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SORBITOL METABOLISM BY APPLE SEEDLINGS

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Key Word Index—*Malus domestica*; Rosaceae; apple; *Laminaria digitata*; Phaeophyceae; $^{14}CO_2$; [^{14}C]fructose; [^{14}C]sorbitol; sorbitol-6-phosphate phosphatase; mannitol dehydrogenase.

Abstract—The aims of this work were to compare the roles of sorbitol and sucrose in seedlings of *Malus domestica*, to discover which tissues synthesize sorbitol and which break it down, and to examine these tissues for enzymes of sorbitol metabolism. The detailed distribution of label was determined after supplying intact seedlings with $^{14}CO_2$, and excised parts of seedlings with [U-^{14}C]fructose and [U-^{14}C]sorbitol. The results showed that appreciable synthesis of sorbitol occurred only in the leaves but did not depend directly on photosynthesis. All tissues examined metabolized sorbitol but metabolism was extensive only in root apices, and in leaves which had been kept in the dark. The above experiments suggest that sorbitol supplements but does not replace sucrose. Extracts of apple leaves showed no trace of either a polyol or a polyol phosphate dehydrogenase but did exhibit sorbitol-6-phosphate phosphatase activity. A limited number of experiments with extracts of the blades of *Laminaria digitata* indicated that they contained mannitol-1-phosphate phosphatase and mannitol dehydrogenase.

INTRODUCTION

Sucrose is remarkable in that, almost universally in higher plants, it plays the three roles of the major soluble product of photosynthesis, the predominant form of translocated carbon, and the major soluble storage carbohydrate of the non-photosynthetic cells. In certain families the above functions are performed, to some extent, by sugar alcohols but the extent to which sugar alcohols are analogous to sucrose, and the way in which they are metabolized are not known. There is evidence that, in plants which do use a sugar alcohol extensively, sucrose is still an important product of photosynthesis [1, 2], a form of translocated carbon [3] and a storage compound [4]. Thus sugar alcohols do not appear to be capable of replacing sucrose completely.

The aims of the present work were to compare the roles of sorbitol and sucrose in apple seedlings, to discover which tissues in the seedlings make sorbitol and which break it down, and then to look in the appropriate tissues for the enzymes responsible for the synthesis and breakdown of sorbitol. First, the detailed fates of photosynthetically produced sorbitol and sucrose were determined in pulse and chase experiments with complete seedlings supplied with $^{14}CO_2$. Previous experiments of this type have used strips [2, 5] or disks of leaves [6], or shoots [7, 8] and do not reveal the role of roots in sorbitol metabolism. Secondly, we determined the distribution of label after supplying excised parts of the seedling with [^{14}C]fructose, and with [^{14}C]sorbitol. Finally, we examined the appropriate tissues for enzymes of sorbitol metabolism.

RESULTS

Metabolism of $^{14}CO_2$

Seedlings were given a 30-min pulse of $^{14}CO_2$ in the light under conditions as nearly natural as was practicable. For the 330-min chase in $^{12}CO_2$, some seedlings were kept in the light and the others were put in the dark. At the end of the pulse, and the chase, seedlings were divided into leaves, shoot and root, which were immediately and separately killed and analysed. Six different plants were given pulses, and for each type of pulse and chase, two different plants were analysed in detail. Although the total ^{14}C recovered per seedling varied with the seedling and the experiment, the detailed distribution of ^{14}C was very similar in plants given the same treatment. The following aspects of these distributions (Table 1) are noted. First, export of recently fixed carbon from the leaves was slow. Less than 0.5% of the total ^{14}C fixed was found in the stem and root at the end of the pulse. Even after a long chase, in the light or in the dark, over 80% of the label in the seedling was still in the leaves. Second, in all parts of the seedling, sorbitol plus sucrose accounted for at least 70% of the label at the end of the pulse. These two compounds still dominated the labelling patterns at the end of the chase regardless of whether this was in the light or dark. Finally, at the end of the pulse there was a very rough equivalence between the labelling of sorbitol and sucrose. During the chase this equivalence largely disappeared as the proportion of the label recovered as sucrose declined, whereas, except in roots in the dark, that in sorbitol either rose or remained the same.

