# Nonstructural Carbohydrate and Crude Protein in Pinegrass Storage Tissues

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**Abstract**

**Nonstructural carbohydrates in storage tissues of pinegrass (*Calamagrostis rubescens* Buckl.) consist of sucrose, glucose, fructosan, and starch. The predominant polymer is a long-chain fructosan. An acid-extractable structural carbohydrate appeared to be xylan. Total nonstructural carbohydrates (TNC) of rhizome plus root tissue decreased during May, reached a minimum value during late May to early June, increased until late June, remained constant until late August, and then increased until November. The TNC level of crown tissue was low during May and early June and reached a peak during July and again in the fall. The crude protein concentration of rhizome plus root tissues was relatively constant throughout the season. Rhizome plus roots accumulated the largest amounts of TNC and crude protein.**

Stored organic compounds, such as carbohydrates, fats, and proteins, serve as food reserves for plants at times when photosynthesis cannot supply sufficient material for maintenance and growth (Trlica 1977, White 1973). These reserves must provide a source of energy and a source of molecules for growth when plants are completely defoliated; however, a definite role in regrowth of partially defoliated plants has not been proved (May 1960, Jameson 1964). In addition to a role for reserves in regrowth of completely defoliated plants, reserves are utilized by perennials during the winter (Menke and Trlica 1981).

Root reserves typically decrease following harvest of aerial growth, and this decrease implies a role for reserves in regrowth. Whether or not reserves are used directly for regrowth, reserve level may serve as a measure of plant vigor following grazing (Trlica 1977).

Grasses can be classified on the basis of the type of nonstructural carbohydrates (NC) that are accumulated: starch or fructosan accumulators (Smith 1968). Furthermore, they are classified according to the type of structural carbohydrate (SC); xylose is the predominant SC component in fructosan containing grasses, while glucose is the predominant SC component in starch containing grasses (Ojima and Isawa 1968).

Pinegrass (*Calamagrostis rubescens* Buckl.) is an important source of forage on the forest ranges of British Columbia (McLean et al. 1969). Simulated grazing studies have established that pinegrass is most sensitive to herbage removal during July (Freyman 1970, Stout et al. 1980), and that a pinegrass stand will deteriorate under simulated intensive grazing practices, such as clipping biweekly from May 15 to September 15 to a stubble height of 5 or 10 cm (heavy and moderate grazing intensities respectively) (Stout et al. 1981).

The objectives of this study were to determine: (1) type of carbohydrates stored; (2) seasonal pattern of carbohydrate and crude protein levels; and (3) morphological distribution of carbohydrate and protein reserves in pinegrass.

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## Material and Methods

### Identification of the Carbohydrate Storage Form(s) in Pinegrass

Pinegrass rhizomes were collected from the field in October and planted in 4-liter plastic pots containing loam, sand, and peat in the ratio of 1:1:1. Tiller growth and development occurred during a 3-month period in a growth chamber with a 16-hr photoperiod, a light energy of 90 $Wm^{-2}$ provided by fluorescent (Vita-Lite, Duro-Test Corp., U.S.A.) and incandescent lamps, a day temperature of 20 ± 1C, and a night temperature of 18 ± 1C. Tillers in 12 pots were then clipped to a 5-cm stubble height at 2-wk intervals during a 6-wk period. Tillers in 12 other pots received no clipping treatment during the 6-wk period. Following the 6-wk treatment period, 3 replicates of each treatment were harvested; each replicate consisted of 4 pots. A completely random design was used for this experiment.

