Is photosynthetic acclimation to low temperature controlled by capacities for storage and growth at low temperature? Results from comparative studies of grasses and trees

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The ability of warm-grown leaves to acclimate their photosynthetic machinery to low, non-freezing temperature was compared for contrasting species of grasses and trees. All trees (Betula pubescens, Salix sp. and Picea abies) and young plants of one of the grasses (Hordeum vulgare) showed acclimation of photosynthesis while the other two grasses (Phalaris arundinacea and Festuca ovina) did not. It was those species that maintained leaf sugar concentrations essentially unchanged that showed acclimation. Trees maintained leaf sugar concentrations essentially unchanged by effectively converting leaf sugar surpluses into storage compounds. Grasses were, by comparison, less effective. However, very young plants of Hordeum maintained leaf sugar concentrations unchanged by continued growth rather than by increased storage. This diversity of low-temperature responses are discussed in relation to possible different priorities of trees and grasses: for grasses to undergo cold hardening by allowing sugars to rise, and for trees to store sugars to allow photosynthesis to operate independently of growth as growth varies with growth rhythm and air temperature.

Introduction

A shift to low temperatures in the chilling range (0–10°C) imposes constraints on the enzymatic reactions of photosynthesis that, in many plants, can be counteracted by physiological adjustments (acclimation). Prominent features of this acclimation are increased capacities of rate-limiting key enzymes, such as ribulose bisphosphate carboxylase (Rubisco) of the carbon reduction and sucrose phosphate synthase (SPS) of the sucrose pathway (Holaday et al. 1992, Hurry et al. 1994). As a result, the rate of photosynthesis is gradually increased from what is initially possible at low temperature (Berry and Björkman 1980).

Not all plants native to cold regions can acclimatize their mature, warm-grown leaves, following a shift to low temperature. Many woody species can acclimatize mature leaves (Berry and Björkman 1980), whereas many species of grasses and herbs cannot (Huner et al. 1993). Although these latter plants can typically acclimatize the new leaves that develop at the lower temperature, full low-temperature competence will not be achieved until all the old leaves have been replaced, a process that will take considerable time to complete for large plants. Theoretically, these differences in acclimation behaviour might reflect different abilities or ‘needs’ to maintain photosynthesis at low temperatures.

Generally, low temperatures limit growth more than photosynthesis (Pollock et al. 1983, Boese and Huner 1990). Reduced demand for photosynthesis because of reduced growth may lead to the accumulation of photosynthetic intermediates and end products. The accumulation of phosphorylated intermediates may suppress photosynthesis on the short-term by sequestering free phosphate needed for continued operation of the carbon reduction cycle (Labate and Leegood 1988, Myers et al. 1999). The accumulation of sugars suppresses photosynthesis more lastingly by suppressing the expression of nuclear encoded photosynthetic genes (Krapp and Stitt 1995, Smeekens 2000). Such suppression of photosynthesis is evidently incompatible with acclimation acting in the opposite direction. In other words, the balance between the activities of sinks and sources, as
reflected by the sugar concentrations, might determine whether acclimation will occur or not. This view is supported by the observation that the higher rate of utilization of carbohydrates at low temperatures by rape, compared to sunflower, was correlated with rape being able to maintain a higher rate of photosynthesis (Paul et al. 1992).

Redirection of assimilates from growth to storage when growth is reduced, should help to keep sugars low and photosynthesis high. For instance, the massive storage of photosynthetically derived starch in coniferous needles in spring, prior to the onset of growth (Little 1970), might serve as a sink complementing growth. Alternative sinks to growth might also operate in trees in late summer when growth stops in response to shortened daylength (Heide 1974) well before photosynthesis stops in response to leaf shedding (deciduous trees) and the arrival of low or freezing temperatures (evergreen trees). Trees therefore differ from many grasses/herbs that continue to both grow and photosynthesize as long as temperatures allow (Sakai and Larcher 1987). Such differences in growth rhythms might be associated with differences in storage capacities/efficiencies. Some support for this supposition comes from observations that perennials store larger amounts than annuals (Rosnitschek-Schimmel 1983).

Another source for variation in acclimation behaviour might be the fact that trees and grasses/herbs do not experience the same temperature environment. Trees experience the full impact of variable air temperatures as they protrude into the turbulent air where thermal equilibration is rapid, particularly so for conifers with rough surfaces and small leaves (Grace 1989). However, grasses/herbs are protected from air turbulence close to the ground and are warmed by incoming solar radiation during the growing season (Körner and Larcher 1988). It is possible that trees, being more exposed, have evolved a more adaptable metabolism for carbon fixation and storage.

