6-1971

Stand density and carbohydrate reserve trends in two varies of red clover (Trifolium pratense L.) subjected to several cutting management regimes

Richard Urban Clark

To the Graduate Council:

I am submitting herewith a thesis written by Richard Urban Clark entitled "Stand density and carbohydrate reserve trends in two varies of red clover (Trifolium pratense L.) subjected to several cutting management regimes." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

John H. Reynolds, Major Professor

We have read this thesis and recommend its acceptance:

Henry Fribourg, David Coffey

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 1, 1971

To the Graduate Council:

I am submitting herewith a thesis written by Richard Urban Clark entitled "Stand Density and Carbohydrate Reserve Trends in Two Varieties of Red Clover (*Trifolium pratense* L.) Subjected to Several Cutting Management Regimes." 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.

John H Reynolds
Major Professor

We have read this thesis
and recommend its acceptance:

Henry H Fribourg

David L. Coffey

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

STAND DENSITY AND CARBOHYDRATE RESERVE TRENDS IN TWO VARIETIES OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) SUBJECTED TO SEVERAL CUTTING MANAGEMENT REGIMES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Richard Urban Clark

June 1971

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to the following:

Dr. John H. Reynolds, his major professor, for the sincere assistance and valuable guidance afforded throughout the course of the author's graduate study;

Dr. Henry Fribourg and Dr. David Coffey for their helpful suggestions and critical reading of the manuscript, and for serving as members of the author's graduate committee;

Mr. Charles Leslie, for his assistance in the preparation of data; and

The University of Tennessee Agricultural Experimental Station for the financial aid of a graduate assistantship.

ABSTRACT

The effects of four cutting schedules on persistence of the plants and concentration of carbohydrates in the roots of two varieties of red clover (*Trifolium pratense* L.) were studied at Knoxville, Tennessee. The two varieties studied were Kenland, a recommended variety in Tennessee, and Kentucky Synthetic A-2, an experimental variety developed at the University of Kentucky. Each variety was subjected to cutting frequencies of two, three, four and six times per year. Stand estimates were made and samples were taken for carbohydrate analysis at selected intervals during the first year after the seedling winter.

Kentucky Synthetic A-2 maintained a greater stand density than Kenland at the termination of the study. At that time there was no significant difference in stand density among cutting treatments within a variety. Stand density of the Kentucky Synthetic A-2 was two to three times greater than was that of the Kenland.

Carbohydrate reserve trends revealed a significantly higher percentage of total nonstructural carbohydrates in the roots of Kentucky Synthetic A-2 than in those of Kenland on three of the seven sampling dates. Yearly trends for all treatments included a slight increase in percentage from June to September, a sharp increase during the late fall months, and a decrease in total nonstructural carbohydrates from a November or December peak into spring.

Correlation coefficients for the relationship between the percentage of total nonstructural carbohydrates on a given date and the stand

density for the same treatment on some later date did not reveal any significant relationship. The correlation coefficient for September carbohydrates with the September to October stand reduction was positive and significant at the .05 level of probability.

TABLE OF CONTENTS

CHAPTER PAGE

I. INTRODUCTION 1

II. LITERATURE REVIEW 2

III. EXPERIMENTAL PROCEDURE 5

IV. RESULTS 8

Weather Conditions 8

Stand Estimates 11

Total Nonstructural Carbohydrates In Roots 15

V. DISCUSSION 26

VI. SUMMARY 30

REFERENCES 32

APPENDIX 35

VITA 37

LIST OF TABLES

TABLE PAGE

1. Average Weekly Maximum and Minimum Temperature and Weekly Total Precipitation for the Year March 1, 1968 to February 28, 1969 9
2. Stand Estimates on 1968-69 Counting Dates 12
3. Analyses of Variance for 1968-69 Stand Estimates on 7 Sampling Dates 13
4. Total Nonstructural Carbohydrates on 1968-69 Sampling Dates 16
5. Analyses of Variance for 1968-69 Total Nonstructural Carbohydrate Percentages on 7 Sampling Dates 17
6. Correlations of Carbohydrate Percentages on Selected Dates With Stand Estimates on Selected Dates 29
7. Cutting Dates for Cutting Frequencies in 1968 36

LIST OF FIGURES

FIGURE		PAGE
1.	Percent of Total Nonstructural Carbohydrates in the Roots of Kenland Red Clover Subjected to Two Cuttings (———), Three Cuttings (----), Four Cuttings (-·-·-·), and Six Cuttings (-x-x-x)	19
2.	Percent of Total Nonstructural Carbohydrates in the Roots of Kentucky Synthetic A-2 Red Clover Subjected to Two Cuttings (———), Three Cuttings (----), Four Cuttings (-·-·-·), and Six Cuttings (-x-x-x)	21
3.	Percent of Total Nonstructural Carbohydrates in the Roots of Kenland (----) and Kentucky Synthetic A-2 (———) Red Clovers Cut Twice	22
4.	Percent of Total Nonstructural Carbohydrates in the Roots of Kenland (----) and Kentucky Synthetic A-2 (———) Red Clovers Cut Three Times	23
5.	Percent of Total Nonstructural Carbohydrates in the Roots of Kenland (----) and Kentucky Synthetic A-2 (———) Red Clovers Cut Four Times	24
6.	Percent of Total Nonstructural Carbohydrates in the Roots of Kenland (----) and Kentucky Synthetic A-2 (———) Red Clovers Cut Six Times	25

CHAPTER I

Introduction

Red clover (*Trifolium pratense* L.) is grown on several hundred thousand acres in Tennessee. It is used for hay, pasture, and as a soil improving crop. Red clover has become increasingly important in recent years because of the need for a replacement for alfalfa. An alfalfa replacement has been sought because of the difficulties encountered in alfalfa weevil control. At comparable stages of growth, red clover approaches alfalfa in nutritional value.

In Tennessee one of the major problems of red clover culture has been lack of persistence. The high cost of stand establishment increases the need for a stand to endure as long as possible. Good management practices are essential in extending the longevity of stand and developing maximum yields and achieving high quality of forage.

Since management is extremely important to the success or failure of red clover production, a knowledge of the effects of certain management practices on persistence of stand was considered to be valuable. The purpose of this project was to study the effects of first-year management practices on two varieties of red clover. Stand counts were chosen as an indicator of persistence, and carbohydrate root reserves were chosen as an indicator of physiological responses.

CHAPTER II

Literature Review

The question of persistence of red clover has been the subject of considerable speculation. In early Tennessee history Killebrew (14) classified the plant as a biennial and recommended cultivating it as such. Other early agronomists recognized the perennial tendency of the plant (1, 18), and Smith (21) found that although red clover behaved as a biennial in most areas, its food storage cycle was that of a true perennial. Studies from some southern locations showed certain varieties of red clover still yielding satisfactorily three, four, and five years from seeding dates (7, 9, 19). A stand nine years old has been reported in Colorado (5).

Many factors may contribute to the lack of persistence of red clover and, consequently, to its behavior as a biennial. Depletion of stands has been shown to occur most rapidly after periods of climatic and/or management stresses from clipping or grazing (6, 13). Other factors which contribute to lack of persistence of red clover stands are diseases and insects. The actual cause of the lack of persistence is not adequately explained by any one factor, but it is probably a combination of factors. Some workers have implicated internal breakdown, a necrosis of the pith of the crown, as a major factor influencing persistence in red clover (4, 8, 16). No specific organisms have been isolated in diseased plants, however, and damage is attributed to interactions of insect damage and root rot. In these studies (4, 8, 16) prolonged survival of individual plants seemed to depend on the development of a secondary root system before the original crown deteriorated. Other work has shown reduced persistence in flowering types as opposed to non-flowering types of the species (2, 22).

