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Translocation and Metabolism of Carbohydrate Fraction of ^{14}C-photosynthates in 'French' Prune, *Prunus domestica* L.[1]

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Abstract. **When $^{14}CO_2$ was administered to leaves on girdled 'French' prune spurs, the label was incorporated into sorbitol, sucrose, glucose, fructose, starch, and amylase-insoluble assimilates. The rates of export of soluble sugars and sorbitol and mobilization of starch from leaves were proportional to the rate of fruit growth. The deposition of amylase-inert assimilates in leaves exceeded that of starch, which may account for the gradual increase in specific leaf weight in prunes. The proportion of sorbitol to total sugars in leaf blades and petioles, stem, and peduncle was nearly constant during the 22-day experimental period but changed abruptly in the fruit. ^{14}C-sorbitol fed to fruits via their peduncles was metabolized to ^{14}C-sucrose, but the reverse reaction was barely detectable.**

Sorbitol, a sugar alcohol, is a common carbohydrate constituent in members of the Rosaceae family. The fruit of the 'French' prune is relatively rich in this compound, especially when ripe (14). Reid and Bieleski (13) identified it in apricots, *Prunus armeniaca* L., and reported that the ripening fruit relied on carbohydrate reserves, rather than on current photosynthates, as carbon sources. They based their interpretation on the inability of the apricot fruit to metabolize sorbitol, a major carbohydrate in the translocation stream, to sucrose. In 'French' prune, a light to moderate crop caused a slight diminution of starch in the stem (14) while a heavy crop in 'Sugar' prune exhausted it (4). This study was undertaken to elucidate the transport and metabolism of soluble carbohydrates and starch produced by 'French' prune leaves after administration of $^{14}CO_2$. Source-sink relationship is particularly important in this cultivar because overcropping often leads to potassium deficiency symptoms under some California conditions (10).

Materials and Methods

$^{14}CO_2$ administration and sampling of treated spurs. On July 5 and 25, 8 spurs having 20 to 70 leaves and bearing 2 to 7 fruits were selected on a 6-year-old 'French' prune tree growing in the University Orchard at Davis, CA. The spurs were girdled at their bases to prevent radioactive contamination of the tree. Some of the leaves, but no fruit, were

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enclosed within a plastic bag containing a vial with $Na_2{}^{14}CO_3$ (21 x 10^6 cpm, sp act 16.4 mCi/mM). An excess of 4N HNO_3 was injected into the vial with a syringe and the fine hole immediately sealed with tape. Two hr later, 0.5 ml of a saturated aqueous solution of $Ba(OH)_2$ was injected into the plastic bag, which was removed 15 min later. Little or no precipitate was noted. Two spurs were sampled at 6, 30, 54 and 528 hr (22 days) after exposure.

The spurs were separated into leaves, stem, and fruits and each part lyophilized. Fruits from the July 25 treatment were pitted and the diced pulp was extracted with boiling 95% ethanol. Freeze-dried samples were pulverized, the leaves in a mortar, and stem pieces in a Wiley mill. Portions of samples were extracted with 80% ethanol and the alcohol-insoluble substances (AIS) dried and re-weighed.

^{14}C-sugar and -sorbitol determination. Ethanolic extracts were evaporated and the aqueous residues were passed through a column of Amberlite IRA 400 (CO_3^{2-} form) to eliminate organic acids (15). Sugars in the aqueous eluate were separated by descending paper chromatography, using Whatmann #1 paper and methyl ethyl ketone, glacial acetic acid, and water saturated with boric acid (9:1:1, by volume) as solvent (12). The radioactive zones on the chromatograms were located with a strip scanner (Actigraph III, Nuclear-Chicago) and the sugars identified by co-chromatography with standards. Their positions on the chromatograms were confirmed by spraying with 2% anisidine:HCl in butanol and heating to 110°C, followed by treatment with saturated KIO_4 solution in acetone (1:4, by volume) (1). The radioactive zones were combusted and the resulting $^{14}CO_2$ was trapped in a scintillation fluid containing phenethylamine (11) and subsequently counted (Packard Tricarb, Model 3375).

