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Temperature effects on partitioning of ^{14}C assimilates in tall fescue (*Festuca arundinacea* Schreb.)

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SUMMARY

Fructan synthesis and carbon partitioning to roots of tall fescue (*Festuca arundinacea* Schreb.) were studied with a ^{14}C-labelling technique at three growth temperatures: 24/17 °C, 16/10 °C and 8/5 °C (day/night). Plants at 16/10 °C and 8/5 °C had an increased proportion of assimilates allocated to the root system; 10 d after exposure at $^{14}CO_2$, the relative carbon partitioning to the roots averaged 12·5, 16·3 and 26·8 % respectively, at 24/17 °C, 16/10 °C and 8/5 °C. Chilling temperatures induced an increase in the amounts of sucrose and fructans in leaves. Interestingly, a similar pattern occurred in roots where low-DP fructans (DP < 8) are the predominant non-structural carbohydrate constituent. Incorporation of $^{14}CO_2$ into root oligo- and polysaccharides showed a progressive movement of radioactivity from sucrose to fructans. These results show that the roots can serve as a carbon storage organ and that fructan synthesis depends on sucrose supply. The function of the fructan stored in the roots is unknown.

Key words: Carbohydrate balance, *Festuca arundinacea*, fructans, low temperature, sucrose.

INTRODUCTION

Perennial grasses in temperate regions are naturally exposed to prolonged periods of low temperatures (chilling temperatures). Fructan is the main polysaccharide reserve in vegetative tissues in most cool-season grasses (Pontis & Del Campillo, 1985; Pollock, 1986; Nelson & Spollen, 1987; Chatterton *et al.*, 1989). Generally, low ambient temperatures lead to an alteration in the balance between carbon assimilation and utilization resulting in a pronounced increase in fructan and sucrose contents in leaves of barley (Wagner & Wiemken, 1989), *Lolium temulentum* (Pollock, 1984), *Lolium perenne* (Arbillot *et al.*, 1991), *Triticum aestivum* (Tognetti, Calderon & Pontis; 1989, Tognetti *et al.*, 1990) and *Poa pratensis* (Solhaug, 1991). As fructan accumulation is correlated with a decrease in growth temperature (Chatterton *et al.*, 1989), it has been suggested that fructans may act as cryoprotectants (Eagles, 1967) and influence the cold hardiness of fructan-accumulating species (Suzuki & Nass, 1988; Pontis, 1989). However, in leaves of *Lolium perenne* (Gonzalez *et al.*, 1990) and *Lolium temulentum* (Pollock, Eagles & Sims, 1988), the depression of freezing point attributable to the accumulation of carbohydrates was not sufficient to account for the freezing tolerance of the plants. As emphasized by Tognetti *et al.* (1990), the role of fructans in cold acclimation remains an open question since their accumulation most likely results from sucrose accumulation (Pollock, 1984; Wagner, Wiemken & Matile, 1986) rather than from low temperatures *per se*.

Compared to aerial parts, little knowledge exists about fructan metabolism in grass roots. Fructan content of roots of wild grasses (*Elymus repens*, *Agropyron repens* and *Holcus lanatus*) varied over the year with a maximum in autumn and minimum in early spring (Steen & Larsson, 1986). Under controlled conditions, low temperatures increased both sugar and fructan concentrations in roots of *Poa pratensis* (Solhaug, 1991), *Agropyron* and *Agrostis alba* (Chatterton *et al.*, 1987).

The dynamics of entry of radiolabelled assimilates synthesized in leaves from $^{14}CO_2$ into root carbohydrates has not been reported. Our objective was to

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determine the effects of growth temperature upon distribution of radioactivity among non-structural carbohydrates, including fructans, in leaves and roots of tall fescue (*Festuca arundinacea* Schreb), a cool-season grass that accumulates fructans (Housley & Volenec, 1988).

