Plant Physiol. (1990) 93, 902–906
0032-0889/90/93/0902/05/$01.00/0

Received for publication October 30, 1989
Accepted February 21, 1990

# Fructan Metabolism in Wheat in Alternating Warm and Cold Temperatures[1]

**Byeong-Ryong Jeong and Thomas L. Housley***

*Agronomy Department, Purdue University, West Lafayette, Indiana 47907*

## ABSTRACT

**The objective of this research was to develop a system in which the direction of fructan metabolism could be controlled. Three-week-old wheat seedlings (*Triticum aestivum* L. cv Caldwell) grown at 25°C were transferred to cold temperature (10°C) to induce fructan synthesis and then were transferred to continuous darkness at 25°C after defoliation and fructan degradation monitored. The total fructan content increased significantly 1 day after transferring from 25°C to 10°C in both leaf blades and the remainder of the shoot tissue, 90% of which was leaf sheath tissue. Leaf sheaths contained higher concentrations of fructan and greater portions of high molecular weight fructan than did leaf blades. Fructan content in leaf sheaths declined rapidly and was gone completely within 48 hours following transfer to 25°C in darkness. In leaf blades the invertase activity fluctuated during cold treatment. The activity of sucrose:sucrose fructosyl transferase increased markedly during cold treatment, while fructan hydrolase activity decreased slightly. In leaf sheaths, however, the activity of invertase decreased rapidly upon transfer to cold temperature and remained low. Trends in sucrose:sucrose fructosyl transferase and hydrolase activity in sheaths were the same as those of leaf blades. Sheath invertase and hydrolase activity increased when plants were transferred back to darkness at 25°C, while sucrose:sucrose fructosyl transferase activity decreased. These results indicate that changing leaf sheath temperature can be utilized to control the direction of fructan metabolism and thus provide a system in which the synthesis or degradation of fructan can be examined.**

Many $C_3$ grasses of temperate origin accumulate fructans as storage carbohydrates (4, 14). Fructans play an important role in photosynthate partitioning in these grasses. Large quantities frequently accumulate in leaves, stems, roots, and seeds, depending on the stage of plant development and on environmental conditions, such as light intensity and temperature (7, 9, 16, 18). Accumulation of fructan is induced by conditions that reduce plant growth and carbon translocation rates more than photosynthetic rates. Fructan concentration and mean mol wt of fructans from leaf and stem tissues of meadow fescue (*Festuca pratensis*) have been shown to vary with season (17). The major period of fructan synthesis was late autumn and early winter when plant growth was restricted. Fructan concentration decreased in spring during regrowth. When plants were transferred from warm (20°C) to cold temperature (5°C) fructan concentrations increased 3- to 10-fold in all plant parts of crested wheatgrass (a fertile hybrid of *Agropyron desertorum* and *A. cristatum*) and redtop (*Agrostis alba* L.) although starch became the most prevalent carbohydrate (5). Cold stress (5°C at night) induced fructan accumulation in leaf blades of barley (*Hordeum vulgare*) and wheat seedlings (20). In *Lolium temulentum* exposure to low temperature (5°C) induced fructan accumulation, especially in the meristematic regions of developing leaves (15). Fructans were synthesized by continuous illumination of excised primary leaf blades of barley and were degraded by transferring leaves to darkness (22, 23).

There is also a wide variation in the degree of polymerization of fructan, depending on the tissues within a plant and on the species (4, 16). Tissues that function as a reservoir of nonstructural carbohydrates stored a greater portion of high mol wt fructans than leaf blades. In field-grown forage grasses (17) extracts of stems contained higher mol wt fructans than those of leaves. The mean mol wt of leaf blade fructan was also smaller than that of the leaf sheath in *Festuca arundinacea* (11).

