FALL HARVEST EFFECTS ON ALFALFA ROOT TOTAL NONSTRUCTURAL CARBOHYDRATES AND PERCENT DRY MATTER

By

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AND PERCENT DRY MATTER

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PREFACE

Fall harvest management effects on root total nonstructural carbohydrates (TNC) and percent dry matter (%DM) of 'Cimarron' alfalfa were studied to further explain the disparity between earlier studies which lead to the recommendation of a 4 to 6 week "resting" period for alfalfa in the fall and later studies which have shown harvesting alfalfa during this time has little or no effect on forage yield in subsequent years. Patterns of fall and winter TNC and %DM were observed and compared with forage yield in the following years.

I am very grateful to my major advisor, Dr. Kevin J. Donnelly, for his invitation to study at Oklahoma State University and the help he extended to me during the course of this study. I am also very grateful to the other members of my committee, Dr. Andrew Mort, Dr. Jimmy Stritzke and Dr. John L. Caddel for their thoughtful input, suggestions and motivation to finish what was begun. Dr. Stritzke and Dr. Caddel have done much in previous studies to lay the groundwork for much of this study.

Mr. Terry O'Brien was of utmost assistance in perfoming much of the laboratory analysis, for which I am very grateful, as well as a great quantity of moral support. Time and space do not permit me to acknowledge the many individuals who assisted me in collection and analysis of field samples, often in the worst of weather.

To my wife and friend, Julianna, I am deeply grateful for her selfless sacrifices of time and effort to provide moral and finacial support during this time. My children, Elizabeth and Nathaniel, have provided great joy during this time. I am also deeply indebted to my parents, James H. and Ruth M. Ogg for their financial and moral support.

"It is the glory of God to conceal a matter,
But the glory of kings to search out a matter."

Proverbs 25:2

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CHAPTER I

INTRODUCTION

Literature Review

Oklahoma produced over 1.5 million tons of alfalfa (Medicago sativa L.) hay on 400,000 acres in 1985, providing an important source of protein for the state's livestock industry (Oklahoma Agricultural Statistics, 1985). Careful harvest management is needed to encourage stand persistence and vigor and sustain optimum production. Timing of the year-end harvest has been shown to be critical in northern states to maintain stand densities, yield levels, and root carbohydrate reserves necessary for cold tolerance, over-winter respiratory needs, and spring regrowth (Feltner and Massengale, 1965; Nelson and Smith, 1969; Smith, 1972). Research conducted in the northern US and Canada has shown the need for a 4- to 6-week regrowth period prior to the first killing frost to maintain yield levels and stand persistence.

The total nonstructural carbohydrate (TNC) cycle in alfalfa roots is an important physiological process allowing the plant to regrow following forage harvest or dormant periods induced by winter or drought. Rapid regrowth following forage harvests during the growing season draws upon carbohydrate reserves stored in the plant's taproot until sufficient leaf area is established to provide carbohydrates by

means of photosynthesis for continued growth and accumulation of carbohydrate reserves in the root tissue (Brown et al., 1972). Excess TNC are transported to the taproot as simple sugars and converted to the storage form--starch--thus replenishing the depleted TNC reserves. The ability of the plant to accumulate sufficient TNC prior to the next harvest is influenced by the frequency of harvests. Numerous harvests which do not permit the accumulation of sufficient TNC between harvests lead to decreased stand and yield (Reynolds, 1971; Brown et al., 1972).

High levels of TNC are needed for hardening, over-winter respiration and spring regrowth (Smith, 1972). Fall harvest management guidelines have been established for the northern US and Canada which allow sufficient time during which environmental conditions are favorable for regrowth and subsequent TNC reserve accumulation between the last fall harvest and the killing freeze (Smith, 1981a). These management recommendations take into consideration cooler temperatures, shorter days, and lower light intensity, all of which contribute to slowed growth of alfalfa in the fall. Smith (1972) cited evidence leading to the recommendation that the timing of the year-end harvest of alfalfa occur four to six weeks prior to the first killing frost (or immediately after) to avoid reduction in root TNC and stand injury. Irvine and McGlunn (1982) found a third harvest before cessation of growth in the fall caused winter injury and reduced yields in southwestern Saskatchewan. These recommendations attempt to avoid the possibility of going into the winter months with low TNC reserve levels.

While the 4- to 6-week regrowth period following the final fall harvest has been shown to allow for accumulation of adequate TNC reserve levels in the northern US and Canada, there is some indication that such

management practices may not apply to the southern US. In Oklahoma, Sholar et al. (1983) reported that only in the case of a very short recovery period (six days) between the final harvest and the first killing freeze (-5 C) was there a significant reduction of root TNC reserves. Total yield the following year was not affected. They suggested that alfalfa grown in the southern US may be more tolerant of late fall harvest than that grown in northern regions. Reynolds (1971) in Tennessee found that root TNC had increased to about the same levels by 1 November under several different harvest schedules having the same final harvest date. He surmised that TNC continued to accumulate in the fall due to the presence of photosynthetically active tissue and favorable climatic conditions. However, in contrast to these other studies, Dougherty and Evans (1982) in Kentucky recommended a fourth and final harvest no later than mid-September or after a freeze down of the topgrowth.

Recent evidence from some researchers in the northern US also suggests that the recovery period may not be as critical to yield and stand persistence when using improved cultivars and soil fertility practices. These studies show results similar to Sholar's. Tesar and Yager (1985) in Michigan found that TNC levels differed during the fall as a result of different fall harvest dates, but were not different by mid-December. In Wisconsin, Walgenbach (1983) has shown that TNC may not be depleted by regrowth in the fall due to the slow rate of growth under cooler temperatures and shorter days. Further research is needed in the Southern Plains to describe the TNC cycle in the fall and winter in response to harvest date and its effect upon yields and stand densities.

In some instances, late fall harvest or grazing of alfalfa for hay may be justified. Nonirrigated alfalfa in Oklahoma may be cut two or three times in the spring and early summer. Often, insufficient moisture during July and August causes alfalfa to go into a summer dormancy during which forage growth is retarded. Often, sufficient precipitation occurs in the fall promoting considerable regrowth. Hall et al. (1986) have shown that alfalfa continued to accumulate TNC when stressed by drought, and regrew when adequate precipitation occurred. In Oklahoma, a significant amount of regrowth may occur following harvests prior to mid-October. Even when there is insufficient forage to justify mechanical harvest, there may be enough forage to permit grazing.

Utilization of the forage during the fall months may be of secondary importance when compared to grazing or removal of fall regrowth as a means of helping control the alfalfa weevil (Hypera postica Gyllenhal). Winter grazing and the introduction of parasitic wasps (Bathyplectes curculionis Thompson) reduced the number of over-wintering alfalfa weevil eggs by 60% (Senst and Berberet, 1980). Removal of the stems decreases the sites for oviposition as well as eliminating plant cover in which adults overwinter. Fick and Lui (1976) found that alfalfa weevil larvae feeding lowered the level of root TNC and slowed the maturity of alfalfa plants. Fick (1976) also found that feeding by weevil larvae delayed the accumulation of root TNC which may have caused subsequent reductions in regrowth rates. By removal of potential oviposition sites through grazing or harvest during the fall and winter, it may be possible to reduce weevil numbers and lessen potential damage by larval feeding.

Standard chemical methods of extracting and determining TNC concentrations in forages have been published (Smith, 1981b). Extraction of the TNC from plant tissue using either acids or enzymes has been compared (Smith et al., 1964). Grotleutchen and Smith (1967) determined that enzymatic extraction of TNC was more specific and accurate than acid extraction. Greub and Wedin (1969) concluded that in species where starch is an important storage compound, enzymatic extraction was superior to extraction with sulfuric acid. Gabrielson et al. (1985) used enzymatic extraction with amyloglucosidase in conjunction with dinitrosalicylic acid as a colorimetric test for reducing sugars in alfalfa and cicer milkvetch (Astragalus cicer L.) root tissue.

Chemical methods are expensive and time-consuming, often requiring personnel trained in specific procedures. Wolf (1978) compiled information from a number of studies over various environments and found a high correlation between percent alfalfa root dry matter (%DM) and root TNC. Root %DM was determined with a gravimetric method using simple and readily available equipment. However, Nelson and Smith (1968) found that a high percentage of the TNC in alfalfa roots during the fall was sucrose. Loss of sucrose might occur using the gravimetric method due to leaching of soluble sugars while obtaining the saturated weight. Both the chemical and gravimetric method should be compared to determine their correlation in the fall and winter.

Objectives

The objectives of this study were to determine levels of TNC in alfalfa roots in the fall and winter, to compare the effect of the timing of fall harvest on yield in subsequent years and stand persistence, to determine the influence of fall harvest on TNC levels when measured on certain dates and finally, to compare and correlate chemical analysis of TNC and gravimetric estimation of TNC levels using %DM.

CHAPTER II

FALL HARVEST EFFECTS ON ROOT TOTAL NONSTRUCTURAL CARBOHYDRATE CYCLES IN THE FALL AND WINTER

Introduction

The ability of alfalfa (Medicago sativa L.) to regrow following repeated harvests during the growing season and to persist from year to year are desirable characteristics which make it the most popular forage legume grown in the United States. Surplus photosynthate is translocated from the leaves to storage organs (the taproot) during times of optimum photosynthesis and stored as nonstructural carbohydrates. These reserves are used for respiration and redistributed for synthesis of new structural components upon inititation of regrowth following harvest or in early spring (Brown, et al., 1972).

Concentration of total nonstructural carbohydrates (TNC) in alfalfa roots follows a cyclical pattern of depletion and replinishment during the growing season (Smith, 1962). Root TNC are rapidly depleted following forage harvest, but begin to recover as the plant develops sufficient leaf area to provide energy for growth of new plant material and storage of TNC in the taproot. Repeated harvests with insufficient time between harvests to allow adequate accumulation of TNC reserves may

cause a general decline in the health of the stand as well as a loss of plants (Reynolds, 1971). Smith (1972) reviewed the importance of root TNC in the fall and winter and concluded that adequate levels of TNC during the fall and winter were critical for stand survival and yield. Feltner and Massengale (1968) found alfalfa plants low in root TNC during the fall had higher disease ratings than plants with higher TNC. Kust and Smith (1961) found that frequent harvests during the growing season reduced TNC in mid-November and that the level of root TNC at that time was closely related to yield the following year. These and other studies have led to the recommendation that the final fall harvest occur four to six weeks prior to the first killing frost to allow sufficient time for root TNC to recover to adequate levels or immediately after the frost so that root reserves are not depleted by rapid regrowth (Smith, 1972).

In the southern US, Mays and Evans (1973) found that the ideal recovery time in Alabama was greater (six to eight weeks) than that recommended for northern states and that TNC levels fluctuated very little during that time, perhaps due to slowed regrowth and decreased respiration during October and November when temperatures were cooler but conditions for photosynthesis were still favorable. A study by Nelson and Smith (1969) showed that cooler temperatures lengthen time of development and reduce respiration, resulting in higher root TNC concentration.

Sholar et al. (1983) in Oklahoma found that in several alfalfa cultivars root TNC generally did not vary among different fall harvest treatments when measured following the first killing freeze. Collins and Taylor (1980) in Kentucky found alfalfa clipped on or after November

1 had little reduction in TNC of roots sampled on 15 November. Tesar and Yager (1985) in Michigan also found that root TNC did not differ by mid-December, even when differences occurred earlier in early and late-fall cutting treatments. They found yield was more closely related to the relative disease resistance of the cultivar and the level of K fertility. These results indicate that the concentration of TNC is not the only critical factor. Root TNC levels may not be as critical with the introduction of numerous improved alfalfa cultivars having high levels of disease resistance and improved soil fertility practices.

Several authors have speculated on the reasons for the late fall-early winter recovery of TNC in alfalfa roots. Reynolds in Tennessee noted that even though the topgrowth had been killed by freezing temperatures, rosette-type leaves near the crown remained green during the winter, allowing a small amount of photosynthesis to occur. Mays and Evans (1973) in Alabama noted that environmental conditions in the fall are favorable for photosynthesis and that viable leaves are present through the entire fall and winter. Sholar et al. (1983) in Oklahoma noted that fall regrowth was prostrate and leafy, and conditions were good for photosynthesis to occur during the fall. It appears that alfalfa may continue to photosynthesize and transport sugars to roots in the fall in the southern US.

The purpose of this study was to further analyze the trends of root TNC during the fall and winter in response to different fall harvest dates as well as to examine fall harvest effects on yield in subsequent years and plant population at the termination of the study.

Materials and Methods

Two experiments were initiated in September, 1984, at the Agronomy Research Station, Stillwater, Oklahoma. One experiment was on an irrigated fine silty, mixed, thermic, Cumulic Haplustoll (Experiment 1) and the other experiment was on a nonirrigated fine loamy, mixed, thermic Fluventic Haplustoll (Experiment 2). 'Cimarron' alfalfa was planted September, 1983, at a rate of 22.4 kg ha^{-1} and 16.8 kg ha^{-1} on Exp. 1 and Exp. 2, respectively. Exp. 1 had a soil pH of 6.7 and soil test levels of 135 kg P ha^{-1} and 299 kg K ha^{-1}. Fifty-two kg K ha^{-1} (as 0-0-62) were applied in the winter of 1985. Exp. 2 had a soil pH of 6.2 and soil test levels of 43 kg P ha^{-1} and 250 kg K ha^{-1}. No additional fertilizer was needed for Exp. 2.

Plots were arranged in a randomized complete block design with four replications. Plot dimensions were 5 x 6 m and 4 x 10 m for Exp. 1 and 2, respectively.

In the fall of 1984 and 1985, four and six fall cutting treatments were imposed on Exp. 1 and 2, respectively (Table I) using a small sickle-type mower. Routine forage harvests were taken during the growing season in 1985 and 1986 from the middle 1 x 6 m strip of each plot in Exp. 1 and the middle 1 x 10 m strip of each plot in Exp. 2 using a flail-type harvester.

All fall harvest treatments were imposed prior to the dates of the first killing freeze (-5 C). On the average, the first killing freeze has occurred about November 16. Temperatures of this magnitude occurred 3 December 1984 (-11 C) and 1 December 1985 (-7 C). However, both years the first effective killing temperatures may have occurred later than

TABLE I

1984 AND 1985 FALL HARVEST TREATMENT DATES

Dates of individual fall harvest treatments			
Irrigated		Nonirrigated	
1984	1985	1984	1985
October 1	September 28	October 3	September 28
October 19	October 16	October 17	October 16
November 3	November 1	October 31	November 1
November 16	November 18	November 16	November 20
--	--	August 31 November 16	August 23 September 28 November 20
--	--	August 31	August 23

these dates due to snow cover at the time of the first lethal air temperatures. Forage growth continued and appeared viable until late December in 1984 and mid-December in 1985.

After each fall harvest treatment was imposed, roots from two 500 cm^2 areas were sampled at weekly intervals from each plot until active growth was no longer apparent (about mid-December each year). Throughout the winter, roots were sampled on a monthly basis. After removal from the soil, whole plants were placed on ice until roots could

be washed to remove soil. Roots were clipped to a length of 10 cm from the crown buds and fine lateral roots were removed. Roots were heated at 100 C for 90 min and then dried at 70 C in a forced-draft oven. Dry root material was first ground through a 2 mm screen and then through a 0.25 mm screen to insure uniform particle size. Total nonstructural carbohydrates were extracted from 200 mg of root material using amyloglucosidase and amylase for 24 h at 54 C (Smith, 1982). In order to insure all sucrose in the samples had been hydrolyzed to reducing sugars, samples were heated in 0.3 N HCl for 30 min at 100 C. Reducing sugars were determined spectrophotometrically at a wavelength of 575 nm using dinitrosalicylic acid as an indicator (Gabrielson et al., 1985). Concentrations of TNC in dry root samples were calculted using regression equations determined by simutaneous analysis of starch standards of known concentrations. Samples from Exp. 2 taken during the fall of 1984 were analyzed in the laboratory by individual sampling dates. Samples from both years of the irrigated experiment and 1985 of the nonirrigated experiment were analyzed by replications to avoid confounding sampling date effects with laboratory run effects.

During the summer of 1985, roots were collected from the border area and analyzed to determine TNC cycles occurring during the regular growing season.

At the termination of both experiments, a 2.5 m^2 area of the yield strip was undercut and plants were counted in order to estimate stand persistence.

Results and Discussion

Alfalfa root TNC levels declined rapidly after each forage harvest during the growing season, and increased until the next forage harvest (Figure 1). Root TNC depletion and accumulation patterns during the fall differed with fall harvest treatment in both irrigated and nonirrigated experiments.

Experiment 1 -- Irrigated

The relative amplitude of the final seasonal root TNC cycles was diminished as the date of fall harvest was delayed (Figures 2 and 3). In both years of the irrigated experiment, harvest dates near 1 October and 15 October resulted in a decline in root TNC followed by an increase in root TNC up to late November or early December. These root TNC cycles were accompanied by regrowth of forage during the fall (Table II). In both years, those treatments cut near 1 October had lower root TNC levels during October and early November than those cut near 15 November. Harvest treatments cut near 1 and 15 November showed some decline in root TNC, but not nearly as pronounced a decrease as seen in the earlier harvest treatments. Less regrowth occurred following fall harvest of these treatments than in those cut in October. There was no recovery of root TNC following the two November harvests, perhaps due to a combination of insufficient leaf area to support photosynthesis, cooler temperatures and shorter daylengths, resulting in an overall decrease in plant activity. All treatments showed a general decline of root TNC throughout the winter.