Metabolism of [U-^{14}C]*fructose*

The extent to which non-photosynthetic parts of the seedling could synthesize sorbitol was investigated by determining their ability to convert [U-^{14}C]fructose to sorbitol (Table 2). The amounts of [U-^{14}C]fructose absorbed and metabolized varied with the type of tissue. In order to compare tissues the label recovered per fraction is expressed as a percentage of the total amount of [U-^{14}C]fructose metabolized by the sample. The latter

Table 1. Distribution of ^{14}C after exposing apple seedlings to $^{14}CO_2$ in the light

	Distribution								
	30 min in $^{14}CO_2$ in light			30 min in $^{14}CO_2$ in light followed by 330 min in $^{12}CO_2$ in the dark			30 min in $^{14}CO_2$ in light followed by 330 min in $^{12}CO_2$ in the light		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
% of ^{14}C in complete seedling recovered per organ	99.7	0.25	0.05	83.7	10.9	5.4	85.6	7.8	6.6
% of ^{14}C in each organ recovered in:									
Water-insoluble material	16	5	2	11	31	15	14	21	14
Water-soluble material	84	95	98	89	69	85	86	79	86
Acidic components	7	4	10	8	6	7	4	5	5
Basic components	4	3	7	7	10	13	1	2	4
Neutral components	66	80	78	64	46	55	76	72	68
Sucrose	26	42	41	10	9	24	20	22	28
Sorbitol	36	31	27	49	31	19	54	42	37
Fructose	1	2	2	1	1	2	1	2	2
Glucose	1	2	2	1	1	1	1	2	2

Total ^{14}C fixed varied with the seedling: the mean value $\pm$s.e. for the water-soluble material of 5 seedlings was 5163 ± 721 dpm $\times 10^{-3}$ per seedling.

Table 2. Metabolism of [U-^{14}C]fructose by apple seedlings

Fraction	Percentage of metabolized ^{14}C recovered per fraction					
	Bark of stem	Wood of stem	Complete root system	Root apices	Leaf disks in the light	Leaf disks in the dark
CO_2	25	24	39	32	3	22
Water-insoluble material	n.d.	n.d.	n.d.	8	17	12
Water-soluble material	75	76	61	60	80	66
Acidic components	n.d.	n.d.	n.d.	13	13	13
Basic components	n.d.	n.d.	n.d.	15	2	3
Neutral components	42	36	22	17	62	46
Sucrose	25	28	13	11	11	8
Sorbitol	2	1	1	2	43	31
Glucose	4	3	2	2	2	2
^{14}C metabolized (dpm × 10^{-3}/sample)	344.9	1086.1	899.4	1153	547.6	632.5

n.d.: not determined.

Metabolized ^{14}C is the sum of the ^{14}C recovered in $^{14}CO_2$, in the water-insoluble and water-soluble material minus that recovered in [^{14}C]fructose, except for the first three tissues where it does not include the label in the insoluble material. Samples were incubated for 6 hr.

figure is obtained by summing the ^{14}C recovered as $^{14}CO_2$, in the water-insoluble material and the water-soluble material, and subtracting from this sum the amount of label recovered as [^{14}C]fructose. All the tissues examined metabolized [^{14}C]fructose readily and 17–60% of that metabolized was converted to neutral compounds. Analyses of the latter showed that in the non-photosynthetic tissues there was no significant labelling of sorbitol and that most of the label was present as [^{14}C]sucrose. Sucrose was also labelled in the leaf disks but such labelling was slight in relation to that of sorbitol which even in the dark accounted for one-third of the total [^{14}C]fructose metabolized.

