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The effect of forage management on carbohydrate reserves of alfalfa and smooth bromegrass

by

Gerard M. Hoppe

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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DATED Oct. 15th 1987

He makes the grass grow for the cattle,
and plants for man to cultivate
bringing forth food from the earth.
Psalm 104:14 (NIV)

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The effect of forage management on carbohydrate reserves of alfalfa and smooth bromegrass submitted by Gerard M. Hoppe in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in AGRONOMY.

Peter D Wallon
.....
Supervisor

G. W. Mathison
.....

Peter Baily
.....

Date 13 Oct. 1987

This thesis is dedicated to the memory

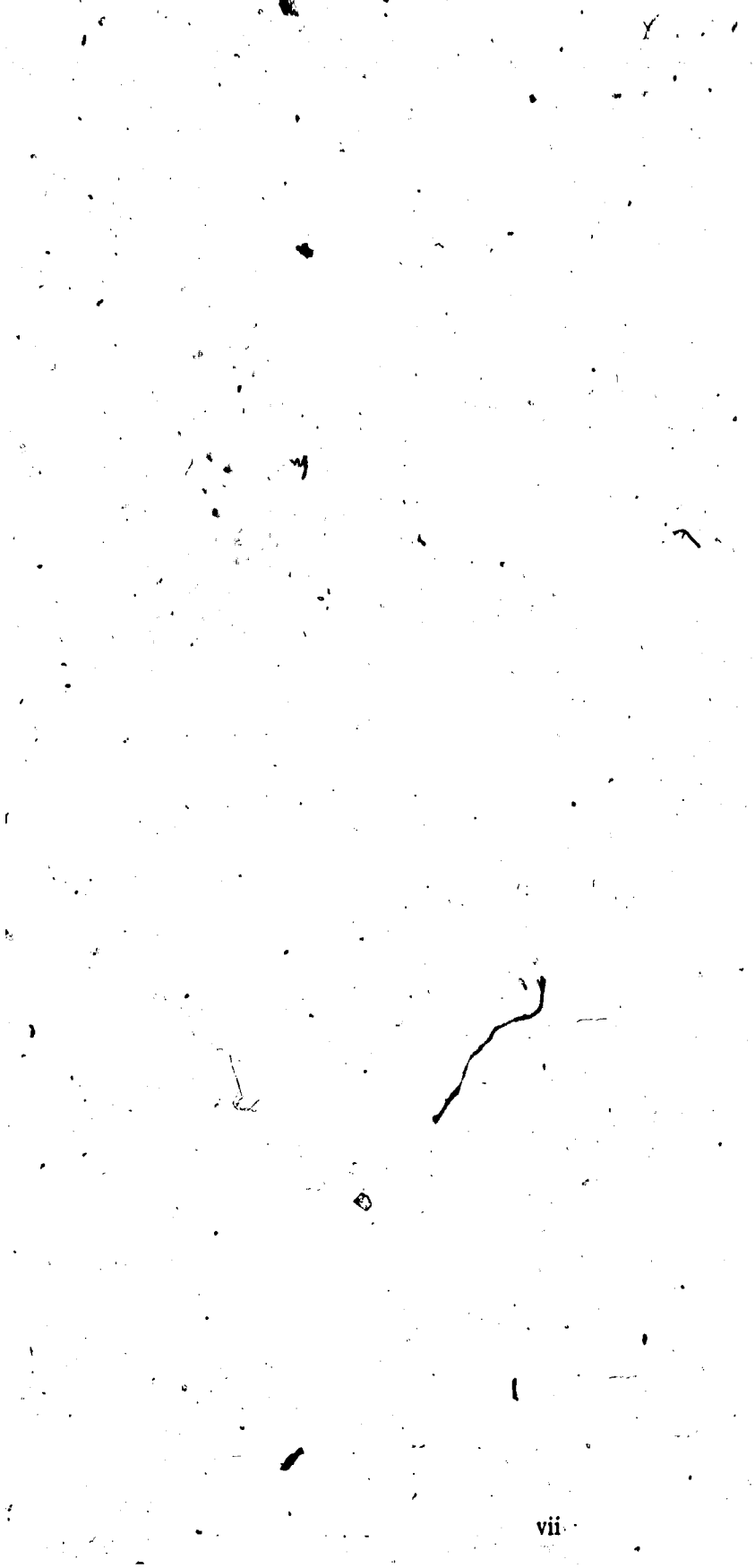
of my grandmother, Ethel Carroll.

ABSTRACT

The effects of summer and fall management on alfalfa and bromegrass carbohydrate reserves under western Canadian environmental conditions have in the past, largely been determined by inference and interpolation. In 1984 to 1985, treatments were imposed on a mixed stand of alfalfa and bromegrass grown under dryland conditions on an eluviated black chernozemic soil. Trial A studied the effects of summer defoliation and nitrogen fertilizer on yield, herbage quality and total nonstructural carbohydrate (TNC) root reserves of alfalfa and bromegrass. Trial B investigated fall cutting on carbohydrate root reserves, the fall plus yield and herbage quality the following year. Near infrared reflectance (NIR) spectroscopy assayed herbage quality (acid pepsin dry matter dissappearance, APDMD; protein; fibre and phosphorus) and root TNC reserves in alfalfa and bromegrass. Both scanning and fixed filter NIR instruments were used.

In trial A, nitrogen fertilizer increased stand productivity and herbage quality. Root carbohydrate reserves increased in alfalfa but decreased in bromegrass with nitrogen application. Four cuts per year increased herbage quality but reduced yield. A two cut system gave high yields and herbage quality and maintained TNC reserves of alfalfa and bromegrass at high levels. Root TNC levels of both species were depleted by frequent defoliation. Sward height and growth stage score of the grass were used to develop yield prediction models with correlation coefficients greater than 0.80. Fall harvests were applied to trial B at weekly intervals from September 7 to October 5, 1984; consistently higher yields and herbage quality (measured as APDMD) were obtained under the two (hay) and four (simulated grazing) regimes in 1985 compared an August 23, 1984 harvest. Fall defoliation affected root TNC of alfalfa but not bromegrass. Reserves increased with a fall cut imposed on a hay regime, whereas under simulated grazing no difference occurred compared to the control. Alfalfa TNC levels were not affected by nitrogen application whereas bromegrass reserves were depleted. Two to four wavelengths were required to develop calibration equations for the quality components. Spectral data was collected and analysed as log(1/reflectance). Calibration

equation coefficients of determination ranged from 0.80 to 0.98.



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1. INTRODUCTION

One of the most important forage mixtures for pastures and rangeland in the Great Plains of Canada and the northern United States is alfalfa-smooth bromegrass. When forage is grown for hay, the objective of most farmers is quantity rather than quality as hay is utilized as a maintenance feed for beef cattle. On the Canadian prairies hay is usually taken as two cuts per year depending on the growing season (McElgun *et al.*, 1972). However, under pasture conditions the management objective is to maximize quality by maintaining plants in a juvenile stage by repeated defoliation. Pasture is therefore utilized for growth and production of dairy and beef cattle. Workers in the United States have studied the trend of carbohydrate root reserves in pure stands of alfalfa (Cooper and Watson, 1968; Nelson and Smith, 1968) or smooth bromegrass (Smith, 1962) under various defoliation and/or nitrogen regimes. However, studies in Canada relating carbohydrate root reserves of alfalfa and bromegrass when grown as a mixed stand are few (Wolf *et al.*, 1964). Therefore in the past, seasonal changes in TNC reserves under western Canadian environmental conditions and the way in which they influence yield have been determined by inference and interpolation.

A knowledge of the trend in total nonstructural carbohydrate (TNC) reserves in perennial forage plants is fundamental to an understanding of the effects of management practices, particularly during regrowth after defoliation and during other times of stress (Smith, 1962; Nelson and Smith, 1968). The TNC reserves may be temporarily stored in all plant parts. However stem bases, stolons and rhizomes are the major areas of storage in grasses whereas in legumes it is the crown and tap root (Graber *et al.*, 1927).

Where defoliation[1] is too early, too late, or too frequent there is a decline in TNC reserves (Granfield, 1935; Chatterton *et al.*, 1974). Low carbohydrate reserves have been associated with reduced production and stand longevity (Hedrick, 1958; Reynolds and Smith, 1962; Blaser *et al.*, 1966). The rapidity of regrowth after dormancy or defoliation is related to the amount of TNC reserves at the time of defoliation. However, the role of TNC reserves

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[1]Defoliation is defined as the removal of part or all of the plant shoot.

following defoliation or dormancy is a continuing matter of controversy (Graber *et al.*, 1927; Weinmann, 1948; White, 1973; Rapoport and Travis, 1984; Richards *et al.*, 1987). May (1960) and Baker (1963) cautioned that the carbohydrate level at the time of defoliation may have little bearing on future growth and productivity. Recent work by Richards *et al.* (1987) has also cast doubt upon the role of TNC concentration or amount in determining regrowth yield. The precise role of TNC reserves of perennial grasses and legumes in regrowth and metabolism has yet to be fully elucidated.

Applied nitrogen has variable effects on root TNC levels ranging from an increase or decrease to no effect (Paulsen and Smith, 1969; White *et al.*, 1972). The response is determined by the level of root TNC at the time of nitrogen application and prevailing abiotic factors. The increased carbohydrate level at the mature stages of growth is a reflection of the greater photosynthetic tissue produced by fertilized plants (Paulsen and Smith, 1968). Where temperature and moisture are favorable, in general plants with low carbohydrate levels at the time of nitrogen application increase reserves, whereas those with initially high levels show a gradual reduction (Cook, 1966).

Fall management of alfalfa is an important aspect of management that is often misunderstood; as it affects the health, vigor, persistence and productivity of the stand. The early fall is considered a critical time for storage of TNC reserves necessary for hardening, winter dormancy, cold resistance and spring growth (Smith, 1960). Smith (1972) called this the 'critical fall harvest period'; which for alfalfa is generally 4 to 6 weeks prior to the first killing frost. Since the first killing frost occurs in mid-October for central Alberta, the critical fall period is considered to extend from early September to mid-October. Harvesting during this fall period results in reduced survival and yield in the following year (Mark, 1936; Johnston, 1966; McKenzie, 1980). Several researchers have concluded that a fall harvest after a killing frost is less likely to result in stand and yield reduction (Brown, 1963; Collins and Taylor, 1980).

The determination of forage quality (ie. digestibility and nutrient content) and TNC of the roots usually requires several laboratory analyses which are time consuming and expensive.

Near infrared reflectance (NIR) spectroscopy meets the requirements of a method that is quick and accurate and is gaining acceptance both in research and industry (Polesello and Giangiacomo, 1983). NIR is being successfully used in plant breeding programs, crop management, plant physiology and ruminant nutrition (Marten, 1985a). In this study, calibration and prediction equations for the assay of herbage quality and root carbohydrates were developed on a fixed and scanning spectrophotometer.

The objectives of this study were two-fold. Firstly, to evaluate the effects of two management regimes on the seasonal trends in root total nonstructural carbohydrate of an alfalfa-smooth bromegrass sward in central Alberta and to relate root TNC to dry matter production and herbage quality. Secondly, to investigate the effects of fall harvest on root TNC in the fall plus dry matter yield and herbage quality the following year.

2. LITERATURE REVIEW

2.1 Total nonstructral carbohydrates (TNC) reserves

The importance of stored organic reserves[1] in perennial legumes and grasses was recognized to be a limiting factor for forage yields early in the century by Nelson (1925). Graber *et al.* (1927) proposed the hypothesis that "new top growth was initiated and developed largely at the expense of previously accumulated organic reserves" and that the roots of alfalfa and the rhizomes of grasses were organs of storage. The implications of their hypotheses to the management of many herbage species is reflected by the large number of research projects on organic reserves in the past 60 years.

In an extensive literature review, May (1960) considered this hypothesis somewhat premature. He accepted that after complete defoliation, the initial synthesis of photosynthetic tissue and root respiration, plus root respiration during a dormant period are maintained at the expense of previously stored reserves. However, the paucity of knowledge on mobilizing hormones and mechanisms of translocation and utilization of carbohydrates at meristems precluded a critical evaluation of the part played by reserves in determining plant growth. Since 1960 numerous experiments have confirmed that reserves accumulated in the stem bases and roots are used by plants for regrowth. Studies using radioactive labelled ^{14}C have revealed the proportions of stored reserves used for regrowth, respiration and tissue maintenance (Hodkinson, 1969; Smith and Silva, 1969; Smith and Marten, 1970; Balasko and Smith, 1973)

Plant tissue contains many organic compounds that can be considered as reserve substances, including carbohydrates, fats, oils and proteins. Research by Weinmann (1948), Cook (1966) and Smith (1972) has shown fats, oils and proteins to play a minor role as reserve substances; a few studies have indicated proteins as possible reserve substances mobilized after a defoliation (Sullivan and Sprague, 1943; Davidson and Milthrope, 1965). Hence, nonstructural

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[1]Synonymous terms in use are; food reserves, labile carbohydrates, nonstructural carbohydrate, reserve foods, total available carbohydrate, total nonstructural carbohydrate.

carbohydrates are considered the most important reserve substance. Smith (1972) has shown that perennial forage grasses may be classified into two groups based on the type of carbohydrate accumulated in their vegetative parts. The study revealed that starch is accumulated in storage otgans of forage species of tropical and subtropical origin, while fructosans are the primary stored carbohydrate in grasses of temperate origin. Smith (1972) found that this latter group can be sub-divided into those that store long- or short-chain fructosans. Bromegrass (*Bromus* spp.) is a short chain fructosan accumulator (Grotelueschen and Smith, 1968; Smith, 1968; Bender and Smith, 1973).

The principle reserve carbohydrates of grasses are sugars, fructosans, dextrins and starch (Wienmann, 1952; Smith and Grotelueschen, 1966). Examination of carbohydrate fractions in different plant parts of bromegrass identified the stem bases (and top two inches of roots) to contain the highest percentage of TNC (Reynolds and Smith, 1962; Okajima and Smith, 1964; Smith, 1967). Cook (1966) compiled an extensive review of storage organs for TNC reserves of numerous forage species.

The tap root of alfalfa is the principle site for carbohydrate reserve storage (Escalada and Smith, 1972). The major components of carbohydrate reserves of alfalfa are starch, sucrose, glucose and fructose (Gaber *et al.*, 1927; Jung and Smith, 1961; Dobrenz and Massengale, 1966; Nelson and Smith, 1968). Starch is the principle storage fraction with sugars as intermediates during starch synthesis and degradation (Ueno and Smith, 1970; Deregibus *et al.*, 1978).

