

VARIATION IN CARBOHYDRATE CONTENT OF ROOTS
OF ANDROPOGON SCOPARIUS AS AFFECTED
BY TIME OF SHOOT REMOVAL

By

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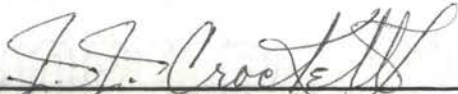
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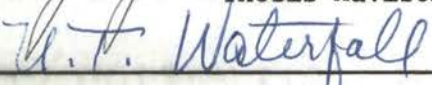
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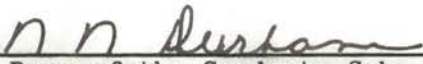
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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. LITERATURE REVIEW	3
III. DESCRIPTION OF STUDY AREA	7
Topography; Soil	7
Climate	7
IV. MATERIALS AND METHODS	11
Plot Design; Clipping Treatments	11
Collection and Preparation of Samples	12
Extraction and Analysis of Ethanol-soluble Sugars	12
Extraction and Analysis of Starch Content	14
Conversion of Klett Readings to Root Carbohydrate Content	16
Statistical Analysis	17
V. RESULTS AND DISCUSSION	18
VI. SUMMARY	26
LITERATURE CITED	28
APPENDIX A	30
APPENDIX B	31
APPENDIX C	35

LIST OF TABLES

Table	Page
I. Clipping Dates	11
II. Duncan's New Multiple Range Test Results	22

LIST OF FIGURES

Figure	Page
1. Monthly Precipitation at Site and Average Monthly Precipitation	9
2. Average Monthly Temperature and "Normal" Average Monthly Temperature	10
3. Reducing Sugars and Sucrose Contents of Samples	19
4. Mean Starch Content of Samples	20
5. Mean Total Carbohydrate Content of Samples	21

CHAPTER I

INTRODUCTION

Throughout the winter, the perennial plants remain in a state of depressed metabolic activity, using but little of their stored reserves for the necessary physiological processes. With the advent of spring, these plants start putting forth new foliage, the initial growth of which is made by expenditure of 75% or more of the carbohydrates stored in the basal organs during the past year (McCarty and Price, 1942). An impairment in the storage of reserves in the preceding growing season will likely be reflected by decreased vigor of the plant in spring growth. It is, therefore, of utmost importance that the plant store sufficient reserves during a growing season to carry it through the period in the succeeding spring when photosynthetic activity is inadequate to meet the demands for food and building materials.

The realization that "the amount of organic food reserves should be indicative of the vigor of the plant" (Aldous, 1930), has led to investigations regarding the relation of the storage process to the annual cycle of plant growth and to such factors as clipping and grazing. While it is true that herbage removal is not the sole factor influencing reserve accumulation, and that soil and climate have variable and interacting influences on the process, it is nevertheless apparent that "harvesting of forage must be controlled

so as to permit plants to carry out their proper growth functions" (McCarty and Price, 1942). For scientific range management, a thorough acquaintance with the effects of herbage removal on the physiological responses of the plants concerned is necessary (Cook, Stoddart, and Kinsinger, 1958). Weinman (1948) puts great emphasis on the root reserve aspect of growth by stating that "the aim of pasture management must be to maintain an adequate level of reserves in the desirable species of a sward."

If the effects of herbage removal on reserve storage are to be really understood, much data is yet to be obtained and interpreted. Many species must be investigated under a variety of conditions. This particular study has involved a determination of the effect of one clipping applied at various dates throughout the growing season on the reserve carbohydrates in roots of Andropogon scoparius Michx.

CHAPTER II

LITERATURE REVIEW

Perennial grasses have been found to exhibit an annual growth cycle: herbage growth, growth of roots, flowering, and secondary herbage growth (McCarty and Price, 1942). Seasonal precipitation and temperature fluctuations cause definite growth rate fluctuations; also, the advent of flowering and seed formation coincide with a decrease in growth rate (McCarty and Price, 1942; Stoddart and Smith, 1955). Not only is growth cyclic, but its periodicity is closely linked to the "annual march of carbohydrates" in the roots, with an inverse proportionality existing between the two throughout the yearly cycle (McCarty, 1938; Kerr, 1966; Hischke, 1961).

With such a close relation between growth and food storage, and between food storage and subsequent plant vigor, it is not unexpected that disruption of the growth processes by herbage removal affects the consequent vigor of the plants. Defoliation, either by clipping or grazing, reduces the amount of leaf surface and, consequently, the income from photosynthesis. At such times of defoliation, when current photosynthesis is not able to supply requirements for current growth and respiration, the reserves must be called upon (Davidson and Milthrope, 1965).

Evidence of the harmful effects of indiscriminate herbage removal is accumulating. Sampson and Malmsten (1926) have shown

drastic effects of repeated clippings on Stipa lettermanii and Agropyron violaceum. When clipped five times a year for three years, these plants yielded in the third year only 12% and 9%, respectively, the amount of herbage which had been produced during the first year. Sampson (1914) found that Festuca viridula plots which were harvested three times a year for three years then were given no treatment during the fourth and fifth years, showed a decrease in vegetative growth, indicating loss of vigor. Reproductive growth was impaired as well. No flower stalks appeared the fourth year, and only a few during the fifth year. Blaisdell and Pechanec (1930) reported that, on plots of Agropyron spicatum and Balsamorhiza sagittata, one clipping applied during the growing season reduced the following year's herbage and flower stalk production. Buckey and Weaver (1939) noted that severe clipping of Andropogon scoparius and A. gerardi decreased the amount of root reserves and resulted in the destruction of the plants in a period of a few years.

McCarty (1935) pointed out that diminution of reserve carbohydrates by certain clipping treatments does not necessarily kill the plants and the remaining reserves can promote fair growth the next season. The decreased carbohydrate level can, however, prevent maximum yields and decrease the number of fruit stalks produced during the subsequent season. Furthermore, the decline in food level resulting from such use tends to be cumulative if the treatment is repeated from year to year.

McCarty and Price (1942) indicated that the amount of carbohydrate root reserves at the end of the season is influenced less by the frequency of clipping than by the time and degree of

clipping. Aldous (1930) found that the damaging effect of frequent clipping is partially eliminated by increasing clipping height.

Most studies seem to indicate that the time of clipping has the most crucial influence on the root reserves. McCarty (1935) and Sampson and McCarty (1930) have shown that in grasses maximum storage of root reserves is attained after the current growth approaches maturity, that is, in the declining phase of herbage growth. Should herbage be removed during the growing season, reserves are used in regrowth and full storage potentials are not reached. The decrease in storage has been said to be proportional to the rate of growth at the time of herbage removal (McCarty, 1935). Blaisdell and Pechanec (1930) observed that apparently the effect of clipping depends on the amount of herbage present during the storage period following growth cessation. McCarty and Price (1942) found that in range plants subjected to one clipping during the growing season the amount of stored carbohydrates decreased as the time of clipping approached the end of the growing season. Those plants clipped when the seed was ripe contained least carbohydrates of all. Not only do clippings made near the time of seed ripening cause a reserve carbohydrate decrease, but apparently cause permanent injury, related to the reproduction process. As Murneek (1925) noted, reproduction leads to a physiological inertia, and, in annuals, to death. In perennials, removal of flower stalks and seeds at this stage comprises an extremely serious loss of material and an excessively long growing period would be required for its replacement.

