [25] and is based on partial least squares regression [26]. Here, daily air temperatures from August (previous year) until July were related to phenological data (leafing or bloom dates), in order to study the temporally different effects of temperatures on plant development [27,28]. PLS-regression indicates that an earlier flowering is related to lower temperatures during dormancy phase (positive PLS-coefficients) and to higher temperatures after dormancy release (negative PLS-coefficients). This method looks graphic, but

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gives no guarantee that the statistically derived parameters for t_1 and C* are physiologically correct. Over and above this, the method does not allow to distinguish between the endo- and ecodormancy phase.

Classical, experimental studies to determine t_1 are rather promising than any modelling approaches. Unfortunately, these experiments are not standardised and focused from bud's meristem, one-node-cuttings to complete shoots and whole trees [2,29,30].

Frequently, twigs from naturally growing trees are taken to observe their development under controlled environmental conditions, which are beneficial for its development [16,31-34]. If twigs develop, after a certain time of forcing conditions, it can be assumed that the chilling amount, which the tree received outside, was sufficient for endodormancy release. The mean time to bud break or the percentage of bud break in a given time interval can be a hint for the depth of dormancy.

A further approach was focussed on changes of flower primordia fresh weight in order to estimate t_1 for apricot (*Prunis armeniaca* L., [17,35,36]) in South France. According to these studies t_1 was related to the date when fresh weight (FW) increased between two successive sampling dates by 15%.

Compared to modelling approaches, experimental studies show a clear physiological response of the samplings to forcing temperatures, and thus are the right method to derive reliable values for t_1 and C*.

A second important parameter in phenological models is the beginning of ontogenetic development (t_1*), which is a fixed date in pure forcing (F)-models, were forcing accumulation starts. In sequential chilling/forcing (CF-) models the accumulation of forcing rates only can start if endodormancy (t_1) is released [5].

In subtropical regions, where the spring is warm and early starts, forcing frequently follows endodormancy release directly, because in these regions beneficial temperatures for plant development are much more frequent than in temperate and boreal climates, so that the ecodormancy phase is very short or even absent. In these climates, sufficient chilling amounts are rather the challenge than the occurrence of beneficial forcing temperatures [18]. However, in temperate and boreal climates the duration of ecodormancy can be very long, mainly if the chilling requirement of the tree is low and fulfilled until the end of the year. But in temperate climates, the annual variability of air temperature frequently leads to mild spells during winter, which do not have the same effect on plant development as similar temperatures in the beginning of spring. For this reason CF-models usually show the tendency to very late dates of t_1, in order to avoid the accumulation of higher temperatures in the beginning or mid of winter [34,37]. This must lead to an overestimation of C*.

Surprisingly, the consideration of photoperiodicity during ecodormancy led to better model results [38,39] and mainly to more realistic dates for t_1 and C* [14,34,37]. Experimental studies showed that some late successional species (e.g. *Abies alba*, *Quercus petraea*, *Tilia cordata, Picea abies, Fagus sylvatica*) have a photoperiodic response [16,32], so that in this case the inclusion of photoperiodicity is physiologically justified. However, a large number of early successional species did not show any photoperiodical sensitivity.

Already Linkosalo et al. [40] claimed that the lower performance of CF-models, compared to pure F-models, could be the result of an inadequately formulated temperature response during the early phase of ecodormancy. For this reason it is absolute necessary to develop phenological models which are process orientated and validated to physiological parameters [17,37,41,42]. In order to do this, we must deeper understand the process of dormancy induction and-release and have to know the plant specific timing of t_1 and t_1*.

For the first time, this study describes the relevant development phases for the sweet cherry cultivar 'Summit' between autumn (previous year) and spring on the basis of basic physiological markers such as bud's Fresh Weight (FW), Dry Weight (DW), Water (WC), Nitrogen (NC) and Carbon Content (CC). Hereby, we were able to show the timing and temporal variability of para-, endo- and ecodormancy phase, the chilling requirement of 'Summit' (C*) and the invisible physiological beginning of ontogenetic development (t_1*) for 6 years. These results will lead to a better understanding of the duration of these relevant phases, which must be realistically integrated in phenological models.

Material and Methods

Experimental site

The study was conducted for 6 seasons (2011/12-2016/17) at the experimental sweet cherry orchard at Berlin-Dahlem (52.47°N, 13.30°E, h=51 m). The orchard (980 m^2) comprises 80 cherry trees of the cultivars 'Summit', 'Regina' and 'Karina', growing in 8 rows with 10 trees each, aligned in N-S direction. 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 prevailing soil type is parabrown soil with weak marks of pale soil, FAO-Classification: Albic Luvisol. It is a silty to medium-loamy sand (surface soil) and silty-loamy sand to sandy clayey loam (sule-soil). The long-term average annual air temperature and precipitation (1981-2010) are 9.9°C and 562 mm, respectively.

Sampling of twigs

In order to determine the date of endodormancy release experimentally, 2 multi-branched twigs from selected 'Summit' trees (length 20-30 cm) were cut off weekly in November and December. After cutting, twigs were 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. Buds were observed daily to determine the time of bud break and beginning of blossom. When twigs started to bloom under controlled conditions we had the indication that the chilling requirement at sampling was fulfilled in the orchard. We assumed that the chilling requirement was sufficient if 3-4 flowers per twig did completely open (BBCH 60), on the first and the following samplings. Afterwards, we calculated the state of chilling $S_c(t)$ in chill portions (CP) from 1 September until the sampling date for the twigs which started to bloom at first.

