Europ.J.Hort.Sci., **68** (5). S. 222–226, 2003, ISSN 1611-4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

Carbon Partitioning and Seasonal Dynamics of Carbohydrates in the Bark, Leaves and Fruits of Apple (*Malus domestica* Borkh.) cv. 'Golden Delicious'

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Summary

Seasonal dynamics of the carbohydrates sorbitol, sucrose, glucose and fructose in fruits, leaves and bark and photosynthetic activity were followed in 12-year-old cv. 'Golden Delicious' apple (*Malus domestica* Borkh.) in response to defruiting in mid August.

Photosynthetic activity of leaves decreased in the trees defruited in August compared to fruiting trees in the second half of August. In the mid-season, leaf carbohydrates were slightly but not significantly increased in defruited trees, which could suggest that the observed photosynthetic response is a result of sink-limitation. There were only minor differences observed when leaf, fruit and bark carbohydrates were compared for fruiting and defruited trees, which indicates that early defruiting in August did not significantly change carbohydrate translocation. Bark carbohydrates increased towards the end of the season, which underlines previous findings that the bark acts as a storage organ for spring growth in deciduous trees, such as apple.

Zusammenfassung

Kohlenstoffverteilung und Kohlenhydratdynamik in der Rinde, Blättern und Früchten von Äpfeln (*Malus domestica* Borkh.) der Sorte 'Golden Delicious' während der Vegetationsperiode. Die Dynamik der Kohlenhydrate Sorbitol, Saccharose, Glucose und Fructose in Früchten, Blättern und in der Rinde 12-jähriger Apfelbäumen der Sorte 'Golden Delicious' wurde während einer Vegetationsperiode verfolgt und der Effekt des Entfernens der Früchte Mitte August bestimmt.

Die photosynthetische Aktivität der Blätter der entfruchteten Apfelbäume nahm im Vergleich zu den fruchttragenden Bäumen in der zweiten Augusthälfte deutlich ab. Die Konzentration der Kohlenhydrate in den Blättern nahm nach dem Entfernen der Früchte Mitte August wenig, aber nicht signifikant zu, was ein Zeichen für die Abhängigkeit der gemessenen photosynthetischen Aktivität von der Sink-Aktivität sein könnte. Die Kohlenhydratkonzentrationen in den Blättern, Früchten und in der Rinde unterschieden sich kaum zwischen den fruchttragenden und entfruchteten Bäumen. Damit wurde gezeigt, dass die Entfruchtung der Bäume Mitte August die Kohlenhydratverlagerung kaum beeinflusste. Die Kohlenhydratkonzentrationen in der Rinde nahmen gegen Ende der Saison zu. Damit konnten einige vorherige Ergebnisse bestätigt werden, dass die Rinde als Reserveorgan für das Wachstum in Frühjahr dient.

Key words. photosynthesis – sorbitol – sucrose – glucose – fructose – leaf – fruit – bark

Introduction

Troughout the year, a bearing apple tree undergoes different stages. At the beginning of the season, an apple tree mainly uses stored carbohydrate reserves and slowly develops its leaf area. After developing its leaf area, photosynthesis per tree increases and declines again with senescence of leaves in the autumn (FAUST 1989). Carbohydrates produced in the leaves of apple trees are transported, mainly as sorbitol and to a lesser extent as sucrose, to sinks like shoot tips, fruit and roots (YOSHIOKA et al. 1988).

Carbon partitioning in a sink organ is influenced by phloem unloading, membrane transport, metabolism and storage of carbohydrates (BERÜTER and STUDER FEUSI 1997). Fruits are strong sinks of assimilates in cropping apple trees and modify biomass partitioning in the plants (ŠTURM and ŠTAMPAR 1999). The sink strength of the apple fruit is not constant during the growing period. From mid June till full ripening, apple fruits become strong sinks and may absorb nearly all assimilates from leaves on the same short shoot (spur) (FUJII and KENNEDY 1984), accumulating them in the form of fructose, glucose, starch and sucrose (BERÜTER et al. 1997). A decline in concentration of starch, glucose and sorbitol and an increase of fructose and sucrose during the season is known (BERÜTER and STUDER FEUSI 1997). After harvest, excess of carbohydrates in the late season is stored in stem bark and root tissues and may play a role in higher chilling and freez-

ing tolerance. Additionally, they may be utilised for energy and material at the early growth stage of shoots and fruit in the following spring (YOSHIOKA et al. 1988).