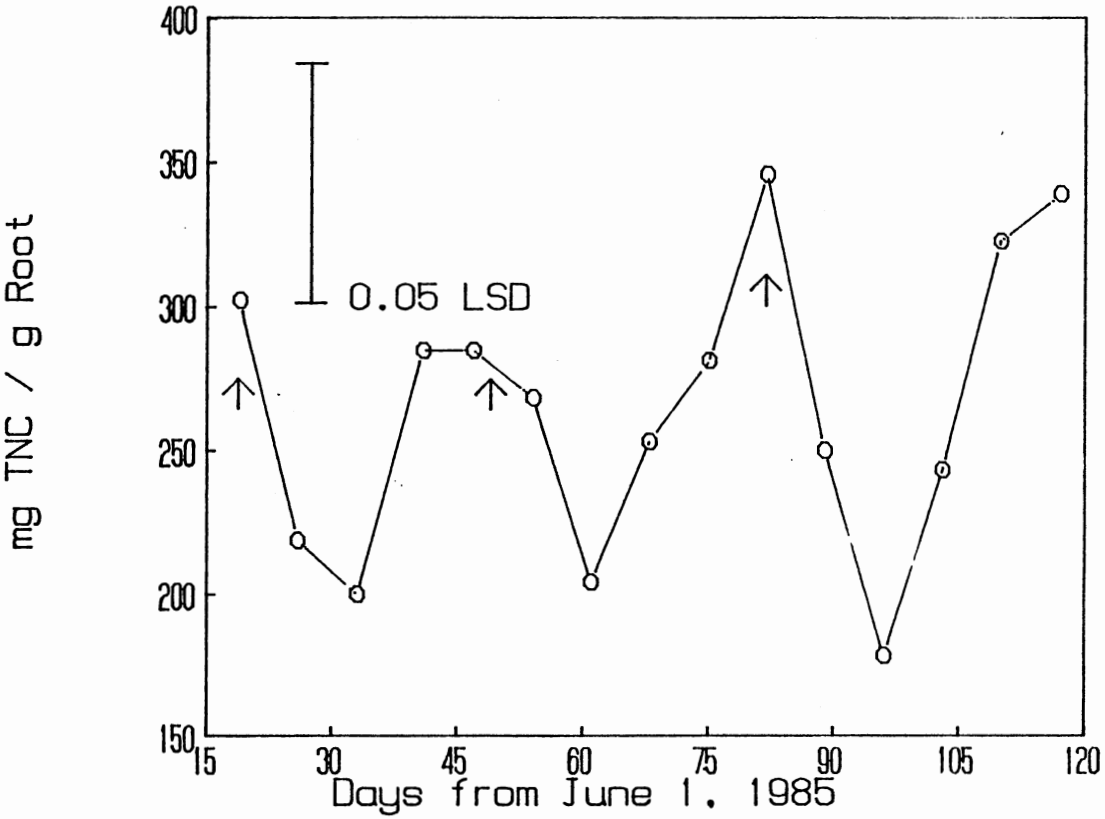

Figure 1. Root TNC levels of plants sampled from border area of the irrigated experiment during the 1985 growing season. Arrows indicate seasonal harvests.

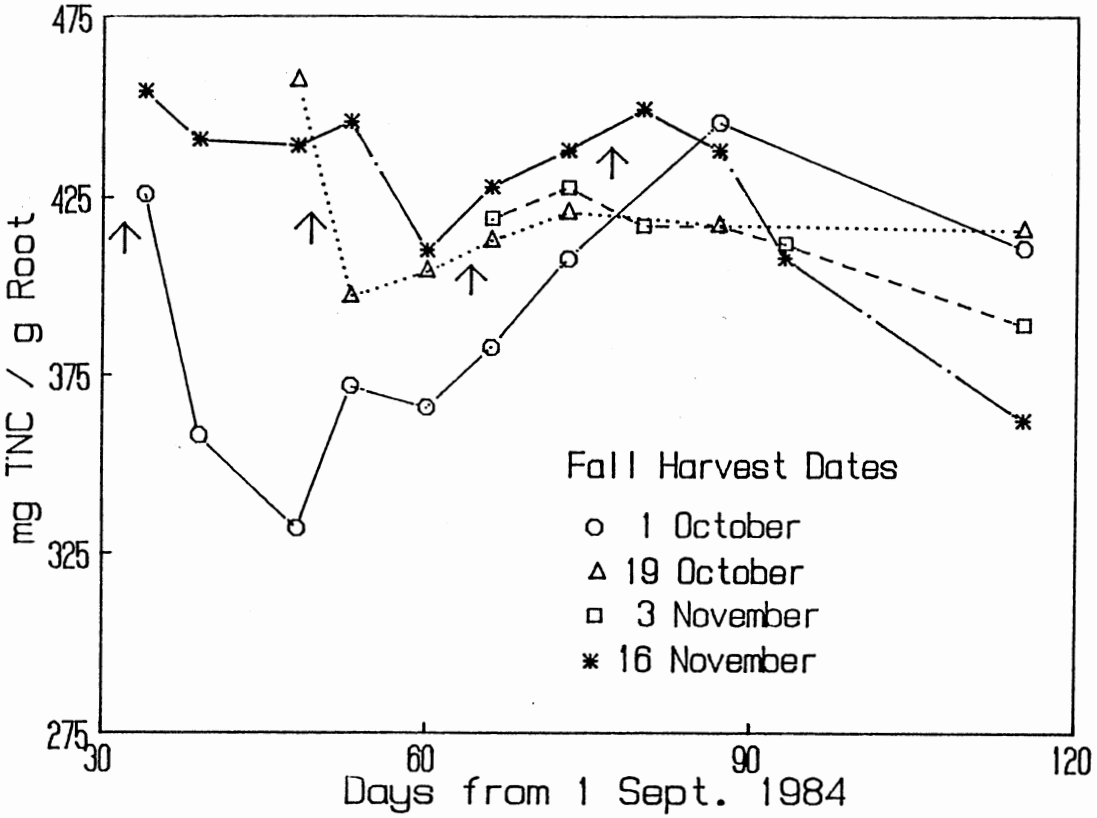

Figure 2. Fall 1984 root TNC trends of irrigated alfalfa in response to fall harvest treatments. Arrows indicate harvest dates.

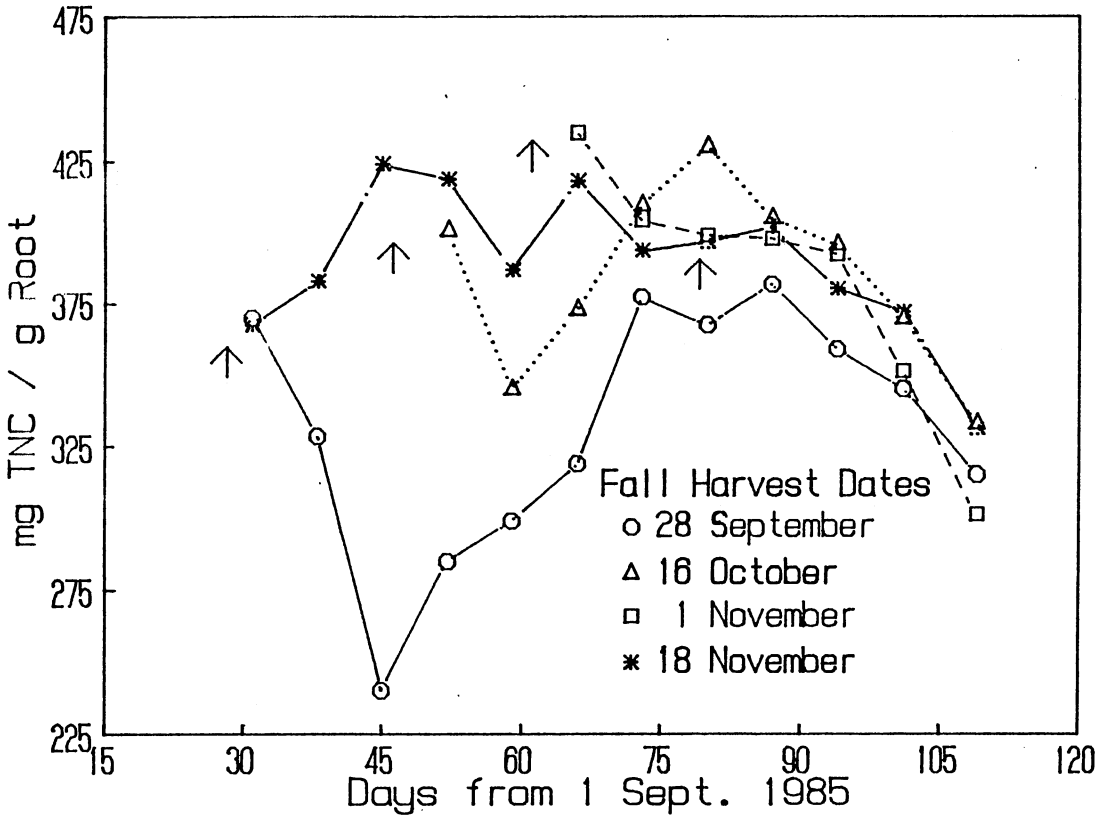

Figure 3. Fall 1985 root TNC trends of irrigated alfalfa in response to fall harvest treatments. Arrows indicate harvest dates.

TABLE II

REGROWTH OF 'CIMARRON' ALFALFA IN IRRIGATED AND NONIRRIGATED EXPERIMENTS AFTER FALL HARVESTS

1984		1985	
Fall Harvest Date	Regrowth by 24 Dec	Fall Harvest Date	Regrowth by 10 Dec
	Irrigated		
	Mg ha^{-1}		Mg ha^{-1}
Oct 1	1.67a*	Sept 28	1.03b
Oct 19	1.35a	Oct 16	1.51a
Nov 3	1.07b	Nov 1	0.69b
Nov 16	0.75b	Nov 18	0.24c
5% LSD	0.53		0.36
CV (%)	27.6		26.0
	Nonirrigated		
Aug 31	1.78a	Aug 23	1.70a
Oct 3	1.80a	Sept 28	0.96b
Oct 17	1.71a	Oct 16	0.97b
Oct 31	1.25b	Nov 1	0.43c
Nov 16	0.96b	Nov 20	0.41c
Aug 31 & Nov 16	0.87b	Sept 28 & Nov 20	0.22c
5% LSD	0.40		0.40
CV (%)	19.2		34.2

* Within columns, means followed by the same letter are not significantly different at the 0.05 level.

Treatment differences in root TNC occurred on six of the eleven 1984 sampling dates prior to the first killing freeze and on all dates following (Table III). Treatment differences in root TNC occurred on six of the nine 1985 sampling dates prior to the first killing freeze and on only one date following (Table IV). However, many of the treatment differences that occurred prior to mid-November may be attributed to the various stages of regrowth that each harvest treatment was in as a result of staggering the harvest dates throughout the fall.

In 1984-85, significant differences in TNC often occurred between treatments harvested near 1 October and those harvested near 15 November (Table III). On 9, 18, 23 and 30 October and 6 November, the treatment harvested 1 October had significantly lower levels of TNC than the treatment harvested 16 November. This was an expected result since the 16 November treatment had not yet been harvested on these dates while the 1 October treatment was going through an active regrowth cycle. From 13 November through 3 December, no significant differences in root TNC were observed. By 24 December and throughout mid-winter, the 15 November treatment had lower root TNC levels than the 1 October treatment.

In the fall of 1985, differences again occurred between TNC levels of the treatment harvested 28 September and that harvested 18 November (Table IV). Root TNC levels of the 28 September treatment were lower than the 18 November treatment on the 8 October and 5 November sampling dates. After this time, there were no significant differences in root TNC levels. While treatments cut earlier recovered, treatments cut later dropped from initially higher levels of TNC, resulting in a convergence of TNC values among treatments in late November (Figure 3).

TABLE III

ROOT TNC MEANS OF THE IRRIGATED EXPERIMENT DURING THE FALL AND WINTER OF 1984-1985

Sampling Date	1984 Fall Harvest Date			
	Oct 1	Oct 19	Nov 3	Nov 16
	---------- mg g^{-1} ----------			
Oct 4	426	-	-	454
9	358b	-	-	441a
18	332b	457a	-	439a
23	372b	397b	-	446a
30	366b	404a	-	410a
Nov 6	383b	413ab	419ab	428a
13	408	421	428	438
20	-	-	417	449
27	446	417	417	438
Dec 3	-	-	412	408
24	411a	416a	389ab	362b
Jan 15	355a	370a	350a	314b
Feb 19	333a	336a	306ab	289b
Mar 15	195ab	210a	183ab	159b

* Within rows, means followed by the same letter are not significantly different at the 0.05 level. LSD = 36.

TABLE IV

ROOT TNC MEANS OF THE IRRIGATED EXPERIMENT DURING THE FALL AND WINTER OF 1985-1986

Sampling Date	1985 Fall Harvest Date			
	Sept 28	Oct 16	Nov 1	Nov 18
	mg g^{-1}			
Oct 1	370	-	-	367
8	328b	-	-	383a
15	240b	-	-	424a
22	285b	401a	-	419a
29	299c	345b	-	387a
Nov 5	319c	374b	435a	418a
12	377	410	404	394
19	367b	430a	399ab	397ab
26	382	405	398	402
Dec 3	359	396	392	380
10	345	370	351	372
18	315	333	301	331
Jan 6	302	307	279	315
Feb 19	276ab	299a	250b	261b
Mar 6	272	269	265	288

* Within rows, means followed by the same letter are not significantly different at the 0.05 level. LSD = 37.

After 26 November, TNC levels of all treatments began to decline, and differences among treatments throughout the winter were generally nonsignificant in contrast to 1984.

Although there were treatment differences among 1984 root TNC levels, there were no differences in any seasonal forage harvest yields the following year (Table V). Lower mid-winter root TNC levels of the late fall harvest treatments apparently did not limit the subsequent year's performance. Differences in yield among treatments did occur at the first forage harvest in 1986 (Table VI). The 18 November fall harvest treatment yielded significantly higher than the 28 September harvest treatment in the first spring harvest of 1986. This difference was also reflected in the total 1986 seasonal yield. However, the 2-year total yield was not significantly different among treatments. All other seasonal harvests had no differences in yield.

Although the 28 September 1985 fall harvest treatment was imposed prior to the six-week critical period, it had significantly lower TNC levels than the other treatments during the critical period (Figure 3). This treatment had significantly lower yields in the first spring harvest of 1986. This may lend limited support to management practices in the Southern US which promote relatively high root TNC levels during the fall to support metabolic processes. However, treatments with low spring yields had higher yields the previous fall, resulting in no significant differences in the 2-year total yield of the experiment (Table VI).

TABLE V

FORAGE DRY MATTER YIELDS FROM 1984 FALL AND 1985 SEASONAL HARVESTS OF THE IRRIGATED STUDY

1984 Fall Harvest Date	1984 Fall Harvest Yield	1985 Forage Harvest Dates				1985 Summer Total
		5/9	6/19	7/19	8/21	
	----------Mg ha^{-1}----------					
Oct 1	1.7c*	5.0	4.1	3.2	2.8	15.1
Oct 19	2.2a	5.1	3.9	2.9	2.8	14.7
Nov 3	1.9b	5.3	4.0	3.1	2.8	15.3
Nov 16	1.7c	5.3	4.0	3.1	2.7	15.1
5% LSD	0.1	NS	NS	NS	NS	NS
CV (%)	3.9	5.2	11.0	9.5	5.7	5.0

NS Not significantly different.

* Within columns, means followed by the same letter are not significantly different at the 0.05 probability level.

TABLE VI

FORAGE DRY MATTER YIELDS FROM 1985 FALL AND 1986 SEASONAL HARVESTS, AND 2-YEAR TOTAL OF THE IRRIGATED STUDY

1985 Fall Harvest Date	1985 Fall Harvest Yield	1986 Forage Harvest Dates					Summer 1986 Total	2-Year Total
		4/24	5/29	6/26	7/30	9/4		
	----------Mg ha^{-1}----------							
Sept 28	2.3a*	2.8c	3.4	3.6	2.6	2.0	14.4c	33.5
Oct 16	2.1a	3.1bc	3.5	3.4	2.7	1.9	14.6bc	33.5
Nov 1	1.8b	3.4ab	3.7	3.8	2.7	1.9	15.5ab	34.5
Nov 18	1.1c	3.6a	3.8	4.1	2.6	2.0	16.0a	33.9
5% LSD	0.2	0.4	NS	NS	NS	NS	1.0	NS
CV (%)	2.8	7.3	5.1	10.5	6.5	6.3	4.0	3.2

* Within columns, numbers followed by the same letter are not significantly different at the 0.05 level.

Experiment 2 -- Nonirrigated

While trends of root TNC in the fall and winter were similar between years in the irrigated experiment, trends during this time in the nonirrigated experiment differed from year to year. From July until mid-October of 1984, there was little precipitation and subsequently, very little growth occurred during this time, while TNC values were relatively high. Most fall regrowth occurred in the last two weeks of October, as treatments cut prior to this time showed no significant differences in regrowth (Table II).

The treatment harvested 31 August 1984 showed only a small shallow cycle in root TNC after cutting (Figure 4). An additional small cycle independent of harvest treatments occurred later, perhaps in response to mid-October precipitation. During the late fall of 1984, TNC cycles of treatments harvested only 31 August and those harvested 31 August and 16 November responded differently. The treatment cut only once in August continued to increase in root TNC after 15 November, while that harvested twice declined after 15 November (Figure 4). The 31 August treatment may have continued to accumulate TNC during December because it already had sufficient leaf area to support photosynthesis and replenishment of root TNC. The treatment cut both in August and mid-November may have declined in root TNC during December due to removal of the canopy and subsequent regrowth initiated in response to the unusually warm weather that occurred in December. Although regrowth did occur, it was not sufficient to support accumulation of root TNC following the mid-November harvest.

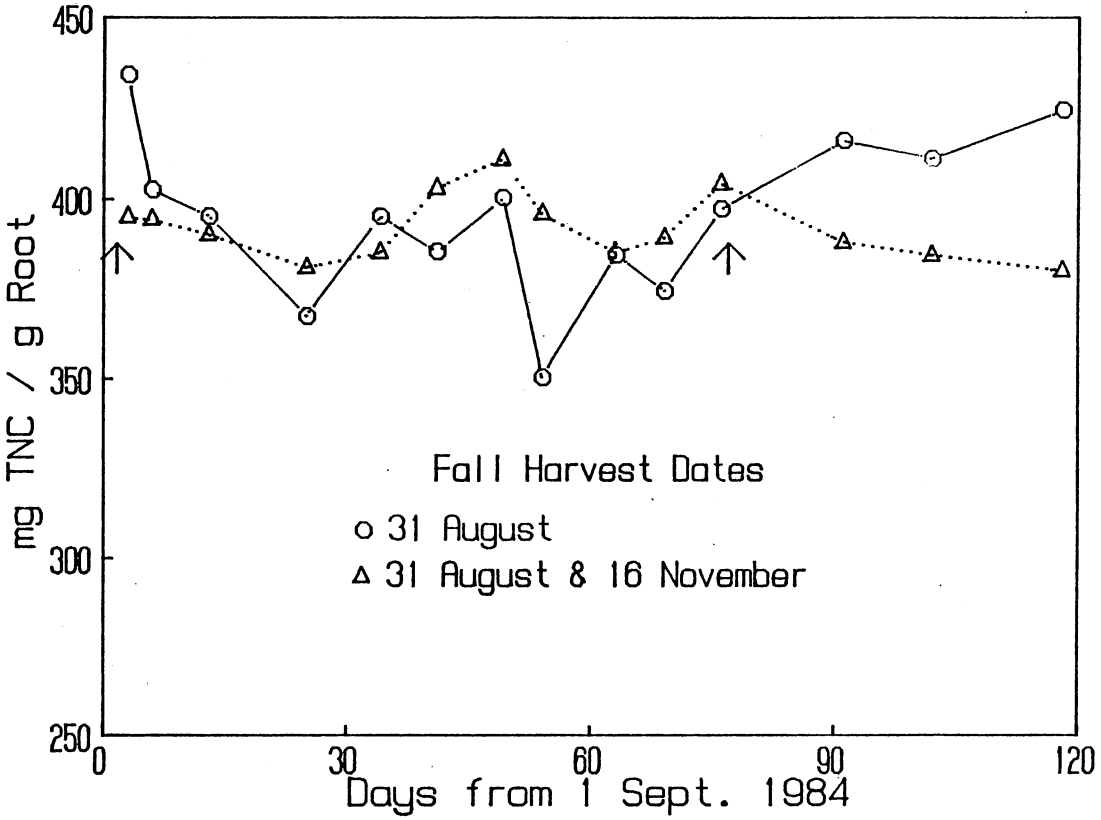

Figure 4. Fall 1984 root TNC trends of nonirrigated alfalfa, in response to one fall cut (31 August) and two fall cuts (31 August and 16 November). Arrows indicate harvest dates.

