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Response of Fructan to Water Deficit in Growing Leaves of Tall Fescue[1]

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Changes in dry matter and water-soluble carbohydrate components, especially fructan, were examined in the basal 25 mm of expanding leaf blades of tall fescue (*Festuca arundinacea* Schreb.) to assess their roles in plant response to water deficit. Water was withheld from vegetative plants grown in soil in controlled-environment chambers. As stress progressed, leaf elongation rate decreased sooner in the light period than it did in the dark period. The decrease in growth rate in the dark period was associated with a decrease in local relative elongation rates and a shortening of the elongation zone from about 25 mm (control) to 15 mm. Dry matter content of the leaf base increased 23% during stress, due mainly to increased water-soluble carbohydrate near the ligule and to increased water-soluble, carbohydrate-free dry matter at distal positions. Sucrose content increased 258% in the leaf base, but especially (over 4-fold) within 10 mm of the ligule. Hexose content increased 187% in the leaf base. Content of total fructan decreased to 69% of control, mostly in regions farther from the ligule. Fructan hydrolysis could account for the hexose accumulated. Stress caused the osmotic potential to decrease throughout the leaf base, but more toward the ligule. With stress there was 70% less direct contribution of low-degree-of-polymerization fructan to osmotic potential in the leaf base, but that for sucrose and hexose increased 96 and 67%, respectively. Thus, fructan metabolism is involved but fructan itself contributes only indirectly to osmotic adjustment.

Water deficit limits crop productivity in part through reduction in leaf growth, which in turn limits whole plant photosynthetic capacity. Leaf growth requires that the expanding cells maintain ψ_w lower than that of the xylem. A decrease in cell ψ_w during water deficit can be promoted by osmotic adjustment, the enhanced accumulation or synthesis of solutes that lowers cell ψ_s and allows the cell to maintain turgor.

Water-soluble carbohydrates may accumulate in elongating regions of grass leaves responding to water deficit (Munns et al., 1979; Barlow, 1986) and enhance osmotic adjustment. Utilization of these compounds for respiration and/or biosynthesis seems to be impeded by stress, but the metabolic steps by which this occurs are unknown. Suc accumulates in these slow-growing tissues, so Suc use is probably more inhibited than Suc supply per se.

The region of cell division and expansion in emerging leaf blades of tall fescue (*Festuca arundinacea* Schreb.) accumulates large amounts of WSC, up to 50% of the dry weight (Volenec and Nelson, 1984). Much of the WSC is fructan, and much of that is of low (<5) DP, contributing 0.2 MPa to the ψ_s of the actively elongating region (Schnyder and Nelson, 1987). Low-DP fructan in tall fescue includes fractions that chromatograph with the inulin series of oligomers, a fructan not usually found in graminaceous plants (Spollen and Nelson, 1988).

It is unclear how fructan metabolism in an actively elongating sink tissue may be involved in Suc unloading, growth, and osmotic adjustment during water deficit. Fructan content might increase with stress as Suc concentration increases because Suc is the substrate for fructan synthesis. However, previous studies indicate that DM deposition rate and fructan synthesis may be coupled (Schnyder et al., 1988), so stress-induced decrease in DM production may be accompanied by decreased fructan synthesis. Changes in fructan concentration could also participate in regulation of growth by altering ψ_s. Understanding the changes in fructan content during water deficit will help elucidate steps in carbon use of growth zones that are affected by stress and further test the hypothesis that Suc import, growth, DM deposition, and fructan synthesis are linked.

Contents of DM components in leaf elongation zones of plants with adequate water supply are known to vary with distance from the ligule and with the environment (Schnyder and Nelson, 1989; Gastal and Nelson, 1994). The demand for carbon and the efficiency of its utilization also vary spatially (J.R. Pearen and C.J. Nelson, unpublished data). In this paper we evaluate changes in the spatial distribution of growth and components of DM in the basal 25 mm of growing leaf blades of tall fescue that occur as plants become water limited. We also evaluate changes in ψ_s of the leaf elongation zone during progressive stress and show how each WSC component contributes to osmotic adjustment.

MATERIALS AND METHODS

Plant Preparation

Vegetative tillers of a tall fescue (*Festuca arundinacea* Schreb.) genotype (V2-29, selected from the cv Alta) selected for rapid leaf elongation were grown in a greenhouse and

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Abbreviations: DM, dry matter; DP, degree of polymerization; *LER*, leaf elongation rate; MLB, mature leaf blade; *REER*, relative elemental elongation rate; ψ_w, water potential; ψ_s, solute potential; SST, sucrose:sucrose fructosyl transferase; WSC, water-soluble carbohydrate.

separated from the mother plant. Leaves and roots on tillers were trimmed to 5 and 1 cm, respectively. Uniform tillers were transplanted into 1300 g of dry soil in 1-L white plastic pots, six tillers to a pot. Soil was a 2:1:1 mix of silt loam topsoil, sand, and peat moss, respectively. Plants were moved that day to a growth chamber with a 14-h photoperiod, constant 19°C at the soil surface, and 92% RH. Photon flux density (400–700 nm) was 500 μmol m^{-2} s^{-1} at the tops of the pots. Plants were watered every other day and fertilized weekly with a modified Hoagland solution or a high-NPK source (Volenec and Nelson, 1984) through week 4 (experiment 1) or week 6 (experiment 2) after transfer to the chamber.

