The Role of Carbohydrates in Active Osmotic Adjustment in Apple under Water Stress

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Abstract. Greenhouse grown 2-year-old potted ‘Jonathan’ apple trees (Malus domestica Borkh.) were subjected to various levels of water stress in February. Midday leaf water potential ($\psi_w$), leaf osmotic potential ($\psi_S$), soluble sugars, and starch contents of mature leaves were measured throughout the development of water stress to determine whether active osmotic adjustment could be detected and whether carbohydrates were involved. Active adjustments of 0.6 MPa were observed 3 and 5 days, respectively, after water stress was initiated. Leaf turgor potential ($\psi_t$) could not be maintained through the osmotic adjustment when $\psi_t$ dropped below $-1.6$ MPa. Sorbitol, glucose, and fructose concentrations increased while sucrose and starch levels decreased significantly as water stress developed, strongly suggesting that sugar alcohol and monosaccharide are the most important osmotica for adjustment. Sorbitol was a primary carbohydrate in the cell sap and accounted for $>50\%$ of total osmotic adjustment. The partitioning of newly fixed $^{14}$C-labeled photosynthates in mature leaves was not affected by water stress immediately after the 30-min $^{14}$CO$_2$ treatment. All the $^{14}$C-labeled carbohydrates decreased in the labeled leaves very rapidly after $^{14}$CO$_2$ labeling. The decrease in $^{14}$C-sorbitol was greater than the decrease in other carbohydrates under both well-watered and stressed conditions. After 24 hours of water stress, however, the percentage of $^{14}$C-sorbitol increased while the percentages of sucrose, starch, glucose, and fructose decreased significantly with increasing levels of stress. The ratio of $^{14}$C-sorbitol in leaves with $\psi_w=\psi_p=-3.5$ MPa to leaves with $\psi_w=\psi_p=-0.5$ MPa was significantly higher than that of $^{14}$C-sucrose, $^{14}$C-glucose, $^{14}$C-fructose, or $^{14}$C-starch.

Water deficit can cause serious losses in most crop plants, including apple trees in arid regions. Water stress has been shown to affect many plant processes, for example, cell growth (Acevedo et al., 1971), ABA synthesis (Cornish and Zeevaart, 1984), stomatal opening (Henson et al., 1989), CO$_2$ assimilation (Robinson et al., 1988), and sugar accumulation (Harada et al., 1983). The responses of plants to water stress have been reviewed by Hsiao (1973), Morgan (1984), Jones et al. (1985), and Turner (1986).

Many plants develop morphological and physiological adaptations to water stress. Some examples of adaptation to water stress are rapid completion of ontogeny, leaf rolling, reduced leaf size, low stomatal conductance, and high root : shoot ratios (Landsberg and Jones, 1981; Morgan, 1984). Through osmotic adjustment, some species actively accumulate solutes during water stress and decrease $\psi_t$, so that $\psi_S$, remains above levels critical for cellular expansion (Hsiao, 1973; Hsiao et al., 1976; Turner, 1979). Long-term osmotic adjustment to periodic stresses and short-term diurnal adjustment both provide an effective mechanism to maintain sufficient turgor for stomatal opening and photosynthetic productivity (Lakso, 1983). Inorganic cations, organic acids, amino acids, and sugars, which are the primary osmotica, bring about osmotic adjustment through either internal synthesis or uptake of osmotically active substances (Muñns and Weir, 1981; Nicolas et al., 1985; Rhodes et al., 1986; Turner, 1979). There is little doubt that soluble sugars can contribute to osmotic adjustment. In agronomic crops an increase in soluble sugar concentration in response to water stress has been found in roots and leaves (Cutler et al., 1977; Drossopoulos et al., 1987; Turner et al., 1978). However, low levels of solute in sorghum (Newton et al., 1986) and of total carbohydrate in maize (Thankur and Rai, 1980) have been associated with increasing water stress.