Metabolism of [U-^{14}C]*sorbitol*

We supplied [U-^{14}C]sorbitol to different parts of the seedling in order to compare their ability to breakdown sorbitol (Table 3). Each part absorbed [^{14}C]sorbitol readily and all of them metabolized it to some extent. This metabolism involved conversion to insoluble substances, sugars, CO_2, and to (probably) amino acids and organic acids. The extent of this metabolism was quite slight except in the root apices. For these apices we compared sorbitol as a respiratory substrate with sucrose, glucose and fructose. We incubated samples of the apices in 0.3 mM substrate (Table 3); $^{14}CO_2$ production from [U^{14}C]sorbitol, [U-^{14}C]glucose, [U-^{14}C]sucrose and [U-^{14}C]fructose in a 6-hr incubation was 17, 30, 29, and 29% of the absorbed label, respectively.

The ability of leaves to metabolize [^{14}C]sorbitol could be increased by keeping the leaves in the dark. If leaf disks were put in [U-^{14}C]sorbitol in the dark, as in the experiments shown in Table 3, $^{14}CO_2$ production for the first 3 hr was 3.5×10^3 dpm, but for the period 24–37 hr from the start of the incubation a value of 43.9×10^3 was obtained. Evidence that this increase was not due to microbial contamination is provided by the fact that no $^{14}CO_2$ production was detected when the leaf disks were removed from the medium at the end of the incubation and the medium alone was then incubated at 25° for a further 12 hr. A similar increase in the extent of [U-^{14}C]sorbitol metabolism was achieved by keeping the

Table 3. Metabolism of [U-^{14}C]sorbitol by apple seedlings

Fraction	Percentage of absorbed ^{14}C recovered per fraction					
	Bark of stem	Wood of stem	Complete root system	Root apices	Leaf disks in light	Leaf disks in dark
CO_2	0.6	0.8	3.3	13.7	0.6	2.2
Water-insoluble material	0.5	0.3	2.3	13.5	4.3	1.9
Water-soluble material	98.9	98.9	94.4	72.8	95.1	95.9
Acidic components	0.6	0.6	2.7	7.7	0.8	1.2
Basic components	0.1	0.5	0.5	7.0	0.2	0.2
Neutral components	98.5	97.2	90.8	50.4	94.5	94.5
Sucrose	0.6	0.5	1.1	2.4	0.5	0.8
Sorbitol	91.9	85.8	77.6	42.0	88.1	85.7
Fructose	0.5	0.9	1.8	0.8	0.5	0.4
Glucose	0.3	0.5	0.9	0.5	0.2	0.8
^{14}C absorbed (dpm × 10^{-3}/sample)	428.3	870.1	344	539.1	852.1	818.4

Samples were incubated for 6 hr.

Table 4. Effects of growth in the dark on metabolism of [U-^{14}C]sorbitol by disks of apple seedling leaves

Fraction	Percentage of absorbed ^{14}C recovered per fraction*	
	Seedlings grown with light	Seedlings kept in the dark for 5 days
CO_2	9	19
Water-insoluble material	5	13
Water-soluble material	86	68
Acidic components	5	8
Basic components	2	6
Neutral components	88	46
Sucrose	3	4
Sorbitol	65	36
^{14}C absorbed (dpm × 10^{-3}/sample)	2479	859

* Samples were incubated in the dark for 27 hr and were then killed and analysed.

seedlings in the dark before sampling the leaves and supplying them with isotope (Table 4). These results show that although uptake was drastically reduced there was a marked increase in the extent to which the absorbed [^{14}C]sorbitol was metabolized.

Enzymes of sorbitol metabolism

The data of Bieleski and Redgwell [2] suggest that in the Rosaceae sorbitol is made via a polyol phosphate dehydrogenase and a sorbitol-6-phosphate phosphatase. It seemed likely that sorbitol breakdown would be via a polyol dehydrogenase. Accordingly we examined leaves for the first two types of enzyme, and roots for the latter.