Experiment 1

Effects of water deficit on *LER* and length of the elongation zone were investigated. Beginning 4 weeks after transfer to the growth chamber, control plants were watered every 2 d while water was withheld from the remaining plants. Leaf length and pot weight were measured daily 4 h into the photoperiod and *LER* was calculated. Leaf length was also monitored continuously on two plants (Schnyder et al., 1987). The tip of the elongating blade was taped to nylon fish line, which was wrapped 1.5 times around the 1.5-cm-diameter wheel of a rotating potentiometer (Helipot model 5101) that was suspended above the plant. Weight (<10 g) was applied to the free end of the line to keep the line tight. Changes in leaf length with time were traced with a chart recorder.

The spatial distribution of elongation was determined as described previously (Schnyder et al., 1987). Two hours into a dark period leaf length was measured on six elongating leaves, and three of these were designated for *REER* measurements using the hole-displacement technique (Schnyder et al., 1987). Briefly, a fine wire was used to pierce small holes at 3-mm intervals through the base of the tiller. About 6 h later leaf length was again measured, tillers with holes were excised, and distances between adjacent holes in the outer sheath (reference for zero time) and the elongating leaf blade were measured. Data on leaves were discarded if the ligule of the elongating leaf was more then 2 mm from the point of attachment.

Lengths of elongating blades ranged from 6.8 to 16.4 cm and averaged 37% (SE = 9%) of the length of the most recently collared blade of that tiller, a stage at which *LER* and leaf dimensions in the elongation zone remain nearly constant for several days (Schnyder and Nelson, 1989). Making holes in well-watered leaves decreases the *LER* (Schnyder et al., 1987); therefore, the effect of holes on *LER* was corrected by comparing, during the previous dark period, *LER* of the three leaves designated to be pierced with the mean *LER* of leaves in the same pot that were not to be pierced. The ratio of these *LER*s was used to correct the *LER* measured in each pierced leaf (Schnyder et al., 1987).

Experiment 2

Mass components and ψ_s were measured using plants 6 weeks after transfer to the growth chamber with conditions similar to those in experiment 1. Leaf lengths and pot weights were measured daily 2 h after the beginning of the dark period. Control pots were watered to the starting weight every 2 d and were fertilized every 4 d with a modified Hoagland solution. Plants were sampled at 7 to 10 h into the dark period for analysis of tissue mass, carbohydrate and water contents, and ψ_s. ψ_w was measured on one MLB per pot using a pressure chamber. Intact tillers were removed from the pot and placed in a humidified box that allowed tissue manipulation. The central 2 cm of the MLB was sampled to represent the mature leaf. The leaf base was exposed and dissected into 5-mm segments starting at the ligule. Each sample contained segments from four to eight tillers.

Segments collected for DM and WSC analyses were quickly placed in preweighed, 500- or 1500-μL polypropylene microcentrifuge tubes and held on ice. Tubes were capped tightly, blotted to remove external water, and weighed. Tubes and samples were then oven-dried (70°C for 40 h), dry weights were measured, and water content of the samples was calculated. Dried segments were frozen in a mortar in a small amount of liquid nitrogen to facilitate pulverizing with a pestle, then extracted in 7 mL of water during grinding. The filtrate (Whatman 40) was collected and analyzed for total WSC by the anthrone procedure (Dimler et al., 1952).

WSC samples and standards were separated using TLC (Spollen and Nelson, 1988). Standards in outside lanes were stained to mark the positions of the hexose, Suc, low-DP (DP < 10), and high-DP fructan in sample lanes. Components were scraped off the plate and eluted from the silica gel with water, and their contents were quantified using the anthrone procedure. Free Glc content of extracts was determined colorimetrically using Glc oxidase (Glc Trinder; Sigma).

ψ_s was determined on samples from at least two leaves per pot harvested at the same time as the samples taken for measurement of mass components described above. Tissue was dissected as above, sealed in microcentrifuge tubes, and immediately frozen in liquid nitrogen and stored at −20°C. Sap was extruded from thawed leaf segments by opening the tube inside a humidified box, pulverizing the tissue in the tube with a glass rod, and then centrifuging the recapped tubes at 11,000*g* for 1 min. After centrifugation, tubes were opened in the humidified box and the supernatant was removed with a capillary tube that had been drawn out to a fine tip with a pipette puller. Immersion oil was taken up in the capillary both before and after the sample to prevent evaporation. Sap was analyzed using a nanoliter osmometer (Clifton Technical Physics, Hartford, NY) with Suc solutions of known ψ_s as calibration standards. Output was linear to −5.00 MPa.

The osmolality of hexose, Suc, and low-DP fructan was determined from their molar content and tissue water content assuming that the solutes behaved ideally. Molar content of low-DP fructan from the chromatography plates was determined from the ratio of Glc, measured with Glc oxidase, to total carbohydrate in an acid hydrolyzate (Spollen and Nelson, 1988), assuming one Glc molecule per oligosaccharide.