Sampling of buds

In order to analyse FW, DW, WC, NC and CC of the cherry buds, 3 flower-bud cluster per tree were taken each week, randomly over 4 trees (replications) during the cold season from beginning of October until April. The single leaf bud from each of the cluster was previously removed. After beginning of ontogenetic development, sampling was done development-related at 'bud swelling' (SB), 'side green' (SG), 'green tip' (GT), 'tight cluster' (TC) and 'open cluster' (OC). The stages picking ripeness of fruit (PR, BBCH 87), complete leaf fall (LF, BBCH 97), and beginning of cherry blossom (BB, BBCH 60) were additionally

recorded. After cutting, clusters were immediately placed in plastic bags in a polystyrene box on ice. Subsequently, the mean FW per bud of each bud-cluster were determined. After drying the buds at 60°C for 48 hrs, DW and WC were determined. In order to analyse NC and CC, buds were ground in a ball mill (Retsch M1, Haan, Germany). NC and CC were measured using an elemental analyser (vario MAX CNS, Elementar-Analysensysteme GmbH, Hanau, Germany).

Statistical analysis

For all sampling dates, mean values and standard deviations of WC, FW, DW, NC, CC (Table 1 and Tables S1-S5) based on 4 replications. Significant parameter changes within a season and between the sampling dates were tested with the Tukey-HSD-test (n=4, $p \leq 0.05$, Tables S1-S5). In order to test significant differences for the mean parameter values over 6 seasons the Scheffé-test were used, because in this case the sample size was variable (n=16-24, Table 1). Statistical relationships between physiological parameters and meteorological conditions were presented by linear and non-linear regression functions.

Results

Identification of paradormancy period for 'Summit'

Each year shortly after picking ripeness of cherries, in the end of June (x=176.6 DOY, s=5.2 d, 2012-2016), the formation of new bud clusters started at the tree. Afterwards, the buds developed and grew until leaf fall (LF) in autumn. Sinking temperatures and shorter days at the end of this period induced LF, which was on average completed on 6 November (310 DOY, s=7.3 d, Table 2). Since flower buds are unable to bloom during this period (PR-LF), we assigned this period to paradormancy, which lasts on average more than 4 months (133 d, s=11.1 d, 2012-2016, Table 3). During this time FW, DW slightly increased while WC already reached a constant value (Figure 1). Since analytic data were only available from beginning of October, parameters changes at the end of this phase were relatively weak.

Identification of endodormancy period for 'Summit'

After completing leaf fall, the tree stopped measurable effects of bud growth. This is exemplarily visible in (Figure 1). From this date, all investigated physiological parameters reached a constant level (LF-t_1: WC=53.5%, FW=61.9 mg/bud, DW=28.8 mg/bud, NC=1.6%, CC=49.6%, mean of 6 season, Table 1), which did not change significantly in all individual seasons until t_1* (Tables S1-S5).

From our climate chamber experiment we derived the date of endodormancy release (t_1) for all years. Twigs, which faced a sufficient number of chill portions in the orchard, started to bloom under controlled conditions. On average, endodormancy was released on 30 November (333.7 DOY, s=7.6 d, Table 2), ranging between 19 November 2013 (DOY 323) and 9 December 2014 (DOY 343).

Parameter	LF-t_1	t_1-t_1*	t_1*+7d	t_1*+14d	t_1*+21d	SB	SG	GT	TC	OC
WC	53.5^{ab}	52.8^{a}	55.3^{a-c}	56.0^{bc}	**57.1^{c}**	60.9^{d}	66.6^{e}	73.2^{f}	77.2^{g}	78.4^{g}
FW	61.9^{a}	62.2^{a}	64.4^{a}	65.3^{a}	79.7^{a}	76.5^{a}	98.1^{a}	**164.9^{b}**	292.9^{c}	383.9^{d}
DW	28.8^{a}	29.2^{a}	28.7^{a}	28.7^{a}	34.1^{ab}	29.9^{a}	32.7^{ab}	**43.2^{b}**	66.6^{c}	82.6^{d}
NC	1.6^{a}	1.6^{a}	1.7^{a}	1.7^{a}	1.9^{ab}	2.0^{ab}	**2.3^{b}**	2.8^{c}	3.4^{d}	3.6^{d}
CC	49.6^{f}	49.3^{ef}	49.3^{ef}	48.8^{d-f}	49.0^{d-f}	**48.2^{c-e}**	47.9^{cd}	47.5^{bc}	46.6^{ab}	46.2^{a}

Table 1: Mean water content (WC in %), fresh weight (FW in mg/bud), dry weight (DW in mg/bud), nitrogen content (NC in %), carbon content (CC in %) of 'Summit' sweet cherry buds during endo- (LF-t_1), ecodormancy (t_1-t_1*) and ontogenetic development (at t_1*+7d until OC) of 6 seasons 2011/12-2016/17. Different letters indicate significant changes (Scheffé-test, $p \leq 0.05$); bold values indicate the beginning of significant changes.