The amount of reserve substances stored by the plant depends on a balance between the relative activity of photosynthesis and respiration at different ontogenic stages. Reserve substances are accumulated in perennial forage plants when synthates are in excess of requirements for growth and maintenance (Graber *et al.*, 1927). TNC reserves are important for winter survival, initiation of spring growth, regrowth after defoliation or at any time photosynthetic production is inadequate to meet growth and maintenance demands. The level of reserve carbohydrates in perennial forage plants can be greatly affected by management

practices and the environment (Smith, 1960).

The influence of applied nitrogen on the level of root TNC depends on the amount of carbohydrate present and its relationship to other nutrients and environmental factors. Low levels or deficiencies of soil nitrogen provoke the accumulation of sugars in the plant (Paulsen and Smith, 1969). Watkins (1940) stated that under low soil nitrogen bromegrass accumulated excess carbohydrates, whereas high soil nitrogen resulted in a greater depletion of reserves after defoliation (Brown and Blaser, 1965; Reynolds, 1971). The apparent reason for this is that high levels of soil nitrogen promote rapid herbage growth, utilization of photosynthate and hence less carbohydrate accumulation. Consequently there is a reduction in reserves when no other factors are limiting (White, 1973).

Fertilization with nitrogen of an alfalfa-brome stand decreased the available carbohydrate content of rhizomes and stem bases of bromegrass throughout the growing season (Watkins, 1940; Van Riper and Owen, 1964). An application of nitrogen to alfalfa caused nodule shedding or a reduction of nitrogen fixation (Moustafa *et al.*, 1969). The detrimental effects of nitrogen application to the legume component of a mixed stand have been documented (Ashford, 1967).

The literature concerned with defoliation and its effect on carbohydrate reserves, production and plant vigor is immense. In general too late, too early, too frequent or too heavy defoliation causes a decline in stored reserves, plant vigor and production. Comprehensive reviews have been made by Weinmann, 1948; May, 1960; Jameson, 1963; Youngner, 1972; White, 1973. Frequent and low cutting height drastically reduce TNC (Smith and Nelson, 1967). Research on the various factors of defoliation confirmed that excessive, frequent defoliations were more damaging to the plant than one severe clipping (Smith, 1962). Successive cuts would cause a continuous depletion of reserves and would not allow the plant to recover. Labile carbohydrate reserves only contribute to regrowth during the first few days after defoliation (Hodkinson, 1969; Richards *et al.*, 1987). The extent of this contribution to regrowth depends on (1) the growth stage of the plant, (2) the severity of defoliation and (3)

the environmental factors influencing growth and photosynthesis. Smith (1981a) suggested that carbohydrate reserves were important only in determining initial regrowth, but that it was the general carbohydrate status of the plant which determined the overall response to defoliation. Richards and Caldwell (1985) and Richards *et al.* (1987) found that the total carbohydrate status (TNC pool) did not account for the regrowth responses of *Agropyron desortum* and *A. spicatum* after defoliation. The study revealed that meristematic limitations were more important than stored carbohydrates or concurrent photosynthates in determining the ability of the Agropyron species to regrow after defoliation. The contribution of concurrent photosynthates was more important to regrowth than stored carbohydrate reserves. They concluded that a species ability to reallocate reserves to its growing points for synthesis of new foliage may be the key to that species ability to tolerate defoliation.

Defoliation of legumes can result in the shedding and death of root nodule tissue with a consequent release of nitrogen to the soil (Whitehead, 1970). The release of nitrogen to the soil may inhibit either additional initiation of legume nodules or the nitrogenase activity of nodules already present. If nitrogenase activity is inhibited the legume component of the sward may become solely dependent upon available soil nitrogen. Grasses grown in association with legumes may absorb the newly released nitrogen thereby reducing the soil nitrogen inhibition of legume nitrogen fixation (Lorenz *et al.*, 1961).

Defoliation at any time of the year usually affects carbohydrate reserve levels. Plants are, however, affected more by defoliation at certain morphological stages of development than at others. It is important for management purposes to know at what stage forage species can be utilized without severe carbohydrate reserve depletion.

When spring growth is initiated the plant exhibits active growth, and the TNC reserves show a sharp decline (Smith, 1962). During active growth, temperature is the most important factor affecting the carbohydrate reserve levels. Low temperatures promote the accumulation of reserves, whereas high temperatures cause a decline in reserve levels (Robinson and Messengale, 1968). The deleterious effects of frequent defoliations on TNC levels are greatest

during periods of warm temperatures. Night temperatures can also greatly affect reserve levels. Low night temperatures favour accumulation of TNC reserves, as fewer sugars are used during respiration.

Drought differentially affects TNC storage, growth and photosynthesis (Rapoport and Travis, 1984). Growth rates are reduced to a greater degree than photosynthesis under low moisture conditions. Consequently, demand for sugars is less and substrates accumulate as reserves (Blaser *et al.*, 1966; Trlica, 1971; Trlica and Cook, 1972). During drought and heat stress frequent defoliation prevents plants from accumulating sufficient reserves and weakens resistance. Favourable soil water conditions that promote plant growth or regrowth tend to lower carbohydrate reserves.

The pattern of change in forage yield, quality and TNC reserves of alfalfa and bromegrass in response to advancing maturity during spring and summer is well established (Smith, 1962, 1964). Nitrogen fertilization of grass and grass-legume mixtures has been used by Archer and Decker (1971) to extend the grazing season into the fall and early winter.

Alfalfa winter survival is affected by harvest management during the growing season; a single fall cutting reduced carbohydrate levels more than three cuttings per season prior to the fall (Kust and Smith, 1961). The early fall is thus considered a critical time for storage of food reserves in alfalfa (Willard *et al.*, 1934; Johnston, 1966; Jung *et al.*, 1969) regardless of developmental stage. Several researchers have concluded that fall harvests after a killing frost are less detrimental than harvests 4 to 6 weeks prior to the killing frost (Brown, 1963; Smith and Rowheder, 1971; Collins and Taylor, 1980). Kust and Smith (1961) found a direct relationship between the amount of TNC reserves present in the fall and the yield of alfalfa hay in the following year. Reynolds (1971) however did not find a correlation between TNC after a fall cutting and next years yield. Sholar *et al.* (1983) in Oklahoma found fall cutting had little effect on root carbohydrate reserves. In central and northern Alberta mid August to early October is considered the critical fall harvest period (MacKenzie, 1980; McKenzie and McLean, 1980). In western Canada there have been no fall defoliation studies of TNC reserves in

grass-legume mixtures.

The accumulation of TNC reserves ceases when alfalfa is cut in the early fall (Fulkerson, 1970) but depletion of reserves will occur as new photosynthetic tissue is produced. This depletion will continue until either the leaf area is sufficient to meet demands, the leaves are killed by frost, or the plant is again harvested. The final fall cut should be timed to allow reserves to either build up prior to freeze-up or when no additional growth will occur. In Ontario Fulkerson (1966, 1970) studied the effect of fall harvests on yield and TNC reserves in alfalfa and found reduced root densities, stand and yield. A fall management map for alfalfa was determined for the area.

Plant height has been the most frequently used criterion for the estimation of numerous crop yields with varying degrees of success (Hussey *et al.*, 1985). Two problems in interpretation of the literature on this subject are: (1) the numerous ways in which plant height has been measured, and (2) published research usually omits the method employed to measure plant height. To standardize height measurements various instruments have been developed (Shrivastava *et al.*, 1969; Baker *et al.*, 1981; Sharrow, 1984). Height has also been combined with other charactors to enhance yield prediction (Michalk and Herbert, 1977; Tan *et al.*, 1977).

2.2 Herbage quality

Forage is ultimately grown for meat, wool and milk production. For good animal management there must be sufficient quality and quantity of forage to supply the nutrient requirements of the particular class of animal. The determination of forage quality (i.e. digestibility and nutrient content) is necessary to prepare a balanced animal diet. Studies into the feed value (or forage quality) of grasses and legumes reveal that grasses usually contain less crude protein and minerals but more fibre than legumes at similar stages of development (Marten, 1985a). The superior feeding quality of herbage containing a grass and legume mixture to pure grass is well known (Donefer *et al.*, 1966; Walton, 1983). The time of

harvesting in relation to plant growth stage has a pronounced effect on forage quality. The stage of growth is the most important single factor controlling forage value. As the plant matures, digestibility, crude protein and mineral values fall, while fibre values rise.

Fertilizers and defoliation are normally used to increase forage production, however, quality can be greatly influenced. The response of an application of nitrogenous fertilizer to a grass-legume sward is such that there is an increase in the grass with a corresponding decrease in the legume. Defoliation has the reverse effect on the sward. The crude protein content of the herbage may remain constant or it may increase. Digestibility, fibre and phosphorus will be differentially affected when the applied nitrogen interacts with moisture and temperature in the mixed sward (Smith, 1962).

The mineral content of herbage will reflect soil deficiencies. In some unfertilized pastures, the P level of grass and legumes may well be below animal requirements. The extent to which the ruminant digestive system can utilize and efficiently convert the increased forage quality consituents when nitrogenous fertilizers are used has not been extensively studied.

The dry matter digestibility of forage may be assayed using various chemical analyses however the in vitro method of Tilley and Terry (1963) has proved to be the most accurate predictor of digestibility. Problems associated with this method are: (1) maintaining a constant supply of rumen fluid from fistulated animals and (2) the variability in activity of the rumen fluid even when animals are fed the same forage. These and other factors may result in large discrepancies between and within digestion runs. Donefer *et al.* (1963) found that forage dry matter disapperance brought about by an acid pepsin solution was highly correlated with in vivo data. Basically, the method is the second stage of the Tilley and Terry (1963) procedure. The acid pepsin technique has been used succesfully by Lema (1972) on smooth bromegrass hays, with correlations of 0.89 between this method and in vivo results. He stressed the point that the two stage Tilley and Terry (1963) in vitro procedure had no apparent advantage over the acid pepsin dry matter disapperance (APDMD) method.

Determinations of forage quality usually require several laboratory analyses which are time consuming and expensive. Near infrared reflectance (NIR) spectroscopy is gaining acceptance as an alternative method of analysis for the estimation of forage quality in both research and industry (Polesello and Giangiacomo, 1983). This method is rapid, accurate and less expensive than conventional laboratory analyses. A brief review of near infrared reflectance spectroscopy follows.

2.3 Near infrared reflectance spectroscopy

Kaye (1954, 1955) was the pioneer of near infrared transmission spectroscopy. Whetsel (1968) reviewed classical spectroscopy in the near infrared region. At the same time near infrared reflectance (NIR) spectroscopy was first shown to have application in agriculture for the prediction of oil, protein and moisture content of grains and oilseeds (Norris and Hart, 1965; Ben Gera and Norris, 1968).

During the past 15 years there has been a rapid advance in the development of NIR spectroscopy technology. Various types of NIR spectrophotometer instruments are currently being used as an alternative to conventional laboratory methods to determine the nutritive value of a wide range of agricultural commodities (Polesello and Giangiacomo, 1983). There are numerous companies marketing NIR instruments, all instruments manufactured can be classified into two main groups:

1. Filter systems, which make use of fixed filters of a specific wavelength to select wavelengths corresponding to the calibration samples, and

2. Spectrocomputer systems, which use scanning monochromators over the NIR region from 1100 to 2500nm and perform wavelength selection through complex mathematical processes following the scanning of calibration samples.

Despite the varying complexity of these systems, the basis of their function is the same. Analysis is based on a beam of NIR monochromatic light shone on the surface of a finely ground sample. The intensity of the light reflected from the sample is collected by optical

detectors and converted to signals processed by the instrument to predict sample composition (Redshaw and Weisenburger, 1983). The type of system selected depends on the particular application to be made. Filter instruments, with multiple filters (varying from 6 to 24) can scan selected portions of the NIR region in approximately 20 seconds compared to 1 or 2 minutes required by monochromators. Their relatively low cost also permits their use for routine analytical determinations (Shenk and Hoover, 1976; Shenk *et al.*, 1976; Burdick *et al.*, 1981). The high cost of monochrometer type instruments may preclude their use for routine analysis.

One of the many uses of NIR in Canada and the USA is in wheat marketing. In addition to grain elevators and the food trade, numerous universities and agricultural research stations are using NIR systems to determine forage quality and animal performance, (Redshaw and Weisenburger, 1983; Marten *et al.*, 1984). The majority of locations have fixed filter systems which are used for routine moisture, protein and fibre determinations. Where spectrocomputer systems are found, research into prediction of amino acids, fibres, in vivo digestibility, animal response and minerals of numerous crops including forages is progressing (Marten *et al.*, 1983a; Redshaw and Weisenburger, 1983; Marten *et al.*, 1984; M[illegible]n *et al.*, 1985).

The NIR procedure is a rapid, nonconsumptive and precise method that has the capability for simultaneous multiconstituent sample analysis. However, it requires calibration with samples of known chemical or nutritional value. Shenk *et al.* (1979) stated four equally important criteria to successfully predict forage quality by NIR. They are: (1) calibration samples should be representative of the population to be predicted, (2) chemical analysis of the calibration samples must be accurate, (3) correct choice of mathematical treatment and (4) correct choice of wavelengths.

Forages and feedstuffs are variable and chemically complex. Norris *et al.* (1976) demonstrated that NIR has the capability of predicting the chemical or nutritional value of these substances with a reasonable degree of accuracy. Research has provided additional

evidence that forage quality in perennial and annual forage plants could be predicted as stated above (Hymowitz *et al.*, 1974; Rinne *et al.*, 1975; Shenk and Barnes, 1977; Shenk *et al.*, 1981; Marten, 1985b). The standard errors frequently found for conventional laboratory analysis of crude protein (CP), acid detergent fibre (ADF) and in vitro digestible dry matter are 0.43, 1.40 and 0.90 g/Kg, respectively. The standard errors for scanning monochromator NIR analysis of these same parameters are 0.56, 0.60 and 0.63 dag/Kg[3], respectively (Templeton *et al.*, 1983), thus the standard errors for prediction are commonly lower than those reported for conventional analysis. Eckman *et al.* (1983) reported that animal (sheep) response (dry matter intake, digestibility and digestible energy intake) were predicted as accurately with a scanning monochrometer instrument as with conventional laboratory analysis. Mathison *et al.* (1985) found similar results with sheep response, whereas standard errors of prediction for cattle response (dry matter intake and digestibility) were lower than conventional chemical prediction.