Fructans are synthesized by the enzymes SST[2] (EC 2.4.1.99) and FFT (EC 2.4.1.100). It is known that the activity of SST, the enzyme that catalyzes the synthesis of the trisaccharide, increased concomitantly with the onset of fructan synthesis (2, 23). FFT catalyzes the transfer of fructose from the trisaccharide to other oligomers and between oligomers (16). However, little is known about this enzyme. Degradation of fructans is accomplished by fructan hydrolases which have been characterized in barley (8) and *Dactylis glomerata* (24) as fructan exohydrolases. In barley, SST and fructan hydrolase were present in the same compartment, vacuoles (21). This implies that fructan synthesis and degradation takes place simultaneously. However, we do not know how the direction of fructan metabolism, synthesis or degradation, is controlled.

To understand the role of fructan in carbohydrate partitioning in wheat, the relationship between fructan synthesis and degradation must be ascertained. Our objective in this study was to develop a system in which the direction of fructan metabolism could be shifted or controlled by alternating cold and warm temperatures.

[1] This research was partially supported by Purdue Agricultural Research Station, USDA Competitive Grants 87-CRCR-1-2438 and 89-37130-4749, NSF Collaborative Research Grant INT 8900004, and was carried out during the tenure of NATO Grant CRG0706/87. AES J. No. 12262.

[2] Abbreviations: SST, sucrose:sucrose fructosyl transferase; FFT, fructan:fructan fructosyl transferase; gfw, gram fresh weight.

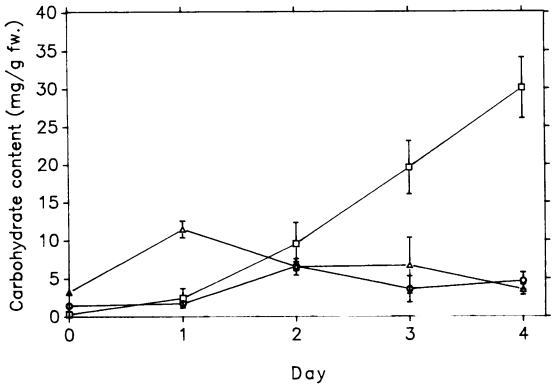


**Figure 1.** Time course of changes in amounts of carbohydrates (fructose equivalents) in wheat leaf blades after transferring from 25°C to 10°C. (○), Fructose; (△), sucrose; and (□) fructan. Values are the means of three replications. The bars represent 1 SD.

## MATERIALS AND METHODS

### Plant Growth

Wheat (*Triticum aestivum* L. cv Caldwell) plants were grown in 20 pots (20 cm diameter, 16 cm height) containing 15 plants and in a controlled environmental growth chamber at 25 ± 1°C. Daylength was 12 h (light period: 8:00 AM to 8:00 PM) with 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density using a mixture of fluorescent and incandescent lamps. Twenty-one d after planting plants were transferred to a growth cabinet at 4:00 PM with the same photoperiod and photosynthetic photon flux density but at 10 ± 1°C for cool temperature fructan induction. The leaf blades were excised at d 4 and the remaining plant tissue was transferred back to 25°C in a darkened growth cabinet. The remaining shoot tissue fresh weight was 90% sheaths and 10% immature leaf material. As a result, the tissue harvested after blade excision was termed 'leaf sheath'. Leaf blades and sheaths were harvested daily at 4:00 PM during cold treatment and warmed darkened sheaths were harvested.

### Fructan Content Determination

Tissues were frozen in dry ice immediately after harvesting and stored at −80°C until used. Frozen tissues were weighed (1 g) and ground in liquid $N_2$ using a mortar and a pestle. Carbohydrates were extracted twice with boiling aqueous 80% (v/v) ethanol and twice with boiling water. The combined extracts were flash evaporated to reduce the volume to 1 mL. Extracts were centrifuged, frozen, thawed, and centrifuged. Sugars were separated by TLC (silica gel, Sigma) in a solution consisting of 1-butanol:acetic acid:water (55:30:15, v/v/v). Standard sugars were separated on both sides of TLC plate and sprayed with urea-HCl. The spots corresponding to standard sugars were cut out and eluted from the silica gel with water. Sugar concentrations were measured with anthrone reagent at room temperature (12).

