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A study of management effects on carbohydrate reserves in Gahi-1 pearlmillet

Richard W. Couch

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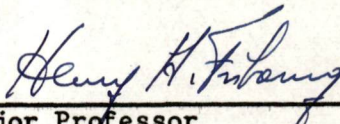
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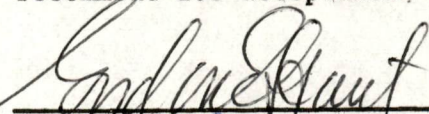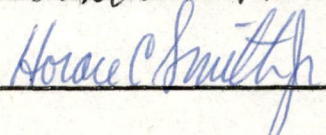
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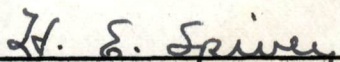
     I am submitting herewith a thesis written by Richard W. Couch entitled "A Study of Management Effects on Carbohydrate Reserves in Gahi-1 Pearlmillet." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

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Dean of the Graduate School

A STUDY OF MANAGEMENT EFFECTS ON CARBOHYDRATE RESERVES

IN GAHI-1 PEARLMILLET

————————

A Thesis

Submitted to

the Graduate Council of

The University of Tennessee

————————

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

————————

by

Richard W. Couch

August 1961

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CHAPTER I

INTRODUCTION

The permanent and semi-permanent pastures composed of perennial grasses and legumes seldom produce adequate amounts of forage during the hot, dry summer months. If these pastures are to be utilized to the fullest extent, some form of summer supplementary forage must be available during these periods of low production of the legume-grass pastures. The use of summer annual supplementary forage species is becoming more popular, especially with the dairy farmer, to partially solve this feed shortage problem.

Pearlmillet (Pennisetum glaucum (L.) R. Br.) is one species that appears to be particularly well-suited for use as a summer supplemental forage grass in Tennessee and other southeastern states. It is a summer annual which grows better than most forage crops during the hot, dry, summer weather. It does not contain any prussic acid-forming glucosides or other known poisonous constituents. At the present time, it is more resistant to the pathogens that affect other plants such as Sudangrass (Sorghum sudanense (Piper) Stapf.). It produces more high quality forage during the summer than does most of the other grasses, and maintains a high level of production under unfavorable climatic conditions that often reduces the quality and production of perennial grasses.

There is, however, a lack of knowledge of the basic management factors necessary to realize the greatest potential from this crop as a pasture, hay, silage, or soilage species. Information is lacking particu-

larly in the area of carbohydrate reserves in relation to regrowth and production of high quality forage, as influenced by various management factors.

Some research has been conducted on the cutting, grazing, and fertilization management of the crop in relation to the influences of management on regrowth, production, morphological development, and nitrogen content of the plants. However, just what influences the carbohydrate reserves have on these factors, or what influences cutting height and nitrogen fertilization have on the reserve carbohydrates, are not known.

With these problems in mind, an investigation was conducted in an attempt to answer the following questions:

1. How close to the soil surface can pearlmillet be cut and still produce good yields of high quality forage?

2. How does different cutting intensities affect total forage yield?

3. How does nitrogen fertilization and cutting intensities affect the rate and amount of regrowth?

4. How does nitrogen fertilization and cutting intensities affect the amount of reserve carbohydrates available for utilization in initial regrowth?

5. Does the carbohydrate content of the basal stubble vary with different heights of stubble and different amounts of nitrogen fertilization?

6. How does cutting frequency affect the carbohydrate reserves?

7. Does carbohydrate reserves affect the rate of regrowth?

8. How accurate and useful is the refractometer in determining the carbohydrate content of plants?

9. How accurate and useful is the anthrone method of carbohydrate analysis for analyzing plant material?

CHAPTER II

REVIEW OF LITERATURE

Cutting Experiments With Pearlmillet

Extensive research by numerous workers (7,21,24,26,27,31,34,37,38) has been conducted on the grazing and cutting management of perennial grasses. However, few people have worked with annual grasses, and even fewer with cutting management of pearlmillet.

One of the first experiments on the pasture potential of pearlmillet was done in South Carolina. Cathcart (9) found that pearlmillet produced 43% more total digestible nutrients per acre under grazing than when harvested for silage. Pearlmillet yielded 740 pounds of total digestible nutrients per acre and 68 cow-days per acre when grazed. The average yield per acre, expressed in terms of alfalfa hay equivalent, was 1,472 pounds under grazing and 1,020 pounds when ensiled.

Hoveland and McCloud (20), and Hoveland[1], working with the Starr variety of pearlmillet in Florida, found the highest yield to be from plants that had been allowed to grow to a height of 54 inches. The protein content of these plants was about 15%. The highest protein content, about 25%, came from plants cut when the height ranged from 12 to 30 inches. These workers also found that a stubble height of 10 to 18

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[1]Hoveland, C. S. Defoliation of oats and pearlmillet as related to herbage yields. Unpub. Ph. D. Thesis, University of Florida, Gainesville, Florida. 1959.

inches produced a leafier, more tender plant which was better for grazing than those plants allowed to grow to a 54-inch height. They concluded that, for pasture, the plants should be allowed to reach a height of 30 inches, then be grazed to a 10- to 18-inch stubble, but for silage or soilage, the 54-inch height would produce more. Hoveland's experiment, run over a 4-year period, led him to conclude that a 50% decrease in root production under a 12-inch cut, as compared to a 30-inch cut, would explain the decreased forage yield under the more severe cutting treatments. He found that a 19-inch row gave the highest yields and resulted in the easiest weed control. Irrigation of some treatments did not increase yields to any extent. The number of live shoots declined throughout the season, irrespective of cutting treatments, but were considerably greater under high nitrogen fertilization.

In a similar experiment, Broyles and Fribourg (8) studied the responses of Gahi-1 pearlmillet, German millet (Setaria italica (L.) Beauv.), and sweet and Piper Sudangrass, under various cutting intensities and levels of nitrogen. The total dry matter produced, percent nitrogen, and total nitrogen amounts in the harvested forage, were measured. Invariably, as the yields of dry matter increased, the percent nitrogen decreased. With nitrogen fertilization rates up to 120 pounds per acre, there was a marked increase in yields of dry matter, nitrogen and percent nitrogen in the harvested forage. Pearlmillet produced the most dry matter and contained more nitrogen in the harvested forage, German millet produced the least, and the Sudangrasses were intermediate. Both Gahi-1 pearlmillet and Piper Sudangrass produced more yields and the best nitrogen percentages when cut

at the 30-inch height to a 10-inch stubble.

Ramaiah,[2] working with Gahi-1 pearlmillet, found that the percentages of primary stems with buds below the scheduled height of cut decreased in all cutting treatments as the season advanced, irrespective of the cutting intensity. This was in general agreement with what Hoveland[1] found in his work in Florida. Ramaiah also found that the percentages of primary stems with apical buds above the scheduled height of cut were generally inversely proportional to cutting heights. The regrowth from the 6- and 10-inch stubble was more rapid than that from the lower stubble heights. In general, tiller production was less under the more severe cutting treatments. Mortality generally was inversely proportional to severity of cutting management. He found no significant differences in total seasonal dry matter production between the nine cutting intensities studied, a fact not supported by other pearlmillet experiments (8,20). In contrast to Hoveland and McCloud's conclusion that a 19-inch row produced more seasonal dry matter, Ramaiah found the 7-inch row spacing with a seeding rate doubled from normal to give the highest production. However, regrowth and tiller production were suppressed by the closer row spacings. Therefore, spacing, plant density, stage of growth at harvest, and stubble heights were important factors in determining regrowth after harvest, leafiness, and productivity of the plants over the whole season.

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[2]Ramaiah, V. The morphological development of Gahi-1 pearlmillet as affected by management. Unpub. M. S. Thesis, University of Tennessee, Knoxville, Tennessee. 1960.

In an experiment in Mississippi, Drapala and Johnson (12) studied the
border and competition effects in Gahi-1 pearlmillet and Greenleaf Sudan-
grass plots subjected to different levels of nitrogen fertilization.
They used rows 6 inches apart in the plots and applied ammonium nitrate at
the rate of 0 and 100 pounds nitrogen per acre in alternate plots. The
outermost rows of individual plots were 3 inches apart, resulting in no
nitrogen rows being 3, 9, 15, 21, 27, 33, and 39 inches away from the high
nitrogen plots, which they termed "contagious plots". They found that the
high nitrogen top-dressing did not produce a border effect on adjacent
plots more than 15 inches away from the edge of the contagious plots.

Alexander and McCloud (2) employed the Leaf Area Index concept in
studying the regrowth of pearlmillet. This LAI represents the relation-
ship between the leaf area of plants and the area of the soil surface
which the plants cover. Therefore, a Leaf Area Index of 2 would mean
there are 2 square feet of leaf area to 1 square foot of soil surface.
By varying the cutting heights from 2 to 48 inches and the row widths
from 4.5 to 36 inches, they obtained Leaf Area Indexes ranging from 1.2
to 48. The optimum LAI for maximum growth was found to be 2.9; this was
obtained in the treatment cut to a 24-inch height of stubble in 36 inch
rows. A 2-week regrowth period seemed to be most efficient for light
utilization, but efficient regrowth was correlated with decreasing LAI.
Introduction of shade on a portion of the experiment reduced the amount of
regrowth of the affected plants, but it did not alter the response of the
various Leaf Area Indexes.

Another species of the genus <u>Pennisetum</u> has been studied quite extensively in the tropics. Napiergrass (<u>Pennisetum purpureum</u> Schumach.), a perennial grass, has undergone several studies on the effects of fertilization and frequency of cutting on the yield and composition of the forage (10,29,40,47). All of these workers agree that longer cutting intervals will increase the dry matter production, but decrease the general quality of the material. The workers also agree that an 8-week cutting interval will insure the best palatability, persistence, and yield. Wilsie et al. (47) found that a cutting interval of less than eight weeks resulted in poor recovery, depleted stands, and greater weed competition. Vincente-Chandler (40), using nitrogen at rates from 0 to 2,000 pounds per acre per year, obtained increases in yields to 800 pounds nitrogen per acre, but found the nitrogen utilization efficiency to fall off at about 400 pounds of nitrogen per acre. With an 8-week cutting interval and 800 pounds of nitrogen per acre, Napiergrass produced 22 tons of dry matter per acre containing 9.7% protein. This extremely high production removed 674 pounds of nitrogen per acre.

Dark-Room Experiments

The early work concerning carbohydrate reserves in plants in relation to their regrowth in the dark was conducted in Wisconsin. Albert[3] made a study of the measurement of root reserves in alfalfa (<u>Medicago</u>

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[3]Albert, W. B. Studies of the measurement on root reserves in alfalfa plants. Unpub. M. S. Thesis, University of Wisconsin, Madison, Wisconsin. 1924.

sativa L.) and found that the majority of the alfalfa roots produced 8 to 25% of their original weight in stem and leaf growth in the dark-room. Plants cut earlier in the season and more frequently in the field averaged less stem and leaf growth than did the more mature plants which were not cut quite so often. There was a general tendency for a greater total loss of weight in the frequently-cut plants, even though the dark-room growth was considerably less. However, much higher "respiration and oxidation rates" in these plants would seem to compensate for this extra loss. Albert found that the larger roots were producing the most dark-room regrowth with a wider variation in the percent growth in the frequently-cut plants.

A later study by Albert (1) provided additional information and further substantiated some of the conclusions of his first study. Dark-room samples drawn from first-year seedlings had a total loss of 47% of the original dry weight. The stem and leaf regrowth accounted for 18% of the loss and he said that the remaining 31% loss probably was due to "respiration, oxidation, and perhaps some leaching". Dark-room samples drawn from three-year-old plants lost 25% of their total original dry weight, but 14% of the weight was recovered in top-growth, leaving a smaller loss due to the other factors. Mature plants produced more dark-room growth with about one-half the loss of plant weight, as compared to plants cut early and more frequently in the season. The top-growth was found to be higher in percent nitrogen than that of plants grown in the field. Albert concluded that plants would produce top-growth in the dark until the soluble carbohydrates were very low, about 3 to 5%.

A bulletin entitled "Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants" summarized much of the work of Albert and included the work of Garber et al. (18). Albert (18) concluded that the yellow stems and leaves grown in the dark-room were definitely produced from stored carbohydrates in the alfalfa plants. Chemical analysis of the plants in this experiment led him to conclude that regrowth in the dark was not as accurate a measure of reserve carbohydrates as is needed for studying their influences upon plant behavior. He felt, however, that the dark-room results were a valuable supplement to chemical analysis of the plants.

After these initial studies, it seemed emphasis was placed entirely upon chemical analyses in studying the various effects of available carbohydrate reserves on plant growth. In 1959, Kendall and Hollowell (21) working with red clover (Trifolium pratense L.) in Kentucky, compared plants which were physiologically reproductive and some which were physiologically vegetative, with respect to growth, carbohydrate content, and survival in a dark chamber. They found the physiologically mature plants grew slightly less, but survived longer in the dark than did the vegetative plants. The rate of growth on a daily basis was greater in the plants which were vegetative. They felt that this might account for the earlier death of the plants in the vegetative stage. They also concluded that survival in the dark was not comparable with persistence under field conditions.

Dixon,[4] working with Kentucky 31 tall fescue (Festuca arundinacea Schreb.), used the dark-room for studying the influence of cutting management upon regrowth in the dark. He varied the clipping interval and left plants in the dark-room from 7 to 21 days, using leaf growth as an index of the regrowth in the dark. He found that the plants making the most growth in the dark contained the most stored carbohydrates when placed in the dark. The differences in the dark-room growth seemed to be a result of clipping frequencies, the frequently-cut plants making less dark-room growth than those plants from plots cut less frequently. Also, less yield generally was obtained from the frequently-cut plants. It seemed that frequent clippings did lower the total available carbohydrates in a plant, thus resulting in less yield and dark-room growth. Dixon concluded that the growth in the dark, especially that of plants with high total available carbohydrates, was not an adequate measure of available carbohydrates, even though it did indicate certain trends and situations.

An intensive study by Mays[5] provided some dark-room results on varieties of Sudangrass and pearlmillet. He grew the plants in cans in the greenhouse under optimum growing conditions. When a height of 18 to 20 inches was reached, the plants were cut to 2-, 4-, 6-, and 8-inch stubbles and placed in the dark chamber. Most of the plant regrowth took

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[4]Dixon, J. D. The effect of frequency of clipping on dry matter and carbohydrate reserve content of Kentucky 31 tall fescue. Unpub. M. S. Thesis, University of Kentucky, Lexington, Kentucky. 1960.

[5]Mays, D. A. Sudangrass and pearlmillet development and productivity as influenced by cutting heights. Unpub. Ph. D. Thesis, Pennsylvania State University, University Park, Pennsylvania. 1960.

place during the first 4 days and, after 10 days, the plants appeared

dead. The length of plant regrowth and weight of dry matter were measured.

Gahi-1 pearlmillet produced more dry matter than the other species at all

cutting heights, but there were no differences in the length of regrowth

among the cutting heights or species. This meant that Sudangrass and

pearlmillet grown in the dark recovered at the same rate regardless of

the cutting height. This led him to conclude that the differences due to

cutting heights observed in the field and greenhouse portions of his ex-

periment were due to differences in amounts of photosynthetic tissue re-

maining after the cutting treatment was made.

Carbohydrate Reserves in Perennial Grasses

Since very little work had been done on the relationship between

carbohydrate reserves and the growth of annual plants, especially the

millets, it seemed that a selected review of the numerous experiments on

carbohydrate reserves in perennial grasses would be beneficial.

Some of the first work dealing with carbohydrate reserves was done

in Missouri by Trowbridge et al. (39). They found a carbohydrate present

in the corm of timothy (Phleum pratense L.), but did not determine its

specific identity. However, they concluded that the weather affected and

influenced carbohydrate metabolism to a large extent.

Extensive work has been done with orchardgrass (Dactylis glomerata

L.) and perennial ryegrass (Lolium perenne L.) by Sullivan and Sprague (34,

37,38). By conducting complete chemical analysis on the plants for all

carbohydrates, they have shown the lower stubble to be the most important part of the plant for carbohydrate reserve storage (34). They also found the water-soluble carbohydrates, glucose, fructose, sucrose, and especially fructosans, to be the main reserve compounds. This was indicated by cutting the plants, then determining the chemical composition for 35 successive days (37,38). The non-water-soluble carbohydrates remained at about the same concentration after cutting, whereas the water-soluble portion greatly decreased the first 3 days, then slowly recovered over the 35-day period. It was shown also that nitrogen fertilization would lower the amount of water-soluble carbohydrates in the plants throughout the season, especially the fructosans which made up the largest portion of these reserve materials (34,38).

Norman (26,27) found the water-soluble carbohydrate content to reach a peak at full emergence of the panicles of perennial ryegrass and orchard-grass. His findings regarding the effect of nitrogen and place of storage on the carbohydrate content agreed closely with those of Sullivan and Sprague (34,38). More total water-soluble fructosans were found in rye-grass than in orchardgrass on the first cut and throughout the season, but both had less water-soluble fructosans on subsequent cuts. The amount of structural material was affected little by nitrogen application or fructosan use.

Several intensive studies were carried out in Great Britain with timothy, orchardgrass, tall fescue, and perennial ryegrass by Waite and Boyd (41,42,43,44). The effects of nitrogen fertilization on the available carbohydrate reserves, times of reserve carbohydrate peaks, and the place

of carbohydrate reserve storage were in agreement with the results obtained
by other workers (7,26,27,30,34,37,38). They concluded that the stage of
growth was the major factor governing the chemical composition of a plant
at any time.

Garber (17) summarized the literature on carbohydrate reserves in his
work with Kentucky bluegrass (Poa pratensis L.). He found the carbohydrate
reserves interrelated with several ecological plant factors. He concluded
that, with abundant nitrogen, the carbohydrate reserves were constantly
consumed in more rapid growth; therefore, they soon became the principal
limiting factor in plant growth. When the bluegrass was cut six times a
year, only 43% as much yield was obtained as when it was cut only once at
maturity. The frequently-cut plots yielded less the next year also,
probably because of lower carbohydrate reserves. This led Garber to con-
clude that high carbohydrate reserves, together with an optimum amount of
fertilizer, are necessary for maximum yield.

McCarty and Price (24) worked with several native mountain forage
plants in Utah and found carbohydrate storage periods and clipping results
similar to those found by workers in other areas of the country. However,
they found sucrose and starch to be the principal reserve carbohydrates in
these plants. They also said that insoluble carbohydrates were converted
to soluble carbohydrates during the winter. These findings were somewhat
unlike those of other workers dealing with temperate climate plants.

The findings of McCarty and Price (24) are even harder to explain
when the studies of De Cugnac (11) and Weinmann (46) are considered. Accord-
ing to De Cugnac, grasses can be divided into two groups, those which

accumulate fructosans in their vegetative organs, usually together with

sucrose, but no starch; and those which store sucrose, with or without

starch, but no fructosans. He suggested that the fructosan-containing

grasses are essentially species native to cool climates, while grasses

accumulating sugars and starch are mostly adapted to warm regions. This

seemed to be confirmed by Weinmann in his work with a variety of grasses

in South Africa. Weinmann (44) did extensive work with carbohydrate re-

serves and summarized it by stating that the seasonal trend of reserve

carbohydrate storage was influenced by the growth rate, climatic and

meteorological conditions, stage of maturity, species, and time of the

year. He felt that repeated defoliation effects were cumulative and would

affect the plant in various ways and degrees, depending on the frequency of

defoliation. Generally, carbohydrate starvation weakened the ability of

the plant to survive adverse conditions. However, he found cutting some-

times did not reduce the amount of stored carbohydrates, but merely caused

a weight loss in storage organs.

Carbohydrate Reserves in Annual Grasses

Mays'[5] experiment with Sudangrass and pearlmillet varieties in

Pennsylvania was involved in greenhouse work along with a 2-year field

experiment in which he studied the effects of various cutting heights and

nitrogen levels on the productivity and development of these annual plants.

Four cutting intensities, 2-, 4-, 6-, and 8-inch stubbles from an 18- to

20-inch growth height, high-nitrogen treatments of 200 pounds per acre,

and low-nitrogen treatments which received no top-dressing during the season, provided the extremes of management. Refractometric determinations of the carbohydrate content of expressed sap from the basal stubble were made on the plants at the time of cutting and 4 days later. The carbohydrate content of pearlmillet and Sudangrass was practically the same at the time of cutting, but there was considerably more variation in the carbohydrate content 4 days after cutting in both species. The average carbohydrate content for the high nitrogen treatments was only 2.9%, as compared to 3.6% for the treatments which received no nitrogen fertilizer. This finding was in close agreement with the reports of others dealing with nitrogen fertilization and carbohydrate reserves in perennial grasses. The carbohydrate content also was lowered correspondingly by the more severe cutting intensities. Differences in the carbohydrate content in 1960 were higher than those in 1959. Most of this increase was encountered in the pearlmillet varieties.

Norman et al. (28) said that there was about 2 to 3% fructosans in Sudangrass at all times, but the amount was subject to seasonal fluctuations. They said that this grass contained an average of 4.1% fructosans in the aerial parts over the period of May 29th to August 5th. The highest percent fructosan content, 7.6%, occurred when the plants were in bloom, and the lowest, 2.3%, occurred with only leafy growth early in the season.

Other studies on perennial plants in the genus Pennisetum have been conducted, but few contribute much as to the effect of available carbohydrate reserves on production. Weinmann (45) defined total available carbohydrates as "all the carbohydrates which can be used in the plant as a

source of energy". He felt that these total available carbohydrates were the compounds that should be analyzed in any carbohydrate reserve study. He analyzed Kikuyugrass (Pennisetum clandestinum Hochst. ex Chiov.) and found sugars, fructosans, and starch in the roots and rhizomes at concentrations of 14 to 16%.

