

Nonstructural Carbohydrates and Root Development in Blue Grama Seedlings

A.M. WILSON

Abstract

The objective of this study was to determine relationships between total nonstructural carbohydrates (TNC) and adventitious root development in blue grama [*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.] seedlings. Levels of TNC were altered by use of shade and recovery treatments in full light. Seedling growth characteristics and TNC, N, and P concentrations were investigated in 5-week-old seedlings during a 3-day root growth test. Leaf mass, crown mass, total N, and total P were not significantly associated with production of adventitious roots. A decrease in amount of TNC in shoots and an increase in root mass indicated that 13% of the root mass was derived from TNC that had accumulated before the test. The percentages of leaf TNC and crown TNC utilized for growth of adventitious roots were 14 and 30, respectively. This study indicates that blue grama seedlings should be managed to maintain adequate levels of TNC. Utilization of TNC for rapid root growth could be an advantage where the soil surface dries rapidly after precipitation.

Blue grama [*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.], the dominant perennial forage species of the Central Plains, generally does not reestablish from seed on semiarid, disturbed lands (Bement et al. 1965, Hyder et al. 1971). Seedling death is attributed to the lack of adventitious root development and subsequent extension into moist soil (Wilson and Briske 1979). Requirements for root development include warm soil temperatures, a moist soil profile, and 3 to 4 days with a continuously moist soil surface. Seedlings that have not developed adventitious roots fail to survive during periods of drought or low temperatures.

The utilization of total nonstructural carbohydrates (TNC) in roots and crowns for shoot regrowth has been extensively investigated (Pearce et al. 1969, Smith and Marten 1970, White 1973, Trlica and Singh 1979), but little information is available on the utilization of TNC in leaves and crowns for root production (Davidson 1969, Evans 1972). The objective of this study was to determine relationships between TNC concentrations and adventitious root development in blue grama seedlings. Understanding its role in root production should improve the establishment of blue grama and aid in managing blue grama stands.

Materials and Methods

General Conditions

An Ascalon sandy loam (fine-loamy, mixed, mesic Aridic Argiustoll) was sterilized with dry heat (100° C for 2 days) and placed in plastic pots (15 cm diameter by 15 cm deep). Twenty-five seeds of 'Lovington' blue grama were planted at a depth of 2 mm. Each pot

was surface irrigated with 250 ml of water, allowed to drain 2 hours, and seeds were covered with 2 cm of dry soil. Seedlings emerged through the dry soil layer, and at 3 weeks were thinned to 10 vigorous, well-spaced seedlings per pot.

Studies were conducted under greenhouse conditions during the months of June through September. Air temperatures varied from 25 to 35° C. Average midday photosynthetically active radiation (PhAR) was 1400 $\mu\text{E m}^{-2} \text{sec}^{-1}$. Daylength was 14 hours supplemented with sodium vapor lamps at the end of the day.

Each pot was weighed on alternate days to estimate the amount

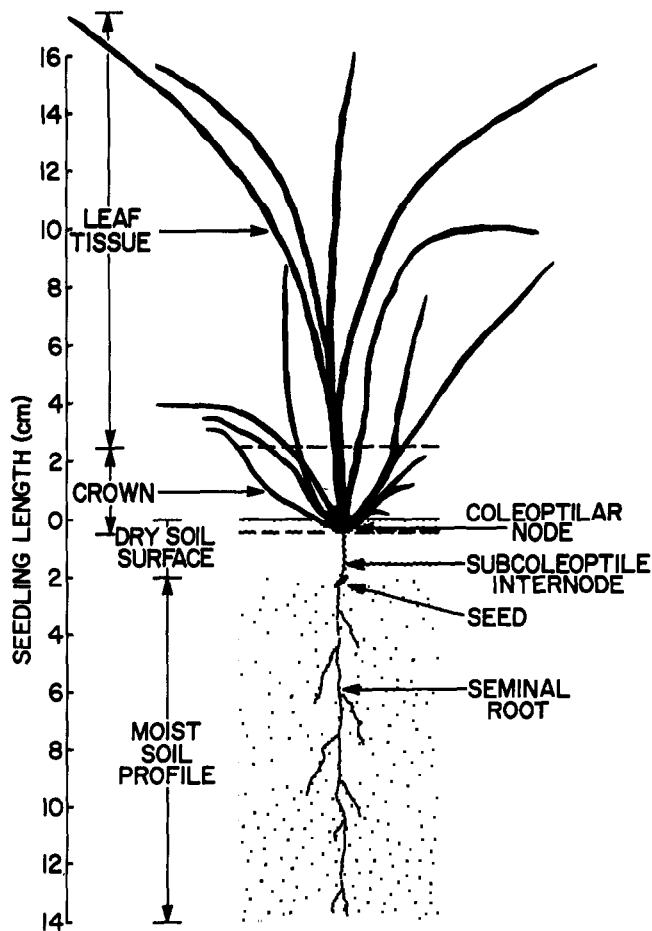


Fig. 1. Five-week-old blue grama seedling before the 3-day root growth test. Seedlings did not develop adventitious roots during the 5-week period because of a dry soil surface. Surface irrigation at 5 weeks resulted in subsequent growth of adventitious roots from the coleoptilar node and from higher nodes of the primary shoot and of tillers.

