Internal management of non-structural carbohydrate resources in apple leaves and branch wood under a broad range of sink and source manipulations

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Summary  Apple (Malus × domestica Borkh.) grown in a Mediterranean climate depends on regular irrigation throughout the growing season. The objective of the current study was to elucidate the changes in carbohydrate storage and utilization by mature, field-grown apple trees in response to water availability to the trees and to the level of cropping. Fourteen-year-old apple trees cv. ‘Golden Delicious’ were grown under various combinations of irrigation rate (11, 33 or 77 l day−1 per tree) and crop level (∼100, ∼300 or >1000 fruits per tree) beginning 47 days after full bloom (DAFB). Non-structural carbohydrate concentrations were measured at 78 (leaves and branch wood), 102 (leaves), 183 (branch wood) and 214 (branch wood) DAFB. Midday stem water potential (SWP) was measured at 2-week intervals between June and October. Trunk cross-sectional area was measured 47 and 265 DAFB. At harvest, 139 DAFB, the fruits of each tree were counted and weighed. SWP at 102 DAFB ranged between −0.6 and −2.7 MPa. Fruit fresh weight at harvest was positively related to SWP measured 37 days before harvest with distinct slopes for light/intermediate and heavy crop levels. Leaf and branch wood starch concentrations 78 and 102 DAFB were positively related to irrigation rate and negatively related to crop level. Mean fruit weight at harvest was positively related to branch wood starch concentration and near maximum at a concentration of 40 mg g−1 dry weight. Branch wood starch concentration recovered after harvest, especially in water-stressed trees. Sorbitol concentration was negatively related to irrigation rate. The sorbitol-to-starch concentration ratio in leaves at 102 DAFB was closely proportional to SWP. It is suggested that branch wood starch concentration represents the overall balance between carbon sources and sinks and may therefore serve as a reliable indicator of photo-assimilate availability. In water-stressed trees, sorbitol is prioritized over starch, probably to support osmotic adjustment, thereby suppressing fruit growth even further.

Keywords: fruit development, Malus × domestica, osmotic adjustment, reserves, sorbitol, starch, water deficit, water potential.

Introduction

Carbon supply and demand relations within growing plants are a key aspect in understanding production patterns of agricultural crops. Modeling carbon balance responses to atmospheric conditions in peach (Prunus persica L.; Grossman and DeJong 1994) and apple (Malus × domestica Borkh.; Lakso et al. 1999, 2001) have resulted in quite realistic estimates of shoot and fruit growth, indicating that carbon availability to sinks is often the limiting factor to crop productivity in commercial orchard operations. Carbon reserves play an important role in perennial plants, in particular deciduous trees, by supplying the required energy for the emergence and growth of new plant organs at the beginning of the growing season (e.g., Myers and Kitajima 2007). The most common reserve carbohydrate in plants is starch, although other carbohydrates, such as hemicellulose and glucans, have also been shown to be mobilized within the plant and utilized as sources of energy (Hoch 2007).

Drought may significantly limit carbon fixation by reducing stomatal conductance (Escobar-Gutiérrez et al. 1998, Naor 1998, 2001) and therefore photosynthetic rates (Beppu et al. 2003, Reyes et al. 2006). The relationship between water availability and carbon balance is fundamental for the understanding of the limitations to crop productivity in arid and semi-arid environments, where
production relies heavily on irrigation. However, temporary water deficits during the growing season are quite common in most apple-growing regions of the world. The mechanisms by which plants adapt to drought have been studied in various plant species, and the maintenance of turgor by osmotic adjustment is an important adaptive mechanism for minimizing the detrimental effects of water deficits (Morgan 1984). Osmotic adjustment has been reported in mature leaves of apple (Wang et al. 1995), where the sorbitol concentration in water-stressed potted trees increased, while the sucrose and starch concentrations decreased. Growth of the fruit requires carbohydrate supply and the existence of turgor pressure. The latter can be maintained either by increasing the overall water potential or by decreasing osmotic potential through osmotic adaptation. Irrigation rate may affect both photosynthesis and turgor, although the relationship between water availability and turgor is not well documented in relation to fruit growth.