Early clippings have been shown to result in the most complete

recovery. In fact, clipping early in the season may result in as much storage as no clipping. Clipping at the end of the growing season results in very little depletion of reserves since storage is virtually complete by that time and little regrowth will occur to deplete the stores (McCarty and Price, 1942).

Herbage removal has been closely linked with pasture deterioration. According to Aldous (1930) the chief causes for the decreasing productivity of Kansas pasture plants are overgrazing and premature grazing. Parker and Sampson (1931) made the point that frequent herbage removal or removal during the period of most rapid growth results in marked decreases in yield and in "decreased longevity of perennial bunch grasses, with a resulting curtailment in the establishment of such desirable species leading to a succession towards the climax or subclimax community."

CHAPTER III

DESCRIPTION OF STUDY AREA

The plots used for this study were located in an almost uniform Andropogon scoparius stand in a tall grass prairie site, 9 miles west and 2 miles north of Stillwater, near Lake Carl Blackwell, Payne County, Oklahoma.

Topography; Soil

Elevation of the area is about 888 feet above sea level. The plots were located on a nearly flat area with a very gentle slope to the north. The soil is principally Kirkland silt-loam.

Climate

Data from the United States Department of Agriculture Hydraulic Laboratory about two miles from the plots indicate a 70-year average annual precipitation of 33.07 inches, with January and February having the lowest averages and May and June the highest. The mean annual temperature is 60.8°F, with the lowest monthly average being 37.9°F in January and the highest, 84.7°F in July. The average growing season is 207 days: from April 4 until October 28.

Data taken at the study site for the six month period from

April to September, 1966,¹ showed an average monthly precipitation of 3.23 inches, compared to the 70-year average of 3.71 inches for the same months. July was a very wet month, receiving 6.03 inches, compared to the average of 3.09 inches for that month. August was also wetter than usual, but the other four months were below average in the amount of precipitation received; September was particularly dry, receiving only 1.03 inches, compared to an average for that month of 3.72 inches. (For a graphic presentation of the precipitation data for the study site in 1966 and the "average" precipitation for the same months, see Figure 1.)

Temperatures during the six month period followed fairly closely the "normal" temperatures for the area as recorded by the United States Weather Bureau (Figure 2).

The thermal growing season for the area was initiated on April 21 and ceased on October 16.

¹Gerald P. Hutchinson, 1966. Unpublished data. Oklahoma State University, Stillwater, Oklahoma.