Photosynthetic productivity of fruit trees depends on internal and external factors (ŠTAMPAR et al. 1999). The main internal factors are sink demand and feedback signals from the sink itself (PAUL and FOYER 2001). Increased leaf starch concentration may lead to a feedback-induced inhibition of leaf and canopy net photosynthesis. Mid-season decline in photosynthesis of 'Braeburn' was associated with an increase in leaf starch concentration as crop load decreased (WÜNSCHE and PALMER 1997). GIULIANI et al. (1997) stated that the afternoon decline of photosynthesis in non-fruiting apple trees is due to lack of vegetative sink demand which causes non-structural carbohydrate accumulation in leaves, limiting phloem loading and assimilate distribution. WIBBE and BLANKE (1995) observed a decline of leaf photosynthesis after defruiting the apple trees of 'Golden Delicious'. This reduction occurred irrespective of the month of defruiting and was most pronounced in the late season.

The response of sugars to different agro-technical measures has been intensively studied mainly in relation to physiological and morphological status of the apple tree. There is, however, little information on the seasonal changes of sugars in different organs in relation to crop load. Therefore, the present work was undertaken to investigate the seasonal carbohydrate composition of fruits from mid June till harvest when fruits act as major sink site on an apple tree. Also the leaf and bark carbohydrates and net photosynthesis of leaves were measured on fruiting and defruited trees to estimate the influence of carbohydrates in the ripening part of the season on photosynthetic leaf performance.

Materials and Methods

Apple trees

The measurements were carried out in summer and autumn 2001 on 12-year-old cv. 'Golden Delicious' apple trees on M9 rootstock, grown in the experimental orchard of the University of Ljubljana. The treatments encompassed four fruiting and four trees defruited on 13th of August 2001 after the control measurement of photosynthesis and control sampling of sugars.

Photosynthesis

The photosynthetic activity and transpiration of bourse leaves on fruiting spurs were measured 5 times in the season. The control measurement (1) was carried out 12 days before the defruiting. The second measurement (2) was carried out 7 days after defruiting and the 3rd, 4th and 5th measurements 16, 37 and 61 days after defruiting, respectively. A portable measuring system Li-6400 (LICOR, Lincoln, USA) was used for the first three measurements. Measurements were taken at PFD of 1500 μmol m^{-2} s^{-1} (6400-02B light source) and at 350 μmol mol^{-1} CO_2 concentration. Leaf temperature was adjusted to 22–23 °C. Due to the technical troubles the last 2 measurements were performed with LCA4 portable measuring system under saturated light conditions (1100–1500 μmol m^{-2} s^{-1} PAR) and ambient temperature and CO_2 concentration of about 400 ppm.

Sugar analysis

Leaves for sugar analysis were sampled five times; the first sampling was carried out on 2nd August, 11 days before the defruiting. The next samples were taken 8, 17, 38 and 50 days after defruiting, respectively. Apple fruits were sampled (fruiting trees) on 14th of June, 5th and 26th of July, 2nd, 21st, on 30th of August, 20th of September and 2nd of October. The last 5 sampling dates coincided with the sampling time of leaves. Bark was sampled twice, first in mid of the growing season (2nd of August) and second at the end of the growing season (6th of November). Leaves and bark for sugar analyses were sampled from one-year old shoots with mature leaves at 10 a.m. each time. For analysis only the lamina of leaves without pedicle was taken. Leaves and bark were shock frozen in liquid nitrogen immediately after excision from the tree. After freeze drying samples were ground and prepared for sugar and sorbitol determination by HPLC (High Performance Liquid Chromatography) according to the DOLENC and ŠTAMPAR (1997) method. This method was slightly modified because only 1 g (leaves) or less (bark) of the sample was used and diluted with bidistilled water to 30 or 50 ml in regard to the sample amount. Also fruit samples were prepared according to the DOLENC and ŠTAMPAR (1997) method.