Early season carbohydrate availability has been shown to affect apple shoot growth and fruit gelatinization (Byers et al. 1985, 1991, 1990, Bepepe and Lakso 1998, Lakso et al. 2001). Differences in available carbon resources early in the growing season may originate from previous-year effects; Greer et al. (2002) demonstrated a positive relationship between photo-assimilate accumulation in potted apple trees at the end of the growing season and the final crop level in the following season.

The distribution of newly fixed carbon among different carbohydrate species largely depends on the physiological status of the tree: a heavy sink load promotes the synthesis of soluble sugars, in apple mainly sucrose and sorbitol, which are readily loaded into the phloem and transported to sink organs (Klages et al. 2001). Conversely, a low demand for carbon enhances the accumulation of starch, first in leaves and then in roots and other woody tissues. While this phenomenon is common to many fruit tree species such as apple (Lakso et al. 2001), peach (Nii 1997) and citrus (Li et al. 2003a, 2003b), in other species such as sweet cherry, photosynthesis and non-structural carbohydrate accumulation may be independent of cropping (Roper et al. 1988).

Crop load, defined as fruit units per trunk cross-sectional area (TCSA) or per light interception by green leaf area, is known to affect carbohydrate production and partitioning in apple. Increases in crop load stimulated the photochemical efficiency of photosystem II in leaf chloroplasts (Urban et al. 2004), leaf chlorophyll concentration (Wünsche et al. 2005), stomatal conductance (Reyes et al. 2006) and subsequently net canopy gas exchange rates (Palmer et al. 1997), while decreasing the starch concentration of source leaves (Wünsche et al. 2005). Post-harvest recovery of leaf starch concentration is promoted by crop load, to the extent that by the end of the season no significant differences are found between trees bearing various crop yields (Wünsche et al. 2005). Soluble non-structural carbohydrates in leaves and phloem sap are affected by crop load to a lesser extent (Klages et al. 2001). Fruit quality attributes such as size, skin color, sugar concentration and flesh firmness are negatively related to the number of fruits on a tree as a result of competition over available carbohydrates (Buwalda and Lenz 1992, Palmer et al. 1997, Wünsche et al. 2005).

The objective of the present study was to assess changes in carbohydrate metabolism in leaves and branch wood of mature apple in response to a broad range of source (irrigation rate) and sink (fruit thinning) manipulations. Of particular interest were the specific effects of water deficit on carbon availability to support fruit growth. The results point to starch concentration, in particular branch wood starch, as a possible indicator for photo-assimilate availability to individual sinks. The interplay between starch, sucrose and sorbitol under water stress conditions is elucidated.

Materials and methods

Plant material and growing conditions

The experimental plot is situated in a commercial apple orchard in northern Israel, 33.1° N, 35.4° E at an altitude of 1000 m above sea level. During June through September, average daily maximum and minimum temperatures are 28 °C and 17 °C, respectively, with little variation (Figure 1). Precipitation does not occur during the summer, and practically all the water requirement of the trees is supplied by irrigation, starting in early May.

The experiment comprised 54 apple trees cv. ‘Golden Delicious’ (Malus × domestica Borkh.) grafted on the local rootstock ‘Hashabi 13-4’ and spaced 2.5 m × 4.5 m. Trees were 14 years old. The trees selected had flowered profusely and had a very high cropping potential. All trees were grown according to commercial practice until early June, except that irrigation and fruit thinning were not applied.

Differential treatments were initiated on 6 June 2006, 47 days after full bloom (DAFB)—at the end of the fruit cell division stage (Figure 1). Mean fruit fresh weight at this stage was 11.2 g. Two factors were examined where each treatment received one of nine irrigation rate–crop level combinations. Irrigation rates of 11, 33 or 77 mm day$^{-1}$ per tree were applied, equivalent to 1, 3 or 7 mm day$^{-1}$, respectively. Fruit counts per tree were set to ~100, ~300 and >1000 (unthinned) by manual thinning on 6 June 2006. A split plot factorial experimental design was used with irrigation rate as main factor. Each combination of irrigation rate × crop level was repeated three times having two trees per replicate, a total of 54 trees (3 irrigation levels × 3 crop levels × 3 replicates × 2 trees). Irrigation was applied daily using a drip system. An effort was made to minimize water percolation beneath the root zone by restricting continuous water supply to 1 mm pulses. Irrigation blocks were surrounded by border trees and rows. In thinned trees, each fruit cluster was allowed to bear one fruit. After harvest, differential irrigation
was maintained at the same rates until the first autumn rainfall, which occurred that year on 15 October.