Strokes et al. (35) studied Napiergrass and Japanese cane (Saccharum officinarum L.) in relation to the effects of irrigation with sewage effluent on the establishment and yield of these grasses. They found carbohydrate and nitrogen reserves in the crowns of plants from irrigated plots to be 3.5 times more concentrated than those in non-irrigated plots. They concluded that early fertilization and irrigation produced more rapid growth and elaboration of carbohydrates. Total carbohydrates accounted for 31% of the total dry weight of the crown, with total sugars comprising 4.8% of this amount.

Refractometer

Brown and Zerban (6) describe the use of the Zeiss refractometer. It is designed for quick, easy determination of the total solid content of plant sap. The total solid content is assumed to be total soluble sugars. To obtain the approximate percent sugar in a plant, a few drops of sap are squeezed onto the prism of the refractometer and the sugar percentage, in percent sucrose equivalent, is read from the refractometer scale. This makes possible a great many refractometric determinations in a short time.

The accuracy of this method of carbohydrate analysis is hard to ascertain. Variation was found to exist between individual stalks, inter-

nodes, and varieties of sugar cane seedlings in an experiment conducted by

Herbert (19). He felt the reliability of this method would depend on the

uniformity of sampling and method of extraction. An average of several

readings seemed to be the best estimate of the actual percent sugar, but

even this was highly variable.

In an experiment with cantaloupes, Barham and McCombs (4) found a

good correlation between refractometer readings and total sugar content,

but whether this relationship could be said to be true for grasses is not

known.

Chemical Analysis for Soluble Carbohydrates

To discuss adequately all the methods for determining various sugars

in plants would require much space, even though many are quite similar.

Many people have analyzed plant tissue for the purpose of relating carbo-

hydrate content to regrowth of the plants. Sullivan (36) summarized some

of his own work and cited some of the outstanding references along this

line of research. He also prepared a mimeographed document[6] in which he

described in detail the series of steps for analysis of forage plants

which is used at the U. S. Regional Pasture Laboratory at University Park,

Pennsylvania. This analysis, even though it is probably one of the best

for plant tissue analysis, is a long, complicated procedure at best, and

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[6]Sullivan, J. T. Methods for analysis of forage plants, with par-
ticular reference to carbohydrate constituents. Personal communication,
Circa, 1960.

requires a well-equipped laboratory for efficient operation.

Dreywood (13) drew attention to a color reaction brought about by the addition of anthrone in sulfuric acid solution to solutions of carbohydrates. The solution was a clear green if a carbohydrate was present, and usually a brown when other organic materials were present. Later, Morris (25) developed a technique using this color reaction for the estimation of carbohydrates. He said that this color was produced with the same intensity by equivalent amount concentrations of all carbohydrates, including reducing sugars, but it was not given by other reducing agents, such as proteins or fats. Morris used an Evelyn photoelectric colorimeter with a 620 millimicron filter for the determinations and it proved very effective. The anthrone-sulfuric acid solution darkened with time. Therefore, fresh reagent had to be prepared daily and a known sugar standard included with each series of unknowns.

Sattler and Zerban (32) have indicated that the color produced in the anthrone reaction is due to a dehydration of the sugar, with the resultant formation of a furfuraldehyde derivative which condenses with the anthrone to give the characteristic color.

Others have used this method for the study and determination of various carbohydrates. Koehler (22) conducted an investigation into the possibilities of adapting the anthrone-sulfuric acid method for the differentiation of the various polysaccharide carbohydrates by the anthrone reaction rate and color intensity. Scott and Melvin (33) conducted an experiment as to the individual effects of the various factors and errors involved in the anthrone method for determining dextrans. Then they

designed a procedure for improving the precision of the method. This was
possible only after all the sources of error were analyzed and carefully
evaluated for magnitude of effect.

Loewus (23) suggested the use of ethyl acetate for eliminating the
necessity of making fresh reagent each day. Since the anthrone-sulfuric
acid reagent darkened with time, he mixed the anthrone with ethyl acetate
and added the mixture to the carbohydrate solution. Sulfuric acid was
then added. Loewus postulated that this eliminated errors due to darkening
of the reagent. The accuracy of the method was not altered by the presence
of the ethyl acetate.

Goss (16) found that careful standardization of the heating and cool-
ing steps in her procedure were necessary for accurate quantitative results.
This was in agreement with the results of other workers (5,22,25,32,33).
She studied the effects of anthrone purity and concluded that the purity
of anthrone was not responsible for variable and inconsistent results.
Even though the anthrone reagent broke down with time, the decomposition
was much slower under refrigeration. Therefore, the reagent would probably
give accurate results over a period of 4 days if kept refrigerated. She
found that it was possible to obtain results on very dilute solutions of
dextrose to concentrations of 5 ppm.

Barnett and Miller (5) described a method for determining the
soluble carbohydrate content of dried samples of grass silage, using the
anthrone reaction. They took 1 gm. of dried silage material, added 100
ml. of 0.25 N sulfuric acid in a 500 ml. conical flask, and simmered for
30 minutes under reflux. An aliquot of this extract then was diluted to

the proper concentration for analysis of sugar content. They obtained

reliable results and felt this method could be used to advantage in plant

tissue analysis.

CHAPTER III

METHODS AND PROCEDURES

The field aspects of this investigation were conducted during the
summer of 1960 at the Koella Farm, Main Agricultural Experiment Station,
Blount County, Tennessee.

The experiment was located on Sequatchie silt loam and Huntington
silt loam soils. These soils are quite similar, both characterized by
high water-holding capacity and high levels of fertility which make for
optimum conditions for pearlmillet production.

General Agronomic Information

Climate

A total of 25.13 inches of rainfall was recorded at the Climatolog-
ical Station on Blount County Farm during the period of crop growth from
May 2nd to October 31st. This station is located approximately 2 miles
from the experimental area. However, it has been observed that during
the summer, in most years, more rain falls on the experimental area than
at the Climatological Station. The distribution of season precipitation
is presented in detail in Appendix D.

The average seasonal minimum and maximum temperatures were 57.5 and
82.0° F., respectively, during the same period. The daily minimum and
maximum temperatures from May 1st to October 31st are presented in Appendix
E.

Fertilizer applications and plantings

The variety of pearlmillet used was Gahi-1. It is characterized by good seedling vigor, high total yields, and generally good recovery after cutting or grazing.

Prior to seeding, a uniform application of 600 pounds per acre of 0-20-20 fertilizer and 33 pounds of nitrogen per acre, as ammonium nitrate, were drilled over the area.

On May 2, 1960, the experiment was seeded at the rate of 20 pounds of certified seed per acre. A "Planet Junior" was used for the seeding. A 0.39-inch rain on May 7th provided adequate moisture for seed germination and seedling emergence, resulting in an excellent stand.

The crop was cultivated only once, the latter part of May, when the plants were approximately 6 inches high. This gave excellent weed control, with the exception of crabgrass in a few of the plots near the end of the growing season.

The crop history of this land revealed that corn was grown the previous season on about two-thirds of the experimental area and soybeans on the other third. The experiment was laid out in seven ranges of eight plots each. The first, second, and part of the third ranges were located on the soybean land, while the remainder of the ranges was located on the corn land. Replication one, consisting of 14 plots, occupied all of range one and three-fourths of range two. Replication two occupied the remainder of range two, all of range three, and one-fourth of range four. Range three was equally divided lengthwise by the corn and soybean land. Therefore, eight of the plots of replication two were markedly affected

by the lengthwise variation in soil fertility due to the previous year's crops. This was clearly evident by taller growth, darker, greener leaf colors and increased vigor of the plants growing on the soybean land. The remainder of the experimental area which had had soybeans as a previous crop also revealed a visual nitrogen effect on the growth of the first cutting, but subsequent cuttings showed no apparent nitrogen effects.

Treatments, Experimental Plot Technique, and Sampling Procedures

Plot size

Each experimental plot consisted of seven 22-foot rows, 21 inches apart. The middle 18-foot section of the center row was harvested for yield. The four rows on either side of the center row were used for dark-room and chemical analysis samples, and the two outer rows served as guard rows. This provided ample space for preventing border effects from nitrogen movement (12).

Treatments

The grass was grown at two nitrogen fertilization levels, in combination with seven intensities of cutting management. The high-nitrogen treatments received 120 pounds of nitrogen per acre as ammonium nitrate 5 days after emergence. In addition, 60 more pounds of nitrogen per acre were applied after each of the first three cuts. This 300 pounds nitrogen per acre top-dressing plus the 33 pounds per acre applied prior to planting made a total of 333 pounds nitrogen per acre for the high nitrogen plots. The low-nitrogen treatments received only the initial 33 pounds of nitrogen

per acre broadcast prior to planting. At each nitrogen level, the grass was cut at seven different management intensities. These are presented in Table 1.

The treatments were replicated four times and arranged in a randomized complete block design. The combination of two nitrogen levels, seven cutting intensities, and four replications made a total of 56 experimental units.

Yield samples

The forage yields were taken when the grass reached the desired heights as determined by a yardstick. The yield rows were trimmed by two feet on each end, leaving a harvesting length of 18 feet. Thus, the area harvested measured 18 feet by 21 inches, or 0.00072 acre.

The plots were cut with a hand sickle. In order to insure that each plot was cut to the correct stubble height, appropriate guide bars were used on all except the 1-inch stubbles. These were close enough to the ground level to make cutting at the correct height a relatively easy task.

The harvested forage from each plot was placed in a cloth bag for oven-drying. In cases where the yield of a plot exceeded that which could be comfortably placed in one bag, the green weight was taken, and a sample for dry matter determinations was collected.

The green forage was oven-dried in a forced-air oven at 60 to 70° C. and then weighed to the nearest 0.01 pound.

Table 1.--Cutting intensity treatments used in the carbohydrate reserve experiment, Koella Farm, 1960

Symbols used in text	Description of cutting intensity
20-1	20-inch growth cut down to a 1-inch stubble
20-3	20-inch growth cut down to a 3-inch stubble
20-6	20-inch growth cut down to a 6-inch stubble
30-1	30-inch growth cut down to a 1-inch stubble
30-6	30-inch growth cut down to a 6-inch stubble
30-10	30-inch growth cut down to a 10-inch stubble
EB-4	Early bloom growth cut down to a 4-inch stubble

Refractometer readings

Immediately after or just before a plot was cut, and 4 days later, a one-half inch section of culm was removed from the stubble at one-half inch above the ground. This provided a uniform sampling height for all the treatments in the experiment.

From this piece of stem, the soluble carbohydrate content of the plant sap was determined by the use of a portable refractometer. This was an Atago refractometer made by Atago Optical Works Co., Tokyo, Japan. It has a range of 0 to 32% sucrose with an accuracy of ± 0.2%.

A drop of sap was squeezed, with pliers, onto the prism of the refractometer and the cell sap concentration, sucrose equivalent, was read directly, as a percent, from the instrument.

Three readings were taken on representative stubble samples from each plot on the day of cutting. These were taken in order to obtain a better average of the actual percent soluble carbohydrates in the plants of each plot (19).

Four days after cutting, three more readings were made in the same manner in each plot. At this time, the plants in most treatments were showing signs of regrowth, an inch or more in length.

Dark-room samples

The dark-room stubble samples were obtained by digging exactly 12 inches of row. A block of soil 6 inches wide, 12 inches long, and 6 inches deep was removed along with the plant stubble. This block included most of the roots.

After tagging the samples for identification, they were brought into the laboratory for further preparation. The soil was first removed by gentle shaking and the remainder was removed by washing. The plants then were weighed and replanted in vermiculite. A nutrient solution was prepared for watering the plants in the vermiculite.

After the plants had been watered, they were moved to the dark-room. This was a small basement room with two small windows. Both windows and the door were sealed off with cardboard, making the room completely light-free. A green light-bulb furnished the necessary light for watering and observation of the plants. This room contained overhead steam pipes which kept the room temperature near 100° F. at all times. The plants died within a week, making very little regrowth. It was concluded that the high temperature was responsible for the premature death of the plants, and that a move to a cooler place was necessary.

A small room was located which had a constant room temperature of approximately 75° F. This was an ideal dark-room, having no windows, and a solid-panel door. Again, a green light bulb furnished the necessary light. At this location, the plants lived longer, but did not make any appreciable regrowth. A close check revealed a fungus growth on the surface of the vermiculite and plant stubble. The fungus proved to be Rhizoctonia spp. and was controlled with pentachloronitrobenzene (PCNB), sold under the trade name of "Terraclor". This allowed for more plant regrowth, but still not enough for a sufficient analysis.

Then, for a time, the soil was left on the roots and the samples were enclosed in a bag to secure the soil and roots. They were weighed

and placed in the dark room. These were treated also for control of the Rhizoctonia spp., and at first appeared to be making good growth. But it soon became apparent that they too were not suitable for analysis because of partial decomposition, withering, and a dried, dead appearance. Therefore, it was decided to abandon this phase of the experiment because of insufficient growth for measurement and analysis. The reasons for the failure of this phase of the experiment are somewhat obscure. However, the transplanting procedure and change in environments were most likely the primary causes of failure. Mays[5] succeeded with his dark-room experiment, but he grew the pearlmillet in crocks in the greenhouse. The plants were cut, and the entire crock was placed in the dark-room. His procedure produced no transplanting shock and there was little change among the two environments.

Chemical analysis procedure

The samples for chemical analysis for soluble carbohydrates consisted of entire plants from 18 inches of row. They were either pulled or dug, vigorously shaken to remove the excess soil, placed in plastic bags, and tagged for identification. They were then moved to a freezer. The entire operation, from the time of removal from the soil to placement in the freezer, was usually less than an hour. The samples were quick-frozen overnight at -20° F., then placed in storage at 0° F.

While the plants were still partially frozen, the roots were removed at the base of the culms. The culms and leaves were removed at varying heights corresponding to the scheduled cutting treatment intensities. The stubble was saved and put immediately into a small paper bag, placed in a forced-air drying oven and dried for 48 hours at 70° C. (5). The samples

then were ground in a Wiley mill and stored in air-tight bottles. From
selected samples, a 0.5 gm. sample was analyzed for soluble carbohydrate
content.

The anthrone method of carbohydrate analysis seemed to be well suited
to the needs of this experiment. Considerable variation existed in the
procedure as used by various workers. Therefore, the method used in this
experiment was adapted from several sources (5,16,22,25,33) and is de-
scribed as follows:

1. Place exactly 0.5 gm. sample of air-dry forage in Soxhlet ex-
   traction thimble.

2. Put approximately 100 ml. distilled water in a 500 ml. conical
   flask and set up Soxhlet extraction apparatus.

3. Extract for 1 hour, cool, and make to volume in 100 ml. volu-
   metric flask.

4. Take a 1 ml. aliquot and dilute it until the final concentra-
   tion is between 0 and 60 ppm.

5. Place in a bottle and store in freezer at 0° F.

6. Prepare anthrone reagent by adding 100 ml. concentration sul-
   furic acid to 0.2 gm. anthrone ($C_6H_4COC_6H_4CH_2$).

7. Pipette 10 ml. of this reagent into Evelyn colorimeter test
   tubes and cool to 0° F.

8. Carefully layer 1 ml. of the extracted solution onto the
   anthrone-sulfuric acid solution.

9. Insert immediately into an ice-water bath until all samples
   have been pipetted.

10. Quickly mix the solutions and place in a boiling water bath for exactly 8 minutes.

11. Remove, cool for 10 minutes, and read in percent light trans- mission with the Evelyn colorimeter.

12. Calculate the percent soluble carbohydrates from a standard sugar curve previously developed.

Samples for nitrogen analysis also were collected. After the yield samples for each treatment at each cutting date had been dried, weighed, and the yields recorded, they were compounded into one composite sample and ground in the Wiley Mill. Each of these samples was mixed thoroughly and a small, random portion was placed in an air-tight bottle. These were later analyzed for total nitrogen by the Kjeldahl-Gunning Method (3). This method is described in detail in Appendix A.

Presentation of Data and Statistical Analysis

Dry matter yields of harvested forage are presented in terms of oven-dry yields in pounds per acre. Nitrogen percentages were determined on the total forage harvested for yield, for each treatment, at each date of cutting. Recovery of nitrogen in pounds per acre, which is the amount of nitrogen in the harvested forage, was computed from the two afore- mentioned measurements. The refractometer readings, an average of three individual measurements, are presented as the percent cell sap concentra- tion, abbreviated as "C.S.C." in text. The heights at the time of cutting are reported in inches, a measurement of the length of the plants from the base of the culms to the top of the leaves bent over in the natural

position. The data for each cutting date and cutting intensity are pre-
sented in Appendix B for the low nitrogen treatments and in Appendix C
for the high nitrogen treatments.

An analysis of variance was conducted on the total seasonal dry
matter yield. For the purpose of differentiating between treatment means,
the multiple range test developed by Duncan (14) was utilized. The means
used in this test are reported as the means of the four replications.
Elsewhere in the text, the dry matter yields, nitrogen and C.S.C. percent-
ages, and nitrogen recoveries are reported as the means of the observations
taken of each treatment, nitrogen level, or cutting intensity.

The dry matter yields, nitrogen and C.S.C. percentages, and nitrogen
recoveries, plotted over time, were analyzed graphically.

In an attempt to determine the relationship of the percent C.S.C.
to the date of cutting, a regression analysis for these variables was
carried out. These data are shown graphically, along with the calculated
regression lines.

The total water-soluble carbohydrates, glucose equivalent, was deter-
mined by the anthrone method of carbohydrate analysis. These are reported
as the percent of total water-soluble carbohydrates in stubble dry weight.
These data are shown for selected treatments and individually compared to
the C.S.C. percentages, at the time of cutting, for the same treatments.

In the discussion of the results, wherever the term "significantly
different" is used, it will be taken to mean "significantly different at
the 0.05 level of probability", unless otherwise qualified. Wherever the
symbol * is used, it will be taken to mean "significant at the 0.05 level

of probability"; the symbol ** will be taken to mean "significant at the 0.01 level of probability".

Whenever the term "initial regrowth" is used in the text, it will be taken to mean "the regrowth taking place during the first 4 days after cutting".

The amount of C.S.C. utilized during the first 4 days after cutting for initial regrowth was obtained by dividing the difference in percent C.S.C. at the time of cutting and that found 4 days later, by the percent at the time of cutting. Whenever the term "amount C.S.C. used" appears in the text, it will be taken to mean the percent C.S.C. as determined by the above computations.

Whenever the terms "reserve carbohydrates or carbohydrate reserves" are used, they will be taken to mean the water-soluble carbohydrates which are readily available for plant use.

CHAPTER IV

RESULTS AND DISCUSSION

Results are discussed according to the individual variables studied.
These variables are: (1) dry matter yield, (2) percent nitrogen in the
dry matter harvested, (3) nitrogen recoveries from the harvested forage,
(4) percent C.S.C. at the time of cutting and 4 days after cutting, and
(5) percent C.S.C. utilized for initial regrowth during the first 4 days
after cutting.

Dry Matter Yields

The summary of the results of the analysis of variance of total
seasonal dry matter yields is presented in Table 2. The combined effects
of nitrogen fertilization and cutting intensities on Gahi-1 pearlmillet
were significantly different at the 0.01 level of probability, as indi-
cated by the F-test. The high level of nitrogen and the less severe
cutting intensities resulted in higher dry matter yields (Table 3). All
of the high nitrogen treatments, except the 20-1 and 30-1 cutting in-
tensities, resulted in significantly larger dry matter yields than did
the low nitrogen treatments. The yields of the low nitrogen treatments
tended to be inversely proportional to the intensity of cutting. The 20-3
and 30-1 cutting intensities were the exceptions. Even though these dry
matter yields were not significantly different from the yields of the
other low nitrogen treatments, they were unusually high and unusually low,
respectively. The reasons for this unusually high dry matter yield for

Table 2.--Analysis of Variance of the Total Seasonal Dry Matter Yields
of Gahi-1 Pearlmillet at Two Levels of Nitrogen and
Seven Intensities of Cutting

Source of Variation	d.f.	Estimate of Variance	F
Replications	3	0.647	0.19
Treatments	13	2.094	6.23**
Error	39	0.336	

C.V. = 24.1%.

Table 3.--Total Seasonal Dry Matter Yield Comparisons Between
Nitrogen Levels and Cutting Intensities

Nitrogen Applied Lbs./A.	Cutting Intensity	No. of Cuts During Season	Dry Matter Yields	
			Pounds Per Acre	Multiple Range Groupings[1]
33	30-1	4	5,218	
	20-1	5	5,614	
333	20-1	5	5,750	
33	20-6	7	5,890	
	30-6	5	6,438	
	30-10	5	6,998	
	EB-4	4	7,000	
	20-3	6	7,028	
333	30-1	4	7,444	
	20-3	7	7,972	
	30-6	6	8,712	
	20-6	8	9,266	
	EB-4	4	9,280	
	30-10	6	9,312	

[1]Any two means having the same perpendicular line beside them are
not significantly different at the 0.05 level of probability.

the 20-3 cutting intensity are not known entirely. However, the regrowth on these plots was observed to be faster and more uniform throughout the growing season than that in most of the other treatments. Also worthy of note is the fact that the nitrogen recovery from this treatment was very low, indicating that the harvested forage contained a large amount of stemmy material. This situation would be conducive to high dry matter yields, but a lower percent nitrogen in the harvested forage. The stand on the 30-1 cutting intensity treatment at the low level of nitrogen fertilization was seriously depleted after the first cut. Therefore, this probably resulted in the unusually low yield.

At the low nitrogen level, cutting intensity seemed to influence dry matter yields to a greater degree than at the high level of nitrogen fertilization.

Seasonal Distribution of Dry Matter Yields, and Nitrogen and Cell Sap Concentration Contents

The dry matter yields, percent nitrogen recoveries, and the percent C.S.C., at the time of cutting, 4 days later, and percent used in initial regrowth for the seven cutting intensities were averaged at each level of nitrogen fertilization. These means are presented in Table 4.

The percent nitrogen content, dry matter yields, and nitrogen recoveries were higher for the high nitrogen treatments than those for the low nitrogen treatments. The percent C.S.C. was lower at the time of cutting in the plants grown under the high nitrogen treatments, but higher than those grown under the low nitrogen treatments 4 days after cutting.