A knowledge of the use and accumulation of reserve carbohydrates by red clover is fundamental to an understanding of management responses, since carbohydrates are the main source of reserve energy stored in the roots of the plant. Smith (21) found that the concentration of food reserves stored in red clover roots was dependent upon the stage of maturity and climatic conditions at the time of sampling. Decreases in total carbohydrates occurred following forage harvest and during dry climatic conditions. Cutting during periods of low carbohydrate reserves was considered to be detrimental to the plant, while cutting the plant at near maturity was considered to be favorable to the accumulation of high carbohydrate reserves. Smith (21) later reported that red clover, when left uncut, exhibited three distinct growths from the crown during the growing season. Carbohydrate reserves were high during October and November and began decreasing in April when spring growth began. Jung and Smith (12) found the carbohydrate fraction of red clover in roots and crowns to increase in content during the fall, reach a peak during fall or winter, and decrease during winter and spring.

Smith (23) reported the fluctuations of carbohydrate root reserves in red clover subjected to cutting treatments of zero, two, and three times. Higher levels of carbohydrates were maintained in the two-cutting than in the three-cutting treatment. However, carbohydrate levels in the fall period of storage were virtually the same for all treatments. Ruelke and Smith (20) found that percentages of total available carbohydrates in red clover roots were highest in plants not fall-cut and were lowest in plants which had been cut, particularly those cut four times during September, October and November. Virtanen and Nurmia (27) observed in second-year red clover stands that minimum root reserves were reached 12 to 15 days after cutting, regardless of the date of cutting. They also found that the original carbohydrate level before cutting was regained much more quickly in the fall than in the June-July period and that the carbohydrate content of the roots at the end of the experiment became higher as the last cutting occurred later. This conclusion is in disagreement with that of Ruelke and Smith (20).

Work by Greathouse and Stuart (10) in 1937 showed that winter hardiness was associated with rate of carbohydrate metabolism. Other studies revealed that a high level of cold resistence was maintained as long as the total available carbohydrate level remains relatively high (11, 12). Bula and Smith (3) found that total available carbohydrates in red clover decreased by as much as 62 percent during winter dormancy and that the rate of metabolic activity was inversely proportional to cold resistance developed.

Carbohydrate reserves have been shown to be important in the capacity of the plant to produce regrowth after cutting and to develop cold resistance. Although most of the researchers agree that red clover should not be cut during periods of low carbohydrate reserves, there is some disagreement about the effect of late fall cutting on carbohydrate reserves that could be involved in developing winter hardiness.

CHAPTER III

Experimental Procedure

'Kenland' red clover and 'Kentucky Synthetic A-2,' an experimental red clover variety developed at the University of Kentucky, were broadcast-seeded by hand at the rate of 9 kg seed/ha on September 1, 1967 at Knoxville, Tennessee. The seeds were covered with a cultipacker. Three tons of lime had been applied on August 14, 1967 to adjust the pH of the plow layer upward from 5.7. Phosphorus was applied at the rate of 44 kg/ha and potassium was applied at the rate of 37 kg/ha.

The soil of the experimental area at the Plant Science Farm was Sequoia silty clay loam. The area seeded was divided into two sections. One section was used as a sampling area for obtaining red clover roots for carbohydrate analyses, and the other section was used for obtaining stand estimates. The same treatments were applied to both sections of the experiment. Plot size was 2.13 m x 7.80 m.

The experimental design was a randomized complete block with a factorial arrangement of treatments. Two varieties undergoing four cutting frequencies were the treatments. Each treatment was replicated four times.

The two varieties, Kenland and Kentucky Synthetic A-2, received cutting treatments of two, three, four and six cuttings during 1968. The cutting dates for the two-cut treatments were June 25 and September 12; for the three-cut treatments May 22, July 16, and September 12; for the four-cut treatments May 7, June 25, July 30, and September 12; and for six cuttings per year were April 24, May 22, June 25, July 16, August 14, and September 12.

Stand estimates were obtained by random placement of a 0.30 m x 0.45 m quadrat in each plot of the stand count section of the experiment. Four readings were taken in each plot. Stand counts were made after each plot had been cut one or more times, after the late summer cutting of all treatments, and during the fall and winter months. Dates of stand estimates in 1968 were June 28, September 23, October 22, November 26, and December 18. In 1969, estimates were made on February 1 and March 5.

Sampling for carbohydrate analysis began in mid-June 1968 when plants were in the pre-bud to early bloom stage depending upon the cutting treatment. Fifteen to thirty plants were dug from each plot on each sampling date. Top growth was removed at the junction of root and stem, and roots were clipped at 10 cm in length. Side roots were not trimmed. Roots were washed free of soil under running tap water. The roots were dried at 100 C for one hour and drying was completed at 70 C. The roots were ground to pass a 40-mesh sieve, placed in glass bottles, and dried at 70 C to constant weight. Bottles were capped and the samples held for analysis. Sampling dates for carbohydrate analyses in 1968 were June 20, September 5, October 15, November 21, and December 18. Sampling dates in 1969 were February 4 and March 21.

An acid hydrolysis (17) was used to remove the total nonstructural carbohydrates from the samples. A weighed sample of red cover tissue and 25 ml of 0.2 N sulfuric acid were placed in a 125 ml Erlenmeyer flask and heated in a boiling water bath for one hour. The sample extract was then filtered through Whatman's No. 1 filter paper and the paper and residue washed with 25 ml of 1.4 N sulfuric acid to bring the solution to 0.8 N. This solution was then heated in a boiling water bath for two hours, cooled, and neutralized with 25% w/v sodium hydroxide. The neutralized solution was transferred to a 250 ml volumetric flask and the flask filled to volume.

A 1 ml aliquot of sample was taken for a colorimetric determination of reducing power by the Nelson-Somogyi method (15, 25). This procedure involved the addition of 2 ml of copper reagent to the test tube containing the sample and then heating the solution for fifteen minutes in a boiling water bath. The solution was then cooled to room temperature and 2 ml of arsenomolybdate reagent were added. The contents of each tube were mixed, 20 ml of water were added, and the absorbance of the solution was read at 510 nm on a Spectronic 20 spectrophotometer. From the absorbance value, the percent of total nonstructural carbohydrates was calculated for each sample with glucose as a standard sugar. Samples were analyzed in duplicate.

CHAPTER IV

Results

I. Weather Conditions

Red clover performs best in relatively cool and moist seasons. Tennessee is located on the southern edge of the red clover belt and the extremely hot and dry climatic conditions that are sometimes present in late summer and early fall are considered unfavorable for the persistence of red clover. Milder winters, however, probably make winterkilling less of a problem in this area than in others farther north.

Temperature and precipitation data presented in Table 1 are in the form of weekly summaries of weather conditions at the Plant Science Farm during the climatological year March 1, 1968 to February 28, 1969. A comparison with long-term normals (26) indicate that this year was near-normal in temperature and somewhat less than normal in precipitation. The year was characterized by intervals in which deviations from the normals occurred and when climatic factors may have become critical to the plants. Late winter and spring of 1968 were warmer than normal with near-normal rainfall occurring at frequent intervals. This period should have been conducive to the persistence of red clover. July had near-normal temperatures but was drier than usual and red clover experienced one period in the first half of the month when sufficient soil moisture may have been lacking. Temperatures for the month of August were higher than normal and the amount of precipitation was only

Table 1. Average weekly maximum and minimum temperature and weekly total precipitation for the year March 1, 1968 to February 28, 1969[a]

Week of	Climatological week	Maximum temperature	Minimum temperature	Precipitation
		---------- C	-----------	mm
Mar. 1	1	9.4	-6.1	1.3
Mar. 8	2	17.2	0.0	59.2
Mar. 15	3	18.8	0.6	18.3
Mar. 22	4	17.8	0.6	24.9
Mar. 29	5	21.6	7.8	44.2
Apr. 5	6	18.8	6.7	23.3
Apr. 12	7	22.2	5.0	22.1
Apr. 19	8	22.2	10.0	17.8
Apr. 26	9	26.1	7.2	24.6
May 3	10	21.7	6.1	1.8
May 10	11	26.1	15.6	26.7
May 17	12	22.8	8.9	15.5
May 24	13	26.7	13.3	51.3
May 31	14	27.2	13.3	53.6
Jun. 7	15	29.4	17.2	16.8
Jun. 14	16	28.9	14.4	15.2
Jun. 21	17	31.1	18.3	21.3
Jun. 28	18	30.0	15.6	3.6
Jul. 5	19	29.4	16.7	0.3
Jul. 12	20	32.8	17.8	10.9
Jul. 19	21	33.3	14.4	52.1
Jul. 26	22	31.1	20.0	22.9
Aug. 2	23	33.3	20.0	8.9
Aug. 9	24	32.7	20.0	16.0
Aug. 16	25	34.4	20.6	3.0
Aug. 23	26	32.8	15.0	0.0
Aug. 30	27	28.9	12.2	1.5
Sep. 6	28	27.2	12.2	26.4
Sep. 13	29	27.2	12.2	17.5
Sep. 20	30	28.9	13.9	8.1
Sep. 27	31	27.2	9.4	0.8
Oct. 4	32	21.7	6.1	30.0
Oct. 11	33	26.7	11.1	0.0
Oct. 18	34	23.3	6.7	10.9
Oct. 25	35	15.0	-1.1	10.2
Nov. 1	36	22.8	7.8	17.3
Nov. 8	37	8.3	-1.1	7.6
Nov. 15	38	13.3	-0.5	17.3
Nov. 22	39	15.6	-2.7	7.6
Nov. 29	40	12.2	2.2	27.2