MATERIALS AND METHODS

Plant culture

Tall fescue (*Festuca arundinacea* Schreb) seedlings were grown in sand culture in a glasshouse and grown for approximately 2 months. Plants received a nutrient solution containing 1·9 mM KNO_3 0·55 mM $Ca(NO_3)_2$, 2·5 mM NO_3NH_4, 0·5 mM $CaCl_2$, 0·1 mM NaCl, 0·5 mM $MgSO_4$, 0·4 mM H_2KPO_4, 0·3 mM HK_2PO_4, plus micro-elements. Three sets of 30 plants were clipped to leave a stubble height of 5 cm, and transferred to growth chambers with 500 μmol m^{-2} s^{-1} PPFR, 12 h photoperiod, and 70% relative humidity. Chambers had day/night temperatures of 24/17 °C, 16/10 °C, and 8/5 °C.

^{14}C labelling

Three weeks after transfer to the growth chambers, each set of 30 plants was transferred to a controlled environment ^{14}C-labelling chamber, adapted from Warembourg & Paul (1973), with a volume of 175 l and a growing area of 0·25 m^{-2}. The PPFR and relative humidity were similar to that experienced in the growth chamber. The CO_2 concentration was maintained at 330 μl l^{-1} ($\pm$25) by connecting an infrared CO_2 analyser through a regulation device to both a CO_2 generating unit using a solution of Na_2CO_3 and a soda-lime CO_2 trapping system. Temperature was maintained ($\pm$1 °C) at the growth temperature for each set of plants.

Plants were exposed to $^{14}CO_2$ throughout a 12 h photoperiod to label the storage carbohydrates. The specific activity of the solution used to generate $^{14}CO_2$ was 18·5 MBq g^{-1} C. At the end of the labelling period, the $^{14}CO_2/^{12}CO_2$ of the air within the chamber was trapped and replaced by $^{12}CO_2$. The chamber was switched from a closed to a semi-open system with an external air flow of 0·13 l s^{-1} in order to minimize reassimilation of respired $^{14}CO_2$. Plants were kept in this chamber for 10 days following exposure to $^{14}CO_2$, unless sampled earlier.

Sampling and total ^{14}C determinations

Seven successive samplings were taken during the course of the experiment. The first sampling started immediately after the end of $^{14}CO_2$ exposure (time 0 in the figures), and the others were 12, 24, 48, 72, 120 and 240 h later. For each sampling, 4 plants were selected randomly and washed free of sand. Shoots and roots were separated, dried at 70 °C in a ventilated oven for rapid dehydration of tissues, and ground. This procedure was reported to cause no alteration in carbohydrate content or composition of samples (Schnyder & Nelson, 1987). Total ^{14}C content was determined by liquid scintillation following dry combustion and CO_2 trapping (Bottner & Warembourg, 1976).

Carbohydrate extraction, separation and radioactivity

Carbohydrates were extracted from dry tissue (0·5 g) in boiling 80% ethanol under reflux for 1 h. The ethanol filtrate was concentrated by rotary evaporation and taken up in 5 ml water. This fraction contained fructose, glucose, sucrose and fructans of low degree of polymerisation (DP). Fructans of high-DP were extracted from the ethanol-insoluble residue with boiling water for 1 h. Each fraction was centrifuged for 20 min at 10000 ***g*** and filtered through a 0·45 μm membrane. Carbohydrates were separated by ion retardation on high performance liquid chromatography (HPLC) using a cation exchange column (Sugar-Pak, Millipore Waters, USA) eluted with water at a flow rate of 0·5 ml min^{-1}. Carbohydrates were detected with a differential refractometer (model 156, Beckman) and quantified using glucose, fructose, sucrose and inulin as external standards and mannitol as an internal standard. Carbohydrates were collected and the radioactivity of each fraction was measured by liquid scintillation spectroscopy.

