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**NONSTRUCTURAL CARBOHYDRATE RESERVES OF TEMPERATE  
PERENNIAL GRASSES IN AUTUMN EARLY GROWTH**

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**Abstract**

The objective of this study was to determine levels of nonstructural carbohydrate reserves of four temperate perennial grasses: Orchardgrass (*Dactylis glomerata* L.), Timothy (*Phleum pratense* L.), Perennial ryegrass (*Lolium perenne* L.), and Reed canarygrass (*Phalaris arundinacea* L.) in their early growth stages during the cool autumn temperatures in northern Japan. At the time of sampling, all grasses were in their vegetative stage, and Reed canarygrass was not forming rhizomes. Fructosan concentration in reed canarygrass roots (8.04%) was 22 times that of the leaf blade (0.36%) and twice that of the stem (3.40%); the concentration in reed canarygrass root was the highest of the four grasses. Timothy stored fructosan in the root at a significantly higher concentration (1.65%) than did the orchardgrass (0.58%) and perennial ryegrass (0.83%). The concentration of fructosan in the timothy was the highest in the stem, the lowest in the leaf blade and intermediate in the root. On the other hand, orchardgrass and perennial ryegrass stored the highest amount of fructosan in the stem, the lowest amount in the root, and an intermediate amount in the leaf blade. In addition, the

root dry weight and the ratio of the root dry weight to the total dry weight were significantly higher in reed canarygrass than in the other three grasses. Timothy was in second place surpassing orchardgrass and perennial ryegrass. We considered that winter survival is the highest in reed canarygrass and second highest in timothy over orchard grass and perennial ryegrass.

**Keywords:** Orchardgrass, Timothy, Perennial ryegrass, Reed canarygrass, NSC, Root, Cool air, Winter survival

### **Introduction**

Wintering of pasture grasses is essential for the stable production of forages in northern Japan. The winter survival of grasses is thought to be closely related to the nonstructural carbohydrate reserves that are stored in the vegetative storage parts in the fall of the year. So far, many studies on the carbohydrate reserves in temperate annual, biennial, and perennial grasses have been carried out, reported, and reviewed, particularly with regard to their taxonomy, changes with maturity, and differences in species and cultivars (Lewis, 1984; Ojima and Isawa, 1967; Smith, 1972; Tamura et al., 1985; Yoshida et al., 1998; Yukawa and Watanabe, 1995). However, the details of the nonstructural carbohydrate reserves of temperate perennial grasses in their early growth stages, when grown under the cool temperatures and short days of fall in northern Japan, are still largely unknown.

In this study, we investigated the growth and accumulation of nonstructural carbohydrates in the leaf blade, stems, roots of four important temperate grasses in the fall of the year during their vegetative stages of growth, approximately 80 days after seedling emergence.

## Material and Methods

Four temperate perennial grasses: Orchardgrass (*Dactylis glomerata* L. cv Natumidori), Timothy (*Phleum pratense* L. cv Kunpuu), Perennial ryegrass (*Lolium perenne* L. cv Yatuyutak), and Reed canarygrass (*Phalaris arundinacea* L. cv Venture) were sown on August 18, 1999, in an experimental field at the Tohoku National Agricultural Experiment Station. The seeding rate was 20 kg per hectare for each species. Fertilizer was initially applied at the rate of 70, 140, and 70 kg per hectare of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O, respectively. The experiment was conducted in a randomized block design with nine replications. Plants were sampled on November 15, 1999 including roots in a 400cm<sup>2</sup>(20×20cm) area of each plot. At that time, Reed canarygrass had not formed rhizomes, and the Timothy was forming haplocorms. The Orchardgrass and Perennial ryegrass were in their vegetative stages. The plants were removed from the soil and litter, washed with tap water, and divided into leaf blades, stems, which included the leaf sheath and stem base, and roots. The fractions were weighed and dried in a forced-draft oven for 20 hours at 70 °C to a constant weight. The dry materials were then weighed, ground, and stored for analysis.

The nonstructural carbohydrates were extracted from 200mg of ground materials that had been shaken for one hour with 50 ml of distilled water at room temperature. Ninety percent ethanol was used to separate the fructosan from the mono- and oligo-saccharides. The carbohydrates were quantitatively determined by the Anthrone method (Yemm and Willis, 1954). Fructose was used as the standard. Results are expressed as a percent of the fresh-weight. The DPs of fructosan are also estimated and compared relatively among species according to a qualitative analysis by HPLC.

## Results and Discussion

Reed canarygrass stored the greatest amount of fructosan in the root, the lowest

amount in the leaf blade, and an intermediate amount in the stem. The concentration of fructosan in the root (8.04%) was 22 times that in the leaf blade (0.36%) and two times that in the stem (3.40%), and it was higher than any of the other three grasses. The amount of fructosan stored in timothy roots was higher than the amounts stored in orchardgrass and perennial ryegrass roots, and the concentration of fructosan was the highest in stem (3.41%), the lowest in the leaf blade (1.04%) and an intermediate in the root (1.65%). On the other hand, the fructosan concentrations contained in orchardgrass and perennial ryegrass were highest in stems, intermediate in leaf blades, and lowest in roots as shown in Figures 1.