Crude extracts of leaves of apple seedlings released phosphate from sorbitol 6-phosphate at pH 7.5 more readily than from a range of sugar phosphates (Table 5). Variation of the pH of the reaction mixture from pH 5.0 to 9.0 suggested peaks of activity with sorbitol 6-phosphate at pH 5.0 and 7.1. We concentrated on the latter as it was more likely to be of physiological significance. In order to obtain some estimate of non-specific phosphatase activity, we compared phosphate release from sorbitol 6-phosphate with that from glucose 6-phosphate and, in some experiments, fructose 6-phosphate. Over the range pH 7.0–7.5, the excess of the former over the latter showed a clear optimum at pH 7.2. Fractionation of crude extracts of leaves of apple seedlings with $(NH_4)_2SO_4$ showed that the fraction precipitating at 40–60% saturation had a much higher ratio of activity with sorbitol 6-phosphate to that with hexose 6-phosphates than was found in the crude extracts (Table 6). Dialysis of the 40–60% fraction for 2.5 hr against 10 mM glycylglycine, pH 7.0, at 4° increased the ratio further to give activities of 4.87 and 0.25 nkat per g fr. wt for sorbitol 6-phosphate and glucose 6-phosphate, respectively. A crude extract of leaves of pear seedlings gave an activity of 5.43 nkat per g fr. wt and no activity was detected towards glucose 6-phosphate. No evidence of sorbitol-6-phosphate phosphatase was detected in crude extracts of either the apical 2 cm of roots of pear seedlings or the complete root system of apple seedlings. However, convincing evidence for an analogous phosphatase that converted mannitol 1-phosphate to mannitol was found in extracts of the blades of the brown alga *Laminaria* (Table 5).

Table 5. Phosphatase activities of extracts of leaves of apple seedlings and blades of *Laminaria digitata*

Substrate	Phosphatase activity (nkat per g fr. wt)	
	Apple	*Laminaria*
Sorbitol 6-phosphate	4.73	—
Mannitol 1-phosphate	1.60	18.6
Glucose 1-phosphate	0.80	2.1
Glucose 6-phosphate	0.92	0.57
Fructose 1-phosphate	0.87	—
Fructose 6-phosphate	0.50	none detected
Fructose 1,6-bisphosphate	1.97	0.13

Extracts were made in medium II at pH 7.5 and centrifuged at 100 000 *g* for 30 min: the supernatants were assayed at pH 7.5.

We found it difficult to detect enzymes of carbohydrate metabolism in extracts of apple leaves. Fewer problems were found with leaves of ash, another plant which uses a sugar alcohol. Thus we examined extracts of apple and ash leaves. We monitored our procedure by determining whether samples of pure glucose-6-phosphate dehydrogenase or phosphofructokinase were inactivated by extracts of the leaves. We did this by adding measured amounts of pure enzyme to an extract and then comparing the activity found in the extract with that predicted from separate measurements of the added activity and the initial activity of the extract. When leaves of apple seedlings were extracted with medium I and polyclar, 60% of the added glucose-6-phosphate dehydrogenase was lost; with ash leaves the loss was slight. Some improvement was obtained by using medium II but we never obtained satisfactory recoveries from extracts of apple leaves. The actual activities of glucose-6-phosphate dehydrogenase and phosphofructokinase detected in extracts of ash leaves extracted in medium II were 8.3 and 5.0 nkat per g fr. wt, respectively. These activities are appreciable when compared to those found in extracts of pea leaves [9]. Extracts of apple leaves in medium I, and of ash leaves in medium I, and also in medium II, were tested for their ability to oxidize 0.15 mM NADH and 0.15 mM NADPH in the presence of the following at 1 mM: fructose, fructose 6-phosphate, glucose and glucose 6-phosphate. The pH was varied from pH 7.0 to 8.5. With extracts of ash we also determined the effects of adding, separately, $MnCl_2$, $MgCl_2$, $ZnCl_2$, $CoCl_2$, NaCl and KCl at 1.0 and 10 mM, $FeCl_2$ at 1.0 and 2.5 mM. In none of the above experiments could we demonstrate consistently any activity of polyol phosphate or polyol dehydrogenase. Attempts to demonstrate polyol phosphate dehydrogenase by assaying in the reverse direction were also unsuccessful. Extracts of apple leaves in medium II were incubated with either 0.15 mM NAD or 0.15 mM NADP, and 1 mM sorbitol 6-phosphate at pH 7.0, 7.5 and 8.5. The effects of $MgCl_2$ and $MnCl_2$ at 1.0 and 10 mM were also studied. No activity was found. No activity of NAD-dependent polyol dehydrogenase was detected when extracts of leaves of ash and apple were