**Midday stem water potential measurements**

Midday stem water potential (SWP) was measured bi-weekly beginning at the onset of differential irrigation treatments (47 DAFB). Two leaves from the shaded portion of the canopy of each tree were sealed while intact with plastic bags wrapped in aluminum foil pre-noon, allowing them to reach equilibrium with the branch wood for at least 90 min; they were removed by a sharp cut through their petioles, covered with a plastic bag and immediately inserted in a pressure chamber (‘Arimad II’; Kfar-haruv, Israel) where pressure was gradually increased using compressed nitrogen. Chamber pressure was recorded at the stage when a droplet formed at the cut end of the petiole (Boyer 1967).

**Non-structural carbohydrate analyses**

Leaves and branch wood were sampled for non-structural carbohydrate (NSC) analysis from two randomly selected trees of each irrigation rate–crop level combination. Leaf samples comprised 10 leaves from non-fruiting spurs on each tree. Branch wood samples comprised mature (4–6 years old) sections 10–20 mm in diameter, situated on two different branches at the shaded, bottom portion of the tree. Samples were collected on 7 July 2006 (78 DAFB—Figure 1; leaves and branch wood), 31 July 2006 (102 DAFB; leaves only), 20 October 2006 (183 DAFB; branch wood only) and 20 November 2006 (214 DAFB; the branch sections only). The same 18 trees were sampled at each measuring date. Samples were collected in the morning and heated in a drying oven to 90 °C for 90 min to eliminate all enzymatic activity (Schädel et al. 2009). Thereafter, the branch sections were cut to 2-mm-thick slices, and both leaves and branch wood were dried at 65 °C for an additional 36 h before they were kept at −18 °C until analysis. Samples were ground to a fine powder. Starch concentration was determined according to the method described by Li et al. (2003b). Samples were repeatedly rinsed with ethanol to remove all soluble sugars and reacted with the enzyme amyloglucosidase to hydrolyze the starch. Thereafter, soluble sugar concentration was determined spectrophotometrically. Sucrose, glucose, fructose and sorbitol concentrations were determined by high-performance liquid chromatography (HPLC) following the method described by Olesinski et al. (1996). All carbohydrate concentrations were determined on a dry weight (dw) basis.

**Starch staining**

Cross-sections of branch wood were sampled and treated as previously described. Previously heated (90 °C for 90 min) branch wood slices were immersed in lugol solution for 20 s and washed under running water. Thereafter, the stained cross-sections were allowed to dry at 25 °C for at least 24 h before they were photographed under magnification using a DC300FX camera (Leica, Solms, Germany).

**Fruit fresh weight**

Trees were picked individually on 7 September 2006 (139 DAFB). The fruit of each tree was counted, weighed and sorted according to diameter categories at 5 mm intervals between 30 and 90 mm using a commercial grader (‘Greefa’; Tricht, The Netherlands). To calculate mean fruit fresh weight gain, mean fresh weight at onset of the differential treatments was subtracted from fresh weight at harvest.

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Figure 1. Daily maximum and minimum temperatures and precipitation rates at the experimental plot during the study, between 6 June and 30 November 2006. Key events are marked with arrows at the top of the chart. Numbers (DAFB) specify the sampling days for carbohydrate measurements.
Table 1. A summary of some key observations—number of fruits per tree, SWP at 102 DAFB, fruit fresh weight gain between 47 and 139 DAFB and TCSA increment between 47 and 265 DAFB for different irrigation rate–crop level combinations.

