EFFECTS OF WATER STRESS ON THE PARTITIONING OF [^<14>C] GLUCOSE, [^<14>C] SUCROSE AND [^<14>C] SORBITOL IN APPLE SHOOTS

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出版情報：BIOTRONICS. 26, pp.73-83, 1997-12. Biotron Institute, Kyushu University
バージョン：
権利関係：

BIOTRONICS **26**, 73–83, 1997

EFFECTS OF WATER STRESS ON THE PARTITIONING OF [^{14}C]GLUCOSE, [^{14}C]SUCROSE AND [^{14}C]SORBITOL IN APPLE SHOOTS

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(Received June 4, 1997; accepted August 22, 1997)

WANG Z. and QUEBEDEAUX B. *Effects of water stress on the partitioning of [^{14}C]glucose, [^{14}C]sucrose and [^{14}C]sorbitol in apple shoots.* BIOTRONICS **26**, 73–83, 1997. The objective of this study was to determine how soluble carbohydrates were partitioned and metabolized in apple mature source leaves, young sink leaves, and stems in response to water stress. A solution of [^{14}C]glucose, [^{14}C]sucrose, or [^{14}C]sorbitol was applied to apple (*Malus domestica* Borkh. 'Red Jonathan') shoots which had previously experienced either water stress or non-stressed conditions. Mature source leaves had higher conversions of labeled [^{14}C]glucose and [^{14}C]sucrose to sorbitol with higher sorbitol to sucrose ratios than young sink leaves and stems. Young sink leaves and stems had higher conversions of labeled [^{14}C]glucose, [^{14}C]sucrose and [^{14}C]sorbitol to fructose than mature leaves. As leaf water potential decreased from −1.5 MPa to −3.0 MPa, the conversions of [^{14}C]glucose to sorbitol increased from 12 to 25% of total ^{14}C-soluble carbohydrate in young sink leaves and from 20 to 30% in mature leaves. Water stress also stimulated the conversion of [^{14}C]sucrose to fructose and glucose, the necessary substrates for sorbitol synthesis. Our results suggest that sorbitol accumulation in water-stressed mature leaves was due to increased rates of glucose conversion to sorbitol.

Key words: apple; ^{14}C labeling; carbohydrate partitioning; leaf water potential; sorbitol; water stress.

INTRODUCTION

Water stress and other abiotic stress can dramatically limit plant growth and productivity (*10, 11*). Many higher plants, fungi, and bacteria develop various physiological adaptations to abiotic stress (*9, 11*). An example of such adaptations is the accumulation of low molecular compounds such as proline, organic acids, sugars, and sugar alcohols (*1, 9, 16*). It has been suggested that the accumulation of these compounds may help plants maintain cell turgor through osmotic adjustment thus minimizing the detrimental effects of water stress (*13, 19*).

Many Rosaceae species including apple, pear, peach, cherry, plum and apricot

produce both sucrose and sorbitol as primary photosynthetic products (*2*). Sorbitol, a sugar alcohol, accounts for 50–80% of the carbon exported from apple leaves (*26*). Unlike sucrose which is synthesized and utilized by leaves of all ages, sorbitol is synthesized primarily in source leaves and metabolized primarily in sink tissues. Aldose–6–phosphate reductase is the key enzyme for converting glucose–6–phosphate to sorbitol in the cytosol in mature source leaves (*7, 8*). The key enzyme for the conversion of sorbitol in young sink leaves is sorbitol dehydrogenase (*14, 27*). Increased sorbitol and decreased starch and sucrose have recently been found in water–stressed mature apple leaves (*22, 24*). The accumulation of other sugar alcohols, such as mannitol in celery (*5*) and pinitol in *Mesembryanthemum crystallinum* (*21*), has also been associated with higher salinity or drought stress. Transgenic tobacco plants that synthesize and accumulate mannitol demonstrate increased salt tolerance (*17*).