Starch analysis. As a measure of ^{14}C distribution and transport, the leaf AIS from both spurs on each sampling date were analyzed for starch. A pre-boiled subsample of 500 mg was treated with 10 mg amylase (CalBiochem. Corp.) in acetate buffer, pH 5.0, for 48 hr with a few drops of toluene. The crude amylase was purified by precipitation with saturated $(NH_4)_2SO_4$; the precipitate was re-dissolved in H_2O and re-precipitated with acetone according to Conn and Stumpf (3). After incubation, the mixture was filtered and the residue placed in a Sohxlet apparatus and extracted for 6 hr with 80% ethanol. The filtrate and the ethanolic extract were combined and an aliquot of the amylase-soluble fraction combusted as described above. The insoluble residue was oven-dried and a subsample also combusted. To obtain an estimate of the amount of starch deposited in stems, 2 portions of spurs which were not exposed to $^{14}CO_2$ on July 5 and collected 54 hr after treatment were analyzed in the above manner.

Metabolism of ^{14}C-sucrose and ^{14}C-sorbitol by plum fruit. On July 27, about a month before harvest, several fruits from potassium-limited trees growing in the Rio Oso district were collected. The peduncles were recut and the ends immersed in water or 0.2% K_2SO_4. The vials containing the plums were placed for 20 hr in a growth chamber at 27°C under continuous fluorescent light. The fruits were then transferred to vials containing uniformly labelled ^{14}C-sucrose (5 x 10^6 cpm, sp act 434 mCi/mM, Amersham/Searle) or ^{14}C-sorbitol (5 x 10^6 cpm, sp act 10 mCi/mM, Amersham/Searle). After a 7-hr exposure, the fruits were returned to their original vials. One and 3 days later the fruits were pitted, and the mesocarp tissue was diced and dropped into boiling 95% ethanol. The pulp was extracted for 16 hr with 80% ethanol with a Sohxlet apparatus. Amounts of sugars and sorbitol in the ethanolic extracts were estimated by combusting the radioactive zones on paper chromatograms as described above.

Results and Discussion

The amount of ^{14}C recovered from the 16 spurs, excluding the non-exposed leaves, ranged from 7.5 x 10^6 to 18.6 x 10^6 cpm with a mean of 13.7 ± 3.1 x 10^6 cpm. The variability is attributed to differential uptake of $^{14}CO_2$ and subsequent respiratory losses. While some loss may have occurred through the girdles which had not healed during the 22-day experiment, no radioactivity was detectable in the stem portion below the girdles. Because of these variations, the amounts of ^{14}C recovered from various parts of the spurs are expressed as percentage of the total recovered for comparative purposes. Six hr after administration of $^{14}CO_2$, 90% of the radioactivity resided in the treated leaves, whereas, 22 days later, 82 and 88% were found in the fruits (Fig. 1). Much of this transport occurred early; 50% of the ^{14}C had moved into the fruits within 60 hr after treatment. The rate of export was faster after the July 25 treatment when the fruits were enlarging rapidly than after the July 5 exposure when the fruits were still in Stage II.

Analyses of exposed leaves revealed that much of the radioactivity in the initial samples was in the alcohol soluble fraction. This consisted primarily of soluble carbohydrates; as they were exported, the amount in the residual AIS increased on a percentage basis (Table 1). Based on the total recoverable radioactivity, the leaf AIS amounted to 8-12% in the first 3 samples, but decreased to 5-6% in the last one. Hydrolysis of the leaf

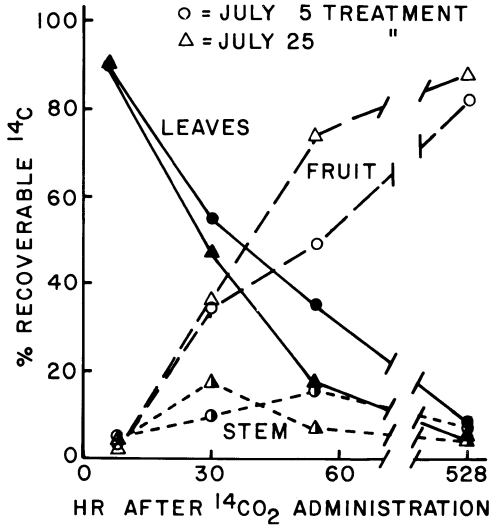

Fig. 1. Percentage of radioactivity recovered from leaves, fruits, and stem of girdled 'French' prune spurs at intervals after administration of $^{14}CO_2$ to the foliage on 2 dates.

Table 1. Percentages recoverable radioactivity in the alcohol insoluble substances in $^{14}CO_2$-treated leaves and unexposed fruits on the same 'French' prune spurs. Values represent duplicate spurs.