Table 1 (continued)

Week of	Climatological week	Maximum temperature	Minimum temperature	Precipitation
		---------- C	----------	mm
Dec. 6	41	6.7	-8.3	0.0
Dec. 13	42	7.2	-7.8	10.7
Dec. 20	43	7.8	-4.4	31.0
Dec. 27	44	7.2	-2.2	37.6
Jan. 3	45	3.3	-6.1	47.0
Jan. 10	46	1.7	-2.8	55.1
Jan. 17	47	13.3	-5.0	0.8
Jan. 24	48	10.0	-4.4	7.3
Jan. 31	49	14.9	-1.7	9.1
Feb. 7	50	5.6	-7.8	0.0
Feb. 14	51	4.4	-10.6	0.8
Feb. 21	52	6.1	-10.0	0.0
Feb. 28	53	7.2	-2.8	9.4

[a]Data compiled by H. A. Fribourg, Agronomy Department, University of Tennessee, Knoxville, Tennessee.

two-thirds of normal. Extremely high temperatures during this period coinciding with little rainfall created climatic, and probably soil moisture, conditions unfavorable to red clover. October was normal in precipitation and temperature while November was slightly below normal in precipitation and minimum temperatures. December, January, and February were cooler than normal, especially the minimum temperatures. This period was characterized by extremely high precipitation in late December and January and unusually low precipitation in February. There was little or no snow cover present during the winter months.

II. Stand Estimates

Stand estimates for 1968-69 are shown in Table 2. Reductions in stand from June 1968 to March 1969 ranged from a high of 80.7 percent in the Kenland 6-cut treatment to a low of 27.1 percent in the Kentucky Synthetic 3-cut treatment. Reduction in stand for this period was higher in all treatments of Kenland when compared with the same cutting treatment of Kentucky Synthetic A-2. In both varieties the largest loss of stand occurred in the 6-cut treatment while the smallest loss was found in the 3-cut treatment. There was no significant difference among the cutting treatments within each variety on the last counting date (Table 3) however, because the 6-cut treatments had a higher plant population at the beginning estimate date and lost more plants than the rest of the treatments.

The interval during which the greatest loss in stand took place was from June 28, 1968 to September 23, 1968. This interval was the period when half of the harvests were made. The 6-cutting treatment

Table 2. Stand estimates on 1968-69 counting dates

Treatment†	Counting Date						
	Jun. 28, 1968	Sep. 23, 1968	Oct. 22, 1968	Nov. 26, 1968	Dec. 18, 1968	Feb. 1, 1969	Mar. 5, 1969
				plants/m^2			
Ken 2-cut	87.8[b,c††]	74.8[a]	49.6[b,c]	36.7[b,c]	33.8[c]	33.1[b]	28.1[b]
Ken 3-cut	54.0[c]	82.7[a]	57.6[b]	48.9[b]	36.2[b,c]	36.0[b]	28.1[b]
Ken 4-cut	84.9[b,c]	71.2[a]	43.2[b,c]	39.6[b,c]	29.5[c]	23.0[b]	18.7[b]
Ken 6-cut	91.4[b,c]	48.2[a]	38.1[c]	33.1[c]	24.5[c]	17.3[b]	17.6[b]
Ky Syn 2-cut	115.8[a,b,c]	82.0[a]	79.9[a]	73.4[a]	64.0[a]	56.8[a]	56.8[a]
Ky Syn 3-cut	89.9[b,c]	80.6[a]	82.7[a]	74.8[a]	59.0[a]	61.2[a]	65.5[a]
Ky Syn 4-cut	120.1[a,b]	97.1[a]	77.7[a]	71.2[a]	43.2[a,b]	57.6[a]	58.3[a]
Ky Syn 6-cut	143.9[a]	67.6[a]	77.0[a]	71.9[a]	57.6[a]	57.6[a]	61.2[a]

†Ken = Kenland; Ky Syn = Kentucky Synthetic A-2.

††Values followed by the same letter within a column are not significantly different at the .05 level of probability.

Table 3. Analyses of variance for 1968-69 stand estimates on 7 sampling dates

Source of variation	Degrees of freedom	Mean squares
Jun. 28, 1968		
Variety	1	11,557.51**
Management	3	2,907.94**
V x M	3	208.78
Sep. 23, 1968		
Variety	1	1,336.18
Management	3	1,151.63
V x M	3	319.09
Oct. 22, 1968		
Variety	1	8,258.31**
Management	3	231.98
V x M	3	65.47
Nov. 26, 1968		
Variety	1	8,773.81**
Management	3	121.14
V x M	3	63.41
Dec. 18, 1968		
Variety	1	5,550.39**
Management	3	189.19
V x M	3	85.57
Feb. 1, 1969		
Variety	1	8,143.87**
Management	3	137.64
V x M	3	176.82
Mar. 5, 1969		
Variety	1	6,049.91**
Management	3	111.35
V x M	3	1,803.73**

**Significant at .01 level of probability.

of Kentucky Synthetic A-2 lost 53 percent of the June stand during this period and the Kenland 6-cut treatment lost 47 percent of the June stand. The 6-cut treatment experienced the greatest loss in both varieties.

Reduction of stand in the Kenland variety was considerable during the period from September 23 to October 22, 1968, whereas there was little or no loss in the Kentucky Synthetic A-2 variety. Losses in stand ranged from 21 percent to 39 percent in the four cutting treatments of Kenland, while only the 4-cut treatment of Kentucky Synthetic A-2 had any appreciable loss.

Stand losses from December 18, 1968 to March 5, 1969, were small and indicated that winter killing was not a serious problem. Only the Kenland 4-cut treatment showed a loss of any magnitude.

The June 28, 1968 stand estimate was taken after all plots had been cut at least once and some as many as three times. The Kentucky Synthetic A-2 variety had the highest plant density on this date. Kentucky Synthetic A-2 cutting treatments were all significantly higher at the .05 level of probability than the Kenland cutting treatments except the Kentucky Synthetic 3-cut treatment. Within each variety, only the Kentucky Synthetic 3-cut treatment showed any significant difference from the rest of the cutting treatments.

The September 23, 1968 stand estimate revealed no significant differences among any of the treatments. This estimate was made after all the cutting treatments for the year had been performed.

Greater stand density in Kentucky Synthetic A-2 was revealed again on the October 22, 1968 counting date. All cutting treatments of the Kentucky Synthetic A-2 variety were significantly higher at the .05 level of probability than were all the cutting treatments of Kenland. There was no significant difference among the cutting treatments of Kentucky Synthetic A-2 and the only significant difference among the Kenland treatments was the difference in plant density between the 3-cut and 6-cut treatments. Although there had been a slight reduction in plant density from the October 22, 1968 counting date on the November 26, 1968 counting date, losses were uniform and the same variation among treatments existed as was found to the October counting date.

On the February 1 and March 5, 1969 counting dates all cutting treatments of the Kentucky Synthetic A-2 variety were significantly higher at the .05 level of probability than were all the cutting treatments of the Kenland variety. There was no significant difference among cutting treatments of each variety.