Little information exists concerning the effects of water stress on the metabolism of sorbitol and other soluble carbohydrates in various apple organs other than mature leaves. The water stress effects on ^{14}C–sugar partitioning were recently reported for mature apple leaves (*24*). Here, we reported the effects of water stress on ^{14}C–sugar conversion and metabolism in various apple organs. The specific objectives of this report were: (1) to compare the metabolism of ^{14}C–labeled glucose, sucrose, and sorbitol in various apple organs including mature source leaves, young sink leaves, and stems, (2) to examine water stress effects on the metabolism of [^{14}C]glucose, [^{14}C]sucrose, and [^{14}C] sorbitol in these organs, and (3) to determine whether sugar conversion in young sink leaves and stems shows the same response to water stress as mature source leaves. This study will provide a better understanding of how water stress affects sorbitol and other carbohydrate metabolism in different leaves and how sorbitol and sucrose are metabolized and partitioned in stems before they are transported to sink tissues. The study will also enable us to further investigate the causes of sorbitol accumulation under water stress conditions.

MATERIALS AND METHODS

Plant Materials and Water Stress Treatments

The growing conditions and water stress treatments were described previously (*24*). Briefly, one–year–old apple plants (*Malus domestica* Borkh. 'Red Jonathan') grafted on EMLA 111 rootstocks were planted in 7–L pots filled with peat–based Professional Growing Medium 300–S (Pro–Gro Products, Elizabeth, NC, USA). The plants were grown in the greenhouse and pruned to two extension shoots per plant, watered daily, and fertilized weekly with Peters' water soluble fertilizer (20.0N–8.6P–16.6K) at a nitrogen concentration of 470 mg L^{-1}. The plants had two actively growing shoots which was approximately 85–90 cm long with ≈45 leaves on each extension shoot at the beginning of the ^{14}C –labeling studies.

Four water stress treatments: well watered control, mildly stressed, moderately stressed, and severely stressed, were created by controlling the

amount of water applied to each pot (*22, 24*). The four water stress treatments produced a range of water stress levels on plants. At 5 days after the imposition of water stress, leaf water potential (Ψ_w) in the four treatments were approximately: −1.0 (well-water control), −1.5 (mildly stressed), −2.0 (moderately stressed), and −2.5 MPa (severely stressed). Slight wilting occurred in young sink leaves at midday in the severely water-stressed treatments, but no wilting occurred in any of the other treatments. The photosynthetic rates of mature leaves remained relatively stable at 9–11 μmol CO_2 m^{-2} s^{-1} when Ψ_w decreased from −1.0 to −2.0 MPa, but decreased to ≈7 μmol CO_2 m^{-2} s^{-1} when Ψ_w was −2.5 MPa. Midday air temperature in the greenhouse during the experiments varied from 28 to 32℃ and the maximum photosynthetic photon flux varied from 1000 to 1400 μmol m^{-2} s^{-1}. The experimental plot design was a randomized complete block with two single-tree replications for [^{14}C]sorbitol labeling experiment or three single-tree replications for [^{14}C]glucose and [^{14}C]sucrose labeling experiments.

[^{14}C]glucose, [^{14}C]sorbitol and [^{14}C]glucose Labeling

The procedures for [^{14}C]glucose labeling have been described previously (*24*). Essentially, a total of 12 shoots, each from the 12 pre-water-stressed plants (i. e., three from each of the four water stress treatments), were excised for [^{14}C] glucose labeling at 5 days after the imposition of water stress. Each excised shoot was placed into a 50 mL test tube containing 740 kBq of [^{14}C]glucose in 3 mL of sterilized, distilled and deionized water. The excised shoots were exposed to the natural light inside the greenhouse and allowed to photosynthesize for the duration of the experiment. Thirty minutes were required to absorb 3 mL of the [^{14}C]glucose solution. Then, for the pre-well-watered control shoots, 40 mL of sterilized, distilled and deionized water was added to each test tube to continue well-watered conditions. For the pre-water-stressed shoots, no water was added and the shoots were allowed to wilt via tissue dehydration and leaf transpiration to simulate water stress. Young sink leaves (positions 1–5 starting from the shoot apex), mature source leaves (positions 10–35), and young stems (upper parts of stems from positions 1–20) were sampled at 4.5 h after the [^{14}C]glucose labeling and frozen in liquid nitrogen for the measurements of ^{14}C-soluble carbohydrates. The Ψ_w of mature leaves of the excised shoots in various treatments ranged from −1.5 to −3.0 MPa at the end of the experiments (4.5 h after labeling).