The author, now deceased, was plant physiologist, USDA-ARS, Crops Research Laboratory, Colorado State Univ., Fort Collins 80523

This study was a cooperative investigation of USDA-ARS and Colorado State Univ. Agr. Exp. Sta. Scientific paper No. 2811.

The author thanks the following who contributed to the study: K. Keatley, determinations of total N and total P; B.L. Oskroba, determinations of total nonstructural carbohydrates; and C.J. Carren, measurements of plant growth characteristics.

Manuscript received December 3, 1982.

of water needed to moisten the soil to about field capacity, but still maintain a dry surface layer to a depth of 2 cm. That amount of water was placed in a plastic dish and was absorbed by the soil through holes in the bottom of the pot. The subirrigation procedure kept the seminal root in moist soil, maintained a dry soil surface, promoted shoot growth, but prevented the initiation of adventitious roots (Fig. 1).

Experiment I: TNC Utilization for Root Growth

At 5 weeks after planting, 5 seedlings were randomly removed from each of 36 pots for determination of dry crown mass, dry leaf mass, and TNC percentage in crowns and leaves. The crown included the lower 3-cm portion of stem base (Fig. 1). The leaf sample included leaf blades, leaf sheaths, and occasionally a reproductive culm which extended above 3 cm. The subirrigation procedure (dry surface), was continued on 18 pots and the remaining 18 pots were surface irrigated (moist surface). Treatments of dry vs. moist surface continued four 3 days. The remaining five seedlings in each pot were then harvested for determination of dry crown mass, dry leaf mass, TNC percentage in crowns and leaves, and dry adventitious root mass produced during the 3-day test. A composite sample of 5 seedlings from each of 18 pots (90 seedlings total) was used for measurement of growth characteristics and for TNC analysis. Experiment I included four replications over time.

Experiment II. Relation Between TNC and Root Production

Five sets of 18 pots (each containing 10 seedlings) received 1 of 5 light treatments beginning on the 27th, 28th, 29th, 30th, or 31st day after planting. Pots in each treatment were maintained under low light intensity ($270 \mu\text{E m}^{-2} \text{sec}^{-1}$ midday PhAR) for 4 days and then received full sunlight for 4, 3, 2, 1, or 0 days respectively. A composite sample of 5 seedlings from each of 18 pots (90 seedlings total) was harvested from each treatment on day 35. All pots were then surface irrigated to promote growth of adventitious roots during a 3-day test. On day 38, the remaining 90 seedlings (5 per pot) were harvested and composited for measurement of seedling growth and determination of TNC, total N, and total P in crowns. Experiment 2 included 6 replications conducted over time.

Analytical Conditions

Plant material was dried at 60°C and ground to pass through a 40-mesh screen. TNC was extracted by the method of Smith et al. (1964) and analyzed on a glucose equivalent basis with use of the iodometric titration of Heinze and Murneek (1940). Reagents were modified as suggested by the Association of Official Agricultural Chemists (1965). Total N was determined by the Kjeldahl method (Isaac and Johnson 1976) and total P by the colorimetric method (Murphy and Riley 1962).

Analysis of variance was used for testing differences among treatment means. Regression analysis was used for determining relationships between days of recovery and adventitious root growth and between TNC and root growth. Differences among treatments are given at $P \leq 0.05$.

Results and Discussion

Experiment I: TNC Utilization for Root Growth

Changes in mass and TNC percentage were investigated in seed-

lings that developed no adventitious roots (dry soil surface) and in seedlings that developed many adventitious roots (moist soil surface) during a 3-day test. The increase ($P < 0.05$) in shoot mass (crown + leaf) was 92 mg per seedling in the dry treatment (367 vs. 459) and 29 mg per seedling in the moist treatment (373 vs. 402). Adventitious root mass in the dry and moist treatments was 0 mg and 57 mg, respectively. Seedling growth rates in the 2 treatments were similar, but a portion of the growth in plants from the moist treatment was partitioned into developing roots.

In the dry treatment, TNC increased from 8.9 to 9.8% in leaves and from 11.9 to 13.1% in crowns ($P < 0.05$). In the moist treatment, TNC decreased from 8.9 to 7.0% in leaves and from 11.2 to 7.3% in crowns ($P < 0.05$).