III. Total Nonstructural Carbohydrates In Roots

The percentages of total nonstructural carbohydrates for the 1968-69 sampling dates are shown in Table 4. The first sampling date was June 20, 1968 after each plot had been cut at least once except the 2-cut treatments. The 2-cut treatments of both varieties and the 4-cut treatment of Kentucky Synthetic A-2 were significantly higher than the other treatments in total nonstructural carbohydrates on this date.

No significant difference was found among the treatments on the September 5, 1968 sampling date (Table 5). On October 15, 1968 the

Table 4. Total nonstructural carbohydrates on 1968-69 sampling dates

Treatment[†]	Sampling Date						
	Jun. 20, 1968	Sep. 5, 1968	Oct. 15, 1968	Nov. 21, 1968	Dec. 18, 1968	Feb. 4, 1969	Mar. 21, 1969
				percent			
Ken 2-cut	12.3[a,b][††]	15.5[a]	18.9[c]	28.6[a]	26.1[a]	22.3[a]	14.7[a,b]
Ken 3-cut	7.3[d]	16.7[a]	21.2[b,c]	29.0[a]	26.6[a]	23.7[a]	19.5[a,b]
Ken 4-cut	9.3[c,d]	15.8[a]	19.7[b,c]	29.4[a]	26.7[a]	16.1[a]	14.3[b]
Ken 6-cut	10.1[c]	12.0[a]	19.1[c]	30.7[a]	30.6[a]	21.3[a]	18.8[a,b]
Ky Syn 2-cut	13.8[a]	16.9[a]	24.2[b]	27.8[a]	29.8[a]	19.3[a]	17.1[a,b]
Ky Syn 3-cut	10.5[b,c]	17.0[a]	23.3[b,c]	30.5[a]	33.1[a]	20.9[a]	18.5[a,b]
Ky Syn 4-cut	12.1[a,b]	17.9[a]	29.3[a]	31.2[a]	30.5[a]	22.5[a]	18.6[a,b]
Ky Syn 6-cut	11.1[b,c]	14.9[a]	19.8[b,c]	32.2[a]	29.3[a]	21.9[a]	21.1[a]

[†]Ken = Kenland; Ky syn = Kentucky Synthetic A-2.

[††]Values followed by the same letter within a column are not significantly different at the .05 level of probability.

Table 5. Analyses of variance for 1968-69 total nonstructural carbohydrate percentages on 7 sampling dates

Source of variation	Degrees of freedom	Mean squares
Jun. 20, 1968		
Variety	1	37.1953*
Management	3	23.3304*
V x M	3	5.0675
Sep. 5, 1968		
Variety	1	23.4441
Management	3	21.0009
V x M	3	2.4865
Oct. 15, 1968		
Variety	1	154.6601**
Management	3	34.7188*
V x M	3	30.7755*
Nov. 21, 1968		
Variety	1	7.7815
Management	3	14.0670
V x M	3	2.9340
Dec. 18, 1968		
Variety	1	80.5181*
Management	3	7.5499
V x M	3	22.1990
Feb. 4, 1969		
Variety	1	0.6845
Management	3	13.1870
V x M	3	38.4889
Mar. 21, 1969		
Variety	1	32.0200
Management	3	31.0743*
V x M	3	9.7034

* Significant at .05 level of probability.

** Significant at .01 level of probability.

Kentucky Synthetic 4-cut treatment was significantly higher than the other treatments in total nonstructural carbohydrates.

No significant differences were found among treatments on the November 21 and December 18 sampling dates in 1968 or the February 4 sampling date in 1969. The only significant difference among treatments on the March 21, 1969 sampling date existed between Kentucky Synthetic A-2 cut six times (high) and Kenland cut four times (low).

The changes in the percentage of total nonstructural carbohydrates in the roots of Kenland red clover cut two, three, four, and six times are shown in Figure 1 for June 1968 to April 1969. The 2-cut treatment had the highest percentage of total nonstructural carbohydrates on the first sampling date. On this date the 2-cut treatment had not been cut, the 2-cut treatment had been cut once, the 4-cut treatment once, and the 6-cut treatment twice. Carbohydrate percentages rose slightly between June and September, when most of the clippings were performed. The 3-cut treatment experienced the greatest increase while the 6-cut treatment increased the least.

The carbohydrate percentages began rising sharply in October and continued to increase into November when a peak in observed values was reached. Little change occurred in the percentage of total nonstructural carbohydrates from November to December. The percentage of carbohydrates began to decline in December and continued to decline into March. On this sampling date, the 3-cut treatment contained the highest percentage of total nonstructural carbohydrates, followed by the 6-cut, 2-cut and 4-cut treatments. Percentages ranged from a low of 7 percent in June

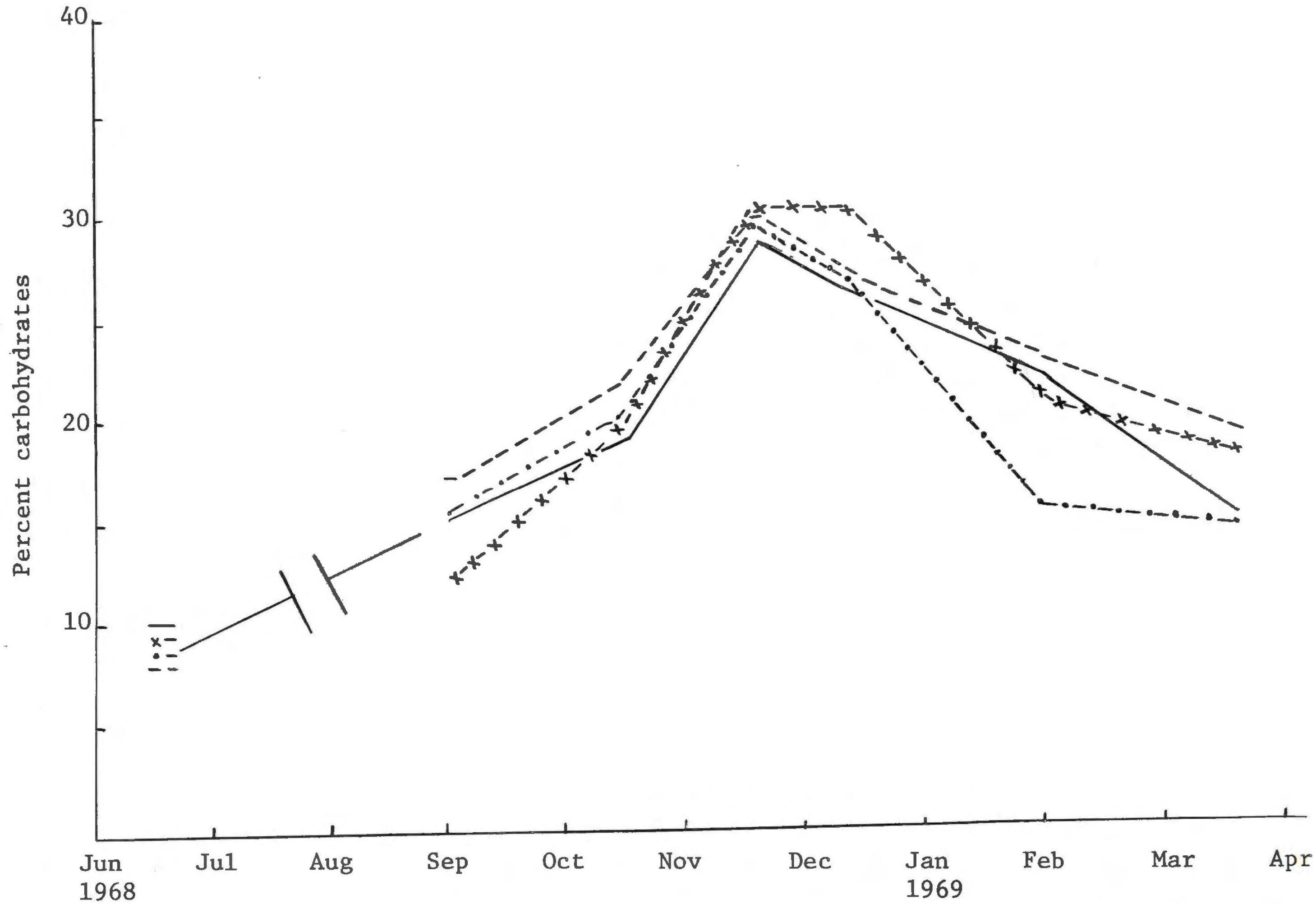

Figure 1. Percent of total nonstructural carbohydrates in the roots of Kenland red cover subjected to two cuttings (——), three cuttings (----), four cuttings (-·-·-·), and six cuttings (-x-x-x).

for the 3-cut treatment to a high of 31 percent in the 6-cut treatment in November.

