https://doi.org/10.17221/123/2020-HORTSCI

Response of sweet cherry buds and twigs to temperature changes – evaluated by the determination of the degradation and synthesis of sucrose

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Citation: Götz K.-P., Chmielewski F.-M. (2021): Response of sweet cherry buds and twigs to temperature changes – evaluated by the determination of the degradation and synthesis of sucrose. Hort. Sci. (Prague), 48: 149–157.

Abstract: This study was undertaken to determine the degradation and synthesis of sucrose (Suc) in sweet cherry buds and the twig tissue response to a sequence of environmental temperature changes (cold (orchard) – warm (controlled temperature of ~22 °C) – cold (orchard)). The results of two years' (2016, 2017) findings were compared with the buds of trees and the buds of twigs in November/December in northeast Germany. The Suc content in the buds of trees and the buds of twigs under natural conditions was stable. Temperatures of ~22 °C resulted in a significant (Suc) degradation (62%, from 39 to 15 mg/g DW) in the buds of twigs after 21 days (day of the year (DOY) 340). The significant re-synthesis (66%, to 25 mg/g DW after 21 days, DOY 361) in the orchard is noteworthy, and highlights the Suc value as a cryoprotective saccharide. The marked changes in the Suc, glucose, and fructose contents of the twigs exposed to a cold-warm-cold sequence (< DOY 319, DOY 319–340, DOY 340–361), lead to the conclusion that this adaptation is the result of tissue- and cold-specific sucrose invertases/synthases. The effect of low-temperature-active enzymes explains the role of Suc in the buds of trees during the winter rest. When using twigs for plant physiological examinations during the winter rest, results on a metabolite level should be considered when drawing conclusions concerning the overall tree physiology.

Keywords: *Prunus avium* L.; buds; trees; twigs; saccharides; air temperature

Acclimation to low temperatures under natural conditions is initiated by decreasing temperatures with the onset of autumn. Cold acclimation is a very complex process, involving physiological, biochemical, and molecular changes (Castède et al. 2015; Parada et al. 2016; Gholizadeh et al. 2017; Perea-Resa et al. 2017; Miki et al. 2019; Xin, Browse 2000; Stitt, Hurry 2002). Many different cellular processes are involved, including osmotic adaptation by sugars, protection of specific macromolecules during dehydration, an energy and hormone balance, cell wall and membrane modifications, and gene regulation (Xin, Browse 2000; Barrero-Gil, Salinas 2013). Plants vary considerably regarding inherent freezing tolerance before cold acclimation, and also when concerning the potential to acquire freezing tolerance during cold acclimation. Accumulation of low molecular weight carbohydrates (sugars) during cold acclimation is well established for many plants, including Arabidopsis, and the time course for sugar accumulation correlates well with the development of freezing tolerance during cold acclimation (Xin, Browse 2000). Certain types of sugars clearly fulfil specific and different functions, especially in the

Supported by the Deutsche Forschungsgemeinschaft (DFG) in the Project "Profiling as method to identify relevant metabolites for phenological modelling purposes" by the Grant No. CH 228/7-1.

https://doi.org/10.17221/123/2020-HORTSCI

sub-cellular compartments (Zuther et al. 2004; Hoermiller et al. 2017).

Research on *Prunus avium* L. (Chmielewski et al. 2017) has demonstrated that the sucrose in the buds of trees (5 seasons, 2011–2016) was the only saccharide, which strongly increased during the endodormancy phase (leaf fall – end of endodormancy (t_1); T_{mean} = 5.8 ± 2.6 °C), and remained nearly constant throughout the ecodormancy period (t_1 – beginning of ontogenetic development (t_1*)). Because sucrose is considered to be a cryoprotective compound which protects buds exposed to freezing conditions during winter, it is not surprising that the maximum sucrose content occurs during ecodormancy, which is considered to be the coldest period, with T_{mean} of 2.2 ± 1.3 °C. With the beginning of the ontogenetic development, the sucrose content decreased markedly until the 'swollen bud' stage, and the T_{mean} concurrently increased during this developmental phase to 4.1 ± 1.7 °C. The strongest linear correlation (r = 0.97, P < 0.05) was found for the sucrose content during ecodormancy and the average minimum temperature was found during endodormancy, at which point the strongest increase of sucrose took place. The correlation coefficient between the sucrose content during ecodormancy and the mean air temperature (r = –0.93), or the maximum temperature during endodormancy (r = –0.84), was lower, emphasising the relevance of sucrose as a cryoprotective compound.