RESULTS

LER

Daily increase in leaf length of control plants remained steady and near 21.7 mm d^{-1} (SE = ±4.1) in experiment 1,

whereas *LER* of stressed plants became less than that of the watered plants about 96 h after the last addition of water and was near zero after 158 h (Fig. 1A). The *LER* of stressed plants decreased during the light period sooner than during the dark period (Fig. 1B). Continuous monitoring of leaf length between 82 and 106 h showed about 8 mm (33%) of the 24.5 mm of leaf elongation took place during the 14-h light period. During the subsequent 24-h period only 1 mm (8%) of the 12 mm of total elongation occurred during the light period. Growth during the light period was near zero after 106 h for stressed plants, but *LER* during darkness began to decrease only after 106 h.

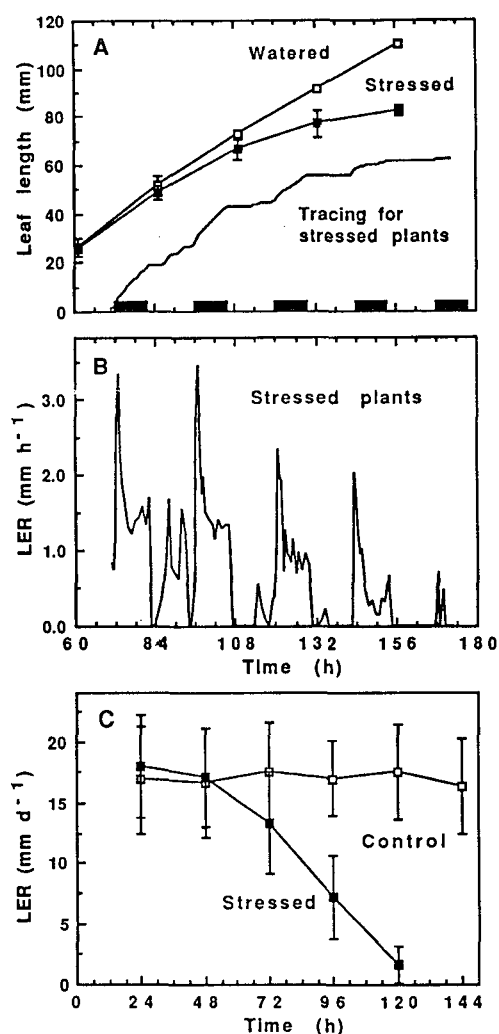

Figure 1. A, Leaf length increased in a nearly linear manner for control plants in experiment 1, but gradually slowed as plants became stressed. The tracing shows that the length of the emerging leaf blades of stressed plants increased faster in darkness (solid bars on x axis) than in light. B, *LER* increased rapidly at the beginning of the dark period, then decreased and maintained a level that was higher than during the preceding light period. C, Daily *LER* of emerging leaves of control and stressed plants in experiment 2. Vertical bars are 2 times the SE.

Each day the *LER* increased rapidly during the first half-hour of darkness, then declined to about half that rate within the next hour (Fig. 1B), even when daily leaf length increment was diminished by stress (Fig. 1A). This short-term enhanced rate was observed previously under well-watered conditions (Volenec and Nelson, 1983; Schnyder and Nelson, 1988) and under conditions of low ψ_w (Parrish and Wolf, 1983). The short-term enhancement above the steady-state rate contributed about 11% of the total elongation during each of the first two dark periods shown (Fig. 1B). Dark *LER* was lower after the initial burst at 118 h, suggesting that supply of water had become limiting. During the last dark period (Fig. 1B) only a small initial burst was observed, with no subsequent growth. In experiment 2, *LER* of controls was 17.7 mm d^{-1} (SE = ±4.4). *LER* of stressed plants began to decrease by 60 h and was near zero after 108 h (Fig. 1C).

Changes in DM Components of the MLB

Water and DM contents of the MLB were lower than those of controls at 126 h, and water content appeared to be declining from 78 h (Fig. 2A). The change in DM at 126 h due to stress resulted primarily from less synthesis of both WSC and WSC-free DM, i.e. DM minus WSC (Fig. 2B). Changes in WSC components had already occurred at 78 h (Fig. 2C), however, indicating that stress had affected carbon metabolism in the MLB, probably via decreased photosynthesis. At this time Suc and low-DP fructan had decreased by about 50% and continued to decline (Fig. 2C). High-DP fructan content had also decreased to 52% of control values by 126 h, indicating the apparent association between high- and low-DP fructan. Conversely, hexose content of the MLB increased by 102 h.

Spatial Distribution of Elongation

Cell elongation in control leaves during darkness occurred within 25 mm of the ligule (Fig. 3), with a spatial distribution of elongation similar to that reported previously (Schnyder and Nelson, 1988). At 128 h after watering *REER* was reduced throughout the elongation zone. By 152 h the magnitude of the *REER* was diminished further, primarily in the distal region, and the elongation zone had shortened to less than 16.5 mm. Growth was slow and erratic (Fig. 1B) during the last dark period measured (176 h), when *REER* had decreased further.