The procedures for [^{14}C]sorbitol labeling were similar to those of [^{14}C] glucose except that the amount of [^{14}C]sorbitol labeled for each shoot was 700 kBq. The procedures for [^{14}C]sucrose labeling were also similar to those of [^{14}C] glucose except that 1 h was required for all shoots to absorb 5 mL of the [^{14}C] sucrose solution and that the experiment was terminated at 24 h after labeling. The radioactivity into sucrose, glucose, fructose, and sorbitol was determined immediately after the complete absorption of the solution and at 24 h after [^{14}C] sucrose labeling.

Leaf Water Potential and ^{14}C-soluble Carbohydrate Analysis

The Ψ_w of mature apple leaves was measured with a pressure bomb (*18, 23*) immediately after sampling and used as an indicator of water stress for mature leaves, young sink leaves, and stems.

[^{14}C]sucrose, [^{14}C]glucose, [^{14}C]fructose, and [^{14}C]sorbitol were analyzed with a LKB 1219 Rackbeta liquid scintillation counter (Wallac Oy, Turku, Finland) as previously described (*23, 24, 25*). Briefly, soluble carbohydrates were first separated with a Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan) on a 300×7.8 mm Bio-Rad HPX-87C carbohydrate column (Bio-Rad, Richmond, CA, USA) at 85℃. Degased, distilled, deionized water at 0.6 mL min^{-1} was used as the mobile phase. Then, an Advantec SF-2120 fraction collector (Advantec Toyo Kaisha, Ltd., Osaka, Japan) was used to collect each soluble sugar into a 7-mL scintillation vial following HPLC separation. 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. After the collection, 3 mL of Beckman Ready Gel liquid scintillation cocktail was added to each vial. [^{14}C]sucrose, [^{14}C]glucose, [^{14}C]fructose, and [^{14}C]sorbitol fractions were counted via a LKB 1219 Rackbeta liquid scintillation counter and corrected for background. Specific radioactivity of total soluble carbohydrate was calculated as disintegrations per minute (dpm) per gram dry tissue. The ^{14}C-radioactivity in each carbohydrate was calculated as % of total ^{14}C-soluble carbohydrate in the extract.

Statistical Analysis

Statistical analysis was performed using SAS procedures (SAS Institute Inc., Cary, NC, USA). Means (n=2 or 3) for ^{14}C-sugar activity in mature apple leaves, young sink leaves, and stems were separated by LSD at $p<0.05$.

RESULTS

[^{14}C]glucose Labeling

Under non-water-stressed conditions ($\Psi_w \approx -1.5$ MPa), the conversion of labeled [^{14}C]glucose to sorbitol was higher in mature apple leaves (19%) than in young sink leaves (14%) and stems (7%) with a [^{14}C]sorbitol/[^{14}C]sucrose ratio of 1.13 in mature leaves at 4.5 h after labeling (Table 1). However, conversion of [^{14}C]glucose to sucrose was ≈85% higher in stems than in mature source and young sink leaves with a lower [^{14}C]sorbitol/[^{14}C]sucrose ratio of 0.22 in stems. Stems and young sink leaves contained higher percentages of [^{14}C]fructose than mature leaves.

Water stress increased the conversion of labeled [^{14}C]glucose to sorbitol from ≈20% of total ^{14}C-soluble carbohydrate at $\Psi_w = -1.5$ MPa to 30% at $\Psi_w = -3.0$ MPa in mature apple leaves at 4.5 h after labeling (Fig. 1A). The conversion of [^{14}C]glucose to fructose in mature leaves showed a quadratic pattern and no change in conversion of [^{14}C]glucose to sucrose in response to water stress was observed. The conversion patterns of labeled [^{14}C]glucose to sucrose, fructose,

Table 1. Conversion of [^{14}C]glucose into other soluble carbohydrates in mature source leaves, young sink leaves, and stems of excised apple shoots under non-water-stressed conditions at 4.5 h after [^{14}C]glucose labeling. Means (n=3) within the columns followed by the same letters are not significantly different (LSD 0.05).