Multiple filter NIR instruments were satisfactory in predicting forage quality in perennial forage plants (Barton *et al.*, 1976; Barton and Burdick, 1979 and 1981; Winch and Major, 1981). Burdick *et al.* (1981) reported standard errors obtained with the NIR filter instrument for CP, ADF and IVDMD were 0.58 to 1.15, 1.07 to 2.47 and 1.78 to 2.54, respectively. Generally, these standard errors compare favourably with those of conventional laboratory methods. However, Winch and Major (1981) reported that in vitro or in vivo digestibility of temperate grasses and legumes were not estimated with an acceptable degree of accuracy by an NIR analyzer equipped with six filters. Burdick *et al.* (1981) used a scanning monochrometer to select the first and second wavelengths and used such filters plus others to enable prediction of IVDMD to an acceptable level. Both fixed filter and scanning monochrometer instruments have the potential when utilized to their full capacity to rapidly and accurately provide estimates of forage quality.

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[3]dag/Kg - decigram per 1000grams

NIR is being used extensively in research in plant breeding programs (Gill *et al.*, 1979), crop production (Park *et al.*, 1983; Blosser, 1985; Coleman, 1985), plant physiology (Rosenthal, 1977; Marten, 1985a) and animal response (Norris *et al.*, 1976; Shenk *et al.*, 1976; Lindgren, 1983; Marten, 1985a).

Studies into TNC root reserves are numerous, with the majority on alfalfa, the link between root TNC and winter kill has generated numerous studies. The conventional laboratory assay (Smith 1969b, 1981b) is expensive, difficult to standardize and time consuming. Although studies utilizing a scanning monochrometer NIR instruments conducted have been limited, the results are favourable for accurate prediction of root TNC (Marten, 1985b; Brink and Marten, 1986). The multiple regression coefficient (R^2) for root TNC determinations in the literature varies from 0.90 (Marten *et al.*, 1983) to 0.95 (Redshaw, 1986 personal communication). The standard errors of calibration reported by Brink and Marten (1986) were equivalent to, or lower than those for laboratory assays. . .

No studies using NIR spectroscopy to estimate root TNC in bromegrass were located by a comprehensive literature review. However, a similar search for studies on the prediction of root TNC in alfalfa resulted in the conclusion that NIR systems can be used to accurately and efficiently evaluate the above (Marten, 1985b; Brink and Marten, 1986).

3. PART I: NEAR INFRARED REFLECTANCE SPECTROSCOPY FOR THE ASSAY OF FORAGE QUALITY AND ROOT CARBOHYDRATES

3.1 Materials and methods

Two sample types were used, herbage and root material of alfalfa and bromegrass. Herbage samples for quality analysis were collected form trial A in August 1984 with root samples of alfalfa and bromegrass collected from all trial A harvests in 1984 . Details of trial A are presented below.

3.1.1 Field trial A location and climate

Field trial A was located at the University of Alberta Ellerslie Research Station (latitude 53° 25' N, longitude 113° 13' W and elevation 694m), on an eluviated black chernozem soil of the Malmo series (Verma and Toogood, 1969; Dudas and Pawluk, 1982). A description of the soil type from an adjacent soil pit site (located 400m north of the trial site in NE 1/4 section 22, Tp 3, R 5, W 4) is presented in Appendix 1. These soils are fertile and well drained.

The climate is cool continental characterized by warm summers and cold winters (Appendix 2). Climatological data was obtained from the Ellerslie weather station, situated 900m northeast of the trial site. The mean annual precipitation is 452mm, rainfall accounts for 75% of the annual precipitation, sixty percent of which falls between May-August and is heaviest (84.5mm) in July (Appendix 3).

The trial site was located within a 30 ha field and was seeded with a mixture of alfalfa (*Medicago media* Pers.) and smooth bromegrass (*Bromus inermus* Leys.) in 1977. It was subsequently harvested annually for hay taken as two cuts. Small amounts of liquid manure were applied, the most recent application occurring in the summer of 1983. Commercial fertilizers were not used prior to this experiment. In the fall of 1983, when the trials were marked out, the stand was vigorous. The exact location of the two field trials in the SE quarter section (24,Tp 51, R 25, W 4) was determined by visually selecting an area that was level,

accessible, had good plant growth and uniform ground cover. Ground cover is defined here as basal cover, in which only that portion of the ground surface occupied by the plant is measured. Basal cover was expressed as a percentage of individual species (Clarke *et al.*, 1942). Two line transect studies carried out in the fall of 1983 confirmed the visual observations of uniform composition of alfalfa and smooth bromegrass (Appendix 4). Based on soil test recommendations fertilizers (phosphate, P and sulphur, S) were broadcast onto the experimental site in October, 1983; phosphate was applied as P_2O_5 at 40Kg/ha and elemental sulphur at 10Kg/ha. A split application of the nitrogen fertilizer treatments were applied with 50% in late October, 1983 and early May 1984; and were repeated in October, 1984 and April, 1985.

3.1.2 Experimental design and treatments

Trial A was a split plot test (Table 3.1). The main plots consisted of three nitrogen (N) applications [0, 200 and 400 Kg/ha applied as ammonium nitrate (34-0-0)] with sub-plots consisting of three defoliation (D) treatments: one cut as a control; two cuts as for commercial hay production; or four cuts to simulate rotational grazing (Appendix 5). There were sixteen replications, six for destructive root sampling and ten for other data collected at each harvest. At each harvest the grass and legume were scored for their growth stage (Table 3.2) and the yield of dry matter, the botanical composition and mean sward height recorded.

3.1.3 Sampling of herbage material

Herbage samples for quality analysis were collected in trial A (Part II) in August 1984 and represented a mixed alfalfa-smooth bromegrass sward at three growth stages and three nitrogen fertilization levels. Samples were collected from the forage harvested for yield (as outlined in Part II, section 4.1.3) by sub-sampling approximately 300g dry weight.

Table 3.1 Partition of degrees of freedom (df) for trial A.

Source		df
Replication(R)	(r-1)	9
Nitrogen(N)	(a-1)	2
Error 1 (E1)	(r-1)(a-1)	18
Defoliation(D)	(b-1)	2
DxN	(a-1)(b-1)	4
Error 2 (E2)	a(b-1)(r-1)	54

Table 3.2 Growth stage score for alfalfa and smooth bromegrass used in trial A in 1984 and 1985.

Score	Alfalfa	Bromegrass
1	Juvenile	Juvenile
2	Prebud	Stem elongation
3	Bud stage	Flag leaf
4	10-49% bloom	Head emergence
5	50-100% bloom	Anthesis
6	Seed set	Seed set

3.1.4 Sampling of root material

Root samples of both species were collected for TNC determination (by conventional laboratory methods) from all harvests in trial A in 1984. Herbage was removed leaving 3cm of stubble. On each collection date smooth bromegrass roots were obtained by using a core (75mm diameter and 175mm deep). Four core samples, randomly located, were taken per sub-plot and stored at -25°C for further processing. The core samples were washed with cold water in the laboratory and stem bases, rhizomes and roots were collected over a series of sieves (2mm and 1mm screen sizes). Root material was bulked from the four cores per sub-plot, dried in a forced draft oven at 70°C for 24 hours and ground on a Willey mill (1mm screen). The samples were thoroughly mixed, reground on a Udy cyclone mill (1mm screen), placed in glass bottles and redried at 70°C to constant weight. The bottles were tightly capped and stored at -10°C for TNC analysis.

Four alfalfa plants were randomly chosen from each sub-plot. Herbage was removed leaving 3cm of stubble. Diseased plants or those with multiple lateral roots were discarded (Escalada and Smith, 1972). Crowns and roots of plants were dug from the soil to a 30cm depth on the day of harvest and stored at -25°C. In the laboratory, the four roots were cleaned with cold water, all lateral roots were removed and a 10cm section of tap root immediately below the crown was retained for TNC reserve determinations. The four processed roots were bulked per sub-plot prior to grinding and TNC analysis. Cleaned samples were dried in a forced draft oven at 100°C for 30 minutes and then drying was completed at 70°C for 48 hours (Smith, 1973). The same method of grinding and storage was used as for bromegrass.

3.1.5 Chemical analysis

3.1.5.1 Herbage quality analysis

Acid pepsin dry matter disappearance was determined in the University of Alberta forage quality laboratory for samples collected in trial A 1984. A modification (Kendal *et al.*, 1970) of the acid-pepsin dry matter disappearance (APDMD) technique of Donefer *et*

al. (1983) was used (Appendix 6). The percentage of dry matter removed from the sample by the digestion procedure was then calculated by subtracting the weight of the forage residue from the initial forage weight and expressing this as a percentage. The APDMD method is only a partial measure of herbage degradebility because it only measures proteins digested by pepsin.

The herbage samples used for APDMD determination were also analyzed by the Soils and Animal Nutritional Laboratory (SAML, formerly Soils and Feed Testing Laboratory, Alberta Agriculture), for three other forage quality parameters under research project number 85-059. The procedure described by Goering and van Soest (1972) was used to determine acid detergent fibre (ADF). The ADF determination measures lignin and hemicellulose. Crude protein (CP) content and phosphorus (P) were analysed using the Technicon autoanalyzer 2C. Residual moisture in the stored samples varied from 5.22 to 7.17%. The amount of forage degradeble dry matter and crude protein per hectare were calculated by multiplying the production per hectare by the decimal fraction of degradebility and crude protein respectively.

All trial A herbage samples analysed in the University of Alberta forage laboratory and SAML were used to calibrate a Technicon 300 model B NIR infraalyzer spectrophotometer for each quality constituent assayed. The calibrated NIR Technicon 300 model B was used to determine quality constituents for herbage samples collected from trial A in 1985.

3.1.5.2 Total nonstructural carbohydrates (TNC)

Root material collected in trial A in 1984 was analysed in the University of Alberta forage quality laboratory. Determination of TNC reserves was made using the enzyme digestion techniques outlined by Smith (1981b) for smooth bromegrass and alfalfa. Residues from water extraction were incubated with α-amylase enzyme of *Aspergillus orizael* origin (Miles Laboratories Inc., USA) solution to obtain TNC values. The clarification stage using Lead Nitrate was omitted as it was found to interfere with

subsequent stages of the assay. This has subsequently been confirmed by Khaleeluddin and Bradford (1986). TNC values are expressed on a dry weight basis as percent glucose for alfalfa and percent fructose for bromegrass (Shaeffer and Somogi, 1934). Alfalfa samples were used to calibrate a Technicon infraalyzer 500 near infrared reflectance spectrocomputer and bromegrass samples to calibrate a Technicon 500 with a filter transfer to a Technicon 300 model B infraalyzer. Samples from trial A in 1985 and trial B 1984 were analysed using NIR spectroscopy. The detailed methodology is presented in the following sections.

3.1.6 NIR Instrumentation and calibration

Both herbage and root sample types were divided into three sets; calibration, bias and prediction samples to enable accurate and reliable equation development for the sample type and constituent being measured. Each sample set is a sub-set of the population and therefore is representative of the population. The herbage group contained 42 samples for calibration, 30 for bias and 38 for prediction. The TNC root samples of alfalfa and bromegrass contained 42 samples for calibration, 30 for bias and 45 for prediction.

Two NIR spectrophotometers were used to determine herbage quality and root material. For both instruments all samples were ground according to specifications (Norris *et al.*, 1976; Casler and Shenk, 1985). Each sample was thoroughly mixed and a 2g sub-sample placed in a sample holder. Samples were illuminated with monochromatic light and the diffuse reflectance collected with lead sulphide detectors. The signal was amplified from the detectors, digitized and recorded as log(1/R), where R = reflectance. Wavelength selection, calibration and prediction equations differed for the two instruments.

3.1.6.1 Filter type spectrophotometer

The Technicon infraalyzer 300 model B; containing eight interference filters in fixed positions, was used in this study. A tilting mirror is used to locate individual filters when samples were scanned. These filters covered selected regions of the NIR spectrum

from 540 to 2336nm [4] (Table 3.3). The 300 model B operates in a double-beam mode using an intergrating sphere to collect the reflected energy (from the sample) as well as to provide the dual beam capacity (Appendix 7), where the interior of the sphere is the reference standard.

To calibrate the instrument, readings at multiple wavelengths were taken on a set of samples and their spectral data (collected as log 1/R) was related to laboratory values using a multiple linear stepwise regression. All eight wavelengths were considered for inclusion in the regression equation. The equation with the lowest standard error and highest R^2 for the analysis of acid pepsin dry matter disappearance (APDMD), acid detergent fibre (ADF), crude protein (CP) and phosphorus (P), was used to calibrate the instrument. No mathematical treatment of the spectral data (log 1/R) were performed as (1) satisfactory calibrations were obtained and (2) this option was not available on the 300 model B instrument.

The calibration constants obtained from the regression equation were stored in the instrument and used to determine the composition of unknown samples by solving the following formula:

$$\% \text{ constituent} = F_0 + F_1 \log 1/R_1 + F_2 \log 1/R_2 + \ldots + F \log 1/R$$

where F = constant from regression for filter #k.
log 1/R = logarithm of inverse of reflectance for filter #k.

Sample particle size, cell packing, equipment and environment can cause variations in reflectance. If the infraalyzer is reading higher or lower than samples of known laboratory value, a bias exists. To correct any bias arising in the data from such sources an adjustment was made to the F_0 constant of the regression equation using samples of known laboratory value (not belonging to the calibration or prediction set). Thirty samples were used for bias adjustment of all constituents measured. When corrected for bias, a new

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[4] nm = nanometers (1000nm = 1μm)

Table 3.3 NIR Technicon 300 filter numbers and corresponding wavelengths.

Filter number	Wavelength, nm
1	2336
2	2310
3	2230
4	Blank
5	2180
6	Blank
7	2100
8	1940
9	540
10	1680

prediction equation was obtained. Samples of known chemical value were tested against this equation in order to determine the standard error of prediction (SEP) and squared simple correlation coefficient (r^2).

3.1.6:2 Scanning monochrometer type spectrophotometer

The Technicon infraalyzer 500C consisting of the Technicon infraalyzer 500S scanning monochrometer and Hewlett-Packard 1000 (model 6) computer, was used to determine percent TNC reserves of alfalfa and smooth bromegrass. The Technicon 500S monochrometer scanned the infrared region from 1100-2500nm. The samples were scanned at 2nm intervals allowing 700 wavelengths to be collected over the spectrum. An intergrating sphere with dual lead sulphide detectors was used to collect the diffuse reflectance which enabled wavelength transformation to the 300 model. The computer program packages allowed for data collection, manipulation and analysis (Shenk *et al.*, 1981 presents details on individual programmes).