Table 6. Phosphatase activity of fractions obtained by treating extracts of leaves of apple seedlings with $(NH_4)_2SO_4$

Fraction	Phosphatase activity (nkat per g fr. wt)		
	Sorbitol 6-P	Glucose 6-P	Fructose 6-P
Crude extract	3.45	1.92	2.1
0–20% satd $(NH_4)_2SO_4$ ppt.	0.12	0.10	none detected
20–40% satd $(NH_4)_2SO_4$ ppt.	0.18	0.02	none detected
40–60% satd $(NH_4)_2SO_4$ ppt.	3.12	0.32	0.37
60% satd $(NH_4)_2SO_4$ supernatant	0.88	0.95	0.93

Extracts were made in medium II at pH 7.5 and centrifuged at 27 000 g for 30 min. The supernatant was treated with $(NH_4)_2SO_4$ and each fraction was assayed at pH 7.1.

assayed at the high substrate concentrations (400 mM hexose or, in the reverse direction, 150 mM sugar alcohol) that have been used to assay such an enzyme in extracts of liver [18].

We were no more successful in our search for sorbitol dehydrogenase in apple roots. Extracts of apple seedling roots showed no activity towards either 0.5 mM NAD and 0.5 mM NADP at pH 7.5 in the presence of 1.5, 15 or 150 mM sorbitol. Similarly, negative results were obtained with extracts of the apical 2 cm of the roots of seedlings of apples and pears, and with extracts of pear fruit. Our extraction technique was good enough to demonstrate an activity of 1.64 nkat per g fr. wt for glucose-6-phosphate dehydrogenase for the apical 2 cm of apple roots. Evidence that the assay was capable of detecting polyol dehydrogenase is provided by the fact that the assay did demonstrate such an enzyme in extracts of the blades of *Laminaria*. Incubation of *Laminaria* extracts with 10–300 mM fructose and 0.5 mM NADPH in 50 mM glycylglycine buffer at pH 7.5 led to appreciable oxidation of NADPH. No activity was found if the fructose was replaced by any of the following: fructose 6-phosphate, fructose 1-phosphate, glucose 6-phosphate, glucose 1-phosphate. Only a trace of activity was found with glucose and none was detected if NADH was used instead of NADPH. *Laminaria* extracts not only catalysed a fructose-dependent oxidation of NADPH but were also capable of a comparable mannitol-dependent reduction of NADP. The pH optimum for fructose reduction was pH 7.5 and that for mannitol oxidation was pH 7.9–8.3. Fructose reduction increased with fructose concentration at least up to 300 mM. Rates of 0.6, 2.7, 4.9 and 9.1 nkat per g fr. wt were obtained at fructose concentrations of 10, 80, 150 and 300 mM, respectively. $MgCl_2$ increased activity and maximum stimulation, 25% of the rate in the absence of $MgCl_2$, was found at 5 mM. The absolute activities found in the extracts of *Laminaria* were appreciable: at pH 7.5, 100 mM fructose, 5 mM $MgCl_2$ and 0.5 mM NADP a value of 16.4 nkat per g fr. wt was detected.

DISCUSSION

Sorbitol is clearly a major product of photosynthesis in apple seedlings as it is in many other Rosaceae [5]. The distinctive feature of apple seedlings is that sucrose was labelled by $^{14}CO_2$ to almost the same extent as sorbitol. In most other studies of this type [2, 7, 8] the labelling of sorbitol has greatly exceeded that of sucrose.

