Carbohydrate Reserves of Intermediate Wheatgrass after Clipping and Etiolation Treatments[1]

PHIL R. OGDEN AND WALTER E. LOOMIS

Professor of Range Management, Department of Watershed Management, University of Arizona, Tucson, Arizona; and Professor of Plant Physiology, Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa.

Highlight

The total water-soluble carbohydrate (TWSC) fraction of intermediate wheatgrass stem bases and roots is depleted with etiolation and is a good measure of the reserve energy of this species. When the TWSC fraction was depleted to about 1% dry weight, vigor of the grass was too poor to recover from a clipping treatment. Root weight was also reduced with etiolation. Late September to early November was a period of active herbage and root growth for intermediate wheatgrass. Growth during this fall period enabled grasses which had been clipped three times at 6-week intervals during the summer to recover to very nearly the level of TWSC and root weight as the check plants.

A knowledge of the pattern of reserve energy use and storage in the stem bases and roots of plants in response to phenological development, climate, and grazing treatments is essential to develop good systems for proper management of range plants. A measure of the total energy reserve rather than specific carbohydrate fractions is usually the information desired for making management decisions, but the fractions present determine what methods of analyses are appropriate.

The carbohydrates which function as reserves in most higher green plants are sugars, fructosans, and starches (Weinmann, 1947). Which of these fractions predominate in the stem bases and roots, however, vary with species. De Cugnac (1931) reported analyses of a number of grasses and classified these according to the distribution of starch, sucrose and fructosans in their stubble. He divided the grass family into two definite physiological groups: (1) the "Sacchariféres," mostly grasses of tropical origin, which never form levulosides (fructosans) in their tissues but contain sucrose, reducing sugars, and starch; and (2) the "Lévuliféres," mostly plants of temperate origin, which are characterized by the presence of levulosides in some of their organs or at certain stages in their development.

Considerable evidence has accumulated which show that grasses belonging to the temperate origin *Agropyron* genus store fructosans as their primary polysaccharide reserve. Julander (1945) reported that tissues of bluestem wheatgrass (*Agropyron smithii* Rydb.) and bluebunch wheatgrass (*Agropyron spicatum* [Pursh] Scribn. and Smith)

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contained fructosans, and the iodine test for starch was negative. Chilcote (1950) and Cook, Stoddart, and Kingsinger (1958) recognized that fructosans were present and starch was not present in the roots of crested wheatgrass (*Agropyron desertorum* [Fisch.] Schult.).

Ogden (1966) found a large portion of the polysaccharide reserve of intermediate wheatgrass (*Agropyron intermedium* [Host] Beauv.) roots and stem bases was extracted in 80% ethanol extract along with the reducing sugars and sucrose. Smith and Grotelueschen (1966) reported that it is primarily short chain fructosans which are readily soluble in 80% ethanol, and Smith (1968 and 1969) lists intermediate wheatgrass as a species which stores primarily short chain fructosans.

The reducing sugars, sucrose, and fructosans are all water soluble, and the data presented in this paper show that analysis of total water-soluble carbohydrates in stem bases and roots of intermediate wheatgrass provides an index to the energy reserves of this species which are depleted by etiolation and are sensitive to fluctuations associated with clipping and phenological development.

Methods

Four treatments (check, clipped, early etiolated, and late etiolated) were studied. The treatments were applied in a randomized block design with three replications. The study site was a pasture at The Utah Agricultural Experiment Station Livestock and Range Field Station, Cedar City, Utah.

Treatments were begun June 28, 1960 after the pasture had been grazed earlier in the spring. At this time, the grass had regrown to early head stage. Clipped plots were cut to a 1-inch stubble height June 28th, August 9th, and September 22nd. Early etiolated plots were covered June 28th, and the plants clipped to a 1-inch stubble height June 28th and August 9th. The late etiolated plots were covered August 11th and clipped to 1-inch on August 11th and again on September 22nd. Check plots were unclipped and not covered.

The covering for etiolated treatments consisted of frames which were covered with Sisal-kraft paper sprayed with silver paint. Temperatures under the frames during August reached 43 C as a daytime maximum and 12 C as a nighttime minimum.

Summer and early fall precipitation was 3.4 inches, and this was supplemented with five irrigations at approximately 20-day intervals during July, August, and September.

A random sample was collected in the field for each treatment replication at each sampling date. Samples were collected at about 6-week intervals to allow etiolation stress to develop and to enable sampling of check plants at early head, June 28, dough stage, August 9, mature seed and beginning fall regrowth, September 22, and on November 5 with abundant fall regrowth evident. Reserve carbohydrate depletion was also expected for the clipping treatments with this interval of cutting. Samples consisted of a 6-inch cube of intermediate wheatgrass sod lifted with a shovel. After clipping herbage to 1-inch stubble height, the sod was placed in soil screens for washing the soil from the roots and stem bases. After most of the soil was removed, samples were taken into the laboratory where additional soil and dead plant material were washed from the sample. Roots and stem bases were separated, and the samples blotted with paper towels to remove excess moisture. Samples were placed in screen baskets, weighed to obtain a wet weight, and killed by holding them in an oven at 100 C for 1 to 2 hours, depending on the amount of material, and then dried at 80 C. They were then ground to pass a 60-mesh screen.

Roots and stem bases collected from all replications and sample dates were analyzed for root weights, reducing sugars, sucrose, and fructosans and data were subjected to analyses of variance. Total water-soluble carbohydrates in stem bases and roots were determined on composited samples of the three replications for each treatment at each date. The carbohydrate data by fractions are not reported in this paper. Data on carbohydrate fractions and comparisons and accuracy of methods of analyses utilized in this study are reported by Ogden (1966).