Changes in DM Components of the Leaf Base

DM content of the leaf base (the 25 mm length of the leaf starting at the ligule and containing the elongation zone) decreased with distance from the ligule at all times sampled (Fig. 4A). This decrease was due, in part, to the relatively greater influx of water than DM into this tissue as it elongated (Schnyder and Nelson, 1987). When stressed, DM content of the entire leaf base increased up to 23% by 126 h. Most of the increase occurred between 78 and 102 h after the last watering, when daily *LER* had already been reduced to about 3 mm d^{-1} (17% of control) (Fig. 1C), and growth occurred primarily during darkness. The increase in DM content was

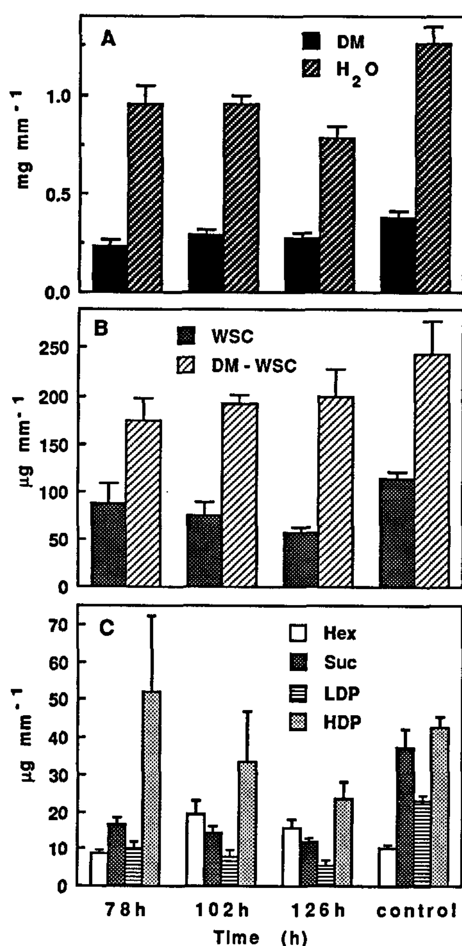

Figure 2. Water and DM content (A), WSC and WSC-free DM content (B), and hexose (Hex), Suc, and low- and high-DP fructan (LDP and HDP, respectively) content (C) of the mature leaf blade. Stressed plants had not been watered for 78, 102, and 126 h, and control plants were well watered and sampled at 150 h.

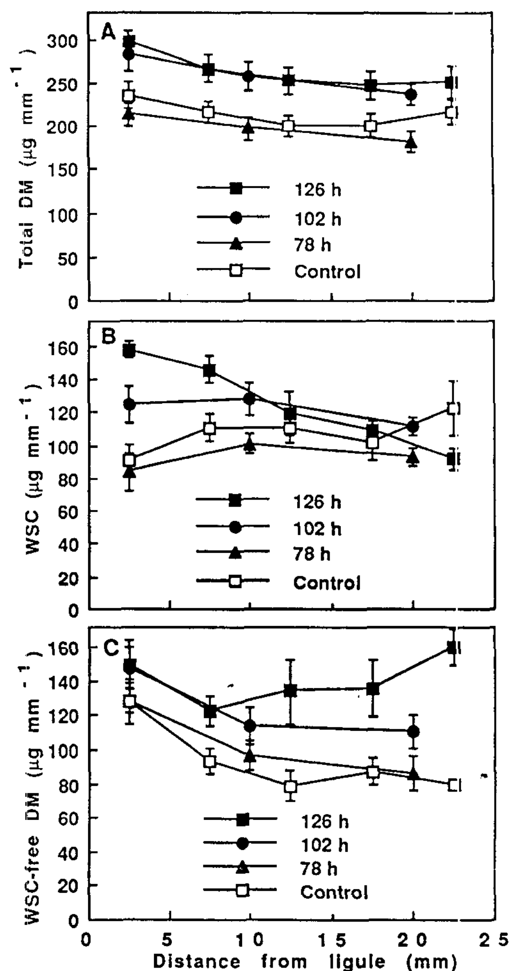

Figure 4. DM content throughout the elongation zone was higher in stressed plants than in the control (A). Content of WSC contributed more to the increase in DM near the ligule (B), whereas content of WSC-free DM contributed more to the increase in DM above 10 mm (C).

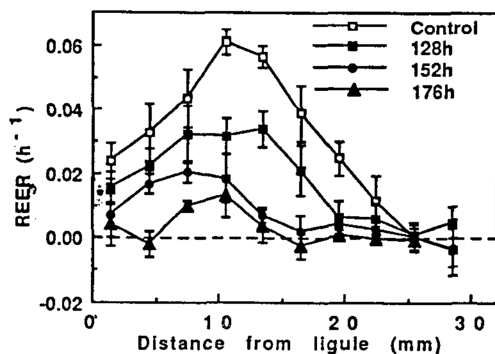

Figure 3. Spatial distribution of *REER* at the base of emerging leaf blades of control and stressed plants at various times after the last addition of water. Four to 12 leaves were used per treatment. Data for the 3-mm segments are plotted at the midpoint of the segments.

accompanied by a proportionately greater decrease in *LER* (Fig. 1C), indicating that net synthesis rate of DM had actually decreased.

Most of the increase in WSC content within the leaf base also occurred between 78 and 102 h, although content near the ligule continued to increase throughout the experiment (Fig. 4B). At 126 h WSC content of the leaf base was 16% higher than in the control, with the greatest increase in the basal 10 mm (50%). Content of WSC-free DM (Fig. 4C) averaged 50% higher by 126 h, but was influenced more toward the distal region, where it was associated with shortening of the elongation zone (Fig. 2) and with initiation of secondary cell wall thickening (MacAdam and Nelson, 1987).