Stage	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	x in DOY	sin d	Date
PR	-	175	179	170	175	184	**176.6**	5.2	26/06
LF	312	304	302	322	307	313	**310.0**	7.3	06/11
t_1	335	332	323	343	328	341	**333.7**	7.6	30/11
t_1*	45	85	35	41	61	45	**52.0**	18.3	21/02
SB	73	99	63	76	89	66	**77.7**	13.9	19/03
SG	80	105	73	86	92	76	**85.3**	11.8	26/03
GT	86	108	83	92	97	82	**91.3**	10.0	01/04
TC	90	112	90	105	102	90	**98.2**	9.5	08/04
OC	96	113	93	107	105	94	**101.3**	8.2	11/04
BB	105	116	95	111	111	97	**105.8**	8.4	16/04

Table 2: Timing of phenological stages from picking ripeness (PR) until beginning of blossom (BB), LF: leaf fall, t_1: endodormancy release, t_1*: beginning of ontogenetic development, SB: swollen bud, SG: side green, GT: green tip, TC: tight cluster, OC: open cluster, x: mean, s: standard deviation, DOY: day of year.

Phase	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	x in d	s in d
PR-LF	-	129	123	152	132	129	**133.0**	11.1
LF-t_1	23	28	21	21	21	28	**23.7**	3.4
t_1-t_1*	75	118	77	63	98	69	**83.3**	20.7
t_1*-SB	28	14	28	35	28	21	**25.7**	7.2
SB-SG	7	6	10	10	3	10	**7.7**	2.9
SG-GT	6	3	10	6	5	6	**6.0**	2.3
GT-TC	4	4	7	13	5	8	**6.8**	3.4
TC-OC	6	1	3	2	3	4	**3.2**	1.7
OC-BB	9	3	2	4	6	3	**4.5**	2.6

Table 3: Duration (days) of phenological phases, paradormancy (PR-LF), endodormancy (LF-t_1) and ecodormancy (t_1-t_1*). x: mean, s: standard deviation.

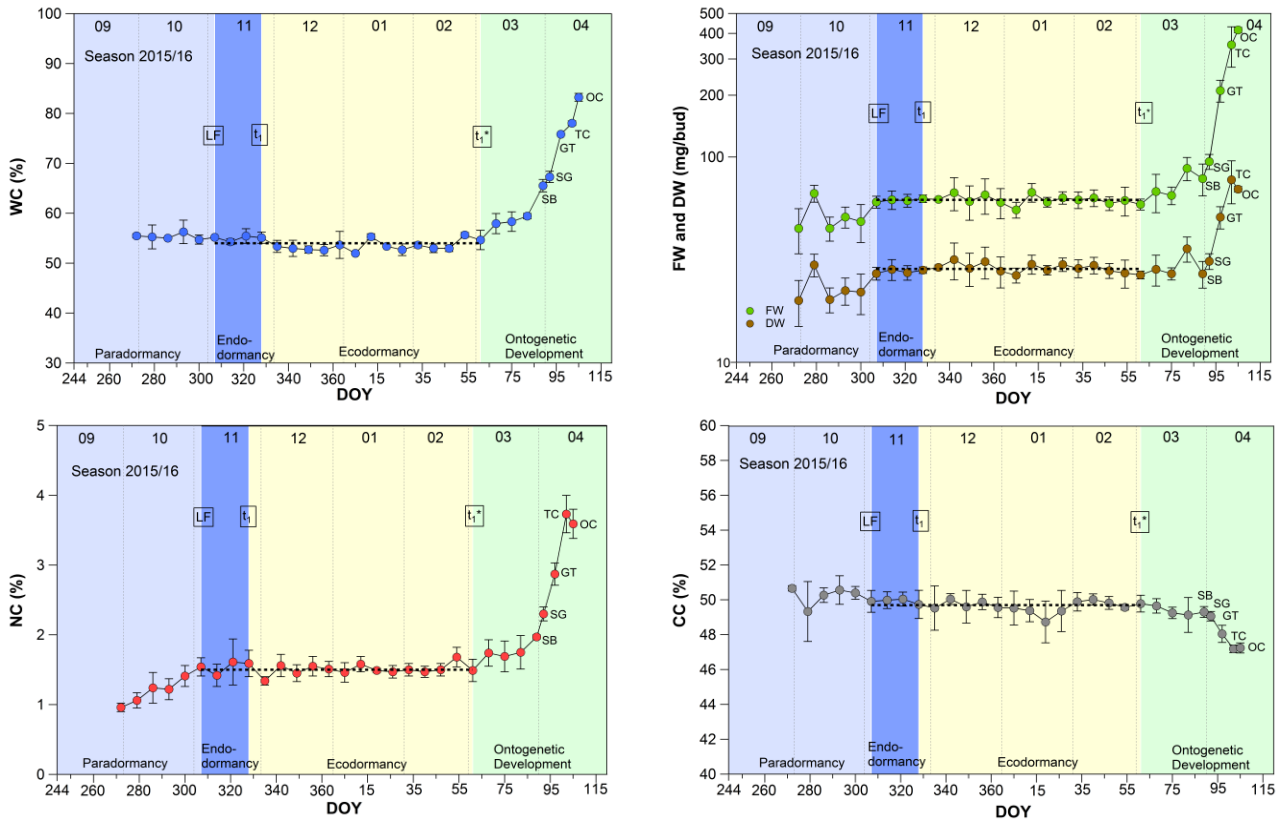

Figure 1: Example for weekly and development orientated (SB, SG, GT, TC, OC) changes of water content (WC), fresh-, dry weight (FW, DW), nitrogen content and carbon content (NC, CC) in 'Summit' sweet cherry buds in the season 2015/16, error bars showing the standard deviation of 4 replications, (Table S1-S5), dashed lines indicate phases with no statistically significant parameter change ($p \leq 0.05$).