Calibration equations for analysis of TNC in alfalfa root samples were developed from 42 samples. Each sample had its spectral optical density data collected as log(1/R). Analysis equations were obtained by multiple linear regression via the "best pair option" (the program selected the first equation in a series of potential equations based on two wavelengths out of a possible 700 wavelengths). The mathematical treatment (logl/R) and the number of wavelengths (to a maximum of six) were selected by choosing the equations with the lowest standard error of calibration (SEC) and largest multiple correlation coefficient (R^2).

Equations for the analysis of smooth bromegrass TNC reserves were derived using the mathematical treatment (log 1/R) on a Technicon 500 infraalyzer. As this instrument became unavailable for further use, the equations were transformed using the program "filter transformation" which converted the scanned optical data by adjusting them to fit a filter instrument. For details of the filter transformation program consult Technicon Instruments Corporation, Tarry Town, New York. To verify the filter transformation one

sample was scanned using the 300 model B and a comparison of wavelength optical data (log 1/R) was made to the filter transformed log(1/R) value. No difference was found hence the same procedure for bias and prediction was followed.

3.2 Results

A reflectance spectra, obtained from root material and a herbage mixture of alfalfa and bromegrass (Figure 3.1), showed little similarity other than common absorption bands of water at 1940nm and fibre at 2336nm. A starch-cellulose peak was prominent at 2100nm in alfalfa, but was absent in bromegrass root and mixed herbage samples. As no difference in spectral scans across years (1984 and 1985) and treatments (nitrogen and defoliation) were found (Figure 3.2), the prediction equations developed from samples collected from trial A 1984 were used to assay all herbage and root samples collected from trial B 1984 and trial A and B 1985.

3.2.1 Herbage samples

The calibration, prediction and unknown sample sets from trial A 1984 (Table 3.8) were found to cover essentially the same range for all quality constituents measured. The multiple correlation coefficients of herbage calibration samples were 0.94 or higher for all four assays, with crude protein being the highest (Table 3.9). The number of wavelengths required to develop equations with a high R^2 value varied from two for crude protein (CP), acid detergent fibre (ADF) and phosphorus (P) to three for herbage degradebility (acid pepsin dry matter disappearance, APDMD). The primary wavelengths for predicting APDMD, CP and P were the same but had different partial regression coefficients (B(1) values, Table 3.9). The primary wavelength for ADF determination corresponded to the fibre wavelength of the instrument.

Verification data for the NIR forage quality prediction equations of Table 3.9 is presented in Table 3.8. The squared coefficients of determination (r^2) for prediction and actual quality values were highest for CP and ADF and lowest for P. Standard errors of prediction (SEP) exceeded standard errors of calibration (SEC) by one third, for all quality constituents except ADF and CP. The standard deviation and mean biases for NIR predicted for all constituents were small (Table 3.8). APDMD generated the largest bias between NIR and laboratory values.

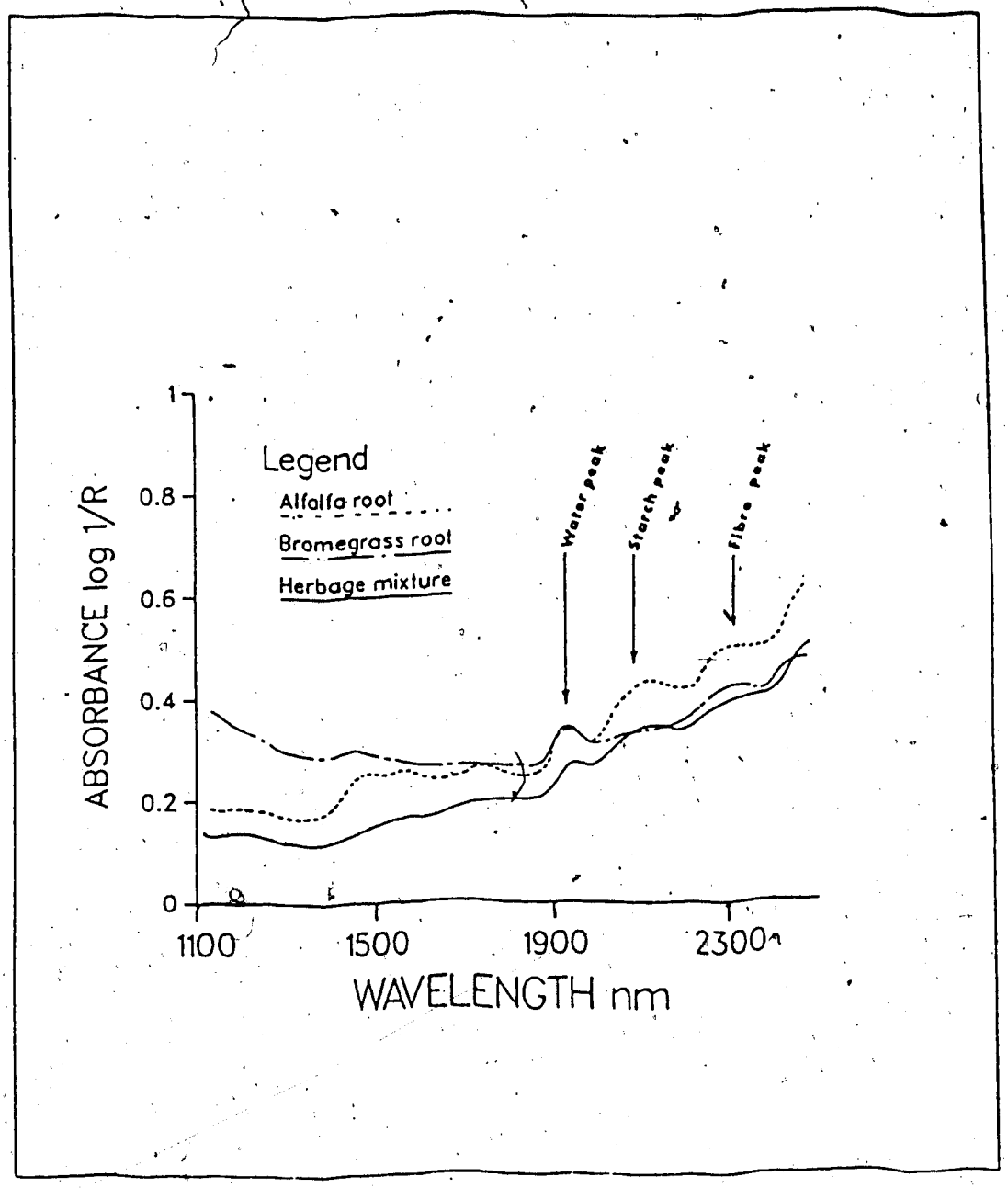


Figure 3.1 Comparison of reflectance spectra for typical alfalfa and smooth bromegrass root material and alfalfa-bromegrass herbage samples. (R = reflectance.)

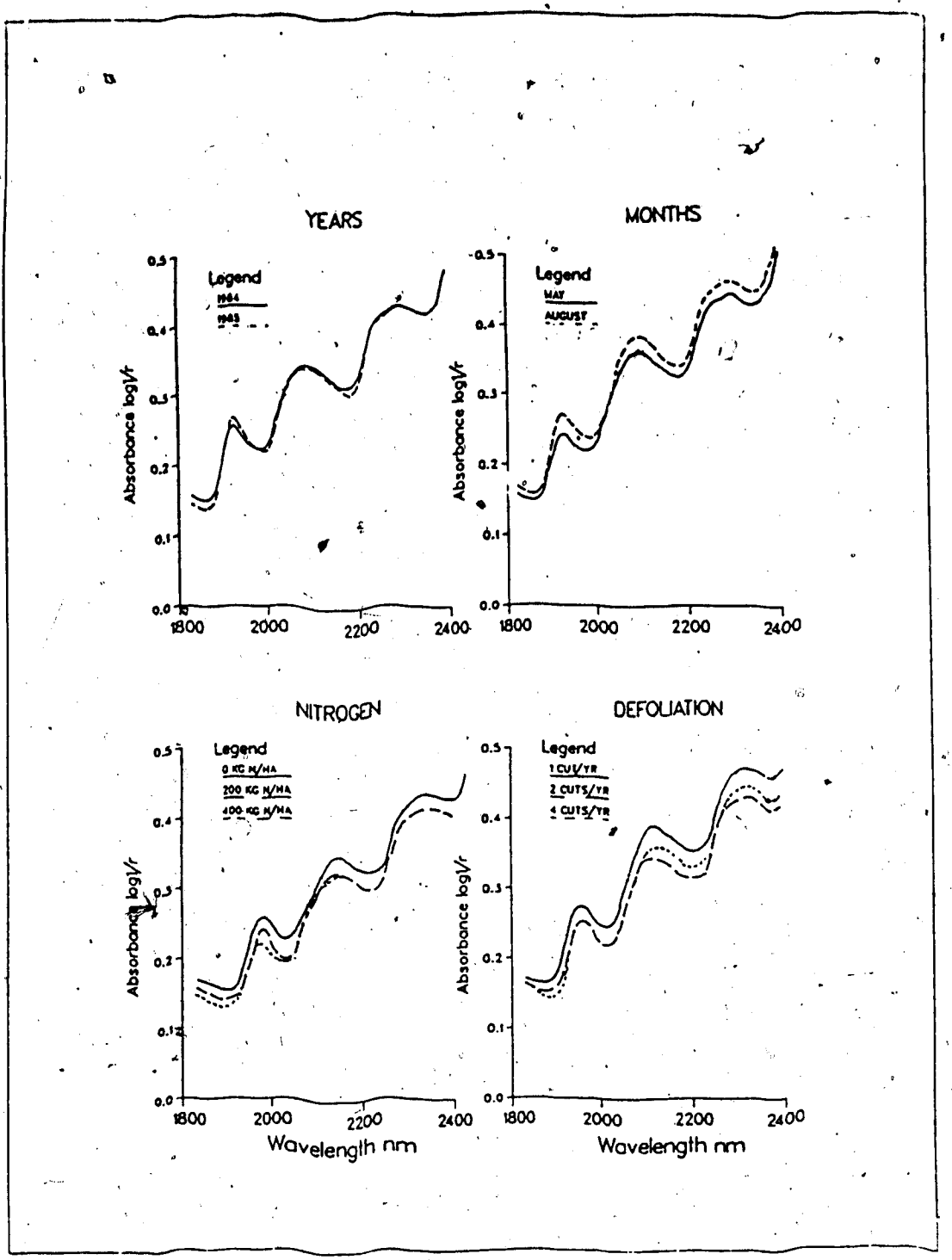


Figure 3.2 Comparison of reflectance spectra for alfalfa samples across years, months, defoliation and nitrogen treatments. (R = reflectance.)

Table 3.4 A comparison of percent composition and degradebility of herbage samples as determined by conventional laboratory methods and NIR Technicon 300.

Forage constituent†	Conventional laboratory		NIR
	Calibration set N=42	Prediction set N=38	Unknown set N=343
APDMD	30.5-58.8	31.3-56.8	24.9-57.8
CP	7.3-27.9	7.5-25.6	6.5-29.0
ADF	16.6-39.3	17.4-37.7	13.0-39.5
P	0.09-0.28	0.09-0.27	0.08-0.31

†APDMD= Acid-pepsin dry matter disappearance; CP = crude protein; ADF = acid detergent fibre; P = phosphorus.

Table 3.5 Correlation data, mathematical treatment and wavelengths needed in equation development for prediction of forage herbage quality using a Technicon 300.

Forage constituent†	Correlation data				Math. treat.Þ	Wavelength,nm þ
	R^2‡	SEC‡‡	x	σ £		
APDMD	0.96	1.84	45.66	9.37	log1/R	2180, 2100, 540
CP	0.98	0.93	17.15	6.20	log1/R	2180, 2100
ADF	0.94	1.73	27.54	7.43	log1/R	2336, 2180
P	0.96	0.01	0.174	0.064	log1/R	2180, 2100
	Prediction equation					
	B(0)	B(1)	B(2)	B(3)		
APDMD	12.06	910.46	-752.28	-44.38		
CP	-3.09	521.00	-488.90			
ADF	65.56	676.70	-942.80			
P	-0.09	5.36	-4.65			

†APDMD= Acid-pepsin dry matter disappearance; CP= crude protein; ADF= acid detergent fibre; P= phosphorus.

‡ Multiple correlation coefficient from the regression of known quality values on NIR values.

‡‡Standard error of calibration (mean square error from regression of known quality values on NIR).

£ Standard deviation of known quality values.

Þ Mathematical treatment for the variable.

þ Wavelengths needed for the best prediction equation.

Table 3.6 Verification data for NIR forage herbage quality prediction equations in Table 3.3 when additional trial A samples were used as test samples.

Forage constituent‡	N	r²Þ	SEPþ	σ†		x̄	
				Lab.	Predicted	Lab.	Predicted
APDMD	38	0.89	2.200	6.11	6.06	45.210	46.670
CP	38	0.94	0.760	9.29	9.03	17.010	17.060
ADF	38	0.94	1.260	7.46	7.36	27.070	27.460
P	38	0.85	0.018	6.43	6.17	0.169	0.173

† Standard deviation of quality values.

‡ APDMD= Acid-pepsin dry matter disappearance; CP= crude protein; ADF= acid detergent fibre; P= phosphorus.

Þ Coefficient of determination of NIR vs. known laboratory values.

þ Standard error of prediction by NIR.

3.2.2 Root samples

Root samples of alfalfa and bromegrass were analysed for total nonstructural carbohydrate (TNC) from trial A and B 1984 and 1985. No differences in the range of chemical composition was evident between the calibration, prediction and unknown sets (Table 3.9). Significant regression equations for root TNC determination were obtained (Table 3.8). The multiple regression coefficients (R^2) of calibration samples were 0.91 and 0.80 for alfalfa and bromegrass, respectively. The number of wavelengths needed to develop equations with the lowest SEC and largest R^2 were three for alfalfa and five for bromegrass. Verification data for the NIR prediction equations are presented in Table 3.9. The squared simple correlation coefficient (r^2) for NIR predicted and known root TNC values of alfalfa and bromegrass were 0.88 and 0.78, respectively. SEP values were lower than SEC values for each species. The standard deviation and mean for NIR assays were similar to laboratory determinations.