Apple (Lakso et al., 1984), orange [Citrus sinensis (L.) Osb.] (Fereres et al., 1979), peach [Prunus persica (L.) Batsch] (Steinberg et al., 1989), cherry (Prunus spp.) (Ranney et al., 1991), and grape (Vitis vinifera L.) (Düring, 1985) have been shown to adjust osmotically in response to drought. Adjustments varying from 0.5 to 3 MPa have been found, but only 0.2 to 0.5 MPa was from active adjustment. The remainder was due to passive dehydration (Davies and Lakso, 1979; Fereres et al., 1979; Goode and Higgs, 1973). However, as high as 2.5 MPa seasonal active osmotic adjustment has been observed in mature field-grown McIntosh apple trees from June to September (Lakso et al., 1984). Fanjul and Rosher (1984) and Goode and Higgs (1973), suggested that photosynthetic accumulation or sugar concentration contributes to this adjustment. The results of Davies and Lakso (1979), however, failed to show active adjustment in the field-grown and potted trees on a seasonal basis. They found that the soluble carbohydrate content of apple leaves did not significantly change during the day. Syvertsen and Albrigo (1980) also failed to observe osmotic adjustment in 20-year-old orange trees. In the literature reviewed, most experiments concentrated on total solutes as osmotica with few reports on total sugar and even fewer on individual sugar level and the role of starch.

In the woody Rosaceae species, sorbitol is the major photosynthetic product and is translocated to developing sink tissues (Bieleski, 1969; Hansen and Grauslund, 1978). Sorbitol, accounting for $60\%$ to $80\%$ of the C translocated in Rosaceae (Webb and Burley, 1962), is reported to be produced in mature leaves and transported to young leaves (Bieleski and Redgwel, 1985). Because sorbitol constitutes a major carbohydrate component in apple trees, we hypothesize that it has an important role in osmotic adjustment during water deficit. The objectives
of this study were to investigate the extent of osmotic adjustment of mature leaves in drought-exposed potted apple trees, the roles that individual carbohydrates play in active osmotic adjustment, the importance of sorbitol in total osmotic adjustment, and the causes of active osmotic adjustment.

Materials and Methods

Plant materials and treatments. The experiments were carried out in February in a greenhouse using 2-year-old ‘Jonathan’/M7 apple trees grown in 3.8-liter pots that were 17 cm in diameter and 17-cm deep and filled with Professional Growing Medium 300-S (Pro-gro Products, Elizabeth City, N.C.). Variation in midday light intensity was 800 to 1000 μmol·m⁻²·s⁻¹, photosynthetic photon flux density for all the treatments, and no supplemental lights were provided. Midday air temperature in the greenhouse was 20 to 25°C. All plants experienced several cycles of water deficit that resulted in midday turgor loss of young leaves, but did not induce terminal bud set or leaf abscission before treatments were started. All trees were watered equally before being treated. Four water stress treatments were initiated on 3 Feb. on actively growing plants with ~40 leaves each on extension shoots. In the nonstressed treatment (control), 1 liter water was applied to each pot every day and \( \psi_w \), was maintained between ~0.5 and ~1.0 MPa at midday under sunny conditions. In the mildly stressed treatment, plants were watered at one-half the volume of water given to control plants. Water potential reached ~1.0 to ~1.5 MPa at midday, 1 day after the initiation of water stress, and then was maintained at that level throughout the experiment by applying small amounts of water as needed. The moderately stressed plants were watered at one-fourth the volume of water given to control plants. Here, \( \psi_w \), reached ~2.0 to ~2.5 MPa at midday 2 days after the initiation of water stress and then was maintained at that level throughout the experiment by misting the soil as needed. The severely stressed plants were not watered during the experiment and \( \psi_w \), was < ~3.0 MPa. The plot design was a randomized complete block with three single-tree replications per treatment.

Photosynthesis (\( P_n \)) and stomatal resistance measurements (\( R_s \)). A mature leaf from a vigorously growing shoot was inserted into a 1/4-liter leaf chamber of LI-6200 (LI-COR, Lincoln, Neb.) portable photosynthesis system before \( \psi_w \) measurement. The leaf was allowed to photosynthesize inside the chamber under full sunlight. \( P_n \) was determined when CO₂ concentration dropped 10 ppm after a steady rate of CO₂ use had been obtained, which typically required 5 to 30 sec per sample, depending on the photosynthetic rate. The leaf area inside the chamber was determined with a LI-3000 portable area meter (LI-COR).