In this study we seek to explain the varying abilities of plants to acclimatize mature leaves to low temperature with respect to their photosynthetic performance by addressing the following questions: (1) What is the range of acclimation amplitude of photosynthesis across contrasting life forms (grasses vs. trees) and across contrasting species of the same life form (slow-turnover vs. fast-turnover plants)? (2) Are differences in acclimation amplitude across diverse groups related to differences in their abilities to maintain growth and increase storage at low temperature, thus avoiding sugar accumulation in leaves? To this end, photosynthesis and carbohydrates were repeatedly analysed following the transfer of plants from 20/10°C to 10/3°C day/night temperature.

Materials and methods

Plant material

The following species of trees and grasses, differing in growth rate and leaf longevity, were used for these studies: *Salix* sp., a fast-growing deciduous tree (clone no. 75 in the Energy project at the Swedish University of Agricultural Sciences), *Betula pubescens* Ehrh. (local provenance from local tree orchard; local refers to N64° E20° throughout), *Picea abies* (L.) Karst. (local provenance from local tree orchard), *Hordeum vulgare* L. (winter cultivar Frost, Svalöf Weibull AB, Sweden), *Phalaris arundinacea* L., a fast-growing perennial grass (Svalöf Weibull AB) and *Festuca ovina* L., a slow-growing perennial grass with long-lived leaves (local clone). Plants were grown from seeds (*Betula, Hordeum and Phalaris*), cuttings (*Salix*), small clonal transplants (*Festuca*) and seedlings (*Picea*). The *Picea* seedlings had been raised at a local outdoor nursery for 1 and 3 years and were in the dormant state at delivery, ready to develop a new set of needles. The 1-year-old seedlings were used in Experiment 1 and the 3-year-old seedlings were used in Experiment 2. Plants were grown individually in volumes of soil ranging from 1 to 7.5 dm³ depending on plant size, ensuring no root limitation. They were watered daily, and once a week with a complete nutrient solution (Superba S+Mikro; Hydro Agri, Landskrona, Sweden). Control conditions were as follows: 20/10°C day/night temperature, 60–70% RH, and an irradiance of approximately 500 μmol m⁻² s⁻¹ (17-h photoperiod), as measured with a quantum sensor (Li-189; Li-Cor Inc., Lincoln, NE, USA), was provided by metal halide lamps (HQI-TS 400 W; Osram, Berlin, Germany). All plants remained in the vegetative state throughout growth and treatment periods.

Experimental design

In Experiment 1, all six species were used. The effects of low temperature on photosynthesis and carbohydrate concentrations were analysed for the most recently developed leaves (the third leaf for *Hordeum and Phalaris*) and the effects of low temperature on rates of extension were analysed for the shoots (further described under Growth analyses below). One leaf per plant, or for *Festuca* and *Picea* having narrow leaves, an assembly of several leaves, was chosen for study. Before transfer from control conditions photosynthetic capacity of this leaf/assembly was assessed, and leaf samples amounting to 0.1–0.5 g were harvested from similar leaves for subsequent carbohydrate analyses. Low-temperature treatment was conducted in a growth room maintained at 10/3°C day/night temperature. Irradiance was reduced by 30% relative to that during control conditions (same lamps) in order to reduce the risks of persistent, low-temperature induced photoinhibition of photosynthesis known to interfere with the fluorescence-based analyses of photosynthesis (Huner et al. 1993). Plants were treated for 21–35 days, except for *Hordeum and Phalaris*, which were treated for 6 and 11 days, respectively, after which they showed early signs of senescence (yellowing of oldest parts). This was probably due to their higher leaf turnover rates reflecting their higher growth rates. Parallel plants were kept under control conditions to establish any
time effect under these conditions (but these plants can evidently not be considered as time controls for the treatment due to the temperature difference).

In Experiment 2, four of the species were used (Hordeum, Phalaris, Salix and Picea). The effects of low temperature on carbohydrate concentrations and growth rates were analysed for the whole plant divided into leaves, stems and roots. For each species, plants were divided into three sets of nine plants each, with similar size distributions of plants within sets. Set 1 was directly harvested whereas set 2 was harvested after 14 days in control conditions. At this point low-temperature treatment started for set 3, using 10°C day/night temperature as above but maintaining growth irradiance as fluorescence was not to be assessed (see above). Set 3 was harvested after 28 days of treatment. Comparisons of sets 1 and 2 were made to establish any time effect.