<table>
<thead>
<tr>
<th>Irrigation rate (1 day(^{-1}) per tree)</th>
<th>Crop level treatment</th>
<th>Crop level (fruit tree(^{-1})) ± SE</th>
<th>Mid-summer SWP (MPa) ± SE</th>
<th>Mean fruit fresh weight gain (g) ± SE</th>
<th>TCSA increment (cm(^2)) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Light</td>
<td>85 ± 7.1</td>
<td>−1.97 ± 0.02</td>
<td>99.4 ± 6.83</td>
<td>3.65 ± 2.05</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>258 ± 0.7</td>
<td>−2.56 ± 0.15</td>
<td>58.5 ± 0.31</td>
<td>−3.75 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>1002 ± 58.0</td>
<td>−2.69 ± 0.22</td>
<td>19.7 ± 1.56</td>
<td>−0.35 ± 0.48</td>
</tr>
<tr>
<td>33</td>
<td>Light</td>
<td>103 ± 8.5</td>
<td>−1.23 ± 0.19</td>
<td>155.3 ± 7.45</td>
<td>2.15 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>292 ± 7.8</td>
<td>−1.44 ± 0.10</td>
<td>137.7 ± 11.36</td>
<td>2.61 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>1048 ± 128.7</td>
<td>−1.54 ± 0.06</td>
<td>66.7 ± 1.93</td>
<td>−0.21 ± 0.74</td>
</tr>
<tr>
<td>77</td>
<td>Light</td>
<td>95 ± 6.4</td>
<td>−1.08 ± 0.01</td>
<td>173.5 ± 1.52</td>
<td>13.5 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>275 ± 2.8</td>
<td>−0.83 ± 0.01</td>
<td>176.8 ± 4.91</td>
<td>2.85 ± 4.95</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>1460 ± 26.9</td>
<td>−0.99 ± 0.08</td>
<td>94.4 ± 1.2</td>
<td>1.25 ± 1.25</td>
</tr>
</tbody>
</table>

Trunk measurements

Trunk circumference at 10 cm above ground was determined for each tree using a measuring tape. Measurements were made at the onset of differential treatments (47 DAFB) and again when trees were dormant (265 DAFB). TCSA was computed for each measurement.

Statistical analysis

Results were analysed using JMP 5.1 software (SAS institution, Cary, NC). As both factors (irrigation level and crop level) are continuous variables, regressions were the predominant analytical procedure. Effects were considered significant at \( P < 0.05 \).

Results

Irrigation and SWP

Cumulative irrigation levels between the onset of differential treatments and harvest (47–139 DAFB) were 7,320, 3,140 and 1,110 l per tree for fully irrigated, mildly stressed and severely stressed trees, respectively. The resulting mean coefficients of evapotranspiration were 1.0, 0.43 and 0.15, respectively. SWP of water-stressed trees decreased over the first 4 weeks, while remaining unchanged in non-stressed trees. In this report, only SWP data from 31 July 2006 (102 DAFB) will be presented, as they were typical of values recorded over July through September. The full SWP dataset was published elsewhere (Naor et al. 2008). SWP values ranged from −0.8 to −1.1 MPa in well-irrigated, −1.2 to −1.5 MPa in mildly water-stressed and −2.0 to −2.7 MPa in severely water-stressed trees (Table 1). There was a trend of decreasing SWP with increasing crop load in all the irrigation treatments, although it was significant only in the low irrigation treatment (Table 1).

Non-structural carbohydrate concentration

Carbohydrate concentrations were measured in leaves and branch wood on several occasions, while at 78 DAFB measurements were performed in both organs. All NSC species reached higher concentrations in leaves compared with branch wood (Table 2). However, while starch concentration was only slightly higher in leaves, sucrose and fructose were twofold higher and sorbitol and glucose fourfold higher in leaves (Table 2). In total, NSC concentration in leaves was approximately double that in branch wood and reached 12–17% of leaf total dry weight. Branch wood had a higher portion of its NSC pool as starch (55.6 and 35.1% for branch wood and leaves, respectively). Leaves, on the other hand, had a higher portion as sorbitol (38.1 and 20.6% for leaves and branch wood, respectively).

Starch concentrations as well as total NSC in leaves decreased substantially in all treatments between 78 and 102 DAFB (Table 2). In branch wood, all NSC species were higher in the autumn than in the summer (Table 2); sucrose concentrations increased most remarkably, fourfold to 10-fold. Starch concentrations increased to the greatest extent in the severely water-stressed and in the high crop level treatments, respectively. In the fully irrigated, low cropping treatment, starch concentration decreased between 183 and 214 DAFB. Sorbitol concentrations reached similar levels in all treatments by leaf fall. Glucose concentrations increased about twofold, and fructose concentrations increased only slightly.