CO₂-labeling. One growing branch of each tree was sealed in a clear plastic bag at 11:30 AM on 4 Feb. Previously generated \( ^{14}CO_2 (3.7 \times 10^{6} Bq) \) was injected into each bag within 1 min for all the treatments, and the leaves were allowed to photosynthesize for 30 min in full light before the bags were removed. The labeled leaves were harvested as described below.

\( \psi_w \) and relative water content (RWC) measurement. Six mature leaves were harvested from each tree immediately after the bags were removed and then again 24, 48, and 96 h later. The leaves from each tree were separated into two groups of three leaves each. One group of leaves was used to determine RWC (Barrs and Weatherly, 1962). The other group was used for \( \psi_w \) measurements by use of a pressure bomb. The amount of water lost during \( \psi_w \) determinations was not measured. Then the samples were sealed into plastic bags, put into an ice chest, and transported to the laboratory for \( \psi_s \), and individual carbohydrate determinations.

\( \psi_s \) and soluble sugar analysis. The leaves were frozen and stored at ~70°C until analyzed. The sap of thawed leaves was expressed with a garlic press. Ten μl of sap was used for \( \psi_s \), determination with a Wescor 5500 vapor pressure osmometer (Wescore, Logan, Utah). Osmotic potential was adjusted to 100% RWC to eliminate passive tissue dehydration using the following equation: \( \psi_s = \frac{100 \times (\psi_s - RWC)/100}{\psi_s - \psi_x} \).

\( \psi_s \) was calculated from the difference between \( \psi_s \) and \( \psi_x \). The contribution of the apoplastic water to \( \psi_s \) was not independently determined so \( \psi_s \) included the contributions of both symplastic and apoplastic water. About 1 ml remaining sap was centrifuged, and the supernatant was diluted lo-fold with 18 MOhm deionized water. One milliliter of diluted sap was filtered through 0.2 μm-nylon sterile filters into 1.5-ml dram vials. Ten microliters were injected into a Shimadzu HPLC (Shimadzu, Kyoto, Japan) for individual sugar analysis. A Shimadzu RID-6A refractive index detector at 30°C was used to quantify sugar content following separation on a Bio-Rad HPX-87C carbohydrate column (300 × 7.8 mm) at 85°C using helium-sparged, 18 MOhm, distilled, deionized H₂O at 0.6 ml·min⁻¹ as the mobile phase. The individual carbohydrates were confirmed by comparing retention times of 10 μl standard sample containing (μg) 20 sucrose, 20 glucose, 20 fructose, and 20 sorbitol. The concentrations of carbohydrates were recalculated to 100% RWC using the equation given for \( \psi_s \), calibration to eliminate the impact of passive tissue dehydration.

Starch analysis. The residue of expressed leaves was dried, weighed, and extracted four times with 10 ml 80% methanol (MEOH). The four MEOH supernatants were discarded and the pellet retained. Five milliliters 18 MOhm water was added to the pellet, and the resulting suspension was boiled for 30 min to swell starch granules. After cooling at room temperature, the solution was digested with 1 ml 1% amyloglucosidase (EC 3.2.1.3) (Sigma Chemical Co., St. Louis, Lot no. 24F-0314) at 55°C in a water bath overnight. The digest was centrifuged and filtered, and then 10 μl of digest was injected into a Shimadzu HPLC for glucose determination as described for the soluble sugar analysis. Starch concentration was determined by converting the glucose equivalent to starch.

C-soluble carbohydrate counting. An Advantec-SF-2120 (Osaka, Japan) fraction collector was used to collect each soluble sugar in a scintillation vial following HPLC separation. Then, 3 ml of Beckman Ready Gel liquid scintillation cocktail was added to each vial and \(^{14}C\)-labeled sorbitol, sucrose, glucose, and fructose fractions were counted via a LKB 1219 Rack-beta (Turku, Finland) liquid scintillation counter.