Plant material was harvested and divided into crown and rhizome plus root fractions. Crown tissue was defined as the 1.5 cm portion of the tiller above its point of attachment to the rhizome. The crown sample included the growing point plus about 8 stem nodes, the attached scale leaves, and sheaths of at least 4 aerial leaves. The rhizome plus root fraction contained the rhizome and all of the attached fibrous root material that could be collected. Crown and rhizome plus root tissues were washed with cold water, freeze-dried, and ground to pass through a 40-mesh screen. Reducing sugars, total sugars, fructosans, starch, and $H_2SO_4$ extractable structural carbohydrates (SC) were then measured. To characterize the relative contribution of rhizomes and roots to the rhizome plus root tissue, on 2 dates the rhizome plus root tissue was separated into rhizome and fibrous root tissues following washing. Dry weight, TNC and crude protein were determined for each type of tissue.

Similar chemical measurements were also made on crown tissue harvested from a native stand of pinegrass in May, June, and October, 1978. For this study, tissue samples from 2 of the 6 replicates of the seasonal trend study were used.

### Seasonal Trend of TNC and Crude Protein in Pinegrass Crowns and Rhizome Plus Roots

In 1978, a 0.2-ha area of land immediately adjacent to the Poison Creek study site (Stout et al. 1980) was fenced to keep out cattle. Six 20 $m^2$ plots were identified within the 0.2 ha area. Samples were collected on 20 dates from May 15, 1978, to October 23, 1979. Pinegrass sods (to a depth of 10 cm in the mineral soil) were dug from a subplot (0.5 to 0.8 $m^2$) within each 20 $m^2$ plot. The subplots were harvested according to a completely random design. The bulk of the adhering soil was removed from the sods and the plant material was put into plastic bags and transported to the laboratory on ice. Sods were washed with cold water and crown and rhizome plus root samples were prepared as above, with the exception that drying was done in a forced air oven at 60°C. TNC and crude protein content were then measured.

### Distribution of Dry Weight, TNC, and Protein in a Pinegrass Tiller

On July 16, 1979, four areas (400 to 900 $cm^2$) of pinegrass sods

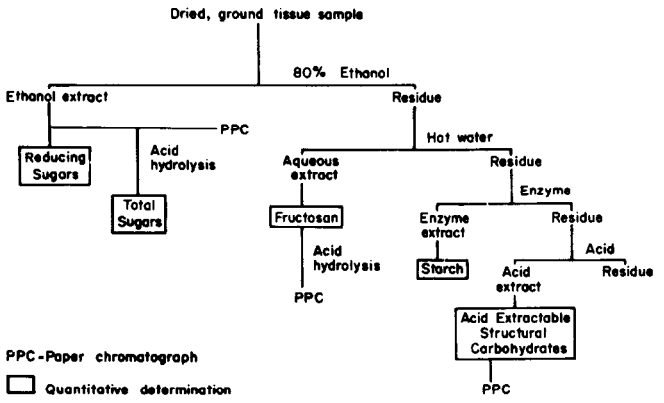


**Fig. 1.** *Diagram of carbohydrate analysis.*

were harvested. The number of tillers in each area were counted. Plant material in each area was then divided into six fractions: rhizome plus root, crown and top tissue sections of 2.5 cm to 5 cm, 5 cm to 10 cm, 10 cm to 15 cm, and greater than 15 cm. Dry weight, TNC and crude protein measurements were made on each fraction.

### Chemical Analysis

Sugars were extracted from freeze-dried and pulverized plant tissues with 80% ethanol (Fig. 1). The reducing powers of the ethanol extract before and after hydrolysis with 0.2N $H_2SO_4$ at 100° C for 1 h were determined by autoanalysis (Suzuki 1971a) for quantification of reducing sugars and total sugars respectively. The alcohol insoluble fraction was extracted with water, and fructosan in the water extract was determined by an automated method (Suzuki 1971b). The plant residue, after removing fructosan, was incubated with a buffer solution containing amyloglucosidase to hydrolyze and extract starch (Dekker and Richards 1971). The reducing power of glucose resulting from the hydrolyzed starch was determined by autoanalysis (Suzuki 1971a). The residue after the enzyme extraction was treated with 1N $H_2SO_4$ to extract acid soluble structural carbohydrates.