The calculated state of chilling $S_c(t)$ from 1 September was relatively constant and reached 23.6 ± 3.3 CP at LF and 40.9 ± 2.9 CP at t_1 (Table 4), so that C* of 'Summit' was about 41 CP. With the identification of t_1 we were able to define the endodormancy period (LF-t_1) which lasts on average 23.7 days and showed a relatively low annual variability between 21 and 28 days (Table 3).

Identification of ecodormancy period for 'Summit'

After the mean date of endodormancy release on 334 DOY (Table 2), low temperatures in the orchard suppressed any growth, so that no physiological response of the buds was visible (Figure 1). All investigated parameters did not significantly change, compared to the endodormancy phase, and stayed constant for nearly 3 months (Figure 1 and Table 1). Altogether, ecodormancy (t_1-t_1*) lasted on average 83.3 days (Table 3). The duration of the phase showed the highest standard deviation, because of the high variability of t_1* (Table 2) which was strongly influenced by the annual course of air temperature.

Identification of ontogenetic development for 'Summit'

The first visible sign of biological activity was a steady rise of the water content in the buds. This was the only parameter which continuously increased from a certain starting date until TC or OC in all seasons (Figure 2 and Table S1). Since liquid water is essential for physiological activity we defined the sampling date, from which a continuous increase of WC was detected, as beginning of ontogenetic development (t_1*). On average, t_1* was observed on 21 February (52.0 DOY, Table 2). SB was detected 2-5 weeks after t_1* (Table 3). Altogether this period lasted 25.7 days. The mean duration of the following developmental phases ranged between 3 and 8 days. The standard deviation of the phenological stages between t_1* and OC reduced continuously (Table 2) and showed that the high variability of t_1* (s=18.3 d) was distinctly compensated during ontogenetic development. This date ranged between 4 February (35 DOY, 2014) and 26 March (85 DOY, 2013) and was related to the annual course of air temperature. The early beginning of ontogenetic development in 2014 and finally the early date of blossom (95 DOY) was the result of mean air temperatures above normal in February (+3.7°C) and in March (+2.9°C, reference period 1981-2010). However, the late dates of t_1* and BB in 2013 (Table 2) were related to an unusually long winter period with negative temperature anomalies in February (-1.4°C) and mainly in March (-5.4°C).

Table 3 additionally highlights that for the climatic conditions at the experimental site, ecodormancy phase (t_1-t_1*) last 3.5-times longer than the endodormancy phase (LF-t_1).

Environmental drivers for parameter changes

In all years the increase of WC in the buds was related to a remarkable rise in air temperature after t_1* (Figure 2). In the seasons 2011/12, 2012/13, 2013/14, 2014/15, 2016/17 temperatures raised from negative values at t_1* to continuous positive temperatures, subsequently. Only in the season 2015/16, the mean air temperature

State of chilling	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	x in CP	s in CP
S_c (LF)	24.0	20.2	20.5	24.7	23.1	29.1	**23.6**	3.3
S_c (t_1)	40.7	41.0	38.0	38.9	40.7	46.3	**40.9**	2.9

Table 4: State of chilling (S_c) at leaf fall (LF) and endodormancy release (t_1), in chill portions (CP), x: mean, s: standard deviation.