Mono- and oligo-saccharide concentrations were significantly different among the grasses and the plant parts. However, the differences were small compared to that of the fructosan. The DP of fructosan was considered to be higher in the order Timothy  $\geq$  Reed canarygrass > Orchardgrass > Perennial ryegrass.

In addition, we observed that the root dry weight and the ratio of root dry weight to total dry weight were higher in reed canarygrass than they were in the other three grasses. Timothy was second place, above orchardgrass and perennial ryegrass (Table 1).

It is generally known that forage grasses are divided into two groups based on the type of nonstructural polysaccharide accumulated in their vegetative parts. Grasses of tropical and subtropical origin accumulate starch, but grasses of temperate origin accumulate fructosans (Lewis, 1984; Smith, 1972; Weinmann and Reinhold, 1946). Ojima and Isawa (1970) have shown that the fructosan levels in the leaf sheath of orchardgrass increase not only in amounts, but also in degree of polymerization with the descending of temperature in autumn. Tamura et al. (1985) and Tamura (1986) reported that species and cultivars of winter annual and biennial grasses that have higher concentrations of nonstructural carbohydrates, especially in roots, have higher rates of winter survival and growth in the following spring. Yukawa and Watanabe (1995) and Yoshida et al. (1998) also clarified that tolerance to snow

mold and freezing in wheat and barley were correlated to the greatest extent with total mono- and disaccharide and fructosan concentration in late autumn. It is considered that nonstructural carbohydrate reserves stored in autumn are essential for wintering and spring growth in temperate grasses.

In the experiment, we found that reed canarygrass is superior in root growth and root nonstructural carbohydrates than the other grasses. Timothy was in second place surpassing orchardgrass and perennial ryegrass. We considered that winter survival is the highest in reed canarygrass and the second highest in timothy.

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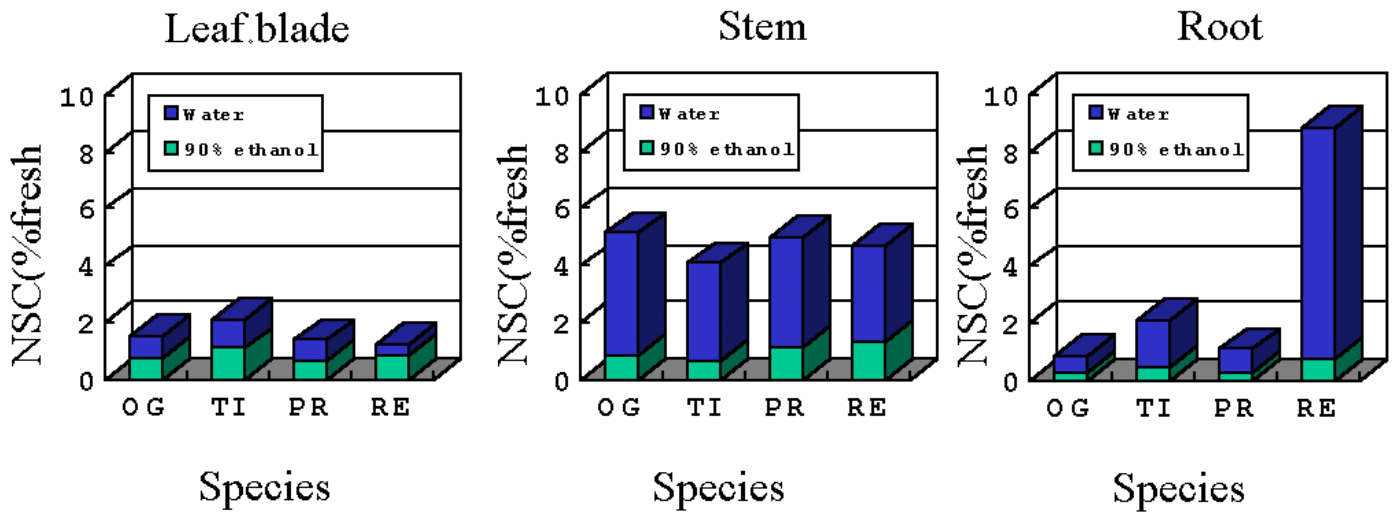
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**Table 1** - Growth of the grasses used in the experiment (Nov. 15, 1999).

Grass species	Plant height cm	Dry matter weight(g/m <sup>2</sup> )				Root/Total
		Total g/m <sup>2</sup>	Leaf g/m <sup>2</sup>	Stem g/m <sup>2</sup>	Root g/m <sup>2</sup>	
Orchard grass	55,0	539,4	263,6	205,8	70,0	0,130 a
Timothy	46,4	475,0	234,7	139,4	100,8	0,214 b
Perennial ryegrass	56,3	789,7	389,2	305,3	95,3	0,122 a
Reed canarygrass	44,2	513,6	188,3	157,2	168,1	0,326 c

Root/Total indicates root/total dry matter weight ratio, and the means followed by the letters are significantly different at  $P < 0.05$ , student t-test.





**Figure 1** - Nonstructural carbohydrate reserves of temperate grasses in early growth stages in fall.

Water; fructosans extracted with water from 90% ethanol extracted residue.

90% ethanol; Mono- and oligo-saccharides extracted with 90% ethanol.

OG; Orchardgrass, TI; Timothy, PR; Perennial ryegrass, RE; Reed canarygrass.