C-starch analysis. A 100-μl aliquot of the digest was injected into a Shimadzu HPLC, the glucose fraction collected, and \(^{14}C\)-glucose counted. The disintegration per minute (DPM) of \(^{14}C\)-starch was calculated from the equivalency of \(^{14}C\)-glucose after starch digestion.

\( \psi_s \) contribution of sorbitol. To determine the contribution of sorbitol to total osmotic adjustment, standard sorbitol solutions ranging from 0 to 100 mg·ml⁻¹ H₂O were prepared and the osmotic potential of each solution was measured using a Wescor 5500 vapor pressure osmometer. The contribution of sorbitol in total osmotic adjustment was calculated following the equation:

\[
\frac{(\psi_{sa} - \psi_{sb})}{(\psi_{sa} - \psi_{sb})} \times 100,
\]

where \( \psi_{sa} \) represents \( \psi_s \) of the standard sorbitol solution at
concentrations found under water stress when $\psi_s$ was from –1.3 to -4.0 MPa; $\psi_s^b$ represents $\psi_s$ of the standard sorbitol solution at concentrations found under well-watered conditions when $\psi_w$ was about –1.0 MPa; $\psi_s^a$ represents $\psi_s$ of leaf samples under water stress when $\psi_w$ was from –1.3 to – 4.0 MPa; $\psi_s^b$ represents $\psi_s$ of leaf samples under well-watered conditions when $\psi_w$ was about –1.0 MPa at midday. The expression ($\psi_s^a - \psi_s^b$) of a sample was considered as total osmotic adjustment imposed by all the osmotica.

**Results and Discussion**

As water stress developed, $\psi_s$ decreased gradually (Fig. 1). Active osmotic adjustment of 0.6 MPa was detected in mature leaves of potted 'Jonathan' apple trees within 3 days of the initiation of water stress as $\psi_w$ decreased from -0.6 to -2.6 MPa. Turgor potential also declined as water potential decreased. When $\psi_w$ exceeded –1.6 MPa, however, active osmotic adjustment in $\psi_s$ maintained $\psi_p > 1$ MPa, but was insufficient to sustain full turgor when $\psi_w$ dropped below –1.6 MPa. After 5 days of water stress, the relationship between $\psi_w$, $\psi_s$, and $\psi_p$, showed similar trends, but with as much as 1.3 MPa active osmotic adjustment detected as $\psi_w$ decreased to –4.0 MPa (data not shown). No active osmotic adjustment was found in young leaves (data not shown).

The amount of active osmotic adjustment we observed in 'Jonathan' is similar to that reported by Davies and Lakso (1979) for field-grown 'McIntosh'/M2 trees on a daily basis, but is substantially lower than the 2.0 to 2.5 MPa seasonal active osmotic adjustment reported for mature 'McIntosh' by Lakso et al. (1984). Our results contrast with those of Davies and Lakso (1979) who found no seasonal adjustment in potted apple trees. Hsiao et al. (1976) reported that a lowering of osmotic potential of >0.2 or 0.3 MPa through dehydration should reduce $\psi_p$ to nearly zero for many crop species. In contrast, a lowering of the same amount of osmotic potential through solute accumulation would still maintain cell turgor and cell volume as well as turgor-mediated processes. Our results indicate that the capacity for osmotic adjustment in apple leaves is limited under a rapidly developed high level of water stress. When water potential was above –1.6 MPa, $\psi_p$ was maintained > 1 MPa through active adjustment, but this active adjustment was insufficient to maintain $\psi_p$ at 1 MPa level when water potential was below –1.6 MPa (Fig. 1). This result is consistent with...
our observation that plants started to show slight wilting in young and old leaves when $\psi_w$ declined to about −2.0 MPa at midday. However, mature leaves in the middle of the shoots did not show any wilting until plants were stressed to $\psi_w$ values below −2.5 MPa.