Sugar components of the acid soluble SC fraction (hemicellulose) as well as of the ethanol-, water- and enzyme-extracts of the NC were identified by paper chromatography (Suzuki 1971b). Polysaccharides in the water extract stayed on the baseline of the chromatogram. The baseline band was cut out, eluted with water, hydrolyzed with 0.2N $H_2SO_4$ and rechromatographed to identify sugar components of the polysaccharides.

The TNC was determined by shaking tissue sample with water for 1.5 h, hydrolyzing the water extract with 0.2N $H_2SO_4$ and measuring the resulting reducing sugars (Nelson 1944). Also, the TNC was calculated by adding the values from the separate analyses for total sugars, fructosan and starch.

Percent nitrogen was determined by a micro-Kjeldahl method (AOAC 1975) and crude protein content was calculated by multiplying percent nitrogen by 6.25 (Heath et al. 1973). Pace et al. (1982) report that 10 to 60% of plant nitrates will be detected by the Kjeldahl method. Thus if nitrate concentration is high, the crude protein level could be over-estimated.

## Results and Discussion

Nonstructural carbohydrates of pinegrass consisted of sugars, fructosan and starch (Table 1). Paper chromatography of the ethanolsoluble fraction revealed that sucrose was the predominant free sugar followed by glucose and fructose, the proportion being approximately 4:2:1. The water extract contained a polysaccharide and paper chromatography after acid hydrolysis indicated that it consisted of fructose moieties with trace amounts of sucrose and glucose, indicating a fructose-polymer or fructosan (Suzuki 1968). Chain length or degrees of polymerization (D.P.) of a fructosan can be estimated by calculating the ratio of fructose to sucrose or glucose. Pinegrass fructosan has a D.P. of approximately 60. Timothy fructosan, phlein, has a D.P. of 88 (Suzuki 1968). Therefore, pinegrass fructosan has a shorter chain length than timothy fructosan but a longer chain length than many other grass species such as *Lolium, Poa,* and *Festuca* spp. (Suzuki 1968). Although a further study is required to measure the actual chain length, pinegrass undoubtedly contains a long-chain fructosan like that of a related species, *Calamagrostis canadensis* (Michr.) Beauv. (Smith 1968).

Pinegrass also contained starch, but it did not exceed 11% of fructosan or 3% of TNC (Table 1). When a statistically significant effect of clipping occurred, it occurred as a decrease in a particular sugar fraction. Most sugar fractions showed a lower mean value for clipped tillers than for nonclipped tillers, and it seems most probable that lack of statistical significance was related to small sample size rather than to lack of a clipping effect. There was no indication that herbage removal would alter the relative relationship between total sugars, fluctosan and starch. In addition, the same relationship between total sugar, fructosan and starch was observed for field grown plants harvested at different times during their growth cycle. Therefore, fructosan is the major carbohydrate polymer storage form in pinegrass, and this will not be greatly altered by growth conditions or stage of plant development.

The major constituent sugar of the acid extractable fraction of SC in temperate and tropical grass species is xylose and glucose respectively (Ojima and Isawa 1968). Paper chromatography showed that the acid extractable SC of pinegrass contained only xylose. Glucose, fructose, galactose, arabinose, or any other possi-

**Table 1. Concentration (percent of dry weight) of nonstructural carbohydrate constituents and acid extractable structural carbohydrates in storage tissues of pinegrass as influenced by clipping and three sampling dates.**