Our results provide very strong evidence that the ability to synthesize sorbitol is largely confined to the leaves in apple seedlings. The data in Table 2 show that all the different tissues metabolized [^{14}C]fructose extensively. The distribution of label strongly suggests conversion of [^{14}C]fructose to fructose 6-phosphate, which then served both as respiratory substrate and precursor of sugars and polysaccharides. Thus any ability of the non-photosynthetic tissues to synthesize sorbitol should have been detected by our experiments. The fact that leaf disks converted [^{14}C]fructose to sorbitol in the dark shows that, although the ability of apple seedlings to synthesize sorbitol is largely confined to the leaves, the synthesis is not obligatorily linked to photosynthesis.

Our experiments indicate the fate of photosynthetically produced sorbitol in apple seedlings. The marked contrast between the rapidity with which label appeared in sorbitol and the slowness with which it disappeared strongly suggests that the prime role of sorbitol in these seedlings is storage of carbon. The data in Table 1 indicate that, once sorbitol had been made, further metabolism by the leaf was slight. This view is confirmed by the fate of [^{14}C]sorbitol supplied to leaf disks. In fact, appreciable breakdown of sorbitol by leaf tissue was only found when the leaves were starved by keeping them in the dark. It is highly probable that sorbitol, as such, is exported from the leaf. A considerable proportion of the label that appeared in the stem and roots after photosynthesis in $^{14}CO_2$ was present as [^{14}C]sorbitol. Some of the label present in the acidic and basic, but not the neutral, components of the water-soluble substances in the stem and root at the end of the pulse was probably due to fixation of $^{14}CO_2$ by phosphoenolpyruvate carboxylase. Thus in the stem and root the proportion of the early products of photosynthesis that were present as sorbitol and sucrose may have been a little higher than is indicated by the data in Table 1. As the stem and root did not synthesize sorbitol at appreciable rates, we suggest that the sorbitol in these tissues came from the leaves. Our results strongly suggest that photosynthetically produced sorbitol is transported to all the major regions of the seedling. With the exception of the root apices, use of sorbitol by the non-photosynthetic tissues appeared to be rather slow. This contrasts with their considerable ability to absorb sorbitol (Table 3). This situation would be expected if sorbitol were predominantly a storage compound.

Our results allow sorbitol and sucrose to be compared in apple seedlings. Both are major products of photosynthesis and both appear to be transported

throughout the plant (Table 1). Every tissue studied readily synthesized sucrose (Table 2) but sorbitol synthesis was localized. The behaviour of sucrose during the cold chase suggests that it was more rapidly used than was the sorbitol that was formed at the same time. Our results are consistent with the view that apple seedlings metabolize and use sucrose in basically the same way as higher plants in general. The apple seedlings are distinguished by their synthesis of sorbitol as well as sucrose during photosynthesis and by the use of this sorbitol as a transport and storage compound that is rather less available than sucrose.

The pathway of sorbitol metabolism in the Rosaceae is still unknown. The kinetics of labelling of sorbitol 6-phosphate by $^{14}CO_2$ [2], and thermodynamic considerations, strongly suggest a key role for sorbitol-6-phosphate phosphatase in sorbitol synthesis. Our results strengthen this view considerably. Although crude extracts of apple leaves showed phosphatase activity towards a range of phosphate esters, that with sorbitol 6-phosphate was clearly the highest. Partial purification yielded a preparation in which activity with sorbitol 6-phosphate was 10 times that with the common hexose 6-phosphates (Table 6). The highest activity that we found with sorbitol 6-phosphate was 7 nkat per g fr. wt of leaf. Although this is certainly not the maximum catalytic activity, the value is close to estimates of the rate of photosynthesis of apple leaves, 460–780 nmol CO_2/min per g fr. wt [11]. Our results from *Laminaria* indicate that this species contains a mannitol 1-phosphatase similar to that reported for other brown algae by Yamaguchi *et al.* [12].