Statistical evaluation

The significance of defruiting was tested. Results were statistically analysed with the program Statgraphics plus 4.0 using the analysis of variance (ANOVA). Differences between treatments were estimated with a multiple range test using least significant difference (LSD) or Duncan test at $\alpha<0,05$.

Results and Discussion

The first gas exchange measurement, performed 12 days before defruiting, revealed no differences in leaf photosynthesis when both groups of trees, later exposed to two different treatments (fruiting, defruited trees), were compared (Fig. 1). One week and 16 days after defruiting the photosynthetic rate was significantly higher in trees with fruits than in leaves of defruited trees. These results confirmed sink-stimulation of photosynthesis by fruits. A month later, at the fourth measurement, the difference between treatments was not significant any more. Our previous measurements (VEBERIC et al. 2002) showed that 'Golden Delicious' fruits are strong sinks till they are full ripe, afterwards their importance as carbohydrate sinks declines. In mid-season photosynthesis can be down regulated in response to a lower crop load and concomitant accumulation of starch in the leaves (WÜNSCHE and PALMER 1997). Similarly, short-term accumulation of carbohydrates, such as starch and sucrose, in the leaves is involved in the reduction in photosynthesis after autumn fruit harvest-

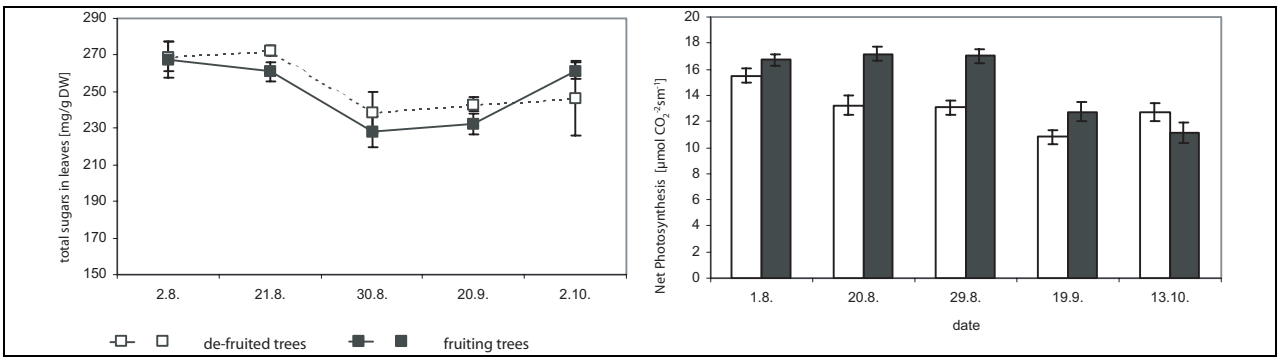


Fig. 1. Total sugar content in leaves (left) and net photosynthesis (right) of the leaves on fruiting and de-fruited 'Golden Delicious' apple trees.

ing (WIBBE and BLANKE 1995). Also in our case slightly higher total sugar content can be detected in the leaves of defruited trees in the period of reduced photosynthetic activity. As this picture is reversed at the end of the season, it is to expect that similar mechanisms to those described by WÜNSCHE and PALMER (1997) and WIBBE and BLANKE (1995) are involved in the regulation of photosynthetic activity.

Contrary to sucrose and starch much lower importance in down-regulation of photosynthesis is attributed to sorbitol (NII 1997). In apple, sorbitol is the most commonly translocated carbohydrate (LOESCHER et al. 1982). In our experiment, sorbitol content in leaves was much higher than in fruits (Fig. 2a). According to LOESCHER (1987) the source tissue, like fully expanded green leaves, contains mainly the NADPH-dependent aldose 6-phosphate reductase that constitutes a synthetic pathway for sorbitol. In sink tissues, like fruits, NAD^+ dependent sorbitol dehydrogenase responsible for oxidation and utilisation of sorbitol can primarily be found. The concentration of sorbitol in fruits decreased from mid June until beginning of August and then arose again until end of the September. Low fruit sorbitol levels measured in the mid-season probably result from the high utilization rate and reflect high sink strength of developing fruits. Similar, sharp decline of