RESULTS

Effect of low temperatures on assimilate allocation from shoots to roots

Low temperatures increased the proportion of assimilates allocated to the root system (Fig. 1). Ten days after exposure to $^{14}CO_2$, the relative carbon

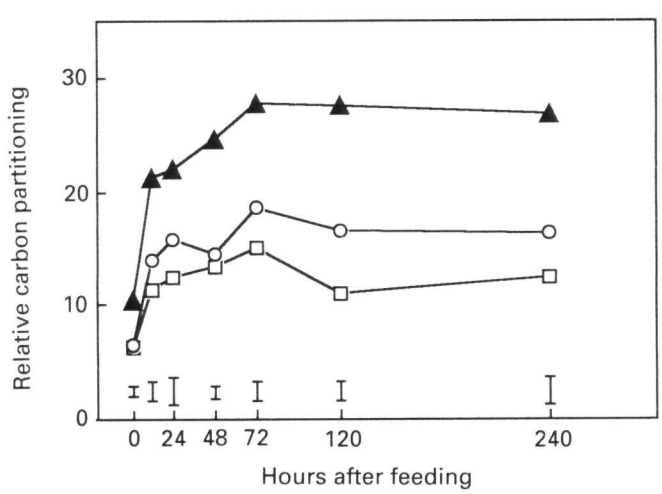

Figure 1. Relative carbon partitioning (percentage of total plant ^{14}C) to the roots of *Festuca arundinacea* grown at 24/17 °C (□), 16/10 °C (○) and 8/5 °C (▲) day/night temperatures. Bars indicate LSD.

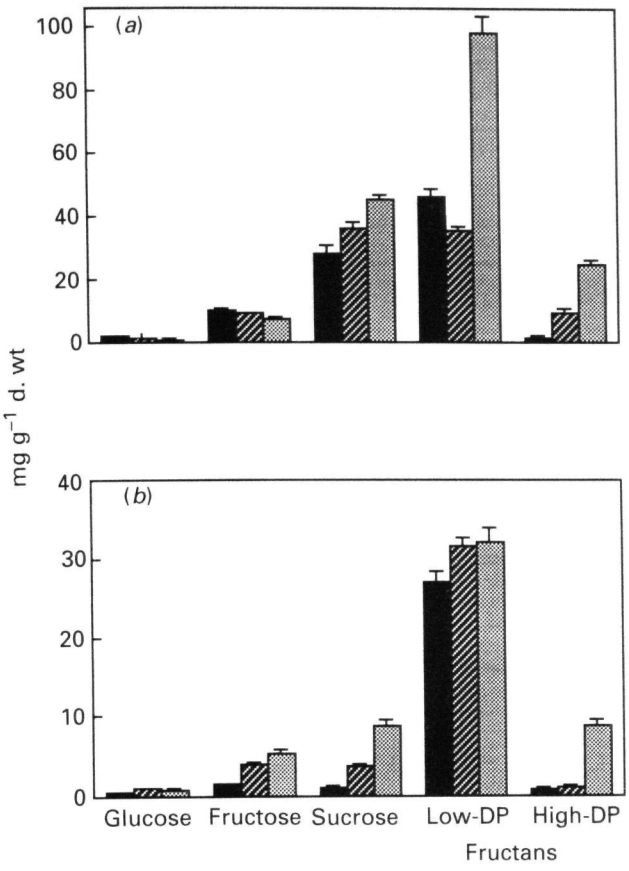

Figure 2. Concentrations of glucose, fructose, sucrose, low-DP (DP < 8) and high-DP fructans in (*a*) shoots and (*b*) roots of *Festuca arundinacea* grown at 24/17 °C (■), 16/10 °C (▨) and 8/5 °C (▩) day/night temperatures. Bars indicate SD, $n = 26$.

partitioning to the roots averaged, respectively, 12·5, 16·3 and 26·8 % of the total plant ^{14}C at 24/17 °C, 16/10 °C and 8/5 °C. These carbon partitioning coefficients were similar to the ratio of root biomass to total biomass, respectively 16·0, 17·0 and 23·1 %.