Experimental research focused on mechanisms concerning the whole tree phenology/physiology is generally possible (Chmielewski, Götz 2016), but may encounter methodological constraints since *in situ* manipulations for all issues are not practicable. Alternatively, twigs are used for phenological observations, including growth and development (bud burst, leaf unfolding; Fu et al. 2013), especially in relation to the impact of abiotic factors (chilling/heat requirement, light quantity/quality (Gariglio et al. 2006; Levizou, Manetas 2007; González-Rossia et al. 2008). Sugars could act in several ways to promote cold acclimation, with hypothetical mechanisms most likely involving interactions with other components of the acclimation response. Clarification concerning the role of sugars in cold acclimation is a major topic for future research (Stitt, Hurry 2002). The aim of this study was to answer the question concerning whether the degradation and synthesis of sucrose in the buds of sweet cherry twigs is influenced by a changing temperature regime – employing the sequence of a cold – warm – cold environment. The results of two years' (2016, 2017) worth of findings were compared with the buds of trees and the buds of twigs under natural conditions over six weeks in November/December in northeast Germany.

MATERIAL AND METHODS

This study was conducted on trees and twigs of the cultivar 'Summit', grafted on GiSelA 5 (Giessener Selektion Ahrensburg 5), a weak- to medium-strong growing stock, at the Experimental Sweet Cherry Orchard (area: 980 m²) at Humboldt-University in Berlin-Dahlem (52°28' Northern latitude and 13°18' Eastern longitude). The long-term (1991–2020) average annual air temperature and precipitation are 10.4 °C and 562 mm, respectively.

Bud sampling. The buds of trees (treatment A) were compared with the buds of cut twigs (placed in plastic flasks), kept for 42 days (six weeks) under the same natural conditions as the orchard (treatment B). Also, the twigs (C) were first kept under controlled conditions at an air temperature of ~22 °C (day/night) for 21 days (3 weeks) and subsequently, the twigs treated this way, were placed in the orchard under natural conditions for 21 days (twigs B, C: in Styrofoam containers and isolation film, in order to prevent the water freezing in the plastic flask). Bud clusters (n = 3/sampling) were taken weekly (2016: 8 November–20 December; 2017: 22 November–3 January 2018), each cluster from 3 trees (A) and from 3 twigs each for B and C. After cutting, the flower buds were selected (exception: the smaller leaf bud in the middle), immediately frozen in liquid nitrogen, stored at –80 °C, and later freeze-dried. All the buds were ground in a ball mill (Retsch M1, Haan, Germany) before analysis.

Twig sampling. In 2017/18, and using the one-year-old twigs, representative pieces of treatment B and treatment C were taken at day of the year (DOY) 347/DOY 3, respectively, immediately frozen in liquid nitrogen, stored at –80 °C, freeze-dried, and ground.

Analysis of saccharides. The analysis of the sucrose (Suc), glucose (Glu), fructose (Fru), raffinose (Raf), and stachyose (Sta) was accomplished for the buds and twigs using High-Performance Liquid Chromatography (HPLC) and Refraction Index (RI) Detection (Weiß, Alt 2017). This HPLC method utilises a Nucleosil column loaded with Pb^{2+} ions, a RI detector, and HPLC-grade water as an eluent, and gives precise and reproducible results regarding the detection of the individual sugars in the plant extracts. This precise and

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quite reliable method permits the detection of other sugar monomers, including maltose, lactose, xylose, galactose, arabinose, ribose, and mannitol.

Statistical methods. Data regarding the bud saccharides for the two years were pooled because of the measurement similarities obtained in the years 2016 and 2017 (mean, standard error (SE), $n = 6$), and analysed using IBM SPSS Statistics 22.0. Afterwards, the mean value, thus obtained, was analysed using a standard analysis of variance (ANOVA), and tested for the significance using Tukey's HSD-test, $P < 0.05$ at the beginning of the experiment (DOY 319), after 21 days (DOY 340), and 42 days (DOY 361) later.

RESULTS AND DISCUSSION

Temperature in 2016 and 2017. The mean air temperature (Table 1) experienced during week 1 through week 3 in the orchard, for the trees (A) and twigs in water (B), was 3.0, 5.7, and 2.5 °C at DOY 326, 333, and 340, respectively. During week 4 through week 6 (A, B, and C), the mean air temperature was 1.5, 5.9, and 3.4 °C at DOY 347, 354, and 361, respectively. The coldest temperatures (T_{min}) during the six-week period ranged between –1.1 (DOY 347) and 3.5 °C (DOY 333, 354), whereas the range for the warmest temperatures (T_{max}) varied between 3.6 (DOY 347) and 8.4 °C (DOY 333).