Analyses for water-soluble carbohydrates were accomplished by pouring distilled water over the 1 g of ground samples in 40-ml centrifuge tubes and holding in a boiling water bath at 92 to 93 C. After holding samples in the water bath for 10 to 15 minutes, they were centrifuged, and the extract decanted into 250-ml volumetric flasks. This was repeated five times.

Twenty-five-milliliter aliquots of the fraction extracted in water were hydrolyzed by adding 0.25 ml of concentrated HCl and holding in a boiling water bath for 30 minutes. The samples were neutralized with sodium hydroxide, cleared with lead acetate and dipotassium phosphate, and reducing substances determined by oxidation with ferricyanide and ceric sulfate titration (Hassid, 1936 and 1937).

Results and Conclusions

Total water-soluble carbohydrates (TWSC) in both stem bases and roots of intermediate wheatgrass increased from early head to dough stage of plant growth (Table 1). Root mortality exceeded growth during this period. The TWSC levels were similar at dough stage and at mature seed stage. The lower level of TWSC on November 5 compared to September 22 corresponded to a period of very active root growth between these dates. The depletion of TWSC associated with this root growth should not be interpreted as detrimental. When the percentage TWSC in roots was multiplied by root weight to get a measure of TWSC in roots per unit of soil, there were more

Table 1. Total water-soluble carbohydrates (TWSC) in stem bases and roots, root weights, and growth stage of intermediate wheatgrass subjected to clipping and etiolation treatments.

Date	Treatment	TWSC in stem bases (% dry weight)	TWSC in roots (% dry weight)	Root weight per 6-inch cube of soil (g, dry weight)	TWSC in roots in 6-inch cube of soil (g glucose equivalents)	Growth stage
June 28	Check	4.8	8.4	11.2 abc[1]	.94	Early head after grazed in early spring
Aug. 9	Check	8.7	13.9	8.4 bcd	1.17	Most seeds not developed but some in dough stage
	Clipped	6.7	15.0	7.8 cd	1.17	Regrowth leaves 9 inches tall
	Early etiolated	1.5	2.2	6.2 d	.14	Regrowth leaves yellow-green, 12 inches tall
Sept. 22	Check	8.8	13.3	11.2 abc	1.49	Mature seeds, abundant new rhizome and root growth
	Clipped	6.1	11.0	8.6 bcd	.95	Regrowth leaves at 2- to 3-leaf stage, 6 inches tall, new root growth evident
	Early etiolated	1.1	1.0	4.7 d	.05	Regrowth leaves few, plant nearly dead
	Late etiolated	1.9	5.6	7.5 cd	.42	Regrowth leaves yellow, 18 inches tall
Nov. 5	Check	7.0	11.4	13.7 a	1.56	Abundant fall leaf regrowth and many new roots
	Clipped	5.8	11.6	11.9 ab	1.38	Abundant leaf regrowth and new root growth
	Late etiolated	1.1	1.4	7.6 cd	.11	Only a few yellow leaves, no root growth

[1] Root weights followed by the same letter are not significantly different at the 95% level.

TWSC on November 5 than on September 22 even if the percentage was less on November 5.

Percentage TWSC in stem bases and roots and weight of roots were depleted with both early and late etiolation treatments (Table 1). When the TWSC of stem bases and roots were about 1% (September 22 for early etiolated and November 5 for late etiolated plants), the vigor of plants was too poor to recover after clipping. Thus, low reserves and reduced root weight were both associated with mortality of the etiolated plants. The TWSC fraction of intermediate wheatgrass was utilized as a reserve and is a good measure of available energy for plant regrowth. The TWSC fraction does not include starch, but Smith (1968) reported that only 0.7% of the total nonstructural carbohydrates of intermediate wheatgrass stem bases was starch. A TWSC analysis should adequately indicate the reserve energy status for intermediate wheatgrass.

By August 9, the plants clipped on June 28 had grown to 9 inches tall with abundant, leafy growth. Stem base TWSC were slightly lower in clipped plants than for check plants, but TWSC in roots were slightly higher in roots of the clipped plants compared to check plants. The TWSC in roots per 6-inch cube of soil (Table 1) were the same for clipped and check plants. Under irrigated conditions, the removal of old top growth at early head stage was only slightly detrimental to the TWSC levels of stem bases and root TWSC levels were replenished by six weeks of growth.

A second clipping on August 9 resulted in reduced TWSC in stem bases and roots of clipped plants compared to the check plants when sampled September 22. Root weights were also significantly lower for clipped plants compared to the check plants on September 22, so that the TWSC in roots per 6-inch cube of soil averaged .95 g compared to 1.49 g for check plants. Plants had only grown 6 inches and were at the 2- to 3-leaf stage in the six weeks from clipping to sampling. A period longer than six weeks would be necessary to provide recovery from this second clipping.

Clipped plants replenished root reserves to very nearly the same percentage level as for check plants by November 5 after the third clipping on September 22. Root weight did not recover to the level of check plants but was not significantly different from the check so that the TWSC in roots in the 6-inch cube of soil samples was only slightly lower than the check plants. The late September to early November period was a very efficient period of root growth and TWSC production for intermediate wheatgrass.

These clipping data indicate that, when irrigated, intermediate wheatgrass could be grazed twice, or perhaps even three times, in a summer at about 6-week intervals if a period of fall growth to replenish TWSC and provide for root growth is allowed.

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