Effect of low temperatures on carbohydrate contents in shoots and roots

Shoots and roots contained glucose, fructose, sucrose and fructans of low-DP (DP < 8) and high-DP (Fig. 2). In both shoots and roots, fructans were the predominant carbohydrate; low-DP fructan content contributed more than 50 and 80 % of the total carbohydrates in shoots and roots, respectively.

In shoots, total fructan concentrations were similar for plants grown at 24/17 °C and 16/10 °C, but were 2·6-fold higher for plants grown at 8/5 °C. A similar difference was reported by Chatterton *et al.* (1989) in *Festuca* leaves grown at 25/15 and 10/5 °C. Sucrose, the proposed precursor for fructan biosynthesis, was at the highest concentration in plants grown at 8/5 °C. These data are in agreement with the generalization that fructan accumulation is not at the expense of any other form of non-structural carbohydrates (Chatterton *et al.*, 1989), especially sucrose. Glucose and fructose, however, were at lower concentration in shoots of plants growing at 8/5 °C than at higher temperatures.

In roots, both low-DP and high-DP fructan contents were higher when plants were grown at 8/5 °C than at 16/10 °C or 24/17 °C. At each temperature sucrose content was lower in roots than in shoots. The 8/5 °C treatment induced an accumulation of sucrose and fructose and a slight increase in glucose concentration compared with 24/17 °C and 16/10 °C.

Effect of low temperatures on $^{14}CO_2$ incorporation into shoot carbohydrates

In shoots, the ^{14}C content of each carbohydrate fraction is expressed as a percentage of total $^{14}CO_2$ assimilated during the labelling period (Fig. 3). At all the temperatures, sucrose was the predominant labelled compound at zero time, immediately after exposure of leaves for 12 h to $^{14}CO_2$. The amount of labelled sucrose declined rapidly during the 12 h dark period after exposure to $^{14}CO_2$, from 15·7 to 2·7 %, 16·7 to 7·5 % and 37·3 to 14 %, respectively, at 24/17 °C, 16/10 °C and 8/5 °C. In the first 2 d after feeding, in plants grown at 16/10 °C and 8/5 °C glucose contained higher percentages of total radioactivity than at 24/17 °C, while labelling of fructose responded little temperature throughout the experimental period. Labelled glucose and fructose decreased rapidly to negligible amounts.

It is generally accepted that sucrose is the precursor of fructans by the concerted action of fructosyltransferases (Pollock, 1986). In plants grown at 24/17 °C, a very low percentage of ^{14}C appeared in either the low-DP or high-DP fructans. However, low-molecular-weight fructans, and to a lesser extent high-molecular-weight fructans, peaked 24 h after labelling when plants were grown at 8/5 °C. The radioactivity in low-DP fructans increased to 3·2 and 10·2 % respectively, at 16/10 °C and 8/5 °C. A significant portion of ^{14}C was invested in long term storage in the shoots when grown at 8/5 °C, in that 6·3 and 2·5 % of the total $^{14}CO_2$ assimilated was still present in low-DP and high-DP fructans, respectively, 10 d after feeding.

Effect of low temperatures on $^{14}CO_2$ incorporation into root carbohydrates

Patterns of labelling of individual carbohydrate fractions differed substantially among growth temperatures (Fig. 4). Roots of plants grown at 24/17 °C had less than 1 % of the total $^{14}CO_2$ incorporated into each fraction. However, in roots of plants grown at 16/10 °C and 8/5 °C sucrose accumulated to 5 and 13 times higher amounts of ^{14}C, respectively. Roots at 16/10 °C and 8/5 °C also contained more radiolabelled glucose and fructose. Before decreasing throughout the time of experiment like in shoots, amounts of labelled monosaccharides and sucrose in roots increased during the dark period following