As in both years of the irrigated study, treatments harvested near 1 November 1984 had no cyclical response to harvest, but only a decline of root TNC (Figure 5). Treatments harvested 3, 17 and 31 October all resulted in post-harvest root TNC cycles of similar amplitude in contrast to the greater amplitude of earlier harvests observed in the irrigated study. Limited regrowth due to moisture stress following the earlier harvests probably explains why larger cycles were not observed. Hall et al. (1986) cited accumulation of photosynthate in roots during periods of drought stress. Available soil moisture and precipitation pattern as well as harvest date appeared to be a factor in influencing root TNC cycles in the fall of 1984. Drought stress may have had a confounding effect during this time.

In the fall of 1985, the nonirrigated experiment responded in much the same way as did the irrigated experiments in 1984 and 1985 (Figure 6). Precipitation in the summer and fall of 1985 was sufficient to allow regrowth following each fall harvest date. Treatments harvested prior to 1 November responded to cutting with a cycle in root TNC followed by a slow over-winter decline. As the date of the final fall harvest was delayed, the amplitude of the TNC cycle decreased. In the 20 November harvest treatment (Figure 7), root TNC levels remained relatively static prior to harvest, resulting in higher initial root TNC values at the time of cutting compared to those treatments harvested before this date. Therefore, in spite of prior difference in TNC among treatments, there were no differences in root TNC levels by late November or early December. Even the treatment harvested twice in the fall of 1985 (cut 28 September and 20 November) recovered to relatively high levels by the time of the 20 November forage harvest, although it

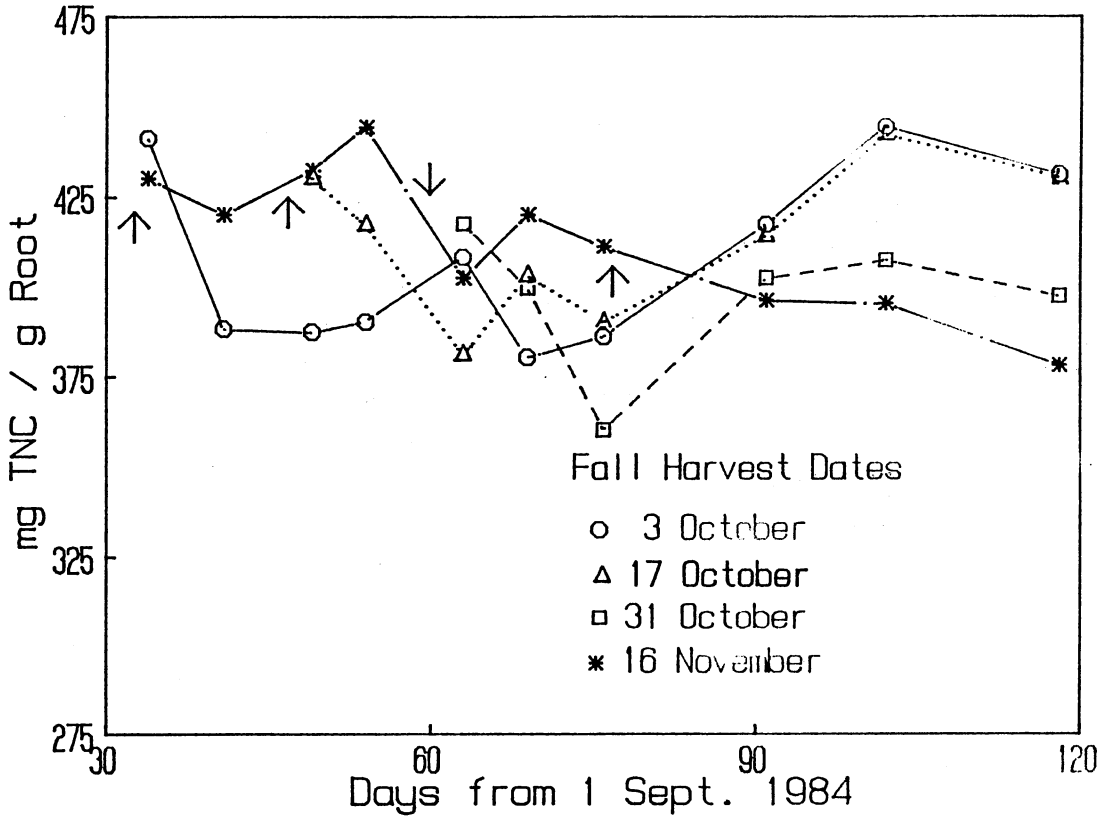

Figure 5. Fall 1984 root TNC trends of nonirrigated alfalfa in response to fall harvest treatments. Arrows indicate fall harvest dates.

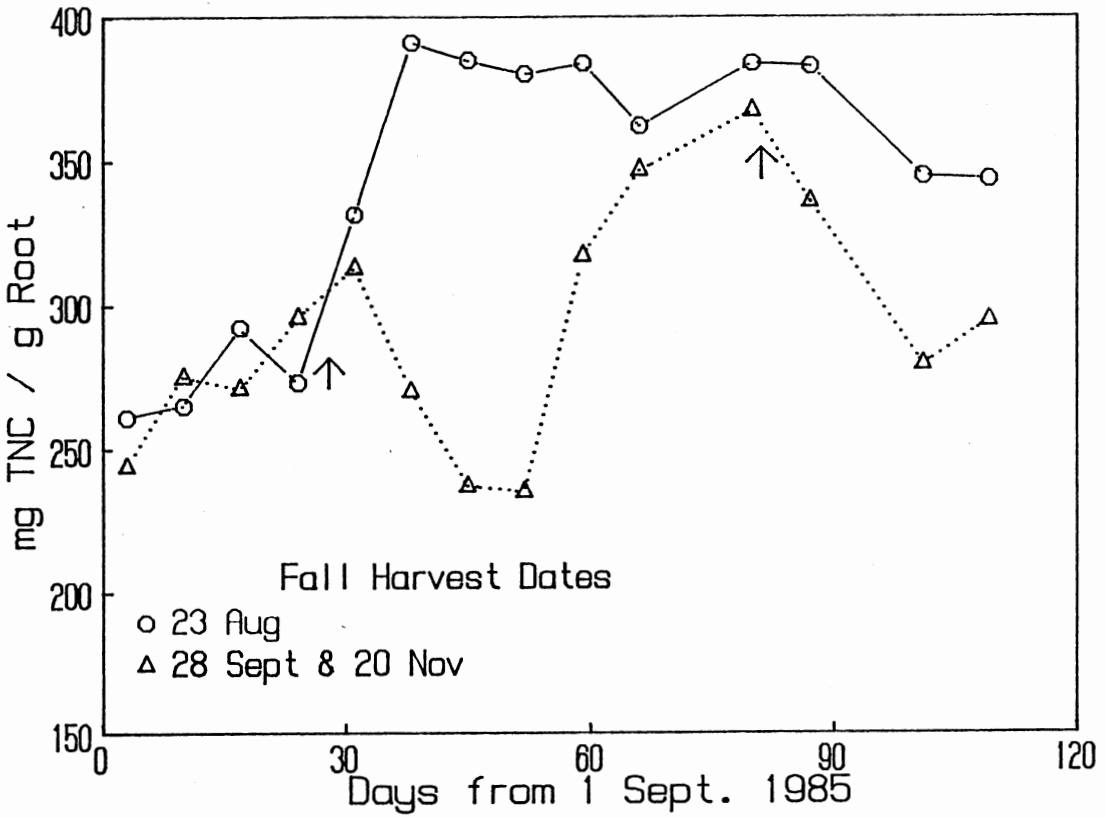

Figure 6. Fall 1985 root TNC trends of nonirrigated alfalfa in response to late summer cut (23 August) and two fall cuts (28 September and 20 November). Arrows indicate harvest dates.

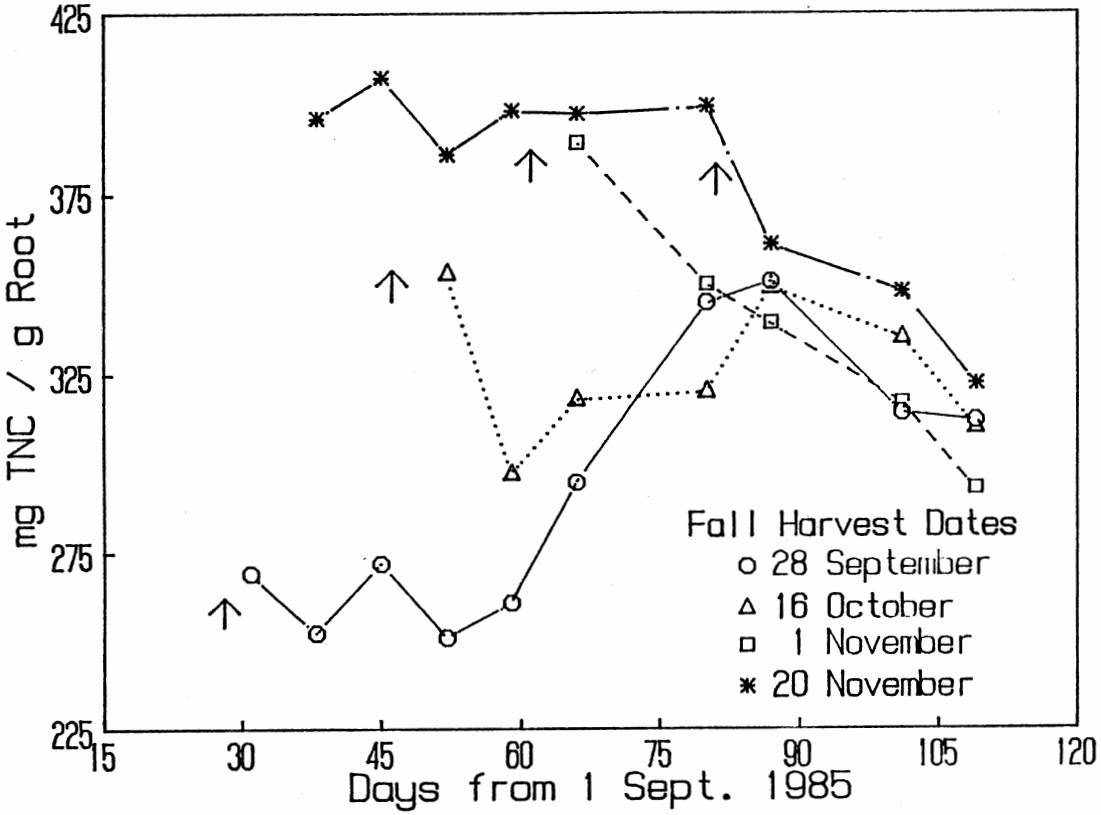

Figure 7. Fall 1985 root TNC trends of nonirrigated alfalfa in response to fall harvest treatments. Arrows indicate fall harvest dates.

declined at a faster rate during the early winter than the treatment with the least severe harvest schedule, that harvested only on 23 August (Figure 6).

Forage yield differences occurred in the nonirrigated experiment in both 1985 and 1986. In the first spring harvest of 1985 (10 May 1985), the treatment last cut 28 August 1984 significantly out-yielded all other fall harvest treatments (Table VII). Although not different from the 3 October 1984 fall harvest treatment, the 1985 seasonal total was highest for the 28 August 1984 fall harvest treatment. This trend was consistent across both years. Treatments last cut in late August 1985 also yielded significantly higher than other treatments in the first harvest of the following year (Table VIII). However, in 1986, total yield of the treatment cut on 16 November did not yield significantly less than the 23 August harvest treatment. This was also reflected in the 2-year total yield (Table VIII).

Significant differences in TNC levels occurred in both years of the nonirrigated experiment (Tables IX and X). However, of the many possible comparisons of yield with root TNC, there was a significant correlation on only a few occasions in both years of the nonirrigated experiment between root TNC levels on certain dates and forage yields in following years. The level of root TNC in the fall was not related to the total yield over two years (including fall harvests) of each experiment. Only on certain individual sampling dates was root TNC correlated with the first spring harvest or the total yield of a growing season (May-August). Lack of consistent relationships between TNC and forage yield in subsequent years throughout this study suggest little

TABLE VII

FORAGE DRY MATTER YIELDS FROM 1984 FALL AND 1985 SEASONAL HARVESTS OF THE NONIRRIGATED STUDY

1984 Fall Harvest Date	1984 Fall Harvest Yield	1985 Forage Harvest Dates				1985 Summer Total
		5/10	6/17	7/19	8/23	
	----------Mg ha^{-1}----------					
Aug 31	0.9b	5.8a	4.5a	2.5	1.9	14.7a
Aug 31 & Nov 16	1.2a	5.1b	4.3ab	2.5	2.1	13.9bc
Oct 3	0.7c	5.2b	4.4a	2.7	2.1	14.5a
Oct 17	0.9b	5.0b	4.5a	2.6	2.1	14.4ab
Oct 31	0.7c	5.1b	4.0b	2.6	2.1	13.8bc
Nov 16	0.7c	5.0b	4.1b	2.5	2.0	13.5c
5% LSD	0.1	0.4	0.4	N.S.	N.S.	0.7
CV (%)	8.3	5.3	5.5	9.7	5.9	3.4

NS Not significantly different.

* Within columns, means followed by the same letter are not significantly different at the 0.05 probability level

TABLE VIII

FORAGE DRY MATTER YIELDS FROM 1985 FALL AND 1986 SEASONAL HARVESTS AND 2-YEAR TOTAL OF THE NONIRRIGATED STUDY

1985 Fall Harvest Date	1985 Fall Harvest Yield	1986 Forage Harvest Dates				1986 Summer Total	2-Year Total
		4/29	6/9	7/8	8/7		
	----------Mg ha^{-1}----------						
Aug 23	1.9b*	4.2a	4.3a	3.7abc	0.9	13.1a	28.7
Sept 28	1.6c	3.1cd	4.0bc	3.4c	0.9	11.4c	28.2
Sept 28 & Nov 20	2.3a	3.0d	3.8c	3.5bc	0.9	11.3c	28.6
Oct 16	1.7bc	3.0d	4.0bc	3.5bc	1.0	11.4c	28.5
Nov 1	1.6c	3.4c	4.1ab	3.9ab	1.0	12.3b	28.4
Nov 20	1.0d	3.8b	4.4a	3.7ab	1.0	12.9a	28.2
5% LSD	0.2	0.2	0.3	0.3	NS	0.6	NS
CV (%)	8.9	4.6	4.9	4.7	7.4	3.0	2.2

NS Not significantly different.

* Within columns, means followed by the same letter are not significantly different at the 0.05 level

TABLE IX

ROOT TNC MEANS OF THE NONIRRIGATED EXPERIMENT DURING THE FALL AND WINTER OF 1984-1985

Sampling Date	1984 Fall Harvest Date						
	Aug 31	Oct 3	Oct 17	Oct 31	Nov 16	Aug 31 & Nov 16	5% LSD
	mg g^{-1}						
Sept 3	395	-	-	-	405	434	N.S.
6	394	-	-	-	432	402	N.S.
13	390	-	-	-	401	395	N.S.
25	381b	-	-	-	439a	367b	26
Oct 4	385b	441a	-	-	430a	395b	31
11	403	388	-	-	420	385	N.S.
19	411	387	430	-	432	400	N.S.
24	350	390	417	-	444	381	N.S.
Nov 2	384	408	381	417	402	385	N.S.
8	374	380	403	399	420	389	N.S.
15	397a	386ab	390ab	360b	411a	404a	32
30	416	417	414	402	396	388	N.S.
Dec 11	411ab	444a	442a	407ab	395b	384b	37
27	424a	431a	430a	397ab	378b	380b	35
Jan 17	325bc	354a	345ab	318cd	295de	287e	23
Feb 26	282a	291a	288a	270ab	246bc	233c	21
Mar 19	163	165	155	150	140	141	N.S.

* Within rows, means followed by the same letter are not significantly different at the 0.05 level

TABLE X

ROOT TNC MEANS OF THE NONIRRIGATED EXPERIMENT DURING THE FALL AND WINTER OF 1985-1986

Sampling Date	1985 Fall Harvest Date					
	Aug 23	Sept 28	Oct 16	Nov 1	Nov 20	Sept 28 & Nov 20
	mg g^{-1}					
Sept 3	261	-	-	-	-	244
10	265	-	-	-	-	275
17	292	-	-	-	-	271
24	273	-	-	-	-	296
Oct 1	332a	269b	-	-	-	313a
8	392a	252b	-	-	396a	270b
15	385a	272b	-	-	407a	237c
22	380ab	251c	353b	-	386a	236c
29	384a	261c	297b	-	398a	317b
Nov 5	362bc	295c	318de	389ab	397a	347cd
19	384a	345bc	320c	350bc	399a	367ab
26	383a	351ab	349b	339b	361ab	336b
Dec 10	345ab	314b	335ab	317ab	348a	279c
18	344a	309ab	316abc	295c	329ab	295c
Jan 6	303a	276ab	276ab	258b	299a	248b
Feb 18	222a	185b	227a	210ab	205ab	189b
Mar 6	188ab	195ab	201a	179ab	210a	165b

* Within rows, means followed by the same letter are not significantly different at the 0.05 level. LSD = 32.

relationship between TNC at these levels and yields under these fall harvest management treatments.

Numbers of remaining plants at the end of the two-year study were not significantly different among treatments at the time of termination of the irrigated and nonirrigated experiments (Table XI). This indicated that these fall harvest management treatments had no effect on alfalfa persistence during the time of this study.