Net photosynthesis during the 3-day test increased leaf and crown mass and increased TNC by 14.9 mg per seedling when grown in soil with a dry surface. In contrast, a moist soil surface resulted in a decrease in TNC of 7.6 mg in the crown and leaf of each seedling. The most likely explanation for the decrease is that TNC was translocated from leaf and crown tissue and utilized in growth of adventitious roots. An estimate of the root mass derived from TNC accumulated before the 3-day test can be calculated from the difference in TNC before and after the 3-day test $[(35.5 - 27.9) (100) / 57.3 = 13.3\%]$.

The leaf TNC and crown TNC utilized for adventitious root growth was 14 and 30%, respectively. The calculations are based on the decrease in amount of TNC per seedling. The estimate of the relative amount of current net assimilate utilized in adventitious root growth during the 3-day test was 58%, which was obtained from the following relationship:

$$\frac{[(\text{Increase in root mass}) - (\text{Root mass derived from TNC})] \times 100}{(\text{Increase in total seedling mass during 3-day test})}$$

It was assumed that current photosynthesis provided materials for leaf and crown growth and for seedling respiration because there was no decrease in TNC in the control treatment (dry soil surface). The estimates were also based on the assumption that biochemical transformations of TNC with pools of other nonstructural compounds in leaf and crown tissue were in a steady-state condition.

Because the seminal root usually represents 5% or less of seedling mass (Nason 1981), it was not considered in the estimates of TNC utilization. Mature plants, however, have an extensive adventitious root system, and translocation from mature roots into new, actively growing roots might occur.

Experiment II: Relation Between TNC and Root Production

Leaf or crown mass was not affected by the 0 to 4 day recovery under full light. Leaf and crown mass, however, were significantly greater (193 vs. 228 mg leaf per seedling and 120 vs. 137 mg crown per seedling) when comparing before and 3-day test data ($P < 0.05$). The length and mass of adventitious roots after the 3-day test were linearly related to length of recovery time in full light. Root length (cm) = $7.05 + 0.27x$ ($r = 0.43$) and root mass (mg/seedling) = $32.52 + 4.27x$ ($r = 0.84$), where x was the days exposed to full light after being held 4 days at reduced light.

Number of days of recovery in full light did not affect the amount of N in crowns. The amount, however, increased ($P < 0.05$)

Table 1. Decrease in total nonstructural carbohydrates (TNC) (mg/seedling), increase in adventitious root weight (mg/seedling), and estimate of percent of root weight derived from TNC in 5-week-old blue grama seedlings during a 3-day adventitious root growth test.

Treatment	TNC/seedling						Difference in TNC per seedling	Adventitious root weight	Estimate of root weight derived from TNC
	Before root growth test			After root growth test					
	Leaf	Crown	Total	Leaf	Crown	Total			
Dry soil surface	18.8 ¹	17.2	36.0	24.7	26.2	50.8	+14.9	0	—
Moist soil surface	18.8	16.7	35.5	16.2	11.7	27.9	-7.6	57.3	13.3 ± 4.1

¹Values are rounded to 3 significant figures.

during adventitious root development in the 3-day test (3.91 vs. 4.17 mg/crown). No conclusions could be made concerning utilization of N compounds in crowns for development of roots because the amount of N taken up by roots during the 3-day test was not determined. The amount of P in crowns decreased ($P < 0.05$) during the 3-day test (0.53 vs. 0.48 mg/crown). This indicates that P was possibly translocated to developing roots. However, the very small decrease in total P probably did not limit root production.

Crown tissue contained 7.5, 12.4, 14.0, 14.1, and 14.6 mg TNC/crown for the 0, 1, 2, 3, and 4 day recovery periods in full light following the 4 days of shade, respectively. This indicates the rapid addition of photosynthate as TNC in crowns when exposed to favorable light conditions ($R = 0.93$). TNC in crowns (%) = $6.67 + 3.45x - 0.529x^2$, where x was the days of recovery in full light. Exposing the seedlings to the added 3-day period of moist surface soil changed the TNC to 11.1, 11.7, 12.8, 13.0, and 12.3 mg/crown, respectively, indicating that crown TNC was used to support adventitious root growth except in the 0 day treatment. In the 4-day recovery treatment, the decrease in TNC (2.3 mg/crown) and the increase in root weight (48.4 mg) provide an estimate of the percentage of root dry weight derived from TNC [(2.3) (100)/48.4 = 4.8%].