Hexose content of control leaves increased with distance from the ligule (Fig. 5A). When stressed, hexose content increased about 3-fold by 126 h, although most of the increase occurred between 78 and 102 h. Glc accounted for 79% of the hexose in the leaf base of control leaves, and its content increased proportionately in the 0- to 10-mm region

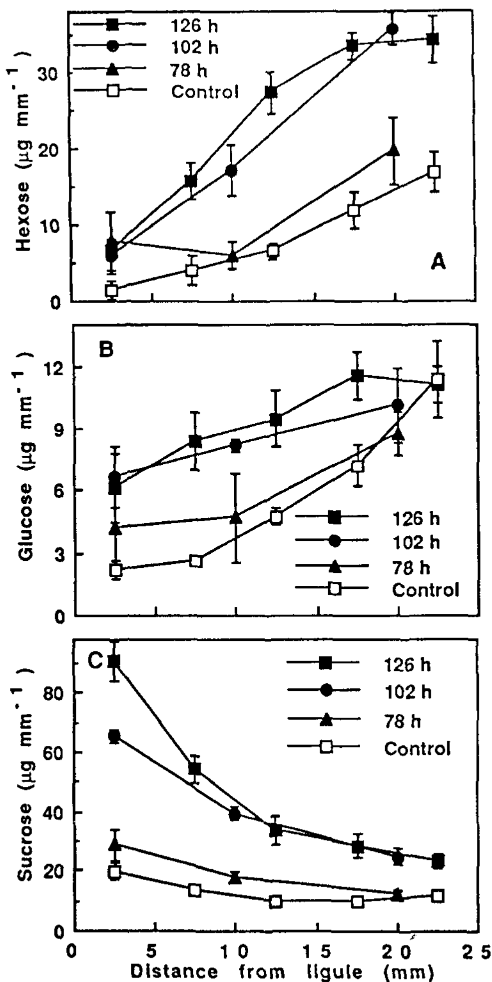

Figure 5. Hexose content was markedly increased in the leaf base (A), especially in the distal region of the shortened elongation zone, in response to stress. Glc (B) accounted for a large fraction of the total hexose (A) in control leaves, but less so in stressed leaves. Suc (C) also increased throughout the leaf base as stress progressed, but the increase was greatest near the ligule.

as stress occurred. Conversely, by 126 h Glc decreased to 33% of total hexose in the region 10 to 25 mm from the ligule (cf. Fig. 5, A and B), where most hexose accumulated, presumably as Fru (Volenec and Nelson, 1984).

Suc content also increased in the leaf base during stress (Fig. 5C), but predominately toward the ligule, where content increased 5-fold. Although most of the increase occurred between 78 and 102 h, Suc continued to increase near the ligule until 126 h.

Low-DP fructan content increased between the ligule and about 10 mm, and remained high at more distant positions in control leaves (Fig. 6A). Low-DP fructan content was decreased throughout as stress progressed. The reduced content remained similar at the proximal end between 78 and 126 h, but continued to decrease in the distal end through 126 h.

Mean fructan DP was similar throughout the leaf base for each sampling date. In contrast, mean DP for the leaf base was 3.8 (SD = 0.1) for control leaves, was slightly increased to 4.1 (SD = 0.1) at both 78 and 102 h, and was increased further to 4.4 (SD = 0.1) at 126 h. The increase in DP by 0.6 units was associated with a new series of oligosaccharides visible on TLC by 126 h (data not shown). This new series of oligomers appeared among, but were distinct from, the inulin-like oligomers found in control plants (Spollen and Nelson, 1988) and may be oligosaccharides of Fru resulting from fructan hydrolysis, which would cause overestimates of mean DP calculated from Fru-to-Glc ratios. For example, one of the bands that appeared in stressed tissue migrated just slower than the DP-3 band and contained Fru, but no Glc was detected with Glc oxidase (W.G. Spollen and C.J. Nelson, unpublished data).

High-DP fructan content paralleled low-DP fructan content in control leaves (Fig. 6B). By 78 h high-DP fructan content had decreased in the leaf base. Nearly full recovery of high-DP fructan content occurred in the basal 10 mm by 126 h. The increase in Suc content near the ligule (Fig. 5C) may have stimulated accumulation of this fructan (Wagner and Wiemken, 1987).

Contribution of Carbohydrates to Osmotic Adjustment

Mature leaf blade ψ_w declined from −0.65 MPa in control leaves to about −2 MPa at 126 h (Fig. 7). Throughout the leaf base of control plants the ψ_s was about −1.5 MPa (Fig. 7). The ψ_s decreased with stress in the leaf base until, at 126 h, the average ψ_s was −3.2 MPa. The ψ_s decreased most in the region near the ligule of stressed leaves, where Suc accumulated the most (Fig. 5C).