Table 4.--Dry Matter Yields, Nitrogen Content of Harvested Forage, and Cell Sap Concentration of Gahi-1 Pearlmillet, as Influenced by Nitrogen Fertilization, Ignoring Cutting Intensities[1]

N Applied Lbs./A.	No. of Observations	Height at Cutting In.	Cell Sap Concentration			D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage, Lbs./A.
			At Cutting %	4 Days After Cutting %	Amount Used %			
33	142	27.8	3.88	1.98	48.8	1245	2.08	25.9
333	157	29.6	3.55	2.14	39.6	1471	3.49	51.3

[1]Means of the total observations taken during the season.

When the difference between these two percentages is divided by the percent at the time of cutting, the percent C.S.C. used in the first 4 days is obtained. This percentage allows comparisons to be made, on an equivalent basis, of the amounts of C.S.C. used for initial regrowth. This difference in C.S.C. percent is assumed to be mostly available reserve carbohydrates. About 10 percent more C.S.C. was used for initial regrowth in the low nitrogen treatments than in the high nitrogen treatments. When nitrogen was not a limiting factor for plant growth, less carbohydrate reserve material was utilized for initial regrowth.

A summary of the effects of cutting intensity on the dry matter yields, percent nitrogen in the harvested forage, nitrogen recoveries, and percent C.S.C., at the time of cutting, 4 days later, and percent C.S.C. used in initial regrowth is presented in Table 5. These figures were obtained by averaging the nitrogen levels for each of the seven cutting intensities. The dry matter yields and nitrogen recoveries generally were inversely proportional to severity of cutting treatment, but the relationship of percent nitrogen in the harvested forage to severity of cutting was not so evident. The data indicate that the percent C.S.C. was influenced by cutting intensity. The percentages of C.S.C., both at the time of cutting and 4 days later, were lowest for the 20-1, 20-3, and 20-6 cutting intensities, somewhat higher for the 30-1, 30-6, and 30-10 cutting intensities, and highest for the EB-4 cutting intensity. When the percent C.S.C. used in initial regrowth is considered, a trend becomes apparent. The 30-10 cutting intensity utilized less reserve carbohydrates than any of the other cutting intensities. It was the least severe cutting

Table 5.--Dry Matter Yields, Nitrogen Content of Harvested Forage, and Cell Sap Concentration of Gahi-1 Pearlmillet, as Influenced by Cutting Intensities, Ignoring Nitrogen Fertilization[1]

| Cutting Intensity | No. of Observations | Height at Cutting In. | Cell Sap Concentration | | | D.M. Yield/ Cutting Lbs./A. | N in Forage % | N in Harvest. Forage, Lbs./A. |
			At Cutting %	4 Days After Cutting %	Amount Used %			
20-1	40	24.9	3.54	1.88	46.9	1136	3.11	35.3
20-3	50	24.0	3.50	1.88	46.2	1200	2.85	34.2
20-6	59	23.8	3.28	1.85	43.6	1028	3.21	33.0
30-1	32	30.8	4.17	2.14	48.6	1583	2.80	44.4
30-6	41	30.9	3.76	2.13	43.4	1478	2.94	43.5
30-10	46	30.5	3.69	2.28	38.1	1418	2.84	40.3
EB-4	31	43.4	4.51	2.54	43.8	2101	2.46	51.7

[1]Means of the total observations taken during the season.

intensity because of the extra amount of leaf area left on the stubble. Therefore, it is logical that it would utilize less reserve carbohydrates. The 30-1 cutting intensity, a very severe cutting intensity, used the greatest amount of reserve carbohydrates, perhaps because of a larger percent C.S.C. at the time of cutting. The 20-1 and 20-3 cutting intensities used practically the same amount of reserve carbohydrates, and somewhat more than the 20-6, 30-6, and EB-4 cutting intensities, which also used practically the same amount. These data indicate that the amount of reserve carbohydrates utilized in initial regrowth is directly proportional to severity of cutting management.

Table 6 presents the dry matter yields, percent nitrogen in the harvested forage, nitrogen recoveries, and the percent C.S.C. with neither nitrogen level nor cutting intensity ignored. The average yields per plot were higher for the high nitrogen treatments and somewhat proportional to severity of cutting management. The percent nitrogen in the harvested forage was higher in the high nitrogen treatments and was fairly uniform over all cutting intensities at each level of nitrogen, but more uniform at the high level of nitrogen. Since the nitrogen percentages were relatively uniform, the nitrogen recoveries would be expected to be largely dependent upon the amount of yield, and this was the case generally. The percent C.S.C. at the time of cutting, and that 4 days later, followed the same general trend that the data in Table 5 reveals. More variability existed, but the 20-1, 20-3, and 20-6 cutting intensities at both levels of nitrogen were still lower in percent C.S.C. at the time of cutting, and 4 days later than the other cutting intensities. The 30-1, 30-6, and

Table 6.—Dry Matter Yields, Nitrogen Content of Harvested Forage, and Cell Sap Concentration of Gahi-1 Pearlmillet, as Influenced by Nitrogen Fertilization and Cutting Intensity[1]

Cutting Intensity	No. of Observations	Height at Cutting In.	Cell Sap Concentration			D.M.Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage, Lbs./A.
			At Cutting %	4 Days After Cutting %	Amount Used %			
33 Pounds Nitrogen Per Acre								
20-1	20	23.6	3.66	1.66	54.6	1123	2.56	28.7
20-3	24	23.0	3.48	1.82	47.7	1171	2.20	25.8
20-6	27	23.3	3.34	1.64	50.6	873	2.27	19.8
30-1	15	29.4	4.34	2.07	52.3	1391	1.93	26.8
30-6	19	30.7	4.14	2.19	47.1	1355	2.15	29.2
30-10	21	29.1	3.93	2.21	43.8	1333	2.03	27.0
EB-4	16	41.4	4.83	2.57	46.8	1750	1.50	26.2
333 Pounds Nitrogen Per Acre								
20-1	20	26.3	3.43	2.10	38.5	1150	3.64	41.9
20-3	26	25.0	3.52	1.94	44.9	1227	3.42	42.0
20-6	32	24.2	3.24	2.03	37.3	1158	3.80	44.0
30-1	17	32.0	4.01	2.21	45.1	1751	3.42	59.8
30-6	22	31.1	3.44	2.08	39.8	1584	3.52	55.8
30-10	25	31.6	3.48	2.34	32.5	1490	3.46	51.6
EB-4	15	45.4	4.17	2.50	40.0	2474	3.19	78.9

[1]Means of the total observations taken during the season.

30-10 treatments were a little higher in percent C.S.C. at both times and nitrogen levels, with the EB-4 treatments still the highest. In general, more reserve carbohydrates were utilized for initial regrowth in the low nitrogen treatments, with the severity of cutting influencing the percent that was used. The 30-10 cutting intensity, at both levels of nitrogen fertilization, used less reserve carbohydrates than the other cutting intensities. The 20-1 and 30-1 cutting intensities used the most reserve carbohydrates at the low nitrogen fertilization level, while the 30-1 and 20-3 cutting intensities used the most reserve carbohydrates at the high nitrogen level. In the low nitrogen treatments, the amount of reserve carbohydrate utilization was proportional to severity of cutting. This relationship, though not quite so evident at the high level of nitrogen fertilization for the 20-1, 20-3, and 20-6 cutting intensities, does continue for the 30-1, 30-6, and 30-10, and EB-4 cutting intensities.

The 20-3 cutting intensity at the low level of nitrogen fertilization was the only cutting intensity at this level of fertilization that did not follow the previously hypothesized relationship concerning reserve carbohydrates and severity of cutting. The yield for this treatment was unusually high, as shown in Table 3. The amount of reserve carbohydrates used in initial regrowth in this trement also was relatively low, indicating that for some unknown reason, this treatment was equal to or better than the 20-6 and 30-6 cutting intensities in reserve carbohydrate utilization efficiency. Since this treatment utilized reserve carbohydrates relatively efficiently, this may explain partly the unusually high total seasonal dry matter yield.

A graphic analysis of the dry matter yields, nitrogen percentages, nitrogen recoveries in the harvested forage, and the percent C.S.C. at the time of cutting and that 4 days after cutting, is presented in Figures 1 through 14.

These graphs show the relationships of these factors as they were affected by treatment and cutting date. The percent nitrogen was relatively uniform throughout the season for all treatments. Thus, the recoveries of nitrogen were affected more by the variation in dry matter yield than by the percent nitrogen at each cutting date throughout the season. Only one nitrogen percentage was determined for each treatment every date that it was cut. If all four replications of a treatment were cut on the same date, the percent nitrogen was determined on a composite sample from these four plots. With only this one average nitrogen percentage for each treatment at each cutting date, the nitrogen recoveries tended to follow the curve of dry matter yields illustrated in Figures 1 through 14. However, as exemplified in Figures 1 and 13, the percent nitrogen sometimes influenced the nitrogen recoveries.

The graphs clearly indicate that the treatments with longer intervals between cuttings and fewer cuts per season contained a larger percent C.S.C. at the time of cutting. However, appreciably more reserve carbohydrates were used during the first 4 days in those cases (Figures 1, 4, 5, 7, 8, 11, and 12).