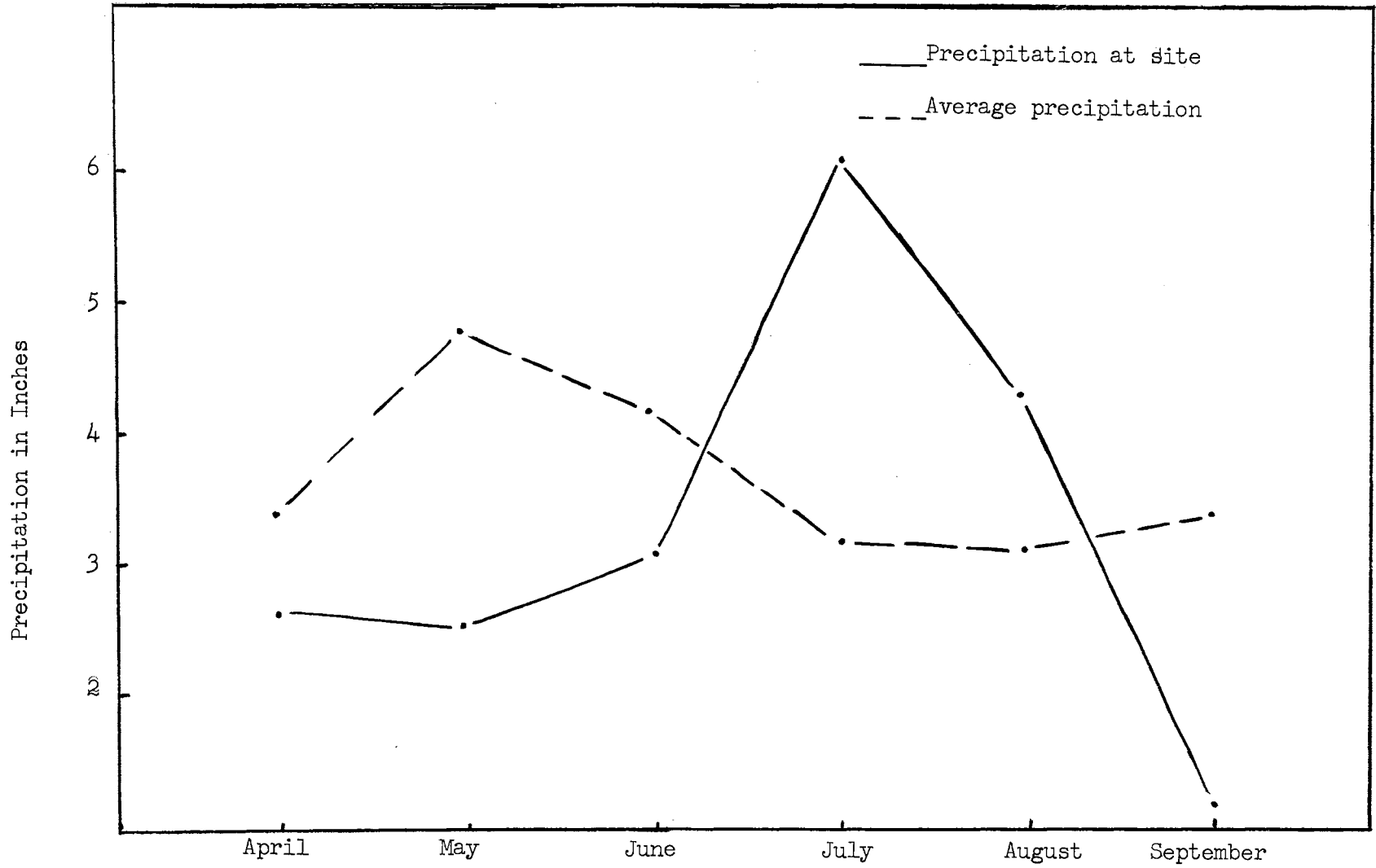


Figure 1. Monthly precipitation at site and average monthly precipitation

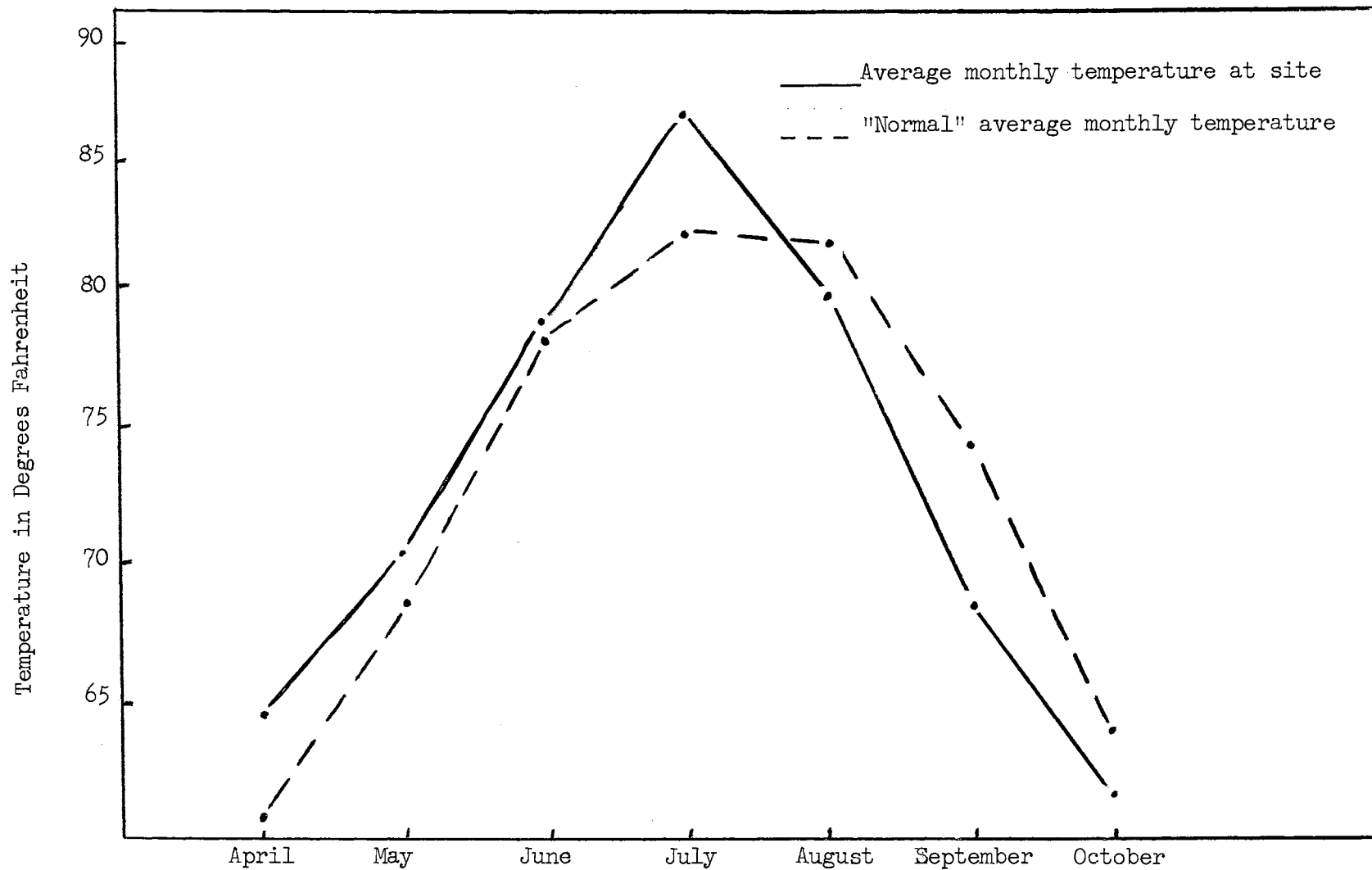


Figure 2. Average monthly temperature at site and "normal" average monthly temperature

CHAPTER IV

MATERIALS AND METHODS

Plot Design; Clipping Treatments

Prior to the beginning of the 1966 growing season, the study area was mowed and raked. Eight plots, measuring 6 ft. by 6 ft. each, were staked out with 1 ft. paths between plots. One of 8 treatments (7 clipping dates, 1 unclipped control) was assigned to each of the plots in a random fashion (Table I).

TABLE I
CLIPPING DATES

Plot	Date	Plot	Date
1	September 1	5	June 1
2	August 1	6	July 1
3	October 1	7	September 15
4	October 15	8	Control

Throughout the season, any plants which appeared other than Andropogon scoparius were removed; this facilitated obtaining unadulterated sods of A. scoparius roots.

On the specified date, each plot was clipped close to the crown (at approximately 1"). Notes were made on herbage growth, and flower and seed production throughout the study.

Collection and Preparation of Samples

During the first week of November, 1966, five sods measuring 4" by 4" by 4" were taken from each of the 8 plots. The roots were carefully washed under a hand faucet; all stems and crowns were removed. Samples were put into labelled paper sacks and subjected to a temperature of 100°C for 2 hours to stop enzymatic activity. Drying was then continued for 24 hours at 70°C. Samples were ground in a Wiley mill to pass a 60-mesh screen. The ground samples were put into small jars and again heated at 70°C for 12 hours to ensure that they were thoroughly dry. The jars were then kept tightly sealed until the chemical analyses were made.

Extraction and Analysis of

Ethanol-soluble Sugars

Two separate determinations were made from the root material from each sod, giving a total of 10 determinations for each plot.

100-milligram samples of ground dry root material were suspended in 10 milliliters of 80% ethanol in a plastic centrifuge tube. The tubes were placed in a gently boiling water bath. After the ethanol had boiled 5 minutes, the tubes were centrifuged 10 minutes at 10,000 rpm; the supernatant was decanted into clean centrifuge tubes. Two other 10 milliliter ethanol extracts were made. The 3 extracts for each sample were combined and the tubes were placed in a boiling water bath to evaporate the ethanol. When the ethanol had evaporated, water was added to bring the volume of each tube to 20 milliliters. The contents of each tube were thoroughly

stirred and then centrifuged 10 minutes at 15,000 rpm; the supernatant was decanted into clean test tubes.

Sucrose was hydrolyzed by mixing 1 milliliter of cleared extract with 1 milliliter of 1N sulfuric acid in clean test tubes and heating the stoppered tubes in a boiling water bath for 30 minutes, after which time 1 milliliter of 1N sodium hydroxide was used to neutralize the acid. The contents of each tube were made to a volume of 10 milliliters by adding 7 milliliters of water. Standards containing 500 micrograms of sucrose per milliliter and blanks of distilled water were included in each run.

The ferricyanide method described by Ashwell (1957) was used for quantitation. Two milliliters of a mixture of equal volumes of ferricyanide and carbonate-cyanide solutions were placed by use of a pumpett into each test tube to be used. To each tube was then added 0.1 milliliter of diluted sample (2-fold dilution for glucose; 10-fold dilution for total sugars). Glucose (50 micrograms/milliliter) and sucrose (500 micrograms/milliliter) standards, as well as water blanks, were included each time. Contents of each tube were mixed; the tubes were stoppered and placed into a boiling water bath for 15 minutes. The tubes were then removed from the bath and 5 milliliters of ferric iron reagent were added to each tube. The tubes were then allowed to stand for 15 minutes, during which time a blue color developed which was read at 640 millimicrons on the Klett Colorimeter.

To obtain an estimate for sucrose, which is probably the predominant non-reducing sugar, the value for reducing sugars for each sample was subtracted from the value obtained for total sugars

for that sample.

Extraction and Analysis of Starch Content

Extraction of the starch content was carried out as outlined by Whistler (1964); quantitative analysis was conducted using a modification of the Dubois phenol-sulfuric acid method (1956).