In both organs, starch concentrations were positively related to irrigation rate and negatively related to crop level at 78 (Table 3) and 102 DAFB (data not shown). In trees suffering severe water deficit and bearing intermediate or heavy crops, a sharp increase in branch wood starch was apparent after the beginning of autumn rains (183, 214 DAFB, Figure 2, Table 2). Figure 3 demonstrates the extent and the spatial distribution of starch concentration in branch wood where it is preferentially deposited in the core, ray and primary xylem tissues, but as its concentration grows, further deposition is detected in secondary xylem as well. In both organ types, sorbitol concentration was negatively related to irrigation rate prior to harvest (Figure 4, Table 3). Inflicting mild water stress on trees increased sorbitol concentrations at 78 DAFB by 14 and 20% compared with non-stressed trees in leaves and branch wood, respectively (Figure 4). Trees suffering severe water
Table 2. Concentrations of specific NSCs expressed in milligrams per gram dry weight in leaves and branch wood of 14-year-old apple trees cv. ‘Golden Delicious’ as measured 78, 102, 183 and 214 DAFB. Values are means ± SE. \( n = 2 \).

<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Crop level group</th>
<th>DAFB</th>
<th>Starch</th>
<th>Sorbitol</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
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<tr>
<td></td>
<td></td>
<td>78</td>
<td>11.2±0.0</td>
<td>11.2±0.0</td>
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<td></td>
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<td>102</td>
<td>21.4±0.3</td>
<td>21.4±0.3</td>
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<td>21.4±0.3</td>
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<td>183</td>
<td>31.2±0.5</td>
<td>31.2±0.5</td>
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<td>31.2±0.5</td>
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<td></td>
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<td>214</td>
<td>40.3±0.7</td>
<td>40.3±0.7</td>
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<tr>
<td>11</td>
<td>Light</td>
<td>78</td>
<td>42.5±2.5</td>
<td>42.5±2.5</td>
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<td>42.5±2.5</td>
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<tr>
<td>11 Light</td>
<td></td>
<td>102</td>
<td>27.0±1.0</td>
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<td>11 Light</td>
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<td>183</td>
<td>35.5±0.5</td>
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<td>11 Light</td>
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<td>78</td>
<td>22.6±0.0</td>
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<td>11 Light</td>
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<td>214</td>
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stress had 43 and 60% higher sorbitol concentrations compared with non-stressed trees in leaves and branch wood, respectively (Figure 4). Sorbitol was negatively related to crop level in leaves but not in branch wood (Table 3). Its concentration in branch wood increased sharply at 183 and 214 DAFB. The ratio between sorbitol and starch concentrations in leaves at 102 DAFB reflected the degree of water stress and was negatively related to SWP (Figure 5). Sucrose concentration was positively related to irrigation in leaves but not in branch wood and was independent of crop level (Table 3). Sucrose concentrations gradually increased over the season in both leaves and branch wood and reached their highest levels close to leaf senescence, 214 DAFB. Glucose and fructose concentrations were not affected by irrigation rate or crop level (Table 3).

Fruit growth

Mean fruit fresh weight at the start of differential treatments in early June (47 DAFB) was 11.2 g in all treatments. Both irrigation rate and crop level had a considerable effect on fruit growth during the period until harvest in early September, 139 DAFB (Table 1): fruit growing on well-irrigated, lightly cropping trees gained, in average, ~176 g (fresh weight), whereas fruit growing on severely stressed, heavily cropping trees gained only ~20 g.

Mean fruit fresh weight gain until harvest was positively and linearly related to SWP at all three crop levels, but it was less responsive to SWP in the high crop load (P < 0.05) (Figure 6).

Mean fruit fresh weight gain between 47 DAFB and harvest correlated with starch concentration at 78 DAFB in a saturation curve, approaching its maximum at starch concentrations of ~60 mg g⁻¹ dw (r² = 0.64) and ~40 mg g⁻¹ dw (r² = 0.91) for leaves and branch wood, respectively (Figure 7). Fruit growth was not significantly related to any other single NSC species. Starch concentrations in leaves and branch wood at 78 DAFB were better predictors of fruit fresh weight than accumulated irrigation, crop level or SWP alone (Table 4), but it was well predicted by irrigation rate and crop level using a multivariate linear model (Table 4). Fruit growth rate was more tightly related to SWP than to irrigation rate alone. This may be accounted for by the effect of crop level on SWP in both deficit irrigation treatments (Table 1).