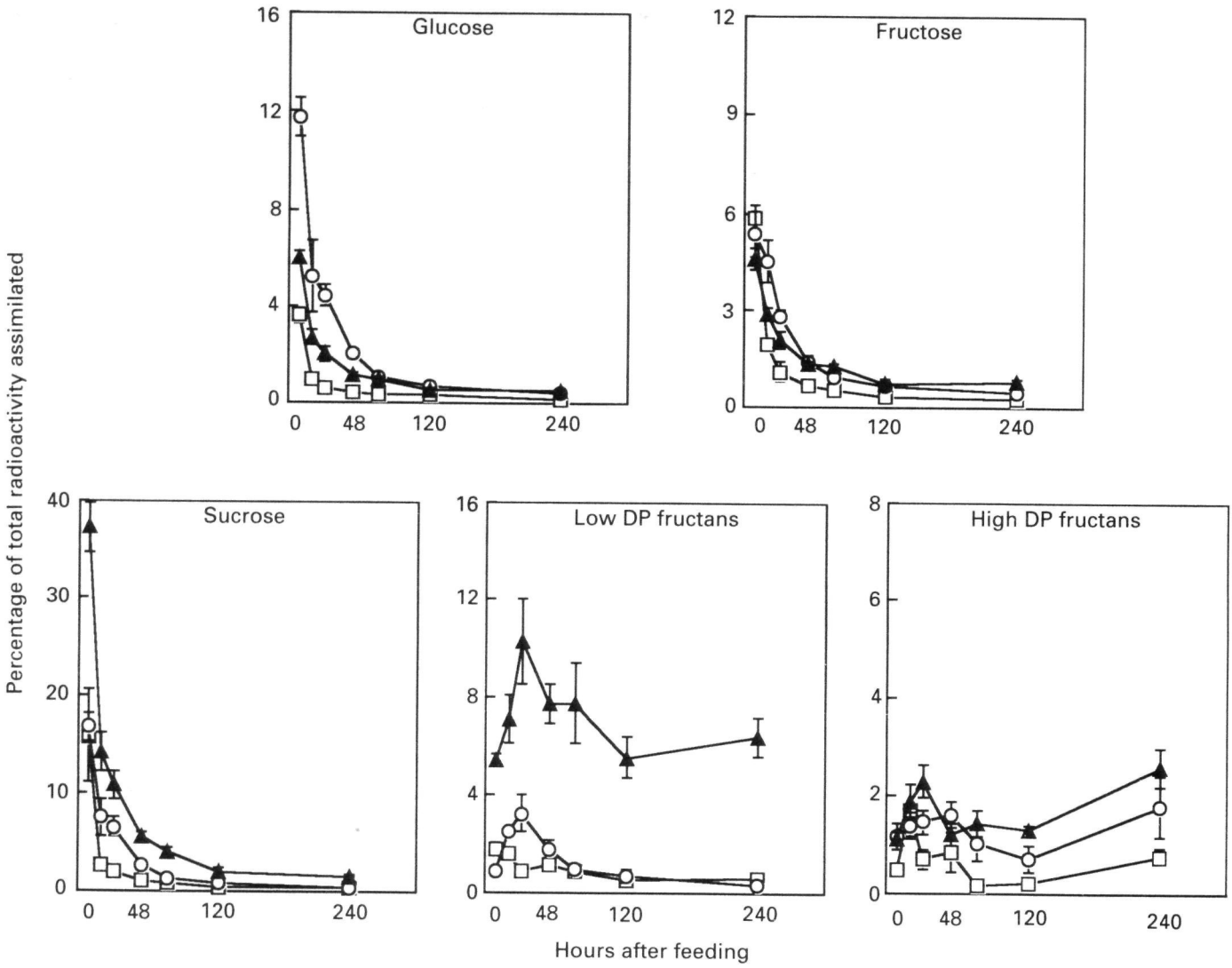

Figure 3. ^{14}C partitioning among water soluble carbohydrates in shoots following incorporation of $^{14}CO_2$ by leaves of *Festuca arundinacea* grown at 24/17 °C (□), 16/10 °C (○) and 8/5 °C (▲). Carbohydrates were separated by HPLC. Values are the means of 4 or 5 determinations; bars indicate SD.