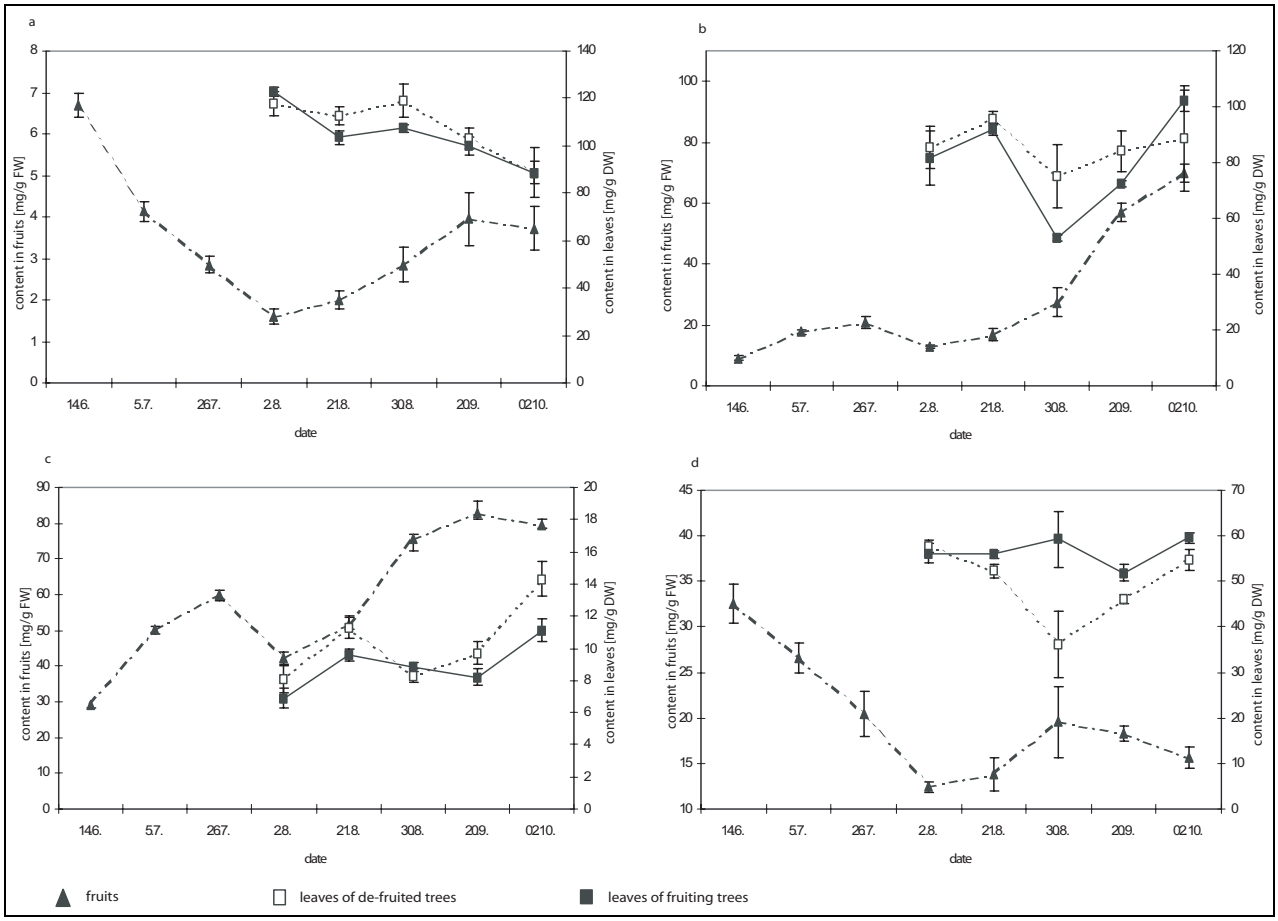


Fig. 2. Content of sorbitol (a), sucrose (b), fructose (c) and glucose (d) in fruits and leaves of fruiting and de-fruited apple trees

fruit sorbitol in the period of May to July was observed by BERÜTER (1985) in cv. 'Golden Delicious' and by ŠTURM and ŠTAMPAR (1999) in 'Idared'. While the increase in fruit sorbitol in the late season was observed in 'Idared' (ŠTURM and ŠTAMPAR 1999), similar to our study, no such recovery was found in the study of BERÜTER (1985).