The percentage of total nonstructural carbohydrates in the roots of Kentucky Synthetic A-2 cut two, three, four, and six times from June 1968 to April 1969 are shown in Figure 2. Trends of the carbohydrate percentages in the Kentucky Synthetic A-2 variety were similar to those in the Kenland variety. The 2-cut treatment contained the highest percentage of total nonstructural carbohydrates in June followed by the 4-cut, 6-cut, and 3-cut treatments, respectively. The 3-cut treatment had the greatest increase from June to September, while the 6-cut treatment increased the greatest. All cutting treatments rose sharply in total nonstructural carbohydrates during October and November and began declining in December. In March, the 3-cut treatment was highest in total nonstructural carbohydrates, followed by the 6-cut, 2-cut, and 4-cut treatments. Percentages ranged from a low of 10.5 in the 3-cut treatment in June to a high of 33.2 in the 3-cut treatment in December.

Figures 3, 4, 5 and 6 show the total nonstructural carbohydrate trends of the two varieties for each cutting treatment. The Kentucky Synthetic A-2 variety maintained a significantly higher level of total nonstructural carbohydrates on three sampling dates. Kenland exceeded Kentucky Synthetic in total nonstructural carbohydrates only on a few sampling dates and at no time was a Kenland cutting treatment significantly higher than was the same cutting treatment for Kentucky Synthetic (Tables 4 and 5, pages 16 and 17).

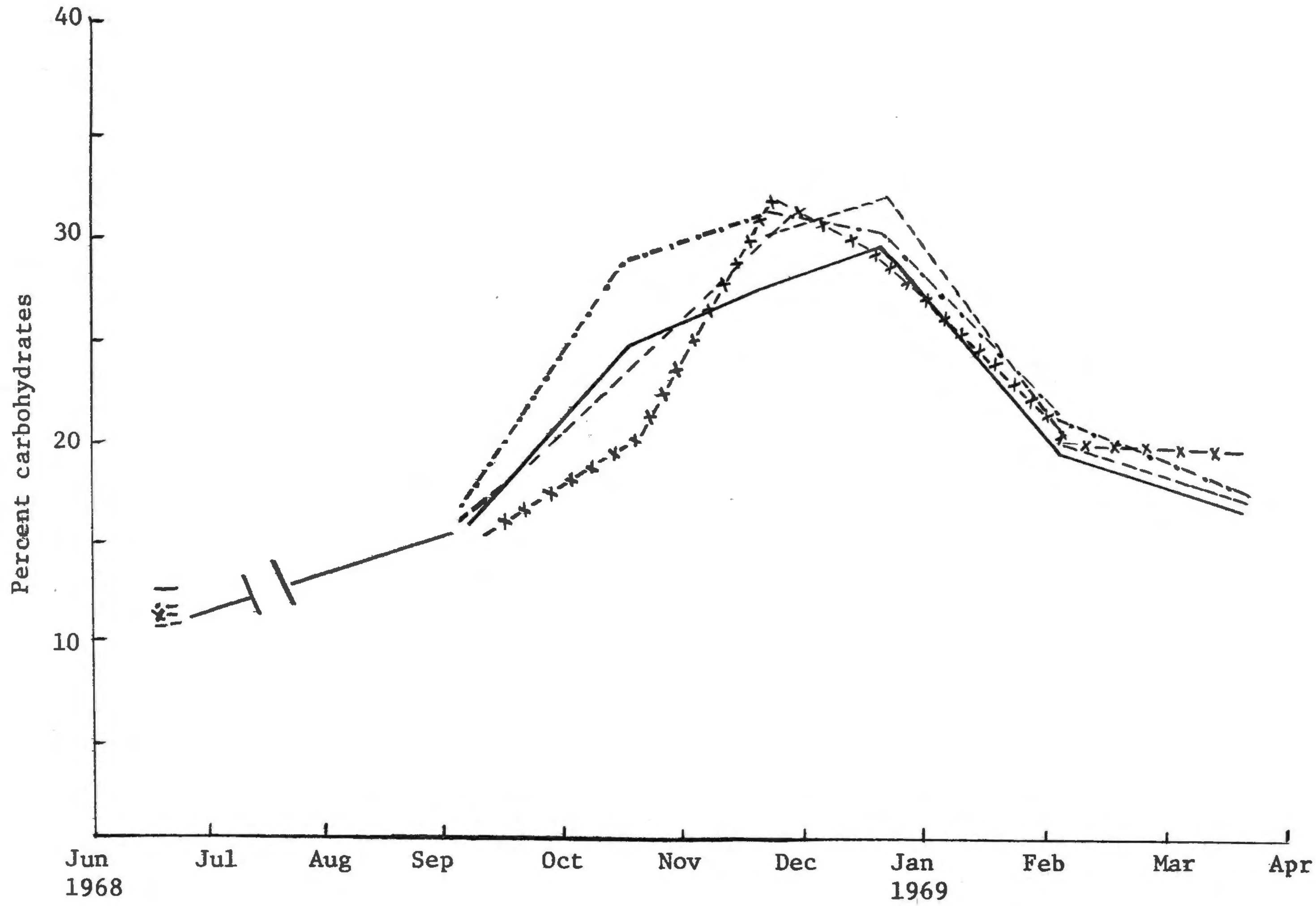

Figure 2. Percent of total nonstructural carbohydrates in the roots of Kentucky Synthetic A-2 red clover subjected to two cuttings (———), three cuttings (----), four cuttings (-·-·-·), and six cuttings (-x-x-x).