Saccharides in buds (means for 2016 and 2017). For the buds of the trees (treatment A), the sum of the saccharides (Suc, Glu, Fru, Raf, Sta) (Figure 1A) were in the range between 96 and 103 mg/g dry weight (DW), and was comparable for each sampling over the observation period – from mid-November (DOY 319) to the end of December (DOY 361). These soluble sugars play a prominent and decisive role in protecting plant cells from damage from low and freezing temperatures in various tissues, including the buds, bark, and wood tissues (Yu et al. 2017, and references therein).

Table 1. Mean weekly air temperature (T_{mean}), minimum (T_{min}), and maximum (T_{max}) in the orchard under natural conditions during the experiment from DOY 326–361 (treatments A, B, C)

DOY	T_{mean} (°C)	T_{min} (°C)	T_{max} (°C)
326	**3.0**	**0.0**	**6.3**
333	5.7	3.5	8.4
340	**2.5**	**0.3**	**5.6**
347	1.5	–1.1	3.6
354	5.9	3.5	8.1
361	**3.4**	**0.6**	**5.7**

DOY in bold: statistical analysis by ANOVA

For the buds of the twigs in the orchard (treatment B, Figure 1A), under natural conditions, the sum of the saccharides rises markedly (by 20%), from 96 mg/g DW (DOY 319) to 115 mg/g DW after 21 days (DOY 340). Setting the twigs in water under low temperatures (T_{mean} between 2.5 and 5.7 °C) results in the buds acting as sinks for carbohydrates, and clearly led to the mobilisation and/or breakdown of sugars and starch from the bark and wood tissues of the twigs, resulting in bud sugar increases, mainly in the Glu and Fru at DOY 340 (Figure 1C,D). This increase was only temporary, because after an additional 21 days in the orchard, the sum of the saccharides (77 mg/g DW (DOY 361)) was well below the value of 96 mg/g DW observed at the beginning of the experiment. This reduction clearly suggests an increasing carbohydrate consumption that involves energy metabolism under low temperature conditions, resulting in a depleting pool of saccharides between DOY 340 and DOY 361.

Concerning the buds of the twigs (Figure 1A), the temperature of ~22 °C (treatment C) resulted in a clear decrease of 50% in the sum of the saccharides, from 96 mg/g DW (DOY 319) to 48 mg/g DW (DOY 340) within 21 days, involving the degradation and utilisation of these saccharides as an energy source. This response was mainly due to the decrease in the Suc, Glu, Raf, and Sta (Figure 1B,C,E,F), while the Fru content (Figure 1D) remained unchanged. Neither bud growth nor bud development was observed during this time. The twigs exposed to a temperature of ~22 °C were subsequently placed (for 21 days) in the orchard, and the sum of the saccharides increased significantly (by 56%) from 48 mg/g DW at DOY 340 to 75 mg/g DW at DOY 361. This is a remarkable response in that, as clearly demonstrated for the twigs under natural conditions (treatment B, DOY 319–340), the low temperatures (T_{mean} between 1.5 and 5.9 °C) led – for a certain time (21 days) – to the mobilisation of sugars from the bark and wood tissues of the twigs. The Suc, Glu, and Raf contents increased until DOY 361 (Figure 1B,C,E), while the Fru and the Sta contents (Figure 1D,F) remained unchanged. The sum of the saccharides in the buds of the twigs in treatment B and treatment C was – after 42 days (DOY 361) — 77 and 75 mg/g DW (equal), and 27% less than the 103 mg/g DW in the buds

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of the trees. Although low sugar levels do not prevent the low-temperature-induced increase of Cold Regulated (COR) transcripts, they do interfere with the development of frost tolerance. This raises the possibility that a low sugar content interferes with the synthesis, post-translational regulation, or operation of the COR proteins (Stitt, Hurry 2002).

Sucrose. A study over five years (Chmielewski et al. 2017) clearly confirmed the cryoprotective role of sucrose during ecodormancy in sweet cherry buds. As is the case of this study, the Suc in the buds of the trees showed a stable content, 39 mg/g DW between DOY 319 and 361 (Figure 1B). The Suc in the buds of the twigs under natural conditions (treatment B) comprised 37 mg/g DW at DOY 319 and DOY 340 (Figure 1B), and tended thereafter to decline to 27 mg/g DW at DOY 361, but was not statistically significant.