In leaves sorbitol concentration decreased in the second half of the season (Fig. 2a). The concentration of sorbitol in leaves of defruited trees shows to be slightly but not significantly higher than in leaves of fruiting trees. It could be expected that sorbitol accumulates in the leaves of defruited trees due to a reduced sink capacity (NII 1997). With the ripening the sink strength of the fruits decreases, in our last measurement the sorbitol concentration in the leaves of fruiting trees is nearly the same as in defruited trees. According to NII (1997) large leaf sorbitol content enhances photosynthesis in peach. Similarly low leaf sorbitol content in the late season was accompanied by decreased photosynthetic activity in our study (Fig. 1 and 2a). For the same cultivar a clear decline in photosynthetic capacity is known for the end September-October period (VEBERIC et al. 2002). On the other hand a decrease of photosynthetic activity of leaves after harvest was not accompanied by sorbitol leaf content reduction in the study of TERHOEVEN-URSELMANS and BLANKE (1999).

Sucrose is the second carbohydrate translocated from the apple leaf into fruit (HANSEN 1970). In the leaves of defruited trees sucrose content had not changed much from August to October (Fig. 2b). See also results by CHEN et al (2002). Fluctuations of leaf sucrose concentration were more pronounced in fruiting trees, which can be explained by sink feedback regulation of leaf metabolism. Our study revealed a clear accumulation of sucrose during fruit development. Prior to uptake into parenchymal cells of the fruit sucrose translocated from leaves into fruit is easily decomposed to glucose and fructose (YAMAKI 1995). Hence, sucrose finally deposited in fruits is not only the translocated sucrose but can also be a re-synthesised one (BERÜTER 1985; BERÜTER et al. 1997). Its accumulation can be explained by the lower activity of acid invertase in the late season (BERÜTER 1985).

In the growing fruit, fructose accumulates steadily, as a result of its restricted partitioning into starch, while glucose remains at the low level during the period of starch synthesis (BERÜTER 1985). Generally, the fructose content of fruits in our study was low when sorbitol concentration was high and increased with decreasing sorbitol content (Fig. 2c). Leaf fructose concentration was similar in both empty and full trees with increasing levels towards the end of the season.

Glucose content was high in young fruits and decreased during summer (Fig. 2d). Although starch content in the fruits was not measured in our study it can be presumed, according to BERÜTER et al. (1997), that glucose is preferentially used in starch synthesis. The glucose content in fruits increases in the late season when the starch level is decreased (BERÜTER 1985).

The measurements of sugar content in bark showed a clear accumulation of sorbitol, sucrose, fructose and glucose at the end of the growing season. Among the sugars analysed sorbitol reached the highest bark concentration both in the mid-season and at the end of the season. According to OLIVEIRA and PRIESTLEY (1988) sorbitol is the major soluble storage carbohydrate in apple tree. Our study revealed the high increase in con-