| Treatment or date collected | Tissue | Nonstructural carbohydrates | | | | | Structural carbohydrates (acid extractable fraction[3]) |
|---|---|---|---|---|---|---|---|
| | | Reducing sugars | Total sugars | Fructosan | Starch | TNC[2] | |
| | | | | Growth-chamber-grown Plants | | | |
| Control | Crown | 4.2 b | 9.6 b | 2.3 ab | 0.23 c | 12.1 a | 9.0 b |
| | Rhizome plus root | 2.5 ab | 7.0 ab | 4.7 c | 0.14 a | 11.9 a | 11.1 c |
| Clipped | Crown | 2.6 ab | 6.6 ab | 1.6 a | 0.18 b | 8.3 a | 8.0 a |
| | Rhizome plus root | 1.5 a | 5.3 a | 3.1 b | 0.14 a | 8.5 a | 9.7 b |
| | | | | Field-grown Plants | | | |
| May 10 '78 | Crown | 0.6 a | 3.8 a | 1.8 a | 0.13 a | 5.7 b | 10.2 a |
| June 07 '78 | Crown | 0.8 a | 2.9 a | 1.2 a | 0.13 a | 4.2 a | 10.9 a |
| Oct 17 '78 | Crown | 0.8 a | 5.0 b | 2.5 b | 0.11 a | 7.6 c | 10.5 a |

[1]For the growth-chamber experiment each value is the mean from three samples and for the field experiment each value is the mean from two samples. Different letters indicate significant differences within a column for a particular experiment at $P = 0.05$ (Duncan's miltiple range test).
[2]TNC = starch + fructosans + total sugars.
[3]This fraction contained a large amount of xylan.

**Table 2. Relative contribution of rhizomes and roots to the dry weight, TNC, and crude protein content of the rhizome plus root tissue.**

| Measurement | Rhizome | | Fibrous roots | |
|---|---|---|---|---|
| | A[1] | B[2] | A[1] | B[2] |
| Dry weight (mg/tiller) | 59 ± 4 | 54 ± 1 | 13 ± 4 | 17 ± 1 |
| TNC concn. (% of dry wt) | - - - | 7.8 ± 0.4 | - - - | 9.3 ± 0.4 |
| Crude protein concn (% of dry wt) | 5.8 | 5.6 ± 0.1 | 4.4 | 4.2 ± 0.4 |

[1]Samples collected from the field August 2, 1978. Values are $\bar{x} \pm$ SE (n = 2) for dry weight and $\bar{x}$ (n = 1) for protein.
[2]Samples collected from the field October 1, 1981. Values are $\bar{x} \pm$ SE (n = 6) for dry weight and $\bar{x} \pm$ SE (n = 3) for TNC and protein.

ble sugar component of acid extractable SC was not detected with the chromatography. A SC polymer consisting of xylose only is xylan (Bailey 1973); thus pinegrass appears to contain xylan. In the growth chamber experiment, the concentration of xylan decreased slightly when pinegrass was clipped (Table 1). However, the field grown plants showed a constant value, about 10% of dry weight, regardless of harvest date.

A water extraction method appears to be better than an acid extraction method for extracting the TNC from pinegrass. Although starch is not extracted with water, it contributes only a small amount to pinegrass TNC, while a much larger amount of xylan will be extracted with an acid, which will cause a greater error in determination of TNC (Table 1).

Rhizome tissue contributed most (76 to 82%) of the dry matter weight to the rhizome plus root fraction (Table 2). Fibrous roots contained a higher concentration of TNC than did rhizomes. This contrasts with bromegrass; Watkins (1940) reported that rhizomes contained more TNC than did roots when plants were not shaded, and that rhizomes and roots contained about the same level of TNC when plants were shaded. Pinegrass rhizomes contained a higher concentration of crude protein than did fibrous roots (Table 2). This observation is consistent with the results reported for shaded bromegrass (Watkins 1940).

Evidence suggests that stem bases, including rhizomes, serve as the main storage organ of perennial grasses and that TNC of roots may not be mobilized for shoot regrowth (White 1973). However, since fibrous roots represented only a small fraction of our rhizome plus root sample, it was not judged necessary to remove the roots.

