Carbohydrate Reserves in Alfalfa Roots During Fall, Winter and Spring

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Determination of total nonstructural carbohydrate (TNC) in roots of alfalfa (*Medicago sativa* **L.) was conducted for samples taken throughout the period between the last cut in autumn and first cut the following spring. Two alfalfa cultivars at different locations under different management practices were used. Root dry matter (DM) was determined as percentage of saturated weight for the samples used in the TNC determination. In general, the TNC root reserves continued to increase until approximately mid-December, when a gradual decline followed. The decline in TNC continued until the end of March when the canopy was re-established, allowing the additional photosynthate to be stored in the roots as indicated by the increased TNC. The trends of percentage DM showed a pattern similar to that of TNC. Since a high correlation between level of TNC and that of DM was obtained at both locations, DM was used to estimate percent TNC. In most cases there were no significant differences between the estimated TNC based on DM and that obtained from carbohydrate analysis.**

INTRODUCTION

The concentration of carbohydrate reserves in alfalfa roots is of prime importance to alfalfa management. Carbohydrate root reserves are an important source of energy for supporting the initiation of regrowth and other metabolic processes. Forage yield has been found to be closely associated with total carbohydrate root reserves (1 — 3). In general, the concentration of total nonstructural carbohydrates (TNC) decreases initially after forage harvest and then increases as photosynthate is translocated to the root (4 — 7).

Bula and Smith (4) found that 48% of available carbohydrates in alfalfa roots present in late autumn were depleted during winter in Wisconsin. In contrast Sholar et al. (8) in Oklahoma, Reynolds (9) in Tennessee, and Mays and Evans (10) in Alabama independently speculated that mild winters permitting the presence of green leaves on alfalfa plants might allow some photosynthetic activity. This could result in adding carbohydrates to the total energy root reserve.

The objective of this study was to determine the changes in TNC in alfalfa roots for the period between the last cut in autumn and the first cut in the following spring. An additional objective was to examine use of the gravimetric method for determining root dry matter (DM) as an alternative procedure for estimating TNC in alfalfa roots.

MATERIALS AND METHODS

The carbohydrate trends in alfalfa roots during the autumn, winter, and spring of 1984 — 85 were examined in two different alfalfa cultivars with different stand ages grown at two locations.

At location one at Stillwater, Oklahoma, a 1.5-year-old stand of 'Riley' alfalfa was grown under irrigated conditions. The plots are located on a nearly flat area and were 30 m long x 20 m wide. The soil is fine-silty, mixed thermic, Cumulic Haplustolls (Port silt loam). Soil analysis indicated the following: pH 6.7, 2.2 kg N/ha, 172 kg P/ha and 325 kg K/ha. The alfalfa in this field was harvested five times; the last harvest was in early October of 1984.

At location two at Perkins, Oklahoma, a 2.5-year-old stand of 'Cody' alfalfa, was grown under dryland conditions. The plots are located on a nearly flat area with a very gentle slope to the east (same dimensions as at location one above). The soil is fine-loamy, mixed thermic, Udic Aggiustolls (Teller loam). Soil analysis showed the following: pH 6.0, 3.4 kg P/ha, and 196.1 kg K/ha. Alfalfa has been routinely harvested

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from this field for hay production. Owing to lack of moisture, this field was harvested only three times; the last harvest for the 1984 growing season was taken in late August.

Alfalfa root samples from both locations were collected at approximately two-week intervals between mid-October 1984 and mid-April 1985. Five alfalfa root samples were dug at random from each alfalfa field, one sample from each plot, on each sampling date. Each sample consisted of twenty roots, cut off at the crown and sized to a length of 10 cm. Root segments were soaked in ice water for approximately 2 hr, then washed in tap water, dried with paper towels, and weighed as specified by the gravimetric method (11). Root samples were oven-dried for 2 hr at 100 °C in an effort to stop enzyme action, then drying was continued at 70 °C for approximately 48 hr. Dry weight (DM) was determined and then expressed as a percentage of saturated weight (11).

The TNC analysis was conducted as follows: the root samples were ground in a Wiley mill to pass through a 1-mm screen. A representative portion of 0.2 g from each sample was placed in a 250-mL beaker with 50-mL of 0.2 HCl and the mixture allowed to boil slowly for 1 hr. The root solution was then filtered into a 100-mL volumetric flask. The beaker and filtrate were washed with 50 mL deionized water, and the solution was then brought to 100-mL volume. Then 0.1 mL of this sample was placed into a 10-mL test tube with 0.9 mL of deionized water. Anthrone reagent, as described by Yemm and Willis (12), was used to determine the TNC content. The anthrone reagent (5.0 mL) was added to the root extract and the mixture agitated. The samples were placed in a hot water bath for 15 min, then for 20 min in a cold water bath. Absorbance of the samples was determined with a spectrophotometer (B and L Spectronic 20) at 620 nm.