$^{14}CO_2$ exposure. Compared to plants grown at 24/17 °C, plants grown at 8/5 °C had 15·4 and 10·1 times higher proportions of ^{14}C allocated to low-DP and high-DP fructans, respectively. As shown in Figure 5, the absolute fluxes of ^{14}C into sucrose, low-DP and high-DP fructans were also higher in plants grown at 8/5 °C than in plants grown in 24/17 °C.

DISCUSSION

During cool temperatures, accumulation of fructan in shoots and roots of *Festuca arundinacea* indicates an excess of carbon fixation over utilization. This response is partly due to the fact that photosynthesis of plants exposed to chilling temperatures is less temperature sensitive than growth processes (Pollock *et al.*, 1983). The data show that the plants acclimate to the change in source–sink balance induced by low temperatures by increasing fructan and also sucrose accumulation in both shoots and roots. Starch is also a storage compound which accumulates at low temperatures; in crested wheatgrass and redtop, leaves and roots accumulated significant amounts of starch at 5 °C (Chatterton *et al.*, 1987). As in roots of *Festuca arundinacea*, starch represented 24% of total non-structural carbohydrates and doubled in amount when plants were grown at 10/5 °C (N. J. Chatterton, personal communication), it would be worthwhile to include starch metabolism in future work of plants subjected to low temperature treatments.

The positive effect of low temperatures on relative carbon partitioning to the roots has already been reported for grass species (Davidson, 1969; Labhart, Nösberger & Nelson, 1983; Pollock *et al.*, 1983). In *Poa pratensis*, low temperatures induced marked differences in growth of different organs, with root production being relatively less affected than growth of leaves, resulting in a decrease of the shoot/root ratio (Solhaug, 1991).

Bucher, Mächler & Nösberger (1987) in *Festuca pratensis*, Borland & Farrar (1985) in *Poa pratensis*, Gordon *et al.* (1982) and Sicher, Kremer & Harris (1984) in barley found a diurnal fluctuation of sucrose content: it increased during the light period and decreased during the following dark period as it was translocated from the leaves. Thus, part of the decrease in labelled sucrose in the shoots was likely