Photosynthesis measurements
Photosynthesis was measured using a fibre-optic-based modulation fluorometer (PAM H.Walz, Effeltrich, Germany). Attached leaves were enclosed in a water-jacketed clamp-on cuvette held at 7°C and flushed with humidified air containing 1500 μmol mol⁻¹ of CO₂. Leaf temperature, as measured by a thermocouple 0.05 mm in diameter, was not significantly altered at any level of measuring light (<0.5°C). Continuous light and saturating flashes of 0.8-s duration (18 mmol m⁻² s⁻¹) were provided by separate halogen lamps (KL 1500, Schott, Mainz, Germany) and directed to the leaf by the fibre. The quantum yield of photosystem (PSII) electron transport was determined as (Fₚ₋ₐ/Fₚ) (Genty et al. 1989), where Fₚ and Fₐ are the fluorescence yields at steady state and at the saturating flash, respectively. The measurements were repeated at stepwise increased irradiance (200, 500 and 1400 μmol m⁻² s⁻¹), allowing steady-state yields to be established at each level. A relative change in the quantum yield of PSII electron transport, induced by treatment and measured at the maximal irradiance level, was taken to represent the same relative change in the photosynthetic capacity. This was justified because at high light and high CO₂ concentration, the quantum yield of CO₂ fixation is linearly related to the quantum yield of PSII electron transport, irrespective of the species (Seaton and Walker 1990), the temperature (Oberhuber and Edwards 1993), and the photosynthetic capacity of the leaf (Cheng et al. 2001). Also, variations in quantum yields translate into variations in rates when the fraction of light absorbed is constant, as was the case here.

Carbohydrate analyses
Plant material was frozen in liquid nitrogen, freeze-dried and ground to powder before sugars were extracted. Samples of 50 mg were extracted with 80% ethanol at 80°C for 2 × 30 min and then again for 2 × 30 min with 50% ethanol, both media containing 4 mM HEPES buffer (pH 7.5). The combined supernatants were made up to 2 ml and stored at −60°C before use. In order to extract long-chained fructans the pellet was additionally washed in 0.5 ml water at 80°C for 2 × 30 min. Aliquots of 2 μl of the ethanol supernatant were assayed for glucose, fructose and sucrose using an enzyme-linked assay (Stitt et al. 1989) as detailed in (Ögren 1999b).

To analyse starch, the pellet was resuspended in 0.5 ml of water and autoclaved for 2 h at 120°C. Aliquots of 50 μl were resuspended in 450 μl of 50 mM Na acetate buffer (pH 4.8) containing 6.3 units of amylglucosidase. After incubation for 16 h at 40°C the glucose released was assayed as described above.

For analysis of fructan, extracts of 30 μl were loaded on 20 × 20-cm silica-gel plates. Raffinose and sucrose were added to the first and last lanes of each plate as references for fructan identification. Plates were developed twice in a mixture of butanol/acetic acid/water in proportions of 55:30:15 (v/v/v) and then sprayed with a mixture of orthophosphoric acid/butanol/urea in proportions of 1:0.8:0.03 (v/v/v) before they were incubated for 5 min at 100°C. All bands representing sugars of higher a degree of polymerization than sucrose, taken to represent fructans, were scraped off and resuspended in 1 ml of water. Fructans were quantified as fructose equivalents using the anthrone method as detailed by Wise et al. (1955). Fructose standards were used for calibration.

Growth analyses
In Experiment 1, rates of shoot elongation were calculated from shoot (= leaf for grasses) length increments between consecutive days. This was done on the day before start of treatment and on the first and last day of treatment. In Experiment 2, growth rates by biomass were calculated from DW increments by comparing sets of plants formed as described above. DWs of leaves, stems and roots were determined after drying at 80°C until constant weight. In both experiments rates were calculated as:

\[
[m_2 - m_1]/[(m_1 + m_2)/2]/[t_2 - t_1]
\]

where m represents length/biomass and t represents time. This approach is valid for plants that grow linearly after shading has become prominent within shoots, as was the case for the majority of plants studied in these experiments.