Organ	Total ^{14}C-soluble carbohydrate* (dpm g^{-1} dry weight)	% total ^{14}C-soluble carbohydrate				Sorbitol to sucrose ratio
		Sucrose	Glucose	Fructose	Sorbitol	
Mature leaves	251,080 a	17.2 b	43.0 a	20.4 b	19.4 a	1.13 a
Young leaves	123,800 c	17.8 b	42.3 a	26.4 a	13.5 b	0.76 b
Stems	187,440 b	32.3 a	34.2 b	26.3 a	7.2 c	0.22 c

*Total ^{14}C-soluble carbohydrate = [^{14}C]sucrose + [^{14}C]glucose + [^{14}C]fructose + [^{14}C]sorbitol. dpm = disintegrations per minute.

and sorbitol in young sink leaves (Fig. 1B) were similar to those of mature leaves. Water stress increased the conversion of [^{14}C]glucose to sorbitol from 12% of total ^{14}C-soluble carbohydrate at $\Psi_w = -1.5$ MPa to 25% at $\Psi_w = -3.0$ MPa in young sink apple leaves. No signficant changes in the conversion of labeled [^{14}C]glucose to other soluble carbohydrates were found in stems as water stress developed (Fig. 1C).

[^{14}C]sucrose Labeling

The labeled [^{14}C]sucrose was readily metabolized and converted to other carbohydrates in the mature leaves of the excised shoots (Fig. 2). Under well-watered conditions, when Ψ_w was -1.0 MPa, $\approx$27% of the labeled [^{14}C]sucrose remained as sucrose, 45% was incorporated into sorbitol, 23% into glucose, and 5% into fructose at the end of the 1-h labeling period. Water stress preconditioning stimulated [^{14}C]sucrose metabolism in the excised shoots, resulting in lower recoveries of [^{14}C]sucrose and increases in the [^{14}C]fructose fraction as Ψ_w decreased. The conversion of [^{14}C]sucrose to glucose was increased while the conversion to sorbitol was decreased when Ψ_w decreased from -1.0 to -2.2 MPa. Further decreases in Ψ_w (from -2.2 to -3.0 MPa), however, resulted in accumulation of [^{14}C]sorbitol with a corresponding decrease in [^{14}C]glucose.

The conversion of labeled [^{14}C]sucrose into sorbitol was $\approx$25% higher in mature and young sink leaves than in stems under non-water-stressed conditions at 24 h after the labeling (Table 2). However, higher conversions of [^{14}C]sucrose into fructose were found in young sink leaves and stems than in mature leaves.

[^{14}C]sorbitol Labeling

When the shoots were supplied with [^{14}C]sorbitol, mature apple leaves contained more than 90% of the labeled [^{14}C]sorbitol and only $\approx$9% of [^{14}C] sorbitol was converted into other soluble carbohydrates at 4.5 h after labeling