Fall root TNC cycles were influenced by timing of final fall harvests in both irrigated and nonirrigated experiments. Early fall harvests (September and October) usually resulted in a depletion of root TNC following the forage harvest and a subsequent accumulation of TNC. Later fall harvest (during November), with the exception of the 1984 nonirrigated harvest taken 31 October, produced no root TNC cycle following cutting, only a decline through the winter. In all cases, root TNC were not significantly different among treatments on at least one date in the late fall or early winter. Staggering the harvest treatments may have caused simultaneous accumulation of root TNC in earlier harvests and depletion of TNC in later harvest treatments resulting in a convergence of TNC values.

Different fall harvest treatments of alfalfa did not create differences in yield over the duration of the experiment. There was some effect on first harvest yield in subsequent years. Harvest during the fall at a time when active regrowth can occur may cause significant reductions in yield in the first spring harvest or the total yield the following year. However, this response does not appear to be closely related to TNC levels.

TABLE XI

PLANT DENSITY AT THE TERMINATION OF THE IRRIGATED AND NONIRRIGATED EXPERIMENTS

Approximate Fall Harvest Date	Plants m^{-2}	
	Irrigated	Nonirrigated
Sept 1	-	114
Sept 1 & Nov 15*	-	99
Oct 1	54	115
Oct 15	54	114
Nov 1	62	109
Nov 15	66	108
Mean	59	110
5% LSD	N.S.	N.S.
CV (%)	14.4	10.2

* Harvested August 30 and November 16, 1984 and August 23, September 28 and November 20, 1985.

Yield differences occurring at individual harvests were offset by yields of the fall harvests. There was not a strong correlation between yield and TNC in the fall although a few individual harvests and sampling dates were significantly correlated. Levels of TNC differed during the fall due to harvest treatment, but the experimental design was responsible for the later harvest treatments having higher TNC levels prior to harvest because of the extended length of recovery between the last seasonal harvest and the fall harvest. Root TNC levels in treatments cut early in the fall usually had a chance to recover. This resulted in a point in time at which there were no significant differences among treatments in the TNC levels. Environmental conditions in Oklahoma during the fall may be such that early fall harvest treatments have sufficient time to recover root TNC after supporting regrowth and later harvests never seriously deplete root TNC because cooler temperatures and shorter days decrease the rate of regrowth. Even though mid-winter root TNC levels were slightly lower on some occasions for later harvest treatments, no negative effects on yield or persistence could be related low TNC levels during this time.

CHAPTER III

A COMPARISON OF ROOT DRY MATTER AND NON-STRUCTURAL CARBOHYDRATE TRENDS IN ALFALFA

Introduction

Nonstructural carbohydrates (TNC) are compounds used by plants for long-term storage of energy produced by photosynthesis. In alfalfa, these compounds are stored in the roots in modified cellular structures called amyloplasts. Analysis of alfalfa root nonstructural carbohydrates has been done by chemical means. This may involve the removal of the TNC from plant tissue using acid hydrolysis, hot ethanol, or enzymatic extraction (Smith et al., 1964; Grotleutchen and Smith, 1967; Smith, 1981b). The extract is then treated with oxidizing agents (copperiodimetric method (Smith, 1981b) or dinitrosalycylic acid (Miller, 1959; Gabrielsen et al., 1985)) or other compounds that form complexes with the sugar units (anthrone (Yemm and Willis, 1954) or parahydroxybenzoic acid hydrazine (Lever, 1972)) which are then measured by titration or colorimetry. Complete hydrolysis is required prior to conducting the reducing test for those agents which react only with reducing sugars (glucose and fructose) that are the basic components of TNC.

Chemical methods may be time consuming, require expensive analytical equipment, use hazardous chemicals, and require personnel with a certain degree of expertise to carry out the analyses. Wolf (1978) has proposed the use of a modified gravimetric method to estimate root TNC using root percent dry matter (%DM) determined on a saturated weight basis by Equation (3.1).

$$\frac{\text{Root Dry Weight}}{\text{Root Saturated Weight}} \times 100 = \text{Root \%DM} \qquad (3.1)$$

The composition of alfalfa root dry matter is complex, including TNC, cell wall components, proteins, lipids, minerals, and other compounds (Bickoff, et al., 1972). Wolf followed the assumption that roots were primarily structural tissue, water and TNC. Therefore, TNC and %DM should be correlated. Indeed, Wolf (1978) found a very significant correlation between root TNC and %DM in a number of studies.

The purpose of this study was to compare the method used by Wolf to estimate TNC using root %DM on a saturated weight basis with the enzymatic/chemical method of quantitative measurement of TNC on alfalfa roots sampled in fall harvest studies.

Materials and Methods

Plant samples from the TNC study were also utilized for the %DM study. After each fall harvest treatment was imposed, roots were sampled at weekly intervals from two different 500 cm^2 areas in each plot until active regrowth was no longer apparent (about mid-December each year). Throughout the winter, roots were sampled on a monthly basis. After removal from the soil, whole plants were placed on ice

until roots could be washed to remove soil. Roots were clipped to a length of 10 cm from the crown buds and fine lateral roots were removed. Roots were soaked for two hours in ice water to achieve saturation, blotted dry, and weighed to the nearest 0.01 g. Roots were heated to 100 C for 90 min and then dried at 70 C in a forced-draft oven. After roots were dry, they were again weighed to the nearest 0.01 g. Percent root dry matter was calculated using Equation (3.1). After conducting an analysis of variance to evaluate treatment differences, fall harvest means for TNC and %DM were correlated with each other and with forage yields from each harvest, yearly totals and 2-year totals.

Root %DM values from each sampling date from both locations were analyzed as separate experiments, giving an estimate of variance for each sampling date (Tables XII and XIII). In the fall of 1984, TNC were also analyzed in this manner. All other TNC samples were analyzed in the laboratory by block in order to confound laboratory run with block and avoid confounding sampling date with laboratory run.

The correlation between root TNC and %DM means was tested for significance for each sampling date throughout the fall and winter.

Results and Discussion

Steele and Torrie (1980) identified the error mean square (EMS) or $\underline{s}^2$ as an estimate of the variation among observations treated alike and a measure of the failure of treatment differences to be the same within a block as well as the failure of observations to equal the estimates of their expected values. The coefficient of variation (CV) may also be used as a measure of the variation between experiments measuring the

same characteristic. By comparing these two parameters among sampling dates, it is possible to identify the relative accuracy of data collected within an experiment.

The alfalfa root %DM data were analyzed by sampling date and if the variance of an individual sampling date differed greatly from the rest, this indicated that the variance of this data was not homogeneous and should not be pooled. Tables XII and XIII identify several sampling dates for which this is the case. Specifically, dry matter data from 24 October and 2 November 1984 and 22 October 1985 of the nonirrigated study and 23 and 30 October 1984 of the irrigated study have been discarded. The EMS and CV on these dates were much higher than those of the other sampling dates.

Root %DM values in 1984 had a tendency to be higher than those in 1985 in both the irrigated and nonirrigated experiments (Tables XIV, XV, XVI, and XVII). This may have been due to a proportional increase in the amount of woody tissue from one year to the next. As younger roots have relatively less woody tissue, it follows that they would have proportionally more tissue devoted to storage of TNC. Older roots have a greater proportion of woody tissue, giving them the capacity to hold relatively more water after soaking.

Overall, root %DM and TNC were correlated in the fall of 1984. However, there were many individual sampling dates on which they were not correlated (Tables XVIII and XIX). Root %DM responded differently than TNC in the Fall 1984 nonirrigated experiment (Figure 8). Treatments cut in late August showed a rather shallow, protracted decline and recovery of root %DM. The treatment harvested mid-November had higher %DM values through November than either treatment harvested

TABLE XII

ERROR MEAN SQUARES AND COEFFICIENTS OF VARIATION FOR %DM AND TNC AT VARIOUS ROOT SAMPLING DATES OF THE NONIRRIGATED STUDY

1984-1985			1985-1986		
SAMPLING DATE	%DM		SAMPLING DATE	%DM	
	EMS	CV		EMS	CV
SEPT 3	.32	1.4	SEPT 3	.74	2.8
6	1.38	3.0	10	3.21	5.4
13	1.40	3.1	17	2.84	4.7
25	.78	2.4	24	3.38	5.9
OCT 4	.39	1.7	OCT 1	3.37	5.8
11	1.66	3.5	8	1.57	3.9
19	2.36	4.1	15	1.12	3.2
24	26.36	11.8	22	10.15	9.7
			29	3.59	5.8
NOV 2	9.16	7.2			
8	3.10	4.1	NOV 5	2.83	4.9
15	2.90	4.6	19	2.29	4.2
30	1.79	3.1	26	2.35	4.4
DEC 11	2.53	4.1	DEC 10	1.56	3.8
27	3.04	5.0	18	.79	2.7
JAN 17	.83	2.9	JAN 6	1.30	3.6
FEB 26	.36	2.0	FEB 18	.39	2.2
MAR 19	.34	2.4	MAR 6	.81	3.1

TABLE XIII

ERROR MEAN SQUARES AND COEFFICIENTS OF VARIATION FOR ROOT %DM OF SAMPLING DATES OF THE IRRIGATED STUDY

1984-1985			1985-1986		
SAMPLING DATE	%DM EMS	%DM CV	SAMPLING DATE	%DM EMS	%DM CV
OCT 4	.18	1.1	OCT 1	5.61	7.7
9	1.29	3.1	8	2.78	5.2
18	.88	2.5	15	4.46	6.6
23	18.14	9.9	22	2.86	5.0
30	8.60	7.2	29	2.84	5.3
NOV 6	2.49	3.5	NOV 5	3.16	5.1
13	4.21	4.7	12	4.76	6.1
27	1.96	3.7	19	1.54	3.5
			26	1.63	3.7
DEC 24	.74	2.5	DEC 3	4.84	6.7
			10	2.44	4.7
			18	2.52	5.0
JAN 15	.41	1.9	JAN 6	1.24	3.5
FEB 19	.27	1.6	FEB 18	1.64	4.6
MAR 15	.73	3.5	MAR 6	1.31	4.1

TABLE XIV

1984-1985 FALL AND WINTER NONIRRIGATED %DM MEANS

Sampling Date	1984 Fall Harvest Date						
	Aug 31	Oct 3	Oct 17	Oct 31	Nov 16	Aug 31 & Nov 16	5% LSD
	% Dry Matter						
Sept 3	38.6b	-	-	-	40.1a	39.3ab	1.0
6	39.2b	-	-	-	41.4a	38.7b	2.0
13	38.0b	-	-	-	41.0a	37.5b	2.0
25	35.7b	-	-	-	40.2a	35.5b	1.5
Oct 4	35.7b	38.6a	-	-	38.9a	35.6b	1.0
11	36.0b	35.3b	-	-	38.9a	36.3b	2.1
19	37.0ab	35.0b	38.4a	-	38.2a	37.6a	2.4
Nov 8	43.3b	41.1b	42.0b	42.9b	46.1a	41.4b	2.7
15	38.2a	36.9ab	35.6b	34.6b	39.0a	39.2a	2.6
30	34.5ab	35.7a	34.4ab	34.3ab	34.9ab	33.6b	1.6
Dec 11	40.3ab	41.5a	38.6bc	38.7bc	39.1abc	37.4c	2.4
27	33.8abc	36.3ab	36.4a	33.5c	33.7bc	33.8bc	2.6
Jan 17	32.1abcd	33.2a	32.8ab	31.1cd	30.9cd	30.3d	1.4
Feb 26	30.8a	30.6a	30.1a	29.0b	28.1b	28.2b	0.9
Mar 19	24.1ab	24.5a	23.8ab	23.7ab	23.5b	23.2b	0.9

* Within rows, means followed by the same letter are not significantly different at the 0.05 level

TABLE XV

1984-1985 FALL AND WINTER
IRRIGATED %DM MEANS

Sampling Date	1984 Fall Harvest Date				5% LSD
	Oct 1	Oct 19	Nov 3	Nov 16	
	% Dry Matter				
Oct 4	38.7	-	-	39.4	N.S.
9	34.1b	-	-	40.5a	2.6
18	32.1b	-	-	39.2a	1.6
23	37.0b	40.6ab	-	47.5a	7.4
30	35.2b	44.8a	-	43.4a	5.1
Nov 6	42.3b	44.1ab	45.4a	46.4a	2.5
13	43.3	42.7	44.2	44.5	N.S.
20	-	-	34.3b	38.4a	3.1
27	38.2	37.3	36.9	38.0	N.S.
Dec 3	-	-	39.1	40.2	N.S.
24	35.6	35.0	34.7	34.0	N.S.
Jan 15	34.5a	34.1ab	33.2bc	32.8c	1.0
Feb 19	33.1a	32.6a	31.2b	30.8b	0.8
Mar 15	24.8	24.4	24.5	24.5	N.S.

* Within rows, means followed by the same letter are not significantly different at the 0.05 level

TABLE XVI

1985-1986 FALL AND WINTER NONIRRIGATED %DM MEANS

Sampling Date		1985 Fall Harvest Date						
		Aug 23	Sep 28	Oct 16	Nov 1	Nov 20	Sep 28 & Nov 20	5% LSD
		% Dry Matter						
Sept	3	31.6	-	-	-	-	30.5	N.S.
	10	32.9	-	-	-	-	33.8	N.S.
	17	35.0	-	-	-	-	37.0	N.S.
	24	30.5	-	-	-	-	32.2	N.S.
Oct	1	32.9	30.5	-	-	-	31.3	N.S.
	8	35.8a	28.8b	-	-	35.3a	28.9b	2.0
	15	37.4a	29.4b	-	-	37.9a	28.5b	1.7
	29	37.2a	29.4b	30.7b	-	36.8a	30.7b	2.9
Nov	5	37.2a	31.2b	31.7b	38.2a	37.4a	32.6b	2.5
	19	39.2a	33.0c	33.5bc	35.4b	39.4a	34.4bc	2.3
	26	37.2a	33.7b	34.0b	33.7b	35.7ab	33.7b	2.3
Dec	10	35.3a	32.5b	32.6b	32.1b	35.0a	29.4c	1.9
	18	34.6a	32.7b	33.3ab	31.1c	33.6ab	29.6d	1.3
Jan	6	34.0a	32.0bc	32.5abc	31.2cd	33.4ab	29.8d	1.7
Feb	18	30.5a	28.2d	29.5b	28.3cd	29.2bc	27.5d	0.9
Mar	6	29.4a	28.1b	29.2ab	28.1ab	29.3ab	28.1ab	1.4

* Within rows, means followed by the same letter are not significantly different at the 0.05 level

TABLE XVII

1985-1986 FALL AND WINTER
IRRIGATED %DM MEANS

Sampling Date	1985 Fall Harvest Date				
	Sept 28	Oct 16	Nov 1	Nov 18	5% LSD
	% Dry Matter				
Oct 1	30.2	-	-	31.7	N.S.
8	29.1b	-	-	35.0a	3.8
15	27.8b	-	-	36.0a	4.7
22	29.1b	35.7a	-	37.2a	2.9
29	29.3b	30.2b	-	36.3a	2.9
Nov 5	30.9b	31.8b	38.9a	37.6a	2.8
12	33.4	34.7	36.9	37.2	N.S.
19	34.2	34.1	35.4	36.7	N.S.
26	33.7b	34.8ab	33.1b	36.5a	2.0
Dec 3	31.0	33.3	33.7	33.6	N.S.
10	32.4	32.7	33.2	34.4	N.S.
18	30.6	33.6	31.3	33.0	N.S.
Jan 6	32.1	31.7	30.7	33.3	N.S.
Feb 18	28.1	28.6	26.6	27.5	N.S.
Mar 6	26.7	28.0	28.0	28.2	N.S.

* Within rows, means followed by the same letter are not significantly different at the 0.05 level

TABLE XVIII

CORRELATION COEFFICIENTS BETWEEN
IRRIGATED ROOT %DM AND TNC
TREATMENT MEANS

1984-1985			1985-1986		
SAMPLING DATE	n	r	SAMPLING DATE	n	r
Nov 6#+	4	.97*	Nov 5#+	4	.94*
13	4	.72	12	4	.46
27+	4	.96*	19+	4	-.06
			26#	4	.50
Dec 24+	4	.98*	Dec 3	4	.88
			10	4	.60
			18	4	.82
Jan 15#+	4	.78	Jan 6	4	.90
Feb 19#+	4	.96*	Feb 19	4	.96*
Mar 15+	4	.01	Mar 6	4	.17
Overall	35	.87*	Overall	56	.90*

* Significant r at the 0.05 probability level.

Dates having significant differences in root %DM among treatments at the 0.05 probability level.

+ Dates having significant differences in root TNC among treatments at the 0.05 probability level.

TABLE XIX

CORRELATION COEFFICIENTS BETWEEN NONIRRIGATED ROOT %DM AND TNC TREATMENT MEANS

1984-1985				1985-1986			
SAMPLING DATE		n	r	SAMPLING DATE		n	r
Oct	4#+	4	.96*	Oct	8#+	4	.99*
	11#	4	.91		15#+	4	.99*
	19#	5	.93*		29#+	5	.98*
Nov	8#	6	.37	Nov	5#+	6	.92*
	15#+	6	.91*		19#+	6	.87
	30#	6	.61		26#+	6	.95*
Dec	11#+	6	.62	Dec	10#+	6	.95*
	27#+	6	.75		18#+	6	.98*
Jan	17#+	6	.97*	Jan	6#+	6	.97*
Feb	26#+	6	.92*	Feb	18#+	6	.80
Mar	19#	6	.94*	Mar	6+	6	.80
Overall		73	.88*	Overall		72	.88*

* Significant r at the 0.05 probability level.

Dates having significant differences in root %DM among treatments at the 0.05 probability level.

+ Dates having significant differences in root TNC among treatments at the 0.05 probability level.