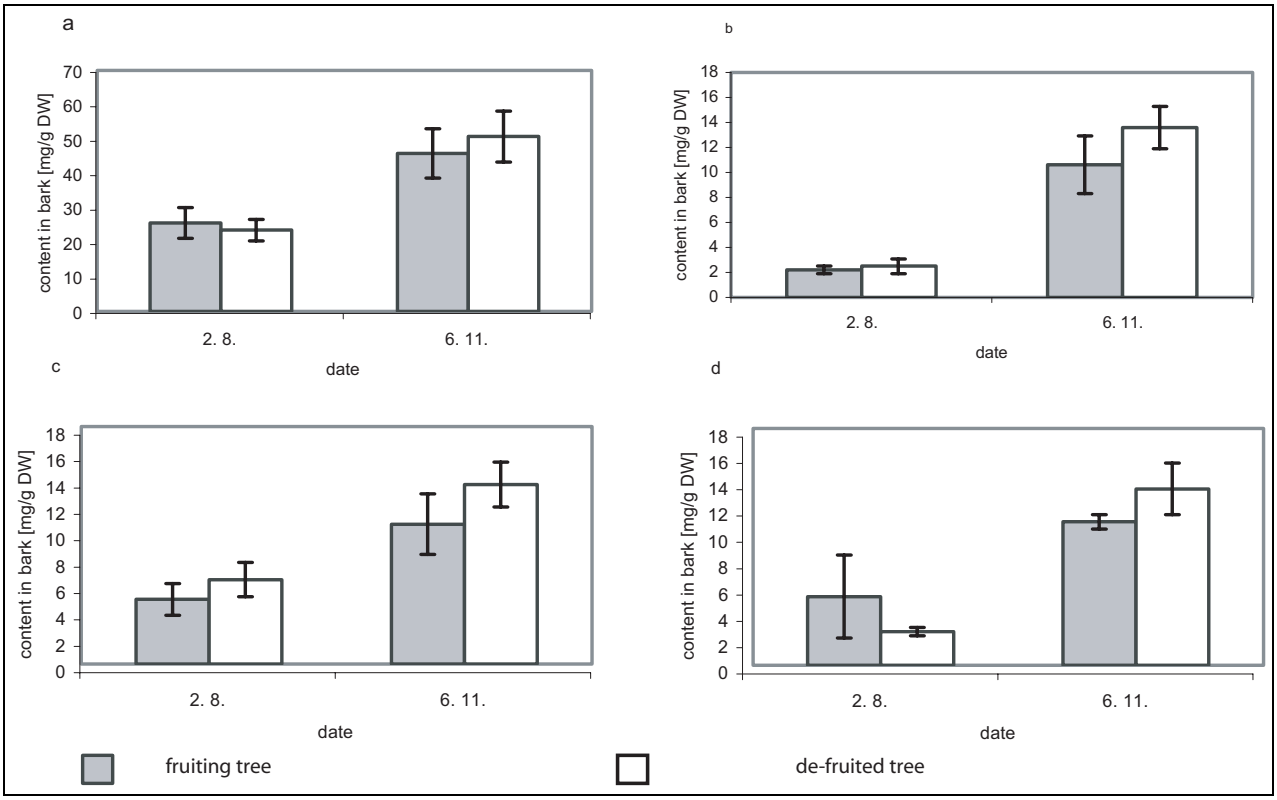


Fig. 3. Content of sorbitol (a), fructose (b), glucose (c) and sucrose (d) in bark of fruiting and de-fruited apple trees.

centration of sucrose and fructose, when August and November sampling periods are compared. This increase was especially evident in defruited trees. Intensive accumulation of sucrose in the period from October to November was documented also for cv. 'Jonagold' (YOSHIOKA et al. 1988).

For single sugars there was no difference in the bark concentration when fruiting and defruited trees were compared. It is to conclude that early fruit removal did not result in a changed carbohydrate translocation pattern. Taking into account the functions of sugars stored in bark (YOSHIOKA et al 1988), it is to expect that the trees subjected to two different treatments in our experiment will not differ in chilling and freezing tolerance and the early spring development in the following season.

Conclusions

Defruiting of trees in mid-August resulted in decreased photosynthetic rates, but only slightly affected leaf carbohydrates. Leaf and fruit levels of sorbitol, sucrose, fructose and glucose showed similar seasonal patterns to those found in previous studies (BERÜTER 1985; BERÜTER and STUDER FEUSI 1997) with no significant difference between both treatments. Similarly, no effect of defruiting on bark carbohydrates was found. In the given experimental conditions no significant effect of fruit removal on carbohydrate partitioning in 'Golden Delicious' was proved.

Acknowledgements

We thank Dr. Gregor Osterc for the translation of the title and summary to the German language.

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Received March 18, 2003 / Accepted May 30, 2003

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