The percent C.S.C. reached a peak at least once during the season for all of the treatments, but the time when this peak was reached varied with the treatment. These reserve carbohydrate storage peaks probably
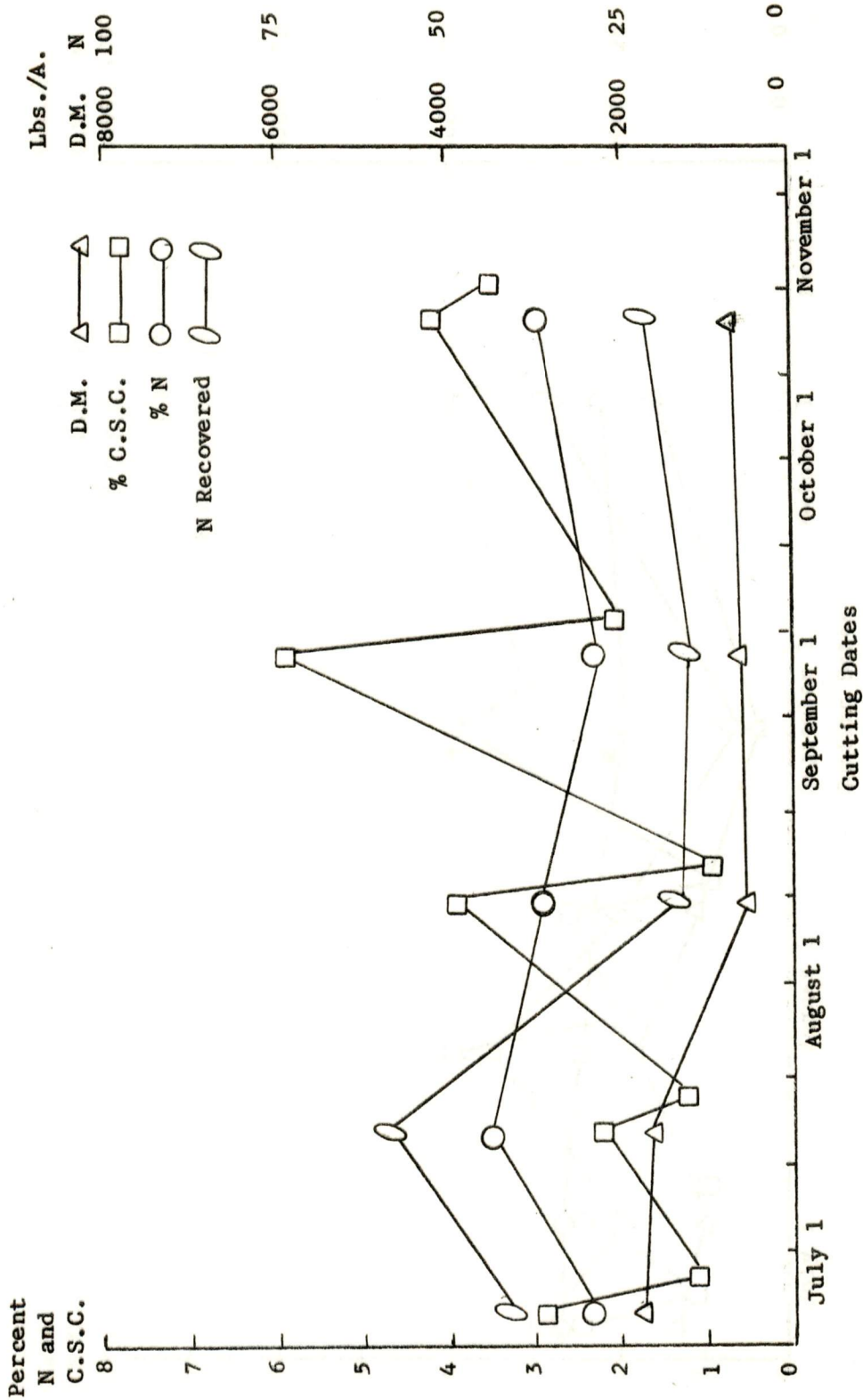
Figure 1--Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 20-1 cutting intensity and low level of nitrogen fertilization.
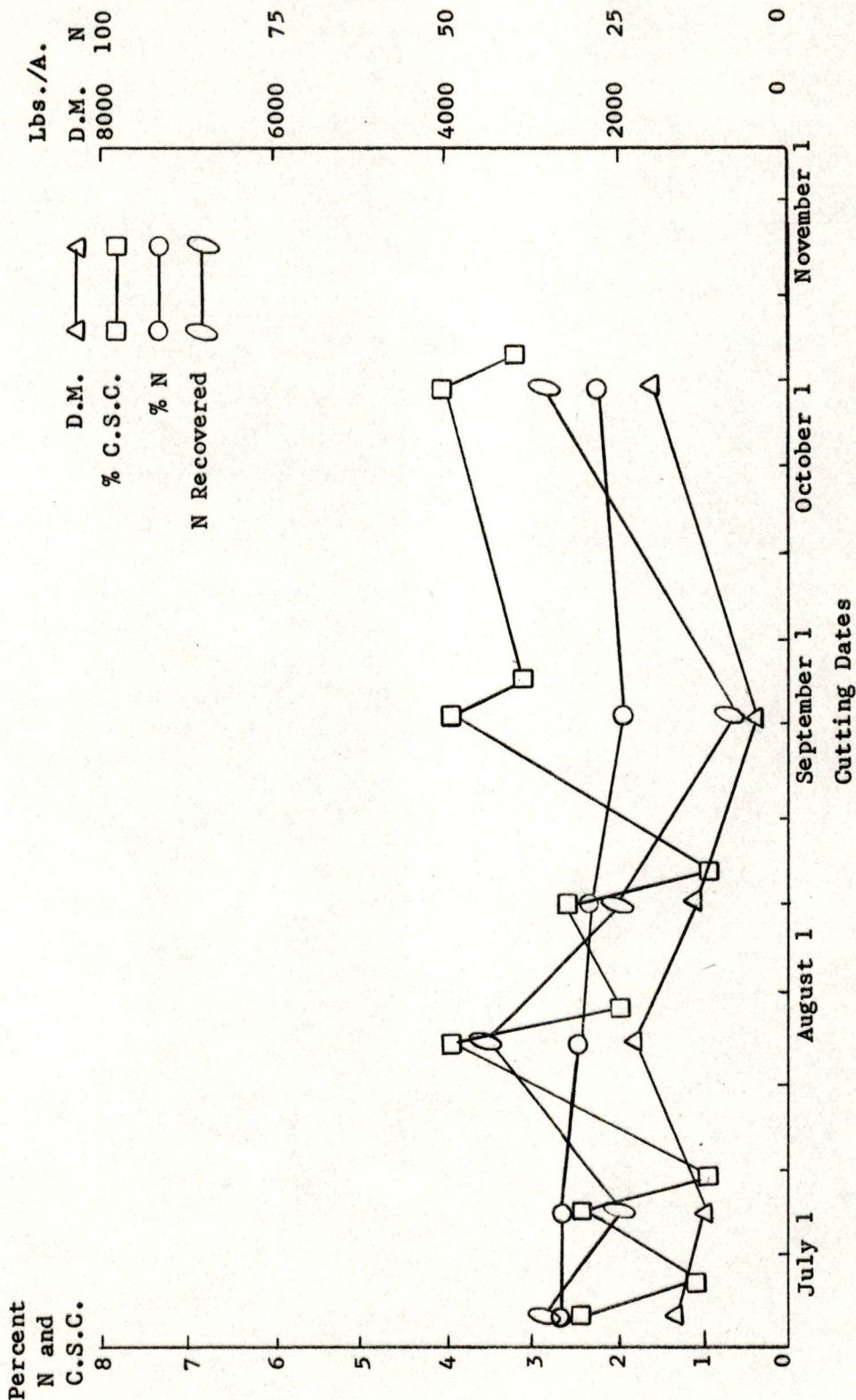
Figure 2—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 20-3 cutting intensity and low level of nitrogen fertilization.
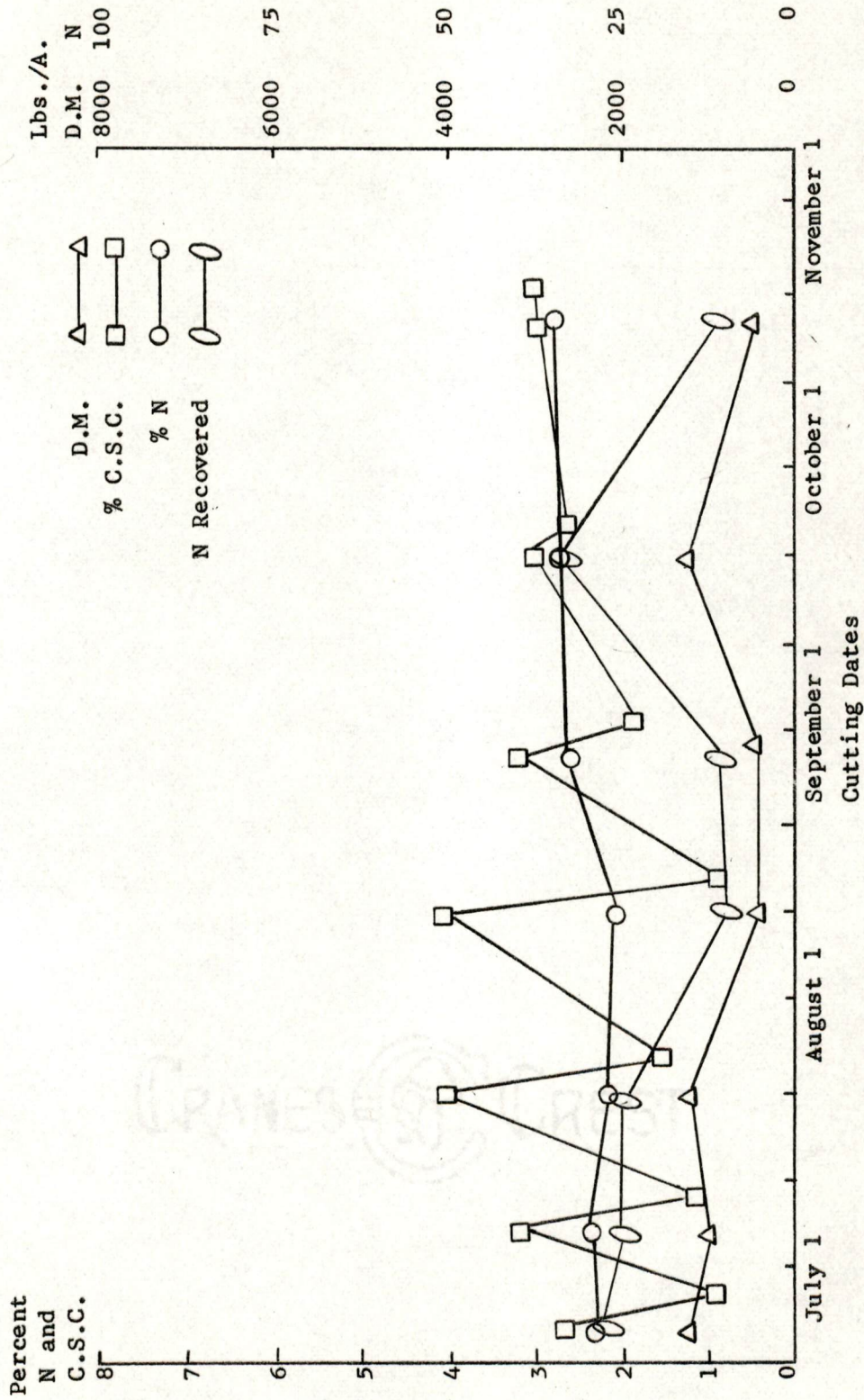
Figure 3—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 20-6 cutting intensity and low level of nitrogen fertilization.
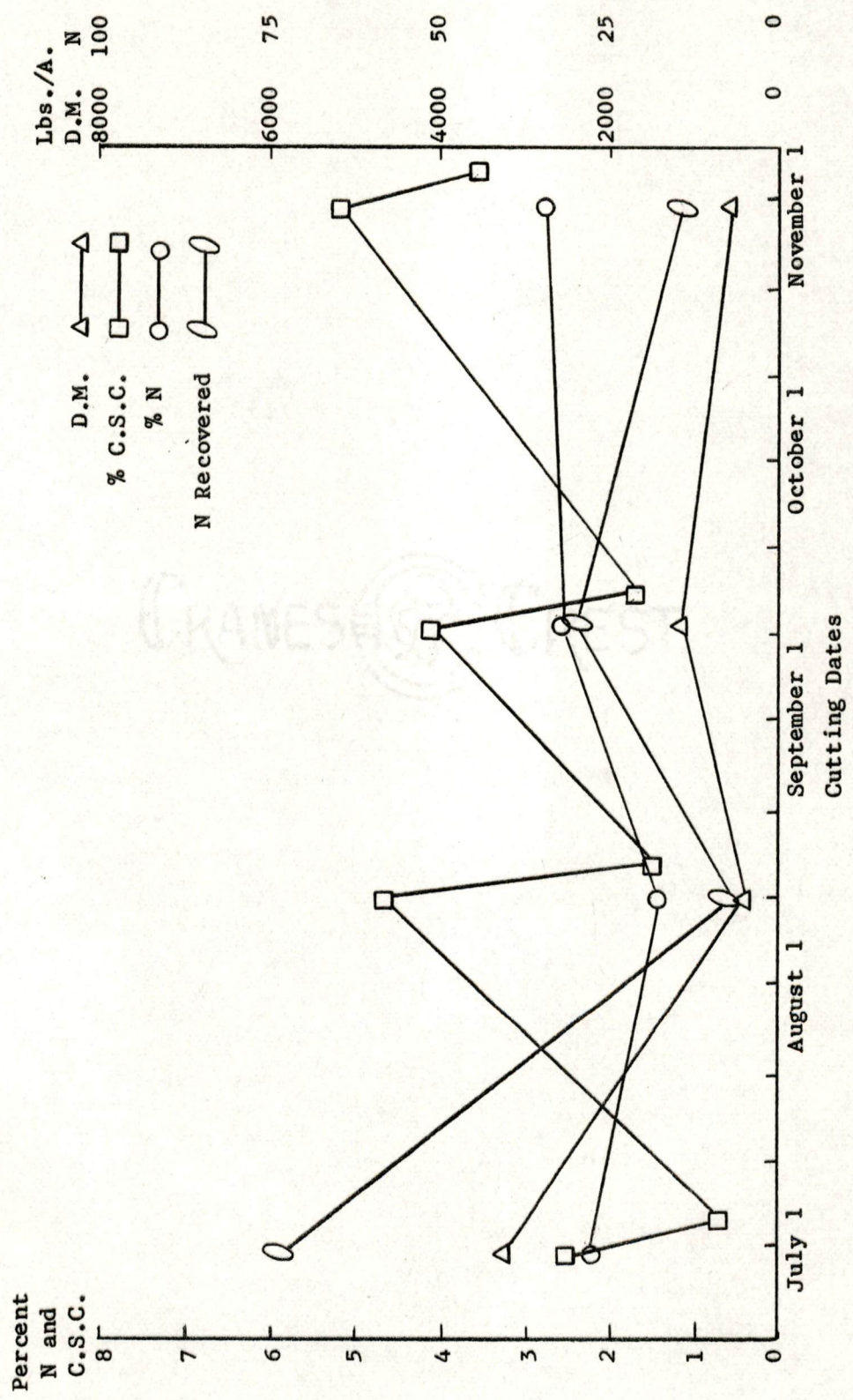
Figure 4.—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 30-1 cutting intensity and low level of nitrogen fertilization.
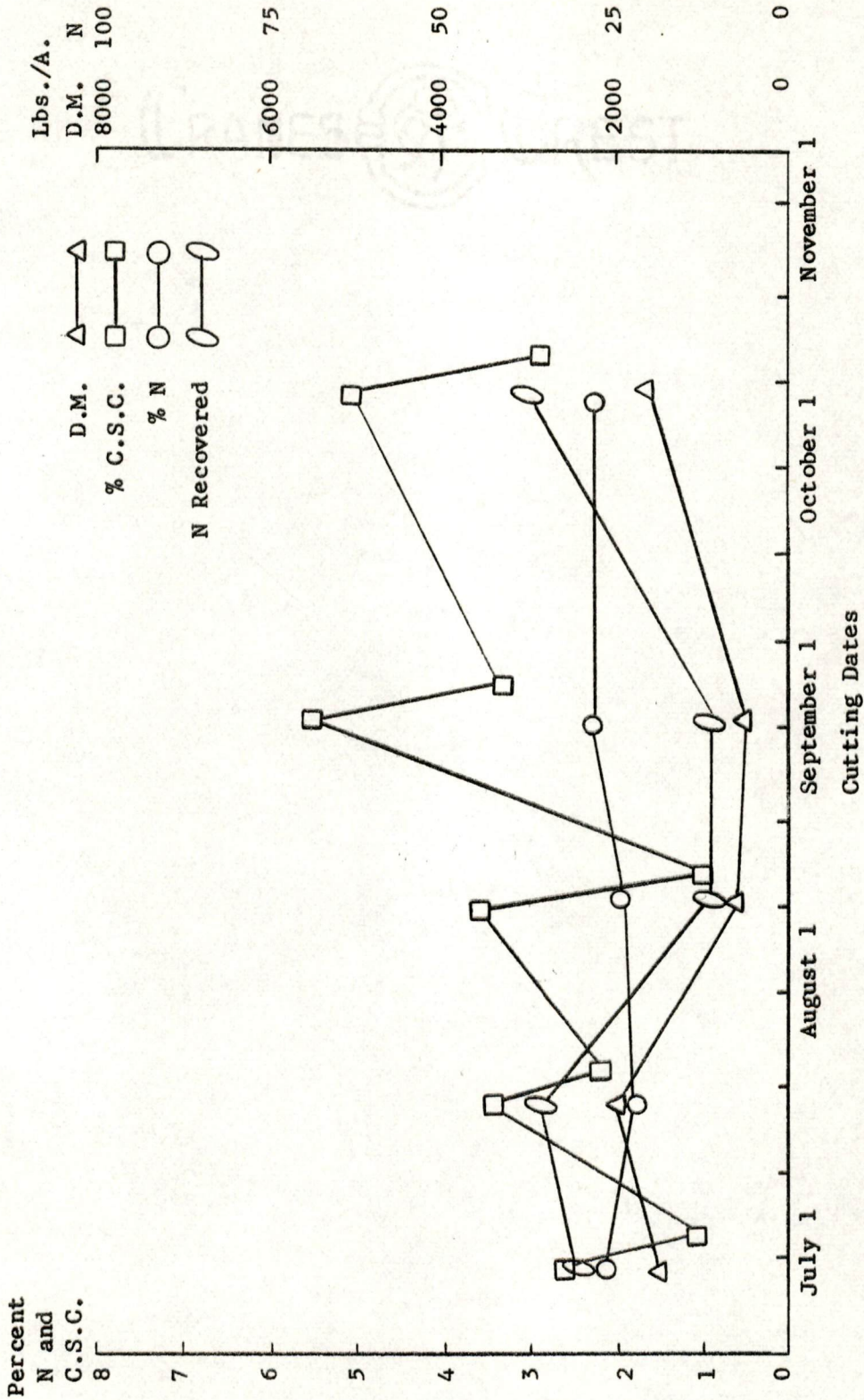
Figure 5--Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 30-6 cutting intensity and low level of nitrogen fertilization.
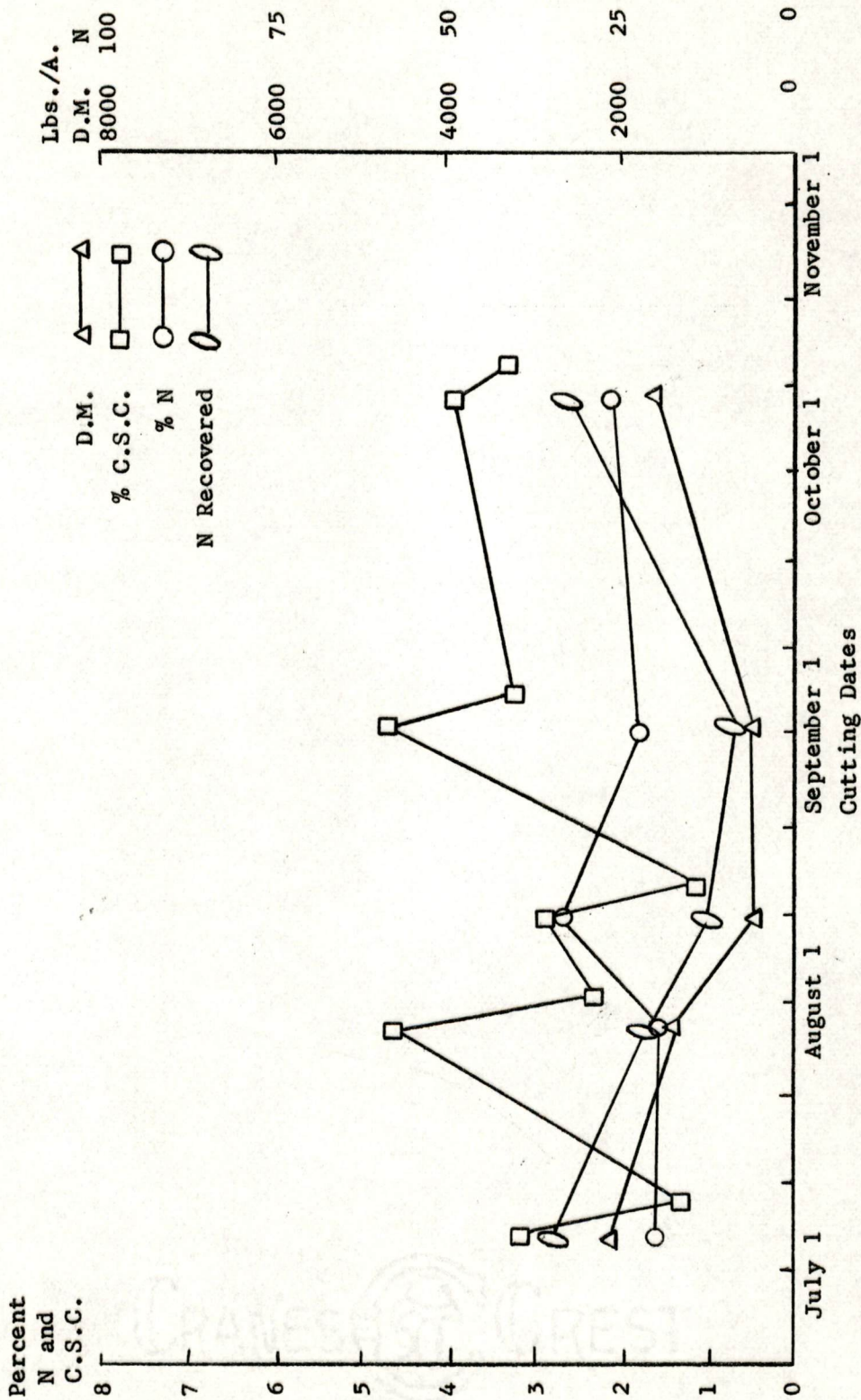
Figure 6--Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 30-10 cutting intensity and low level of nitrogen fertilization.
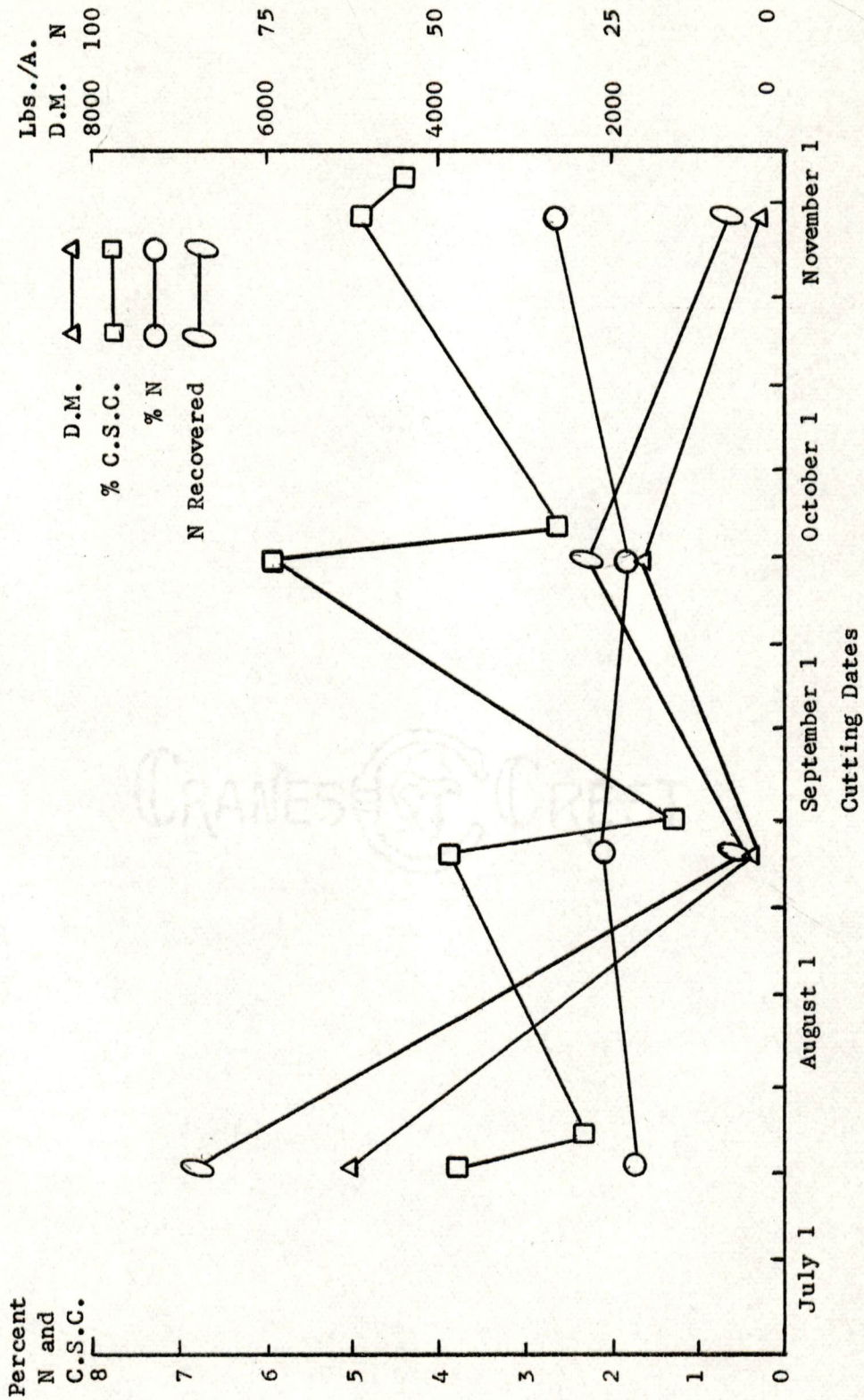
Figure 7—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the EB-4 cutting intensity and low level of nitrogen fertilization.
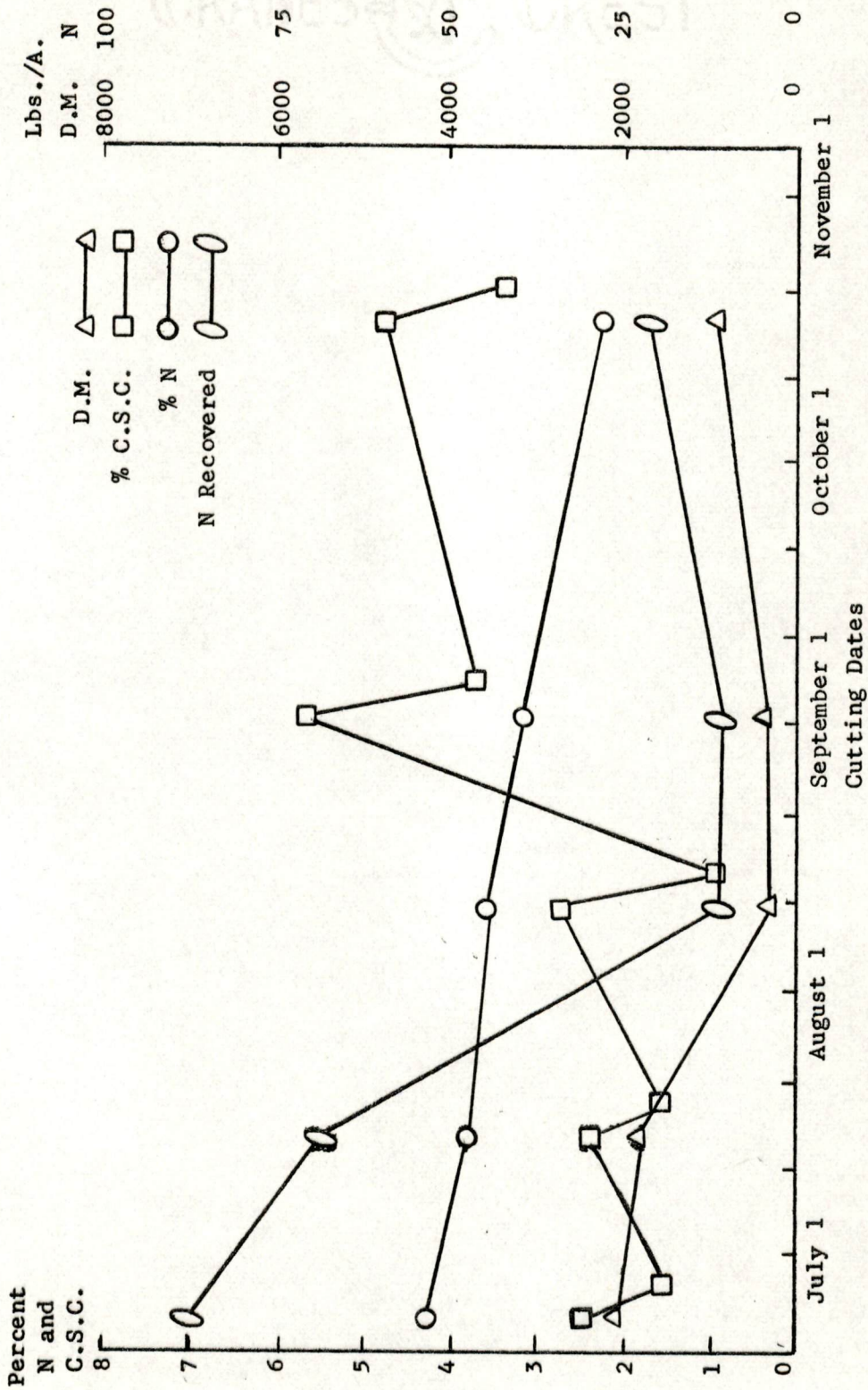
Figure 8—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 20-1 cutting intensity and high level of nitrogen fertilization.
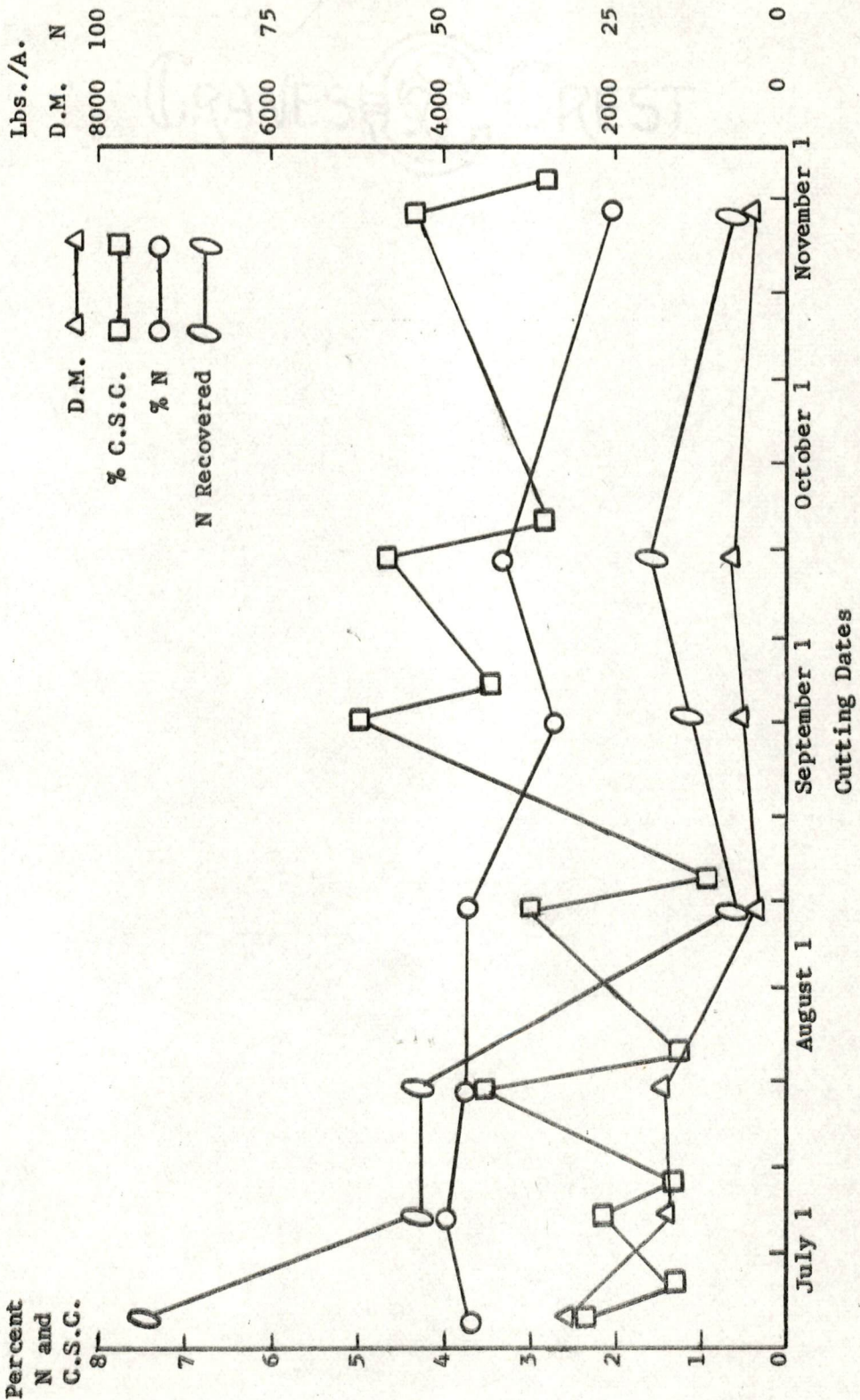
Figure 9—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 20-3 cutting intensity and high level of nitrogen fertilization.
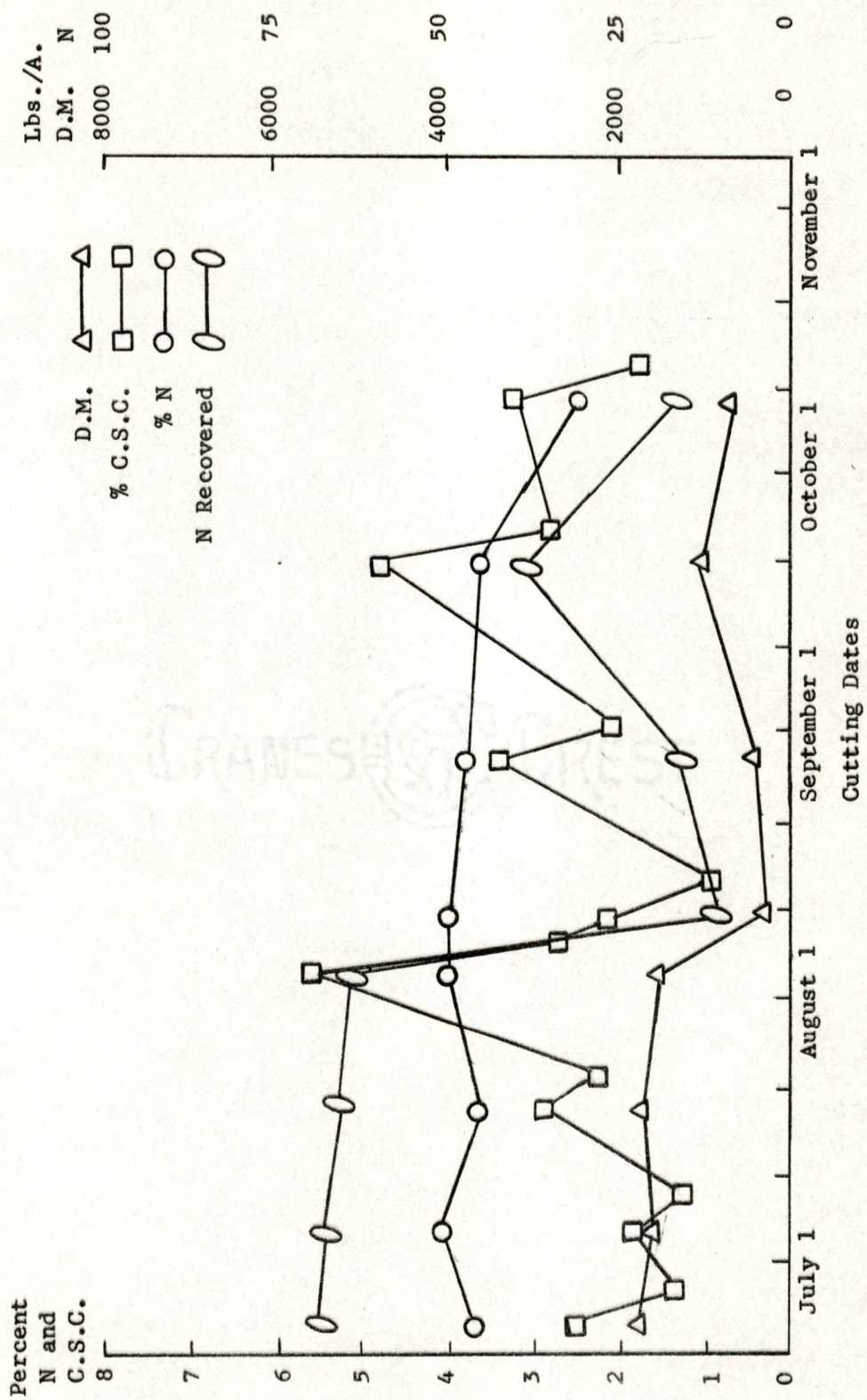
Figure 10--Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 20-6 cutting intensity and high level of nitrogen fertilization.