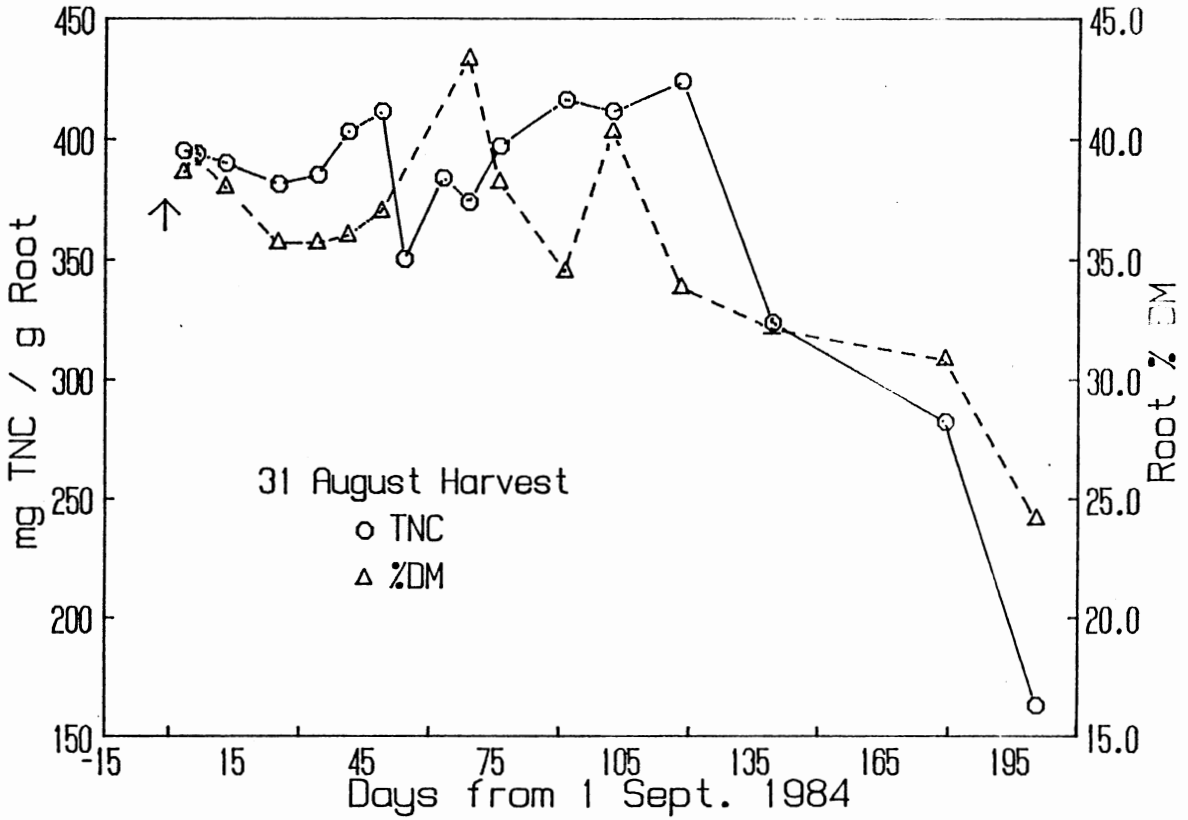

Figure 8. Comparison of nonirrigated 1984 fall and winter TNC and %DM trends in response to fall harvest. Arrows indicate harvest dates. (a) Response to 31 August harvest date.

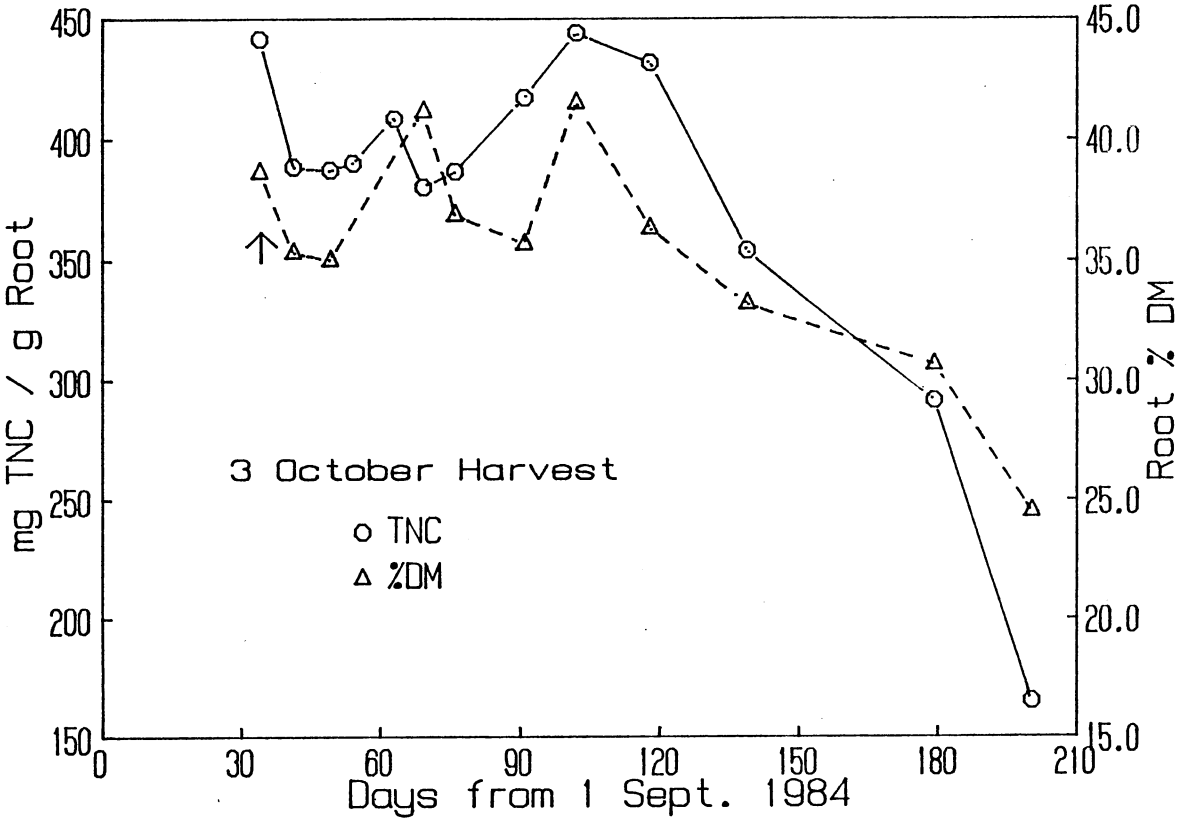

Figure 8. Continued. (b) Response to 3 October harvest date.

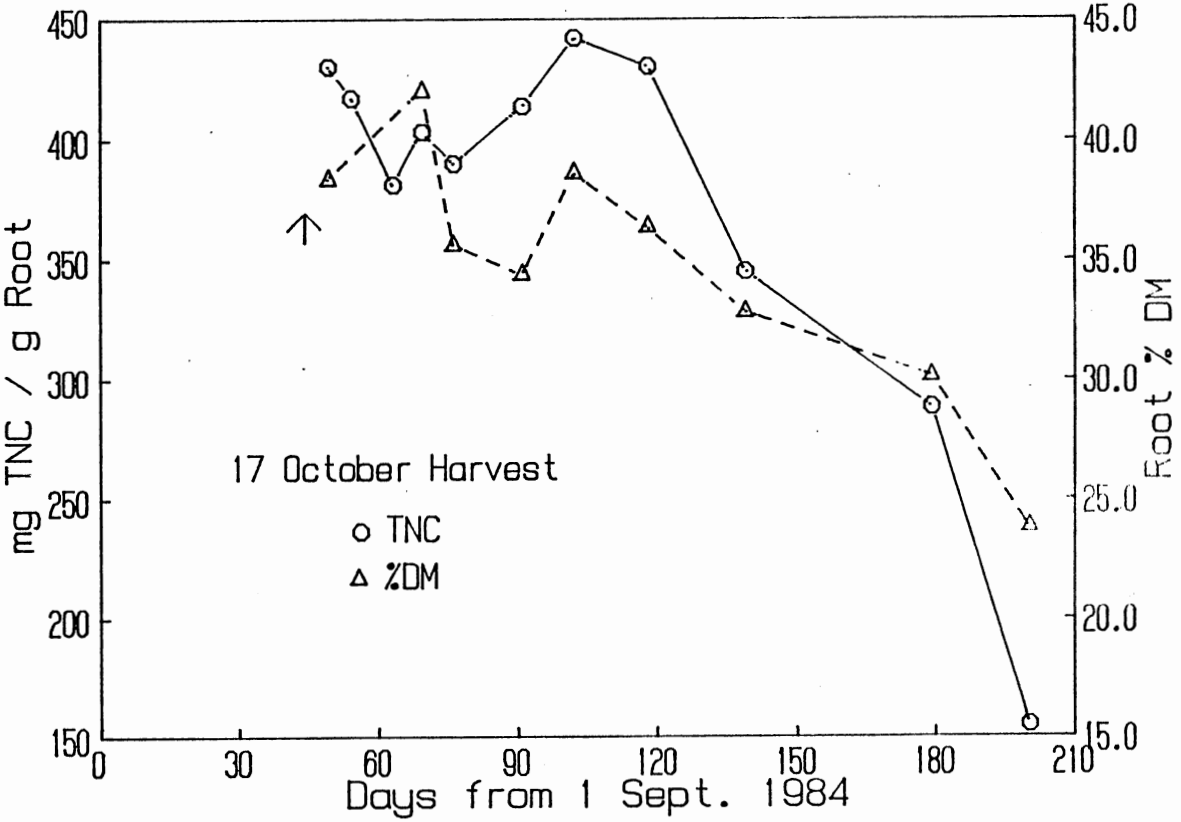

Figure 8. Continued. (c) Response to 17 October harvest date.

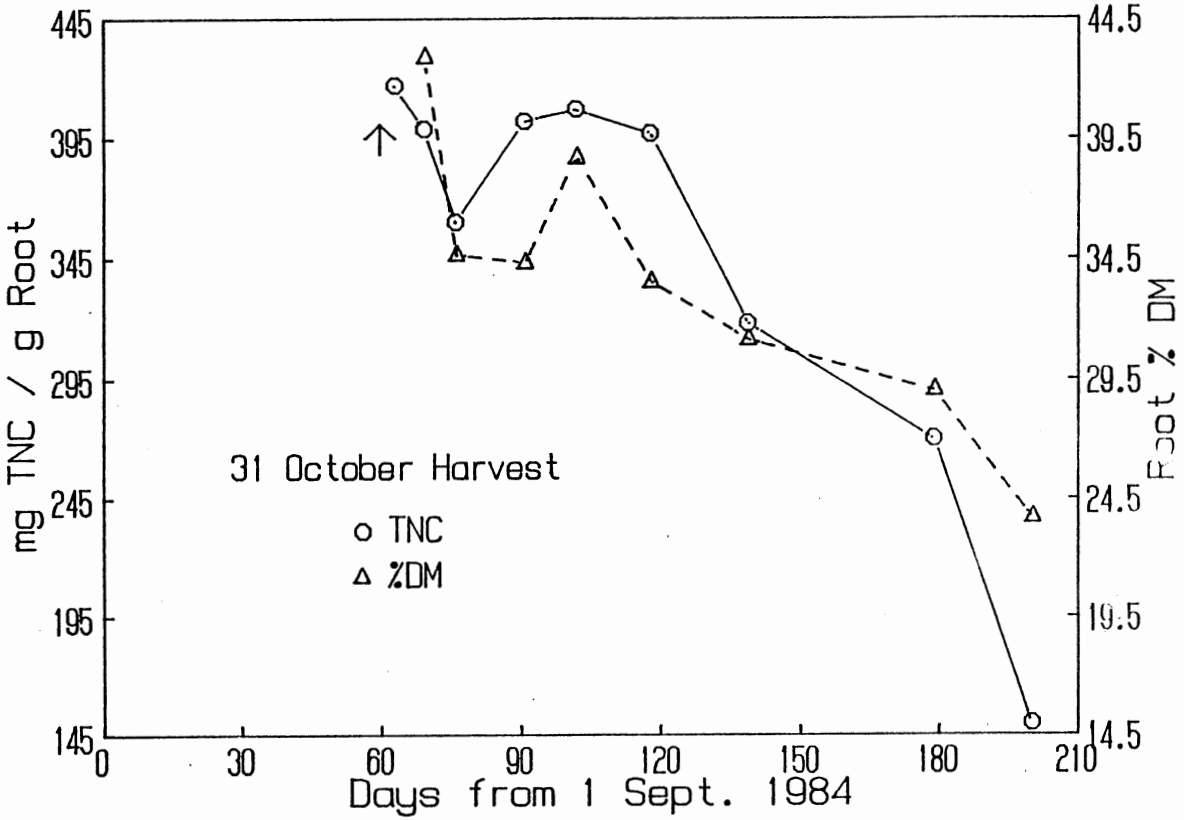

Figure 8. Continued. (d) Response to 31 October harvest date.

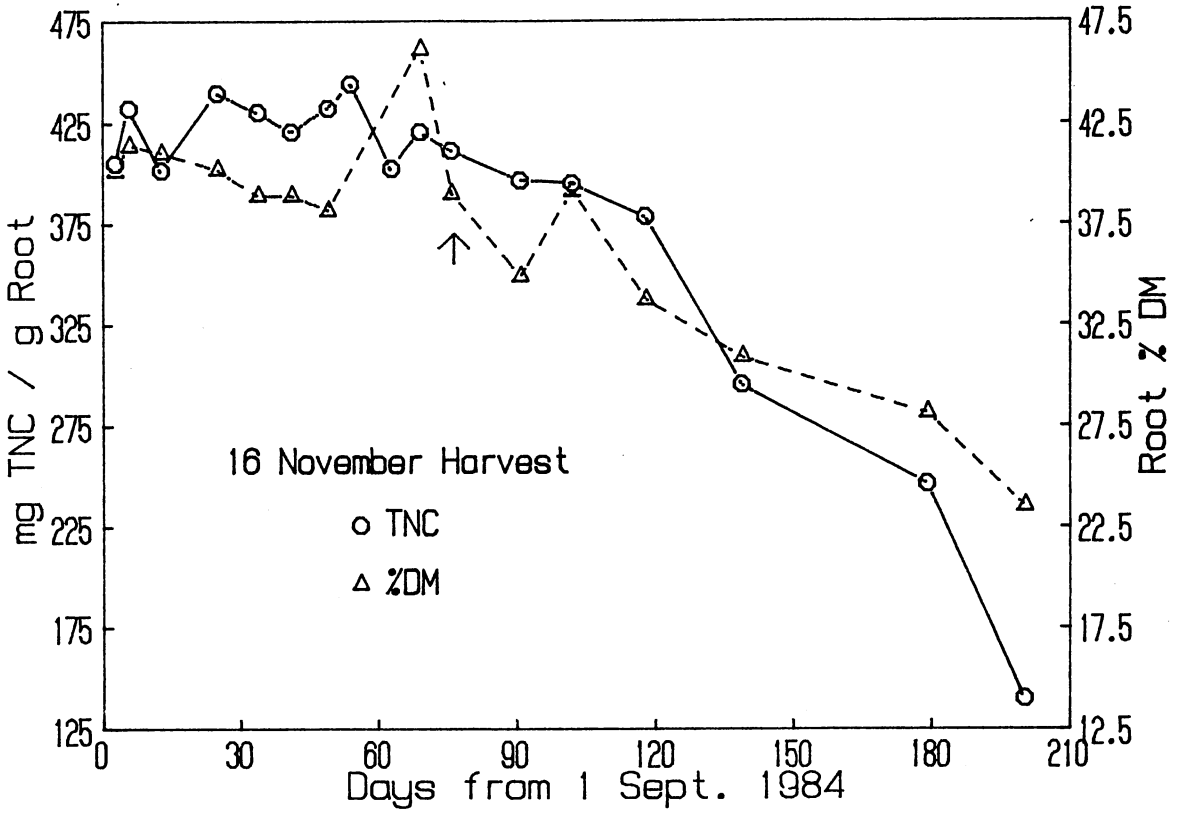

Figure 8. Continued. (e) Response to 16 November harvest date.

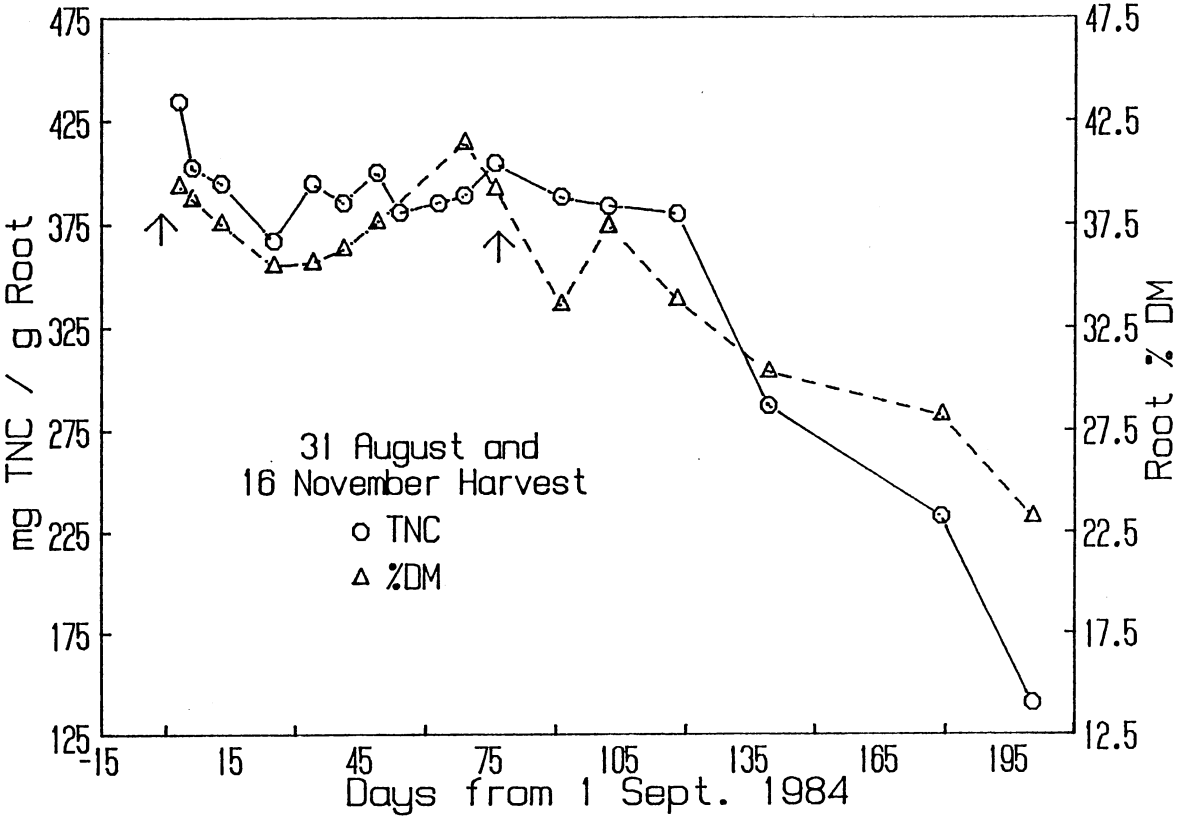

Figure 8. Continued. (f) Response to 31 August and 16 November harvest date.

in August. All treatments showed a decline in %DM during November, regardless of the date of fall harvest, while the TNC trends did not always reflect this. This had a tendency to mask the effect of fall harvest date, particularly in treatments cut after mid-October. This may have been a response to precipitation and mild temperatures during this time. Percent dry matter declined at a greater rate than TNC during November and December in treatments harvested 31 August, 16 November, or on both of these dates. There was a slight recovery of %DM in mid-December in these treatments, while TNC in the treatments harvested 16 November continued to decline following harvest.