Five milliliters of water were added to each centrifuge tube containing the pellet left after 3 ethanol extractions had been performed to remove the sugars. The tubes were heated for 20 minutes at 100°C to gelatinize the starch. When the tubes were cool, 6 milliliters of 52% perchloric acid were added to each; contents were stirred frequently with a glass rod for 20 minutes. Tubes were then centrifuged 10 minutes at 10,000 rpm. The supernatant was poured into clean test tubes; pellets were resuspended in 6 milliliters of perchloric acid and again stirred for 20 minutes. Five milliliters of water were used to rinse each glass rod and were added to the tubes.

Each tube of supernatant from the first spin was filtered with suction; the corresponding extract from the second perchloric acid treatment was filtered through the same paper. Five milliliters of water were used to rinse the tubes and the residue on the filter paper. The combined filtrates for each sample were poured into clean tubes and centrifuged 10 minutes at 15,000 rpm to remove any material which had passed through the filter paper. The supernatant was poured into clean 125 milliliter flasks, to each of which were then 10 milliliters of 20% sodium chloride and 5 milliliters of iodine-potassium iodide reagent. Contents of each flask were mixed and

allowed to stand for 30 minutes. The solutions were then centrifuged batchwise and most of the supernatant was discarded. Three milliliters of ethanolic sodium chloride were added. The suspension was centrifuged and the supernatant discarded. This step was repeated once.

Two milliliters of ethanolic sodium hydroxide were then added to each tube; the tubes were shaken until the black color disappeared. The suspension was centrifuged 10 minutes at 10,000 rpm, and the supernatant discarded.

Each white starch pellet was then suspended in 5 milliliters of ethanolic sodium chloride and centrifuged; this step was repeated once, the supernatant being discarded each time. Five milliliters of water were then added to each tube and the contents were heated until the starch dissolved. The starch solutions were poured into clean tubes and made up to 20 milliliters each with water. A 2-fold dilution was made before the samples were analyzed.

For quantitation, 2 milliliters of 5% phenol were added to each of the tubes to be used. One milliliter of sample was added to each tube. A standard containing 200 micrograms of glucose per milliliter, and a water blank were included each time. Five milliliters of concentrated sulfuric acid were added directly and rapidly onto the surface of the mixture in each tube. Thirty minutes later absorbance was determined at 500 millimicrons, using the Klett Colorimeter.

Conversion of Klett Readings to
Root Carbohydrate Content

The ferricyanide method for quantitation of sugar content gives results consistent with Beer's Law; consequently, the conversion of Klett readings to micrograms per milliliter could be done mathematically by use of the formula $(\text{Klett reading})(X) = \text{micrograms/milliliter}$. X was determined for each set of samples by using the readings obtained on the standard solutions run with that set. From the formula, then, the sugar concentration in each sample was derived. To convert micrograms per milliliter to milligrams of sugar per gram of dry root material, the conversion factor of 0.2 was used. (The original carbohydrate extract was 20 milliliters from each 100 milligrams of sample material. The conversion factor was obtained thusly: $(\text{micrograms/milliliter})(1 \text{ milligram}/1000 \text{ micrograms})(20 \text{ milliliters}/100 \text{ milligrams})(1000 \text{ milligrams}/\text{gram}) = 0.2 \text{ milligrams}/\text{gram}$.)

A standard curve was prepared for use with the phenol-sulfuric acid test results by plotting mean readings obtained from 2 series of glucose standards containing from 10 to 200 micrograms of glucose per milliliter (Appendix A). Sample readings were adjusted to account for the dilution, then values for the sample concentrations were obtained by comparing each reading with the standard curve and then multiplying by 0.2 (since 20 milliliters of starch solution were originally prepared from each sample) to obtain concentrations in milligrams of carbohydrate per gram dry weight of root material. Values were multiplied by 0.9 to account for the difference in water content between starch and glucose.

Statistical Analysis

A one-way analysis of variance, using the various clipping dates as the criterion for data classification, was used to test for a significant difference between the means of the total carbohydrates for the 8 plots.

Duncan's New Multiple Range Test (Steel and Torre, 1960) was run on the means of total carbohydrates to determine specifically which means were different from each other at the 5% level of protection.

CHAPTER V

RESULTS AND DISCUSSION

Tabulated data of the results of the chemical analysis of each sample are presented in Appendix B.

The mean levels of reducing sugars and sucrose for each of the 8 plots are shown in Figure 3; mean starch contents are shown in Figure 4. As can be readily observed, the major fluctuations in total carbohydrates (Figure 5) correspond closely to the fluctuations in starch content. The drastic decline in starch content is likely attributable to its conversion into reducing sugars and subsequent utilization.

Reducing sugars remained fairly constant for all samples; however, the sucrose content increased as the date of clipping was delayed. Sampson and McCarty (1930) regarded the relatively constant sucrose concentration in roots throughout the annual herbage cycle and the large excess of sucrose at the end of the season as indicative that, while sucrose is not very important in the metabolism of the actively growing herbage, it is very important as an accumulation product. McCarty (1935) and McCarty and Price (1942) noted an increase of sucrose near the end of the growing season and proposed that this is the result of enzymatic activity stimulated by the lower fall temperatures, and resulting in a change in the