Statistical analyses
Differences in photosynthesis, carbohydrates and growth were analysed using various analyses of variance (ANOVA) with the SPSS 10.0 package. Repeated measure ANOVA was used to test the significance of difference between start and end values of photosynthesis and of shoot elongation, thus taking into account the interdependence of values on the individual level. The data of carbohydrates and growth, being expressed as proportions, were arcsine square-root transformed before analyses.
Results

Experiment 1: photosynthesis and carbohydrates of leaves

The trees (Betula, Salix and Picea) and one of the grasses (Hordeum) were able to acclimate their fully developed leaves to low temperature: photosynthetic capacity at low-temperature increased by 80% (Hordeum), 50% (Salix and Picea) and 30% (Betula) during low-temperature treatment (Fig. 1). The maximal rate of acclimation, as judged from the slopes of the lines connecting data points in Fig. 1, was about 3-fold higher for Hordeum than for the next best species. Acclimation seems to have started without delay in these species except for Betula, which showed an initial depression in photosynthetic capacity.

In contrast, leaves of Phalaris and Festuca were not able to acclimatize their fully developed leaves to low temperature as evidenced by unchanged low-temperature photosynthetic capacities at the end of low-temperature treatment (Fig. 1). Both species showed an initial depression in photosynthesis that was particularly pronounced and persistent in Festuca.

A repeated measure ANOVA revealed that species responded differently to low temperature (Wilks’ Lambda, $P < 0.001$) and that the latter group of species (Phalaris and Festuca) differed significantly from the former group (Betula, Salix, Picea and Hordeum) (Tukey’s test, $P < 0.05$, Fig. 1) [Parallel plants kept at control conditions showed unchanged values over the time intervals used for treatments (the result of ANOVA tests; data not shown) but these plants cannot be considered as true time controls for the treatment due to the temperature difference].

Those species that showed low-temperature acclimation (Betula, Salix, Picea and Hordeum) were also those that showed essentially unchanged, or somewhat lowered (Picea) leaf sugar concentrations (Fig. 2) (ANOVA, Betula

![Fig. 1](image1.png)

Fig. 1. Photosynthetic low-temperature capacities of warm-grown leaves plotted as a function of duration of low-temperature treatment and expressed relative to the start of treatment. Parentheses denote Hordeum leaves that had started to senesce. Mean ± SE values are given for 4–6 replicate plants. Significant interspecific differences in low-temperature responses are indicated by different letters ($P < 0.05$).

![Fig. 2](image2.png)

Fig. 2. Concentrations of major leaf sugars (sum of glucose, fructose and sucrose), black fills, and leaf storage carbohydrates (sum of starch and fructan), grey fills. Leaves, parallel to those analysed for photosynthesis in Fig. 1, were assessed at the start and end of low-temperature treatments (varying in length as shown in Fig. 1). Mean ± SE values are given for 5–6 replicate plants. Significant differences between end and start values are indicated by asterisk(s): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, $P > 0.05$. Physiol. Plant. 119, 2003
Concentrations of leaf storage carbohydrates showed a more variable pattern: whilst they rose in Betula and Salix from an already high level they stayed essentially constant at a low level in Hordeum and even decreased somewhat in Picea (Fig. 2). Storage carbohydrates comprised only starch in the trees. In the grasses most of the variation in leaf storage carbohydrates was accounted for by a variation in fructans; starch concentration remained within 1–2% of DW in both this and the second experiment (data not shown).

The species that were unable to acclimatize their mature leaves (Phalaris and Festuca) showed increased leaf sugar concentrations: these increased by 159% and 64%, respectively (ANOVA, Phalaris \( P < 0.001 \) and Festuca \( P < 0.001 \)). Concentrations of leaf storage carbohydrates either increased (Phalaris) or remained constant (Festuca).

Carbohydrate values from intermediate time points (data not shown) were consistent with the patterns outlined above: sugar concentrations remained unchanged until day 6 for Betula \( (P = 0.97) \) and until day 13 for Picea \( (P = 0.25) \) but starch concentrations increased by 74% \( \text{for Betula} \ (P < 0.05) \) and decreased by 27% \( \text{for Picea} \ (P = 0.07) \); sugar concentrations increased by 112% \( \text{for Betula} \ (P < 0.001) \) and 22% \( \text{for Picea} \ (P = 0.14) \) until day 2 for Phalaris and Festuca, respectively, but fructan concentrations remained unchanged for both \( (P = 0.20 \) and 0.99); Hordeum and Salix were not measured.

**Experiment 1: shoot elongation**

Rates of shoot elongation decreased markedly with low-temperature treatment. When data for all species were combined, except for Picea which had completed current-year shoot growth, rates of shoot growth after the first day of treatment were only 5–16% of the rates in control conditions (Table 1). However, growth rates recovered partially in Hordeum and Betula, compared to the rest of the species, indicative of acclimation. On the final day of low temperature treatment Hordeum had significantly higher growth rate, 44% of its control rate, than the rest of the species (Tukey’s test, \( P < 0.05 \), Table 1).