We could not detect dehydrogenases for either polyol phosphates or polyols in any of the higher plants we examined. It is not clear whether this reflects the absence of such enzymes from the leaves. Our techniques were good enough to allow us to discover and demonstrate quite easily on NADP-linked mannitol dehydrogenase in *Laminaria*. In addition, we were able to demonstrate, in leaf extracts, enzymes of carbohydrate metabolism that would be expected to be present at much lower activities than those responsible for sorbitol synthesis. Nonetheless, on balance, it seems more likely that our results were due either to inactivation of the dehydrogenases during extraction, or to a failure to provide the right conditions for assay. We showed that it is difficult to extract even a relatively robust enzyme such as glucose-6-phosphate dehydrogenase from apple tissues. Further, Hirai [13] has recently reported that loquat fruits contain a sorbitol-6-phosphate dehydrogenase. However, synthesis of sorbitol via sorbitol-6-phosphate dehydrogenase is not yet proven. The activities reported by Hirai are minute: they appear to be about 0.08 nkat per g fr. wt. Further, the reported pH optimum of pH 9.1 seems well outside the range expected at the site of sorbitol synthesis. Consideration of our results with root extracts in the light of the above arguments means that we can draw no conclusion about the role of polyol dehydrogenase in sorbitol breakdown in apples. However, our demonstration of an NADP-linked mannitol dehydrogenase in *Laminaria* suggests that such an enzyme may be responsible for mannitol breakdown in this species.

EXPERIMENTAL

Materials. Isotopes were from The Radiochemical Centre, Amersham, and substrates, enzymes and co-factors were from Boehringer or Sigma. Seeds of apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.), obtained from locally grown fruit, were soaked in 5% (v/v) HOCl for 15 min, washed in running H_2O for 4 hr, drained, mixed with damp sterile peat, put in a polythene bag and kept at 4° for 70–90 days. The germinated seeds were then planted, one per pot (8 cm diam), in a mixture (1:7:4 by vol.) of loam, peat and sand to which we added chalk (0.06% w/v) and Vitex Q4HN (0.03% w/v). The pots were kept in a well ventilated greenhouse in which the temp. was maintained above 17°, and were illuminated for 16 hr each day by daylight supplemented by artificial light that gave a fluence rate of 10 W/m^2 at the soil surface. Experiments were done with seedlings which had 14 expanded leaves and were about 12 weeks old. Leaf disks (1 cm diam) were punched from seedling leaves with a cork borer. Samples of bark, and of wood, were obtained by peeling the bark off the central stem and then cutting the bark and wood into 1.5 cm lengths. Examination with a microscope showed that the bark contained epidermis, cortex and phloem, and the wood contained xylem and pith. Roots were excised at ground level and samples were prepared from complete root systems. When root tips were needed, germinated seeds were transferred from the cold, put on moist filter paper in a Petri dish and incubated for 4–6 days at 18–20°, after which the apical 1–2 cm of the roots were excised and used at once. Leaves of mature trees of apple, pear and ash (*Fraxinus excelsior* L.) were picked from locally grown trees just before they were needed. Plants of *Laminaria digitata* (L.) Lamour were collected from the Dorset coast, kept in the dark in aerated sea water at 4° and the blades were excised and used within 10 days of collection.