There were differences in the response of TNC and %DM trends to fall harvest treatments in the 1984 irrigated experiment. In the treatment harvested 1 October, both TNC and %DM followed the same trend early in the fall. However, by early November, TNC had reached a high level and maintained this level through December while %DM had begun to decline during this time (Figure 9a). The 17 October harvest showed a similar trend of declining TNC with a slight recovery into December while %DM declined without recovery (Figure 9b). In the 3 November harvest treatment, %DM declined at a faster rate than TNC, but had a slight recovery that TNC did not have (Figure 9c). Similar to the 3 November harvest, %DM response to the 16 November harvest showed a decline with a slight recovery while TNC showed a steady decline (Figure 9d).

The 1985 irrigated and nonirrigated %DM and TNC trends appeared to better correlated than those in 1984. The 1985 nonirrigated %DM and TNC cycles had a clear separation of treatments that were cut 28 September, 16 October, and both 28 September and 20 November from the others

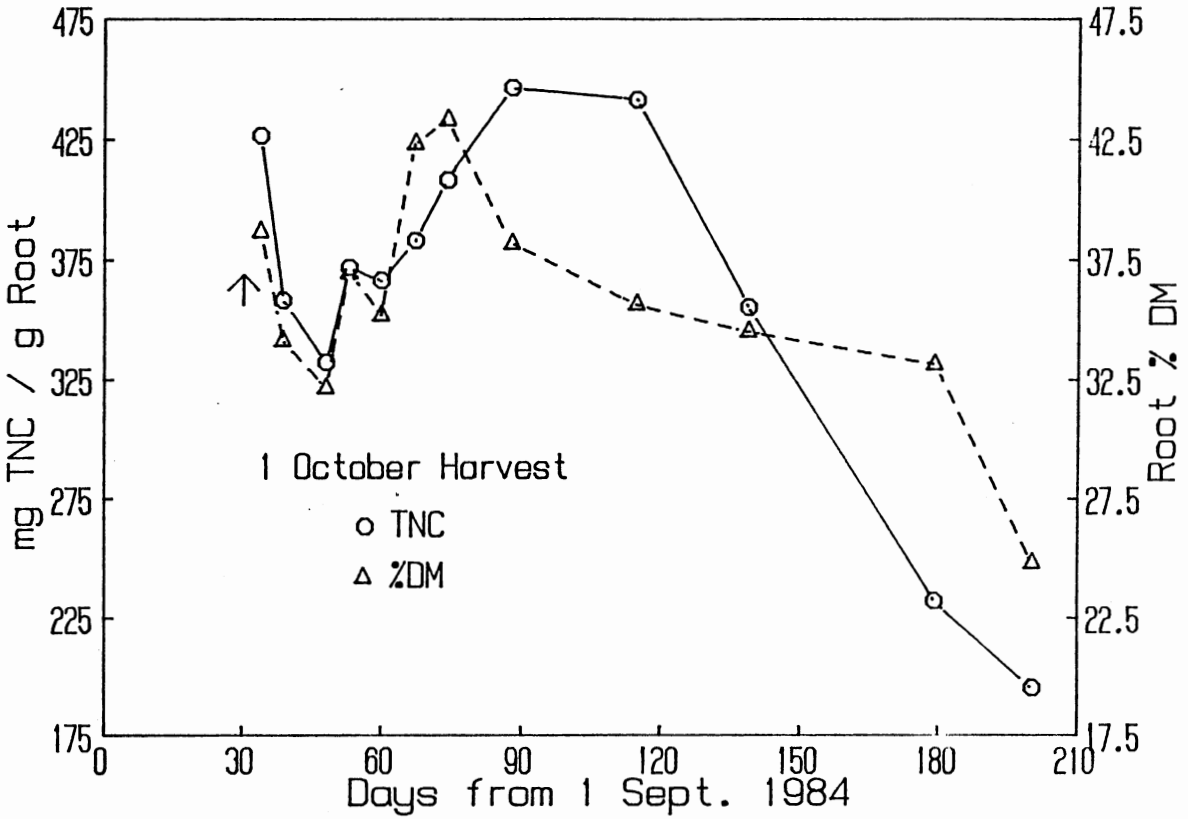

Figure 9. Comparison of irrigated 1984 fall and winter TNC and %DM trends in response to fall harvest. Arrows indicate harvest dates. (a) Response to 1 October harvest date.

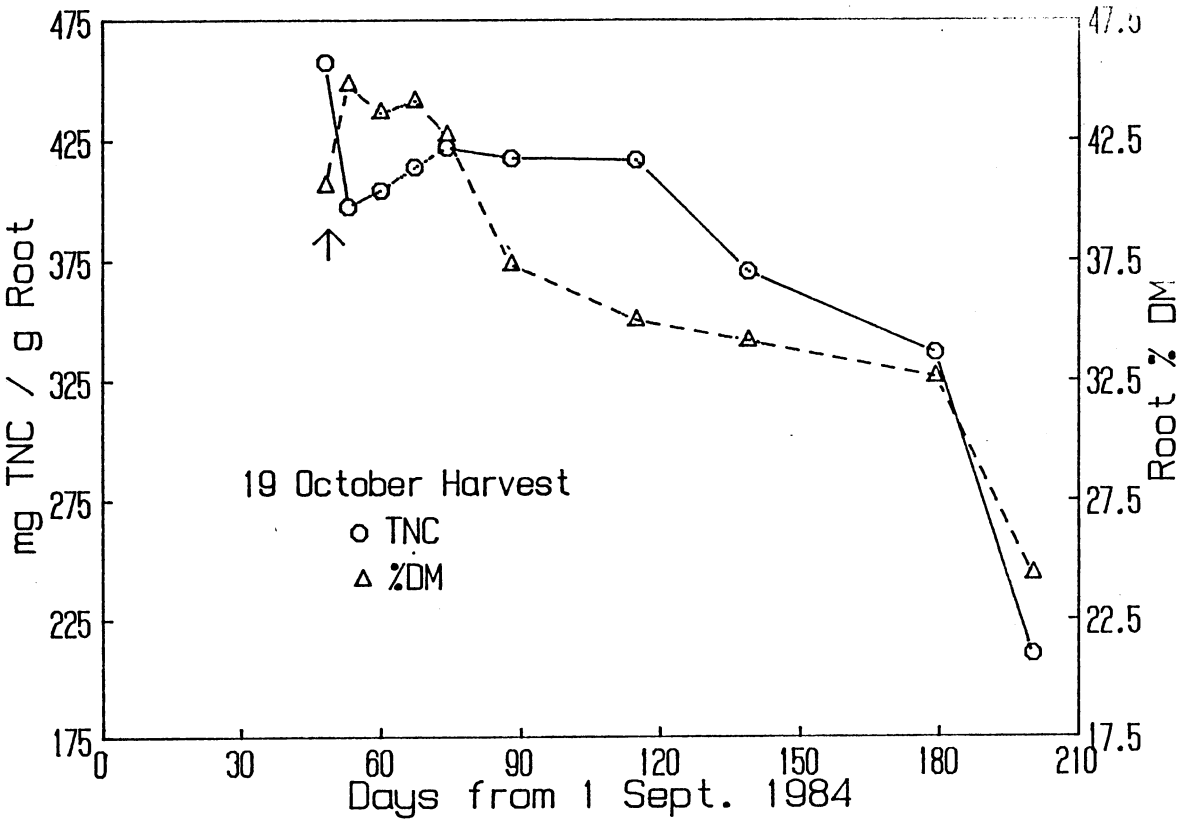

Figure 9. Continued. (b) Response to 19 October harvest date.

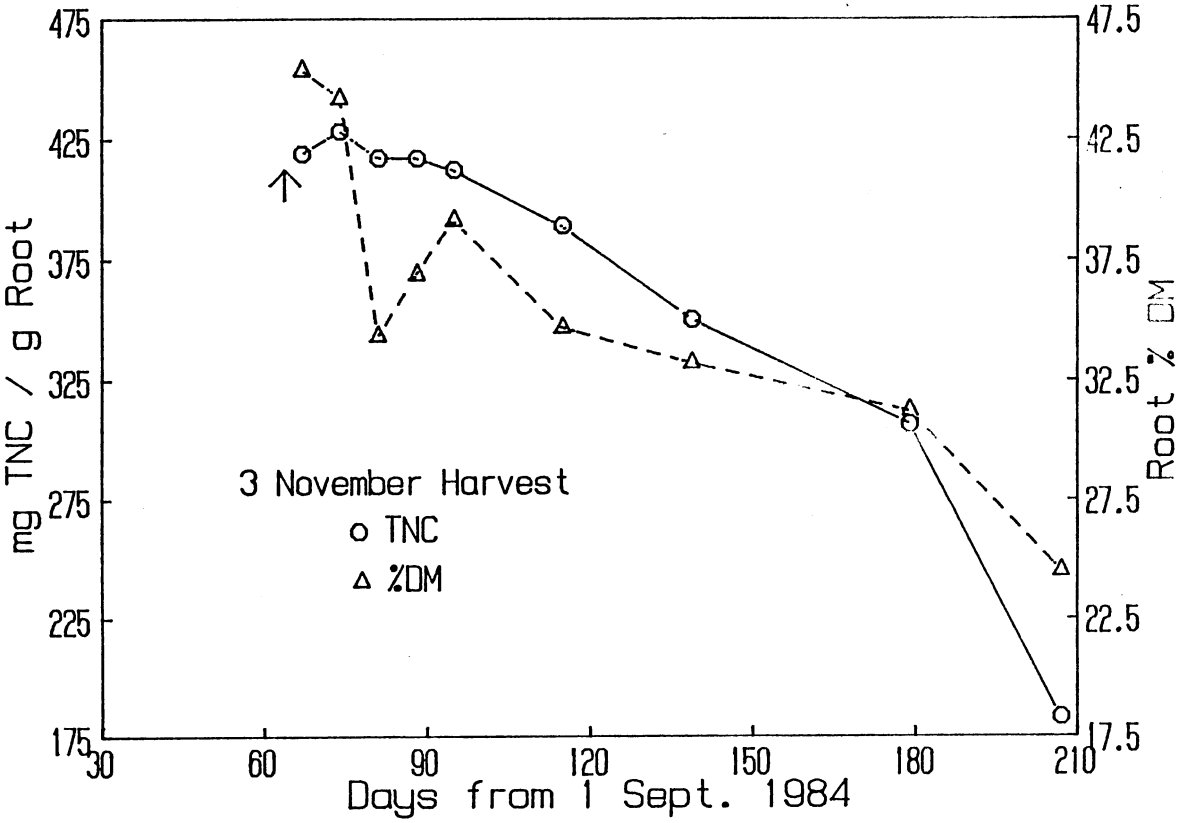

Figure 9. Continued. (c) Response to 3 November harvest date.

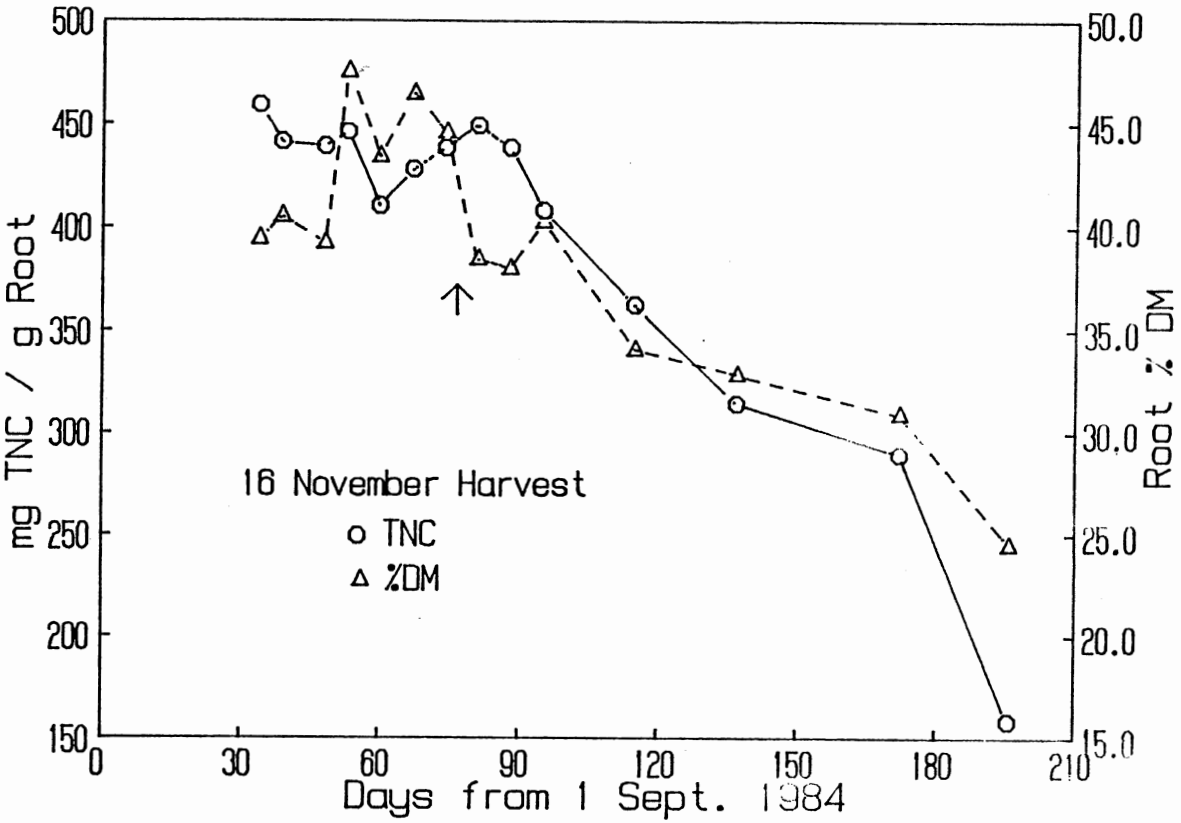

Figure 9. Continued. (d) Response to 16 November harvest date.

(Figure 10). These treatments had low %DM values following the time they were harvested to the time they peaked in late November compared to the other treatments, as did the TNC. In contrast to the treatments showing some recovery of root %DM and TNC, those treatments harvested on 1 November and on 20 November had rather high %DM and TNC values initially (accumulation from 23 August to each harvest) and declined without recovery. The treatment cut twice in the fall (28 September and 20 November) also showed a more rapid decrease of %DM and TNC following the later harvest compared to the other treatments. The three treatments which had low %DM during October and November were also those that yielded significantly less than the other treatments the following spring. Root %DM did not decline as rapidly as TNC through the winter. Continued plant respiration during this time may have used TNC while other DM components (especially structural) remained unchanged.

Root %DM and TNC trends in the irrigated experiment in the fall of 1985 differed from those in 1984. Overall, in 1985, TNC and %DM were significantly correlated (r=.90), but on individual dates there were fewer significant correlations (Tables XVIII and XIX). There was no common peak among treatments as in 1984 (Figure 11). Both the 28 September and 16 October harvest dates had %DM and TNC cycles following the harvest, but that of 16 October was of shorter duration and smaller amplitude. The treatments harvested 1 November and 18 November showed no cycle in %DM following harvest, but only a gradual decline through the winter. While the 28 September and 16 October harvests dates had a decline and recovery of %DM values following harvest, the 1 November and 18 November treatments had higher initial %DM values which declined

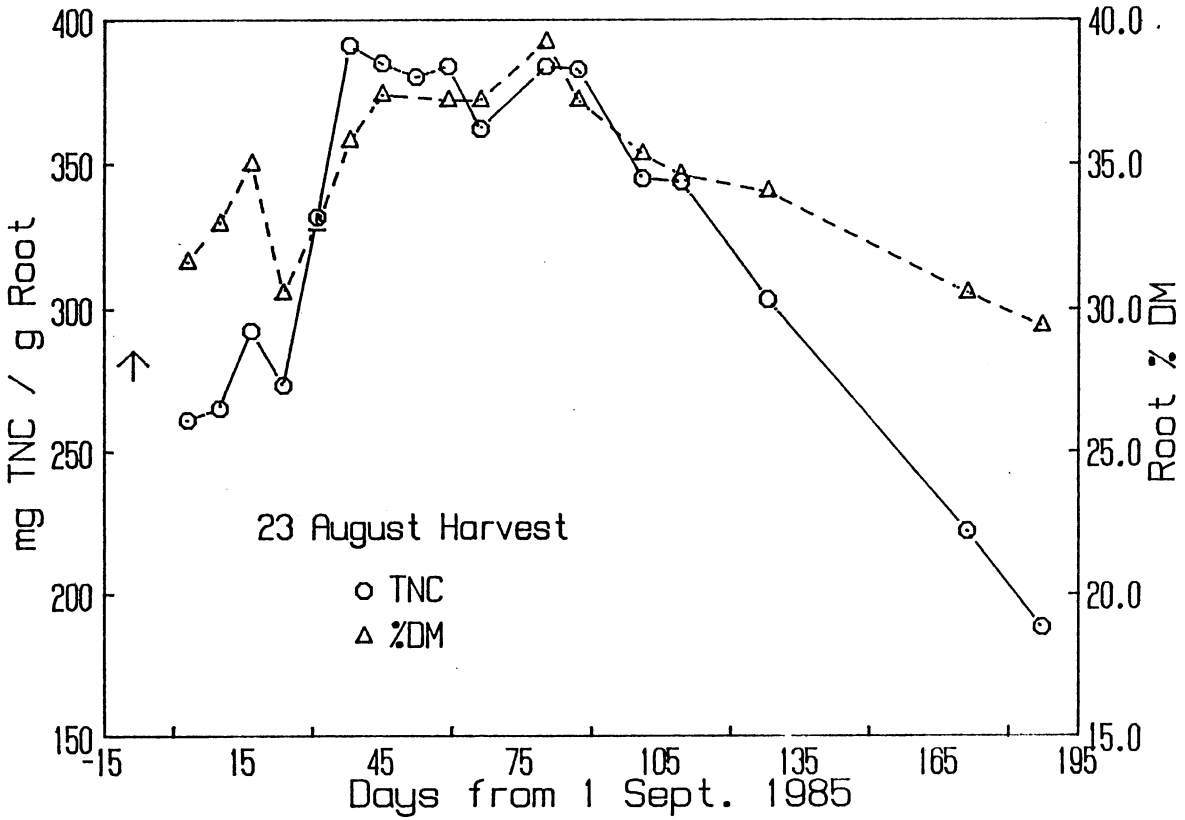

Figure 10. Comparison of nonirrigated 1985 fall and winter TNC and %DM trends in response to fall harvest. Arrows indicate harvest dates. (a) Response to 23 August harvest date.