Rhizome plus root TNC of pinegrass decreased during May, reached a minimum level during late May to early June, then increased during June, and then reached a summer maximum during the latter part of June. At this study site, pinegrass dry matter production is known to cease during the first half of July (Stout et al. 1980). Pinegrass flowers infrequently and thus TNC levels are not easily related to phenological development. At this site leaf number/tiller and leaf area reach a maximum by mid-June. From June the TNC level remained constant until late August. It then increased from late August until November; fall

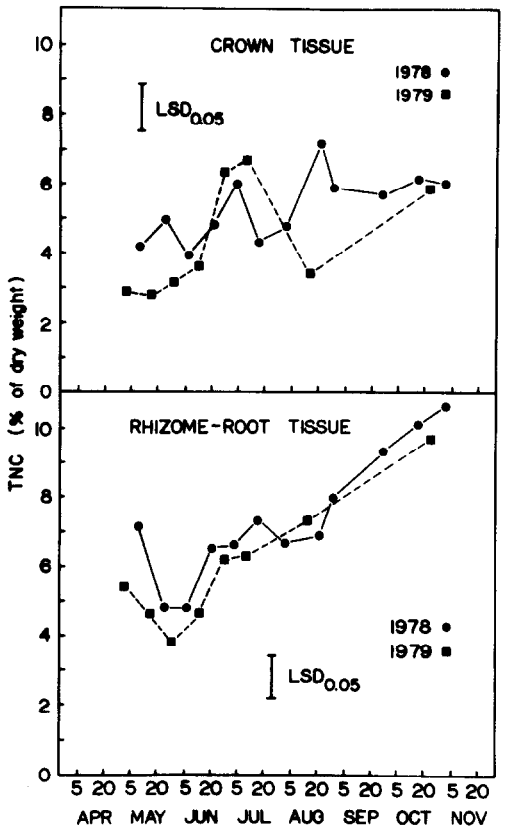


**Fig. 2.** *Seasonal trend of TNC concentration in pinegrass crowns and rhizome plus roots. Each value is the mean of 6 replicates.*

regrowth typically occurs during this period (Stout et al. 1980). The lower level of TNC on May 10, 1979, compared to that on November 1, 1978, indicates that pinegrass carbohydrate reserves are depleted during winter and during spring regrowth.

The TNC seasonal pattern of crowns was quite erratic, although it showed some similarities to the TNC pattern of rhizome plus roots (Fig. 2). Both years, the lowest TNC levels occurred in May and the highest levels tended to occur in the fall. Menke and Trlica (1981) reported similar TNC patterns for roots and crowns of all species studied, including *Poa* and *Agropyron* spp. However, TNC in rhizome plus roots of pinegrass were higher than in the crowns (Fig. 2). It is interesting that during 1978 the TNC level tended to increase during July and August, while in 1979 the TNC level decreased during July and August. July rainfall and temperatures were very similar during July of 1978 and July of 1979, but August of 1979 was drier and hotter than August of 1978.

Crown tissue crude protein was high early in the spring, reached

**Table 3. Distribution of dry weight, TNC, and crude protein among pinegrass tissues.**

| Tissue category[1] | Dry wt. | | TNC | | | Crude protein | | |
|---|---|---|---|---|---|---|---|---|
| | mg/ tiller[2] | Dist'n. (% of total) | % of dry wt.[2] | mg/ tiller | Dist'n (% of total) | % of dry wt.[2] | mg/ tiller | Dist'n (% of total) |
| Top Tissues | | | | | | | | |
| above 15 cm | 25 ± 4 | 18.5 | 7.7 ± 0.4 | 1.93 | 22.3 | 5.4 ± 0.3 | 1.35 | 22.2 |
| 10 to 15 cm | 10 ± 1 | 7.4 | 6.7 ± 0.5 | 0.67 | 7.8 | 3.4 ± 0.3 | 0.34 | 5.6 |
| 5 to 10 cm | 10 ± 1 | 7.4 | 7.5 ± 0.8 | 0.75 | 8.7 | 2.7 ± 0.2 | 0.27 | 4.4 |
| 1.5 to 5 cm | 8 ± 1 | 5.9 | 8.0 ± 0.4 | 0.64 | 7.4 | 3.7 ± 0.1 | 0.30 | 4.9 |
| Crown | 10 ± 1 | 7.4 | 7.5 ± 0.4 | 0.74 | 8.6 | 3.4 ± 0.2 | 0.34 | 5.6 |
| Rhizome plus roots | 71 ± 20 | 53.4 | 5.5 ± 0.5 | 3.91 | 45.2 | 4.9 ± 0.6 | 3.48 | 57.3 |
| Total | 134 | 100.0 | | 8.64 | 100.0 | | 6.08 | 100.0 |