In contrast, the concentration of individual carbohydrates in young leaves did not change with water stress and no active adjustment in young leaves was detected. This lack of change may be due to the use of carbohydrates imported by young leaves for growth metabolism, not solute accumulation (Lakso et al., 1984). The lack of osmotic adjustment in the young apple leaves and shoot tip is effective in reducing leaf area in response to stress while the mature leaves adjust to the stress to maintain photosynthetic production (Lakso, 1983). These results are in agreement with those reported by Swietlik and Miller (1983) for PP333-treated apple seedlings. In contrast, Steinberg et al. (1989) reported active osmotic adjustment in young, but not in mature, peach leaves.

The use of potted greenhouse trees as models for studying environmental stresses has been questioned because water stress develops much more rapidly in this system than in field-grown trees. We attempted to precondition the trees before treatment to simulate field conditions. However, water stress still developed in this experiment within 1 to 2 days after the initiation of treatments due to the limited soil volume and low water-holding capacity of the soil. We do not know how this affected the osmotic responses of the leaves.

The variation in the reported occurrence and intensity of active osmotic adjustment in apple could be associated with cultivar, time of year, previous stress level, rate of stress development, and duration of stress. In preliminary experiments (unpublished) using 4-year-old potted ‘Staymen’/M7a, we observed 0.8 MPa of active osmotic adjustment in response to water stress in October, but no active osmotic adjustment in the same trees in August. Further, the technique used to separate active from passive osmotic adjustment may affect the accuracy of results since rehydrating the leaf to 100% RWC may result in a change of solute concentration during the several hours of rehydration (Lakso et al., 1984). Measuring $\psi_w$ in early morning when RWC is believed to be 100% may also be inappropriate because leaves usually do not show osmotic adjustment in the early morning (Lakso et al., 1984). The method we used may minimize these shortcomings since active osmotic adjustment was consistently detected.

The concentrations of individual carbohydrates in mature leaves responded differently to the development of water stress. The concentrations of sorbitol, glucose, and fructose increased as $\psi_w$ become more negative, while the concentrations of starch and sucrose decreased significantly (Fig. 2). The relationships between $\psi_w$ and the concentrations of individual carbohydrates showed similar trends, except that the changes in sucrose were statistically nonsignificant as $\psi_w$ became more negative (Fig. 3). Sorbitol was the most abundant soluble carbohydrate (30 to 90 mg·ml sap) in mature leaves. Highly negative correlations between $\psi_w$ and sorbitol : sucrose ratio ($R^2 = 0.85**$) or sorbitol : starch ratio ($R^2 = 0.90**$) were also observed (Fig. 4). The high ratio of sorbitol to both sucrose and starch, as water stress developed, suggests the possible conversions of starch and/or sucrose to sorbitol. When the concentration of sorbitol to total osmotic adjustment was quantified (Fig. 5), sorbitol accounted for ≈60% to 70% of total change in solute potential under low to moderate stress, i.e., when $\psi_w$ was more than −2.5 MPa. However, under severe water stress, when $\psi_w$ was less than −2.5 MPa, the total active adjustment increased from 0.5 to 1.3 MPa, and sorbitol accounted for ≈50% of the total osmotica.

These results are in partial agreement with those of Ranney et al. (1991) on cherry and by Zhang and Archbold (1991) on strawberry (Fragaria ×ananassa Duch.). Ranney et al. (1991) found that the change in sorbitol concentration alone accounted for the total solute accumulation in potted young cherry and that the concentrations of glucose, fructose, and sucrose did not change. In strawberry, the major solutes detected, in order of their relative contributions to osmotic potential, were glucose, fructose, sucrose, and myo-inositol (Zhang and Archbold, 1991). These results suggest that water stress favors a functional role for sorbitol as an osmoticum.

There are several possible physiological explanations for the effect of water stress on sorbitol accumulation. Water stress 1) may increase the partitioning of newly fixed carbon into sorbitol; 2) may induce the enzymatic pathways that break down starch and/or sucrose, which increase the substrate (glucose-6-
Sor : Suc: $y = 3.31 + 1.79x + 0.68x^2$, $R^2 = 0.85** (P < 0.01)$.