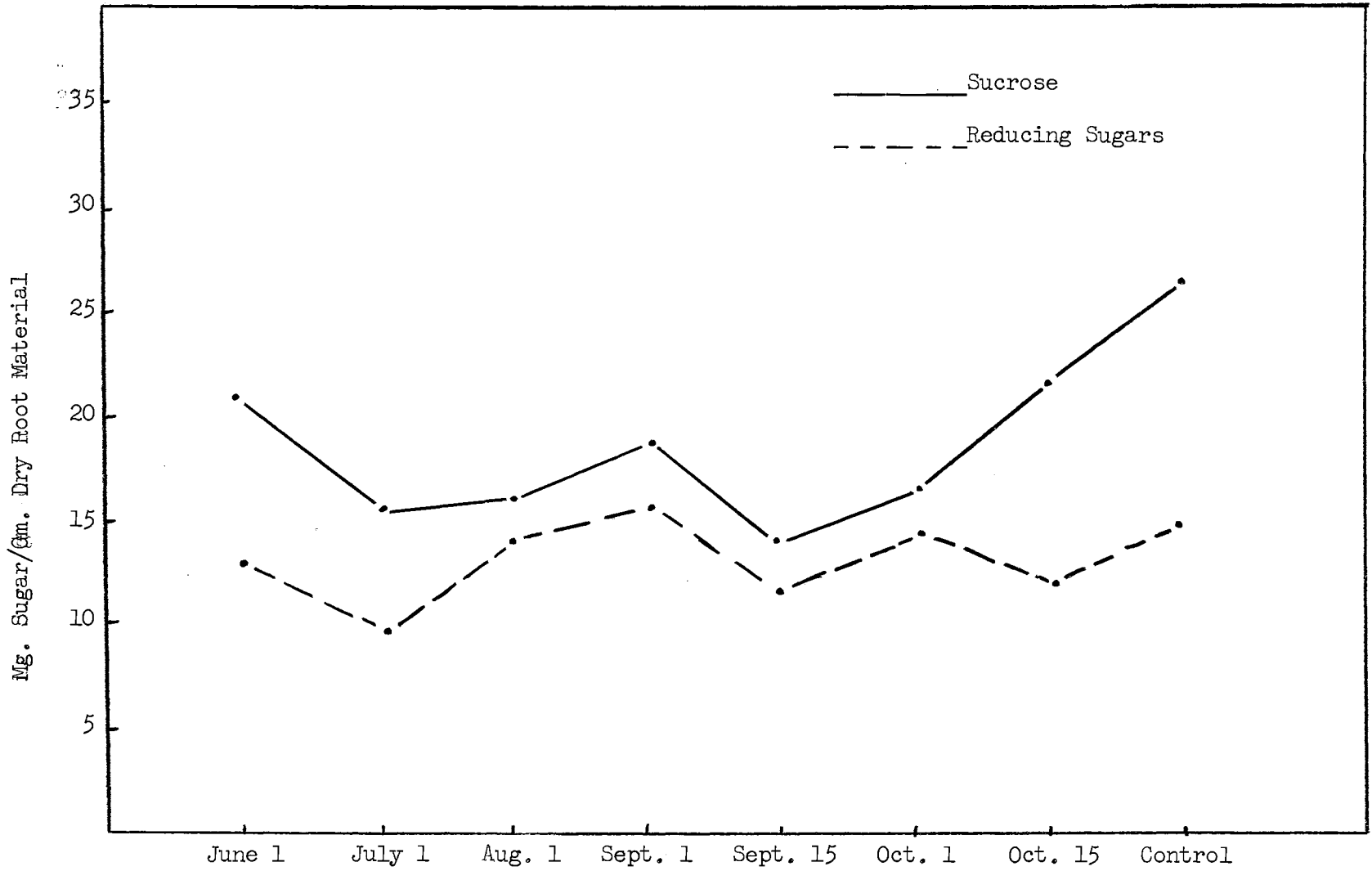


Figure 3. Reducing Sugars and Sucrose Contents of Samples

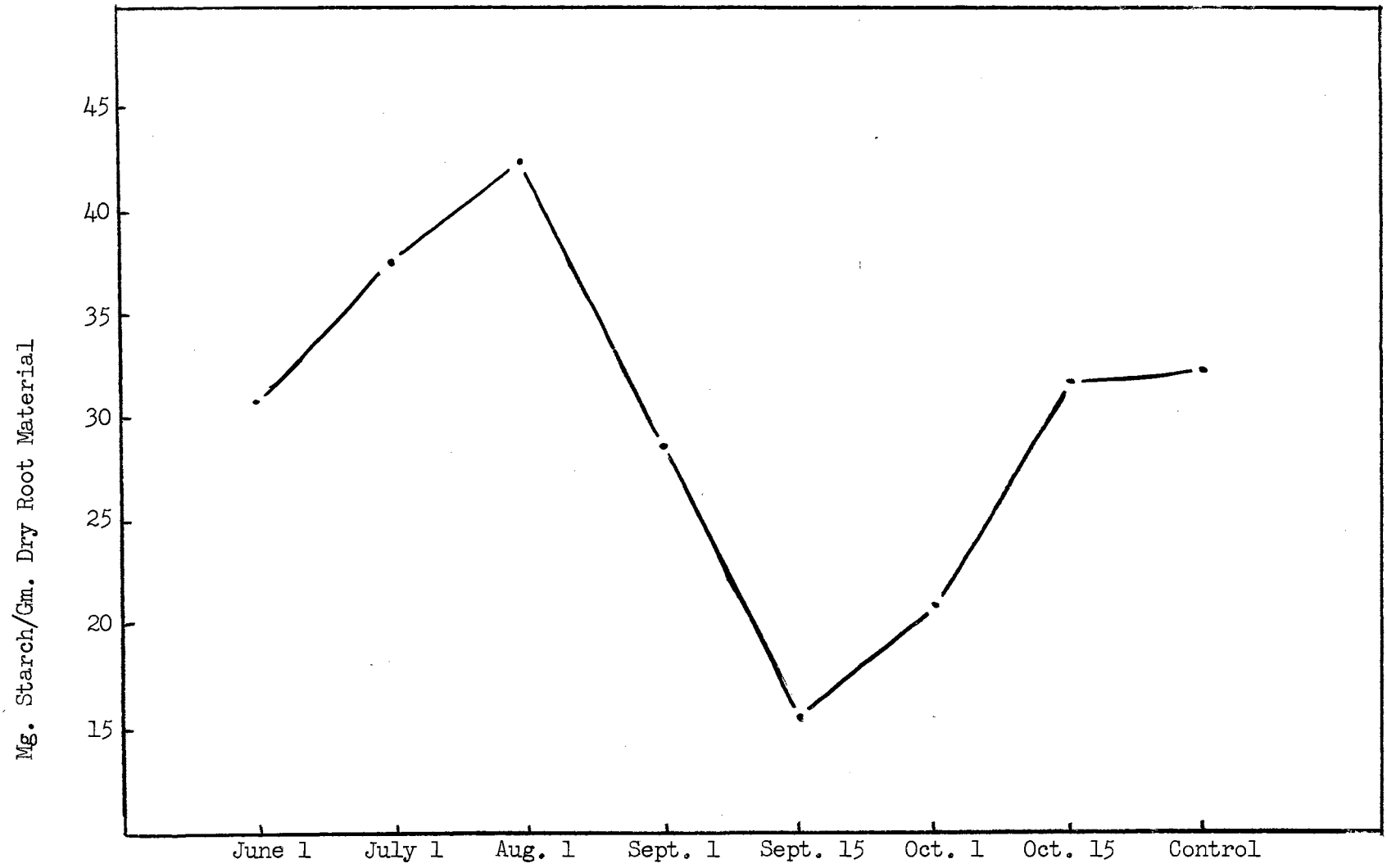


Figure 4. Mean Starch Content of Samples

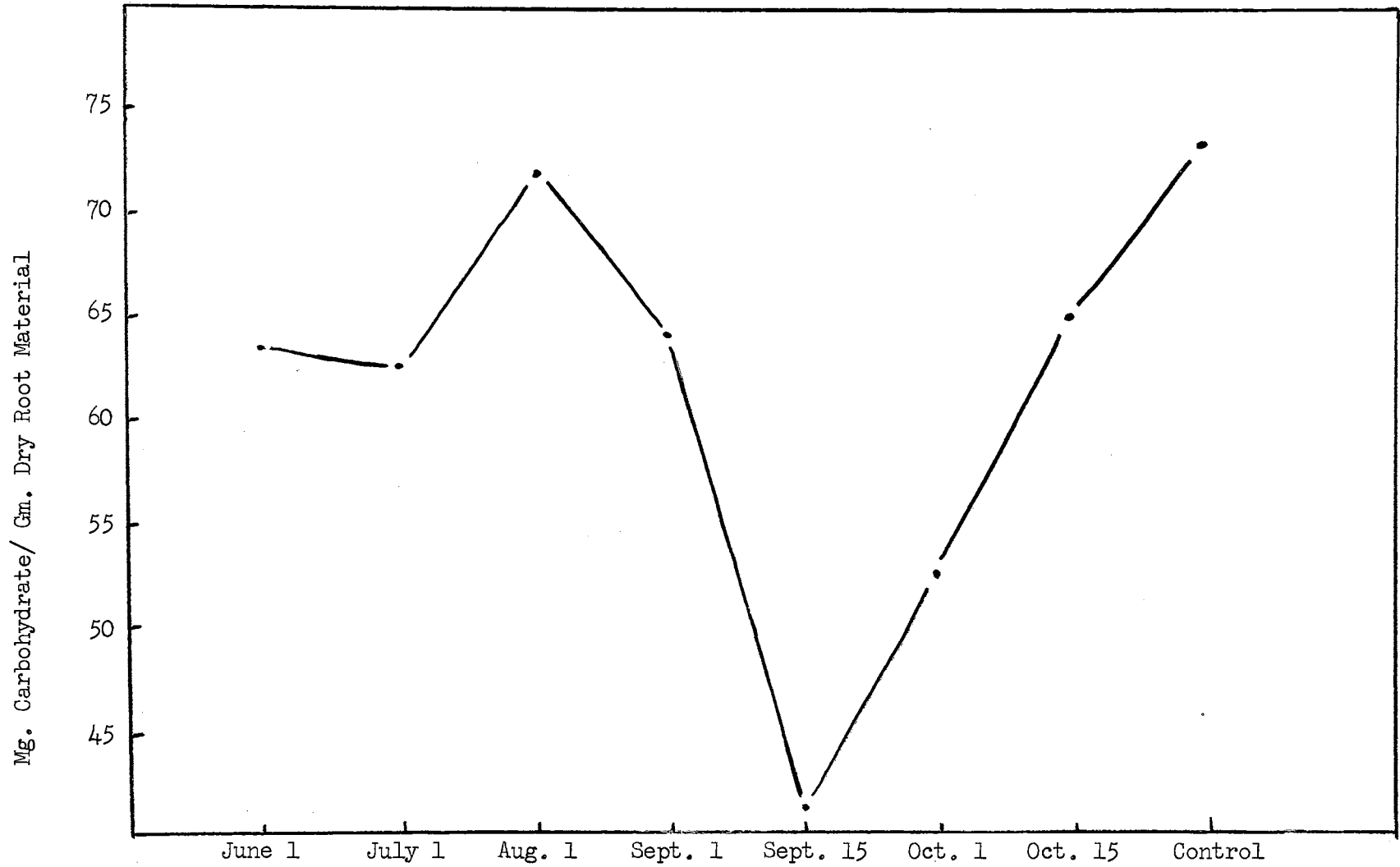


Figure 5. Mean Total Carbohydrate Content of Samples

carbohydrate reserves from the insoluble to the soluble form--a change probably related to the hardening process which prepares the plants to survive low winter temperatures.

A one-way analysis of variance on the mean total carbohydrate contents of the 8 plots (Appendix C) indicated that, at the 5% level of significance, a difference existed among the 8 treatment means.

The Duncan's New Multiple Range test (Table II and Appendix C) indicated, at the 5% protection level, that the total carbohydrate means of the plot clipped September 15 and the plot clipped October 1 were significantly different from each other and were different from (lower than) the means of any other plot. The means of the plot clipped August 1 and the control plot, while not significantly different from each other, were shown to be different from (higher than) the means of any other plot.

TABLE II

DUNCAN'S NEW MULTIPLE RANGE TEST RESULTS

Clipping date	9/15	10/1	9/1	6/1	7/1	10/15	Con.	8/1
Carbohydrate \bar{X}	40.94	51.79	62.68	63.28	63.37	64.35	<u>71.70</u>	<u>72.56</u>

(Underscoring denotes means not significantly different from each other at the 5% protection level.)

Observations made at the study site after the last clipping showed that the control plot had more seed heads than any other plot. The plot clipped June 1 was second in number of seed heads produced, followed by the plot clipped July 1, and next by the plot clipped August 1. The plot clipped October 15 had approximately the same

number of seed stalks before it was clipped as did the control plot. The other plots remained in the vegetative stage. This agrees with the observation of Cook, Stoddart, and Kinsinger (1958) that with crested wheatgrass, as the date of clipping was delayed, the number of spikes produced was decreased.

The failure of the plot clipped August 1 to produce as many spikes as did the plots clipped June 1 and July 1 probably was the major factor accounting for its larger amount of reserve carbohydrates at the end of the season. The generally high levels of all 3 plots clipped early in the season tend to support McCarty's (1935) thesis that early clipping results in a more complete recovery than do later removals. However, the substantially higher carbohydrate level for the plot clipped August 1 seems to indicate that this, rather than an earlier date, may be the optimum time for herbage removal.

According to Mueller (1964), the major flowering period for A. scoparius in this region is from late August to early or mid-September; this agrees with observations made at the study site. Immediately following this would be the major storage or accumulation period for root carbohydrate reserves. The extremely low carbohydrate level of the plot which was subjected to herbage removal on September 15 is consistent with the work of McCarty and Price (1942), which showed carbohydrate storage in mountain brome to be at a minimum during the reproductive stage and subsequent storage to be markedly diminished by close utilization during that time. Also, McCarty (1935) found that one clipping applied to Agropyron smithii or Stipa pluchra at the beginning of the carbohydrate storage interval