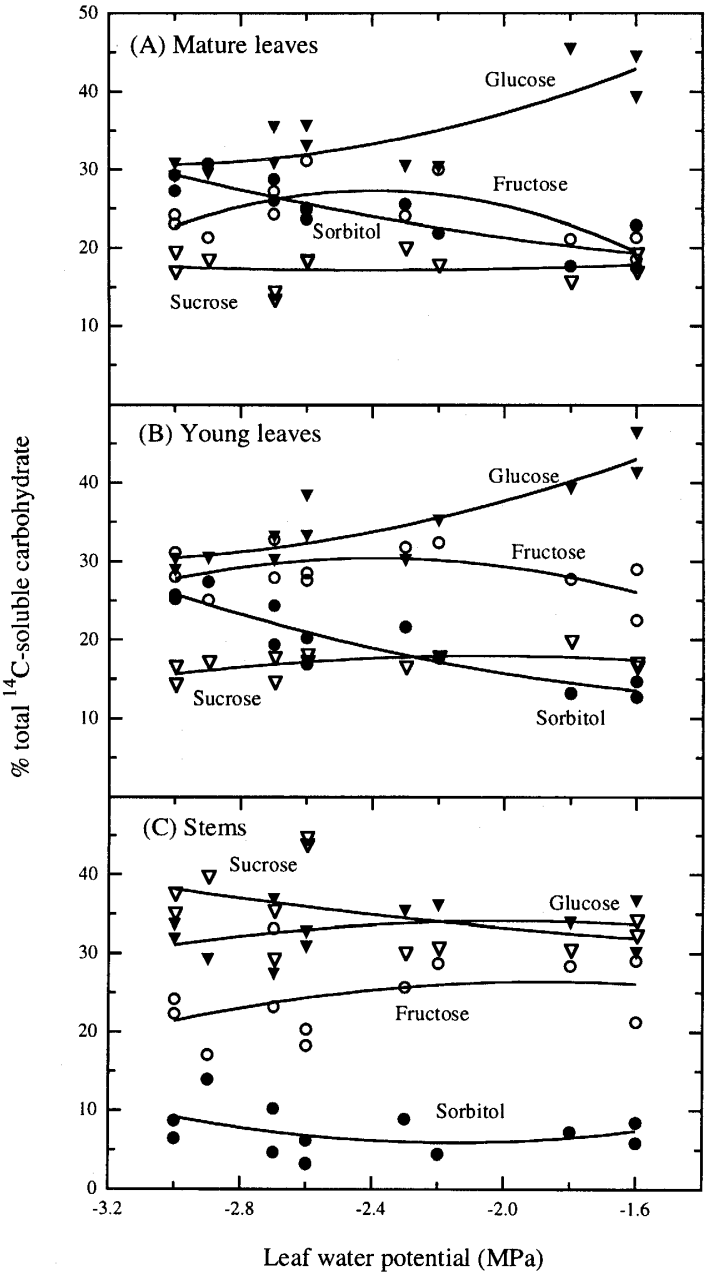

Fig. 1. Conversion of labeled [^{14}C]glucose into other soluble carbohydrates in mature source leaves, young sink leaves, and stems of excised apple shoots as a function of leaf water potential (Ψ_w) at 4.5 h after [^{14}C]glucose labeling. Total ^{14}C-soluble carbohydrate = [^{14}C]sucrose + [^{14}C]glucose + [^{14}C]fructose + [^{14}C]sorbitol. dpm = disintegrations per minute.

(Table 3). However, 20–25% of the labeled [^{14}C]sorbitol was converted in young sink apple leaves and stems, of which 6–10% was recovered in fructose. Due to the low conversion, it is difficulty to conclude how water stress affected the partitioning of [^{14}C]sorbitol into other carbohydrates in different apple organs (data not shown).