TCSA increment

TCSA increment was positively related to irrigation rate and negatively related to crop level (Table 1). A quantitative analysis of the effect of branch wood starch concentration on TCSA increment indicates that significant growth in trunk area occurred only in those trees where branch wood starch concentration in early July exceeded a threshold of approximately 40 mg g⁻¹ dw (Figure 8). Decreases in TCSA along the season, which were reported for five trees, are attributed to measurement errors.

Discussion

In the present study, we examined the response of apple trees to manipulations made to both source (deficit irrigation) and sink (fruit thinning) capacities, which created a broad range in the degree of availability of assimilates at tree level. This platform allowed us to explore the responses of various NSC species in apple leaves and branch wood to those manipulations and to identify which NSCs may serve as indicators for the availability of assimilates for fruit growth. We relied on fruit numbers to represent the sink capacity of the tree, ignoring other sinks. This approach greatly simplifies the complex carbon allocation patterns existing in apple trees (Lakso et al. 2001); its justification derived from the fact that as much as 70% of all carbon fixed by apple trees throughout a growing season is stored or respired by developing fruit (Buwalda and Lenz 1992, Palmer et al. 2002). This proportion increases even further during the period of cell elon-
Figure 2. Starch concentrations in branch wood as measured at 78, 183 and 214 DAFB in various irrigation and crop level treatments. Vertical bars represent standard errors. $n = 2$. 
The effect of source-sink balance on NSC concentrations

Starch is the only substance, out of the five analyzed, the concentration of which increased with irrigation rate and decreased with increasing crop level (Table 3, Figure 2). The quantity of starch stored in perennial branch wood was related to leaf starch concentration as measured in the morning ($r^2=0.77$; data not shown). Leaves, as a primary source tissue, actively export most of the carbohydrates they produce. Starch pools in leaves are transient and change dramatically on a diurnal basis, their size reaching maximum at dusk and minimum at dawn (Nii 1997). Perennial branches serve mainly as rigid structures supporting the plant vascular system. As such, their growth is relatively slow, and their demand for carbon is minimal. Their proximity to the phloem makes them an ideal host for a carbon ‘buffer pool’ which would be available to growing sinks. The size of this carbon reservoir would be dictated by the balance between carbohydrate supply and demand. While it is not expected to be subject to a prominent diurnal pattern (Chaumont et al. 1994, Klages et al. 2001, Thorpe et al. 2005, Schädel et al. 2009), it may well change over a time scale of several weeks, reflecting the carbohydrate availability throughout the tree (Chapin et al. 1990, Thorpe et al. 2005, Schädel et al. 2009).

Photo-assimilate metabolism in source leaves has been extensively studied in relation to starch storage in chloroplasts (e.g., Zeeman et al. 2004, Bondada and Syvertsen 2005). Defruiting of peach (Nii 1997) and citrus (Li et al. 2003) resulted in a sharp increase in leaf starch concentration after a few days, demonstrating its sensitivity to whole-tree sink load. Correspondingly, CO$_2$ enrichment (Pan et al. 1998), prolonging the photoperiod (Wang et al. 1997, 1998) and girdling (Zhou and Quebedeaux 2003) increased starch accumulation in leaves and branches, demonstrating its sensitivity to the source capacity of the tree. At low crop levels, as carbon assimilation exceeds the demand posed by active sinks, chloroplasts may store starch to the point where internal photosynthetic membranes are physically disrupted or even disintegrated (Schaffer et al. 1986, Pammenter et al. 1993, Lombardini et al. 2004, Bondada and Syvertsen 2005, Wünsche et al. 2005). These changes may cause a reduction in the photosynthetic capacity of the tree, as manifested in the present study by the smaller post-harvest accumulation of starch in branch wood of fully irrigated trees compared with mildly water-stressed ones (Figure 2). Alternatively, this observation could be accounted for by an earlier post-harvest mobilization of carbon reserves to roots in well-irrigated, low cropping trees (Perry 1971, Ögren 1999, Barbaroux et al. 2003).