### Measurement of Enzyme Activity

To extract proteins, samples were weighed (1 g), placed in a mortar, flooded with liquid $N_2$, and homogenized with a pestle. The powder was ground in 50 mM Na-acetate buffer (pH 5) with a ratio of 5 mL buffer/gfw. The homogenate was centrifuged for 10 min at 4°C. Enzyme preparation procedures were conducted at 0 to 4°C. Proteins in the supernate were precipitated with 60% ammonium sulfate. After addition of ammonium sulfate, the extract was stirred overnight and centrifuged for 5 min. The pellet was resuspended in 0.5 mL extraction buffer (pH 5). The resulting fraction contained over 85% of invertase, SST, and fructan hydrolase activities (10).

For SST and invertase activity, sucrose (0.1 M final concentration) was added to 100 μL enzyme solution in a final volume of 120 μL. The reaction mixture was incubated for 1 h at 30°C and heated at boiling for 3 min. Glucose and fructose in the reaction mixtures were quantified by observing the formation of NADPH in a coupled enzyme assay (10). The excess amount of glucose over fructose was used to estimate SST activity and the results were reported as micromoles fructose transferred per gram fresh weight per hour (μmol/gfw/h). Invertase activity was determined by measuring the increase in fructose in the reaction mixture (10).

To examine wheat fructan hydrolase, fructans were extracted from wheat plants for substrate. Carbohydrates were chromatographed using a 110 × 1.5 cm column of Bio-Gel P-2 (200–400 mesh) (3), with distilled water as eluant. Fractions containing more than six residues were combined and concentrated. The wheat fructan substrate concentration was 90 mg fructose equivalent/mL solution. The substrate (20 μL) was added to enzyme solution (100 μL) to examine fructan hydrolase activity. Amount of free fructose produced after reaction was used to estimate the wheat fructan hydrolase activity (8).

## RESULTS AND DISCUSSION

Transferring wheat plants to cold temperature (10°C) resulted in the accumulation of fructan in both leaf blades (Fig.

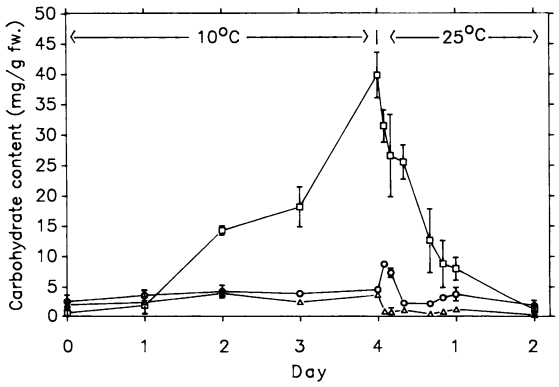


**Figure 2.** Time course of changes in amounts of carbohydrates (fructose equivalents) in wheat leaf sheaths. Plants grown at 25°C were transferred to 10°C and then back to 25°C in darkness after excision of leaf blades at d 4. Values are the means of three replications. Symbols are same as in Figure 1.