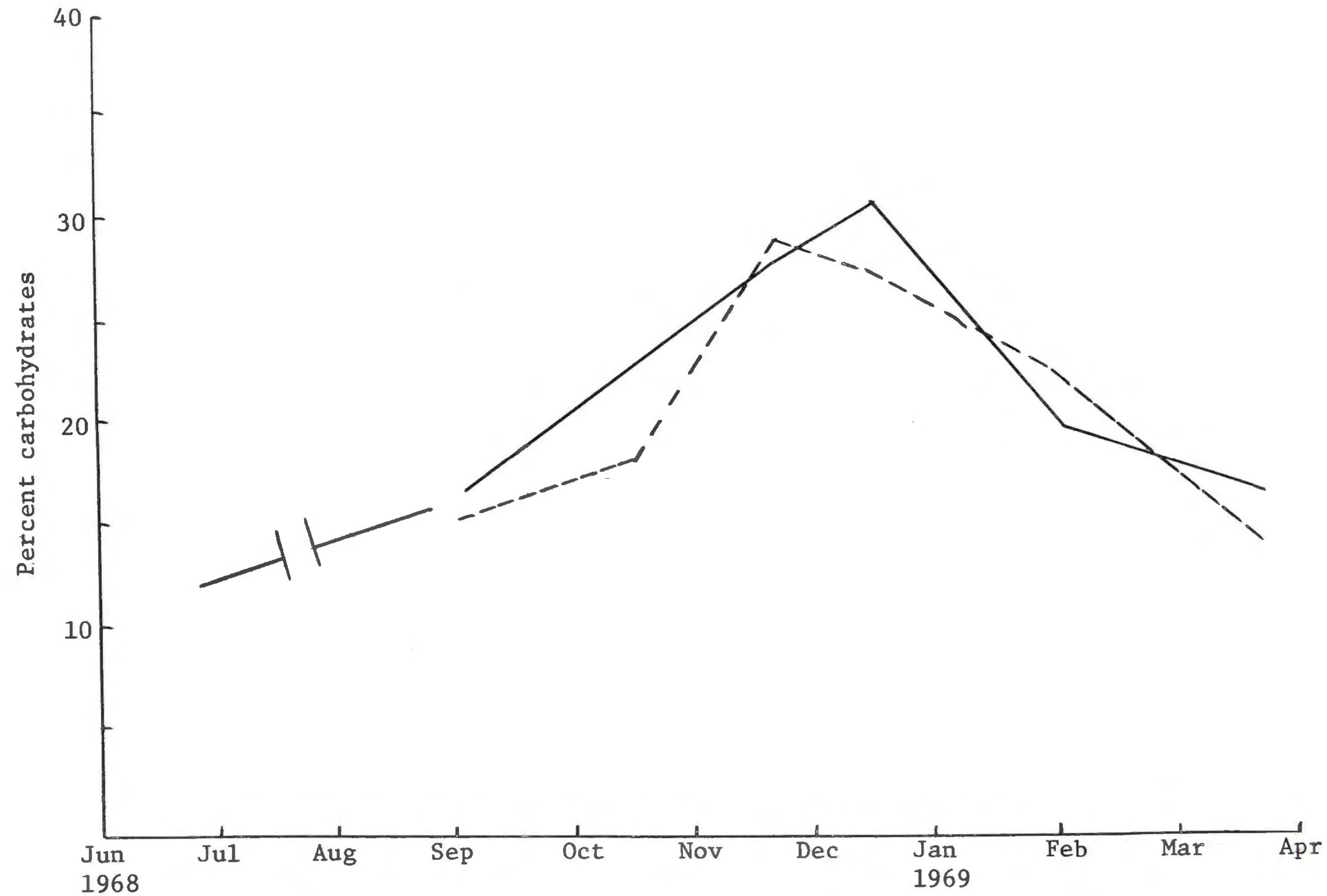

Figure 3. Percent of total nonstructural carbohydrates in the roots of Kenland (----) and Kentucky Synthetic A-2 (——) red clovers cut twice.

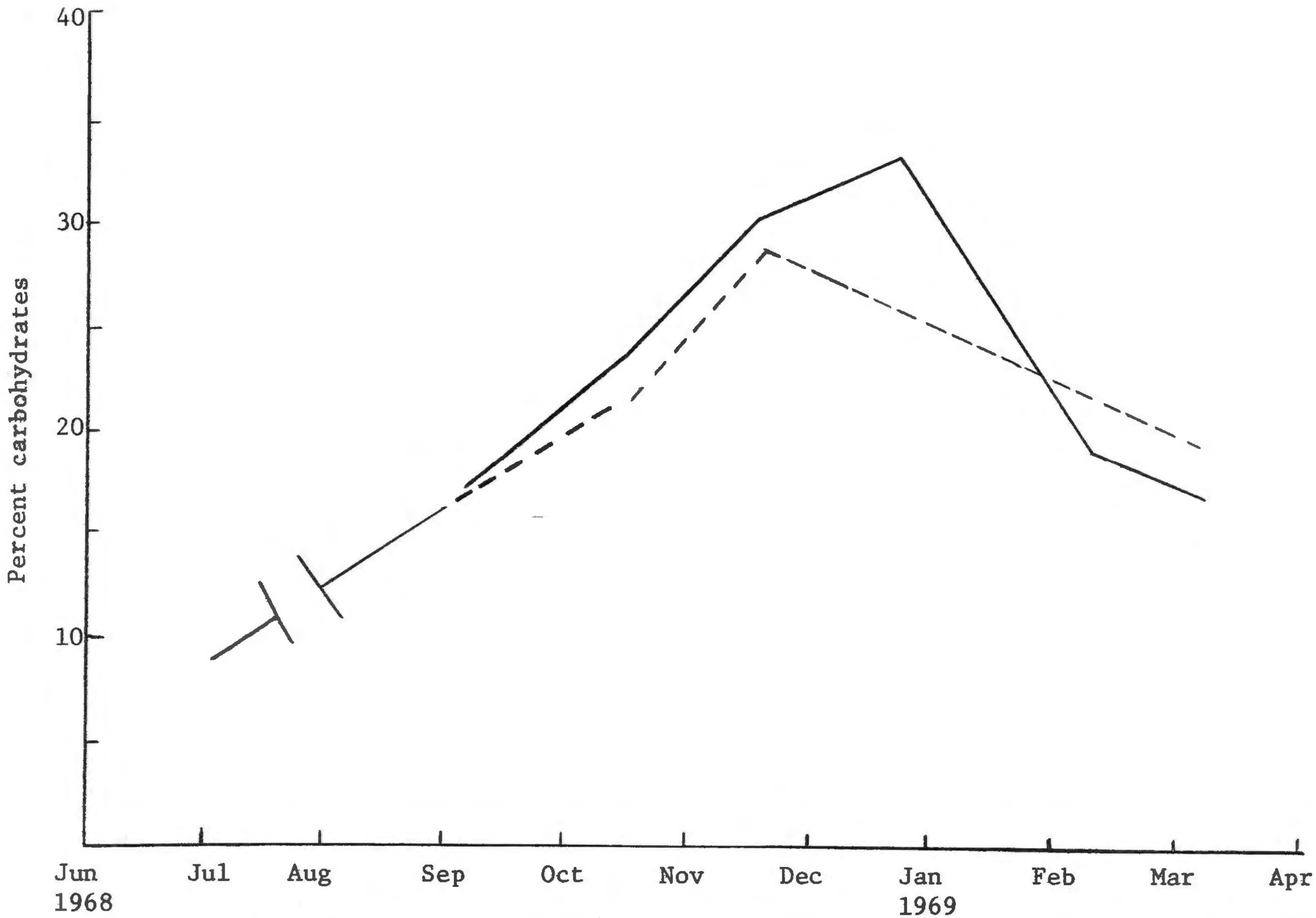

Figure 4. Percent of total nonstructural carbohydrates in the roots of Kenland (----) and Kentucky Synthetic A-2 (———) red clovers cut three times.