Treatment date	Recoverable radioactivity (%) Time after administration of $^{14}CO_2$ 6 hr	30 hr	54 hr	528 hr
		Leaf		
July 5	11.4	17.5	37.2	65.0
	8.5	16.2	29.4	78.7
July 25	14.8	21.3	35.2	72.6
	10.5	19.9	49.6	65.4
		Fruit		
July 5	1.2	1.6	2.7	7.6
	3.9	1.9	3.4	9.9
July 25	2.9	1.1	1.1	5.5
	1.1	0.8	0.9	3.4

Table 2. Percentage recoverable radioactivity in amylase-soluble fraction after enzymatic hydrolysis of the alcohol insoluble substances in prune leaves collected at intervals following administration of $^{14}CO_2$. Values represent leaves from separate spurs.

Treatment date	Recoverable radioactivity (%) Time after administration of $^{14}CO_2$ 6 hr	30 hr	54 hr	528 hr
July 25	25	21	18	6
	26	15	17	16
July 25	18	25	13	8
	15	13	5	8

Table 3. Percentage distribution of recoverable radioactivity among alcohol soluble carbohydrates in extracts of 'French' prune spur parts collected at intervals after exposure of leaves to $^{14}CO_2$. Carbohydrates were separated by paper chromatography.[z]

Organs	Time after exposure (hr)	Recoverable radioactivity (%) Unknown	Sucrose	Glucose	Fructose	Sorbitol
Leaves	6	1.9	16.7	3.0	2.4	76.0
Leaves	30	2.4	15.7	5.9	3.8	72.2
Petioles	30 & 54[y]	1.9	10.0	5.9	4.9	77.3
Peduncles	30 & 54[y]	2.0	11.2	7.3	5.9	73.6
Fruits	6	1.6	26.5	8.5	7.7	55.7

[z]Solvent consisted of methylethyl ketone, glacial acetic acid and water saturated with boric acid (9:1:1, by volume).

[y]Organs combined on 2 sampling times due to small sample size.

AIS with amylase resulted in large variations in starch content, averaging 26 and 17% radioactive soluble substances on AIS basis in the 6-hr samples of July 5 and 25 treatments, respectively; 22 days later, the average percentages had decreased to 11 and 8%, respectively, (Table 2). Paper chromatography of this fraction revealed 2 radioactive peaks, a faster moving one at the Rf of glucose and the other at that of maltose. Of the ^{14}C accumulated by fruits, a small fraction was incorporated into the AIS (Table 1).

Table 4. Distribution of alcohol soluble carbohydrates in prune fruits supplied ^{14}C-sorbitol and ^{14}C-sucrose as substrates through their peduncles. The values are sample means after 1- and3-day exposures.

Substrate	Recoverable ^{14}C (%) Unknown	Sucrose	Glucose	Fructose	Sorbitol
^{14}C-Sorbitol[z]	5.1	48.9	7.1	5.5	33.4
^{14}C-Sorbitol[y]	3.1	25.0	4.2	4.5	63.2
^{14}C-Sucrose	6.4	59.2	19.7	13.1	1.6

[z]20 hr prefeeding with water prior to administration of substrate.

[y]20 hr prefeeding with 0.2% K_2SO_4 prior to administration of sorbitol.

The stem contained relatively small amounts of radioactivity compared with the rest of the spur parts (Fig. 1). The variation between spurs collected on the same date could be attributed to: a) the difference in the size and number of leaves exposed to $^{14}CO_2$; b) the number of fruits on the spurs serving as sink; and c) the stage of fruit development. These variables would affect the gradient between the source and sink, allowing the stem to temporarily accumulate the transient photosynthates. Stem cells, being relatively weak competitors for carbohydrates compared with fruit (9, 14), did not incorporate much ^{14}C. Amylase treatment of alcohol insoluble stem tissue taken from an unexposed portion of 2 spurs collected 54 hr after treatment on July 5 resulted in alcohol soluble hydrolyzates containing 29 and 13% recoverable radioactivity; the rest remained in the solid residues. This indicates that stem cells are depositing cell wall substances and starch in competition with the fruit cells which are accumulating much of the current photosynthates. However, this may be attributable to the girdle; normally, some of the sorbitol and sucrose would have been exported to the adjacent scaffold branch.

The alcohol soluble photosynthates in leaves consisted of 76% sorbitol and 16% sucrose, with the remainder in hexose and an unknown constituent which remained at the origin of the chromatogram (Table 3). In the petioles and peduncles, the proportion of sucrose to hexoses changed relative to that of the leaves, but the sorbitol level remained unchanged. The proportion of labelled sorbitol to sugars is greater than that published earlier (14), but this may be due to the difference in the rate of ^{14}C labelling of sugars and/or their interconversion.