The relatively low estimates of percentage of root weight derived from TNC (13.3 and 4.8% in the two studies) might suggest that TNC was of little importance in root development. Regression analysis, however, indicated that a change of 1 mg in amount of TNC in crowns resulted in an increase of 1.7 mg in dry root mass ($r = 0.74$). Because only small amounts of TNC apparently were translocated and utilized for root growth, there may have been alternative mechanisms by which recovery in full light promoted root growth. One possibility is that TNC influenced leaf area expansion rate which then affected root growth. Methods used in the present study may not have been sensitive enough to detect leaf growth differences among the recovery treatments. A second possible explanation is that light treatments caused biosynthesis of root growth hormones or other substances which then promoted root growth (Scott 1972). A third alternative is that TNC exerted a positive effect on the initiation of adventitious roots. Rapid initiation of new roots or development of a greater number of roots would provide a sink for photosynthate and a greater allocation to root growth. Thus, TNC might affect translocation, carbohydrate allocation, and root growth relationships without being utilized to a great extent as a part of root structure. In a related study (Wilson, unpublished manuscript), seedling leaf area and TNC concentration in crowns were positively associated with numbers of adventitious roots that were initiated.

Utilization of TNC for rapid initiation of roots could be an advantage under conditions where the soil surface dries rapidly after a period of precipitation. But utilization and depletion of crown TNC for root production could be a disadvantage because it would then leave crown tissue susceptible to the injurious effects of drought (Khan 1980). Blue grama apparently has mechanisms for preventing depletion of crown TNC for production of roots. For example, seedlings with a low level of TNC did not use TNC for root production; rather, the TNC in crowns increased during the 3 days of the test (7.5 vs. 11.1 mg/crown).

The conclusion that can be drawn from this study and a related study (Khan 1980) is that TNC functions in several roles relative to establishment and persistence of seedlings. The roles probably include: (1) promotion of rapid initiation of roots, (2) translocation and use for root growth, and (3) protection of crown tissue from the injurious effects of dehydration. TNC levels can be maintained by protecting seedlings from grazing and by controlling competing species which cause excessive shade. Although the mechanisms by which TNC functions in blue grama and other range plants are incompletely understood, a large number of studies indicate that plant productivity and persistence require management practices which maintain adequate levels of TNC (Trlica and Singh 1979).

Literature Cited

- Association of Official Agricultural Chemists. 1965. p. 489-499. Official methods of analysis. 10th edition. Washington, D.C.
- Bement, R.E., R.D. Barmington, A.C. Everson, L.O. Hylton, Jr., and E.E. Remmenga. 1965. Seeding of abandoned croplands in the Central Great Plains. J. Range Manage. 18:53-58.
- Davidson, R.L. 1969. Effect of edaphic factors on the soluble carbohydrate content of roots of *Lolium perenne* and *Trifolium repens*. Ann. Bot. 33:579-589.
- Evans, P.S. 1972. Root growth of *Lolium perenne* L. III. Investigation of the mechanism of defoliation induced suppression of elongation. N.Z.J. Agr. Res. 15:347-355.
- Heinze, P.H., and A.E. Murneek. 1940. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Missouri Agr. Exp. Sta. Res. Bull. 314.
- Hyder, D.N., A.C. Everson, and R.E. Bement. 1971. Seedling morphology and seeding failures with blue grama. J. Range Manage. 24:287-292.
- Isaac, R.A., and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue, using a block digester. J. Ass. Off. Anal. Chem. 59:98-100.
- Khan, S.M. 1980. Nonstructural carbohydrates and tolerance of dehydration in blue grama. Ph.D. Diss., Colorado State University, Fort Collins.
- Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.
- Nason, D.A. 1981. Spikelet weight, seedling water uptake selection, and shoot weight in blue grama (*Bouteloua gracilis*). M.S. Thesis, Colorado State Univ., Fort Collins.
- Pearce, R.B., G. Fissel, and G.E. Carlson. 1969. Carbon uptake and distribution before and after defoliation of alfalfa. Crop Sci. 9:756-759.
- Scott, T.K. 1972. Auxins and roots. Ann. Rev. Plant Physiol. 23:235-258.
- Smith, D., G.M. Paulsen, and C.A. Raguse. 1964. Extraction of total available carbohydrates from grass and legume tissue. Plant Physiol. 39:960-962.
- Smith, L.H., and G.C. Marten. 1970. Foliar regrowth of alfalfa utilizing ^{14}C -labeled carbohydrate stored in roots. Crop Sci. 10:146-150.
- Trlica, M.J., and J.S. Singh. 1979. Translocation of assimilates and creation, distribution and utilization of reserves. R.A. Perry and D.W. Goodall (eds.) p. 537-571. In: Arid-land ecosystems: Structure, functioning and management. Volume I. International Biological Programme 16. Cambridge University Press. Great Britain.
- White, L.M. 1973. Carbohydrate reserves of grasses: A review. J. Range Manage. 26:13-18.
- Wilson, A.M., and D.D. Briske. 1979. Seminal and adventitious root growth of blue grama seedlings on the Central Plains. J. Range Manage. 32:209-213.