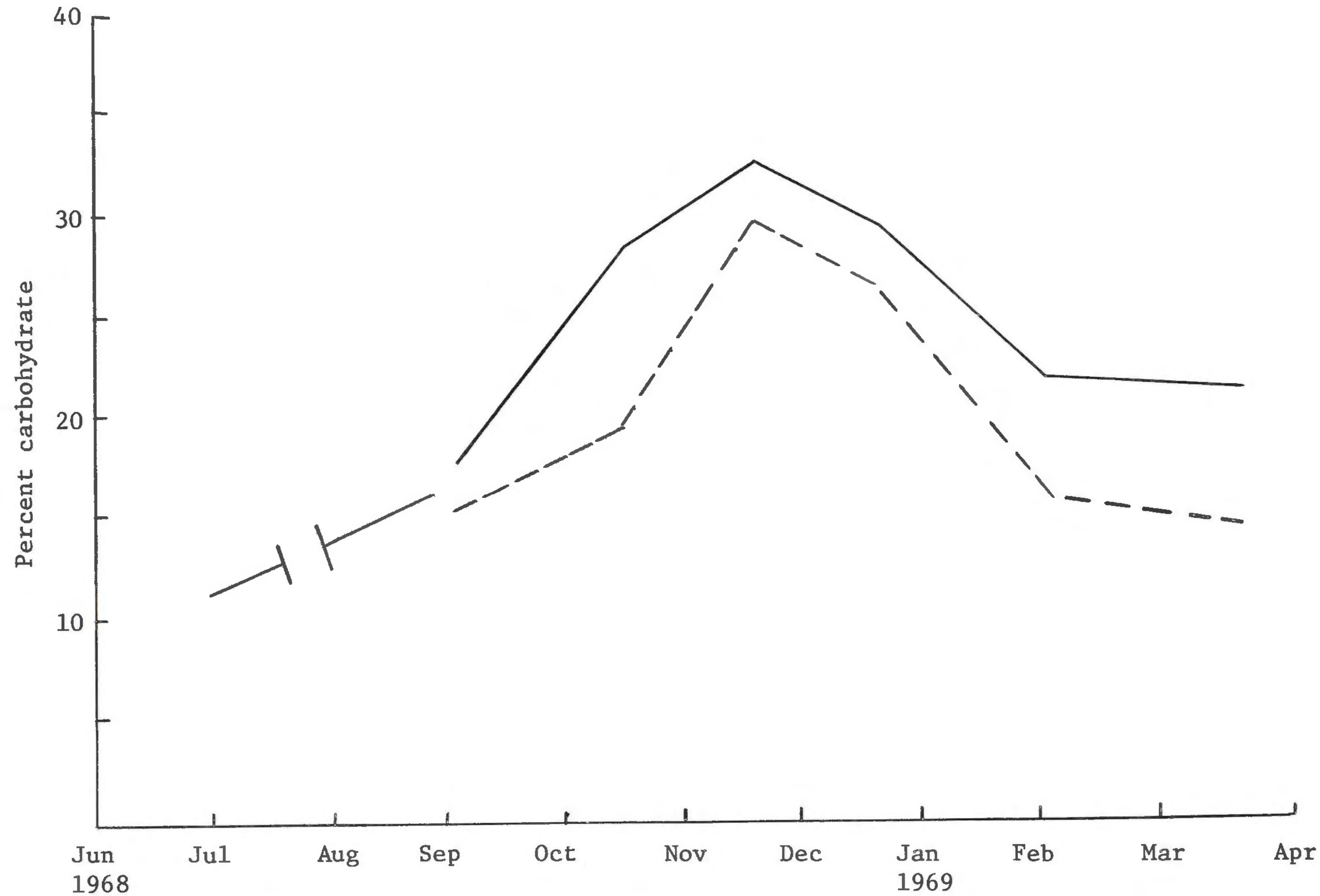

Figure 5. Percent of total nonstructural carbohydrates in the roots of Kenland (----) and Kentucky Synthetic A-2 (———) red clovers cut four times.

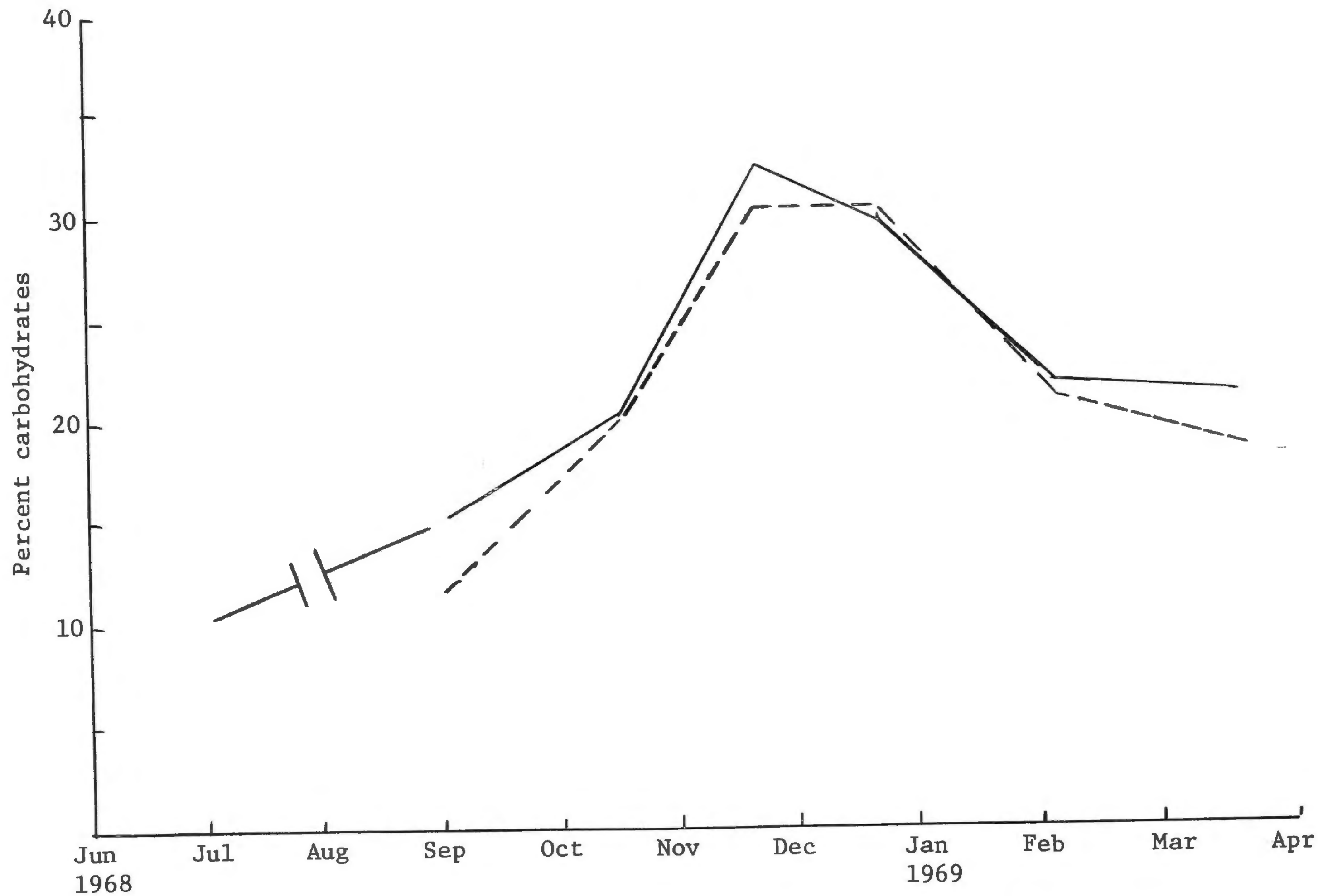

Figure 6. Percent of total nonstructural carbohydrates in the roots of Kenland (----) and Kentucky Synthetic A-2 (———) red clovers cut six times.

CHAPTER V

Discussion

Kentucky Synthetic A-2 proved to be the more persistent of the two varieties of red clover tested. On the last date of stand estimates all cutting treatments of the Kentucky Synthetic A-2 were significantly higher than all the cutting treatments of Kenland. Plant densities on this date were about twice as high in the Kentucky Synthetic A-2. All the significance in plant density was attributed to varieties except for a cutting treatment response on the first estimate date and a significant interaction between varieties and cutting practices on the last estimate date (Table 3, page 13).

Kentucky Synthetic A-2 had a greater plant density than Kenland in June of the first year. This could possibly be due to a higher resistance to crown rot (*Sclerotinia trifoliorum* Erikss.) by Kentucky Synthetic A-2 than by Kenland. Crown rot was prevalent during the first winter after seeding. The 6-cut treatments of both varieties had greater plant densities than the rest of the cutting treatments on the first counting date. This could possibly be attributed to the early cutting reducing the competition between red clover and weeds or other red clover plants.

Weather conditions at certain intervals coupled with intensive cutting treatments contributed to heavy losses in stand between June 28, 1968 and October 22, 1968. Two intervals of especially critical climatic conditions were a period in the middle of July when precipitation was below normal and a period at the last of August and first of September when precipitation was below normal and temperatures above normal. Reduction in stand was especially high in the 6-cut treatments, probably because the cutting dates were too frequent to allow for sufficient accumulation of carbohydrates to be used in regrowth (Figures 1 and 2, pages 19 and 21).

Kentucky Synthetic A-2 plants maintained significantly higher percentages of total nonstructural carbohydrates than did Kenland plants on three of the seven sampling dates. On the first sampling date the Kentucky Synthetic 4-cut, Kentucky Synthetic 2-cut and Kenland 2-cut treatments were significantly higher in total non-structural carbohydrates than the rest of the treatments.

On the September 5, 1968 sampling date, no significant difference was found among the treatments. This sampling date was immediately after a period of climatic stress. This stress possibly caused all the plants to be uniformly low in total nonstructural carbohydrates.

Carbohydrate percentages in all treatments began increasing in October and continued to increase until December. On October 15, 1968, the Kentucky Synthetic 4-cut treatment was significantly higher in total nonstructural carbohydrates than the other treatments, but after this date there was little or no significant difference in total nonstructural carbohydrates among any of the treatments. Possibly after this date all the plants that would be low in carbohydrates had died, leaving only the more hardy plants to be sampled. Sharp gains in total nonstructural carbohydrates in all cutting treatments in late fall are in agreement with those of Smith (23), who reported an inverse relationship between total available carbohydrates and number of cuts performed, however.