resulted in a maximum decrease of root reserve carbohydrates at the end of the growing season. This clipping treatment not only removed great amounts of material in the form of the seed heads, but left the plant with no herbage for carbohydrate manufacture; what reserves were in the roots at the time of clipping were further depleted by regeneration growth. This new herbage did not have time before the end of the growing season to replenish through photosynthesis the root carbohydrate supply.

The plot clipped October 1 had little regeneration growth. As McCarty (1935) noted, the amount of growth made by plants subjected to clipping at several different dates is proportional to the number of days remaining in the growing season following each clipping. The level of reserves in the plants clipped October 1 is somewhat higher than that of the September 15 clipping, but is still significantly lower than the levels of the other plots. Not enough of the storage period was experienced, and some of the reserves which were accumulated were utilized in regeneration growth following clipping.

The September 1 carbohydrate level was fairly high; this clipping occurred before the tremendous depletion required for seed set had taken place.

The level detected in the grass clipped October 15 was somewhat above that for the September 1 clipping. While it is true that much material was removed from this plot in the seed heads, the root carbohydrate level was not extremely decreased because the plants had gone through the major portion of the accumulation period with full herbage, and, consequently, had been able to store a large amount of reserves. Little of the reserve supply was used in regrowth

because the clipping treatment was applied only one day before a 26°F night temperature brought the thermal growing season to a close.

It should be kept in mind that these results are for a definite species under a given set of edaphic and climatic conditions. It has been shown that, while general trends may be the same, the exact carbohydrate levels and timing of peak contents differ among species (Kerr, 1966). Differences are, therefore, almost certain to exist in species other than A. scoparius treated the same way as were the plants in this study, or even in this particular species given a different set of conditions.

CHAPTER VI

SUMMARY

The effect of the time of herbage removal on the seasonal carbohydrate reserves in the roots of Andropogon scoparius was investigated. Eight experimental plots were located in an almost uniform stand of A. scoparius in a tall grass prairie site. Clipping treatments were assigned at random to the 8 plots; throughout the growing season the clippings were made and observations were made on the vegetative and reproductive development of the plants.

After the close of the growing season, 5 sods were removed from each of the plots and the roots were analyzed for content of reducing sugars, sucrose, and starch.

Statistical analysis of the means of total carbohydrates for the 8 plots revealed a difference at the 5% level of significance. A Duncan's New Multiple Range test on the means indicated, at the 5% protection level, that the plots clipped on September 15 and October 1 were different from (lower than) the means of all other plots, and were different from each other. While the means of the plot clipped August 1 and the control plot were not significantly different from each other, they were different from (higher than) the means of all other plots.