Metabolism of labelled substrates. Seedlings were exposed to $^{14}CO_2$ individually. For each exposure, a single potted seedling, 5 hr after the start of the 16 hr photoperiod, was placed in a perspex box (29.5 × 22.5 × 22.5 cm) at 20° and illuminated as during growth. $^{14}CO_2$ was generated in the box by adding lactic acid to 50 μCi Na_2 $^{14}CO_3$ (sp. act. 58.5 mCi/mmol). After 30 min the box was flushed with air and the seedling was either removed (pulse) or left in the box for a further 330 min (chase) in either the above light or in darkness. At the end of the pulse, and the chase, the leaves were quickly pulled off the seedlings, then the stem was cut off at ground level, and the roots were washed free of soil to give the samples referred to as leaves, stem and root, respectively. These samples were killed at once with boiling 80% aq. EtOH. In the experiments in which labelled sugars were fed, samples of leaf disks, root tips (0.5 g fr. wt), bark, stem and root (1.0 g fr. wt) were suspended in 5.0 ml 0.02 M KH_2PO_4 (pH 5.2) that contained either 0.3 mM [U-^{14}C]fructose (4.6 μCi) or 0.3 mM [U-^{14}C]sorbitol (8 μCi). The incubations were in the dark at 25° in 150-ml flasks fitted with a centre well, which contained 10% KOH to absorb respired $^{14}CO_2$. At the end of the incubation the suspending medium was removed and the samples were given 4 successive rinses, each with 10 ml 0.02 M KH_2PO_4, pH 5.2, at 2°. For each tissue the amount of ^{14}C removed in each successive rinse was plotted as in ref. [14]. The plots showed that the rinses effectively removed ^{14}C from the free-space of the samples. The total label left in the tissues after the fourth rinse plus that recovered as $^{14}CO_2$ is summed to give estimates of the uptake of labelled substrates. After the fourth rinse the tissue was killed with boiling 80% aq. EtOH. After killing, all tissues, whether fed $^{14}CO_2$ or labelled sugars, were extracted by boiling, successively, in 80 and 50% aq. EtOH and then H_2O. The residue is called the water-insoluble material and its ^{14}C content was determined as in ref. [15] except for the samples of root and stem. These were first incubated with tissue solubiliser (Koch-Light TS 1) for 48 hr and then the residue was combusted to CO_2 as above. The ^{14}C released by the two treatments is summed to give the total label in the insoluble fraction. The extracts of the samples were

combined, reduced to 4–6 ml at 28° to give the water-soluble substances which were fractionated into their acidic, basic and neutral components by ion-exchange chromatography as in ref. [9]. The sugars in the neutral components were separated by PC as in ref. [16]. Losses during analyses were slight. The ^{14}C recovered in the acidic, basic and neutral components amounted to 94 ± 1% (mean ± s.e. of 28 analyses) of that originally present in the water-soluble material. Recovery from chromatograms of the neutral components was 96 ± 1% (mean ± s.e. of 23 chromatograms) of the activity added. ^{14}C was measured as in ref. [9].

Measurements of enzymes. Samples of tissue (1–2 g fr. wt) were ground in a pestle and mortar with 3–5 vol. extraction medium together with 0.25–1.0 g polyclar (insoluble polyvinylpyrrolidone) per g fr. wt. Extraction medium I was 50 mM glycylglycine (pH 7.5), 20 mM EDTA, 20 mM cysteine–HCl and 20 mM sodium diethyldithiocarbamate. Medium II was similar except that it lacked EDTA. Unless otherwise indicated, extracts were centrifuged at 100 000 g for 30 min and the supernatant, called the crude extract, was assayed at once. Extracts were made at 4° and kept at 1°. For assay of phosphatase 0.5 ml extract was incubated at 25° for 20 min with 3 mM $MgCl_2$, 5 mM substrate, and buffer in a final vol. of 3 ml. The buffer was 50 mM sodium citrate for pH 5.0–6.5, 50 mM glycylglycine for pH 6.7–7.8, and 50 mM Tris–HCl for pH 8.0–9.0. The reaction was stopped by the addition of 4 ml 12% (w/v) TCA and the phosphate was measured as in ref. [17]. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and phosphofructokinase (EC 2.7.1.11) were measured as in ref. [18]. For the assays of polyol and polyol phosphate dehydrogenases the vol. of the reaction mixture was 3 ml and the oxidation of NAD(P)H or reduction of NAD(P) was followed spectrophotometrically at 340 nm. The reaction mixtures contained 0.1–0.2 ml extract together with substrate, co-enzyme, and buffer. The range and concn of buffers were as described for the phosphatase assays; the concentrations of co-enzymes and substrates are given in the text.

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