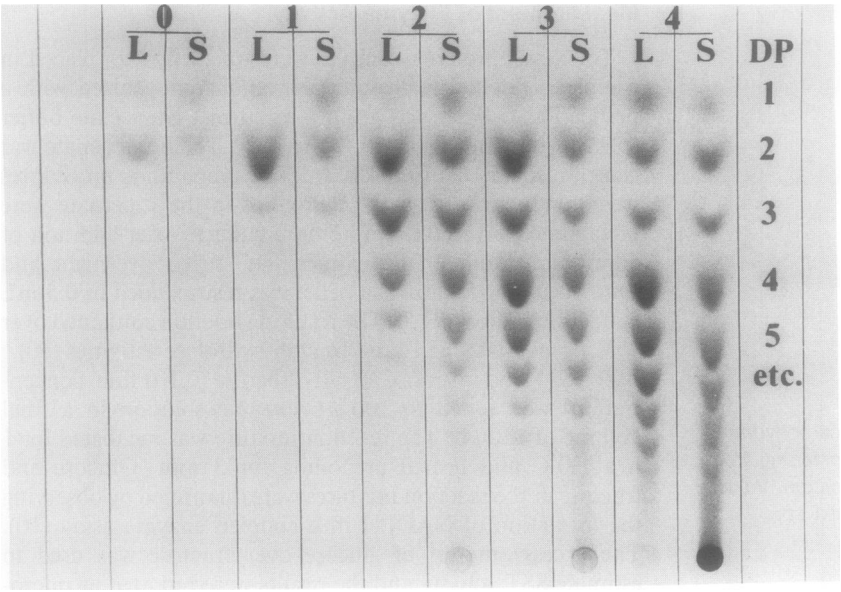


**Figure 3.** Accumulation of carbohydrates in leaf blades (L) and leaf sheaths (S) of wheat. Carbohydrates were separated by TLC. Comparable volume loaded per lane, so the amount of carbohydrate loaded per lane is similar to that of Figures 1 and 2. TLC plate was sprayed with urea-HCl. Numbers refer to days after transferring to 10°C. Degree of polymerization (DP) refers to the number of sugar moieties; DP2 is sucrose.

1) and leaf sheaths (Fig. 2). Sucrose content of leaf blades increased markedly 1 d after transferring to cold temperature and then, declined slowly. Fructans, however, started to accumulate rapidly after a lag of 24 h. Fructose also accumulated during cold treatment. These results were similar to those reported for field-grown wheat (6), *Lolium* (15), and barley (20), although the timing of fructan accumulation was different. In cold-treated *Lolium temulentum* (5°C), sucrose content increased after 24 h and this increase continued for 2 to 3 d and then sucrose content decreased slowly (15). Fructans started to accumulate after sucrose concentration had begun to stabilize. However, no marked change in fructose content was detected. In field-grown wheat, soluble carbohydrate content including fructose increased within 1 d during hardening (6). Wagner *et al.* (20) also reported in wheat and barley seedlings that cool nights (5°C) induced sucrose accumulation first, then rapid fructan synthesis after which the level of sucrose remained constant.

In contrast to leaf blades, sucrose and fructose concentrations increased slowly in leaf sheaths during cold treatment (Fig. 2). Fructan content, however, increased as rapidly as in leaf blades after the first day of cold treatment. To examine fructan degradation in leaf sheaths, which function as a storage tissue for nonstructural carbohydrates, leaf blades were excised at 4:00 PM of d 4 and plants with only leaf sheaths were warmed to 25°C in the dark. Fructan and sucrose contents declined rapidly upon transfer to 25°C in the dark and fructans were nearly gone within 2 d (Fig. 2). In contrast, the content of sucrose in excised leaf blades of barley declined rapidly upon transfer to the dark, whereas the fructan content continued to increase for 8 h then declined (23). In terms of the regulation of photosynthetic carbon metabolism, actively photosynthesizing leaf blades store carbohydrates for short periods whereas leaf sheaths function as a long term reservoir of nonstructural carbohydrates for use during regrowth (11). Therefore, the composition of fructan in leaf sheaths may be different from that of leaf blades. Apparently leaf sheaths contained a higher concentration of fructan as reserve carbohydrate and a greater proportion of higher mol wt fructans when compared with the leaf blades (Fig. 3). Housley and Volenec (11) reported that leaf sheaths contained significantly more nonstructural carbohydrate and a greater percentage of high mol wt fructan than did leaf blades of tall fescue. Similar results were reported in Jerusalem artichoke (*Helianthus tuberosus*) (13).