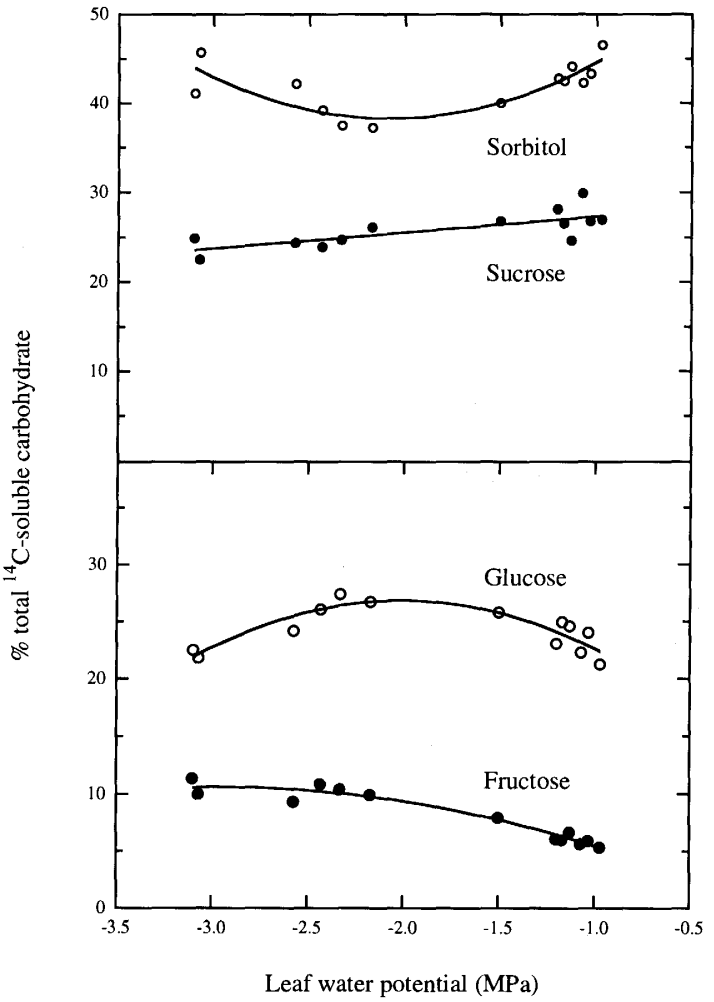

Fig. 2. Conversion of labeled [^{14}C]sucrose into other soluble carbohydrates in mature source leaves of excised apple shoots as a function of leaf water potentials (Ψ_w) immediately after 1-h[^{14}C]sucrose labeling. Total ^{14}C-soluble carbohydrate = [^{14}C]sucrose + [^{14}C]glucose + [^{14}C]fructose + [^{14}C]sorbitol. dpm = disintegrations per minute.

DISCUSSION

Conversion of [^{14}C]glucose, [^{14}C]sucrose, and [^{14}C]sorbitol in Mature Leaves

Our ^{14}C results obtained here with excised shoots are similar to our previous intact plant studies that show sorbitol accumulation in mature apple leaves under water stressed conditions (*23*). The conversion of labeled [^{14}C]glucose into sorbitol in mature apple leaves of the excised shoots was increased by water stress from 20% of total ^{14}C-soluble carbohydrate at $\Psi_w = -1.5$ MPa to 30% at $\Psi_w = -3.0$ MPa at 4.5 h after labeling (Fig. 1A). A significant incorporation of [^{14}C]glucose into sorbitol was also reported to occur in mature apricot (*3*) and apple leaves (*15*) under non-water-stressed conditions. Based on our ^{14}C-

Table 2. Conversion of [^{14}C]sucrose into other soluble carbohydrates in mature source leaves, young sink leaves, and stems of excised apple shoots under non-water-stressed conditions at 24 h after [^{14}C]sucrose labeling. Means (n=3) within the columns followed by the same letters are not significantly different (LSD 0.05).

Organ	Total ^{14}C-soluble carbohydrate* (dpm g^{-1} dry weight)	% total ^{14}C-soluble carbohydrate				Sorbitol to sucrose ratio
		Sucrose	Glucose	Fructose	Sorbitol	
Mature leaves	102,630 b	22.3 b	34.0 a	9.4 b	34.3 a	1.54 a
Young leaves	46,200 c	23.2 b	29.8 b	13.8 a	33.2 a	1.43 a
Stems	212,850 a	32.1 a	26.7 c	14.0 a	27.2 b	0.85 b

*Total ^{14}C-soluble carbohydrate=[^{14}C]sucrose+[^{14}C]glucose+[^{14}C]fructose+[^{14}C]sorbitol. dpm=disintegrations per minute.

Table 3. Conversion of [^{14}C]sorbitol into other soluble carbohydrates in mature source leaves, young sink leaves, and stems of excised apple shoots under non-water-stressed conditions at 4.5 h after [^{14}C]sorbitol labeling. Means (n=2) within the columns followed by the same letters are not significantly different (LSD 0.05).

Organ	Total ^{14}C-soluble carbohydrate* (dpm g^{-1} dry weight)	% total ^{14}C-soluble carbohydrate				Sorbitol to sucrose ratio
		Sucrose	Glucose	Fructose	Sorbitol	
Mature leaves	400,330 a	2.0 c	2.8 c	4.1 c	91.2 a	46 a
Young leaves	149,570 b	3.7 b	6.3 b	10.2 a	79.8 b	22 b
Stems	349,050 a	8.3 a	9.6 a	6.3 b	75.8 b	9 c

*Total ^{14}C-soluble carbohydrate=[^{14}C]sucrose+[^{14}C]glucose+[^{14}C]fructose+[^{14}C]sorbitol. dpm=disintegrations per minute.

labeling study, it appears that once [^{14}C]sorbitol is synthesized in mature leaves, it is rarely metabolized (<10% metabolized) (Table 3). The results indicate that [^{14}C]sorbitol accumulation in water-stressed mature leaves results from increased [^{14}C]glucose conversion (Fig. 1A) and that the enhanced conversion of sucrose under water stress (Fig. 2) increased glucose and fructose, the necessary substrates, for sorbitol synthesis.