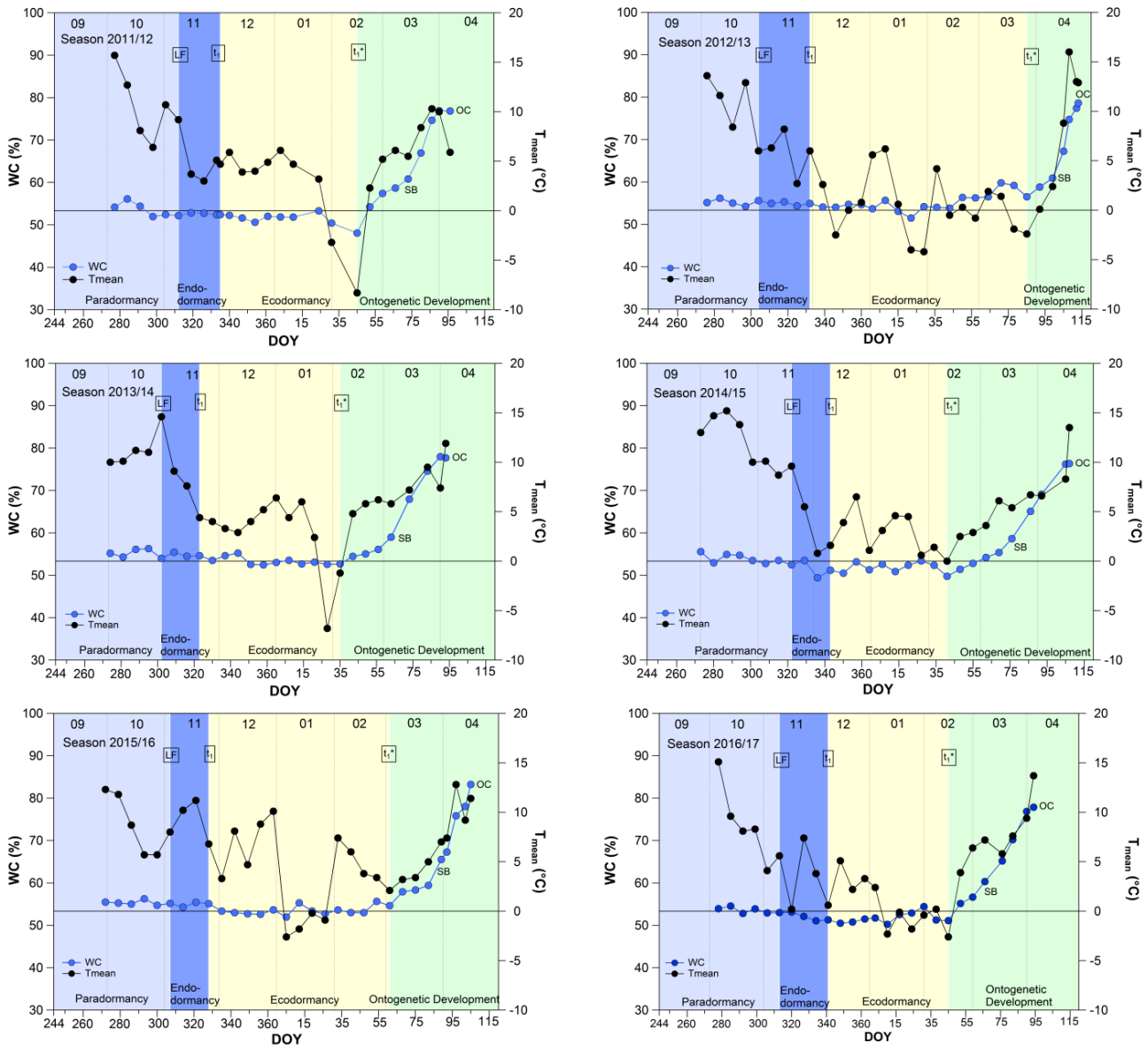

Figure 2: Weekly and development orientated (SB until OC) changes of water content (WC) and mean air temperature (T_{mean}) 7 days before sampling or within phenological phases between SB and OC, 2011/12-2016/17 (abbreviations, Table 2).

had already positive values at t_1*, but also rose permanently after this date.

In 4 out of 6 seasons, WC was significantly higher 7 days after t_1* (t_1*+7, Table S1). On average 3 weeks after t_1* (t_1*+21 d), bud's WC rose significantly from 53% during dormancy phase to about 57% (Table 1). Between SB and OC, it further increased from 61 to nearly 80%. FW and DW did not significantly change between LF and SG with mean values for 6 seasons of 74.3 mg/bud and 30.3 mg/bud, respectively. Following, FW reached nearly the double, quadruple and fivefold and DW the one-and-a-half, double and triple amount at GT, TC and OC (Table 1). NC stayed constant between LF and SB (1.8%) and significantly increased from SG (2.3%) until OC (3.6%). Compared to the NC, CC significantly decreased between SB (48.2%) and OC (46.2%).

Figure 3 shows that the rise of WC and NC in the buds was already forced by daily temperatures above the freezing point. However, the increase of FW and DW was related to higher air temperatures >5°C and >7°C, respectively. This explains the order of significant parameter changes in Tables 1 (mean of 6 seasons), firstly detected by the water content at t_1*+21 d (57.1%), followed by the nitrogen content at SG with 2.3% and the fresh- (164.9 mg/bud) and dry weight (43.2 mg/