Hexose contribution to total ψ_s increased from 2% near the

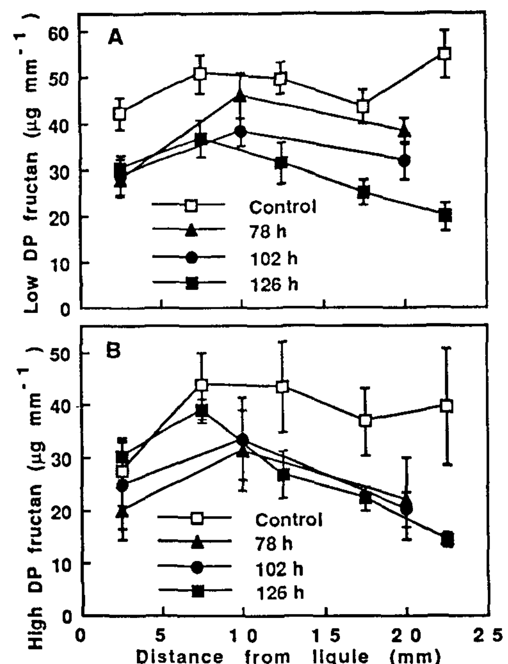

Figure 6. Content of low- (A) and high-DP fructan (B) decreased in the leaf base, especially the distal portion, as stress progressed. Low-DP included DPs 3 to 9, high-DP included DPs >9.

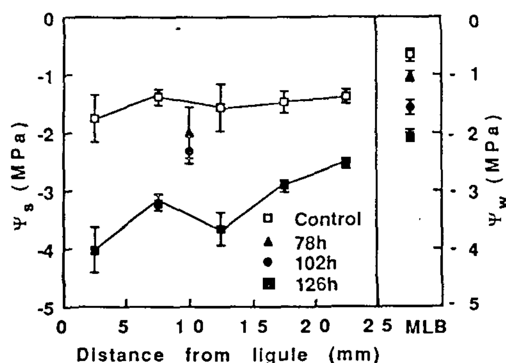

Figure 7. Spatial distributions of ψ_s in the base of the emerging leaf blade for control and stressed plants at 126 h after last watering. The ψ_s of the region from 5 to 15 mm from the ligule at 78 and 102 h after last watering is also shown. The ψ_w of MLB for each treatment is shown on the right. Vertical error bars are 2 times the SE.

ligule to 15% at 20 to 25 mm from the ligule in leaves of control plants (Fig. 8A). At 126 h hexose contribution to tissue ψ_s in stressed plants had about doubled within 20 mm of the ligule. Conversely, Suc contribution to ψ_s of control leaves decreased from 10 to 5% with distance from the ligule. Stress increased the Suc contribution to ψ_s about 2-fold in the leaf base, with the largest increase occurring within 10 mm of the ligule.

Low-DP fructan contributed about 13% to the ψ_s throughout the leaf base in control plants (Fig. 8B), more than any other WSC component. By 126 h, however, its contribution had decreased to about 4%. Although hexose, Suc, and low-DP fructan each contributed significantly to the total ψ_s, the spatial pattern of growth (Fig. 3) did not correlate with concentration of any single carbohydrate species.

DISCUSSION

Despite enhanced Suc content near the ligule during water deficit, content of low-DP fructan was reduced and content of high-DP fructan was reduced or remained near control levels. Total fructan in the leaf base decreased with stress to about 69% of the control by 126 h. The two fructan pools showed generally similar behavior in response to stress, but the timing of these changes and their magnitudes were not always the same. This points out differences in regulation of the sizes of these two pools.

Our data confirm earlier reports (Schnyder and Nelson, 1987) that net fructan synthesis is not simply related to bulk tissue Suc concentration because the content of fructan decreased while that of Suc increased. Although DM content of the bulk tissue increased with stress, net import rate of DM was inhibited, as shown by the fact that most of the 23% increase in DM content of the leaf base occurred between 78 and 102 h but was proportionately less than the approximately 50% decrease in daily *LER* during this period. Therefore, net DM deposition rate must have decreased. Similar considerations indicate that net rate of fructan synthesis was also decreased, which confirms earlier results showing a correlation between growth and net rate of fructan synthesis and extends that observation to include growth under conditions of water deficit.

One consequence of stress was a decreased contribution of fructan to tissue ψ_s (Fig. 8). The net decrease in fructan was very likely linked with increases in Suc and hexose, which contribute more ψ_s per unit mass than does fructan. Thus, the decrease in fructan content due to stress may benefit leaf growth indirectly. However, hexose content increased more than that of WSC-free DM. Assuming that the hexose was available to the phosphorylating enzymes in the cytoplasm, the supply of hexose appears to limit growth less than does the rate of hexose usage.

Growing regions of leaves of maize and bean have decreased acid invertase activity and hexose concentrations, but greater Suc concentrations, when subjected to salt stress (Hawker and Walker, 1978). This suggests that species that store fructan in expanding cells may be able to generate more hexose when sucrolysis is impaired by stress. The increased hexose concentration may promote lower ψ_s and more glycolysis during stress than occurs in species that cannot store fructan. At high ψ_w, rapid flower petal expansion in the ephemeral daylily (*Hemerocallis* hybrid cv Cradle Song) is initiated with concomitant fructan hydrolysis and increased hexose accumulation (Bieleski, 1993), supporting a role for fructan turnover in growth promotion by generation of osmotica and substrates for glycolysis.