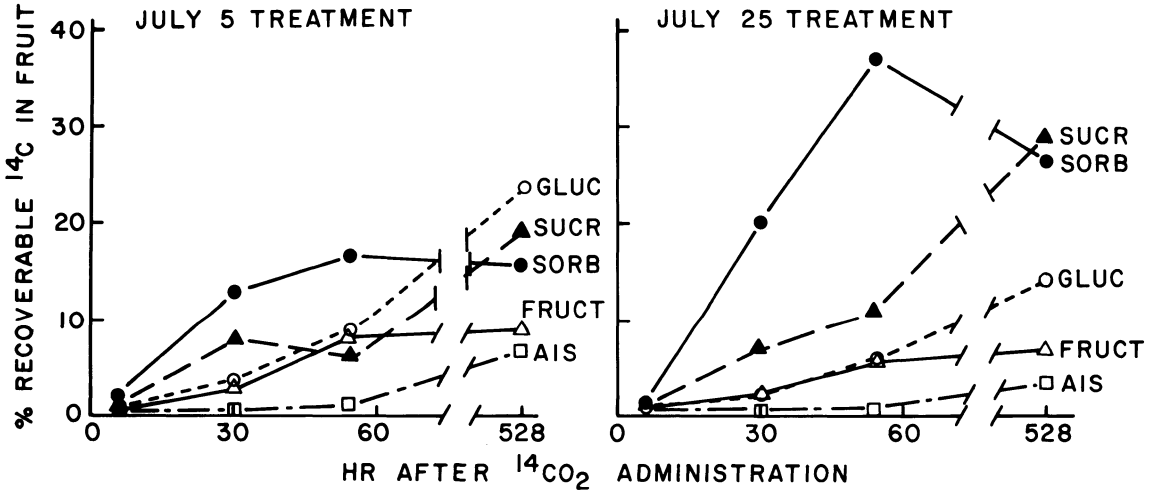

Fig. 2. Percentage of radioactive carbohydrates and alcohol insoluble substances (AIS) found in 'French' prune fruits based on the total radioactivity recovered from the same girdled spurs. Fruits were collected at intervals after administration of $^{14}CO_2$ to the foliage. Small amounts of unidentified ethanol-soluble substances are not plotted.

Table 5. Distribution of recoverable ^{14}C in individual 'French' prune fruits on spurs to which $^{14}CO_2$ was administered to 50% and 25% of the leaves. Fruits were collected 22 days after exposure.

Spur no.	Leaves exposed, percent	^{14}C in individual fruits (%)						
1	50	4.4	25.1	11.2	18.5	6.5	29.0	5.3
2	25	31.5[z]	29.7[z]	15.8[y]	19.6[y]	1.1	2.3	–

[z,y]Represent halves of the same fruit.

These findings support the idea that sorbitol and sucrose are the principal mobile carbohydrates in 'French' prunes, as reported for other stone and pome fruit species (2, 5, 8, 13).

Once sorbitol and sucrose entered the fruit, they were readily converted to other sugars (Fig. 2). Feeding experiments in which ^{14}C-sorbitol and ^{14}C-sucrose were introduced into fruits via their peduncles confirmed an earlier postulation that sorbitol was readily metabolized to sucrose (Table 4). ^{14}C-sucrose was hydrolyzed to glucose and fructose and an unknown substance rather than to sorbitol (Table 4) as in plums and apples (6, 8). The addition of potassium with ^{14}C-sorbitol apparently inhibited its conversion to sucrose, but the number of replicates was small and variability large. Hence, additional studies are needed to clarify this point. Individual fruits on the same spur varied considerably in the amount of radioactivity they contained (Table 5). While this may indicate relative sink strength, a large part of the variability is probably due to the proximity of the fruit to the ^{14}C-exposed leaves, and the vascular anatomy and phyllotaxy of the spurs. Those fruits low in radioactivity may well have been supplied with photosynthates from unexposed leaves, as reported for apples (7).

These findings indicate that when the fruit is accumulating dry matter rapidly in late July and early August, the rate of export of photosynthates from leaves is correspondingly faster, and the leaf starch content does not build up as much as when the fruit is not growing rapidly. This may account for the specific leaf weight being smaller on bearing than nonbearing branches (unpublished data).

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