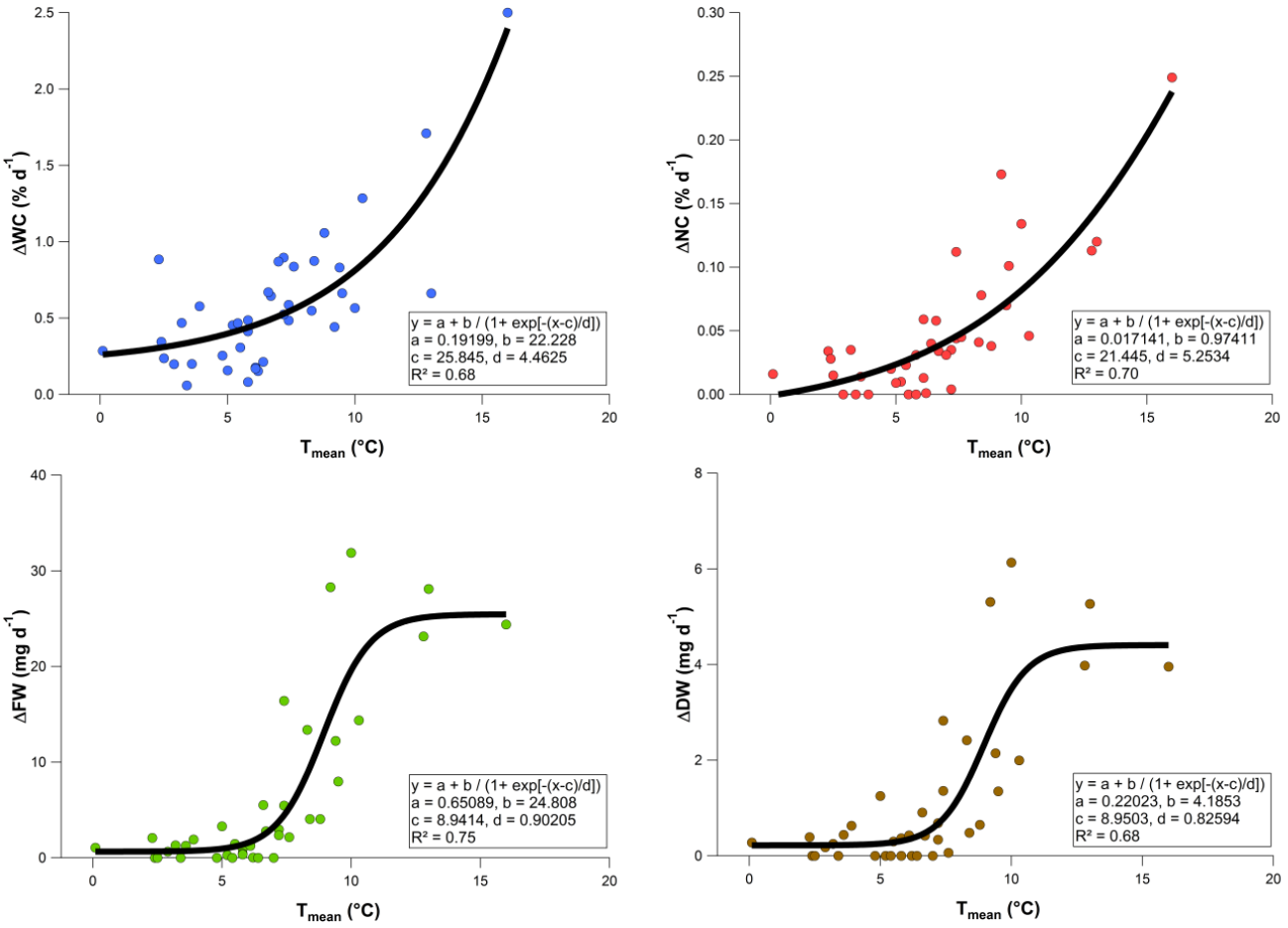

Figure 3: Relationship between mean air temperature (T_{mean}) 7 days before sampling or within phenological phases (SB until TC) and daily changes of water content (ΔWC), nitrogen content (ΔNC), fresh weight (ΔFW) and dry weight (ΔDW) for all samplings between beginning of ontogenetic development and tight cluster, coefficient of determination (R^2) significant at $p \leq 0.05$.

bud), both at GT. For these parameters the statistical relationship to the air temperature was significant with R^2-values between 0.68 and 0.75 ($p \leq 0.05$). However, no significant relationship was found between CC and air temperature.

The annual course of air temperature additionally influenced the timing of endodormancy release and the beginning of bud swelling (Figure 4). Significant negative correlations coefficients ($p \leq 0.05$) indicate that, for the current climate conditions at the experimental site, higher temperatures after LF and after t_1* force the dates of endodormancy release and bud swelling.

Discussion

Physiological interpretation of the results

This study has shown that dormancy period as well as ontogenetic development was strongly related to the WC in the buds, with constantly low values during endo- and ecodormancy (WC=53%). Only after beginning of ontogenetic development it started to rise, up to nearly 80% at OC (Table 1).

Water is the most important polar molecule, both within cells and in their external environment. Water molecules interact strongly with each other and with other charged and polar molecules. These interactions, and the lack of interactions with non-polar molecules (benzene or other hydrocarbons to water break bonds and produces 'holes' or disorganized areas in the structure which are surrounded by areas with tighter structure [43]) are the most important factors in establishing biological structures. Water bound to proteins, cell walls, and other hydrophilic surfaces has important effects on their physiological activity. The biological activity of plant tissues depends on the physiological state of water and changes in the mobility of water are expected to accompany or to precede the physiological changes that occur in buds of woody plants during ecodormancy release [44]. For this reason we found a continuous increase in WC, starting at t_1*. However, 2-5 weeks were necessary to detect first visible changes on the buds (Table 3). This time was shortened by temperatures above normal (Figure 4).

In the search for the mechanism of dormancy control in buds, attention was also drawn to changes in water status during dormancy. It is reported that water in buds went through a significant change during dormancy [45]. Magnetic resonance imaging (MRI) revealed that in dormant buds of apple, blueberry and grape [45], water is mostly bound and it is freed only when chilling accumulation is sufficient for breaking endodormancy. The concept of bound water, originated from observations that a variable fraction of water in both living