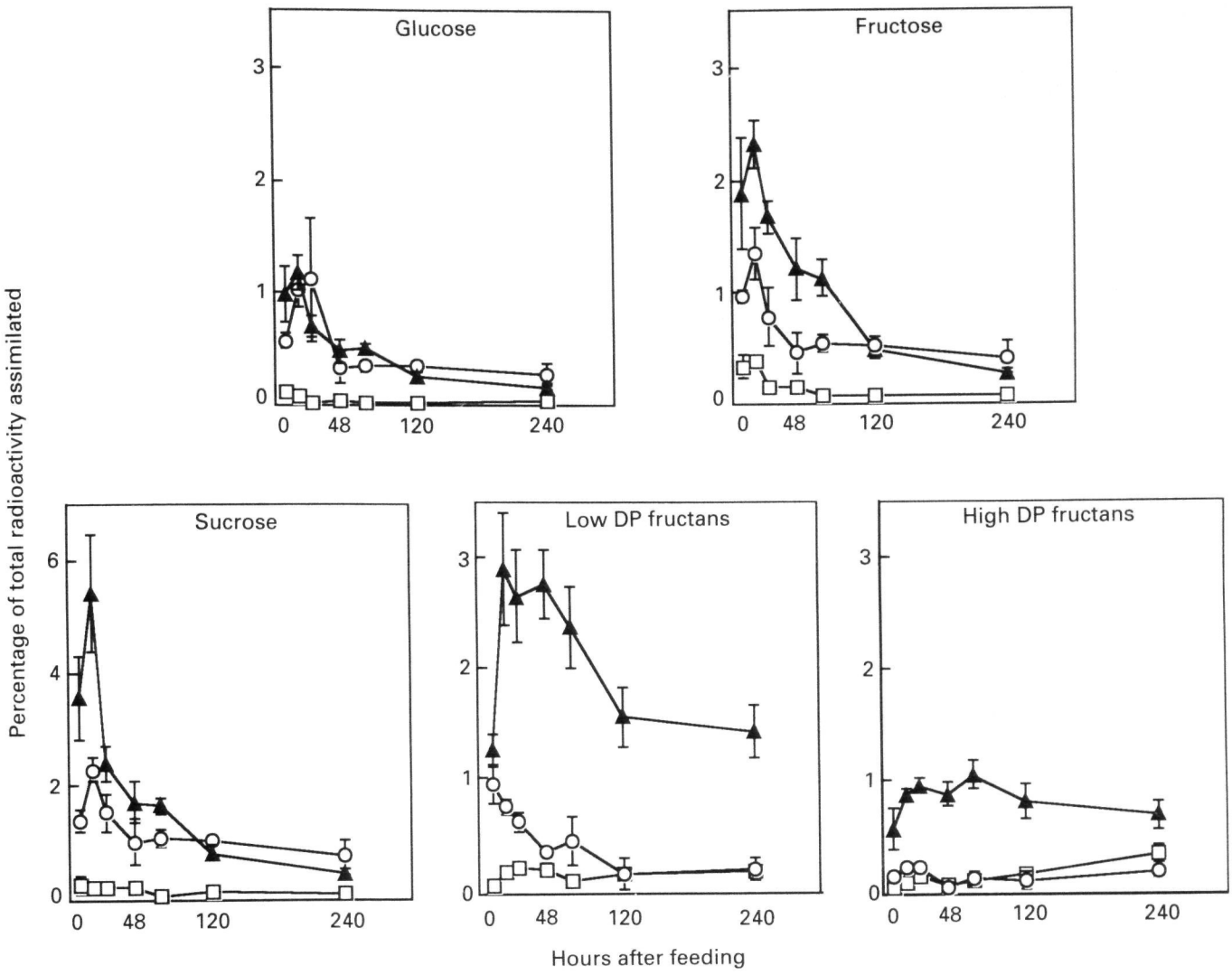

Figure 4. ^{14}C partitioning among WSC in roots following incorporation of $^{14}CO_2$ by leaves of *Festuca arundinacea*. Symbols are as described in Figure 3.

due to its nighttime translocation to the roots. Part of the decline in radioactivity in sucrose was also due to its hydrolysis since glucose and fructose of the shoots accumulated label during the 12 h feeding period.

During the dark period following the 12 h $^{14}CO_2$ exposure, radioactive sucrose of the roots increased only in plants grown at low temperatures (16/10 °C and 8/5 °C). As mentioned above, the rise in radioactive sucrose in the roots probably reflects its nighttime translocation from source to sink. Low temperatures have never been reported to increase the rate of translocation. Instead, a fall in temperature generally leads to a decrease in the rate of translocation, and in some cases such as *Lolium temulentum*, temperatures near zero has no or very little depressive effect (Farrar, 1988). Therefore, low night temperatures (10 and 5 °C) allowed translocation to occur at a faster rate than utilization of carbohydrates in the roots.

In roots, growth at 8/5 °C induced a more than 5-fold increase in proportional and absolute fluxes of ^{14}C to sucrose, low-DP and high-DP fructans in shoots and in roots compared with 24/17 °C. The presence of fructans in leaves may be an advantage for cool-season grasses in that sucrose produced in excess can be sequestered as fructans in the vacuoles (Wagner, Keller & Wiemken, 1983) allowing photosynthesis to continue. Large reserves of fructans may be important for rapid regrowth in the early spring (Pollock & Jones, 1979) or following defoliation (Volenec, 1986). By way of contrast, few studies are concerned with the role of fructans in roots of perennial grasses. Accumulation of fructans in vacuoles of roots may provide a carbohydrate sink, and facilitate the continuous unloading of sucrose by maintaining a suitable turgor potential gradient between leaves and roots. Like in leaves, fructans may also function in roots as an accessible reserve to provide carbon skeletons to sustain the increase of growth processes when ambient temperatures rise.