Marked changes in the content of fructan were assumed to be indicative of changes in the activities of the enzymes involved in fructan synthesis and degradation. The activity of SST increased in parallel with the accumulation of fructans in both leaf blades (Fig. 4) and leaf sheaths (Fig. 5). Activity of SST in the leaf blades increased rapidly and reached a maximum value 3 d after transfer to cold temperature. Simultaneously, fructan hydrolase activity dropped a little. Invertase activity in leaf blades remained relatively constant during cold treatment. In contrast to leaf blades, invertase and hydrolase activities dropped substantially in leaf sheaths 1 d after exposure to cold temperature.

Activities of degradative enzymes were higher in leaf sheaths than in leaf blades. During degradation of fructans at 25°C, invertase activity increased slowly, but did not reach its initial value. Hydrolase activity increased within 16 h to its initial activity, while the activity of SST decreased as others have reported (19, 23). However, the activity change of fructan hydrolase was relatively small, in alternating cold and warm temperature, especially in leaf blades, when compared to that of SST. Tognetti *et al.* (19) showed that when cold-adapted wheat plants were moved back to 23°C the activities of invertase and fructan hydrolase increased to those of controls kept in warm temperature (23°C), while SST activity decreased. In excised barley leaves, SST activity increased 20-fold within 24 h in the light and then disappeared upon transfer to darkness. In contrast, activities of fructan hydrolase and invertase changed only little during the light/dark cycle (23).

The data presented in this study, in contrast to the leaf blade system of Wagner *et al.* (23), indicate that the activity of fructan metabolic enzymes in sheaths changes markedly during both cold treatment and after rewarming in darkness. The ratio of sheath fructan synthetic activity to hydrolytic activity was initially 0.66, then increased to 2.8 on the 4th d of cold treatment and decidely favored fructan synthesis. Further, the increase in the rate of fructan content (410 μg fructose equivalents/gfw/h, Fig. 2) was similar to the net synthetic activity (753 [SST] − 244 [hydrolysis] = net synthesis of 509 μg/gfw/h, Fig. 5) at d 4. By placing the plants in the cold, fructan metabolism was directed toward synthesis of fructans, particularly in sheaths. Although the leaf net synthetic activity (SST-hydrolase = 485 μg/gfw/h, Fig. 4) was similar to that of the rate of fructan accumulation (310 μg/gfw/h, Fig. 1), the greater rate and amount of fructan accumulation in sheaths suggest these to be a more interesting system to examine the control of fructan metabolism. This is particularly true with regard to fructan degradation. In 4 d the sheaths accumulated approximately 40 mg/gfw of fructan, but in less than 20 h, 30 mg/gfw had been hydrolyzed (Fig. 2). During fructan degradation, the rate of loss of fructans (1330 μg/gfw/h, Fig. 2) exceeded the activity of the hydrolase (700 μg/gfw/h, Fig. 5), but the direction of metabolism was obviously altered toward fructan hydrolase. The ratio of synthetic to hydrolytic activity, 24 h after warming in the dark, was 0.37, a ratio favoring net hydrolysis. These results indicate that changing leaf sheath temperature can be utilized to control the direction of fructan metabolism and thus provide a system in which synthesis or degradation of fructan can be examined. Furthermore, these tissues are enriched in enzymes of fructan metabolism and should provide tissue suitable for isolating these proteins which is one of the future directions of our research.

During the preparation of this paper, Bancal and Gaudillère (1) published results of fructan synthesis in wheat seedlings after chilling. They showed that chilling (5°C) induced fructan accumulation in 2-week-old wheat seedling leaf blades and bases. Our results are similar to theirs with a few exceptions.

