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Short Communication

Changes in carbohydrate levels and relative water content (RWC) to distinguish dormancy phases in sweet cherry

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ABSTRACT

Perennial trees require chilling, i.e. a period of cold temperature in the winter, for flowering next spring; sweet cherry is particularly prone to lack of chilling. The objective of this study is to identify possible transition points to clearly distinguish dormancy phases by relating carbohydrate and relative water content (RWC) in reproductive buds to concomitant chilling fulfilment.

This contribution proposes the use of four transition points between the dormancy phases and their characterization in terms of carbohydrates, water contents in combination with chilling values and may allow upscaling to other dormancy studies in trees; two groups of cherry varieties were defined based on their different initial sorbitol and starch level in the autumn. The first separation between para- and (deep) d-endo-dormancy is characterized as a transition from a decrease (variety group 1) or a constant level (variety group 2) to a sharp increase in hexoses and sorbitol and a drop of starch content. The second transition point (d-endo- to f-endo-dormancy) is characterized as the changes in both hexoses (increase) and starch (decrease) terminate and ca. 650 Chilling Hours (CH), i.e. insufficient chilling in the concomitant forcing experiment with cut branches. This third transition point (f-endo- to eco-dormancy) was characterized by ca. 1000 CH, the minimum chilling requirement and restrained flowering (cut branches). The fourth transition point (forcing initiation) marked an increase in water content at ca. 1550 CH, optimum chilling for cherry and coincided with natural flowering.

A ratio of hexoses (glucose plus fructose) to starch content (< 2:1) appeared to be a potential indicator of the beginning of chilling (para-dormancy) and a ratio of 14–20:1 typical for endo-dormancy, whereas the release from dormancy was associated with a decline to less than 10:1 at the end of winter (eco-dormancy).

To our knowledge, this is the first time that transition points are identified based on constituents (carbohydrates and relative water content) in floral buds related to current chilling status and dormancy phases and are also presented in a schematic diagram. The understanding of these changes in relative water content and carbohydrate levels may contribute to manage insufficient chilling in the orchard and support climate change studies with trees.

1. Introduction

Climate change including global warming is associated with warmer winters in most parts of the world (IPCC, 2013), particularly in the stone fruit growing regions. Many perennial, deciduous trees undergo a dormancy period during winter, when they shed their leaves after translocation of their nutrients such as carbon and nitrogen skeletons into the woody perennial parts of the tree (Tartachnyk and Blanke, 2004). Chilling, i.e. a cold period during winter, is a prerequisite for flower initiation in spring in perennial plants (Coville, 1920; Lang et al., 1987; Meir et al., 2016; Vegis, 1964) and maybe hampered by climate change (Blanke and Kunz, 2009; Luedeling et al., 2013). Release from dormancy is associated with chilling fulfilment. Carbohydrates play a major role in the control of bud growth and development during dormancy and dormancy release (Bonhomme et al., 2005; Cottignies, 1986; Hillmann et al., 2016; Marquat et al., 1999). To our knowledge, no study combined the physiology of carbohydrate metabolism, relative water content (RWC) in floral buds and chilling accumulation with the dormancy phases introduced earlier by Lang et al. (1987). Cherry as one of the temperate-zone tree species most affected by global warming, e.g. warmer winter, and therefore lack of winter chill in fruit growing regions (Kaufmann and Blanke, 2017a,b) is used as model crop. This research paper is based on the hypothesis that changes in chilling status are associated with changes in the carbohydrate dynamics, relative water content in reproductive buds, thereby relating mechanism to functioning.

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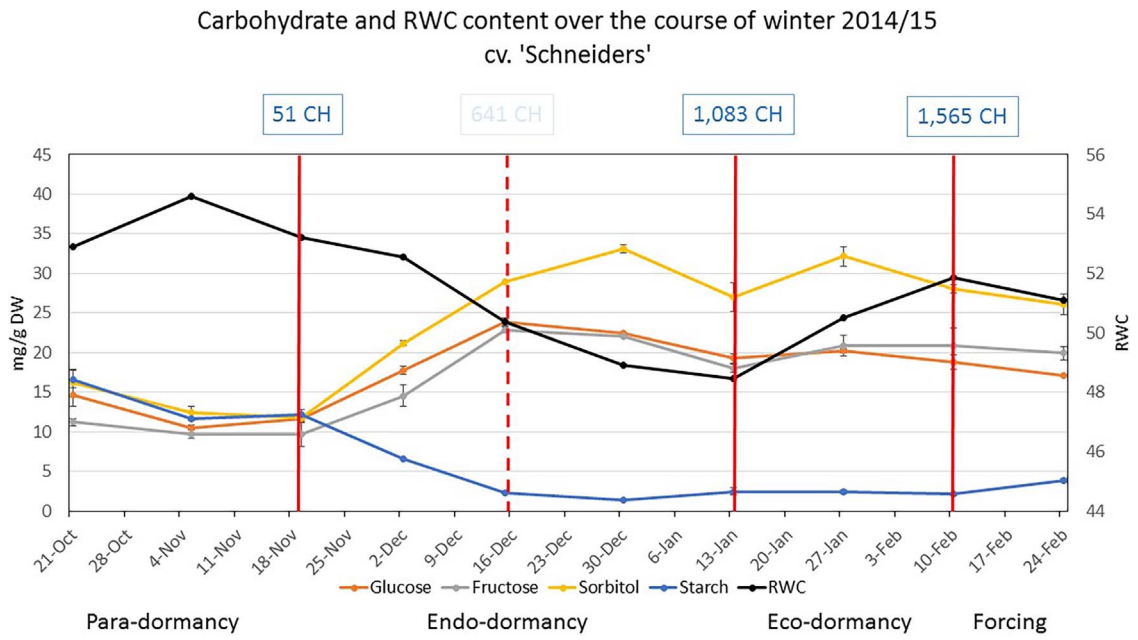