[1]Sods of 650 ± 150 cm² having 1362 ± 239 tillers per m² were dug on July 16, 1979. The top tissues were divided into the indicated sections, measuring from the crown. The crown tissues included the lowest 1.5 cm section of tillers.
[2]The values are $x \pm$ SE for n = 4 plots.

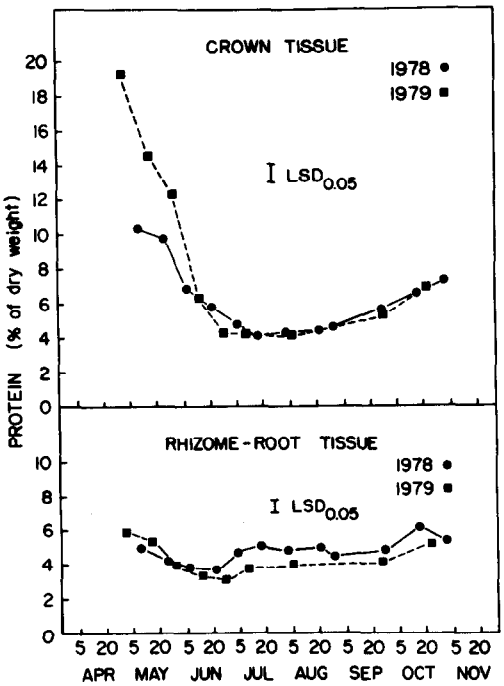


**Fig. 3.** *Seasonal trend of crude protein concentration in pinegrass crowns and rhizome plus roots. Each value is the mean of 6 replicates.*

a minimum in late June, and then increased moderately in the fall (Table 3). Therefore crown crude protein shows the pattern typical for leaves (McLean et al. 1969). The range of crude protein change for rhizome plus roots was small; however, the crude protein tended to correspond to the TNC pattern for rhizome plus root tissue (Table 3).

The distribution of dry weight, TNC and crude protein among pinegrass tissues is shown in Table 3. The dry weight of rhizome plus roots was greater than that of all the other tissues combined, in spite of the fact that during sample collection some rootlets could not be separated from the duff, a problem also reported by George and McKell (1978). Therefore the actual dry weight of rhizome plus roots must be greater than the values shown in the table. Tissue sections containing leaf material had a higher concentration of TNC than did the rhizome plus root tissue. However, the largest amount of TNC, 45.2% of the total TNC, was recovered from the rhizome plus root tissue. The concentration of crude protein in all tissues was relatively low because the sod sample was taken in July (Table 3). A slightly higher concentration of crude protein was found in the uppermost part of the tillers and in the rhizome plus root tissue than in other parts of the tiller. Like TNC, the largest amount of protein, 57.3% of the total crude protein, was found in the rhizome plus root tissues.

In conclusion, the TNC of pinegrass consisted of sugars, fructosan, and starch. A long-chain fructosan was the predominant polymer. The acid soluble SC of pinegrass was xylan and its concentration was constant during the growing season while sugar and fructosan concentrations varied. The summer maximum level of rhizome plus root TNC occurred at or before the critical grazing period in July (Stout et al. 1980); however, crown TNC reached a peak in July. The crude protein concentration of crown tissues was at a minimum level in July, while the rhizome plus root crude protein concentration increased to a summer maximum during July.

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