The very low levels of the plots clipped September 15 and October 1 were probably due to the fact that the herbage was removed

when reserve carbohydrates had been depleted by flower and seed formation, and the plants therefore passed through the period when accumulation is generally most rapid with very little photosynthetic area. The low levels encountered in these plots emphasize the vital importance of late seasonal growth in carbohydrate accumulation.

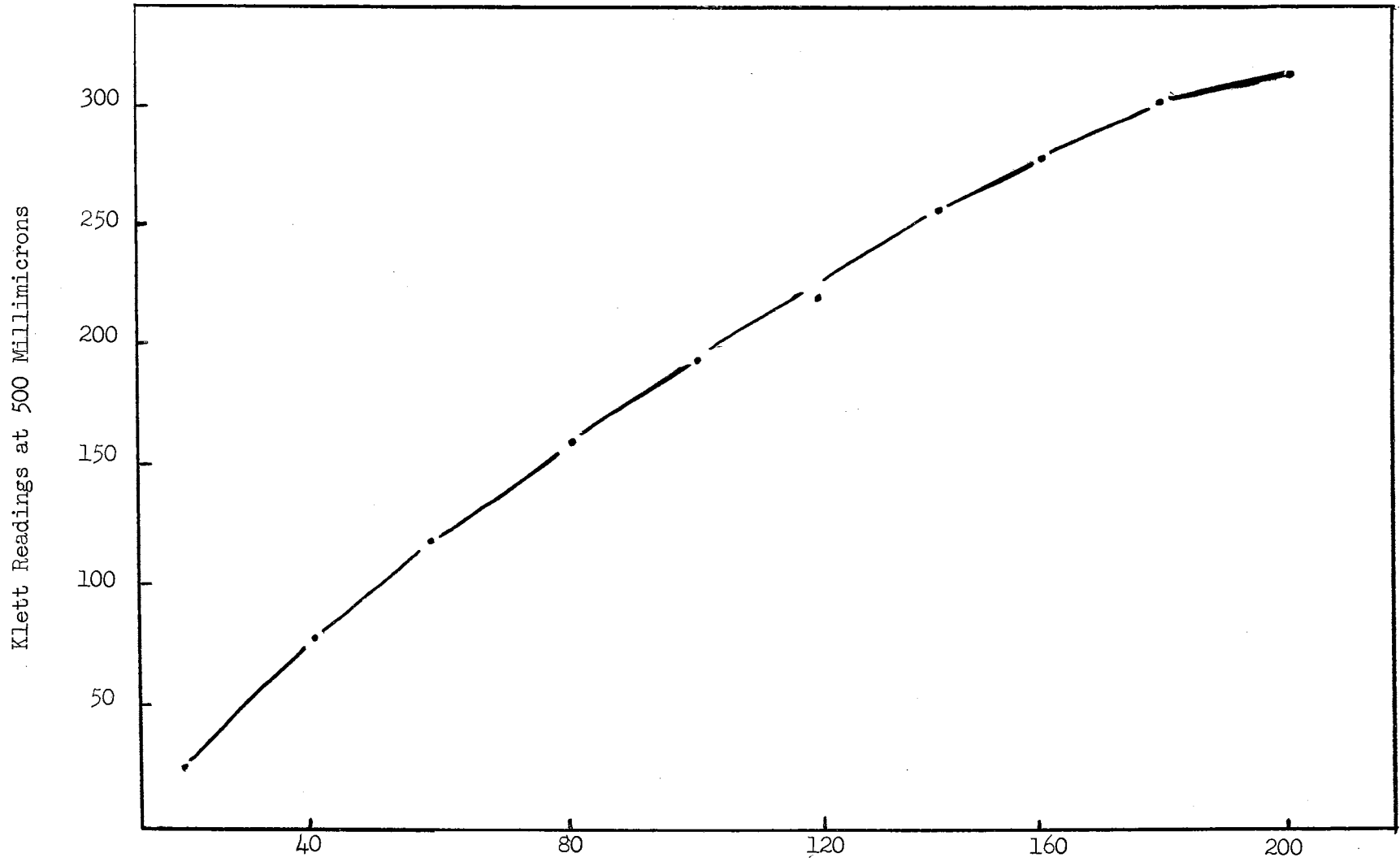
The high level in the plot clipped August 1 was likely due to the fact that while a good herbage regrowth was attained, very few seed stalks were formed, and hence, the carbohydrate reserve was not drastically depleted.

The starch curve closely followed that of the combined carbohydrates. Reducing sugars remained fairly constant in all plots, but sucrose content increased as the clipping date was delayed, possibly due to enzymatic conversion of the insoluble carbohydrate fraction to a soluble form, a process related to the hardening process.

LITERATURE CITED

- ① 1. Aldous, A. E. 1930. Effect of different clipping treatments on the yield and vigor of prairie grass vegetation. *Ecology* 11:752-759.
2. Ashwell, G. 1957. Colorimetric analysis of sugars. *Methods in Enzymology*, vol. III. ed. S. P. Colowick and N. O. Kaplan. Academic Press. New York. pp. 73-105.
3. Blaisdell, James P., and Joseph F. Pechanec. 1930. The effects of herbage removal at various dates on the vigor of bluebunch wheatgrass and arrowleaf balsamroot. *Ecology* 30:298-305.
- ④ 4. Buckey, I. S., and S. N. Weaver. 1939. Effects of frequent clippings on the underground food reserves of certain prairie grasses. *Ecology* 20:246-252.
- ⑤ 5. Cook, C. Wayne, L. A. Stoddart, and Floyd E. Kinsinger. 1958. Responses of crested wheatgrass to various clipping treatments. *Ecol. Monog.* 28:237-272.
6. Davidson, J. L., and F. L. Milthrope. 1965. Carbohydrate reserves in the regrowth of cocksfoot (Dactylis glomerata L.). *J. Brit. Grass. Soc.* 20:15-18.
7. Dubois, Michel, K. A. Gilles, S. K. Hamilton, P. A. Rebers, and Fred Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350-356.
8. Hischke, Jr., Herbert H. 1961. Carbohydrate root reserves of three native prairie grasses. Unpublished master's thesis. Fort Hayes State College. Fort Hayes, Kansas.
9. Kerr, Val Kenneth. 1966. Seasonal fluctuation of ethanol-extractable water-soluble carbohydrates in three native range grasses. Unpublished master's thesis. Oklahoma State University. Stillwater, Oklahoma.
10. McCarty, Edward C. 1935. Seasonal march of carbohydrates in Elymus ambiguus and Muhlenbergia gracilis, and their reaction under moderate grazing use. *Plant Physiol.* 10: 727-739.