Sorbose concentration in leaves was positively related to irrigation rate (Table 3), indicating an increasing photosynthetic rate by source leaves as water status improves through a direct effect on stomatal conductance (Chavez 1991). How-
ever, sucrose level in leaves was less sensitive to the source–sink balance than the levels of starch and sorbitol, suggesting a rather complex regulation of phloem loading by sorbitol and sucrose (Wang et al. 1997, Zhou et al. 2001). The marked increase in branch wood sucrose towards the end of the growing season is probably related to the mobilization of above-ground carbohydrate reserves into the root system (Barbaroux et al. 2003).

Sorbitol has been shown to play a double role in apple and other fruit trees of the Rosaceae family: like sucrose, it functions as a major transport sugar (e.g., Nii 1997, Nosarszewski et al. 2004). Our data demonstrate an inverse relationship between sorbitol concentration in leaves and crop level in the well- and intermediately irrigated treatments (Figure 4), suggesting increased translocation at lower crop load. However, sorbitol has also been shown to act as a key player in osmotic regulation (Wang et al. 1995), allowing trees to maintain some photosynthetic activity under water deficiency. In the present study, this carbohydrate species constituted over half of the soluble carbohydrate fraction in leaves in all treatments. Its concentration in both leaves and branch wood increased in response to water stress (Figure 4, Table 3) opposite to the other soluble carbohydrates (Table 3). This finding may support the hypothesis of the role of sorbitol in osmotic adjustment (Wang et al. 1995).

Glucose and fructose concentrations in leaves and branch wood are generally lower than those of starch, sorbitol and sucrose. These reducing hexoses are chemically active and are involved in primary metabolism, especially in leaves; thus, they do not seem to act as part of the photo-assimilate reserves in apple trees. Indeed, they did not respond to the source and sink manipulations (Table 3). Moreover, Cheng et al. (2005) showed that blocking sorbitol synthesis in apple leaves results in increased starch accumulation while sucrose, glucose and fructose concentrations remained essentially unaffected.

Figure 5. The relationship between midday SWP and the sorbitol:starch concentration ratio in leaves of apple trees cv. ‘Golden Delicious’. Both variables were measured 102 DAFB. $r^2 = 0.91$.

Figure 6. The effect of midsummer midday SWP as measured 102 DAFB on fruit fresh weight gain between 47 and 139 DAFB at various irrigation rate–crop level combinations. Each point represents a single tree. $r^2$ values for linear relations are 0.97, 0.97 and 0.95 for light, intermediate and heavy crop levels, respectively.
The primary storage sites for carbon in deciduous woody plants seem to shift during the growing season; roots have been shown to host the bulk of NSC over the dormant season in apple (Khan et al. 1998, Greer et al. 2002) and in some forest species (Barbaroux et al. 2003, Landhäusser and Liefers 2003), whereas during the growing season, the fruit often contains the largest NSC reservoir (Lakso et al. 2001). The non-structural carbon pooled in the tree scaffold is not directly utilized (as is the case in growing shoots and roots) or irreversibly deposited (as is the case in fruit). Thus, branch wood starch can be viewed as a carbon reservoir available to support growth of other tree organs. The mobile nature of starch pools in branch wood tissue is also suggested from Figure 3, which demonstrates the anatomical patterns of starch distribution across the sapwood: starch is preferentially deposited in the core, in xylem rays and in the youngest xylem, adjacent to the cambium. As surplus carbohydrate keeps accumulating, starch deposition expands to older tracheal elements and fills the entire cross-section of the branch. Tracheal tissues do not maintain metabolism of any kind, and thus this starch could not be utilized to support energetic demands within the storing cells.

Table 4. A comparison of various terms as predictors of mean fruit fresh weight (g) at harvest of 14-year-old apple trees cv. ‘Golden Delicious’. Midday SWP was measured in mid-summer (102 DAFB). Carbohydrate concentrations in leaves and branch wood were measured 78 DAFB.