55
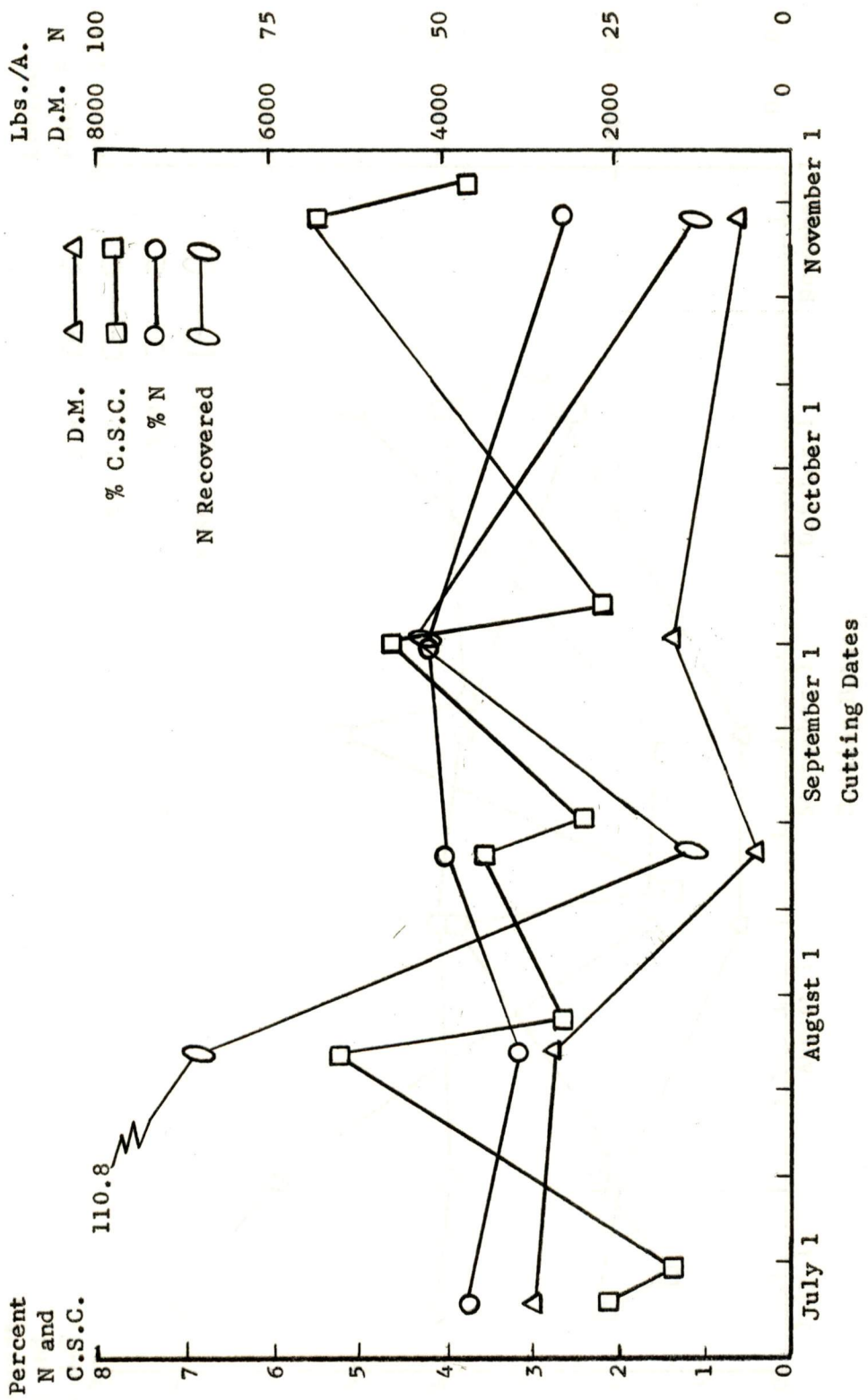
Figure 11—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 30-1 cutting intensity and high level of nitrogen fertilization.
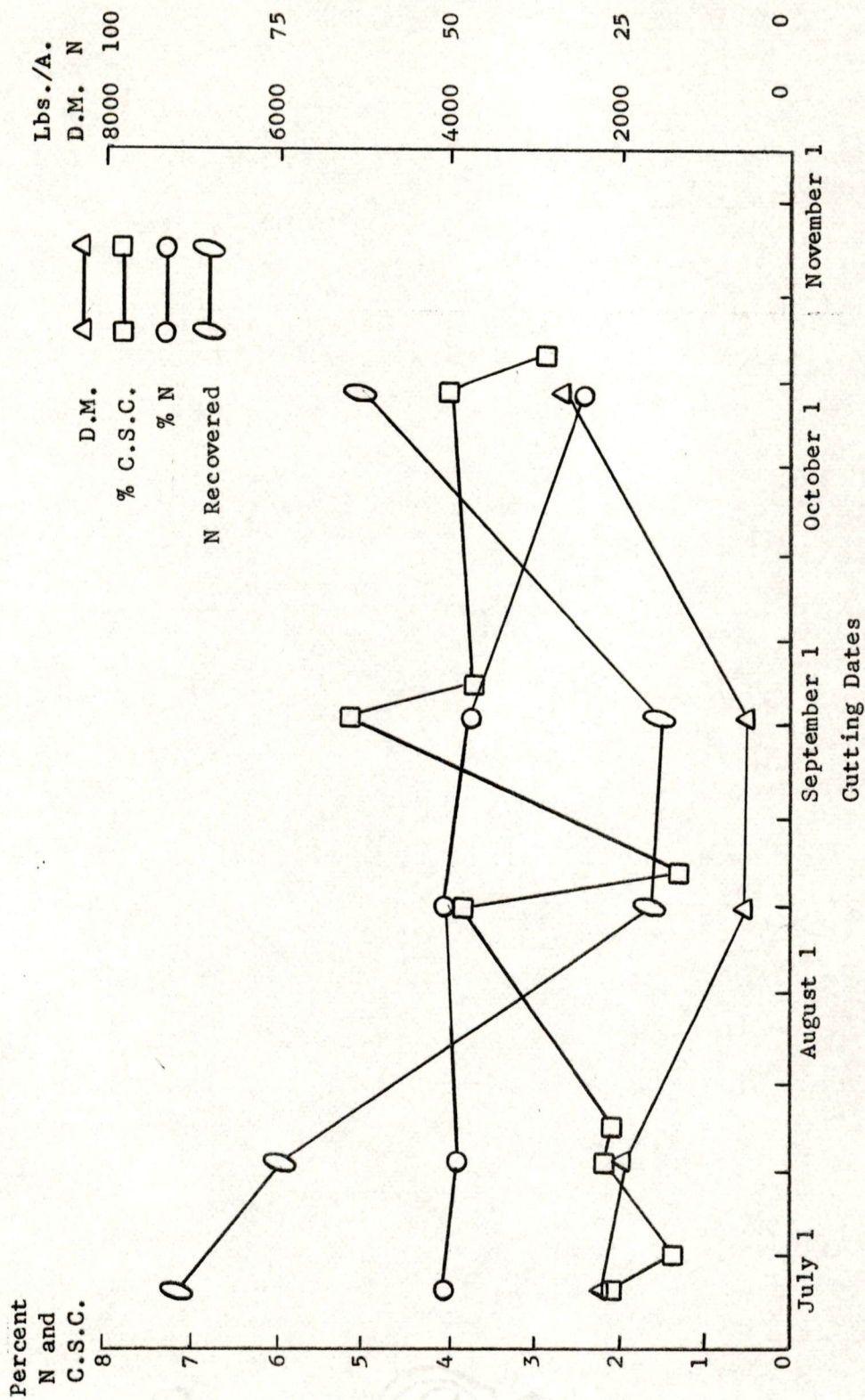
Figure 12--Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 30-6 cutting intensity and high level of nitrogen fertilization.
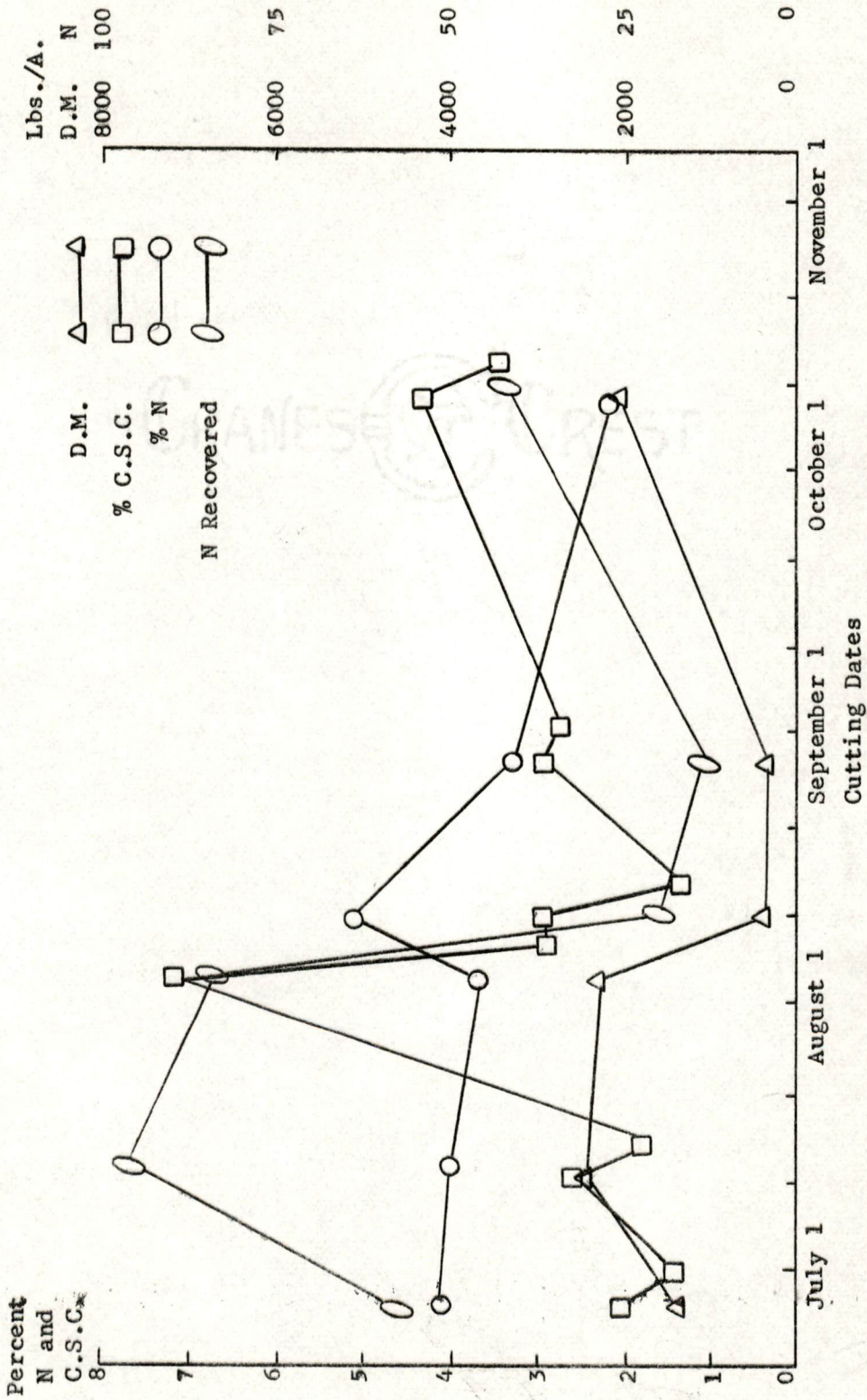
Figure 13.—Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the 30-10 cutting intensity and high level of nitrogen fertilization.
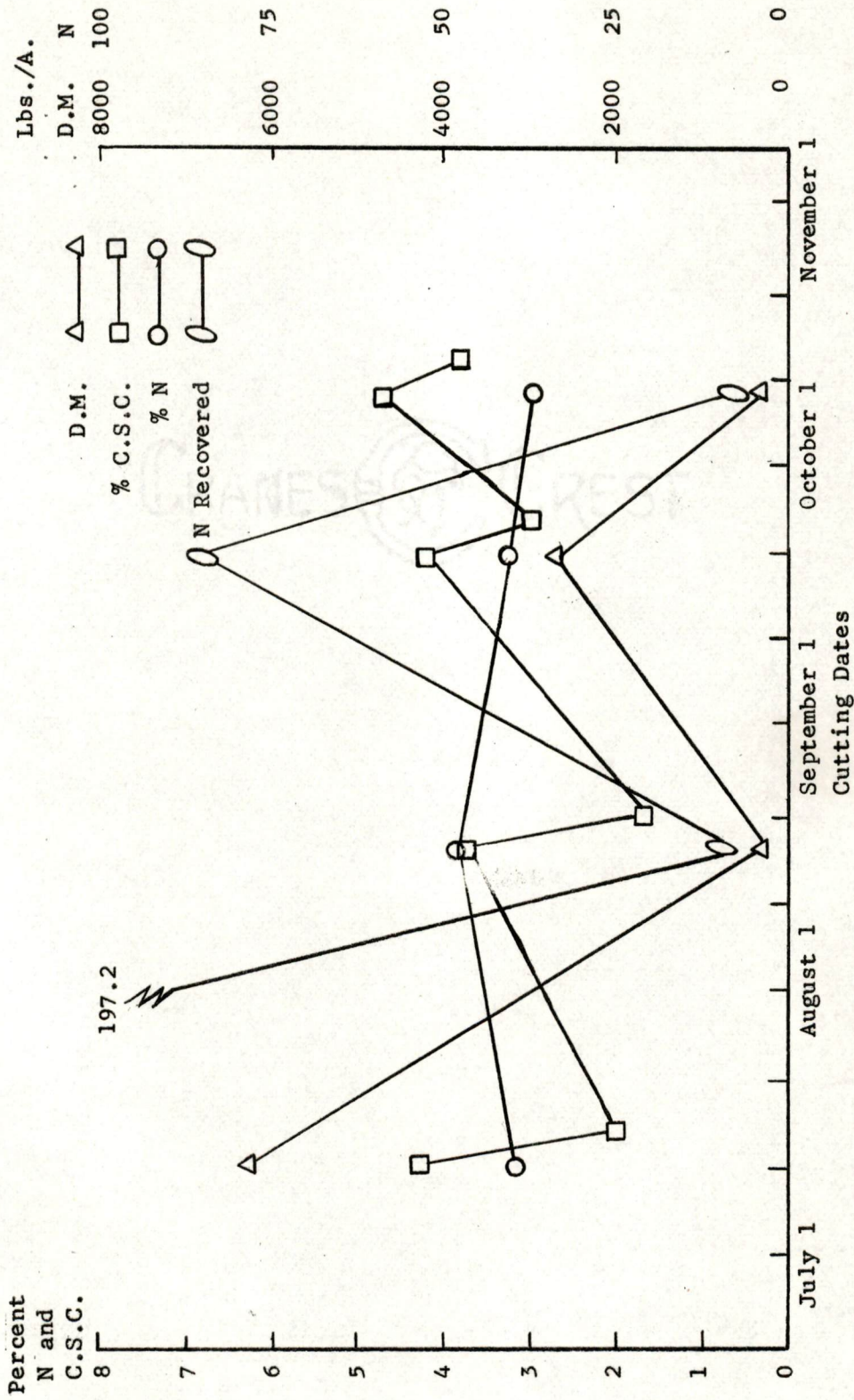
Figure 14--Seasonal distribution of dry matter yield, percent nitrogen, nitrogen recovery from harvested forage, and percent cell sap concentration at cutting and 4 days later, as affected by the EB-4 cutting intensity and high level of nitrogen fertilization.

were due to several reasons. The most logical one, and the one supported by evidence from the literature (21,24,26,27,41,42,43,44) is that the peaks come at about the time of emergence of the inflorescense. The EB-4 treatments were cut at about one-fourth bloom, thus the plants in these treatments might be expected to be relatively high in percent C.S.C. at each cutting date. However, the low nitrogen, EB-4 cutting intensity reached a higher peak on the third cut in the month of September than on any other cut. Therefore, some factors other than maturity alone apparently were involved.

In an attempt to determine the relationship between the date of cutting and the C.S.C. distribution over the season, the percentages of C.S.C. at the time of cutting those measured 4 days after cutting, and the amounts used in initial regrowth were averaged across cutting intensities for each cutting date. These were plotted against cutting dates on three separate graphs for each nitrogen level. The percentages of C.S.C. at the time of cutting for the low and high nitrogen levels are shown in Figures 15 and 18, respectively. The percent C.S.C. began to increase at the beginning of the season, reached a peak during the month of September, then decreased slowly. This indicates that September is the time of highest percent C.S.C. for an average of all the treatments at each level of nitrogen fertilization.