Table 3.7 A comparison of total nonstructural carbohydrate (TNC) in the roots of alfalfa and bromegrass as determined by conventional laboratory methods and NIR Technology.†

	Conventional laboratory		NIR
TNC sample	Calibration set N=42	Prediction set N=45	Unknown set N=353
Alfalfa	6.6-28.3	6.9-19.9	6.2-29.08
Bromegrass	8.0-18.0	8.7-21.3	9.2-22.0

†Technicon 500C for Alfalfa; Technicon 300 for Bromegrass.

Table 3.8 Correlation data, mathematical treatment and wavelengths needed in equation development for prediction of percent total nonstructural carbohydrate in the roots of alfalfa and bromegrass using NIR Technology.β

TNC sample	Correlation data				Math. treat.Þ	Wavelength,nm þ
	R^2‡	SEC‡‡	x	σ£		
Alfalfa	0.91	2.53	17.4	7.5	log 1/R	2208, 22224, 1856
Bromegrass	0.80	1.88	15.9	8.3	log 1/R	2336, 2180, 2100, 1940, 1680

	Prediction equation†					
	B(0)††	B(1)	B(2)	B(3)	B(4)	B(5)
Alfalfa	39.44	9497.3	-9376.5	-272.2		
Bromegrass	5.05	-383.5	-516.5	913.3	129.0	-77.5

β Technicon 500C for Alfalfa; Technicon 300 for Bromegrass.
‡ Multiple correlation coefficient from the regression of known TNC values on NIR values.
‡‡Standard error of calibration (mean square error from regression of known TNC values on NIR).
£ Standard deviation of known quality values.
Þ Mathematical treatment for the variable.
þ Wavelengths needed for the best prediction equation.
† Based on known quality samples from laboratory analysis.
†† B(0)= intercept.

Table 3.9 Verification data for NIR 500C and 300 for total nonstructural carbohydrate (TNC) in the roots of alfalfa and bromegrass prediction equations in Table 3.6 when additional trial A samples were used as test samples.

TNC sample	N	r^2Þ	SEPþ	σ†		x̄	
				Lab.	Predicted	Lab.	Predicted
Alfalfa	45	0.88	1.05	6.28	6.01	17.51	18.09
Bromegrass	45	0.78	2.19	7.25	7.20	14.65	14.87

† Standard deviation of quality values.

Þ Coefficient of determination of NIR vs. known laboratory values.

þ Standard error of prediction by NIR.

3.3 Discussion

A preliminary qualitative investigation of spectral scans of the material to be analysed can prove to be a useful aid to quantitative work. For example, samples containing high amounts of starch may require measurement be made at 2100nm and that this term be included in the calibration equation (Whetsel, 1968; Mercier, 1985 personal communication). This was found to be the case where the fibre peak measured at 2336nm (used primarily for grain fibre assay) was selected as the primary wavelength for acid detergent fibre (ADF) determination. An examination of the spectra in Figure 3.1 showed that there were marked differences in absorption for each sample type, therefore, individual calibration equations were necessary. Spectral comparisons of samples from different years and treatments are an important aspect of NIR technology that should not be ignored if accurate analysis is to be obtained (Redshaw, 1985 personal communication).

To verify if the calibration equations developed from trial A 1984 could be successfully used to assay 1985 samples, scans were compared for years, months, nitrogen and defoliation treatments (Figure 3.2). As there were no major differences within the spectral scans between the sample combinations tested, the calibration equations developed for each sample-constituent type from trial A 1984 could be used to predict the same constituents for samples collected in 1985.

3.3.1 Herbage samples

For the quality constituents measured, the range in chemical composition and digestibility values of calibration, prediction and unknown sets were essentially the same (Table 3.8) and were in agreement with the criteria for accurate NIR analysis (Shenk *et al.*, 1978a, 1978b, 1979; Shenk and Westerhaus, 1985). The multiple regression coefficient (R^2) values and standard errors of calibration (SEC) in this study were similar to those reported by Marten *et al.* (1984) and Shenk *et al.* (1981, 1985). Consistent with other research findings of Barton *et al.* (1976) using NIR for in vitro dry matter disappearance (IVDMD), acid pepsin dry matter

disappearance (APDMD) required more wavelengths for equation development than crude protein (CP), acid detergent fibre (ADF) and phosphorus (P). The additional wavelengths required for calibration of APDMD was expected as digestibility is a complex determination in terms of chemical analysis.

The squared simple correlation coefficient (r^2) of quality constituents was equivalent to or higher than those reported by Burdick *et al.* (1981) and Templeton *et al.* (1983). An r^2 of 0.89 between NIR and laboratory analysis was obtained for APDMD. This was lower than commonly reported for IVDMD (Marten *et al.*, 1983a, 1983b) and can possibly be explained by the lower precision associated with APDMD determination due to differences in chemical analysis. The major differences between the IVDMD method (Terry and Tilley, 1963) and APDMD are as follows; the use of rumen liquor containing numerous enzymes with a subsequent digestion by acid pepsin in the former compared to acid pepsin alone in the latter. A consequence of the greater number of enzymes in rumen liquor digestion is an increased breakdown of the forage before protein degradation occurs by the acid pepsin. Hence the lower precision in the APDMD determination. The larger difference between the laboratory and NIR mean values for prediction after bias adjustment (Table 3.8) may be explained by the variable amounts of nitrogen chemically bound to lignin that are totally indigestible that would be included in the spectral data. The SEP for APDMD and P exceeded the SEC by one third or more which was in agreement with reports on fixed filter instruments (Shenk *et al.*, 1981). As previously stated the high value for SEP of APDMD assay may be in part due to the inherent inaccuracies of its determination.

Minerals are not measured directly by NIR but by their association with other plant components. Therefore, the phosphorus component association was not be measured as accurately as other quality determinants.

3.3.2 Root total nonstructural carbohydrate

Literature on the determination of organic compounds in particular root total nonstructural carbohydrate (TNC) in forage species using NIR spectroscopy is limited (Marten *et al.*, 1983a; Brink and Marten, 1986). One of the major constraints stated by Shenk *et al.* (1978a) for accurate NIR analysis is that the chemical composition of the calibration and prediction sets be congruent. This constraint was adhered to in this study (Table 3.9).

The procedure of Smith (1981b) for the determination of TNC is costly, complex and difficult to standardize with the method for root TNC assay of bromegrass being more difficult than alfalfa. This is due to the additional analytical steps required for the breakdown and release of fructosans into solution to obtain TNC concentration. This was reflected in the larger standard error of laboratory (SEL) assay of 1.45 for bromegrass as compared to 1.30 for alfalfa. Three wavelengths were required to produce the best equation for analysis of root TNC of alfalfa for the mathematical treatment log(1/R). The R^2 values and standard error are inter-related. An increased R^2 correlates to a lower standard error. The squared coefficients of multiple determination (R^2) of known TNC values on NIR values was 0.91. The standard errors of calibration (SEC) were higher than those reported by Brink and Marten (1986).

The NIR standard error of prediction (SEP) was substantially lower at 1.05 for alfalfa, but higher at 2.19 for bromegrass (Table 3.9). The coefficient of determination ($r^2=0.88$) for alfalfa root TNC and SEP (1.05) was lower than reported in the literature (Brink and Marten, 1986). The standard error of laboratory (SEL) obtained by using the method of Smith (1981b) was higher than that reported by Brink and Marten (1986) who used a modified version of Smith (1969b). This may be a reflection of the lowered precision associated with the 1981b method due to the modifications made by Smith to the 1969 method. These modifications entailed the reduction of analysis time by approximately 48 hours. This was achieved by (1) a reduction in digestion time from 44 hours (1969b) to 24 hours (1981b) and (2) the removal of the deleading stage after the clarification of the sugar extract. Khaleeluddin and Bradford (1980) have shown that deleading with lead nitrate as Smith recommends in the 1969 method interferes with the copper-iodimetric titration. Whether the reason for the removal of the

deleading stage in Smith's 1981 method is the same as Khaleeluddin and Bradford is not known.

Shenk *et al.* (1985), working on quality constituents used by the forage and feed industry, have studied the limitations of NIR utilization due to the requirement that each instrument be individually calibrated. The proposed solution was the transfer of NIR calibration equations by computer from one instrument to another. As the author was no longer able to use the Technicon 500 instrument to determine root TNC of bromegrass a filter transformation of the calibration equation was made to the Technicon 300 model B at the University of Alberta, Parkland Farm. A filter transformation was possible because both instruments operate in a double beam mode using an intergrating sphere to collect the reflected light.

The low squared simple correlation coefficient ($r^2=0.78$) and large SEP (2.19) for bromegrass root TNC assay can be attributed to three causes. (1) The drastic reduction in the number of wavelengths available for calibration (from 700 to 8) due to the filter transformation from the scanning monochrometer to the fixed filter instrument. This reduced the R^2 from 0.93 to 0.78 for the monochrometer and filter type instruments, respectively. (2) The fixed filter instrument used is primarily for cereal grain analysis of fibre, protein and moisture. (3) The inherent inaccuracies in the chemical procedure of Smith (1981b) for the determination of fructosans. No comparisons of the data obtained in this study can be made for the bromegrass root TNC, filter transformation or equation development, as there is no published literature on this species available.

An important part in interpreting changes in pasture and range condition, is the determination of botanical composition. Hand separation has been found to be the most reliable method in determining botanical composition. Samples should be randomly taken and representative of the pasture or range. However this has proven costly and laborious. Coleman *et al.* (1985) used NIR to predict various plant species using artificial mixtures and commercial hay samples. Results indicated NIR was more accurate in the prediction of species in the artificial mixtures than in commercial hays. The difficulty in prediction of certain species was

related to (1) interference between species, (2) sampling error and (3) methodology error.

The herbage samples used for the quality assays in this study were also used to test NIR prediction of alfalfa and bromegrass percentage composition. The percentage grass and legume for each sample was related to the spectral data from the Technicon 300 model B using the same procedures outlined previously. Calibration equations with coefficients of determination (R^2) of 0.32 for alfalfa and 0.10 for bromegrass were obtained. Analysis was not taken any further due to the extremely low R^2 values. These findings may be the result of three factors; (1) interference from species other than those being predicted ie. weeds, (2) the small sample size (N=42) used for the cailbration and (3) the use of a fixed filter instrument designed for the assay of moisture, protein and oil in cereals ie. the lack of specificity of the NIR spectrum to species composition).

3.4 Summary and Conclusion

(1)NIR technology is an analytical method that is inexpensive and rapid for the assay of numerous agricultural commodities,

(2) The fixed filter NIR instrument used in this study provided an accurate and rapid analytical method for the measurement of forage quality (measured as acid pepsin dry matter disappearance, protein, fibre and phosphorus). The limitations of this instrument became evident when root TNC of bromegrass was determined.

(3)The spectro-computer used in this research demonstrated that NIR analysis was better for repeatability than conventional laboratory analysis when used to assay root TNC of alfalfa.

4. PART II: EFFECTS OF SUMMER DEFOLIATION AND APPLIED NITROGEN ON FORAGE PRODUCTION

4.1 Materials and methods

4.1.1 Field trial location, climate and design

Field trial location, soil type, climatological information for the region and site history are presented in Part I (section 3.1.1, page XXX). For further detailes on the experimental design and treatments used in trial A consult Part I , page 16.

4.1.2 Field observations

In trial A, dry matter yields were recorded at each harvest in 1984 and 1985 (Appendix 5). Sub-plots measured 8m x 2.5m. The forage was harvested with a modified Mott harvester by clipping an area 4.2m² (6.88m x 0.61m) per sub plot. The clipping height was 4cm and dry matter yield was determined by drying all the forage harvested at 50°C to a constant weight. A sub-sample of approximately 300g dry weight was collected for forage quality analysis in August 1984 and for all harvests in 1985.

Herbage samples were collected from each sub-plot at all harvests in 1984 and 1985. At the first harvest in 1984 a single herbage sample using a rectangular quadrat (1m x 0.25m) was collected for the determination of botanical composition (Kershaw, 1964). However, uneven and sparse regrowth at subsequent harvests made it desirable to double the sample area by harvesting two 1m x 0.25m quadrates per sub-plot. Samples were stored in a freezer at -10°C until hand separations could be made to determine the percentage by weight of alfalfa, smooth bromegrass and other herbage.

The mean height of the sward was determined to the nearest centimetre at each harvest date in both years. Three measurements per sub-plot were recorded and the mean value calculated. Alfalfa density was recorded at each harvest in 1985. To quantify the observation

alfalfa density was recorded in 1985. The number of alfalfa plants in two 1m x 1m quadrats were counted per sub-plot.

4.1.3 Field sampling of root material

Root samples of alfalfa and bromegrass were collected at each harvest in 1984 and 1985 in trial A (Appendix 5) to study seasonal variation in total nonstructural carbohydrates (TNC) concentration levels. The procedure used for sampling and processing alfalfa and bromegrass roots prior to grinding and TNC analyses are presented in detail in Part I (section 3.1.4, page 19).

4.1.4 Chemical analysis

Herbage and root material were used in separate analyses. Prior to analysing herbage and root samples using NIR spectroscopy, a sub-set of each were analysed by conventional laboratory procedures as outlined in Part I (section 3.1.5, page 19). The Technicon 300 model B (containing eight interference filters in fixed positions) was calibrated and subsequently used to assay all remaining of herbage samples. The Technicon 500S was used to estimate root total nonstructural carbohydrate in alfalfa. A calibration equation to assay percent TNC of smooth bromegrass was also developed on the 500C with a filter transformation to the Technicon 300 model B. Further information is presented in Part I (section 3.1.5, page 20).

4.1.5 Data analysis

Trial A was a split plot design. Analysis of variance tests were performed to determine if there were significant effects of nitrogen, defoliation and year on all parameters collected. Sample means were tested for differences using Duncan's New Multiple Range test at $p=0.05$ (Steel and Torrie, 1980). Model predictions for total dry matter yield were generated from a stepwise multiple regression equation (Steel and Torrie, 1980).

Correlations between dry matter yield and root TNC levels of alfalfa and bromegrass were determined for data collected in 1984, 1985 and a combined analysis. These data included all treatment combinations (ie. nitrogen by defoliation) with samples collected periodically (Appendix 5) from May to August. As yield data was collected from ten replicates and TNC reserves from two replicates, mean values were used for correlation analyses.

4.2 Results

This section examines the effects of nitrogen, defoliation, fallcutting and their interactions for the parameters recorded under three sub-sections. Results are set out for field data, herbage quality and root TNC reserves. The first table in each sub-section lists the parameters recorded; each parameter is taken through the statistical analyses used, once completed the next parameter follows in the same pattern. This format is retained for the remaining sub-sections.