**Experiment 2: biomass and carbohydrates of whole plants**

The question of whether changes in leaf carbohydrate status reflected changes in growth and storage, outside the leaves, was addressed by separately analysing leaves, stems and roots for Hordeum, Phalaris, Salix and Picea. All plants were analysed after the same low-temperature period (28 days).

Rates of growth in biomass decreased to about the same extent across leaves, stems and roots and across species: low-temperature growth rates varied between 17 and 42% of control values, combining all data except for Picea (Table 1). Since biomass changes were measured for separate individuals these responses could not be tested for significance of difference. Treatment effects could not be assessed for Picea because its growth rate in control conditions were only a few percent of rates for other species (e.g. Salix). It should be noted that growth in biomass also include accumulation of carbohydrates and other compounds, in contrast to growth in length (volume), the unit used in the first experiment, thus precluding direct comparisons of data.

As for leaf carbohydrates, the result from Experiment 1 that Phalaris accumulated leaf sugars as opposed to Salix and Picea was confirmed (ANOVA, Phalaris \( P < 0.001 \), Salix \( P = 0.89 \), Picea \( P = 0.89 \)). However, in contrast to these results, Hordeum also accumulated leaf sugars \( (P < 0.001) \), possibly reflecting the greater depression in growth in Experiment 2, as well as the difference that developing leaves contributed to responses in Experiment 2 but not in Experiment 1. Interestingly, these trends in changing sugar concentrations in leaves were paralleled by essentially the same trends for stems and roots, indicative of an integrative carbohydrate metabolism encompassing all plant parts. Noteworthy is also the high capacity for storage by the grasses Hordeum and Phalaris, suggesting that the previous result of larger sugar accumulation for the grasses than the trees (excluding Hordeum for the above reason) was not explained by lower storage capacities per se for the grasses.

**Discussion**

Not all of the species studied showed low-temperature photosynthetic acclimation of fully developed leaves. While the three tree species (Salix, Betula and Picea) and one of the grasses (Hordeum) did show this acclimation response, the other two grasses (Phalaris and Festuca) did not (Fig. 1). This diversity of acclimation coincided with a diversity of leaf sugar responses: low-temperature acclimation was only observed in those species that maintained leaf sugar levels essentially unchanged (cf. Figs 1 and 2). This situation resembles the many situations where photosynthetic capacity is modulated by levels of photosynthetic end products. For instance, cooling of leaf petioles (Krapp and Stitt 1995) and increasing the CO2 supply to leaves (Stitt 1991), typically increases leaf sugar concentrations and eventually decreases photosynthetic capacities. Molecular studies have indicated that these effects are causally linked: increased concentrations of hexoses and probably also sucrose will set off a signalling sequence leading to the repression of photosynthetic genes (Krapp and Stitt 1995, Smeekens 2000). More seldom increased end product concentrations in the form of starch might reduce photosynthesis as starch grains may obstruct the diffusion of CO2 to the site of carboxylation (Nakano et al. 2000). Supplying elevated CO2 can compensate for this effect. This was done in the present study in order to assess intrinsic photosynthetic capacities independent of CO2 diffusion and photorespiration, and to acknowledge the fact that photosynthetic low-temperature acclimation is most strongly expressed when assessed at saturating
light and CO₂ (Hurry et al. 1995, Martindale and Leegood 1997).

It thus appears that leaf sugar concentrations must be maintained if mature leaves are to acclimatize to low temperatures, a prerequisite that would be met if growth was unaffected by low temperature. However, all vigorously growing species, excluding Picea that had completed the current-year shoot growth, showed strongly depressed growth rates, particularly with respect to shoot elongation. Growth by cell expansion seems particularly sensitive to low temperatures (Pritchard 1994) and more accurately predicts growth than growth by biomass accumulation, which also includes storage. Young Hordeum plants were exceptional by recovering to as much as 44% of the pretreatment rate of shoot growth, but this achievement was only transitory. Biomass growth of Hordeum was affected by low temperature to a similar extent as the other species when the cold treatment was prolonged in Experiment 2 (Table 1). The superior initial ability of Hordeum might be related to the fact that the plants used were very young (the third-leaf stage). Such young plants may be able to adapt more rapidly to environmental variations than older ones.