The temperature treatment of ~22 °C (treatment C) (Figure 1B) resulted in a significant Suc degradation (62%), from 39 mg/g DW to 15 mg/g DW in the buds of the twigs (treatment C) after 21 days (DOY 340). The significant resynthesis (by 66%, to 25 mg/g DW after 21 days (DOY 361)) in the orchard with T_{mean} 1.5, 5.9, and 3.4 °C at DOY 347, 354, and 361, respectively, is noteworthy, and strongly suggestive of the role of Suc as a cryoprotective saccharide, and with an effective time adjustment of 21 days under low temperature conditions. In comparison, the transfer of warm-grown *Arabidopsis* plants to 4 °C leads to the post-translational activation of sucrose phosphate synthase (SPS) within only 30 minutes. This is detected as a small shift in the apparent molecular weight and an increase in the substrate affinity of SPS (Stitt, Hurry 2002). Suc is channelled into various pathways (in different subcellular compartments), leading to energy production (adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH), via glycolysis, including the tricarboxylic acid cycle), which is required for the biosynthesis of the primary metabolites, and, therefore, for tissue growth and development (Xin, Browse 2000).

Glucose and fructose. The Glu content in the buds of the trees remained at a constant 26 mg/g DW between DOY 319 and 361 (Figure 1C). The Glu in the buds of the twigs under natural conditions (treatment B) was 27 mg/g DW at DOY 319, and increased (significantly) by 30% after 21 days, to 35 mg/g DW at DOY 340 (Figure 1C). The Glu content in the buds subsequently declined by 55% after 21 days in the orchard to 16 mg/g DW at DOY 361. The temperature of ~22 °C (treatment C) (Figure 1C) resulted in a significant Glu decrease (by 48%) from 27 mg/g DW to 14 mg/g DW in the buds of the twigs (treatment C) after 21 days (DOY 340), in response to the energy-consuming metabolism. After, when these twigs were subsequently placed for 21 days in the orchard, the Glu content in the buds increased markedly, to 22 mg/g DW (DOY 361). The use of Glu under low temperatures under natural orchard conditions is likely the result of the sugar storage in the vacuoles, osmoregulation, and the response to cold stress following a period of high temperatures, and also in the cytoplasm, involving the biosynthesis of the primary and secondary compounds, respiration, and gene expression (Xin, Browse 2000), all fulfilled by the mobilisation from the twig tissues.

The Fru in the buds of the trees (Figure 1D) increased significantly, from 15 mg/g DW (DOY 319) to 28 mg/g DW between DOY 340 and 361. The Fru content in the buds of the twigs under natural conditions in the orchard (treatment B), and under changes in the environment (21 days ~22 °C, 21 days orchard, treatment C), were 20 mg/g DW and 17 mg/g DW, respectively, and subsequently resulted in a stable pool between DOY 319 and DOY 361 (Figure 1D).

Raffinose and stachyose. The Raf in the buds of the trees declined significantly (by 50%) from 6 mg/g DW at DOY 319 (Figure 1E) to a mean of 3 mg/g DW at DOY 340 and DOY 361. The Raf in the buds of the twigs under natural conditions in the orchard (treatment B) consistently remained at 7 mg/g DW between DOY 319, DOY 340, and DOY 361 (Figure 1E), and could play a definitive role in the interaction with the water in which the twigs had been placed, and also in response to the low temperatures, thus providing cold adaptation if water is present for use by the twigs and buds. The Raf is significantly reduced (by 50%) at a temperature of ~22 °C (treatment C) (Figure 1E), reaching 3 mg/g DW after 21 days (DOY 340), which is equal to the Raf content in the buds of the trees. Interestingly, this value increased by 33%, to 4 mg/g DW after 21 days (DOY 361), in the orchard under natural conditions, and suggests the possibility of a limited resynthesis and/or reallocation under low temperatures.

The tetra-saccharide Sta in the buds of the trees declined significantly (by 60%) from 10 mg/g DW at DOY 319 (Figure 1F), to a mean of 4 mg/g DW (for DOY 340 and DOY 361). The Sta content in the