Sor : Starch: $y = 16.22 + 13.59x + 5.36x^2$, $R^2 = 0.90** (P < 0.01)$.

P) for sorbitol synthesis; 3) may decrease the rate of transport of sorbitol, relative to sucrose, from the leaf.

It is well established that starch is synthesized in the chloroplast, and significant amounts of newly fixed photosynthates are initially partitioned into chloroplast starch (Vassey and Sharkey, 1989). Both sucrose (Daie, 1985) and sorbitol (Loescher, 1987) are the end products of cytosolic enzymatic pathways. The following results suggest how water stress caused the accumulation of sorbitol in the leaves.

Photosynthesis was inhibited and Rs increased as water stress developed in mature apple leaves. A highly positive correlation between $\psi_w$ under stressed conditions and $\psi_w$ under well-watered conditions. Percent of sorbitol in TOA is the osmotic adjustment as determined by standard sorbitol solutions divided by TOA of leaf samples.

Fig. 6. Effects of water stress on Pn and Rs in mature apple leaves. Pn: $y = 10.43 - 1.45x - 1.11x^2$, $R^2 = 0.85** (P < 0.01)$. Rs: $y = 6.21 + 6.36x + 2.24x^2$, $R^2 = 0.94** (P < 0.01)$.

seedlings grown in the greenhouse. West and Gaff (1976) found that Rs in apple leaves was independent of $\psi_w$ until $\psi_w$ fell
below –1.9 MPa, a similar level as in our result, after which R\textsubscript{s} increased dramatically. The ability of apple leaves to maintain P\textsubscript{n} rate at \(\psi_w\) down to –2.0 MPa (Davies and Lakso, 1978; West and Gaff, 1976), or even down to –3.0 MPa (Lakso, 1979), is well established.

We did not observe a significant effect of water stress on the partitioning of \(^{14}\text{C}\)-CO\(_2\) into \(^{14}\text{C}\)-sucrose, \(^{14}\text{C}\)-glucose \(^{14}\text{C}\)-fructose, \(^{14}\text{C}\)-sorbitol, or \(^{14}\text{C}\)-starch (Fig. 7) immediately following \(^{14}\text{C}\)-labeling for 30 min. About 70% of the recovered \(^{14}\text{C}\) was in sorbitol, 15% in starch, and 5% in sucrose. Glucose and fructose accounted for 6% and 4% of recovered \(^{14}\text{C}\), respectively. The effect of water stress on the metabolism of \(^{14}\text{C}\)-carbohydrates during the 30 min pulse was not determined.

In contrast, significant correlations between leaf \(\psi_w\) and the intraleaf partitioning of \(^{14}\text{C}\) were found at 24, 48, and 96 h after \(^{14}\text{C}\)-CO\(_2\) labeling. Since the trend was similar at all sampling periods, only the results after 96 h (Fig. 8) are presented. There were highly significant positive correlations between \(\psi_w\) and the percentage of \(^{14}\text{C}\) in sucrose, glucose, fructose, and starch, and a highly significant negative correlation between \(\psi_w\) and the percentage of \(^{14}\text{C}\)-sorbitol. At high \(\psi_w\) (more than –1.0 MPa), approximately 30% of the \(^{14}\text{C}\) was found in starch and another 30% in sorbitol. Glucose and fructose combined accounted for 25% of the label, with the remaining 15% being found in sucrose. At \(\psi_w\) of –3.5 MPa, 70% of the label was recovered in sorbitol. The percentage of \(^{14}\text{C}\) found in sucrose, glucose, fructose, and starch was reduced to <10% each. In \textit{Phaseolus vulgaris} (L.), Vassey and Sharkey (1989) observed a similar decline in partitioning of carbohydrates to starch but not to sucrose.