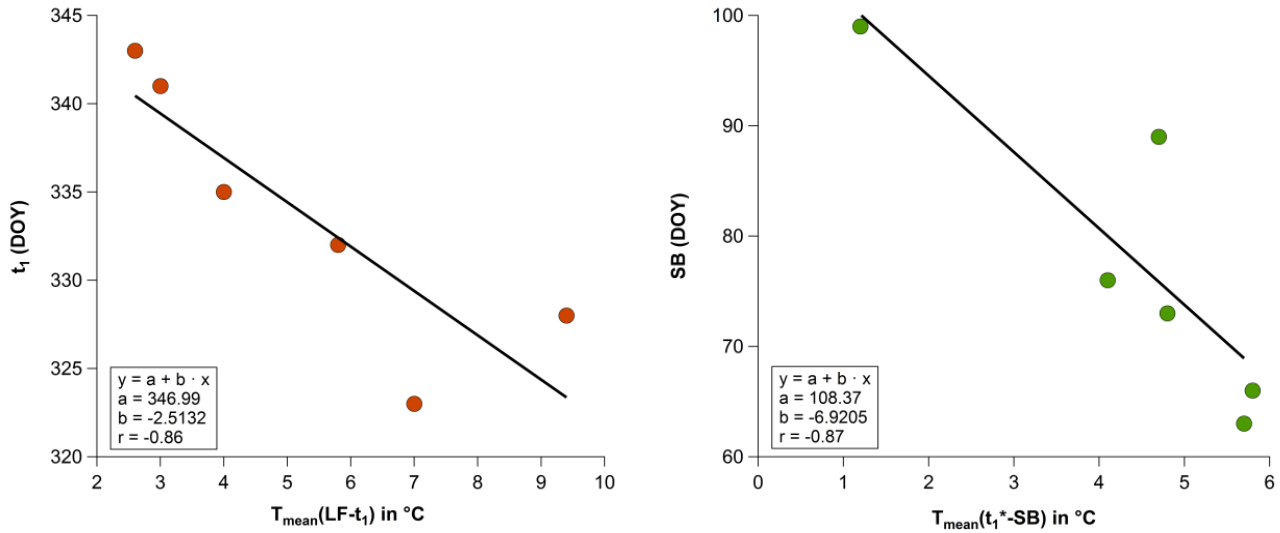

Figure 4: Relationship between mean air temperature during endodormancy phase $T_{mean}(LF\text{-}t_1)$ and date of endodormancy release t_1 and mean air temperature from endodormancy release until SB, $T_{mean}(t_1\text{*-SB})$ and beginning of bud swelling (SB), correlation coefficient (r) significant at $p \leq 0.05$.

and non-living systems has a lower vapour pressure, which remains unfrozen at-20°C to-25°C, does not function as a solvent, and seems to be less physiologically available than bulk water [43]. Maintaining hydrated molecules under extreme dehydrating conditions of low temperatures is a mechanism, developed for survival of the exposed tissues and organs. Keeping the water in a bound condition allows the maintenance of hydration levels at temperatures much lower than the freezing point. It is clear, however, that before growth can be resumed water must be converted into the free-state. This suggestion is backed by the known abundance of dehydrins in dormant tissues. Dehydrins are a hydrophilic family of proteins those are involved in protecting cells against cold conditions [46].

The transition of a dormant meristem to a reproductive one during winter is an irreversible process that involves changes in the pattern of morphogenesis and cell differentiation at the bud apical meristem. The process can be divided into the 'induction phase', involves biochemical modifications which commit the bud to the formation of a reproductive structure, and the 'differentiation phase' during which the tissues of flower organs are formed. The onset of bud development during early and late adaptation to short days, about 2 and 3 weeks, resp., but also during ontogenetic development (t_1*) is linked with variations in almost all metabolic pathways [47,48]. Meristem identity genes and floral organ identity genes must be activated for the floral meristem and floral organ growth and development. As reproductive development commences, increased cell division rates occur in the bud apical meristem. In the dividing cells intense metabolic activity takes place. Concerning proteins, elevated concentrations are present in dividing cells, because of increased synthesis of many enzymes, histones, cyclin depended kinases, as well as structural proteins for daughter cells.

Bud break is preceded by a rapid rise in respiration rate, and gain in the bud's moisture content back up to nearly 80% (Table 1). The increase in respiration rate likely indicates renewed meristem activity (i.e. cell division), and the increase in water content is linked to renewed cell expansion. The outward sign of this spring awakening is the swelling of the buds that culminates in bud break. Because cambium reactivation occurs well before the new leaves will begin supplying assimilates, the energy, nitrogen and carbon requirements of the dividing cells have to be met by the degradation and remobilization of stored nutrients from the permanent structures [49-51].

During winter, tree buds have high levels of cryoprotectant substances that increase their tolerance to cold and freezing temperatures and carbon rich substances, like carbohydrates, which are generally involved as adaptive biochemical factors in osmoprotection during abiotic stress. Spring brings warmer and longer days and the carbon content decreases from the beginning of bud swelling (Table 1). This providing utilization and remobilization of stored resources for the plant to support leaf and stem growth independently of an external supply of nutrients during the active period of growth at flushing time [52].

After changes of WC in the buds, the following increase of the NC at SG can be seen as an indication for stepwise rising sink activity by the initially translocation of essential elements, as well known for N [53]. On this basis, metabolism starts in general to increase and resulted subsequent in markedly assimilate accumulation, like FW and DW, which reflected the strong growth, differentiation and development of different organs of cherry flowers inside the bud, starting at GT (Table 1).

Relevance for phenological modelling

For modelling purposes multi-annual data are absolutely necessary. At the first time, for 6 seasons and in weekly resolution, physiologically relevant parameters were presented in order to divide the bud's dormancy period of 'Summit' into 3 sub-phases, including the beginning of ontogenetic development. We are aware that the transition between the phases is gradual [54], so that the dates presented here for t_1 and t_1* are transitional values.

The low chilling requirement of 41 CP for 'Summit', which is in agreement with the chilling requirement of different cherry cultivars in Spain (30-60 CP [31]) led to a relatively short period of endodormancy (24 d) and a very long phase of ecodormancy (83 d, Table 3). The chilling requirement until LF (24 CP, beginning of endodormancy) and t_1 (41 CP, end of endodormancy) were relatively constant in all years (Table 4), which allows to calculate the endodormancy phase in phenological models by the dynamic approach [55,56].