Fig. 1. Carbohydrate, relative water content chilling availability and dormancy phases during the dormant period (2014/15) of floral buds of cherry cv. 'Schneiders' representing group 1 (n = 100 floral buds per sampling date and SDs).

Hence, the objective of the present work was to investigate the changes in carbohydrate levels and water relations of cherry buds to identify and distinguish the dormancy phases (para-, endo, eco-dormancy) and to determine transition points between dormancy stages using ca. 10,000 flower buds from nine varieties and bi-weekly sampling over two years. To our knowledge, no study of this kind has been reported in the literature, linking physiological processes of dormancy to concomitant temperature records and chilling status in the orchard. The synthesis of carbohydrate dynamics, changes in relative water content (RWC) in floral buds and chilling status is used to calculate carbohydrate ratios, and to elaborate four transition points as thresholds and representatives of the current dormancy stage and integrated them in a schematic of the changes in relative water content and carbohydrate that drive dormancy and dormancy release.

2. Materials and methods

2.1. Plant material

Ten-year-old sweet cherry (*Prunus avium* L.) trees cv. 'Titan', 'Regina', 'Chelan', 'Kordia', 'Skeena', 'Burlat', 'Schneiders späte Knorpelkirsche', 'Benton' and 'Rubin' on dwarfing Gisela 5 rootstock were grown on the experimental orchard of the University of Bonn, Campus Klein Altendorf, Germany (50°N). Temperatures were recorded using Datahog 2 (Skye Ltd., Pontys, Wales, UK) over the winter 2014/15 and 2015/16 and used to calculate the available chilling (Weinberger, 1950). Overall, ca. 10,000 flower buds were sampled bi-weekly from two-year-old wood in one to two meter tree height from these nine sweet cherry varieties from leaf drop to bud break in spring, weighed for subsequent assessment of RWC and stored at −25 °C until further processing.

Three 50 cm long branches of each variety were taken concomitantly to the bud samples for carbohydrate analyses in bi-weekly intervals. The cut branches were kept in a flask of water (recut and water changed twice a week) and were forced in a heated greenhouse (> 16 °C) as a reference, if the chilling requirement was fulfilled.

2.2. Carbohydrate analysis of cherry flower buds

Carbohydrates were analysed in complete flower buds according to Saied et al. (2005). Samples were freeze-dried (Christ Gamma 1–16 LSC, Osterode, Germany), ground (Retsch MM200, Retsch GmbH, Haan, Germany) and dissolved in distilled water as solvent (0.3–0.5 g DM in 5 mL H_2O 0.1 g/mL). The extract was incubated for 60 min in a water bath at 60 °C (stirred after 30 min) and the solution centrifuged (Heraeus Multifuge X3 FR, Thermo Fischer Scientific, Darmstadt, Germany) at 4500*g* for 15 min. The supernatant was used for sugar analysis and the pellet was kept for starch analysis. For the sugar determination, 4 mL chloroform was added to the supernatant, stirred and centrifuged for 15 min at 2000*g*. The supernatant was stored at −25 °C until analyzing via HPLC. Samples were thawn and centrifuged at 16,100*g* in a benchtop centrifuge (Eppendorf 5415R, Eppendorf AG, Hamburg, Germany). This supernatant was analysed for sucrose, glucose, fructose, raffinose, sorbitol and starch using HPLC (ChemStation, Agilent Technologies, California, USA using Software B.02.01.SR2) with a column (type Carbohydrate CA2 + 300 × 8 mm) and a pre-column heated to 75 °C with a flow speed of 0.8 mL/min at 30 bar pressure and examined in a RI detector. The starch pellet was dissolved with 2 mL of H_2O, stirred and centrifuged at 4500*g* for 15 min twice and the supernatant was discarded. The pellets were rehydrated with 2 mL of distilled water and heated for two hours in a water bath at 100 °C. Centrifuge tubes were cooled down and 2 mL of acetate buffer (0.2 M pH 4.6) and 50 µL amyloglucosidase (250 mg in 10 mL acetate buffer) was added, stirred and kept in a water bath for 20 h at 60 °C. This solution was centrifuged at 4500 rpm for 15 min. The supernatant was treated like the sugar samples with chloroform as described above. Representative changes in carbohydrate levels of the two years are presented in Figs. 1 and 2 using one cultivar for each group in the same year.

3. Results

The objective of the present work was to investigate the changes in relative water content (RWC) and carbohydrate dynamics of cherry buds to identify and distinguish the dormancy phases (para-, endo, eco-dormancy) on a plant physiology base and to determine transition points between dormancy stages for orchard management practices.

3.1. Carbohydrate dynamics of cherry flower buds during dormancy

Based on these 10,000 flower buds and their carbohydrate