Figures 17 and 20 show the amounts of reserve carbohydrates used in the period of initial regrowth. These amounts are the differences between the refractometer values at the time of cutting and those taken 4 days later. These data followed the same curvilinear shape as the per-
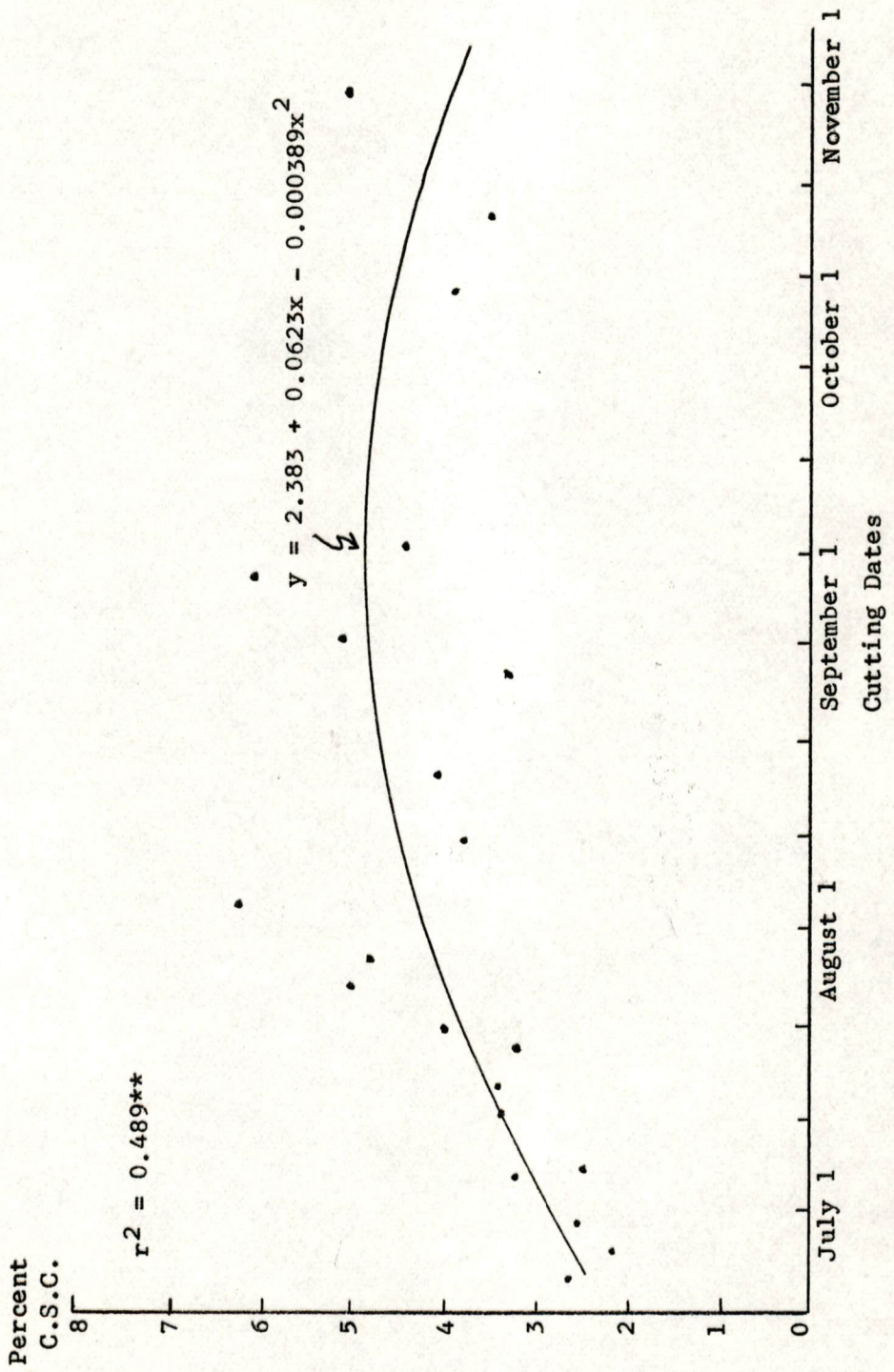
Figure 15--Seasonal distribution of cell sap concentration at the time of cutting for the low level of nitrogen fertilization, ignoring the effects of cutting intensities.
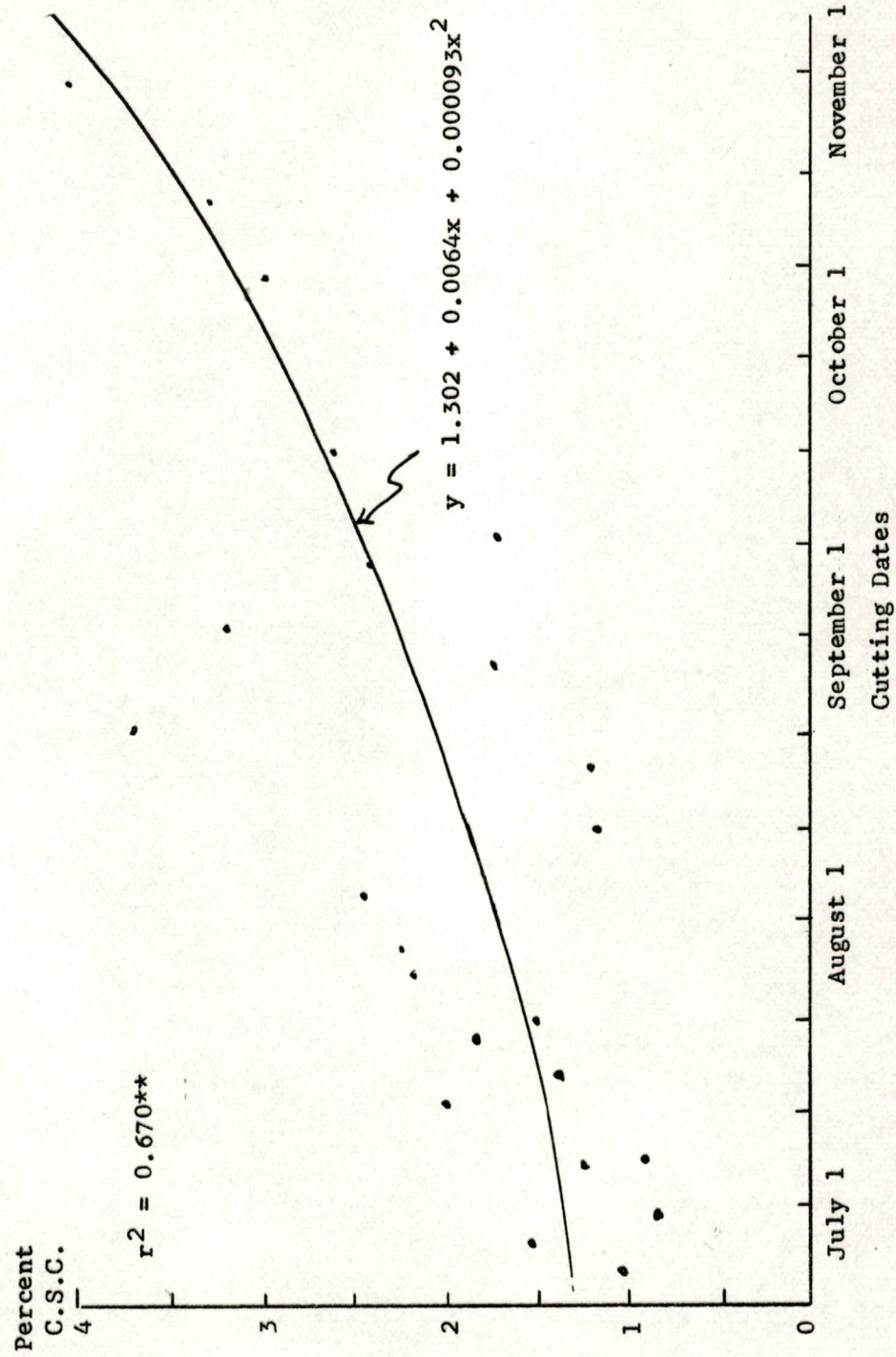
Figure 16--Seasonal distribution of cell sap concentration 4 days after cutting for the low level of nitrogen fertilization, ignoring the effects of cutting intensities.

Percent
C.S.C.

$r^2 = 0.529**$

$y = 1.081 + 0.0559x - 0.000482x^2$

Cutting Dates

July 1   August 1   September 1   October 1   November 1
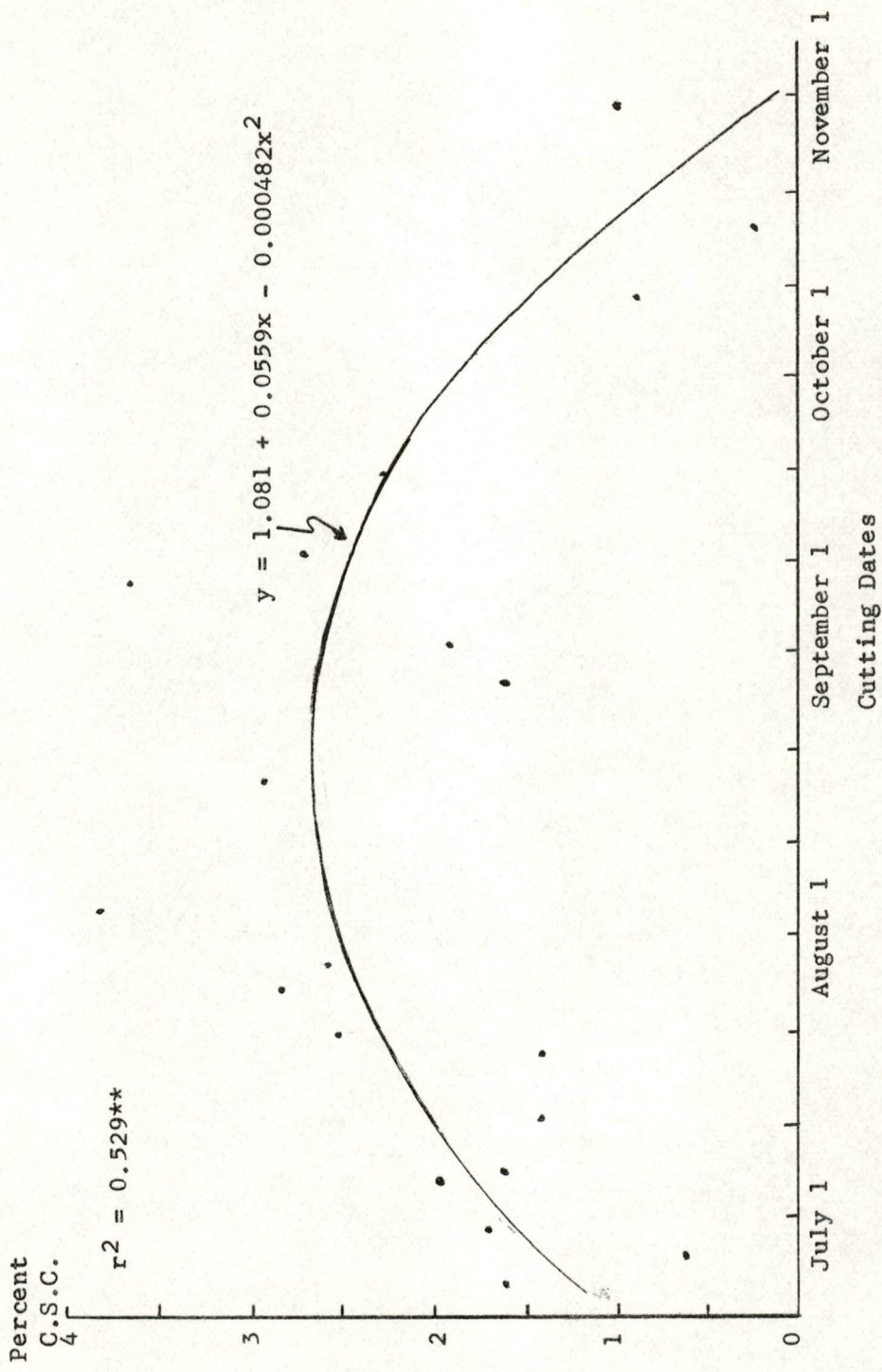
Figure 17--Seasonal distribution of cell sap concentration used in the first 4 days after cutting for the low level of nitrogen fertilization, ignoring the effects of cutting intensities.
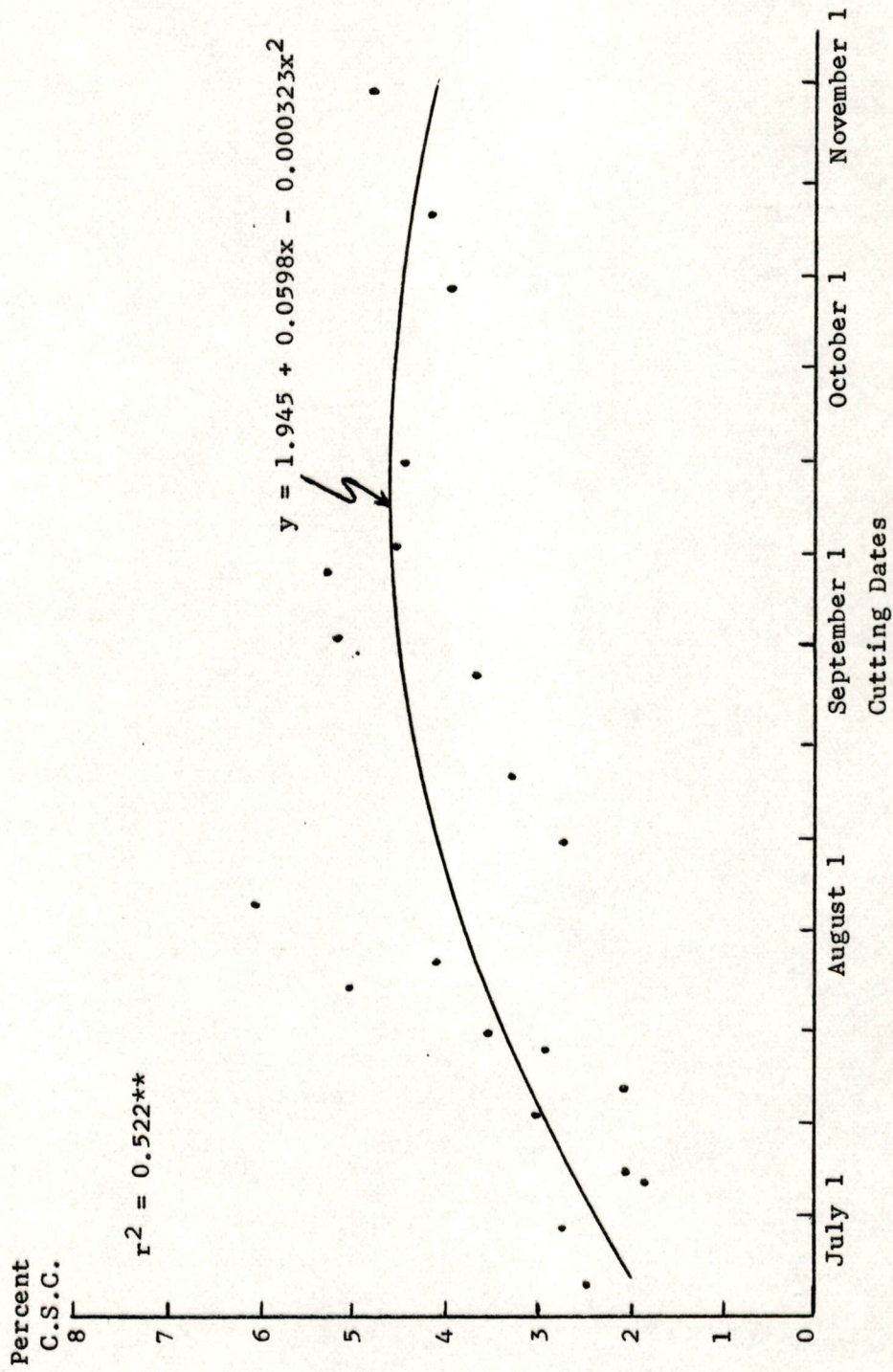
Figure 18--Seasonal distribution of cell sap concentration at the time of cutting for the high level of nitrogen fertilization, ignoring the effects of cutting intensities.
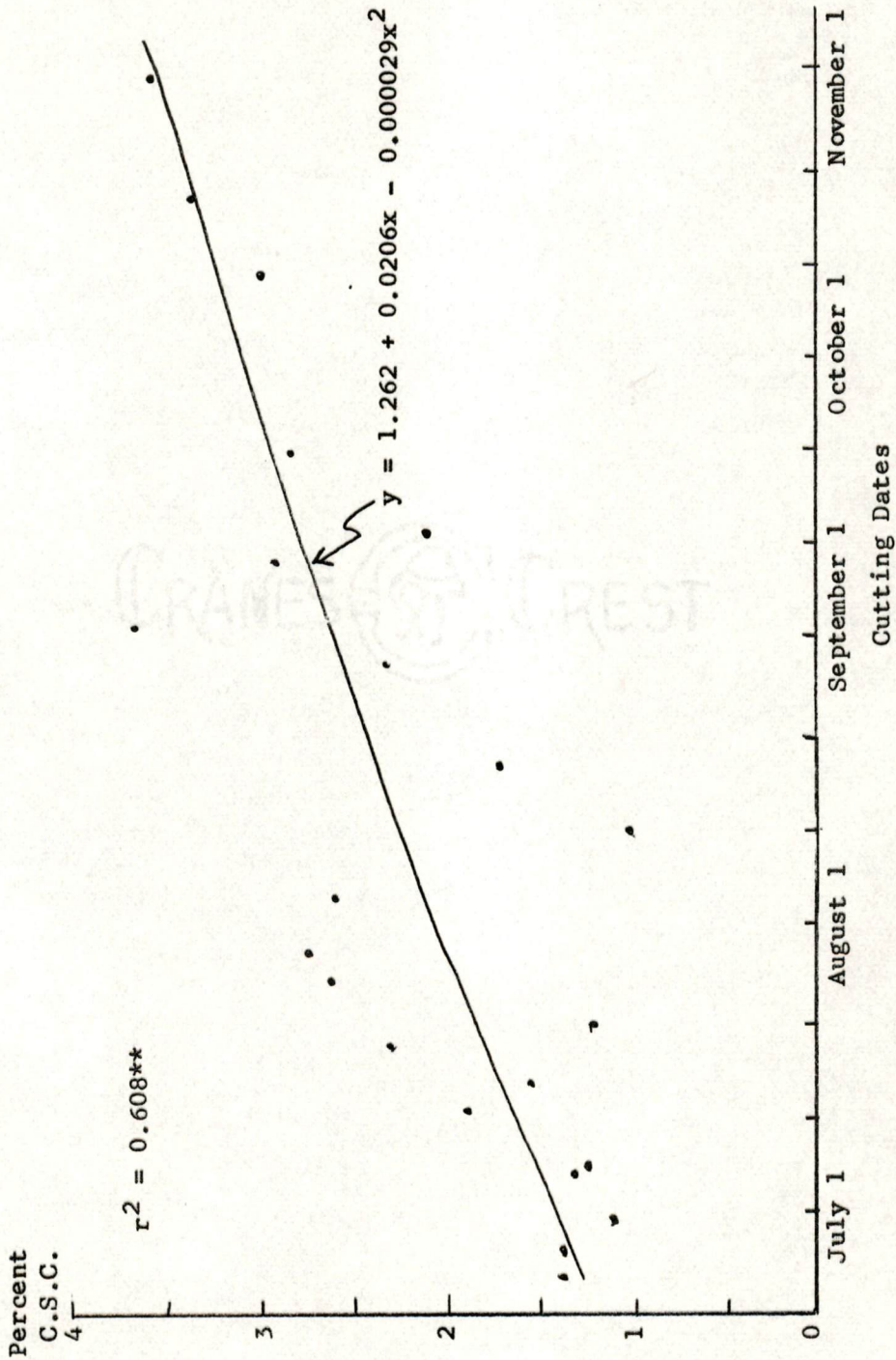
Figure 19--Seasonal distribution of cell sap concentration 4 days after cutting for the high level of nitrogen fertilization, ignoring the effects of cutting intensities.

Percent C.S.C.

4

3

2

1

0

$r^2 = 0.291$ *

$y = 0.683 \times 0.392x - 0.000283x^2$

July 1   August 1   September 1   October 1   November 1
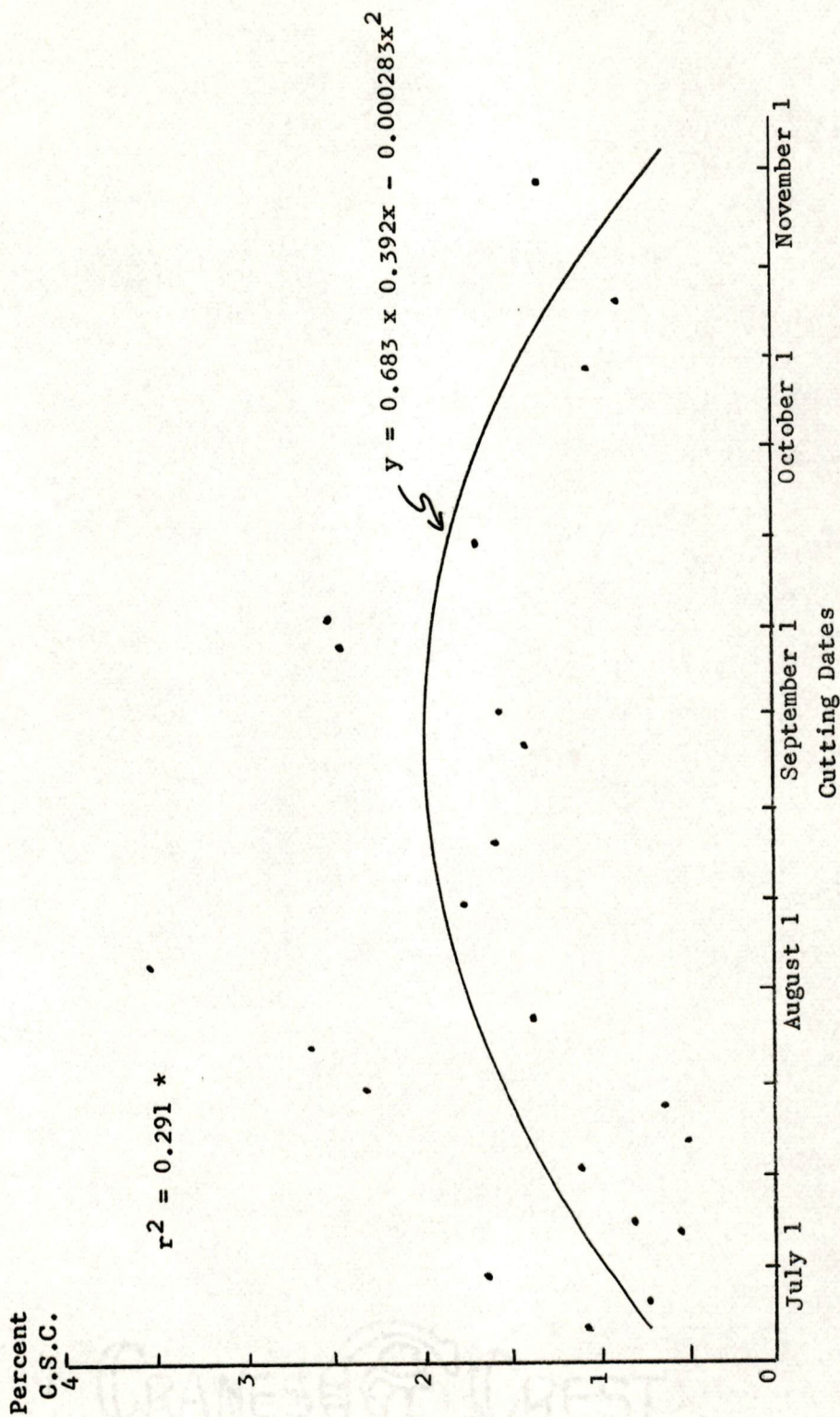
Cutting Dates

Figure 20--Seasonal distribution of cell sap concentration used in the first 4 days after cutting for the high level of nitrogen fertilization, ignoring the effects of cutting intensities.

cent C.S.C. at cutting date, but decreased more toward the end of the season, indicating that less reserve carbohydrates were utilized at the beginning and end of the season than at other times.