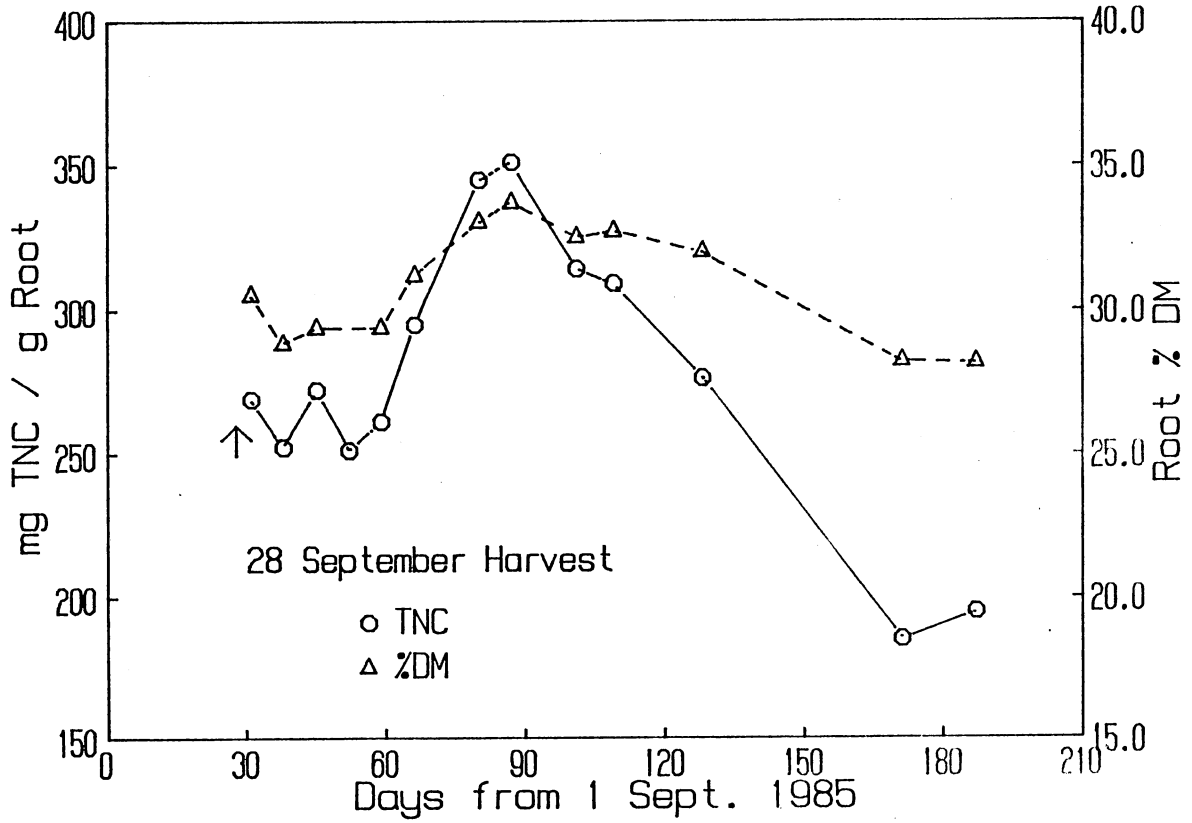

Figure 10. Continued. (b) Response to 28 September harvest date.

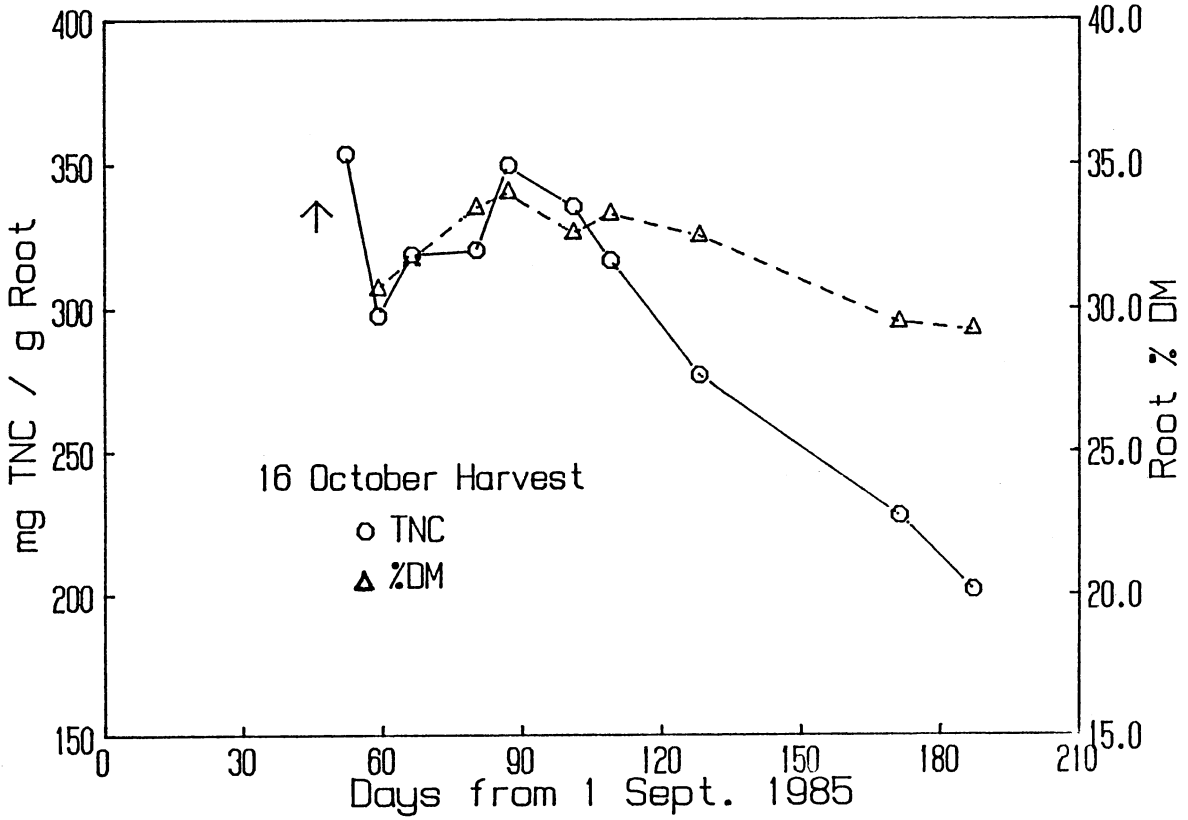

Figure 10. Continued. (c) Response to 16 October harvest date.

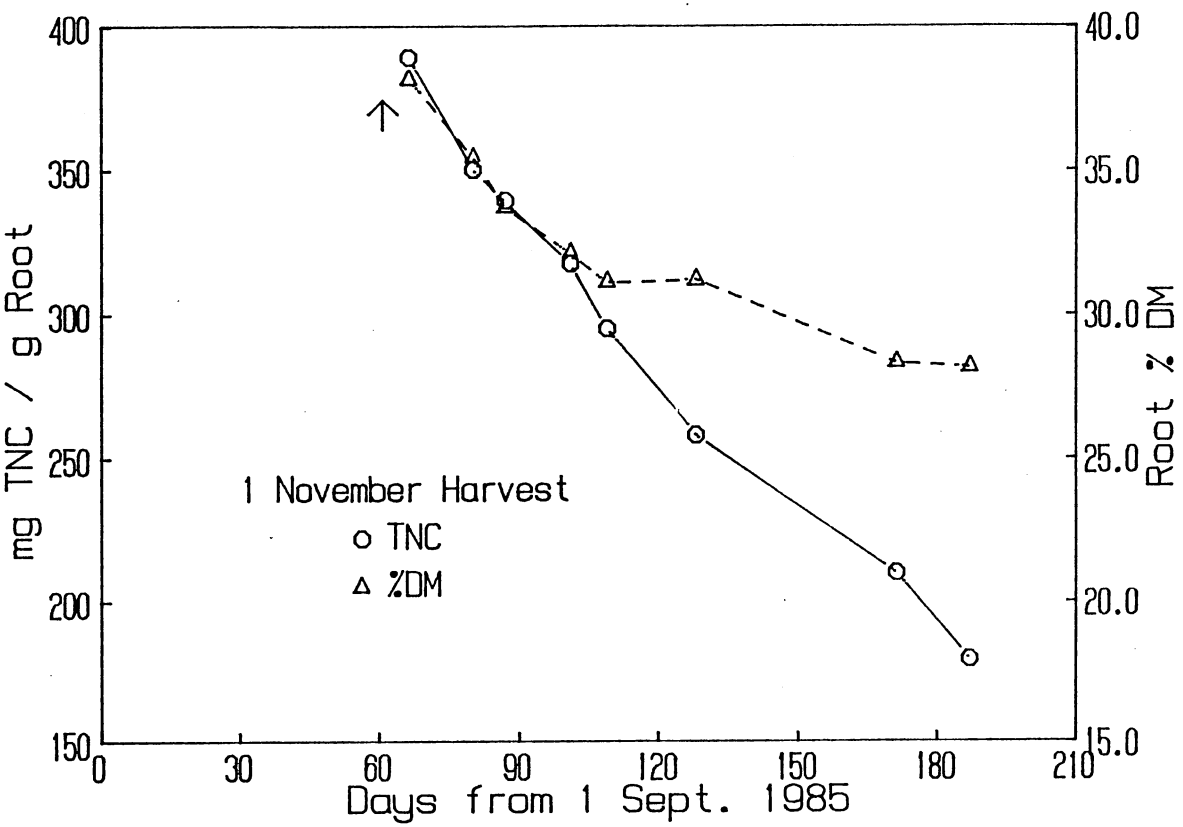

Figure 10. Continued. (d) Response to 1 November harvest date.

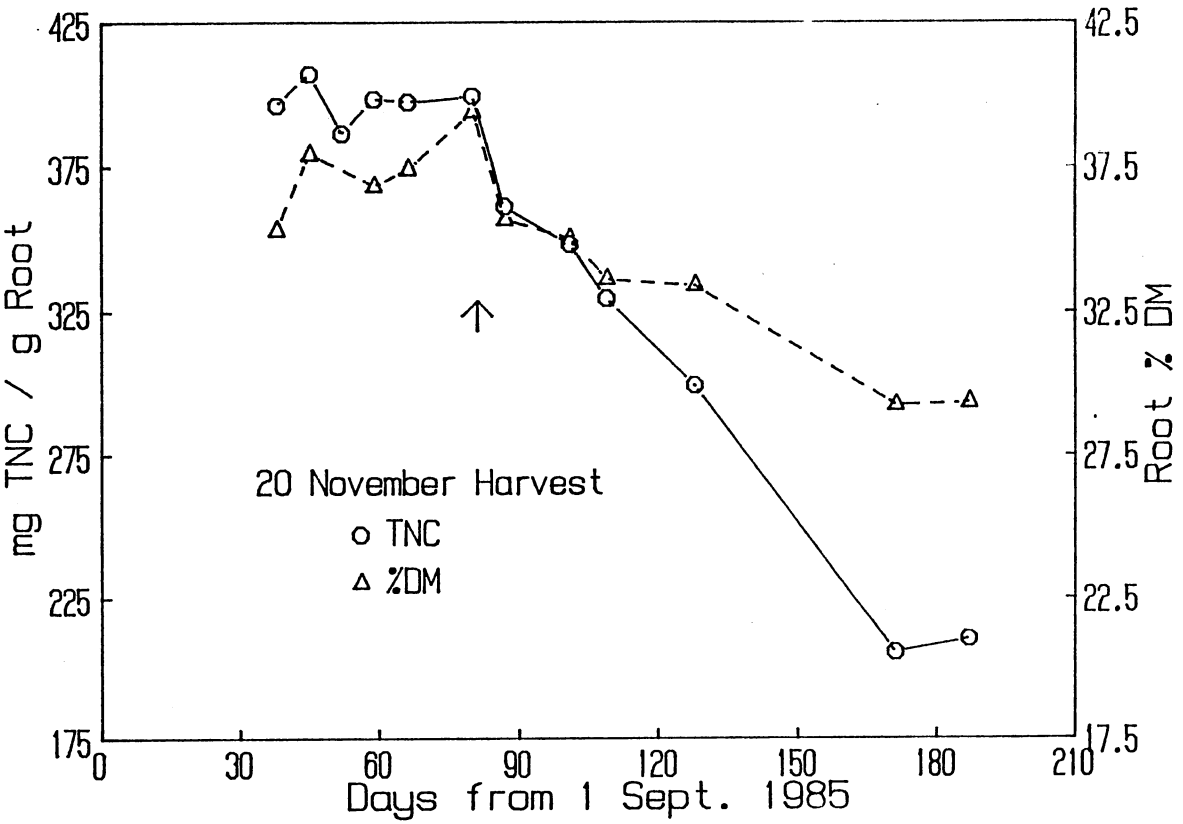

Figure 10. Continued. (e) Response to 20 November harvest date.

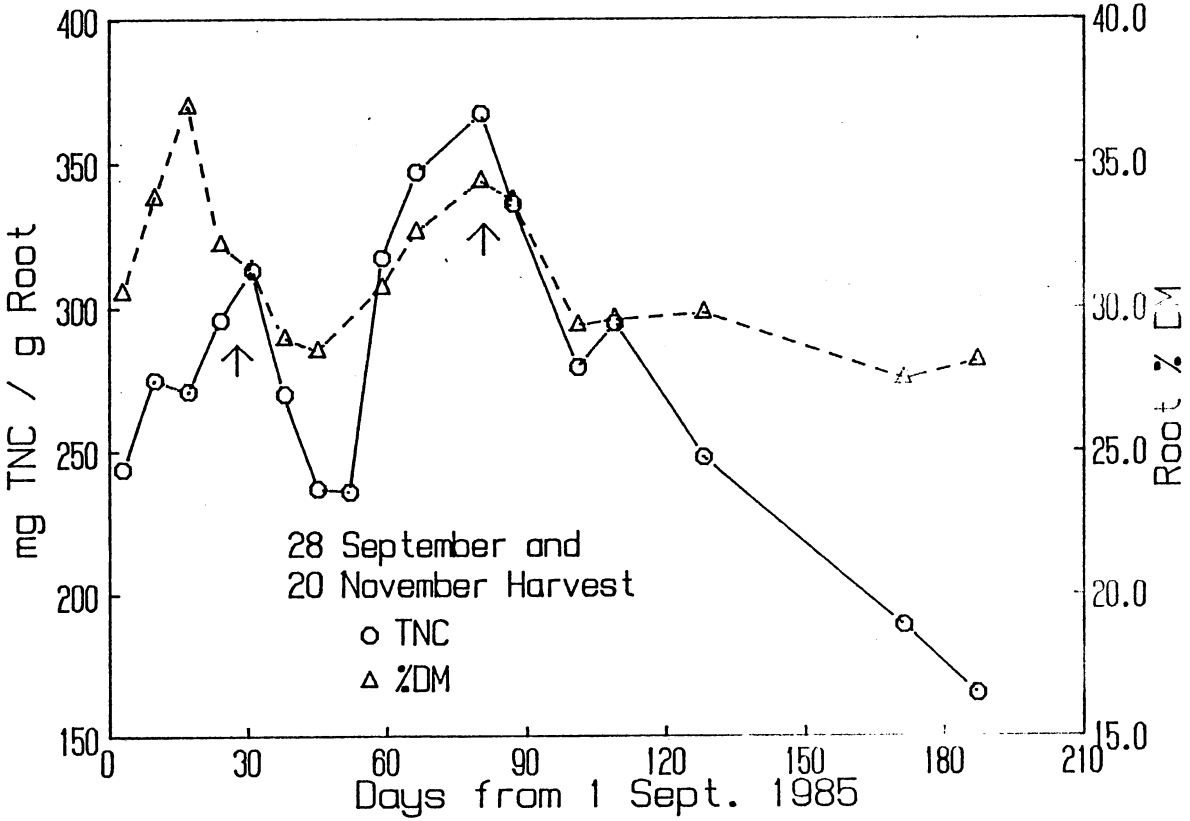

Figure 10. Continued. (f) Response to 28 September and 20 November harvest date.

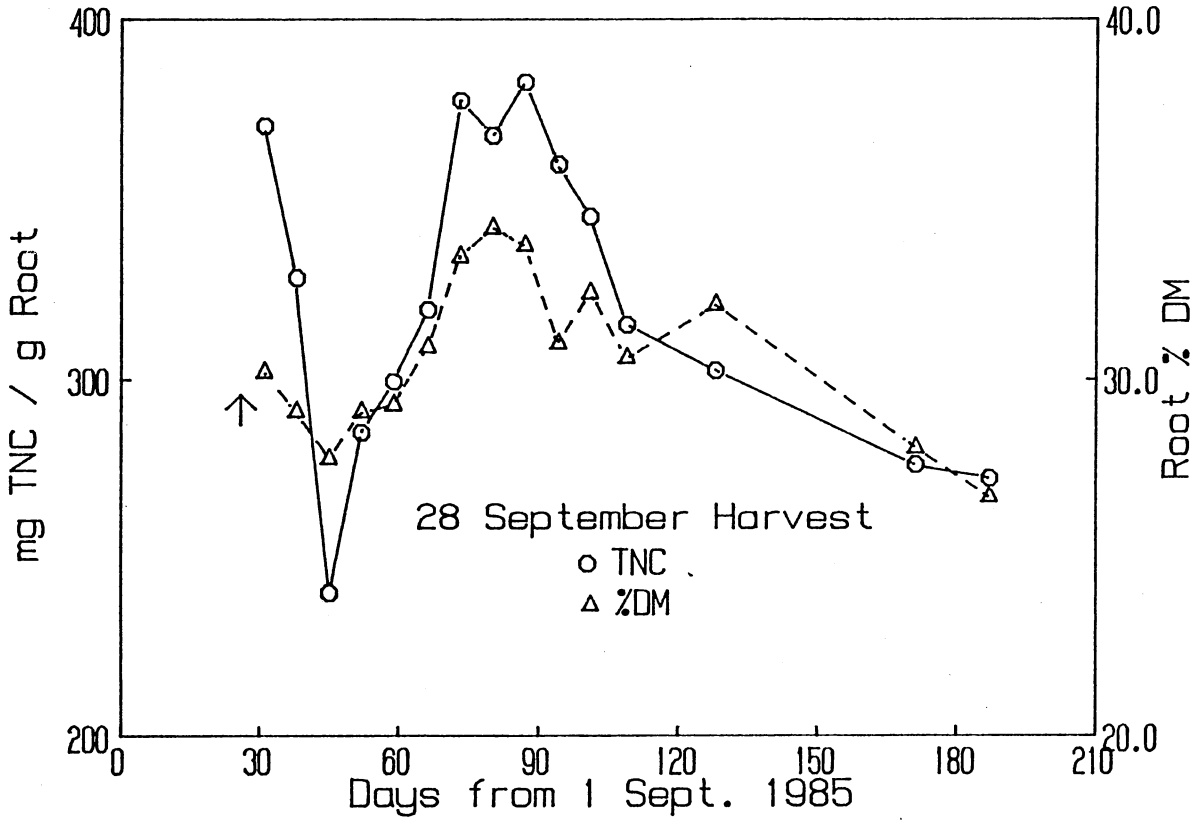

Figure 11. Comparison of irrigated 1985 fall and winter TNC and %DM trends in response to fall harvest. Arrows indicate harvest dates. (a) Response to 28 September harvest date.