Total nonstructural carbohydrates declined considerably from December 18, 1968 to March 21, 1969. This indicated that the carbohydrates had been involved in metabolic activity (possibly respiration and development of cold hardiness). Stand densities did not decline appreciably during this period and climatic conditions were colder than normal with little or no snow cover. These facts indicate that winter killing in the first-year stand was not a major problem in this experiment.

Simple correlation coefficients between carbohydrate percentages and stand estimates on selected dates are presented in Table 6. It was thought that the concentration of carbohydrates in plants of a treatment on a given date might be related to stand density for that treatment on some later date. No correlation coefficient was significant at the .05 level of probability. A sampling of only the surviving plants for carbohydrates at some of the later dates may have removed some of the relationship that may have existed earlier between the two variables. A correlation coefficient relating the September carbohydrate percentages with the reduction in stand from September to October was positive and significant at the .05 level of probability, although this coefficient would be expected to be negative.

Table 6. Correlations of carbohydrate percentages on selected dates with stand estimates on selected dates

Carbohydrate percentage vs. Stand estimate	Correlation coefficient
June carbohydrates vs. Sept. stand	-0.13 NS
Sept. carbohydrates vs. Oct. stand	0.28 NS
Sept. carbohydrates vs. Dec. stand	0.15 NS
Oct. carbohydrates vs. Mar. stand	0.32 NS
June carbohydrates vs. June-Sept. stand reduction	0.00 NS
Sept. carbohydrates vs. Sept.-Oct. stand reduction	0.44*

*Significant at the .05 level of probability.

CHAPTER VI

Summary

The effects of four cutting schedules on persistence of the plants and concentration of carbohydrates in the roots of two varieties of red clover (*Trifolium pratense* L.) were studied at Knoxville, Tennessee. The two varieties studied were Kenland, a recommended variety in Tennessee, and Kentucky Synthetic A-2, an experimental variety developed at the University of Kentucky. Each variety was subjected to cutting frequencies of two, three, four and six times per year. Stand estimates were made and samples were taken for carbohydrate analysis at selected intervals during the first year after the seedling winter.

Kentucky Synthetic A-2 maintained a greater stand density than Kenland at the termination of the study. At that time there was no significant difference in stand density among cutting treatments within a variety. Stand density of the Kentucky Synthetic A-2 was two to three times greater than was that of the Kenland.

Carbohydrate reserve trends revealed a significantly higher percentage of total nonstructural carbohydrates in the roots of Kentucky Synthetic A-2 than in those of Kenland on three of the seven sampling dates. Yearly trends for all treatments included a slight increase in percentage from June to September, a sharp increase during the late fall months, and a decrease in total nonstructural carbohydrates from a November or December peak into spring.

Correlation coefficients for the relationship between the

percentage of total nonstructural carbohydrates on a given date and the stand density for the same treatment on some later date did not reveal any significant relationship. The correlation coefficient for September carbohydrates with the September to October stand reduction was positive and significant at the .05 level of probability.

REFERENCES

REFERENCES

1. Aicher, L. C. 1917. The production of clover seed under irrigation in southern Idaho. Idaho Agr. Exp. Sta. Bull. 100.

2. Bird, J. N. 1948. Early and late types of red clover. J. Agr. Sci. 28:444-453.

3. Bula, R. J., and Dale Smith. 1954. Cold resistance and chemical composition in overwintering alfalfa, red clover, and sweet clover. Agron. J. 46:397-401.

4. Cressman, R. M. 1967. Internal breakdown and persistence of red clover. Crop Sci. 7:357-361.

5. Fergus, E. N., and E. A. Hollowell. 1960. Red clover. Adv. in Agron. 12:365-436.

6. ________, and W. D. Valleau. 1929. A study of clover failure in Kentucky. Kentucky Agr. Exp. Sta. Res. Bull. 269.

7. Gish, P. T., T. J. Smith, and A. S. Williams. 1961. Evaluation of forage crop varieties in Virginia. Virginia Agr. Exp. Sta. Bull. 528.

8. Graham, J. H., C. L. Rhykerd, and R. C. Newton. 1960. Internal breakdown in crown of red clover. Plant Dis. Reporter. 44:59-61.

9. Graves, Charles R. 1968. Performance of field crop varieties. Tennessee Agr. Exp. Sta. Bull. 450.

10. Greathouse, G. A., and N. W. Stuart. 1937. Enzyme activity in cold hardened and un-hardened red clover. Plant Physiol. 12:685-702.

11. Jung, G. A. and Dale Smith. 1960. Influence of extended storage at constant low temperature on cold resistance and carbohydrate reserves of alfalfa and medium red clover. Plant Physiol. 35:123-125.

12. ________, and ________. 1961. Trends of cold resistance and chemical changes over winter in the roots and crowns of alfalfa and medium red clover. I. Changes in certain nitrogen and carbohydrate fractions. Agron. J. 53:359-366.

13. Kendall, W. A., W. H. Stroube, and N. L. Taylor. 1962. Growth and persistance of several varieties of red clover at various temperature and moisture levels. Agron. J. 54:345-347.

14. Killebrew, J. B. 1898. Grasses and forage plants. Tennessee Agr. Exp. Sta. Bull. 2, 3 and 4.

15. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 155:375-380.

16. Newton, R. C., and J. H. Graham. 1960. Incidence of root feeding weevils, root rot, internal breakdown, and virus and their effect on longevity of red clover. J. Econ. Ent. 53:865-868.

17. Pearce, R. B., G. W. Fissel, and G. E. Carlson. 1968. Carbon movement and utilization in alfalfa. Presentation at 60th annual meeting of the American Society of Agronomy. New Orleans, La.

18. Pieters, A. J. 1923. Red clover culture. USDA Farmers Bull. 1339.

19. Reynolds, J. H. 1969. Persistence and reseeding of red clover. Tennessee Farm and Home Sci. 72:14-17.

20. Ruelke, C. O., and Dale Smith. 1956. Overwintering trends of cold resistance and carbohydrates in medium red, ladino, and common white clover. Plant Physiol. 31:364-368.

21. Smith, Dale. 1950. Seasonal fluctuation of root reserves in red clover. (*Trifolium pratense* L.). Plant Physiol. 25:702-710.

22. ________. 1957. Flowering response and winter survival in seedling stands of medium red clover. Agron. J. 49:126-129.

23. ________. 1962. Carbohydrate root reserves in alfalfa, red clover, and birdsfoot trefoil under several management schedules. Crop Sci. 2:75-78.

24. ________. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wisconsin Agr. Exp. Sta. Res. Rep. 41.

25. Somogyi, M. 1952. Notes on sugar determination. J. Biol. Chem. 195:19-23.

26. U.S. Department of Commerce. National Oceanic and Atmospheric Administration. 1970. Local Climatological Data, 1970. Annual Summary with Comparative Data, Knoxville, Tennessee.

27. Virtanen, A. I., and M. Nurmia. 1936. Studies on the winter hardiness of clover. I. Effects of cutting on the carbohydrate reserves of red clover roots. J. Agr. Sci. 26:288-295.

APPENDIX

Table 7. Cutting dates for cutting frequencies in 1968

Cutting frequency	Cutting number					
	1	2	3	4	5	6
6	Apr. 24	May 22	Jun. 25	Jul. 16	Aug. 16	Sep. 12
4	May 7	Jun. 25	Jul. 30	Sep. 12		
3	May 22	Jul. 16	Sep. 12			
2	Jun. 25	Sep. 12				

VITA

Richard Urban Clark was born in Pulaski, Tennessee on July 19, 1945. He attended elementary and junior high school in Lynnville, Tennessee. He graduated from Jones High School in Lynnville, Tennessee in 1963.

In September 1963 he entered the University of Tennessee, and in June 1967 he received a Bachelor of Science degree in Agriculture. In September 1967 he entered the Graduate School at the University of Tennessee. In April 1969 he entered the United States Army and was honorably discharged in November 1970. He received the Master of Science degree in Agronomy in June 1971.