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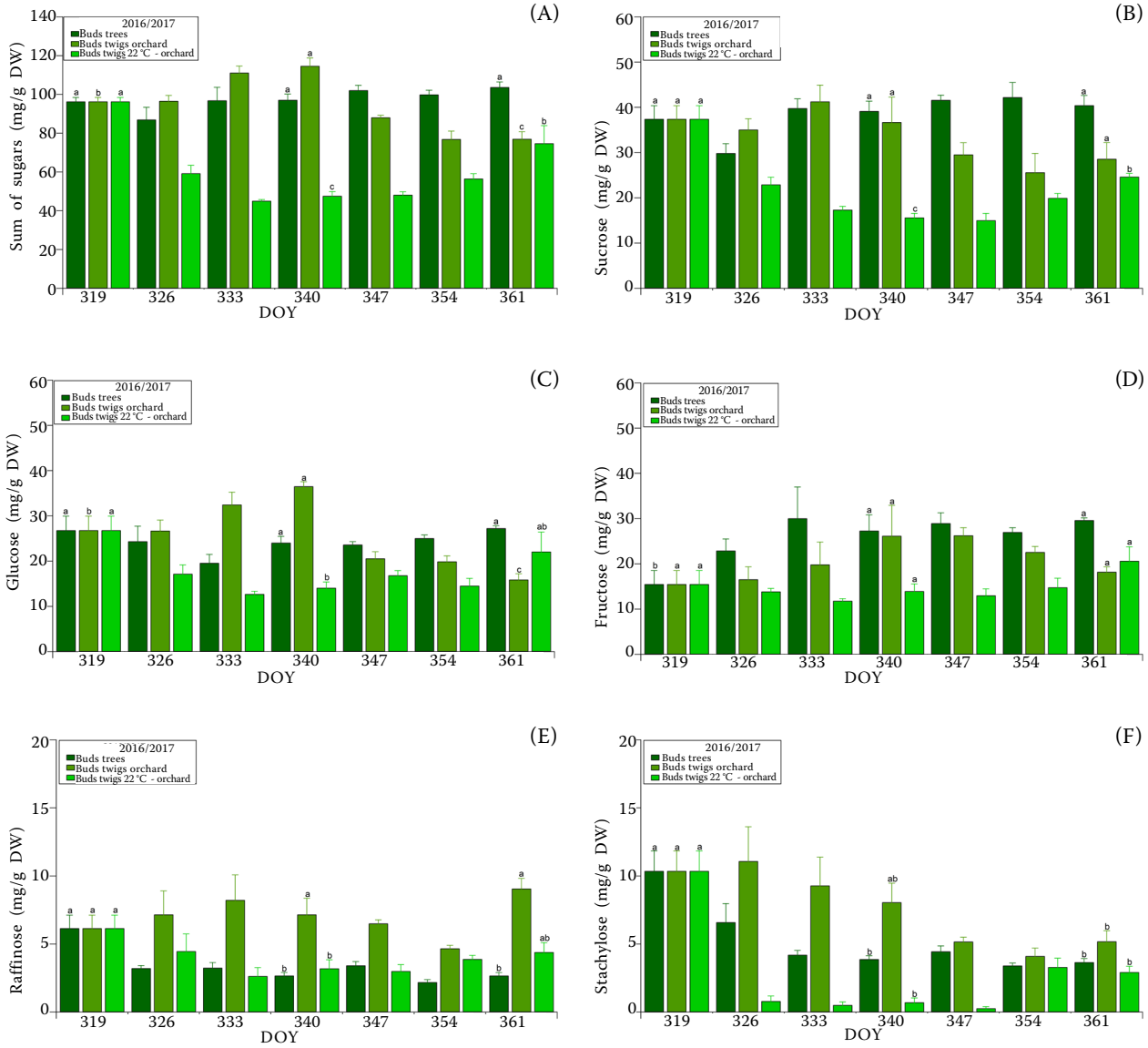

Figure 1. Sum of the sugars (A), and the sucrose (B), glucose (C), fructose (D), raffinose (E), and stachyose (F) contents of the sweet cherry buds of the trees (DOY 319–361), the buds of the twigs kept in water under natural conditions in the orchard (treatment B) (DOY 319–361), and the buds of the twigs kept in water for 21 days at ~22 °C (DOY 319–340) followed by replacement in the orchard (DOY 340–361). Mean of 2016 and 2017; $n = 6$

Different letters indicate different homogeneous groups ($P < 0.05$; ANOVA) for treatment A, B, and C between DOY 319, 340, and 361

buds of the twigs under natural conditions decreased gradually, from 20% to 8 mg/g DW and from 50% to 5 mg/g DW at DOY 340 and DOY 361, respectively, compared to the value at DOY 319. The Sta, therefore, clearly is not an active saccharide to counteract the low temperatures in the buds of the twigs, but will release, following hydrolysis, 2 mol galactose and 1 mol each of Glu and Fru, all of which can be used as energy sources, and/or provide osmotic adjustment within the buds. Sta, remarkably, is greatly reduced (by 93%) at temperatures of ~22 °C (treatment C) (Figure 1F), and declines to 0.7 mg/g DW after 21 days (DOY 340). Interestingly, the Sta content increased 4.3-fold (to 3 mg/g DW) after 21 days (DOY 361) in the orchard under natural conditions, a value equal to the Sta content in the buds of the trees.