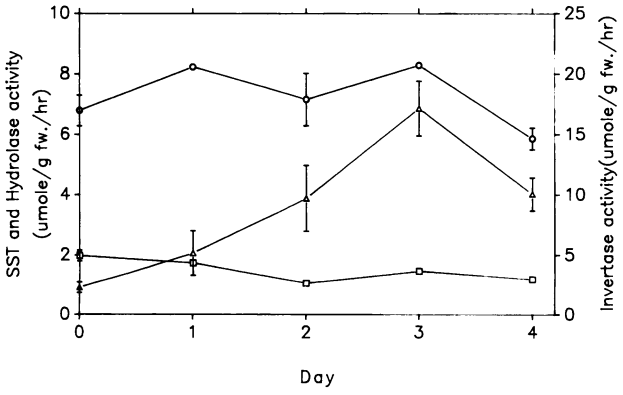


**Figure 4.** Changes in activities of invertase, SST, and fructan hydrolase (μmol hexose/gfw/h) in wheat leaf blades during cold treatment. (○), Invertase; (△), SST; (□), fructan hydrolase. Values are the means of three replications. The bars represent 1 SD.

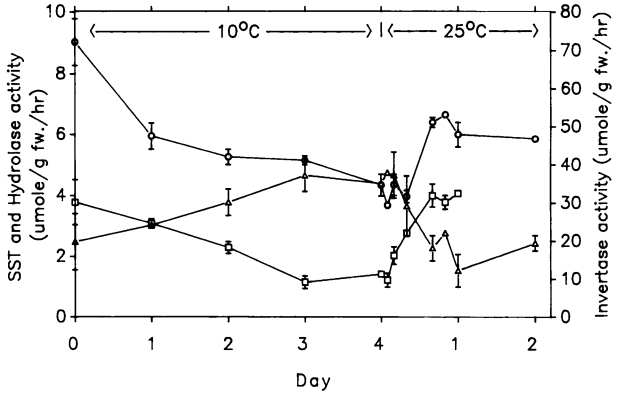


**Figure 5.** Changes in activities of invertase, SST, and fructan hydrolase in wheat leaf sheaths in alternating warm (25°C in darkness) and cold (10°C) temperature. Values are the means of three replications. Plant system and symbols are same as in Figures 2 and 4, respectively.

Their final leaf blade fructan concentration (45.70 mg/gfw) was greater than we found (30.09) but lower (17.62) than our results (39.82) for leaf bases. Their fructan accumulation rate (9.14 mg/gfw/d) in leaf blades was higher than ours (7.51) and lower (3.52) in leaf bases when compared to our results (9.95). The differences are likely due to a greater movement of photoassimilates from leaf blades to leaf sheaths in our system. It is possible that the cooler temperature of Bancal and Gaudillère reduced sink strength of leaf bases, altering transport to the bases that resulted in lower fructan concentrations. Indeed, Bancal and Gaudillère's array of oligosaccharides at d 5 corresponds to d 2 or d 3 in our system.

It is hypothesized that fructan, SST, and fructan hydrolase are located exclusively in the vacuole. This hypothesis requires the possibility for concomitant fructan synthesis and degradation in the same compartment. It will be interesting to learn how the direction of fructan metabolism is regulated. In any case, using wheat sheath tissue we can influence the direction of metabolism by increasing or decreasing the ratio of synthesis to degradation, a system that can be used to examine fructan regulation.

## LITERATURE CITED

1. **Bancal P, Gaudillère JP** (1989) Rate of accumulation of fructan oligomers in wheat seedlings (*Triticum aestivum* L.) during the early stages of chilling treatment. New Phytol **112:** 459–463
2. **Cairns AJ, Pollock CJ** (1988) Fructan biosynthesis in excised leaves of *Lolium temulentum* L. II. Changes in fructosyl transferase activity following excision and application of inhibitors of gene expression. New Phytol **109:** 407–413
3. **Carpita NC, Kanabus J, Housley TL** (1989) Linkage structure of fructans and fructan oligomers from *Triticum aestivum* and *Festuca arundinacea* leaves. J Plant Physiol **134:** 162–168
4. **Chatterton NJ, Harrison PA, Bennett JH, Asay KH** (1989) Carbohydate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. J Plant Physiol **134:** 169–179
5. **Chatterton NJ, Harrison PA, Bennett JH, Thornley WR** (1987) Fructan, starch and sucrose concentrations in crested wheatgrass and redtop as affected by temperature. Plant Physiol Biochem **25:** 617–623