Our results confirm the fact that in temperate climates a distinct time lag between t_1 and t_1* can exist [17,37]. The date of endodormancy release for 'Summit' in the end of November or beginning of December is in agreement with a European Custom on St. Barbara's Day (4 December). According to this custom, cherry twigs, which are cut on 4 December, should be able to start to bloom under forcing conditions at Christmas (20 days later). In our controlled experiments for dormancy release we found that 'Summit' twigs, which were released from endodormancy, needed 15-20 days until beginning of blossom.

We are absolutely agree with Linkosalo et al. [40] that to date, most phenological models are not able to handle the long period of ecodormancy, which we also found for 'Summit'. Parallel or alternating models try to avoid a too early start of ontogenetic development by the extension of endodormancy far into the ecodormancy phase. Thus, these models overestimate t_1 and C*. Studies by Fu et al. [41] confirmed that the sequential model approach is the most appropriate one to predict bud burst data for warmer climate conditions.

The photothermal-time approach, suggested by Blümel and Chmielewski [13], did successfully regulate the accumulation of forcing temperatures during the long ecodormancy phase and lead to much more realistic model parameters for C*, t_1, T_{BF} (base temperature for forcing accumulation) and F* (forcing requirement) [14,34,37]. However, some species do not show any photoperiodicity during ecodormancy [16,32]. For this reason the handling of ecodormancy in phenological models, must be revised and further improved.

WC in the bud was a simple, but very graphic marker to define t_1*. The constant level of water in the buds during endo- and ecodormancy of 53% (Table 1) gradually increased up to nearly 80% with continuously rising air temperatures above the freezing point in the end of winter. Similar values for the water content during endodormancy were reported by Kaufmann and Blanke [57] for 9 further sweet cherry cultivars, ranging between 49% and 53%. Changes in WC would allow to introduce a new temperature-depended definition of t_1* in phenological models. Depending on the annual temperature course, this date was found on average 52 days before any visible changes at the buds were observed (SB). This time is obvious necessary to start and coordinate all physiological processes in the buds which lead to the time shifted start of growth and development [58].

Changes of the bud's water content could be also a physiologically based marker to detect endodormancy release under forcing conditions in climate chambers. Primarily investigations for 'Summit' (data not presented in this study) showed that under controlled conditions, endodormant buds (23°C day/night, 12 h light) cannot or only slightly increase their WC (<5%) within 15 days after sampling. However, samplings which were released from endodormancy in the orchard were able to increase its WC by 10%, within 15 days and reached at the same time the stage GT. This confirms our findings in the orchard, were the bud's water content also significantly increased between t_1* (53%) and SB (61%) by 8%. Because of the lower temperatures outside, this period lasted on average 26 days (Table 3). Additionally, we found that the Relative Water Content (RWC), which indicates the hydration state of the bud, did not change in dormant buds, but was significantly reduced in buds which were released from endodormancy. This is a sign that the buds started to transpire. This would be an alternative method to estimate endodormancy break dates for a large number of woody species, such as suggested by Chuine et al. [17].

Generally, we are agreeing with Campoy et al. [2], Richardson et al. [42], Basler [37] and Chuine et al. [17] that much more work is necessary to better understand the physiological processes which control spring phenology in order to implement them into models. In this study we presented our results on the basis of classical physiological parameters, which were helpful to better understand the dormancy phase of 'Summit' sweet cherries. Nevertheless, parameters presented in this study, did also not allow separating endodormancy from ecodormancy phase. To overcome this situation multi-year identification of suitable metabolites as abscisic acid, a prominent member of the group of plant hormones, his precursors, carotenoids like violaxanthin and neoxanthin, and different sugars is necessary. First results were already presented in Götz et al. [53].

Summary and Conclusion

This study provided an example how the phases of para-, endo- and ecodormancy can be defined and separated for deciduous trees, such as sweet cherries. It additionally sowed how the beginning of ontogenetic development can be physiologically defined.

We can conclude that during paradormancy growth and development of cherry buds took place until the end of leaf fall. In our case, endodormancy was characterised as a relatively short period without any growth and development. During the relatively long period of ecodormancy no visible growth was observed, however moderate bud development in spells with favourable temperatures could be possible. However, we did not detect any changes of WC, FW, DW, NC and CC in the buds. Only continuously rising air temperatures, which exceed the freezing point in the end of winter, led to rising water contents in the buds and enabled active growth and bud development. This study highlights the importance of the bud water content to define the dormancy phases as well as the beginning of ontogenetic development.

We can list the following highlights of our study:

1. Beginning of endodormancy for 'Summit' sweet cherries at 310 DOY (BBCH 97) was relatively constant at a chilling requirement of C*=24 CP.
2. Endodormancy was released at 334 DOY, when buds accumulated C*=41 CP.
3. Ecodormancy phase for Summit (83 d) lasted 3.5-times longer than endodormancy phase (24 d).
4. During endo- and ecodormancy phase no significant change of water-, nitrogen-, carbon content, fresh- and dry weight was measured.
5. Beginning of ontogenetic development was related to continuously rising water contents in the buds, starting on average 26 days (range 14-35 days) before bud swelling was observed and was induced by continuously rising air temperatures above freezing point.

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