The amount of \(^{14}\text{C}\)-carbohydrates recovered from the leaves declined rapidly after labeling (Table 1). These data are consistent with active transport of sorbitol from mature leaves. \(^{14}\text{C}\)-sorbitol disappeared rapidly whether under well watered or stressed conditions, followed by \(^{14}\text{C}\)-sucrose and \(^{14}\text{C}\)-starch, while \(^{14}\text{C}\)-glucose and \(^{14}\text{C}\)-fructose declined more slowly. Under well-watered conditions, only 5% of sorbitol remained in the labeled leaves at 96 h after labeling, but 24% in sucrose and starch, and approximately 28% in glucose and fructose, respectively. Water stress induced more \(^{14}\text{C}\)-carbohydrates to accumulate in the labeled leaves. At 96 h, 30, 40, 40, 51, and 56% of sorbitol, sucrose, starch, glucose, and fructose, respectively, were recovered in water-stressed plants. When compared with well water controls, however, the relative amount of \(^{14}\text{C}\)-sorbitol remaining in the labeled leaves under water stress was higher than that of any other \(^{14}\text{C}\)-carbohydrates. The ratio of \([^{14}\text{C}]\text{CHO}_{\text{in}}/[^{14}\text{C}]\text{CHO}_{\text{out}}\) was \(~6\) for sorbitol, 2 for sucrose and starch, and \(~2\) for glucose and fructose.

The \(^{14}\text{C}\)-labeling results indicate that water stress does not

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|c|}
\hline
Carbohydrate & \(\psi_{w(-0.5)}\) & \(\psi_{w(-3.5)}\) & \(\psi_{w(-0.5)}\) & \(\psi_{w(-3.5)}\) & \(\psi_{w(-0.5)}\) & \(\psi_{w(-3.5)}\) & \\
\hline
Sucrose & 48 a & 48 ab & 32 a & 46 a & 24 a & 40 a & 0.8 a & 1.4 b & 1.7 b \\
Glucose & 48 a & 59 a & 36 a & 55 a & 28 a & 51 a & 1.1 a & 1.5 b & 1.8 b \\
Fructose & 44 b & 44 b & 33 a & 60 a & 27 a & 56 a & 1.0 a & 1.8 b & 2.1 b \\
Sorbitol & 41 b & 59 a & 15 b & 48 a & 5 b & 30 b & 1.4 a & 3.2 a & 6.0 a \\
Starch & 49 b & 62 a & 26 a & 44 a & 24 a & 40 a & 1.3 a & 1.7 b & 1.7 b \\
\hline
\end{tabular}
\caption{Relative amounts of \(^{14}\text{C}\)-carbohydrates remaining in mature leaves at 24, 48, and 96 h after exposure to a 30 min \(^{14}\text{C}\)-CO\(_2\) pulse.}
\end{table}
affect the partitioning of newly fixed assimilates among the sucrose, sorbitol, and starch pathways (Fig. 7), and as such, are not consistent with the hypothesis that water stress increases the partitioning of newly fixed C into sorbitol. The increase in sorbitol was not associated with rate of C assimilation since Pn decreased as water stress developed (Fig. 6). Further, the increase in sorbitol concentration was not associated with the reduced conversion of sorbitol during water stress because sorbitol dehydrogenase (EC 1.18.3.1) activity, the enzyme which converts sorbitol to fructose, is very low in mature leaves (Loescher et al., 1982; Yamaki and Ishikawa, 1986).

However, the increased partitioning of 14C into sorbitol after 96 h of water stress (Fig. 8, Table 1) is consistent with the hypothesis that water stress decreases the rate of transport of sorbitol, relative to sucrose, from the leaf and the hypothesis that water stress induces the enzymatic pathways that break down starch and/or sucrose, which increase the substrate (glucose-6-P) for sorbitol synthesis. We could not determine whether the sorbitol accumulation under water stress was due to the reduced transport of sorbitol or the increased breakdown of starch and sucrose. The evidence of decreases in concentrations (Fig. 2) and percentage of 14C-sucrose and 14C-starch (Fig. 8) under water stress, suggest that the breakdown of starch was stimulated by water stress. More breakdown of starch and sucrose into glucose could be a potential factor for further sorbitol synthesis. Nevertheless, the further conversion from glucose to sorbitol under water stress is not clear. If the conversion to sorbitol synthesis were inhibited under water stress, the reduced sorbitol transport from the mature leaves could be the primary source for sorbitol accumulation.

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