11. McCarty, Edward. 1938. The relationship of growth to the varying carbohydrate content in mountain brome. U. S. Dept. Agr. Bull. 598.
12. McCarty, Edward C., and R. Price. 1942. Growth and carbohydrate content of important mountain forage plants in Central Utah as affected by clipping and grazing. U. S. Dept. Agr. Tech. Bull. 818.
13. Mueller, Dale M. J. 1964. A phenological comparison of mowed and unmowed tall grass prairie sites in north-central Oklahoma. Unpublished master's thesis. Oklahoma State University. Stillwater, Oklahoma.
14. Murneek, A. E. 1925. Correlation and cyclic growth in plants. Bot. Gaz. 79:329-333.
15. Parker, K. W., and A. W. Sampson. 1931. Growth and yield of certain Gramineae as influenced by production of photosynthetic tissue. Hilgardia 5(10):361-381.
16. Sampson, A. W. 1914. Natural vegetation of range lands based upon requirements and life history of the vegetation. J. Agr. Res. 3:93-147.
17. Sampson, A. W., and Edward C. McCarty. 1930. The carbohydrate metabolism of Stipa pulchra. Hilgardia 5:61-100.
18. Sampson, A. W., and Harry E. Malmsten. 1926. Grazing periods and forage production on the National Forests. U. S. Dept. Agr. Bull. 1405:1-54.
19. Steel, Robert G. D., and James H. Torre. 1960. Principles and Procedures of Statistics. McGraw Hill. New York. pp. 107-114.
20. Stoddart, Lawrence A., and Arthus D. Smith. 1955. Range Management. McGraw Hill. New York. pp. 98-105.
21. United States Weather Bureau. 1966. Climatological data for Oklahoma. Washington, D. C.
22. Weinman, H. 1948. Underground development and reserves of grasses. J. Brit. Grass. Soc. 3:115-140.
23. Whistler, Roy, ed. 1964. Methods of Carbohydrate Chemistry IV. Academic Press. New York. pp. 33-36.



APPENDIX A. STANDARD CURVE (Micrograms/Milliliter of Glucose)

APPENDIX B

MICROGRAMS OF REDUCING SUGARS, SUCROSE, STARCH,
AND TOTAL CARBOHYDRATES PER GRAM
OF DRY ROOT MATERIAL

Plot Clipped June 1

Sod	Trial	Reducing Sugars	Sucrose	Starch	Total Carbohydrates
1	1	9.90	25.48	40.7	76.1
	2	9.55	27.76	39.2	76.5
2	1	13.05	18.10	28.1	59.2
	2	13.32	18.99	29.2	61.5
3	1	12.88	22.89	32.4	68.2
	2	13.40	20.44	33.5	67.3
4	1	13.14	17.63	24.5	55.3
	2	13.23	18.69	25.6	57.5
5	1	13.14	17.24	24.8	55.2
	2	13.14	17.63	25.2	56.0

Plot Clipped July 1

1	1	9.58	12.10	34.6	56.3
	2	9.10	14.29	33.5	56.9
2	1	9.87	15.54	39.2	64.6
	2	10.36	16.25	40.7	67.3
3	1	9.20	13.79	34.9	57.9
	2	9.68	12.50	33.8	56.0
4	1	10.65	17.58	35.6	63.8
	2	10.75	15.46	34.2	60.4
5	1	9.39	17.62	43.6	70.6
	2	9.97	19.46	43.6	73.0

Appendix B (Continued)

Plot Clipped August 1

Sod	Trial	Reducing Sugars	Sucrose	Starch	Total Carbohydrates
1	1	12.68	15.25	43.6	71.5
	2	12.87	15.81	43.6	72.3
2	1	13.65	15.41	41.0	70.1
	2	13.17	14.01	42.5	69.7
3	1	15.49	16.97	45.7	78.2
	2	15.18	15.01	47.5	77.7
4	1	13.65	15.54	39.6	68.8
	2	13.94	17.01	40.7	71.6
5	1	13.55	15.51	38.9	68.0
	2	13.84	16.35	38.9	69.1

Plot Clipped September 1

1	1	15.42	24.21	35.6	75.2
	2	15.93	24.85	34.6	75.4
2	1	15.34	18.37	28.8	62.5
	2	15.68	18.03	28.8	62.5
3	1	17.04	18.89	32.4	68.3
	2	16.61	20.43	33.5	70.5
4	1	14.12	19.58	22.7	56.4
	2	13.95	18.64	22.3	54.9
5	1	16.26	14.29	24.1	54.6
	2	16.35	12.91	24.1	53.4

Appendix B (Continued)

Plot Clipped September 15

Sod	Trial	Reducing Sugars	Sucrose	Starch	Total Carbohydrates
1	1	15.49	13.02	22.3	50.8
	2	15.10	11.34	23.4	49.8
2	1	9.00	17.03	16.2	42.2
	2	9.20	15.18	16.2	40.6
3	1	10.36	18.98	14.8	44.1
	2	10.07	18.86	15.5	44.4
4	1	12.59	12.60	14.0	39.2
	2	13.08	9.43	15.1	37.6
5	1	9.58	13.56	9.0	32.1
	2	9.20	11.46	7.9	28.6

Plot Clipped October 1

1	1	14.48	9.78	24.8	49.1
	2	14.57	11.53	25.9	52.0
2	1	15.34	18.12	17.3	50.8
	2	15.51	20.15	18.4	54.1
3	1	13.55	24.32	16.6	54.5
	2	14.14	24.46	16.2	54.8
4	1	15.08	17.53	16.6	49.2
	2	14.74	18.97	17.3	51.0
5	1	14.40	9.78	28.1	52.3
	2	14.06	8.66	27.4	50.1

Appendix B (Continued)

Plot Clipped October 15

Sod	Trial	Reducing Sugars	Sucrose	Starch	Total Carbohydrates
1	1	11.90	21.67	47.5	81.1
	2	12.28	22.82	46.1	81.2
2	1	9.52	18.13	23.4	51.0
	2	9.71	19.46	22.7	51.9
3	1	12.00	22.10	31.0	65.1
	2	12.47	23.89	31.0	67.4
4	1	11.33	19.63	25.9	56.9
	2	11.81	19.64	27.0	58.4
5	1	12.09	22.00	30.2	64.3
	2	12.47	23.14	30.6	66.2

Control Plot--Not Clipped

1	1	10.79	23.56	38.5	72.8
	2	10.96	25.67	37.4	74.0
2	1	15.56	26.42	35.6	77.6
	2	16.09	23.60	34.2	73.9
3	1	18.56	31.81	38.0	88.4
	2	18.83	34.21	37.4	90.4
4	1	14.23	25.07	24.8	64.1
	2	14.67	22.72	24.1	61.5
5	1	14.26	23.62	23.8	61.7
	2	14.52	22.22	24.5	61.2

APPENDIX C

ANALYSIS OF VARIANCE TABLE

Source of Variation	DF	SS	MS	F
Total	79	11846.94	_____	
Treatment	7	7593.21	1084.74	
Error	72	4253.73	59.08	18.36*

* indicates difference at 5% level of significance

DUNCAN'S NEW MULTIPLE RANGE TEST

$$s_{\bar{x}} = 3.812$$

P	2	3	4	5	6	7	8	
SSR	2.83	2.98	3.08	3.14	3.20	3.24	3.28	
LSR	6.88	7.24	7.48	7.63	7.78	7.87	7.97	
Clipping date	9/15	10/1	9/1	6/1	7/1	10/15	8/1	Con.
Carbohydrate \bar{X}	40.94	51.79	62.68	63.28	63.37	64.35	<u>71.70</u>	<u>72.56</u>

Underscoring denotes means not significantly different from each other at the 5% level of protection.

VITA

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