Sucrose metabolism under water stress in mature apple leaves differs from that in non-Rosaceae species. In mature apple leaves, sucrose concentrations either remain the same or decrease as water stress develops (*22, 23*). The conversions of [^{14}C]sucrose to other soluble carbohydrates were also increased in the water-stressed conditions (Fig. 2). In non-Rosaceae species such as wheat (*4*) and bean (*20*), however, water stress increased sucrose concentrations. Water stress also increased sucrose concentrations in carrot cell suspensions (*6*). The difference may be species specific. In Rosaceae species, that includes apple, sorbitol is the primary photosynthetic product (*26*) and sorbitol is the

carbohydrate that contributes most to osmotic adjustment (*22*). In non-Rosaceae species, however, sucrose is the primary carbohydrate. Therefore it is not surprising that sucrose levels increased in species where sucrose plays a major role in osmotic adjustment but not in Rosaceae species.

Conversion of [^{14}C]glucose, [^{14}C]sucrose, and [^{14}C]sorbitol in Young Sink Leaves

Our results indicate that young sink apple leaves accumulated less [^{14}C] sorbitol, but more [^{14}C]fructose than mature leaves under both well-watered and water-stressed conditions when apple shoots were labeled with [^{14}C]glucose or [^{14}C]sorbitol (Tables 1 and 3; Fig. 1). The [^{14}C]fructose level in young sink leaves was 2.5 times higher than that of mature leaves when the shoots were labeled with [^{14}C]sorbitol (Table 3). A similar result was obtained in young apricot leaves in which labeled [^{14}C]glucose was readily converted into sucrose, but not into sorbitol under non-stressed conditions (*3*). The lower sorbitol and higher fructose in young sink leaves than in mature leaves may be attributed to higher activity of sorbitol dehydrogenase (SDH), an enzyme converting sorbitol to fructose, in young sink leaves (*14, 27*). The lower [^{14}C]sorbitol in young sink leaves under water stress may also be due to reduced export of sorbitol from the water-stressed mature leaves.

Like mature leaves, young sink apple leaves accumulated more sorbitol under water-stressed than under well-watered conditions. The sorbitol converted from the labeled [^{14}C]glucose accounted for 12% of total ^{14}C-soluble carbohydrate at $\Psi_w = -1.5$ MPa to 25% at $\Psi_w = -3.0$ MPa at 4.5 h after [^{14}C] glucose labeling (Fig. 1B). The results of sorbitol accumulation in water-stressed young leaves from the current ^{14}C-labeling studies are consistent with those obtained from non-labeling studies (*23*). These results indicate that sorbitol accumulation in both mature and young sink apple leaves play an important role in apple plant's adaptation to water stress.

Conversion of [^{14}C]glucose, [^{14}C]sucrose and [^{14}C]sorbitol in Stems

Less [^{14}C]sorbitol and more [^{14}C]fructose were recovered from stems than from mature leaves when the shoots were labeled with [^{14}C]glucose, [^{14}C]sucrose or [^{14}C]sorbitol (Tables 1-3). The sorbitol/sucrose ratio was lower in stems (0.85) than in young sink leaves (1.43) and in mature leaves (1.54) when the shoots were labeled with [^{14}C]sucrose (Table 2). The low sorbitol and higher fructose levels in the stems may be due to the conversion of sorbitol to fructose in young stem tissues. The lower sorbitol levels in stems may also be due to the preferential unloading of sorbitol into leaf sinks (*12*).

In conclusions, sorbitol accumulation in water-stressed mature apple leaves may be due to (a) increased breakdown of starch (*22*) and sucrose (Fig. 2) thus providing glucose for sorbitol synthesis, (b) increased conversion of glucose to sorbitol (Fig. 1), and (c) lower sorbitol conversion (Table 3). Sorbitol can also be converted into other carbohydrates, especially into fructose, in stems and young sink leaves (Table 3) and can be accumulated in young sink apple leaves under water stress (Fig. 1). Our results suggest that sorbitol accumulation and

its subsequent effect on osmotic adjustment in both mature and young sink apple leaves may increase this plant's tolerance to water stress.

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