Comparison of saccharides in buds (mean 2016, 2017) and twigs (2017, 2018). The sum of the saccharides of the twigs in the orchard (treatment B; Table 2) was 87 mg/g DW at DOY 340,

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Table 2. Comparison of the saccharides in the buds (mean ± SE of 2016, 2017) and twigs (mean ± SE, 2017, 2018) at DOY 340, 361 (2016, 2017) and at DOY 347, 3 (2017, 2018) under natural conditions in the orchard (treatment B)

	DOY	Buds orchard	DOY	Twigs orchard	Twigs compared to buds
Sum saccharides	340	114.7 ± 4.1	347	86.6 ± 5.0	minus*
	361	77.1 ± 3.7	3	74.3 ± 4.4	equal
Sucrose (Suc)	340	36.7 ± 5.5	347	32.8 ± 1.4	equal
	361	28.6 ± 3.6	3	4.0 ± 1.4	minus*
Glucose (Glu)	340	36.5 ± 1.0	347	17.5 ± 2.0	minus*
	361	15.9 ± 1.3	3	29.6 ± 2.4	plus*
Fructose (Fru)	340	26.2 ± 6.7	347	23.8 ± 2.3	equal
	361	18.2 ± 1.1	3	37.0 ± 2.9	plus*
Raffinose (Raf)	340	7.2 ± 1.2	347	5.0 ± 0.2	equal
	361	9.1 ± 0.7	3	1.2 ± 0.2	minus*
Stachyose (Sta)	340	8.1 ± 1.4	347	7.6 ± 0.2	equal
	361	5.2 ± 0.8	3	2.6 ± 0.5	minus*

*indicates significant changes ($P < 0.05$); DOY – day of the year

and 24% lower than the buds (115 mg/g DW). This was mainly due to the significantly lower Glu content (18 mg/g DW (DOY 340)) in the twigs, which was 51% lower than in the buds. The Suc, Fru, Raf, and Sta contents of the twigs were equal to the contents for the buds at DOY 340. The sums of the saccharides for the buds and twigs after 42 days in the orchard at DOY 361 (treatment B) were comparable (77 and 74 mg/g DW). Remarkably, the significant decrease in the Suc content — from 33 mg/g DW to 4 mg/g DW— of the twigs in the orchard (between DOY 340 and DOY 361, respectively), was 86% lower than for the buds (29 mg/g DW). By comparison, the twig's Glu content, and especially the Fru content, increased significantly between DOY 340 and DOY 361, from 18 mg/g DW to 30 mg/g DW, and 24 mg/g DW to 37 mg/g DW, respectively, and the contents were markedly higher in the twigs than in the buds. This clearly is a result of the Suc being cleaved by invertases (INV) in the twigs. The Suc is partitioned into different pathways by multiple isozymes in different subcellular compartments (Tiessen, Padilla-Chacon 2013). The different routes of sucrose degradation are not interchangeable, because the subcellular levels of hexoses and sucrose produce different signals that activate different metabolic pathways (Tiessen, Padilla-Chacon 2013, and references therein). Further, Sturm (1999) provides an interesting overview of invertases, their primary structure, their function, and role in plant development and sucrose partitioning. The Raf and Sta content of the twigs decrease clearly between DOY 340 and DOY 361 from 5 mg/g DW to 1 mg/g DW, and from 8 mg/g DW to 3 mg/g DW, respectively (Table 2). At DOY 361, these values were well below those in the buds. The reason for the decrease most likely relates to the water availability for the twigs. The raffinose family of oligosaccharides plays a role in carbon storage, signal transduction, and osmoprotection (Zuther et al. 2004; da Silveira Falavigna et al. 2018).

The sum of the saccharides was 48 mg/g DW after 21 days at a temperature of ~22 °C in both the buds and twigs (DOY 340, treatment C; Table 3). After placing the buds/twigs under natural conditions in the orchard, the sum of the saccharides of the buds and twigs significantly increased at DOY 361, to 75 mg/g DW and 65 mg/g DW, respectively. The Suc contents of the twigs were 4 mg/g DW and 5 mg/g DW, and found to be 80% lower than the contents for the buds at DOY 340 and DOY 361, respectively. The direct allocation of Suc (as an intact molecule) into the buds cannot be assumed, as the Glu and Fru contents of the twigs increases, suggesting INV activity. Using transformed tobacco, *Arabidopsis thaliana*, *Ricinus communis* L., it is believed (Sin'kevich et al. 2008, and references therein) that the sugars accumulated because of the active acid insoluble invertase hydrolysed sucrose available for translocation, and the hexoses, thus, produced could not enter the phloem. Returning to the mesophyll cells, these compounds are phosphorylated by hexokinase and fructokinase, and engage in the pathway of sucrose (re)synthesis, because these enzymes become active at low temperatures.