4.2.1 Field data

The nitrogen and defoliation treatments, plus their interaction showed significant differences at $p=0.001$ for legume and grass dry matter yield. and The nitrogen x defoliation intreaction for total production (Table 4.1) was significant at $p=0.05$. Differences between years for all dry matter yields except total herbage production were also significant. The interactions; year by nitrogen, year by defoliation and year by nitrogen by defoliation showed significances at various levels of probability for total, legume and grass dry matter production. No significant difference for the legume dry matter interaction year by nitrogen was recorded. An analysis of variance for 1984 and 1985 (Table 4.2) showed significant F values for total dry matter production, legume dry matter and grass dry matter production for 1984 and 1985 nitrogen fertilizer treatments, with the exception of legume dry matter in 1984. Cuts per year and the interaction (nitrogen by defoliation) showed significance at various levels of probability for all yield variables in both years.

Main treatment effects differed significantly among total dry matter, legume dry matter and grass dry matter production in 1984 and 1985 (Table 4.3). An increase in nitrogenous fertilizer application usually resulted in an increase in total dry matter yield of the sward (Figure 4.1). In 1985 sward production was increased by the three levels of nitrogen fertilizer applications, whereas nitrogen application from 200 to 400Kg/ha did not significantly affect yield in 1984 (Table 4.3). Defoliation frequency resulted in the same trend in both years,

Table 4.1 Mean square values from a combined (1984 and 1985) analysis of variance for total, legume and grass dry-matter yields, percent legume and grass, growth stages of the grass and legume, and mean sward height in trial A.

		Dry matter (Mg/plot)			Percent (%)		Growth stage♭		Height
Source	df	Total	Legume	Grass	Legume	Grass	Legume	Grass	(cm)
Replication(R)	9	12.23	334	21	1327	124	0.03	0.02	53
Nitrogen(N)	2	1311.80 ***	5701 ***	1284 ***	0.44 ***	4537 ***	0.28 **	0.12 **	761 **
Error a†	18	11.40	54	8	2.59	5.7	0.02	0.01	24
Defoliation(D)	2	1843.70 ***	5566 ***	8464 ***	694.82 ***	2378 ***	296.82 ***	239.88 ***	32712 ***
ND	4	19.78 *	317 ***	37 ***	30.54 ***	86.66 *	0.19 ***	0.06 *	21
Error b	54	56.70	63	8	4.30	2.87	0.02	0.01	20
Years(Y)	1	11.92	23311 ***	562 ***	783.55 ***	758.99 ***	19.20 ***	11.30 ***	358 ***
YN	2	155.40 ***	140	81 ***	14.40 *	2.88	0.09 *	0.00	6
YD	2	20.71 **	1192 ***	98 ***	36.17 ***	98.52 ***	6.85 ***	2.98 **	1188
YND	4	34.47 ***	171 **	18 **	15.90 ***	26.97 **	0.04	0.01	38
Error c	81	9.52	100	4	5.14	3.79	0.03	0.01	38

*,**,*** Significant at 0.05, 0.01 and 0.001, respectively.

† Error a=RxN; Error b=DR/N; Error c=YR/N

♭ Scored 1= vegetative, 6= seed set (Table 3.2).

Table 4.2 Mean square values from analysis of variance by year for total, legume and grass dry-matter yields, percent legume and grass, growth stage of the grass and legume, mean sward height and alfalfa density in trial A.

			Dry matter (Mg/plot)			Percent (%)		Growth stage‡		Height	Alfalfa
Year	Source	df	Total	Legume	Grass	Legume	Grass	Grass	Legume	(cm)	density‡
1984	Replication(R)	9	15.2	12.7	0.027	260	37	0.05	0.003	36	
	Nitrogen(N)	2	283.1 ***	9.9	0.354 ***	3582 ***	376 **	0.35	0.072 *	317 **	
	Error a†	18	12.1	4.1	0.010	74	58	0.04	0.006	38	
	Defoliation(D)	2	737.6 ***	480.6 ***	0.748 ***	4699 ***	241 ***	119.84 ***	97.652 ***	11509 ***	
	DN	4	16.6 *	39.0 ***	0.039 **	432	142	0.21 **	0.039 **	37	
	Error b	54	6.0	4.8	0.009	6	37	0.05	0.008	26	
1985	R	9	5.4	4.5	7.076	147	194	0.60	0.026	48	80.6
	N	2	1184.1 ***	4.9 *	1003.0 ***	2262 ***	2836 ***	0.03	0.58	451 ***	5.5
	Error a	18	3.9	0.9	4.320	43	45	0.00	0.021	6	29.6
	D	2	1126.9 ***	250.3 ***	520.120 ***	2059 ***	2227 ***	183.48 ***	145.40 ***	22391 ***	336.9 ***
	DN	4	37.6 ***	7.4 **	31.177 ***	56	85	0.00 **	0.031	2	48.5
	Error b	54	3.2	1.8	5.596	36	63	0.00	0.027	8	22.1

*,**,*** Significant at 0.05, 0.01 and 0.001, respectively.

†Error a=RxN; Error b=DR/N

‡ Number of plants per $2m^2$.

Table 4.3 Comparison of main treatment effects for two years at three levels of nitrogen and three defoliation frequencies for total dry matter (TDM) yield, legume dry matter (LDM) yield, percent legume (L%) and grass (G%), growth stage of grass (GSG) and legume (GSL), mean sward height (Ht) and alfalfa density (AAD) in trial A.

		TDM		LDM		GDM		L %		G %		GSG†		GSL†		Ht (cm)		AAD‡
Treatment		1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1984	1985	1985
		Mg/ha																
Nitrogen	0	3.45b	2.47c	2.14a	0.91b	1.17b	1.45c	60a	34a	34b	59b	3.7a	3.1a	4.4b	3.9a	42b	44b	10.5a
Kg/ha	200	4.61a	4.73b	2.01a	1.08a	2.17a	3.50b	44b	20b	47a	74a	3.8a	3.2a	4.5a	4.0a	47a	50a	10.5a
	400	4.81a	5.30a	1.97a	1.11a	2.30a	4.07a	39c	20b	48a	70a	3.9a	3.2a	4.5a	4.0a	48a	52a	10.5a
Defoliation	1	4.40b	4.67b	1.37b	0.78b	2.77a	3.48b	34b	20b	63a	79a	6.0a	6.0a	6.0a	6.0a	63a	76a	12.5a
cuts/yr	2	5.41a	5.57a	3.10a	1.79a	2.16a	3.67a	58a	34a	40b	63b	3.4b	2.0b	5.0b	4.3b	50b	49b	10b
	4	3.06c	2.66c	1.55b	0.47c	0.77c	1.86c	51a	20b	25c	67b	2.1c	1.5b	2.5c	1.6c	25c	22c	9b

a-c, Means within a column for each treatment followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range Test.

† Scored, 1=vegetative to 6=seed set (Table 3.2).

‡ Number of plants per m².

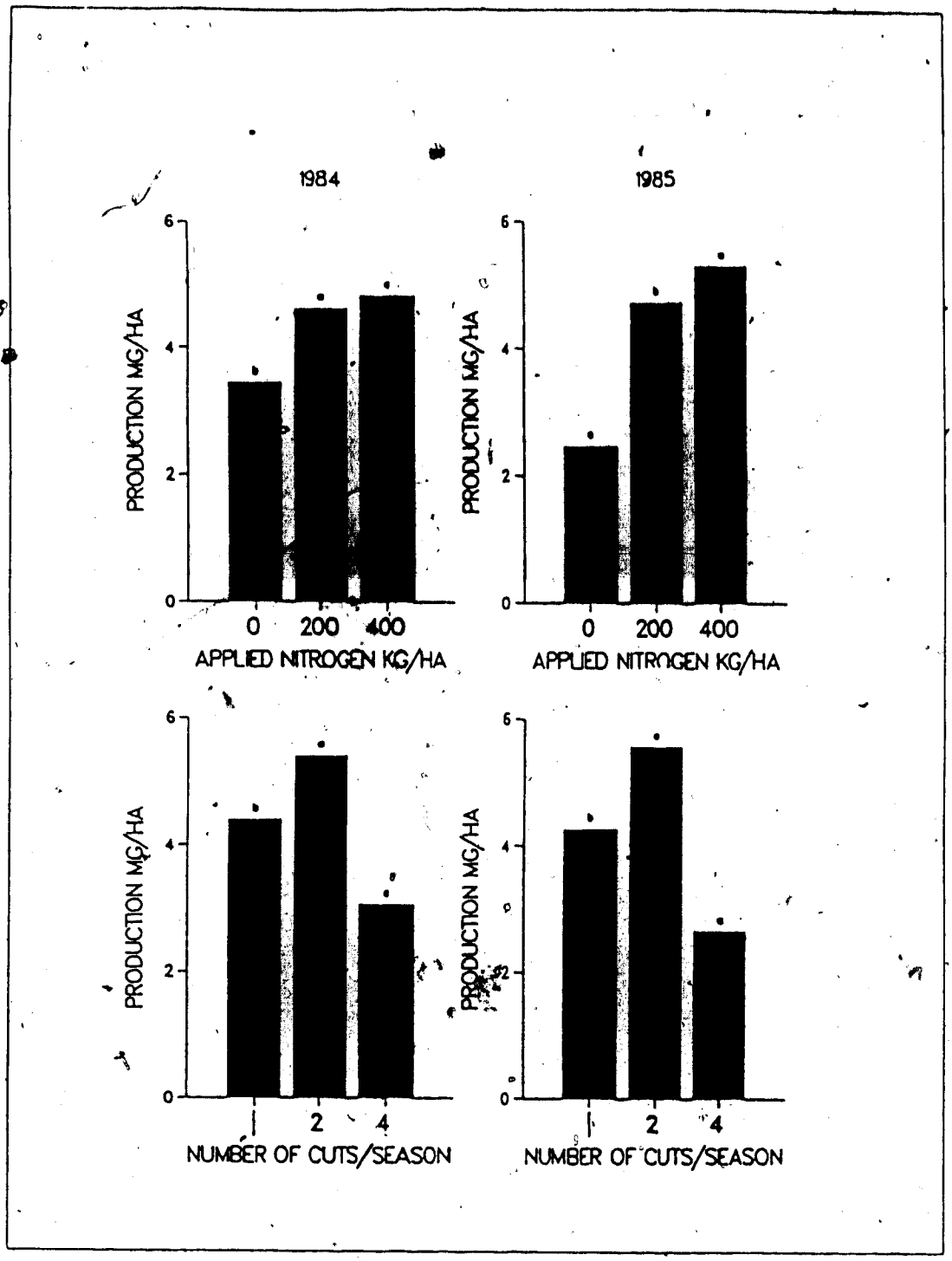


Figure 4.1 Total herbage dry matter production in a grass-legume sward under three nitrogen and three defoliation regimes in 1984 and 1985 in trial A. (Means within each treatment x year histogram followed by the same letter are not significantly different at p=0.05 according to Duncans Multiple Range Test.)

where significant total dry matter yield differences occurred among all treatments (Figure 4.1).

Total herbage dry matter yield differences for nitrogen levels within cuts were more pronounced in 1985 and in the 1984-85 total than 1984 (Table 4.4). Nitrogen application increased yield under all defoliation treatments with the exception of the 1 cut treatment in 1984. Yield in the second year was reduced for all defoliation treatments under zero nitrogen fertilization. In general, production was increased in 1985 compared to 1984 for each harvest regime when nitrogen was applied (Table 4.4). In 1984 nitrogenous fertilizer did not affect alfalfa dry matter yield (Figure 4.2). In the second year (1985) both nitrogen applications increased production over the control treatment. No difference in yield trends were found between 1984 and 1985 defoliation schedules. In contrast alfalfa dry matter yield in 1985 was affected by all defoliation regimes, with the least yield under the four cut system. Nitrogen application increased grass dry matter production in 1984 and 1985 (Figure 4.3). Substantial yield increases were recorded for the 200 and 400 Kg/ha nitrogen treatments in 1984 and 1985 compared to the control, but no significant difference between the 200 and 400 KgN/ha.

Significant differences for nitrogen, defoliation and their interaction were revealed in an analysis of variance of percentage legume and grass in the sward (Table 4.1). There were significant differences for year, year by nitrogen (except for percent grass), year by defoliation and year by defoliation by nitrogen. The analysis of variance for 1984 and 1985 revealed that the percent composition of the sward was significantly affected in both years for both species by nitrogen and defoliation treatments (Table 4.2). A comparison of main treatment effects (nitrogen and defoliation) showed that changes in the percent composition by weight in 1984 and 1985 were different (Table 4.3). Changes in legume and grass percent composition of the sward were greater in 1984 than 1985. Legume percent was reduced by nitrogen application and frequent defoliation in 1985, the reverse pattern was found for 1984. For both years the percentage grass in the sward increased with increased nitrogen fertilization and decreased with increased defoliation.

Table 4.4 Total herbage dry matter yields for an alfalfa-bromegrass sward harvested as one, two and four cuts per year and three levels of nitrogen in trial A.

Harvest type	N level Kg/ha	Dry matter (Mg/ha)		
		1984	1985	Combined
1 cut/yr	0	3.6a	2.8b	3.2b
	200	4.8a	5.0a	4.9a
	400	4.8a	4.9a	4.9a
2 cuts/yr	0	4.9b	3.4c	4.2c
	200	5.4a	6.1b	5.8b
	400	5.9a	7.2a	6.6a
4 cuts/yr	0	1.9b	1.1c	1.5c
	200	3.5a	3.1b	3.3b
	400	3.7a	3.7a	3.7a

a-c, Means within a column followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multilpe Range test.