<table>
<thead>
<tr>
<th>Term</th>
<th>Slope</th>
<th>$r^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation rate (l per tree day$^{-1}$)</td>
<td>1.32</td>
<td>0.43</td>
<td>0.0032</td>
</tr>
<tr>
<td>Crop level (fruit tree$^{-1}$)</td>
<td>-0.066</td>
<td>0.37</td>
<td>0.0128</td>
</tr>
<tr>
<td>Irrigation rate–crop level (multiple regression)</td>
<td>-</td>
<td>0.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Water potential (MPa)</td>
<td>63.6</td>
<td>0.63</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leaf starch concentration (mg g$^{-1}$ dw)</td>
<td>5.15</td>
<td>0.64</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Branch wood starch concentration (mg g$^{-1}$ dw)</td>
<td>5.40</td>
<td>0.91</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
The effect of water stress on NSC concentrations

Water stress in apple trees has been hypothesized to affect fruit growth through two possible mechanisms: (i) reduction in cell turgor which directly attenuates cell elongation (Green et al. 1971) and (ii) limiting carbohydrate availability to growing sinks due to a decline in stomatal conductance, which reduces the photosynthetic rate (McQueen et al. 2004). Water potential is known to affect turgor (Boyer 1968); however, osmotic adjustment may interfere with this effect. In the present study, leaf sorbitol concentration increased with decreasing SWP (Figure 5), suggesting active osmotic regulation in stressed trees (Chavez 1991, Wang et al. 1995), which would be required to maintain some stomatal conductance even at extremely sparse water availability.

The best single parameter to predict fruit growth was branch wood starch ($r^2 = 0.91$); the correlation was far higher than other single parameters (Table 4) but similar to the combined effect of irrigation rate and crop level on fruit growth ($r^2 = 0.88$; Table 4). Both water status and crop load affect starch concentration (Table 3), and it seems that branch wood starch concentration integrates the effects of both irrigation and crop load on the availability of assimilates to support fruit growth.

The large differences in water status in the three irrigation rates (Table 1) did not alter the response of fruit growth to starch concentration (Figure 7), indicating that deficit irrigation did not change fruit turgor to an extent that may affect fruit growth. Starch concentration, affected by both source and sink capacities (Table 4), is a superior predictor of carbohydrate availability to sinks compared with water potential, which reflects mainly the source capacity. These results provide evidence of carbohydrate supply, rather than turgor, being the predominant limiting factor to fruit growth in water-stressed apple trees. Similar conclusions have been drawn for tomato (Liu et al. 2007).

The priority of carbohydrate reserves

Maintaining a non-structural carbon pool within the tree structure may represent an evolutionary survival strategy. In the present study, even heavily cropping, water-stressed trees had a branch wood starch concentration exceeding 10 mg g$^{-1}$ dry weight throughout the summer (Figure 2). On the other hand, under the same conditions TCSA increment was completely eliminated (Figure 8). Furthermore, Figure 2 demonstrates rapid replenishment of the starch pool in the branch wood of severely water-stressed, heavily cropping trees in the autumn, probably as a response to rain (Figure 1). Even then, in the absence of a crop, with sufficient soil water and with moderate temperatures, there was no apparent shoot growth in those trees. It is possible that the removal of fruit promotes leaf senescence and winter dormancy (Paul and Foyer 2001). As the growing season progresses, carbohydrate reserves seem to develop substantial sink strength and actively compete with growing organs (Roper et al. 1988, Chapin et al. 1990, Wyka 1999).

The pattern of the response of fruit growth rates (Figure 7) and TCSA accumulation (Figure 8) to branch wood starch concentration after the cessation of spring vegetative growth demonstrates a situation where fruit has an absolute priority over tree structure as far as carbon allocation is concerned: TCSA did not significantly increase until fruit growth rate reached its full potential at a starch concentration of $\sim 40$ mg g$^{-1}$ dry weight. This finding fits the ‘hierarchical’ approach of carbon allocation (Bustan and Goldschmidt 1999), where a ‘predefined’ set of priorities within the plant dictates the relative growth rate of each sink type (Lakso et al. 2001). Such ‘central control’ of carbohydrate allocation may provide a mechanism for adjustment of tree dimensions through the balance between fruiting and available carbon resources (Naschitz and Naor 2005). Fresh weight gain of fruit varied ninefold from the most carbohydrate-deprived trees to...
those where carbohydrate reserves were most abundant. At the same time, starch concentration increased only fourfold in branch wood and twofold in leaves (Figure 7). These results imply that fruit has a higher priority than reserves in the apple tree during the second half of the fruit development period (e.g., Lakso et al. 2001). However, the situation changes abruptly after harvest, with reserve pools in roots (Wyka 1999) and branches acquiring higher priority.

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References