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Table 3. Comparison of the saccharides in the buds (mean ± SE of 2016, 2017) and twigs (mean ± SE, 2017, 2018) 21 days at ~22 °C (DOY 340, 361 (2016, 2017) and at DOY 347, 3 (2017, 2018)) followed by 21 days under natural conditions in the orchard (DOY 361) (treatment C)

	DOY	Buds ~22 °C, orchard	DOY	Twigs ~22 C, orchard	Twigs compared to buds
Sum saccharides	340	47.7 ± 2.2	347	47.5 ± 1.3	equal
	361	74.8 ± 9.1	3	65.3 ± 3.0	equal
Sucrose (Suc)	340	15.7 ± 0.9	347	3.5 ± 0.9	minus*
	361	24.7 ± 0.7	3	5.1 ± 0.4	minus*
Glucose (Glu)	340	14.1 ± 1.3	347	14.3 ± 1.3	equal
	361	22.1 ± 4.3	3	24.7 ± 0.9	equal
Fructose (Fru)	340	14.0 ± 1.4	347	22.7 ± 1.7	plus*
	361	20.7 ± 3.1	3	31.4 ± 1.2	plus*
Raffinose (Raf)	340	3.2 ± 0.6	347	3.3 ± 1.4	equal
	361	4.4 ± 0.7	3	1.6 ± 0.3	minus*
Stachyose (Sta)	340	0.7 ± 0.3	347	2.8 ± 0.8	plus*
	361	2.9 ± 0.4	3	2.5 ± 0.5	equal

*indicates significant changes ($P < 0.05$); DOY – day of the year

The Glu content of the twigs was 14 mg/g DW at DOY 340, and increased markedly under natural conditions in the orchard to 25 mg/g DW at DOY 361. Also, both values were similar to those for the buds. The Fru content of the twigs was 23 mg/g DW at DOY 340, and also significantly increased under the natural conditions, to 31 mg/g DW at DOY 361, and was higher than in the buds {14 mg/g DW and 21 mg/g DW, respectively}. Regarding the low temperature twig responses in the orchard, as for the former period (prior to 21 days earlier) (< DOY 319), the compartmental protective processes most likely proceeded at the tissue, cell, and organelle levels. Marked changes for the Suc, Glu and Fru contents of the twigs within a cold-warm-cold period, before DOY 319, between DOY 319 and DOY 340, and between DOY 340 and DOY 361, collectively lead to the conclusion that this adaptation is a result of the tissue- and cold-specific sucrose invertases/synthases of the sweet cherry cultivar 'Summit'. These enzymes lead to the significant resynthesis of Suc of 25 mg/g DW in the buds at DOY 361 under natural orchard conditions, with a T_{mean} of 1.5, 5.9, 3.4 °C at DOY 347, 354, 361, and these results occur after a time of high saccharide consumption and metabolism at ~22 °C for 21 days (DOY 319 to DOY 340). This also explains the constant Suc content in the buds under natural orchard conditions (treatment B). These responses clearly explain the course of the Suc in the buds of the trees during the winter rest, especially during the different phenological phases, endodormancy (rise), ecodormancy (plateau), and ontogenetic development (decrease).

Sucrose is the major transport form of carbohydrates in higher plants. However, it is often not clear how much of the sucrose is accessible by invertase in the vegetative tissues, such as the source leaves, since different forms of invertase are distributed among the cellular compartments, including the apoplast, cytoplasm, and vacuoles (Lattanzi et al. 2012, and references therein). The cleavage of sucrose is catalysed by either the sucrose synthase (EC 2.4.1.13) or invertase (EC 3.2.1.26). The invertases (B-D-fructofuranosidase, soluble acid, neutral, and cell wall-bound acid) catalyse the irreversible hydrolysis of sucrose to glucose and fructose. Invertases normally reside within the cell wall or vacuole, and provide higher osmoticum to cold-acclimated cells. Sucrose synthase (UDP-D-Glc: D-Fru 2-alpha-glucosyltransferase) is a cytoplasmic enzyme that catalyses the reversible cleavage of sucrose with uridine 5′-diphosphate (UDP) to form UDP-glucose and fructose. Although capable of synthesising sucrose, sucrose synthase functions primarily in the direction of sucrose degradation (Turhan, Ergin 2012, and references therein).