Significance of treatment effect on TNC and DM was determined by conducting an analysis of variance (ANOVA) for a completely random design; *F* and least significant difference (LSD) tests for significance used the 0.05-probability level. Comparison between the TNC values and DM values obtained by the gravimetric method was obtained by conducting an ANOVA test for a split-plot design, with the main plot as a completely random design. Treatments means were subjected to LSD test.

RESULTS AND DISCUSSION

At the Stillwater location, TNC in alfalfa roots continued to increase from two weeks after the last harvest until approximately mid-December. At this time, a decline in TNC root reserves began and continued until the end of March. By the end of March, a canopy was re-established, allowing additional photosynthate to be stored in the roots (Table 1). The reduction in TNC during January and February most likely resulted from respiration within the root and from supporting any growth that occurred during that period. The sharp decline in TNC during March was probably due to carbohydrates being used for the initiation of regrowth for the new season.

In general, the same result was obtained from the analysis of the root samples taken from the Perkins location, except that reductions began in mid-November (Table 2). This was most likely due to the different management practices used at these locations. The last harvest at the Perkins location (unirrigated) was in late August compared to early October in Stillwater (irrigated). This allowed the initiation of new growth of the unharvested plants during late October. The shading of the regrowth at the base of the mature plant could result in reduction in photosynthetic activity, causing such regrowth to be more dependent on carbohydrate root reserves.

The trends for percent TNC and DM in alfalfa roots were generally similar at both locations (Tables 1 and 2). In addition, the correlations between percent TNC and DM were very high; i.e. $r^2 = 0.94$ for the Stillwater location, and $r^2 = 0.92$ for the Perkins location. Similar correlations between TNC and DM were obtained by Wolf (11). This correlation was further amplified by Rapoport and Travis (13). They found that root cambium activity was also correlated with TNC level.

TABLE 1. Percent total nonstructural carbohydrate (TNC) and percent dry matter (DM) in alfalfa roots during the period between October and April at Stillwater.

Sampling date	DM	TNC	
		Anthrone method	Gravimetric method
	%		
Oct. 15	33.32	38.05**	40.93**
Oct. 29	33.95	40.38*	42.09*
Nov. 14	34.38	44.38*	42.56*
Nov. 30	35.74	46.34*	44.42*
Dec. 14	35.56	45.48	44.21
Jan. 8	34.84	44.67	43.22
Feb. 16	31.74	38.96	38.36
Mar. 2	29.58	35.31	34.47
Mar. 16	24.94	26.05	24.79
Mar. 31	25.15	27.02	26.04
Apr. 12	29.80	32.97	34.11
Apr. 28	32.44	37.47	38.39
LSD (0.05)+	0.93	2.37	1.57
LSD (0.01)+	1.24	3.16	2.10

*,** Within sampling date, difference between % TNC obtained from anthrone method and that obtained from gravimetric method was statistically significant at the 0.05 and 0.01 levels of probability, respectively, based on LSD test.

\+ LSD for difference between means within a column.

TABLE 2. Percent total nonstructural carbohydrate (TNC) and percent dry matter (DM) in alfalfa roots during the period between October and April at Perkins.

Sampling date	DM	TNC	
		Anthrone method	Gravimetric method
	%		
Oct. 19	40.83	41.68*	43.44*
Nov. 2	42.51	44.89	44.38
Nov. 16	41.00	43.35	43.46
Dec. 1	38.15	41.47	40.92
Dec. 14	37.38	40.06	40.21
Jan. 8	35.10	38.32	37.94
Feb. 16	31.96	36.32*	34.32*
Mar. 2	28.86	28.76	29.95
Mar. 16	25.94	22.19	23.43
Mar. 31	26.00	25.07	25.19
Apr. 12	28.26	31.38*	28.82*
Apr. 28	32.26	36.08	25.90
LSD (0.05)+	1.03	3.14	1.41
LSD (0.01)+	1.38	4.19	1.89

* Within sampling date, difference between % TNC obtained from anthrone method and that obtained from gravimetric method was statistically significant at the 0.05 level of probability based on LSD test.

\+ LSD for difference between means within a column.

The results showed that the carbohydrate levels in alfalfa roots continued to decline through winter. We believe that the small regrowth observed on alfalfa plants during winter contributes to further reduction in TNC root reserves.

Based on the relationship between percent TNC and percent DM, a regression equation was obtained with intercept -17.10 and slope 0.92 for the Stillwater location, and with intercept -13.00 and slope 0.75 for the Perkins location. As Wolf (11) proposed, the percent DM was used to calculate weight of TNC (Formula 1) and then percent TNC was calculated (Formula 2).

$$\text{g TNC} = \text{Intercept} + \text{Slope} \times \%\ \text{DM} \quad [1]$$

$$\%\ \text{TNC} = (\text{g TNC}/\%\ \text{DM}) \times 100 \quad [2]$$

On most dates, there was no significant difference between percent TNC obtained from the anthrone method and that obtained from the gravimetric method (Tables 1 and 2) and in the few cases where differences were detected, there was no systematic trend.

Therefore, the data indicate that the gravimetric method can be used as a simpler alternative method for estimating TNC.

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