A progressive movement of radioactivity occurred from sucrose to low-DP and high-DP fructans at all growing conditions. These data are consistent with the hypothesis that not only in leaves, as suggested by Pollock *et al.* (1989), but also in roots, low temperatures can stimulate fructan synthesis by increasing the accumulation of sucrose translocated from the leaves. As free hexose generally does not accumulate during fructan synthesis, Pollock & Cairns (1991) suggested that glucose released from sucrose was recycled. In agreement with this assumption, accumulation of radioactivity into fruc-

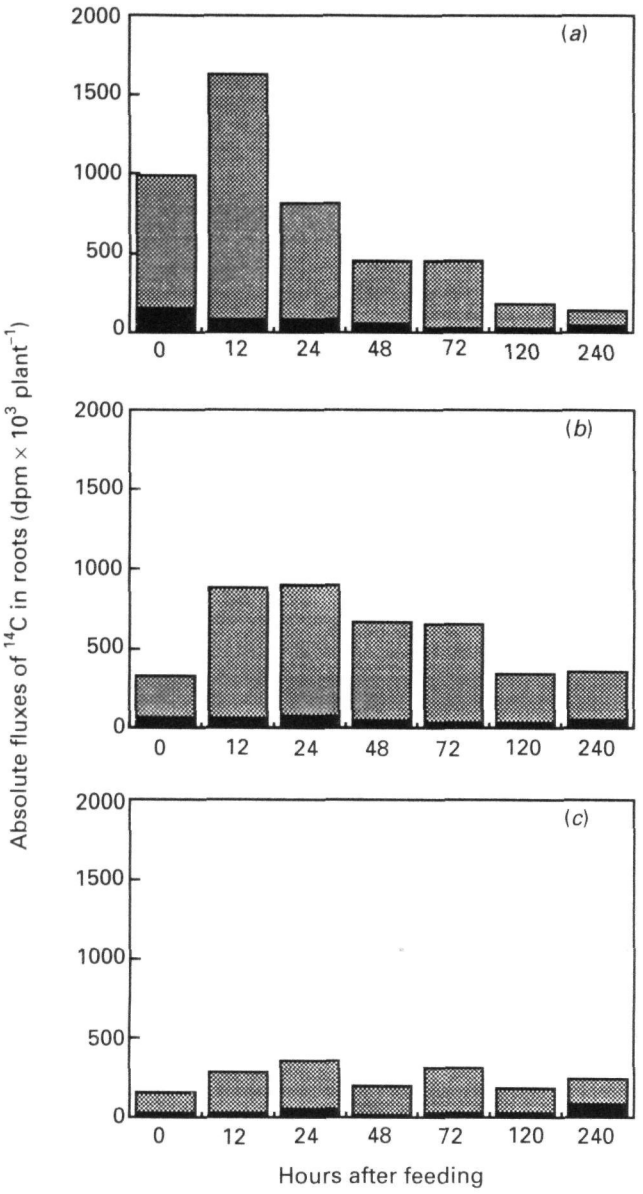

Figure 5. Absolute fluxes of ^{14}C (dpm/plant) in (*a*) sucrose, (*b*) low-DP and (*c*) high-DP fructans of roots of *Festuca arundinacea* grown at 24/17 °C (■) and 8/5 °C (▨).

tans of the shoots or fructans of the roots was not paralleled by a concomitant increase of labelled glucose. Accumulation of fructans and sucrose may be ascribed partly to a decrease in demand by photosynthates, and partly to the differential sensitivity that enzymes of carbohydrate metabolism show to temperature (Pollock & Lloyd, 1987; Pollock *et al.*, 1989). Future experiments will aim to study the enzymes of sucrose and fructan metabolism in roots of plants subjected to cool treatments.

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