6. **Green DG, Ratzlaff CD** (1975) An apparent relationship of soluble sugars with hardiness in winter wheat varieties. Can J Bot **53:** 2198–2201
7. **Hendrix JE, Linden JC, Smith DH, Ross CW, Park IK** (1986) Relationship of pre-anthesis fructan metabolism to grain numbers in winter wheat (*Triticum aestivum* L.). Aust J Plant Physiol **13:** 391–398
8. **Henson CA** (1989) Purification and properties of barley stem fructan exohydrolase. J Plant Physiol **134:** 186–191
9. **Housley TL, Daughtry CST** (1987) Fructan content and fructosyltransferase activity during wheat seed growth. Plant Physiol **83:** 4–7
10. **Housley TL, Kanabus J, Carpita NC** (1989) Fructan synthesis in wheat leaf blades. J Plant Physiol **134:** 192–195
11. **Housley TL, Volenec JJ** (1988) Fructan content and synthesis in leaf tissues of *Festuca arundinacea.* Plant Physiol **86:** 1247–1251
12. **Jermyn MA** (1956) A new method for determining ketohexoses in the presence of aldohexoses. Nature **177:** 38–39
13. **Malmberg A, Theander O** (1986) Differences in chemical composition of leaves and stem in Jerusalem artichoke and changes in low-molecular sugar and fructan content with time of harvest. Swed J Agric Res **16:** 7–12
14. **Meier H, Reid JSG** (1982) Reserve polysaccharides other than starch in higher plants. *In* FA Loewus, W Tanner, eds, Plant Carbohydrates I. Encyclopedia of Plant Physiology (New Series), Vol 13A. Springer-Verlag, Berlin, pp 418–471
15. **Pollock CJ** (1984) Sucrose accumulation and the initiation of fructan biosynthesis in *Lolium temulentum* L. New Phytol **96:** 527–534
16. **Pollock CJ** (1986) Fructans and the metabolism of sucrose in vascular plants. New Phytol **104:** 1–24
17. **Pollock CJ, Jones T** (1979) Seasonal patterns of fructan metabolism in forage grasses. New Phytol **83:** 9–15
18. **Pontis HG, Campillo E Del** (1985) Fructans. *In* PM Dey, RA Dixon, eds, Biochemistry of Storage Carbohydrates in Green Plants. Academic Press, New York, pp 205–227
19. **Tognetti JA, Calderon PL, Pontis HG** (1989) Fructan metabolism: reversal of cold acclimation. J Plant Physiol **134:** 232–236
20. **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
21. **Wagner W, Wiemken A** (1986) Properties and subcellular localization of fructan hydrolase in the leaves of barley (*Hordeum vulgare* L. cv Gerbel). J Plant Physiol **123:** 429–439
22. **Wagner W, Wiemken A** (1987) Enzymology of fructan synthesis in grasses: properties of sucrose-sucrose-fructosyltransferase in barley leaves (*Hordeum vulgare* L. cv Gerbel). Plant Physiol **85:** 706–710
23. **Wagner W, Wiemken A, Matile P** (1986) Regualtion of fructan metabolism in leaves of barley (*Hordeum vulgare* L. cv Gerbel). Plant Physiol **81:** 444–447
24. **Yamamoto S, Mino Y** (1985) Partial purification and properties of phleinase induced in stem base of orchardgrass after defoliation. Plant Physiol **78:** 591–595