When the percentages of C.S.C. 4 days after cutting were graphed, an entirely different relationship was obtained (Figures 16 and 19). There was a general increase throughout the season, indicating that the percent C.S.C. 4 days after cutting was influenced, not only by the amount of the time of cutting, but also by the amount used by the plant in initial regrowth.

After the data presented in Figures 15 through 20 was plotted, a second degree polynomial of the form $y = a_o + a_1x + a_2 x^2$ was hypothesized. It was used to compute a curvilinear regression analysis with each of the six sets of data. In each case, the cutting dates were the independent variables, and the C.S.C. percentages at the time of cutting, those 4 days after cutting, and the amounts used in initial regrowth were the dependent variables, at each nitrogen level.

Variation in date of cutting explained only about 50% of the variation in percent C.S.C. at the time of cutting for both levels of nitrogen (Figures 15 and 18) and only 53% of the variation of C.S.C. used in initial regrowth for the low nitrogen level (Figure 17). An r^2 of 0.29 for the amount of C.S.C. used in initial regrowth for the high nitrogen fertilization (Figure 20) indicates that the variation in date of cutting had much less influence on the amount of C.S.C. used in initial regrowth at this level of nitrogen than at the low level of nitrogen fertilization. This finding explains more fully the data presented in Table 6 for the high

nitrogen treatments. The statement was made, concerning the data in this table, that the percent C.S.C. used in initial regrowth in the high nitrogen treatments was not influenced by cutting intensity to the extent that it was in the low nitrogen treatments. It can now be said that the amount of C.S.C. used in initial regrowth was also too variable to be explained by date of cutting. This substantiates the conclusion reached earlier that, with abundant nitrogen, the reserve carbohydrate material was not used in initial regrowth to the extent that it was with limited nitrogen. It could be postulated that the longevity of plants is directly related to the level of soil fertility, because the drain on reserve carbohydrates, or plant food reserves as they are commonly termed, is less severe when fertility is not a limiting factor. Many workers (26,27,34, 38,41,42,43,44) agree that nitrogen fertilization suppresses the amount of carbohydrate storage in a plant. The fact that high nitrogen fertilization promotes faster regrowth would indicate that the plant carbohydrates could not accumulate to any extent under these conditions. In fact, Garber (17) postulated that, with abundant nitrogen, carbohydrate reserves soon became the principal limiting factor of plant growth. However, with fast regrowth, restoration of photosynthetic tissue would be somewhat more rapid.

F-tests were conducted on the six calculated regression lines and all were significant at the 0.01 level of probability, indicating that the regression lines drawn from these samples were significantly better predictors of the true populations than were the sample means.

A t-test was conducted also for each of the partial regression coefficients. The t-tests on the partial regression coefficients of C.S.C. percentages at the time of cutting, and of the amounts of C.S.C. used in initial regrowth, at both the low and the high levels of nitrogen fertilization (Figures 15, 17, 18, and 20) were significant at the 0.05 level of probability. These significant t values indicate that the hypothesized theoretical equations fit these distributions of data at this level of probability. The t values for the partial regression coefficients of the C.S.C. percentages 4 days after cutting (Figures 16 and 19) were not significant at the 0.05 level of probability, even though the r^2's were the highest of the six analyses. These non-significant t values indicate that the hypothesized theoretical equations used, and upon which the regression analyses were conducted, were not the best possible for the distribution of these two sets of data.

The data of the C.S.C. percentages 4 days after cutting indicate, for each curve, two nearly linear relationships. The first one goes from the beginning of the season until about the first of August, increasing gradually in C.S.C. from about 1 to 2.7%. The other line begins immediately thereafter, again at about 1% C.S.C., and extends upward to about 4% by the end of the growing season. Should another regression analysis be conducted on these two sets of data using a third degree polynomial of the form, $y = a_0 + a_1x + a_2x^2 + a_3x^3$, it is possible that the calculated partial regression coefficients then might be significant.

Chemical Analysis of the Plant Stubble

A chemical analysis was conducted to determine the amount of water-soluble carbohydrates in the plant stubbles. It was hoped that the results obtained from this analysis could be related in some way to the C.S.C. of plant stubbles from the same plots.

Table 7 presents the data obtained from selected samples and treatments by this chemical analysis. The percentages of total water-soluble carbohydrates, as determined by the anthrone method, are shown, along with the individual percentages of C.S.C. at the time of cutting for the same plots. The water-soluble carbohydrate percentages were extremely variable, ranging from 17.5 to 42.5%. The data indicate that there was little relationship between the results obtained by the chemical analysis and those obtained with the refractometer.

Much work was done in the laboratory in an attempt to reduce the existing variability in technique and results. It was found, through a considerable amount of trial and error, that very careful standardization of steps 7 through 10 of the procedure presented on page 30 (in the Methods and Procedure chapter) was necessary for reliable results. The darkening of the anthrone reagent was found to account for much of the total variation in the standard and unknown results, but variation of time and temperature during the sugar-anthrone reaction also produced unreliable results. Therefore, two standards were included with each set of unknowns, and the time and temperature were controlled as closely as possible. More reliable results then were obtained on the standard solutions, but the results obtained from the unknowns (Table 7) were still extremely variable.

Table 7.--Chemical Analysis by the Anthrone Method as Compared to
Refractometer Readings at the Time of Cutting Gahi-1 Pearlmillet

Cutting Intensity	Nitrogen Applied Lbs./A.	Cutting Date	Rep. No.	Carbohydrates[1] %	C.S.C.[2] %
30-10	33	June 27	1	38.7	2.57
		July 5	2	35.0	2.77
		5	3	33.0	3.17
		5	4	30.2	3.43
		6	1	20.5	2.43
		29	2	37.5	4.57
		29	3	37.5	5.03
		29	4	42.5	4.17
		26	1	38.0	5.13
		Aug. 11	2	27.5	2.43
		11	3	29.0	3.20
		11	4	21.3	2.73
		11	1	20.5	6.70
		29	4	22.0	4.00
		Oct. 10	2	25.3	3.47
		10	3	24.5	5.13
		10	4	32.5	3.17
		10	1	26.5	3.53
20-6	333	June 24	1	33.5	2.60
		24	2	29.0	2.37
		24	3	30.0	2.53
		24	4	19.0	2.67
		July 5	1	41.0	1.50
		5	2	3.55	1.77
		5	3	34.0	1.77
		5	4	37.0	2.33
		19	1	37.0	2.73
		19	2	34.0	2.93
		19	3	29.5	3.17
		15	4	26.5	1.17
		Aug. 4	1	32.5	6.53
		4	2	36.0	4.70
		4	3	32.5	5.63
		July 29	4	38.5	4.20
		Aug. 11	1	26.0	2.33
		11	2	26.5	1.80
		11	3	24.5	2.13
		11	4	31.0	2.33

Table 7 (Continued)

Cutting Inten- sity	Nitrogen Applied Lbs./A.	Cutting Date	Rep. No.	Carbohydrates[1] %	C.S.C.[2] %
20-6	333	Aug. 29	1	41.5	5.00
		29	2	42.0	3.53
		29	3	41.5	2.93
		29	4	41.5	2.23
		Sept.21	1	37.0	5.40
		21	2	36.5	4.07
		21	3	19.0	5.27
		21	4	23.0	4.43
		Oct. 18	1	32.0	3.80
		18	2	36.0	3.33
		10	3	20.5	3.13
		10	4	17.5	3.33

[1]Percent total water-soluble carbohydrates, glucose equivalent, of the air-dry weight of the plant stubbles, extracted with 0.25 N sulfuric acid solution in a reflux condenser for 30 minutes.

[2]Cell sap concentration as measured by the refractometer at the time of cutting.

It was thought that much of this unaccountable unknown variation was due to the extraction procedure. Consequently, the data presented in Table 8 are the results of an effort to determine the best method of extraction. Even though the sub-samples, A and B, were somewhat variable, it was observed that the duplicates from each sub-sample were sometimes even more variable than the sub-samples. This indicates that a greater portion of the total variability was due to the chemical analysis, and a smaller portion to the extraction method. Nevertheless, the data and observations obtained from this experimentation supported the hypothesis that water or alcohol extraction was better than sulfuric acid solutions, provided the variability in the anthrone procedure could be controlled. Efforts were made to control more of the anthrone variability than that controlled by time, temperature and reagent darkening, but all proved fruitless.

There seems to be two logical explanations, other than inherent variability of the method, for this phase of the experiment not having produced meaningful, explainable results. The anthrone method of sugar analysis is sensitive to 5 ppm. (16) and the working range of 0 to 80 ppm. was rather narrow. The results obtained from this analysis were used to calculate the percent total water-soluble carbohydrates in the air-dry plant stubbles. With such a sensitive method, one very small error in dilution of the extracts, or in the analysis procedure, would have magnified the error in the final result.

On the other hand, the portable refractometer used in this study measured the percent total solid content of the sap in sucrose equivalents.

Table 8.--Extraction of Soluble Carbohydrates from Samples of Dried Plant
Tissue by Alcohol, Water, and Sulfuric Acid Solutions

Extraction Solution	Extraction Time	Extraction Method	Sub-Sample	Percent Carbohydrate[1]
75% Ethanol	24 hours	Soaking	A	16
			B	13
Dist. Water	1 hour	Soxhlet	A	18
			B	13
Dist. Water	24 hours	Soaking	A	20
	1 hour	Soxhlet	B	16
0.05 N Sulfuric Acid	1 hour	Soxhlet	A	30
			B	29
0.1 N Sulfuric Acid	1 hour	Soxhlet	A	34
			B	30
0.15 N Sulfuric Acid	1 hour	Soxhlet	A	31
			B	31
0.25 N Sulfuric Acid	1 hour	Soxhlet	A	37
			B	35

[1]Percent water- or alcohol-soluble carbohydrates, glucose equivalent, of the air-dry weight of the plant stubbles, an average of duplicate determinations for each sub-sample.

This instrument has an accuracy range of $\pm$ 0.2% and is not nearly as sensitive as the anthrone method of analysis. The refractometer reading percentages were based on the fresh weight of the plant stubbles, whereas the percentages obtained from the chemical analysis were based on the air-dry weight of the plant stubbles. This situation allowed for no common weight basis upon which to compare the relationships between these two sets of data. The absence of this common weight basis, plus the presence of wide differences in the sensitivities of these two methods of carbohydrate measurement, practically eliminated the possibilities of obtaining a meaningful relationship between refractometer readings and chemical analysis measurements, even with the best possible results from both.

General Discussion

The results of this study must be considered tentative at the present time for a number of reasons: (1) the dark-room and chemical analysis phases failed to give very meaningful results. The dark-room samples did not live when placed in the dark. The reasons for this are somewhat obscure, but experimental technique may have been a contributing factor. The variability of the results from the chemical analysis rendered them practically useless also. Thus, both of these phases of the study produced little useful information as to the reliability of the refractometer for studying carbohydrate reserves in Gahi-1 pearl-millet; (2) the extreme variation of soil fertility in the experimental area at the beginning of the season resulted in a higher coefficient of variation than was desirable; and, perhaps (3) improper sampling technique

for the refractometer determinations, due to a lack of information on the correct procedures.

Regardless of the limitations of this experiment, some general trends have been established and these permit certain conclusions.

The cutting intensities of 30-10, EB-4, 30-6, and 20-6, at the high level of nitrogen fertilization, gave the most dry-matter yield and nitrogen recoveries; this is in agreement with the findings of others (8,35,40). Nitrogen fertilization depressed reserve carbohydrate storage and less carbohydrates were utilized for initial regrowth. However, nitrogen fertilization increased the percentage of nitrogen in the harvested forage. These findings are supported also by other workers (34,38,42). The high nitrogen treatments contained less reserve carbohydrates at the time of cutting than did the low nitrogen treatments and utilized about 10% less for initial regrowth. Therefore, it seems that high nitrogen fertilization promoted more efficient plant regrowth with less drain on the reserve carbohydrates.

In general, the utilization of reserve carbohydrates for initial regrowth was directly proportional to severity of cutting, regardless of nitrogen fertilization level. The generally recommended management for cutting or grazing Gahi-1 pearlmillet is to leave a relatively high stubble regardless of the use. For soilage or silage, the recommended management is to allow the plant growth to reach a height of 30 inches, or up to early bloom, then cut it down to 6- to 10-inch stubbles. The utilization of carbohydrates is in general more efficient, and more yield usually is produced with these high stubble cutting intensities. Therefore, it

would seem to indicate that maximum efficiency of carbohydrate utilization is absolutely necessary for maximum forage production.

Even with the rather limited results from this study, it is the feeling of this writer that the refractometer has a great potential for additional studies of this type. Farkas and Pratt (15) also believe that the refractometer has a real potential in determining the irrigation needs of a growing crop. This indicates that the usefulness of the refractometer is not limited just to carbohydrate studies, but can be used advantageously for studying other growth problems as well.

If another study of this type was contemplated, the findings reported herein concerning the experimental procedure, should be used as a guide. However, it would be advisable to make the following corrections: (1) take fresh weight samples of the same part of the plant upon which the percent C.S.C. was determined. This would put the refractometer results on a more comparative basis with chemical analysis and other refractometer results, and (2) determine the exact substances measured by the refractometer; this, in turn, would provide a check on the validity of the refractometer. With these things accomplished, the results from such an experiment would be a valuable research contribution to the study of carbohydrate reserves in plants and their effect on the productivity of grasses.

CHAPTER V

SUMMARY AND CONCLUSIONS

An investigation of the combined effects of nitrogen fertiliza-
tion and cutting intensities on the reserve carbohydrates in Gahi-1
pearlmillet was conducted at the Koella Farm, Main Agricultural Experi-
ment Station, Blount County, Tennessee, during the summer of 1960. The
objectives of this investigation were to determine the relationships of
these factors to the regrowth and carbohydrate reserves of Gahi-1 pearl-
millet, and to study the methods needed for this type of investigation.

The effects of the treatments were measured in terms of dry matter
yields, percent nitrogen, nitrogen recoveries from the harvested forage,
and percent cell sap concentration at the time of cutting and 4 days
later. From this study, the following general conclusions may be drawn:

1. Dry matter yields generally were inversely proportional to
severity of cutting, but proportional to amounts of nitrogen fertiliza-
tion.

2. The nitrogen percentages in the harvested forage were rela-
tively uniform for all cutting intensities at each nitrogen level, but
higher at the high level of nitrogen fertilization.

3. The nitrogen recoveries were largely dependent upon the amount
of dry matter produced.

4. The percent C.S.C. used for initial regrowth generally was
proportional to severity of cutting, but inversely proportional to
amounts of nitrogen fertilization.

5. Cutting intensity seemed to have a larger effect on both yield and percent C.S.C. used in initial regrowth at the low level of nitrogen fertilization than when 10 times as much nitrogen was applied.

6. High nitrogen fertilization favored more efficient reserve carbohydrate utilization for initial regrowth.

7. The plants managed under the 30-10 cutting intensity were the most efficient users of reserve carbohydrates at both levels of nitrogen.

8. The plants managed under the 20-1 and 30-1 cutting intensities, at the low and high levels of nitrogen fertilization, respectively, were the most inefficient users of reserve carbohydrates.

9. The longer the interval of time between cuttings, the more the reserve carbohydrate storage was increased.

10. The highest reserve carbohydrate storage occurred in September.

11. The anthrone method of carbohydrate analysis was too variable, with the present procedure, to produce reliable results.

12. It appears that the refractometer has a great potential for carbohydrate reserve studies. This is indicated by the findings and general trends reported in this writing. Therefore, by using the findings and suggestions set forth in this study as a guide, more and better information can be obtained from similar experiments in the future.

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APPENDIXES

APPENDIX A

PROCEDURE FOR DETERMINING THE NITROGEN CONTENT OF PEARLMILLET BY

THE KJELDAHL-GUNNING METHOD

1. Place a weighed sample of approximately 1.0 gm. of oven-dry forage in a Kjeldahl flask.

2. Add 10 gm. catalyst (K_2SO_4 and $CuSO_4 \cdot 5H_2O$ in ratio of 100:3.2) and 25 ml. of concentrated sulfuric acid.

3. Heat below boiling until frothing ceases, then raise temperature to the boiling point, and continue for 30 minutes after digestion is complete.

4. Cool; add 200 ml. of tap water.

5. Place 500 ml. Erlenmeyer flask containing 100 ml. of 4% boric acid containing methyl red-methylene blue indicator mixture beneath the delivery tube of the condenser.

6. Add a pinch of granular zinc and enough 45% sodium hydroxide to the digested material to make strongly alkaline.

7. Connect Kjeldahl flask to the condenser immediately after addition of sodium hydroxide.

8. Distill over approximately 300 ml. into boric acid.

9. Titrate the distillate using 0.1 normal hydrochloric acid.

10. Calculate percentage nitrogen.

APPENDIX B

DRY MATTER YIELDS, NITROGEN PERCENT AND RECOVERIES IN HARVESTED

FORAGE, AND C.S.C. PERCENTAGES OF GAHI-1 PEARLMILLET SUB-

JECTED TO LOW NITROGEN TREATMENT

Cutting Inten- sity	Cutting Date		Rep. No.	Height at Cutting In.	Cell Sap Conc.		D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
					At Cutting %	4 Days After Cutting %			
20-1	June	24	1	22	2.33	1.17	1646	2.31	38.0
		24	2	20	2.93	1.00	2668	2.31	61.6
		24	3	18	2.80	1.13	1382	2.31	31.9
		24	4	17	3.60	1.07	1314	2.31	30.3
	July	15	1	19	1.90	1.43	1480	3.49	51.6
		15	2	19	1.90	1.17	2296	3.49	80.0
		19	3	22	3.13	1.27	3278	1.44	47.2
		15	4	22	2.77	1.03	1230	3.49	42.8
	Aug.	11	1	23	5.27	0.73	886	2.89	25.6
		11	2	27	2.60	1.00	318	2.89	9.2
		18	3	39	2.90	0.87	222	2.37	5.2
		11	4	22	3.77	1.13	414	2.89	11.8
	Sept.	12	1	25	5.00	1.70	622	2.08	12.8
		9	2	23	7.17	1.77	360	2.25	8.0
		9	3	20	4.93	2.00	540	2.25	12.0
		9	4	20	5.47	2.37	470	2.25	10.6
	Oct.	18	1	28	3.80	3.40	248	2.93	7.2
		18	2	33	5.40	3.37	1092	2.93	31.8
		10	3	25	2.20	1.80	1258	2.82	35.4
		18	4	27	3.33	3.73	732	2.93	21.4
20-3	June	24	1	21	2.00	1.03	1286	2.66	34.2
		24	2	20	2.13	1.03	1452	2.66	38.6
		24	3	18	3.07	1.40	1300	2.66	34.4
		24	4	17	2.40	1.07	1452	2.66	38.6
	July	6	1	23	2.67	0.77	746	2.38	17.7
		6	2	19	2.43	1.23	1148	2.38	27.2
		6	3	19	2.40	1.17	1010	2.38	24.0
		6	4	20	1.87	0.70	1216	2.38	28.8
		26	1	22	5.43	1.63	2018	2.39	48.2
		26	2	20	4.43	2.33	1660	2.39	39.6
		29	3	21	5.43	2.00	2222	1.67	37.0
	Aug.	4	4	34	5.33	2.63	2242	1.35	30.2