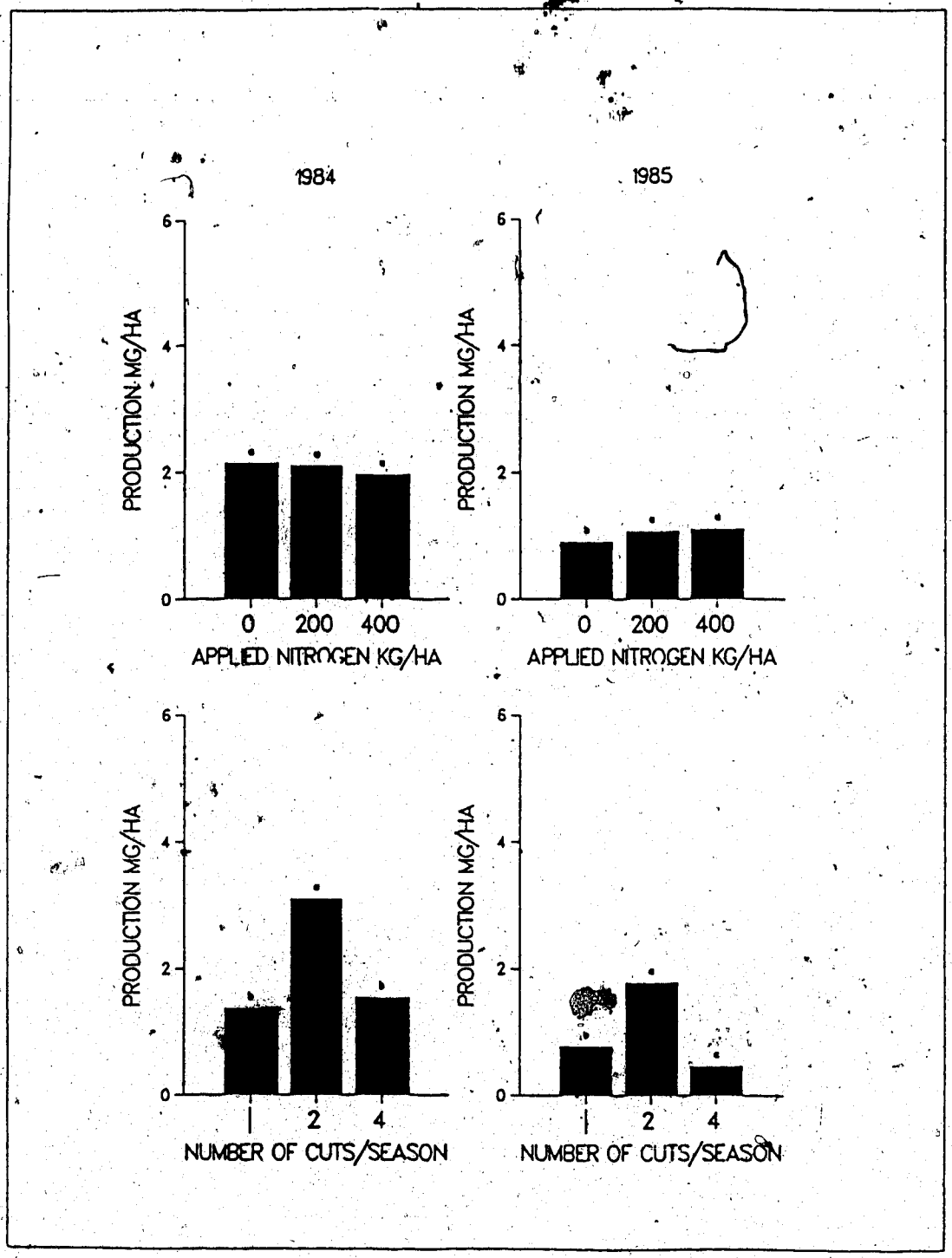


Figure 4.2 Alfalfa dry matter production in a grass-legume sward under three nitrogen and three defoliation regimes in 1984 and 1985 in trial A. (Means within each treatment x year histogram followed by the same letter are not significantly different at p=0.05 according to Duncans Multiple Range Test.)

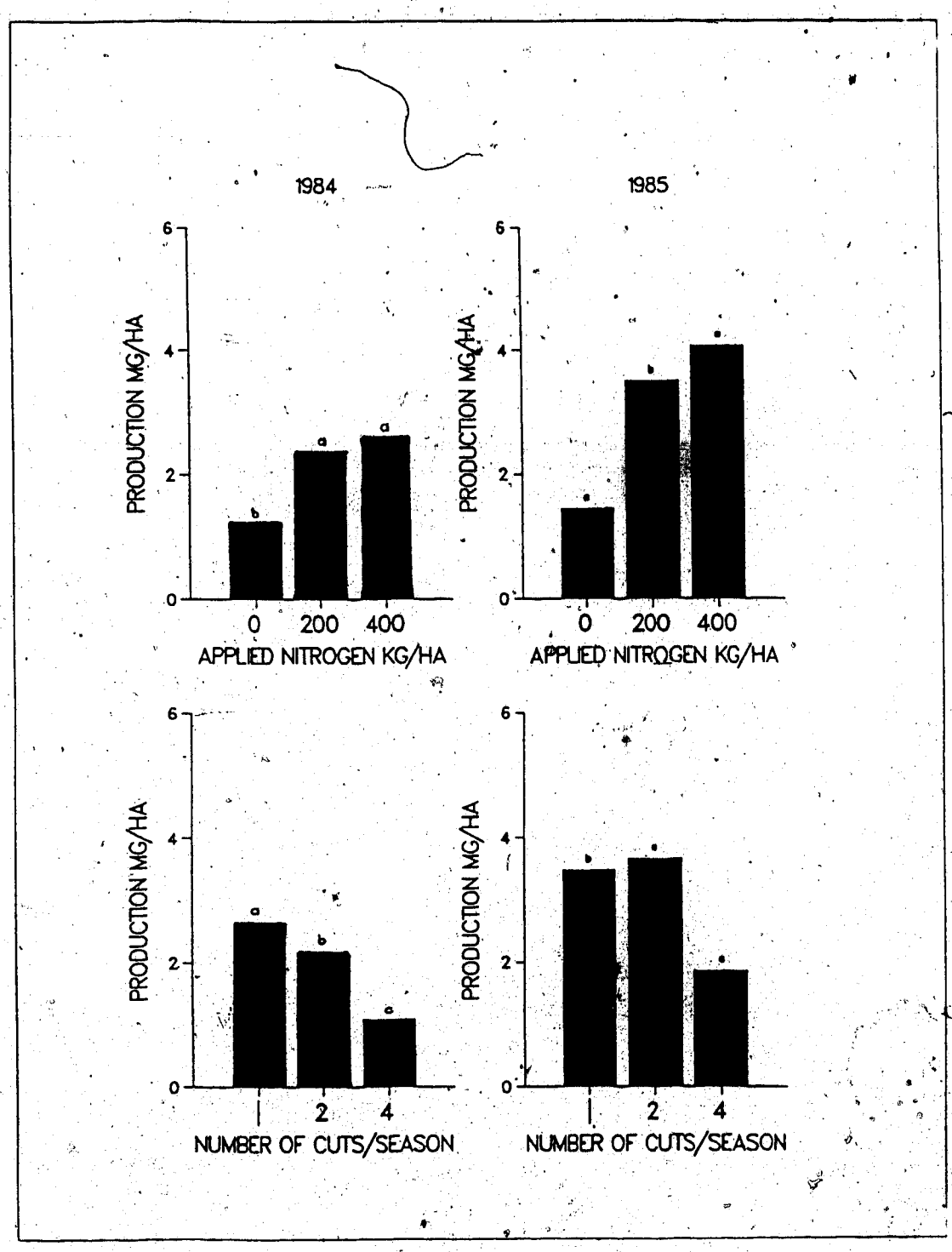


Figure 4.3 Bromegrass dry matter production in a grass-legume sward under three nitrogen and three defoliation regimes in 1984 and 1985 in trial A. (Means within each treatment x year histogram followed by the same letter are not significantly different at p=0.05 according to Duncans Multiple Range Test.)

The growth stage of the grass (GSG) and legume (GSL) was significantly affected by nitrogen, defoliation, their interaction, years and year interactions as shown in the 1984-85 analysis of variance (Table 4.1). An analysis by individual years showed significant values for defoliation in both years although nitrogen was only significant for GSL in 1984 (Table 4.2). The interactions of nitrogen by defoliation for GSG and GSL in 1984 and 1985 were highly significant ($p=0.01$ or higher) with the exception of GSL in 1985. Main treatment comparisons for 1984 and 1985 are set out in Table 4.4. In 1984 under dry conditions (Appendix 3) applied nitrogen increased plant maturity and mean sward height. However, under the moist conditions of 1985 no differences between nitrogen treatments occurred for maturation but were present for plant height. As the number of defoliations increased the growth stage of the legume and grass decreased. Significant differences between defoliation treatments for GSG and GSL were the same in both years.

An analysis of variance of the mean sward height for 1984-85 showed significant differences for nitrogen, defoliation and years (Table 4.1). The analysis of variance for height revealed significant differences for the nitrogen and defoliation treatments in both years (Table 4.2). In 1984 and 1985 the application of nitrogen (at either 200 or 400Kg N/ha) increased the mean sward height compared to the zero treatment (Table 4.3) The more frequent the defoliation the shorter the sward. An increase in the number of defoliations from 1 to 4 per year reduced the alfalfa plant density, however, there was no difference between the 2 and 4 cut systems.

4.2.2 Models for yield prediction.

A simple yield prediction model was developed to simulate the dry matter production by a grass-legume sward under various management regimes. Multiple regression correlations relating plant height, growth stage of the grass and legume to dry matter yield were performed in 1984, 1985 and a combined analysis. The principles of the models are similar to that of Hussey *et al.* (1985) but the model contained an additional variable to account for the

phenology of the grass and legume component of the sward. Significant correlations were found for height and growth stage of the grass for estimating dry matter yield in 1984, 1985 and in the combined analysis (Table 4.5). Mean sward height and growth stage of the grass accounted for 72-78% of the variation in yield of the brome-alfalfa sward during the study. Including the growth stage as a variable with height in the prediction model, increased the percentage of variation in yield accounted for from 88 to 91% in 1984, 92 to 94% in 1985 and from 83 to 90% for the combined 1984-85 analysis.

4.2.3 Herbage quality

The mean square values and significance levels of acid pepsin dry matter disappearance (APDMD) for herbage material harvested in August, 1984 and 1985 are presented in Table 4.6. Additional soil nitrogen did not affect APDMD whereas differences were observed for defoliation treatments in both years. The interaction of nitrogen by defoliation was highly significant in 1985. A closer scrutiny of the main defoliation treatment effects (Table 4.7) revealed the same trend in 1984 and 1985. As the number of harvests increased there was an increase in APDMD. Nitrogen treatments produced no significant changes in APDMD in 1984 (Table 4.6) or 1985 (Table 4.8). Both years received below average rainfall during the growing season (Appendix 3).

An analysis of variance for crude protein (CP) content of the sward in August, 1984 and 1985 showed nitrogen and defoliation treatments were significantly different except for nitrogen in 1984 (Table 4.6). The interaction of nitrogen by defoliation was significant for 1985 only. A comparison of treatment main effects for defoliation on the CP content of the herbage (Table 4.7) indicated the less frequent the defoliation the lower the CP content. The same trend in percent CP was found in both years with the highest protein under the 4 cut and lowest under the 1 cut system. Nitrogen treatments produced no differences in 1984 whereas in 1985 significant differences ($p=0.05$) were present for all treatments (Table 4.8). As the nitrogen application rate increased the CP content also increased.

Table 4.5 Regression equations illustrating the relationship between total dry matter yield (Y), mean sward height and the growth stage of the grass in trial A.

Year	Regression equation
1984	$Y = -467.01 + 23.34X_1 + 122.59X_2$ $R^2 = 0.825$ * (n=208) Standard error of estimate of Y = + 1.36
1985	$Y = -366.56 + 34.61X_1 - 133.87X_2$ $R^2 = 0.883$ * (n=207) Standard error of estimate of Y = + 1.22
Combined	$Y = -406.59 + 29.99X_1 + 19.19X_2$ $R^2 = 0.818$ * (n=415) Standard error of estimate of Y = + 0.72

X_1 = Mean sward height.
X_2 = Growth stage of the grass.
* Significant at 0.05 level of probability.

Table 4.6 Mean square values from analysis of variance for August 1984 and 1985 for acid pepsin dry matter disappearance (APDMD), crude protein (CP), acid detergent fibre (ADF) and phosphorus (P) in trial A.

		APDMD (%)		CP (%)		ADF (%)		P (%)	
Source	df	1984	1985	1984	1985	1984	1985	1984	1985
Replication(R)	10	23.1	0.3	7.4	0.1	0.8	1.2	6.2	0.3
Nitrogen(N)	2	2.7	3.3	26.5	45.3***	3.6	16.9***	21.8	49.4**
Error a†	4	7.2	2.2	11.7	0.6	3.7	0.2	9.4	1.0
Defoliation(D)	2	1116.6***	1744.7***	366.6***	739.6***	752.6***	1149.0***	377.5***	795.2***
DN	4	1.4	16.9***	5.1	3.1**	1.8	5.7**	6.9	3.1**
Error b	12	2.8	1.4	3.9	0.4	2.4	0.7	7.2	0.6

,* Significant at 0.01 and 0.001, respectively.
†Error a=RxN; Error b=DR/N

Table 4.7 A comparison of treatment main effects of three defoliation treatments on herbage quality measured as percent acid pepsin dry matter disappearance (APDMD), crude protein (CP), acid detergent fibre (ADF) and phosphorus (P) in August 1984 and 1985 in trial A.

Date	Treatment	APDMD	CP	ADF	P
		%			
August	1	33.6c	10.5c	36.6a	0.106c
1984	2	47.5b	16.3b	28.1b	0.159b
	4	55.6a	23.3a	18.3c	0.234a
August	1	34.2c	11.1c	32.5a	0.125c
1985	2	46.3b	19.3b	23.7b	0.209b
	4	49.2a	23.2a	19.8c	0.250a

a-c, Means within a column followed by the same letter are not significantly different at the p=0.05 according to Duncan's New Multiple Range test.

Table 4.8 A comparison of main treatment effects of nitrogen application (Kg/ha) on herbage quality as measured by acid pepsin dry matter disappearance (APDMD), crude protein (CP), acid detergent fibre (ADF) and phosphorus (P) in August 1985 trial A.

Treatment	APDMD	CP	ADF	P
		%		
0	42.7a	15.8c	26.6a	0.173b
200	43.4a	18.6b	25.2b	0.203a
400	43.8a	19.2a	24.3c	0.207a

a-c, Means within a column followed by the same letter are not significantly different at $p=0.05$ according to Duncan's New Multiple Range test.