The decreased fructan content can be attributed to decreased synthesis, increased depolymerization, or both. The increased Suc content strongly suggests inhibition at the first step of fructan synthesis, which is catalyzed by the enzyme SST (EC 2.4.1.99). SST converts two molecules of Suc into

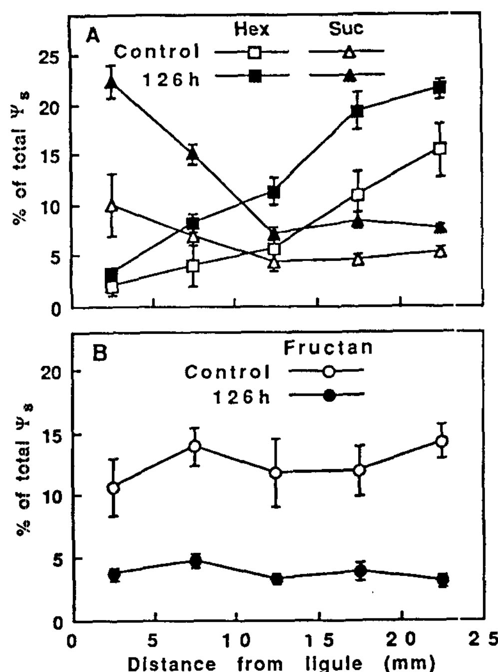

Figure 8. Contribution of hexose and Suc (A) and low-DP fructan (B) to the total tissue ψ_s of bases of control leaves and of leaves 126 h after last watering.

fructan trisaccharide and Glc, is regulated by Suc supply in mature barley (*Hordeum vulgare* L.) leaves (Wagner and Wiemken, 1987), and may be similarly regulated in the elongation zone of tall fescue leaves (Schnyder and Nelson, 1987). Suc synthase (EC 2.4.1.13) and invertase may also be functioning, but 79% of the hexose present in control leaves was Glc. If the remainder is Fru, and usage of hexoses is not preferential, then Suc synthase and invertase would together contribute no more than 42% of total sucrolytic activity. Since fructan is depolymerized in the elongation zone via fructan exohydrolase (EC 3.2.1.26) to generate Fru, especially in the distal positions (Allard and Nelson, 1991), this estimate of Suc synthase and invertase activity is probably too high. Recent measurements (M. Lüscher and C.J. Nelson, unpublished data) indicate that acid invertase activity is very low in the leaf base compared with that of SST.

Two mechanisms might explain a decrease in SST activity during stress. Increased concentrations of potassium, such as occur during water deficit in growing regions of leaf blades of wheat (*Triticum aestivum* L.) (Munns et al., 1979), can inhibit in vitro SST activity from the same species (Chevalier and Rupp, 1993). This suggests that potassium accumulation may be a mechanism for regulating SST in vivo. Second, SST in tall fescue requires Suc to prevent inactivation (M. Lüscher and C.J. Nelson, unpublished data), consistent with regulation of SST activity by Suc supply. Sensitivity to stress of Suc transport to the cellular compartment containing SST, presumably the vacuole (Wagner et al., 1983), could thus be another mechanism regulating SST.

Enzymes that catalyze conversion of fructan trisaccharide to molecules with higher DP were probably not more sensitive than SST to stress. This was inferred by observing that the intensity of staining of the trisaccharide on TLC plates changed in concert with that of other fructan oligosaccharides separated on TLC (not shown). Furthermore, the general relationship between high- and low-DP fructan remained relatively stable over a range of contents.

It is unclear if the rate of fructan depolymerization to free Fru is increased by stress. The Glc fraction of the total hexose decreased with stress, especially with distance from the ligule. Fru presumably made up the majority of the remaining hexose (Volenec and Nelson, 1984), and increased with stress as evidenced by the denser staining of the hexose bands on TLC plates (visualized with a ketose-specific spray). The decrease in fructan content in the distal regions of the leaf base demonstrates that fructan exohydrolase was active. All of the increase in hexose could be accounted for by loss of fructan (cf. Figs. 5A and 6). This also suggests that fructan in the distal region of the leaf base may be an important source of stored hexose when Suc supply is limited.

The shortening of the elongation zone due to stress (Fig. 2) suggests that the content of DM components might be better expressed per elongation zone (e.g. for the 0–15 mm region for 126 h, and the 0–25 mm region for the control). This leads to the same interpretation, however, because hexose and Suc still increase and fructan decreases due to stress.

The different WSC components in the MLB behaved similarly to those in the leaf base with the exception of Suc. Suc decreased in the MLB, presumably because photosynthesis was inhibited. Suc accumulated in the leaf base, however, as demand for it decreased.

When MLB ψ_w reached about −2.0 MPa (126 h), leaf growth had essentially stopped, yet the ψ_s of the growing region (estimated from the basal 15 mm) was −3.6 MPa (Fig. 7). If the MLB ψ_w approximates the xylem ψ_w in the elongation zone, which is reasonable for plants at the end of a dark period, then a turgor of 1.6 MPa is estimated, greater than the turgor of 0.85 MPa estimated in the control leaves. Turgor in the elongation zones of leaves of maize (Michelena and Boyer, 1982), barley (Matsuda and Riazi, 1981), and wheat (Barlow, 1986) was maintained at high levels despite complete inhibition of leaf growth by water deficit, so the high estimate of turgor in the present experiment has precedent. This suggests that limitations to carbon usage incurred by water deficit may contribute more significantly to growth limitation than water supply per se.