Low-temperature-active enzymes (Zhou et al. 2016) can show high catalytic activity at low temperatures, and are associated with low stability at intermediate and high temperatures. A glycoside hydrolase (GH) family 32 invertase from Bacillus sp. HJ14 was expressed in *Escherichia coli*. The purified recombinant enzyme 'rInvHJ14' showed typical bio-

https://doi.org/10.17221/123/2020-HORTSCI

chemical properties of a low-temperature-active enzyme, as its optimal activity is 30–32.5 °C, has a high catalytic activity at 0 and 10 °C, and is thermolabile at 30 °C or higher temperatures.

Turhan and Ergin (2012) investigated bark tissues of sweet cherry trees (cv. 0900 Ziraat and Lambert, grafted on Gisela 5 and Mazzard rootstocks), in both cold-acclimated (CA, January, average temperature of 5 °C) and non-acclimated (NA, July, average temperature of 26 °C) stages. The acid invertase enzyme activity was significantly greater in the NA stage than in the CA stage for all the graft combinations. The contents of the reducing sugars and sucrose were significantly greater in the cold-acclimated stage compared to the non-acclimated stage for all the graft combinations.

Gholizadeh et al. (2017) studied the activity of acid invertase and the activity of alkaline invertase in the lateral buds of walnut (*Juglans regia* L.) cultivars with contrasting high ('Hartley') and low ('Serr') chilling requirements. The activity of the acid and alkaline invertases in the 'Hartley' buds increased parallel with the increasing air temperature of 7 °C, 10 °C, 15 °C, and 22 °C from January to April, respectively, which were greatest from March to April. In the 'Serr' buds, however, the increase appeared delayed by one month starting in February and was the greatest by March. Thus, the 'Hartley' and 'Serr' buds differed regarding the invertase activity development, demonstrating cultivar specific differences in terms of the acid and alkaline invertase. Summarising, both the buds and twig tissues have various receptors, such as different invertases/synthases, whose activity is triggered by different thermal environmental stimuli.

In addition to sucrose, some invertases also hydrolyse fructose-containing oligosaccharides and fructans, such as raffinose and stachyose (Zhou et al. 2016, and references therein). The mean Raf contents of the twigs were (DOY 340/361) about ~2 mg/g DW, and were markedly lower (1.6 mg/g DW) compared to the buds (4.4 mg/g DW) at DOY 361. The Sta content of the twigs was about ~3 mg/g DW (DOY 340/361), and, statistically, markedly higher (2.8 mg/g DW) than in the buds (0.7 mg/g DW) at DOY 340. The sucrose, glucose, fructose, raffinose, and stachyose levels in the bark and trunk tissues of two peach cultivars (*Prunus persica* L.) were measured from September until March (Yu et al. 2017). Of the soluble sugars detected, sucrose was predominant, and the individual and total soluble sugar contents were significantly higher in the bark tissues than in the wood tissues irrespective of the cultivar. Some soluble sugars, such as raffinose and stachyose, which are present only in winter, are thought to prevent the crystallisation of sucrose, thus facilitating glass formation within the cell and protecting the cellular membrane. Raffinose and stachyose were also detected under cold acclimation, as was the case for the sweet cherry buds and twigs in our experiment, but only at minor levels. The contents of most individual soluble sugars were higher in the bark tissues than in the wood tissues irrespective of cultivar. Much of the raffinose and stachyose were contained in the bark tissues (Yu et al. 2017). Raffinose and stachyose can replace starch as a storage carbohydrate in some plants. Also, raffinose, rather than sucrose, is used in some species as a transport substance in the phloem sieve tubes.

CONCLUSION

Changes in the saccharides (Suc, Glu, Fru) of the buds and twig tissue within a cold-warm-cold period is a result of the tissue- and cold-specific sucrose invertases/synthases of the sweet cherry cultivar 'Summit'. The effect of these enzymes explains the constant Suc content in the buds of the twigs kept in water under natural environmental conditions. Moreover, these enzymes also lead to a significant resynthesis of Suc at low temperatures, this occurring after a time of high saccharide consumption and metabolism at ~22 °C. Low-temperature-active enzymes explain the course of the Suc metabolism in the buds of the trees during the endodormancy, ecodormancy, and ontogenetic development. When using twigs for plant physiological examinations during winter rest, characterised by invisible growth and development, the results, on the level of the metabolites, should be considered when drawing conclusions concerning the overall tree physiology.

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Received: August 20, 2020
Accepted: March 24, 2021