Low temperatures generated carbohydrate surpluses in all species, also for Hordeum when treated equally long as the other species (Picea showed surpluses only in Experiment 2; Figs 2 and 3). Trees and grasses, however, handled these surpluses differently. While the trees (Betula, Salix and Picea) effectively converted surpluses into storage polysaccharides, significant amounts remained as soluble sugars in the grasses (Phalaris, Festuca and Hordeum) (Figs 2 and 3). The less effective

| Table 1. Rates of growth during control condition and low-temperature treatment: Experiment 1, growth in shoot length (= leaf length for grasses) in control conditions and on the first and final days of treatment (treatments were of varying length as depicted in Fig. 1), and, Experiment 2, growth in biomass of leaves, stems and roots during the final 2-week period of control conditions and during the whole 4-week period of treatment. Mean ± SE values are given for 5–6 and 9 replicate plants in Experiment 1 and 2, respectively (the same plants as analysed in Figs 1, 2 and 3, respectively). Significant interspecific differences in low-temperature responses are indicated by different superscripted letters (P < 0.05). |

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Rate of growth in length (mm mm⁻¹ day⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Hordeum</td>
<td>1.92 ± 0.10</td>
</tr>
<tr>
<td>Phalaris</td>
<td>1.22 ± 0.06</td>
</tr>
<tr>
<td>Festuca</td>
<td>0.09 ± 0.02</td>
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<td>Betula</td>
<td>0.74 ± 0.07</td>
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<tr>
<td>Salix</td>
<td>1.05 ± 0.07</td>
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<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>Rate of growth in biomass (g g⁻¹ day⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Hordeum</td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>0.136 ± 0.022</td>
</tr>
<tr>
<td>Stems</td>
<td>0.160 ± 0.014</td>
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<tr>
<td>Roots</td>
<td>0.149 ± 0.027</td>
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<tr>
<td>Phalaris</td>
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<tr>
<td>Leaves</td>
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<tr>
<td>Stems</td>
<td>0.169 ± 0.028</td>
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<tr>
<td>Roots</td>
<td>0.147 ± 0.028</td>
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<tr>
<td>Salix</td>
<td></td>
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<tr>
<td>Leaves</td>
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<tr>
<td>Stems</td>
<td>0.080 ± 0.011</td>
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<tr>
<td>Roots</td>
<td>0.073 ± 0.008</td>
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conversion of sugars into storage polysaccharides by the grasses was not explained by these having lower capacities for storage because similar maximal amounts were stored by the grasses **Hordeum** and **Phalaris** as by the trees **Salix** and **Betula** (cf. maximum values of Figs 2 and 3). Thus, the less effective sugar conversion by the grasses is because of either a higher sugar level to trigger storage and/or a lower rate of storage. There is some support for the former possibility. The primary polysaccharide stored by the grasses was fructan and not starch (data in text) and fructan synthesis does not begin until the concentration of sucrose—the building block for fructan—is considerably elevated (Bancal and Gaudillère 1989, Pollock and Cairns 1991). This offers an explanation for the observation that fructan levels of *Hordeum* leaves did not rise in Experiment 1 where sucrose levels were maintained unchanged.

Although the elevated sugar levels prevented grasses from acclimating their photosynthetic machinery, they may have benefited from an increased cold resistance. This effect can be expected given the close correlation that exists between levels of cold resistance and levels of leaf sugars during cold hardening in the autumn for grasses/herbs (Wanner and Junthila 1999) and trees (Ögren 1999b, 2001). For grasses/herbs cold hardening is triggered solely as a result of lowered temperature but for trees it is triggered primarily as a result of shortened day-length (Heide 1974, Ögren 1999a). Responding to low temperatures by cold hardening seems particularly apt for grasses/herbs as grasses/herbs are effectively warmed by solar radiations as long as solar elevation is high, i.e. in the growing season, a consequence of the slow plant-to-air heat transfer in the unstirred bottom layer of the atmosphere (Körner and Larcher 1988). Encountering low temperatures might therefore indicate the arrival of autumn conditions and the need to start cold hardening for grasses/herbs.

Trees, by contrast, encounter low temperatures more frequently in the growing season because of their more exposed position in the turbulent atmosphere (Grace 1989) but evidently not freezing temperatures. Trees should therefore benefit from being able to rapidly convert carbohydrate surpluses into starch so as to allow photosynthesis to operate independently of growth as growth varies with temperature and regulatory constraints (growth rhythm). The differential responses of grasses and trees reported here provide circumstantial evidence for the existence of such differential strategies of grasses and trees.

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