Cutting Inten- sity	Cutting Date	Rep. No.	Height at Cutting In.	Cell Sap Conc. At Cutting %	Cell Sap Conc. 4 Days After Cutting %	D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
20-3	Aug. 11	1	21	3.03	0.53	346	2.27	7.8
	11	2	20	2.60	1.10	374	2.27	8.4
	11	3	22	2.13	1.20	540	2.27	12.2
	29	4	20	3.43	1.53	456	1.74	7.9
	Sept. 2	1	23	5.07	2.50	346	1.93	6.7
	2	2	24	4.67	3.27	456	1.93	8.8
	2	3	28	4.93	3.50	456	1.93	8.8
	Oct. 10	4	34	3.23	2.17	2032	2.19	44.4
	10	1	32	3.07	2.53	1646	2.19	36.0
	Sept. 21	2	17	3.47	1.93	898	2.03	18.2
	Oct. 10	3	34	4.93	3.80	2032	2.19	44.4
	10	2	22	3.40	2.73	774	2.19	16.8
20-6	June 24	1	23	2.60	0.87	1562	2.26	35.2
	24	2	18	2.43	1.03	1258	2.26	28.4
	24	3	20	2.63	0.93	954	2.26	21.4
	24	4	17	3.00	0.93	1038	2.26	23.4
	July 5	1	24	3.63	1.17	844	2.36	19.8
	5	2	23	2.67	1.07	1244	2.36	29.2
	6	3	23	2.57	1.00	996	1.82	18.0
	6	4	20	3.20	0.87	940	1.82	17.1
	21	1	24	4.33	1.13	1424	2.08	29.6
	21	2	20	3.93	2.13	1162	2.08	24.0
	19	3	21	2.93	1.80	1064	2.05	21.8
	21	4	19	3.80	1.30	1038	2.08	21.4
	Aug. 11	1	27	2.53	0.87	442	2.05	9.0
	11	2	27	4.47	1.37	374	2.05	7.6
	11	3	30	5.00	0.53	498	2.05	10.2
	11	4	26	4.21	0.80	442	2.05	9.0
	29	1	24	4.33	1.57	428	2.58	11.0
	29	2	26	3.47	2.17	428	2.58	11.0
	29	3	23	2.30	1.57	456	2.58	11.6
	29	4	23	2.43	1.97	402	2.58	10.2
	Sept. 21	1	21	3.87	2.70	1204	2.69	32.2
	21	2	22	3.57	2.67	1244	2.69	33.4
	Oct. 10	3	36	3.07	2.53	1562	2.06	32.0
	Sept. 21	4	19	4.53	2.50	1272	2.69	34.2
	Oct. 18	1	26	3.13	3.07	484	2.75	13.3
	18	2	21	2.40	2.73	290	2.75	7.8
	18	4	26	3.07	3.13	512	2.75	14.0

Cutting Inten- sity	Cutting Date	Rep. No.	Height at Cutting In.	Cell Sap Conc.		D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
				At Cutting %	4 Days After Cutting %			
30-1	June 30	1	31	2.73	0.87	2890	2.25	65.0
	July 5	2	31	3.90	0.97	3374	1.40	47.2
	June 30	3	29	2.23	0.50	3638	2.25	81.8
	July 5	4	31	2.93	1.97	3388	1.40	47.4
	Aug. 11	1	36	5.47	1.73	470	1.43	6.6
	18	2	26	6.33	1.33	318	1.16	3.6
	11	3	27	5.27	1.23	498	1.43	7.1
	11	4	32	3.20	1.43	442	1.43	6.3
	Sept.12	1	20	4.40	1.47	830	2.49	20.6
	Oct. 10	2	35	4.47	4.03	1382	1.62	22.2
	Sept.21	3	30	4.67	3.00	580	2.49	14.4
	21	4	21	3.87	1.93	1508	2.49	37.4
	Oct. 31	1	34	5.53	2.90	554	2.75	15.2
	31	3	31	5.13	4.57	608	2.75	16.6
	31	4	27	4.93	3.10	388	2.75	10.7
30-6	June 27	1	29	1.77	1.33	2476	3.22	79.6
	30	2	30	2.50	1.00	1426	2.11	30.0
	30	3	28	2.77	1.10	1536	2.11	32.4
	July 5	4	30	3.37	1.17	1880	1.56	29.2
	12	1	27	2.67	1.47	1826	2.36	43.0
	19	2	29	4.23	2.30	2046	1.79	36.6
	19	3	29	2.57	1.97	1936	1.79	34.6
	Aug. 4	4	35	7.17	2.27	2338	2.33	54.4
	11	1	44	3.20	1.00	996	1.91	19.0
	11	2	31	3.97	1.03	374	1.91	7.0
	11	3	22	3.53	0.93	414	1.91	7.9
	Sept. 2	4	36	5.27	3.63	526	2.24	11.8
	9	1	27	6.70	3.47	540	1.90	10.3
	2	2	27	5.13	2.47	498	2.24	11.1
	2	3	34	5.90	4.17	498	2.24	11.1
	Oct. 10	4	32	4.27	3.13	774	2.24	17.3
	18	1	33	3.47	3.40	1424	1.78	25.3
	10	2	31	5.27	3.13	1534	2.24	34.2
	10	3	29	4.87	2.67	2710	2.24	60.6
30-10	June 27	1	29	2.57	1.80	2794	3.10	86.6
	July 5	2	33	2.77	1.00	1742	1.62	28.2
	5	3	30	3.17	1.53	2310	1.62	37.4
	5	4	33	3.43	1.27	2350	1.62	38.0

Cutting Inten-sity	Cutting Date	Rep. No.	Height at Cutting In.	Cell Sap Conc. At Cutting %	Cell Sap Conc. 4 Days After Cutting %	D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
30-10	July 6	1	24	2.43	0.73	2370	2.44	57.8
	29	2	31	4.57	1.90	1674	1.53	25.6
	29	3	32	5.03	3.17	1148	1.53	17.4
	29	4	33	4.17	1.90	1406	1.53	21.4
	26	1	32	5.13	2.57	1992	1.82	36.2
	Aug. 11	2	19	2.43	1.07	540	2.67	14.4
	11	3	21	3.20	1.13	360	2.67	9.6
	11	4	19	2.73	1.20	456	2.67	12.2
	11	1	27	6.70	3.47	526	2.67	14.0
	Sept. 2	2	25	4.53	2.90	526	1.78	9.2
	2	3	33	4.87	3.60	484	1.78	8.6
	Aug. 29	4	33	4.00	1.60	566	2.02	11.4
	Sept. 2	1	24	5.53	2.60	442	1.78	7.8
	Oct. 10	2	34	3.47	2.47	1078	2.08	22.4
	10	3	36	5.13	3.17	1992	2.08	41.4
	10	4	30	3.17	4.37	1922	2.08	39.8
	10	1	33	3.53	2.93	1314	2.08	27.2
EB-4	July 12	1	80	3.60	3.20	5822	1.69	98.2
	12	2	68	3.97	1.40	4300	1.69	72.6
	15	3	58	5.73	1.70	4370	0.86	37.4
	15	4	60	4.90	1.57	4840	0.86	41.6
	Aug. 18	1	37	3.47	1.33	222	2.08	4.6
	18	2	42	3.80	1.53	194	2.08	4.0
	18	3	28	3.40	1.00	290	2.08	6.0
	18	4	40	4.80	1.13	428	2.08	8.9
	Sept. 21	1	33	6.70	2.60	898	1.82	16.3
	21	2	44	5.07	2.67	1880	1.82	34.2
	21	3	31	4.87	2.97	1866	1.82	33.8
	21	4	36	7.07	2.27	1674	1.82	30.4
	Oct. 31	1	28	5.60	4.30	138	2.64	3.6
	31	2	26	4.80	4.47	484	2.64	12.6
	31	3	27	3.73	3.27	152	2.64	4.0
	31	4	25	5.73	5.73	442	2.64	11.7

APPENDIX C

DRY MATTER YIELDS, NITROGEN PERCENT AND RECOVERIES IN HARVESTED

FORAGE, AND C.S.C. PERCENTAGES OF GAHI-1 PEARLMILLET SUB-

JECTED TO HIGH NITROGEN TREATMENT

Cutting Inten-sity	Cutting Date	Rep. No.	Height at Cutting In.	Cell Sap Conc.		D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
				At Cutting %	4 Days After Cutting %			
20-1	June 24	1	24	2.37	1.27	2406	4.24	102.0
	24	2	20	2.40	1.63	1992	4.24	84.4
	24	3	20	2.30	1.93	1854	4.24	78.6
	24	4	18	2.83	1.16	2032	4.24	86.0
	July 15	1	21	2.33	1.30	1728	3.82	66.0
	15	2	20	2.10	1.80	2350	3.82	89.6
	12	3	19	1.90	1.27	2324	3.56	82.6
	15	4	21	2.70	1.50	1314	3.82	50.0
	Aug. 11	1	27	2.40	0.80	304	3.55	10.8
	11	2	25	2.67	0.63	290	3.55	10.2
	11	3	32	2.80	1.20	346	3.55	12.3
	11	4	27	3.07	1.27	346	3.55	12.3
	Sept. 9	1	20	4.90	3.13	526	3.05	16.0
	2	2	25	7.17	4.63	276	3.15	8.7
	2	3	25	5.60	3.20	374	3.15	11.8
	2	4	23	4.33	3.27	402	3.15	12.7
	Oct. 10	1	30	3.87	2.57	1134	2.75	31.0
	18	2	45	5.27	3.07	886	2.25	19.9
	18	3	45	4.27	3.67	1010	2.25	22.6
	10	4	38	3.27	2.80	1106	2.75	30.4
20-3	June 24	1	26	2.23	1.30	1922	3.73	71.6
	24	2	24	1.97	1.10	2240	3.73	83.4
	24	3	22	2.27	1.33	2172	3.73	81.4
	24	4	21	2.83	1.40	3664	3.73	136.6
	July 6	1	22	1.70	1.17	1010	3.94	39.6
	6	2	22	2.33	1.43	968	3.94	38.0
	6	3	20	2.47	1.63	1314	3.94	51.6
	6	4	21	2.20	0.83	2116	3.94	83.2
	21	1	22	3.10	1.17	1204	3.76	45.3
	21	2	19	4.47	1.47	1356	3.76	50.8
	21	3	20	3.07	1.17	1522	3.76	57.2
	21	4	21	3.53	1.13	1604	3.76	60.2

Cutting Inten-sity	Cutting Date	Rep. No.	Height at Cutting In.	Cell Sap Conc. At Cutting %	Cell Sap Conc. 4 Days After Cutting %	D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
20-3	Aug. 11	1	28	2.60	0.63	304	3.70	11.2
	11	2	27	3.63	0.90	346	3.70	12.8
	11	3	31	2.50	0.90	346	3.70	12.8
	11	4	28	3.20	1.20	304	3.70	11.2
	Sept. 2	1	21	4.73	2.73	566	2.70	15.3
	2	2	25	6.07	3.13	428	2.70	11.5
	2	3	33	3.60	2.17	580	2.70	15.6
	2	4	34	5.47	5.60	622	2.70	16.8
	21	1	22	4.07	3.03	746	3.28	24.4
	Oct. 10	2	34	5.37	3.47	2946	2.30	67.6
	10	3	34	4.07	3.77	1784	2.30	40.4
	Sept.21	4	24	5.17	2.53	428	3.28	14.0
	Oct. 18	1	33	4.47	2.43	1038	3.11	32.2
	31	4	15	4.43	2.80	360	2.06	7.4
20-6	June 24	1	21	2.60	1.63	1866	3.72	69.4
	24	2	29	2.37	1.30	1992	3.72	74.0
	24	3	23	2.53	1.37	2310	3.72	85.8
	24	4	20	2.67	1.20	1258	3.72	46.6
	July 5	1	27	1.50	1.30	1812	4.13	74.8
	5	2	23	1.77	1.33	1432	4.13	59.0
	5	3	26	1.77	1.27	1452	4.13	59.8
	5	4	22	2.33	1.33	1894	4.13	78.2
	19	1	21	2.73	1.87	1894	3.64	68.8
	19	2	23	2.93	2.87	1866	3.64	67.8
	19	3	22	3.17	2.13	1728	3.64	62.8
	15	4	20	1.17	1.73	872	4.14	36.0
	Aug. 4	1	37	6.53	3.47	1658	4.00	66.2
	4	2	20	4.70	1.90	1120	4.00	44.8
	4	3	23	5.63	2.73	2102	4.00	84.0
	July 29	4	22	4.20	3.20	1494	4.30	64.2
	Aug. 11	1	20	2.33	1.00	290	3.97	11.4
	11	2	19	1.80	1.23	304	3.97	12.0
	11	3	24	2.13	0.43	290	3.97	11.4
	11	4	20	2.33	1.10	304	3.97	12.0
	29	1	29	5.00	2.23	428	3.79	16.2
	29	2	33	3.53	1.90	456	3.79	17.2
	29	3	29	2.93	2.00	388	3.79	14.6
	29	4	28	2.23	2.23	428	3.79	16.2
	Sept.21	1	24	5.40	2.53	1328	3.66	48.6
	21	2	21	4.07	3.07	1024	3.66	37.4
	21	3	18	5.27	2.96	774	3.66	28.2
	21	4	36	4.43	2.56	1092	3.66	39.8

Cutting Inten-sity	Cutting Date	Rep. No.	Height at Cutting In.	Cell Sap Conc. At Cutting %	4 Days After Cutting %	D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
20-6	Oct. 18	1	22	3.80	3.47	1024	2.91	29.6
	18	2	26	3.33	4.07	844	2.91	24.4
	10	3	25	3.13	2.07	872	3.51	30.6
	10	4	21	3.33	1.43	470	3.51	16.4
30-1	June 27	1	33	1.77	1.73	3526	3.76	132.4
	27	2	34	1.97	1.23	3402	3.76	127.8
	27	3	29	2.30	1.26	2282	3.76	85.8
	27	4	28	2.37	1.16	2572	3.76	96.6
	Aug. 4	1	44	6.03	1.63	2572	2.85	73.2
	11	2	33	2.13	0.86	470	2.85	13.4
	July 26	3	30	5.27	2.57	2600	3.13	81.2
	26	4	30	5.20	2.67	2890	3.13	90.4
	Aug. 29	1	30	5.73	2.60	402	3.70	14.9
	Sept.12	2	20	5.60	1.77	1148	4.13	47.4
	Aug. 18	3	44	4.23	3.70	360	3.93	14.0
	18	4	38	2.83	1.03	332	3.93	13.0
	Oct. 10	1	37	3.80	3.50	3498	2.58	90.2
	31	2	29	5.73	3.00	498	2.46	12.2
	Sept.12	3	21	4.27	1.80	1604	4.13	66.2
	12	4	32	3.93	2.67	1134	4.13	46.8
	Oct. 31	3	32	5.07	4.33	484	2.46	11.9
30-6	June 27	1	31	1.77	1.47	2544	4.06	103.3
	27	2	28	2.30	1.33	1840	4.06	74.7
	30	3	30	2.73	1.10	3222	4.47	144.0
	27	4	28	2.10	1.17	2130	4.06	86.5
	July 12	1	29	2.93	1.93	1812	3.85	69.8
	12	2	28	2.03	2.23	1286	3.85	49.5
	15	3	30	1.90	1.43	1894	3.91	74.1
	12	4	30	1.47	2.03	2656	3.85	102.3
	29	1	28	4.40	2.07	2032	3.91	79.5
	29	2	32	4.23	2.80	1660	3.91	64.9
	Aug. 11	3	43	3.03	1.30	388	4.02	15.6
	11	4	41	4.53	1.17	456	4.02	18.3
	11	1	18	2.67	1.13	650	4.02	26.1
	18	2	36	2.73	1.20	124	4.15	5.1
	Sept. 2	3	33	4.60	2.67	512	3.73	19.1
	2	4	33	5.60	4.73	470	3.73	17.5
	Aug. 29	1	28	4.40	1.90	388	3.98	15.4
	Sept. 9	2	20	5.80	2.70	332	3.68	12.2
	Oct. 10	3	31	4.20	2.63	3014	2.38	71.7
	10	4	38	3.73	3.07	2696	2.38	64.2

Cutting Intensity	Cutting Date	Rep. No.	Height at Cutting In.	Cell Sap Conc. At Cutting %	Cell Sap Conc. 4 Days After Cutting %	D.M. Yield/ Cutting Lbs./A.	N in Forage %	N in Harvest. Forage Lbs./A.
30-6	Oct. 10	1	40	5.57	2.87	3056	2.38	72.7
	10	2	29	3.00	2.73	1688	2.38	40.2
30-10	June 27	1	35	1.37	1.60	1508	4.12	62.1
	27	2	28	2.60	1.17	1522	4.12	62.7
	27	3	28	1.73	1.67	1356	4.12	55.9
	27	4	27	2.57	1.27	1176	4.12	48.5
	July 12	1	30	2.30	1.63	1978	3.98	78.7
	12	2	29	2.87	1.80	2848	3.98	113.4
	6	3	28	1.50	1.17	2656	4.09	108.6
	12	4	34	2.20	1.90	2448	3.98	97.4
	29	1	33	3.53	2.87	1784	3.09	55.1
	Aug. 4	2	32	7.07	2.87	2600	3.64	94.6
	July 19	3	30	2.90	2.37	1660	4.15	68.9
	Aug. 4	4	39	7.30	2.93	2586	3.64	94.1
	11	1	23	3.43	0.87	402	5.15	20.7
	11	2	19	2.47	1.73	402	5.15	20.7
	4	3	43	5.30	2.47	1812	3.64	66.0
	18	4	32	2.13	1.93	138	4.02	5.5
	29	1	35	3.20	2.77	414	3.28	13.6
	29	2	28	2.67	2.77	414	3.28	13.6
	18	3	33	2.87	0.93	180	4.02	7.2
	Sept. 2	4	29	4.53	4.43	470	3.43	16.1
	Oct. 10	1	35	4.27	3.60	2046	2.02	41.3
	10	2	29	3.40	3.27	2144	2.02	43.3
	Sept. 2	3	32	5.67	3.73	442	3.43	15.2
	Oct. 10	4	37	5.40	3.53	2614	2.02	52.8
	10	3	43	3.67	3.30	1646	2.02	33.2
EB-4	July 12	1	70	5.07	1.97	4702	3.17	149.1
	12	2	64	3.00	1.80	4826	3.17	153.0
	12	3	64	3.83	1.93	8768	3.17	277.9
	12	4	62	5.10	2.30	6582	3.17	208.6
	Aug. 18	1	47	3.67	2.23	166	3.74	6.2
	18	2	45	2.80	1.50	166	3.74	6.2
	18	3	53	4.43	1.33	208	3.74	7.8
	18	4	49	3.80	1.50	360	3.74	13.5
	Sept.21	1	40	3.83	2.50	2668	3.20	85.4
	21	2	46	5.13	2.90	1866	3.20	59.7
	21	3	44	4.23	3.70	3084	3.20	98.7
	21	4	25	3.50	2.53	2986	3.20	95.6
	Oct. 31	1	29	5.53	3.83	180	2.92	5.3
	31	2	21	3.33	3.30	332	2.92	9.7
	31	3	22	5.33	4.17	222	2.92	6.5

APPENDIX D

DAILY PRECIPITATION IN INCHES, CLIMATOLOGICAL STATION, BLOUNT COUNTY

FARM, MAIN EXPERIMENT STATION, KNOXVILLE, TENNESSEE, 1960

Day	May	June	July	August	Sept.	Oct.
1						
2						
3		0.80	0.19			
4		0.01	0.18		0.40	0.58
5		0.23		0.76		0.13
6				0.24		0.50
7	0.39					0.02
8				0.21	0.25	0.42
9		0.14		1.45		2.00
10				0.01	0.08	
11	0.22		2.20	0.50	1.35	
12		0.10	0.16	0.16		
13				0.29		
14				0.01		
15		0.62				
16						
17		0.80			0.59	
18		0.16			0.07	
19					0.01	
20	0.04					1.23
21						
22		0.10		0.31		
23		0.56		0.62		
24	0.11	0.63		0.01		
25		1.25				
26	0.51	0.04				
27	0.26	0.02	0.12		0.14	0.02
28	0.22				0.23	0.25
29		0.29			0.83	
30	0.87			0.10	0.08	
31						
TOTAL	2.68	5.75	2.85	4.67	4.03	5.15
Long-term Mean[1]	3.58	3.47	4.72	3.43	2.53	2.63

[1]Period 1931-1955, Knoxville Airport, U. S. Weather Bureau.

APPENDIX E

DAILY TEMPERATURES IN DEGREES FAHRENHEIT, CLIMATOLOGICAL STATION,

BLOUNT COUNTY FARM, MAIN EXPERIMENT STATION, KNOXVILLE, TENNESSEE,

1960[1]

Day	May Min.	May Max.	June Min.	June Max.	July Min.	July Max.	Aug. Min.	Aug. Max.	Sept. Min.	Sept. Max.	Oct. Min.	Oct. Max.
1	38	65	52	81	68	88	62	94	65	92	55	79
2	40	68	54	83	69	90	62	92	61	86	53	80
3	42	74	59	82	68	90	67	96	61	87	58	82
4	48	78	61	93	64	92	68	95	64	90	55	82
5	48	77	63	86	67	87	68	92	62	89	53	74
6	52	76	63	88	64	84	70	92	63	91	54	76
7	40	69	60	88	60	82	70	91	63	94	60	76
8	38	60	58	83	62	84	67	91	65	93	61	78
9	40	63	59	75	63	85	65	96	64	89	58	74
10	44	64	57	82	68	85	65	88	65	88	58	74
11	42	50	58	83	66	82	66	95	64	89	52	75
12	42	54	65	82	65	85	66	80	62	81	47	76
13	36	66	68	89	62	84	65	74	55	79	48	79
14	43	73	65	88	62	90	67	82	45	76	51	77
15	50	82	57	83	59	84	64	83	45	74	53	80
16	---	---	55	82	62	85	60	86	45	79	52	81
17	51	83	54	92	63	89	60	88	61	74	56	79
18	60	88	56	79	65	81	64	89	62	73	43	75
19	63	90	59	82	65	90	64	87	59	78	43	74
20	61	88	61	90	64	89	64	84	59	81	48	71
21	64	92	64	90	64	87	65	88	54	85	33	57
22	49	86	66	89	64	89	67	86	54	84	32	59
23	54	89	63	92	64	92	67	78	58	86	34	64
24	56	88	63	84	65	89	62	86	60	80	39	67
25	58	93	62	93	67	89	62	84	53	82	29	67
26	59	90	65	78	67	93	62	84	49	80	29	64
27	60	90	61	77	68	91	61	84	50	77	35	53
28	52	78	62	85	63	90	64	85	53	70	43	56
29	52	79	66	77	63	90	66	88	59	70	36	62
30	60	83	68	87	61	89	63	88	61	72	38	67
31	55	85	---	---	61	90	63	91	---	---	49	72
Ave.	49.9	77.4	60.8	84.8	64.3	87.6	64.7	87.6	58.0	82.3	46.9	71.9

[1]Temperatures taken 5 feet above the ground over common bermudagrass sod.