When this experiment was repeated, but with plant parts of tall fescue harvested also at the end of the light period, similar patterns of accumulation of DM and DM components were obtained (W.G. Spollen and C.J. Nelson, unpublished data). We included timothy (*Phleum pratense* L.) in this experiment, a species that accumulates only high-DP fructan (Spollen and Nelson, 1988). In timothy, no low-DP fructan was ever detected in MLB or leaf bases at high or low ψ_w at the end of either the light or the dark period (data not shown). Like in tall fescue, the high-DP fructan in the leaf base of timothy decreased in content while hexose and Suc increased. The spatial distribution and time course of accumulation of hexose and Suc in timothy were also similar to the data presented here. This suggests a similar role of fructan in the leaf elongation zone of timothy even though it has only a high-DP form. Clearly, however, the direct effect of low-DP fructan on ψ_s would not be a factor.

Earlier, Barlow (1986) made a preliminary report of a decrease in low-DP fructan content as ψ_w decreased in leaf bases of wheat. In contrast with tall fescue, wheat contains a different low-DP fructan in its leaf elongation zone, and considerably more high-DP fructan accumulates in the leaf base of wheat than in the MLB (Spollen and Nelson, 1988). These data, from three species with diverse fructan composition, show that hexose and Suc contribute more directly to tissue osmotic adjustment in the leaf base than does fructan, although fructan metabolism is closely associated, most likely as a short-term storage molecule. The inhibition of leaf expansion during limited water supply may be due, in part, to the reduced hydrolysis of Suc, perhaps by the inhibition of fructan synthesis. The contribution of SST to the total Suc hydrolyzed throughout the elongation zone is the subject of ongoing studies.

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LITERATURE CITED

Allard G, Nelson CJ (1991) Photosynthate partitioning in basal zones of tall fescue leaf blades. Plant Physiol **95:** 663–668

Barlow EWR (1986) Water relations of expanding leaves. Aust J Plant Physiol **13:** 45–58

Bieleski RL (1993) Fructan hydrolysis drives petal expansion in the ephemeral daylily flower. Plant Physiol **103:** 213–219

Chevalier PM, Rupp RA (1993) Inhibition of sucrose:sucrose fructosyl transferase by cations and ionic strength. Plant Physiol **101:** 589–594

Dimler RJ, Schaeffer WC, Wise CS, Rist CE (1952) Quantitative paper chromatography of D-glucose and its oligosaccharides. Anal Chem **24:** 1411–1414

Gastal F, Nelson CJ (1994) Nitrogen use within the growing leaf blade of tall fescue. Plant Physiol **105:** 191–197

Hawker JS, Walker RR (1978) Effect of sodium chloride on expansion rates and invertase activity of leaves. Aust J Plant Physiol **5:** 73–80

MacAdam JCW, Nelson CJ (1987) Specific leaf weight in zones of cell division, elongation, and maturation in tall fescue leaf blades. Ann Bot **59:** 369–376

Matsuda K, Riazi A (1981) Stress-induced osmotic adjustment in growing regions of barley leaves. Plant Physiol **68:** 571–576

Michelena VA, Boyer JS (1982) Complete turgor maintenance at low water potentials in the elongating region of maize leaves. Plant Physiol **69:** 1145–1149

Munns R, Brady CJ, Barlow EWR (1979) Solute accumulation in the apex and leaves of wheat during water stress. Aust J Plant Physiol **6:** 379–389

Parrish DJ, Wolf DD (1983) Kinetics of tall fescue leaf elongation: responses to changes in illumination and vapor pressure. Crop Sci **23:** 659–663

Schnyder H, Nelson CJ (1987) Growth rates and carbohydrate fluxes within the elongation zone of tall fescue leaf blades. Plant Physiol **85:** 548–553

Schnyder H, Nelson CJ (1988) Diurnal growth of tall fescue leaf blades. I. Spatial distribution of growth, deposition of water, and assimilate import in the elongation zone. Plant Physiol **86:** 1070–1076

Schnyder H, Nelson CJ (1989) Growth rates and assimilate partitioning in the elongation zone of tall fescue leaf blades at high and low irradiance. Plant Physiol **90:** 1201–1206

Schnyder H, Nelson CJ, Coutts J (1987) Assessment of spatial distribution of growth in the elongation zone of grass leaf blades. Plant Physiol **85:** 290–293

Schnyder H, Nelson CJ, Spollen WG (1988) Diurnal growth of tall fescue leaf blades. II. Dry matter partitioning and carbohydrate metabolism in the elongation zone and adjacent expanded tissue. Plant Physiol **86:** 1077–1083

Spollen WG, Nelson CJ (1988) Characterization of fructan from mature leaf blades and elongation zones of developing leaf blades of wheat, tall fescue, and timothy. Plant Physiol **88:** 1349–1353

Volenec JJ, Nelson CJ (1983) Response of tall fescue leaf meristems to nitrogen fertilization and harvest frequency. Crop Sci **23:** 720–724

Volenec JJ, Nelson CJ (1984) Carbohydrate metabolism in leaf meristems of tall fescue. I. Relationship to genetically altered leaf elongation rates. Plant Physiol **74:** 590–594

Wagner W, Keller F, Wiemken A (1983) Fructan metabolism in cereals: induction in leaves and compartmentation in protoplasts and vacuoles. Z Pflanzenphysiol **112:** 359–372

Wagner W, Wiemken A (1987) Enzymology of fructan synthesis in grasses. Properties of sucrose:sucrose fructosyl transferase in barley leaves (*Hordeum vulgare* L. cv Gerbel). Plant Physiol **85:** 706–710