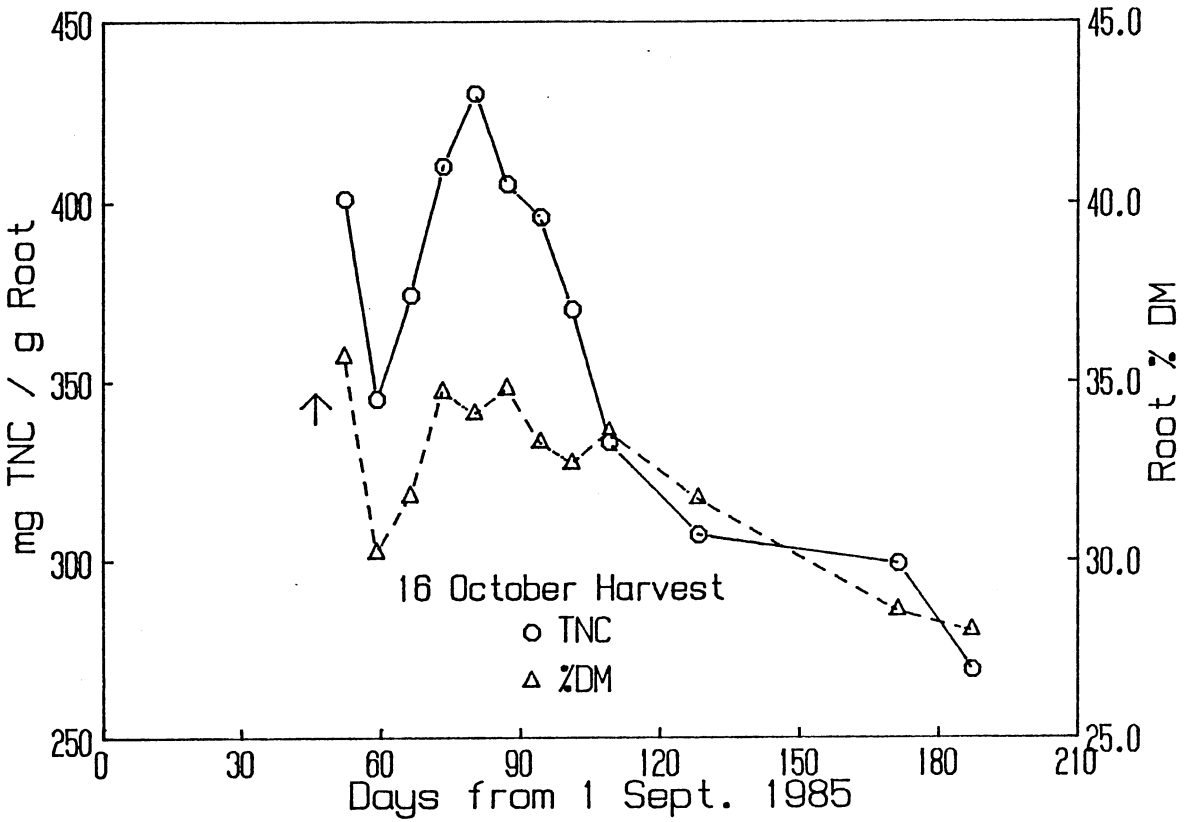

Figure 11. Continued. (b) Response to 16 October harvest date.

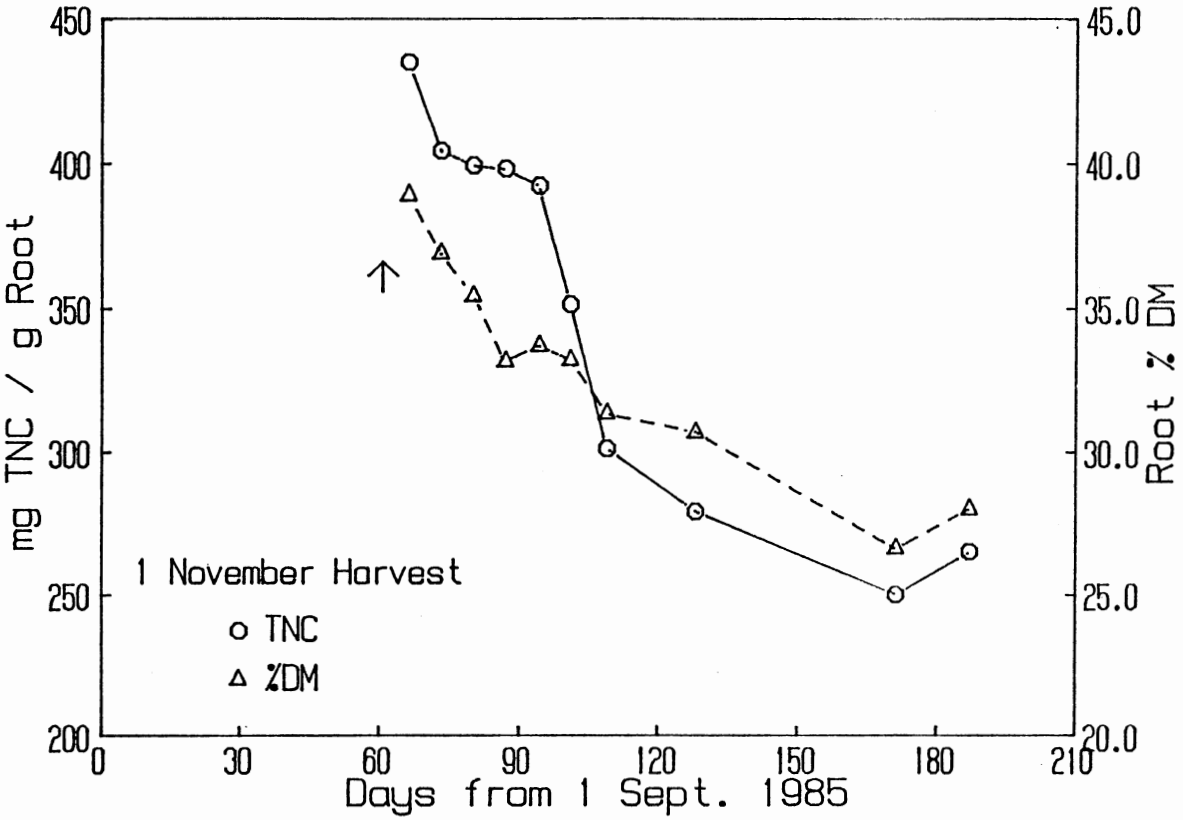

Figure 11. Continued. (c) Response to 1 November harvest date.

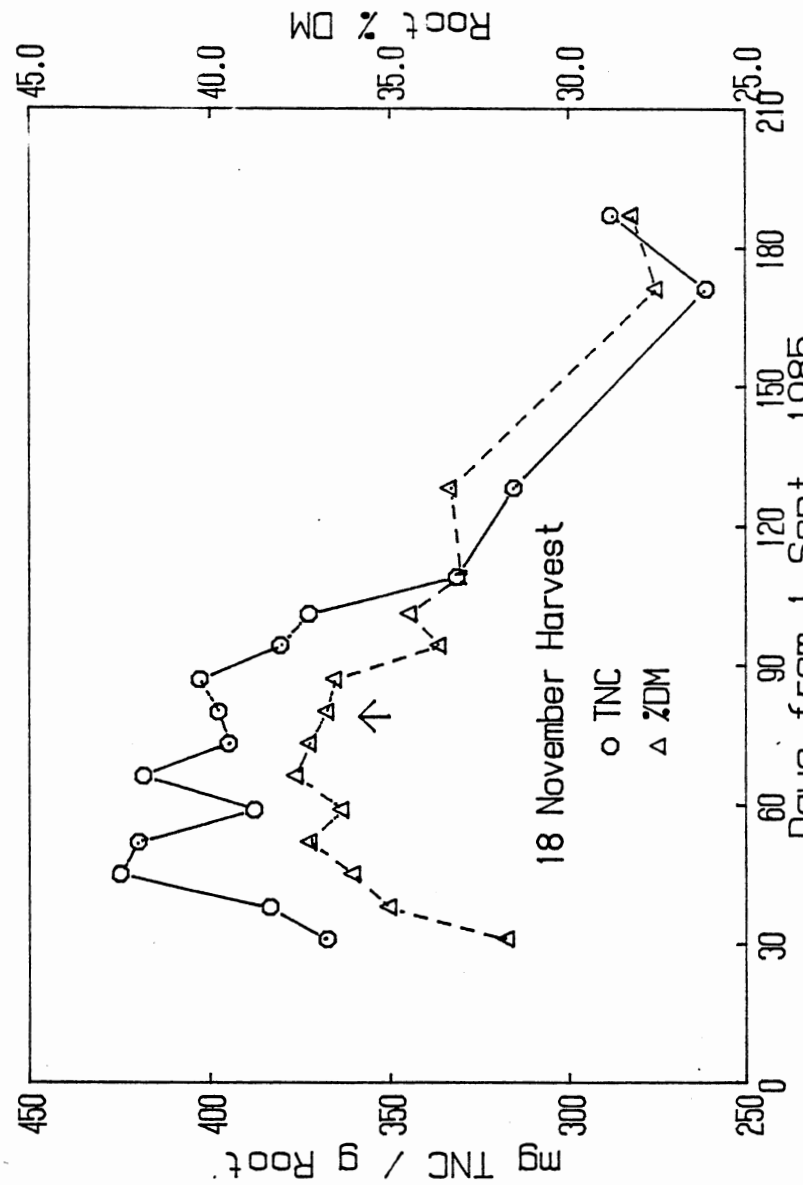

Figure 11. Continued. (d) Response to 18 November harvest date.

without recovery after harvest occurred. Root %DM continued to accumulate in treatments until they were harvested.

When considering the overall correlation between treatment means, there was a significant positive relationship between TNC and %DM during the fall and winter of 1984 and 1985. However, there were several individual dates on which the correlation was not significant, leading to the conclusion that although TNC and %DM may be significantly correlated over a period of time, the correlation may not hold on specific dates within that period. Generally, %DM and TNC trends were similar in the fall. This would be expected as the TNC fraction is a component of %DM. Lack of correlation between %DM and TNC on any individual date may be partially explained by the complex nature of root dry matter. It is possible that another fraction may be confounding the correlation of TNC with %DM. However, Fishbeck et al, (1987) reported that the nitrogen (N) fraction comprised no more than 2% of root DM. Large changes in the N fraction or any other fraction would be necessary to confound any changes of TNC.

The technique used to determine %DM may have also contributed to the poor correlation of %DM and TNC on individual dates. As sucrose is a major fraction of TNC during the fall (Nelson and Smith, 1968), and is very soluble, some may have been leached out of the samples during the soaking process.

Forage yields in 1985 and %DM levels during the previous fall were not significantly correlated in either the irrigated or nonirrigated experiments. However, root %DM levels on several dates in the fall of 1985 were significantly correlated with yields the following year (Table XX). Treatments having low %DM during October and November tended to be

TABLE XX

SIGNIFICANT CORRELATION OF ROOT %DM AND FORAGE YIELD

Experiment	Sampling Date	Forage Harvest Date	Correlation Coefficient
			---r---
Irrigated	29 October 1985	24 April 1986	.94
"	"	1986 Seasonal Yield	.96
Nonirrigated	29 October 1985	24 April 1986	.84
"	"	1986 Seasonal Yield	.96
"	19 November 1985	24 April 1986	.94
"	"	1986 Seasonal Yield	.95
"	26 November 1985	24 April 1986	.94
"	"	1986 Seasonal Yield	.86

those treatments which had significantly lower yields the following spring.

When significant correlations between yield and either root TNC or %DM did occur, TNC and %DM were not often significantly correlated. Both TNC and %DM were significantly correlated with yield only on one root sampling date in the irrigated or nonirrigated experiment; the 29 October 1985 sampling date of both experiments and the April 1986 forage harvest. Even though overall correlations between TNC and %DM were significant for both the irrigated and nonirrigated experiments, they were not both correlated with yield. Furthermore, although trends of %DM and TNC may be correlated over time, the correlation may not be consistent within the period described. There were 20 dates on which both %DM and TNC differed among harvest treatments and were significantly correlated compared with a total of 39 sampling dates. The inconsistencies between TNC and %DM may arise from TNC being a fraction of %DM and its changing more rapidly during the fall and winter relative to other fractions (proteins, cell wall components, minerals, etc.).

CHAPTER IV

SUMMARY

The timing of the final fall harvest of alfalfa affects root TNC and %DM cycles in the fall, but did not affect total yield or persistence over the duration of the study. In some cases, forage yield of individual harvests was affected in subsequent years. Previous studies have indicated that timing of the final fall harvest may be a critical factor in maintaining stand persistence and optimal yields. Previous studies have defined a critical period of 4 to 6 weeks prior to the first killing freeze during which alfalfa should not be harvested. Although some fall harvest studies in the South have failed to show differences in root TNC on a single date in the late fall or yield in subsequent years, this study has show that Cimarron alfalfa grown in north central Oklahoma did exhibit some differences in TNC in the fall due to fall harvest date prior to and after the date of the killing freeze as well as forage yield differences the following year.

Root TNC and %DM cycles were affected by delaying the date of the final fall harvest. Harvests occurring under normal growing conditions prior to late-October exhibited typical regrowth and associated depletion of root TNC and %DM followed by accumulation of root TNC and %DM as the plant developed sufficient photosynthetic capacity. Cycles of the treatments harvested in October reached a low point in TNC and

%DM during the 4 to 6 week period prior to the killing freeze. Generally, as the date of harvest was delayed, two effects were seen. First, the amplitude of the TNC and %DM cycles decreased to the point of having only a decline of TNC and %DM without any recovery or accumulation. This may have been due to the lack of photosynthetic capacity of the canopy due to slowed regrowth associated with the onset of winter dormancy caused by cooler temperatures and shorter daylengths. Secondly, TNC and %DM continued to accumulate during the fall in treatments not yet harvested, resulting in higher TNC and %DM values at the time of fall harvest than treatments previously harvested. In spite of some differences during the fall, treatments were not significantly different at some point during the late fall or early winter in both years of both studies. This may have been an effect of staggering the harvest dates, causing recovery of earlier treatments to coincide with depletion of later treatments and a convergence of root TNC and %DM values. This may be important in future studies to avoid obscuring treatment differences by sampling only at one point in time.

Nonirrigated treatments last harvested in August showed complete recovery of root TNC and %DM prior to the 4 to 6 week critical period prior to the first killing freeze. These treatments also had significantly higher forage yields in subsequent years. In the second year of the study, the treatments allowed to grow through the fall and harvested in mid-November also had high TNC and %DM during the critical period and were at times not significantly different in yield from treatments cut only in August. Irrigated treatments showed no yield differences following the first fall, but did have significant differences among treatments the second year. Treatments allowed to

grow undisturbed through the fall and then harvested in mid-November had higher forage yields the following year.

These trends seem to indicate that utilization of alfalfa forage in the fall should be considered carefully. Some potential for loss of forage yield in later years exists if alfalfa is harvested during the fall. However, there is no strong evidence from this study that directly linked the occasional yield losses that did occur to the level of TNC or %DM during the fall. These results indicate that cutting during the latter part of this critical period may actually be less harmful than cutting prior to the critical period. Other studies have shown adequate root reserves are needed at this time to promote effective hardening in preparation for winter. Although yield was affected in the first year of the nonirrigated study and the second year of both studies, no apparent treatment effects were noted in either experiment on total yield over years and final plant density of both experiments.

Although overall correlation of root TNC and %DM were significant in the fall and winter, they were not always correlated on individual sampling dates. They also failed to be consistent when declaring differences among treatments. This may be due in part to the complex nature of root dry matter, of which TNC is a fraction. One would expect structural components to remain relatively static during the fall and winter when plants are not actively growing. Changes in TNC levels relative to the structural tissue may have caused lack of consistent correlation between TNC and %DM. Other DM components may have also been changing at this time, perhaps in a way that would confound changes in TNC. Other DM components are often only a proportionally small fraction

of DM when compared to TNC. For example, Fishbeck et al, (1987) reported that the nitrogen (N) fraction comprised no more than 2% of root DM. Graber et al. (1927) found that the N fraction was less than 3% of the total root DM, and TNC was almost half (44%).

A number of questions remain to be answered concerning fall harvest management of alfalfa. Foremost may be that of the recovery of root TNC to high levels in the fall and winter, regardless of harvest date. Careful measurement of canopy photosynthesis during the fall in conjunction with measurements of root reserves would help answer this question. Jung and Larson (1972) reviewed many of the different factors relating to cold tolerance, including levels of lipids, amino acids and carbohydrates. Certainly other metabolic processes occurring during the fall should be studied further, especially those related to key mineral (especially K, as was done by Tesar and Yager) and protein components (especially key enzymes needed to convert simple sugars to starch in alfalfa roots). Another area of study would be the effect of differing levels of fall dormancy among alfalfa cultivars on metabolic processes in the fall and winter. These questions, when answered, may help to answer the final and most important question concerning fall harvest management, that of economics. Utilization of fall growth of alfalfa as forage or even as a control measure for pests may be practical and economical as long as forage yield or persistence are adversely affected in subsequent years. The results of this study seem to indicate that although yield differences due to differing dates of fall harvest occurred at individual harvests over the course of time, there was not a significant treatment effect on total yield or persistence over the duration of the experiment.

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