The mean square values and significance levels of acid detergent fibre (ADF) for August, 1984 and 1985 are presented in Table 4.6. As for crude protein, the nitrogen fertilization and defoliation treatments were significantly different ($p=0.001$) in both years with the exception of nitrogen in 1984. The nitrogen by defoliation interaction was significant only in August 1985. The comparison of main treatments for defoliation (Table 4.7) revealed a reverse order of magnitude in ADF content compared to APDMD, protein or phosphorus. The maximum fibre content was obtained under the 1-cut and least under the 4-cut system in both years. Under zero nitrogen (Table 4.8) the fibre content was the highest and application of nitrogenous fertilizer caused a decrease in the acid detergent fibre. It should be noted that if the data presented in Tables 4.7 and 4.9 for forage degradebility (ie. APDMD) and fibre are totaled there is a balance of 20 to 30% not accounted for. The cell wall contents were estimated using ADF which does not measure hemicellulose or pectin and these substances can account for 11 to 50% of the cell wall contents. Similarly APDMD is not an absolute measure of cell contents but is an "indicator" of digestibility. Hence ADF and APDMD combined are not expected to total 100%.

An analysis of variance for herbage material harvested in August 1984 and August 1985 for phosphorus (P) content (Table 4.6) showed the same trend as observed for protein and fibre. The nitrogen treatments produced significant differences in 1985 only. Defoliation regimes produced substantial changes in P content (significant at $p=0.001$) in both years. The interaction of nitrogen by defoliation altered the P content only in 1985. A comparison of the defoliation treatment means showed the highest P content under the 4 cut system and least under the 1 cut system (Table 4.7). Nitrogen application was not significant in August 1984 but resulted in significantly higher herbage P content compared to the zero nitrogen treatment but not between nitrogen applications of 200 and 400Kg/ha in August 1985 (Table 4.8).

Forage degradeble dry matter (DDM) yield in Mg/ha was calculated from total dry matter yield multiplied by the decimal fraction for APDMD (Table 4.9). As previously stated the APDMD values are used as an "indicator" of digestibility (ie. acid pepsin degradebility)

Table 4.9 A comparison of forage degradeble dry matter yield (Mg/ha) under three levels of nitrogen application and three levels of defoliation in trial A in 1985.

Nitrogen Kg/ha	Defoliation treatment (cuts/ year) 1	2	4	Nitrogen mean
0	0.9	1.5	0.5	1.0
200	1.7	2.8	1.5	2.0
400	1.6	3.1	1.9	2.3
Defoliation mean	1.4	2.5	1.2	

hence the true feeding value would be higher than the values in Table 4.9. Nitrogen application also increased the DDM yield. However, the yield response from an application of 200 to 400 Kg N/ha was less than observed for the 0 to 200 Kg N/ha treatments. These results indicate that above 200 Kg N/ha factors other than notrogen are limiting. The nitrogen treatments doubled or quadrupled the DDM yield depending upon the cutting regime. The greatest increase in DDM production by the addition of 0 to 200 Kg N/ha was obtained under the 4 cut regime. Substantial increases were recorded (from 2.8 to 3.4 Mg/ha) for the 2 cut system by applying N above 200 Kg/ha.

The crude protein yield (Table 4.10) increased with the application of nitrogen fertilizer from zero to 400 Kg N/ha. The CP yield was greater for the 2 cut system followed by the 4 and 1 cut regimes. The 2 cut regime yielded about two and three times the CP compared to the 1 and 4 cut systems respectively. Thus if the aim is to manage the sward for CP, the 2 cut system is the most productive harvest regime studied. The responses to nitrogen within each defoliation system were similar for the 2 and 4 cuts as CP increased with each addition of nitrogen fertilizer.

4.2.4 Root total nonstructural carbohydrate

The root total nonstructural carbohydrate (TNC) for alfalfa revealed significant variation by year and by defoliation treatment (Table 4.11). Defoliation was highly significant for alfalfa in 1984 and 1985 (Table 4.12). The effects of defoliation and nitrogen on alfalfa root TNC in 1984 and 1985 are presented in Table 4.13. Nitrogen application generated no differences in alfalfa root TNC in 1984 and 1985, however decreased root TNC levels were observed as the frequency of defoliation increased. The stored reserves of alfalfa for each nitrogen and defoliation treatment were depleted to a greater extent in 1985. As the number of defoliations per year increased root carbohydrate levels decreased in 1984 and 1985. The root TNC of alfalfa revealed significant variation for months, defoliation and their interaction as presented in Table 4.14. The trends in root TNC of alfalfa were similar for the one and two

Table 4.10 A comparison of crude protein yield (Mg/ha) under [illegible]e levels of nitrogen application and three levels of defoliation in trial A in 1985.

Nitrogen Kg/ha	Defoliation treatment (cuts/year) 1	2	4	Nitrogen mean
0	0.2	0.5	0.2	0.3
200	0.6	1.1	0.7	0.8
400	0.5	1.5	0.9	1.0
Defoliation mean	0.4	1.0	0.6	

Table 4.11 Mean square values from a combined 1984, 1985 analysis of variance for percent total nonstructural carbohydrate reserves of alfalfa and smooth bromegrass in trial A.

Source	df	Alfalfa	Bromegrass
Replication(R)	2	5.24	14.51
Nitrogen(N)	2	15.16	55.41 **
Error a†	4	1.98	1.20
Defoliation(D)	2	655.76 ***	11.59 *
DN	4	10.68 *	4.13
Error b	12	2.12	2.63
Years(Y)	1	68.23 **	1.50
YN	2	6.30	7.74 *
Error c	6	2.82	6.24
YD	2	0.83	10.44
YDN	4	15.74	0.91
Error d	12	2.16	2.69

*, **, *** Significant at 0.05, 0.01 and 0.001, respectively.
† Error a=RxN; Error b=DR/N; Error c=YR/N; Error d=YRD/N.

Table 4.12 Mean square values from an analysis of variance by year for percent total nonstructural root carbohydrate in alfalfa and smooth bromegrass in trial A.

Source	df	Alfalfa		Bromegrass	
		1984	1985	1984	1985
		x10⁴			
Replication(R)	2	3.18	2.12	29.09	1.36
Nitrogen(N)	2	7.75	3.71	11.44	51.71 ***
Error a†	4	1.30	4.88	2.42	0.18
Defoliation(D)	2	304.99 ***	351.61 ***	21.84 *	0.19
DN	4	7.45 *	18.97 *	1.07	3.97
Error b	18	1.40	2.88	4.26	1.06

*, **, *** Significant at 0.05, 0.01 and 0.001, respectively.
† Error a = RxN; Error b = DR/N

Table 4.13 A comparison of percent TNC reserves at three levels of nitrogen and defoliation for alfalfa and bromegrass roots in 1984 and 1985 trial A.

		Nitrogen			Defoliation	
Year	Treatment	Alfalfa	Bromegrass	Treatment	Alfalfa	Bromegrass
1984	0	18.12a	15.28a	1	25.08a	15.60a
	200	18.68a	13.56a	2	18.14b	13.90b
	400	19.93a	13.16a	4	13.51c	12.49c
1985	0	16.22a	16.23a	1	23.30a	13.78a
	200	17.40a	13.27b	2	15.80b	13.50a
	400	16.37a	11.49c	4	10.89c	13.71a

a-c, Means within a column followed by the same letter are not significantly different at $p=0.05$ according to Duncan's New Multilpe Range test.

Table 4.14 Mean squares from analysis of variance by months for percent total nonstructural carbohydrate in the roots of alfalfa and smooth bromegrass in 1985 in trial A.

Source	df	Alfalfa		Bromegrass	
Replication(R)	2	2.27		3.86	
Nitrogen(N)	2	5.27		121.09	***
Error a†	4	10.99		1.19	
Defoliation(D)	2	312.46	***	9.53	
DN	4	12.17		3.69	
Error b	12	7.55		3.41	
Months(M)	3	305.46	***	175.03	***
MN	6	5.96		6.76	**
Error c	18	6.94		1.09	
DM	6	77.99	***	1.20	
DMN	12	27.31		2.56	
Error d	36	6.31		1.97	

, * Significant at 0.01 and 0.001, respectively.
† Error a=RxN; Error b=DR/N; Error c=MR/N; Error d=MRD/N.

cuts per season for 1984 and 1985 (Figure 4.4) but differed for the four cut system. With the exception of July 1985, alfalfa root carbohydrate levels were not significantly different for the one and two defoliation treatments in both years (Table 4.15). The four cut system had significantly lower root reserves from June to August in both years.

Table 4.11 showed root TNC of bromegrass to have significant differences among the nitrogen and defoliation treatments. The analysis of bromegrass root TNC by individual years showed significant differences for defoliation in 1984 and nitrogen in 1985 (Table 4.12). Differences in bromegrass TNC were found in 1985 between each nitrogen application. The highest level was recorded for the zero KgN/ha treatment and the lowest at 400KgN/ha (Table 4.13). No difference in the TNC reserves were noted [illegible] the defoliation treatments for bromegrass in 1985. The effect of the three defoliation regimes on the trend of bromegrass carbohydrate reserves in 1984 was similar to that observed for alfalfa (Table 4.13), where reserve levels were reduced by frequent defoliation. To examine any differences in root TNC accumulation in bromegrass an analysis of variance by months (Table 4.14) revealed a significant variation in bromegrass root reserves for months, nitrogen and their interaction was recorded. The trend of bromegrass root TNC at each nitrogen levels were different in 1984 and 1985 (Figure 4.5, Table 4.16). A slight increase in bromegrass root reserves were found in 1984, in contrast to a decrease in 1985. The percent TNC of bromegrass was found to be highest for the zero nitrogen treatment through May to August, 1984 and 1985. An increase in nitrogen application from zero to 200Kg/ha reduced root TNC levels substantially (Table 4.16). Differences between the 200 and 400 KgN/ha only occurred during May 1984 and May and August 1985. The standard error of the mean at each harvest increased as the season progressed in 1984 and 1985 (Table 4.16).

4.2.5 Correlation of TNC reserves and yield

Correlations between dry matter yield of alfalfa and its TNC percent on a dry weight basis at the time of harvest were significant (Table 4.17). Correlation coefficients were positive

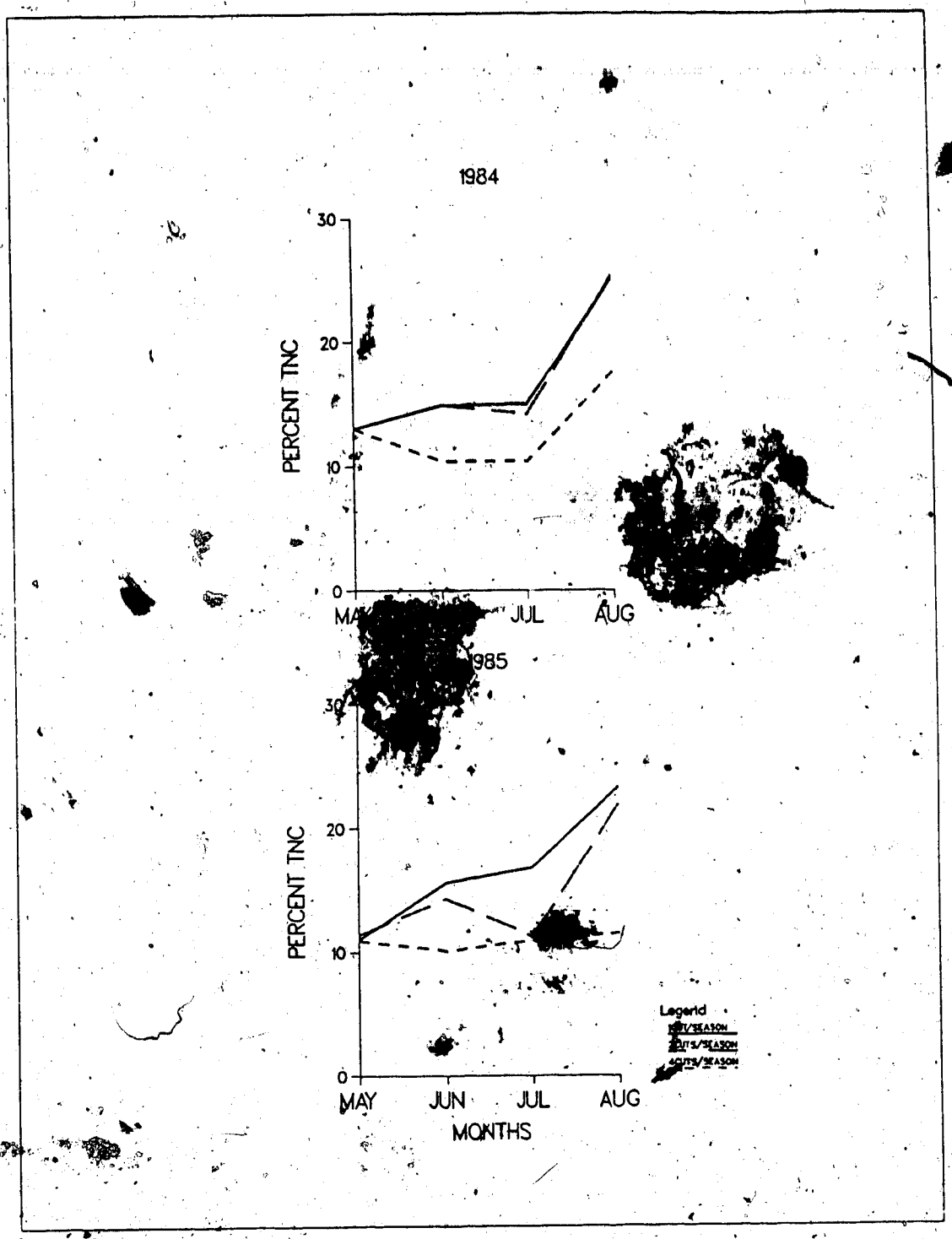


Figure 4.4 Root total nonstructural carbohydrate over the growing season for alfalfa under three defoliation regimes in two years in trial A.

Table 4.15 Total nonstructural carbohydrate reserve percentage (and least significant difference) in alfalfa roots under three defoliation regimes for the growing season† of 1984 and 1985 in trial A.

Year	Month	Number of cuts/year			SE♭
		1	2	4	
1984	May	13.0a	13.0a	13.0a	(-)‡
	June	14.9a	14.9a	10.4b	(1.3)
	July	15.0	14.2a	10.4b	(2.7)
	August	25.1a	25.4a	17.6b	(3.1)
1985	May	11.0a	11.4a	10.9a	(0.2)
	June	15.6a	14.3a	10.1b	(2.0)
	July	16.8a	11.2b	10.9b	(2.4)
	August	23.3a	21.9a	11.5b	(4.5)

† Growing season = May to August.
♭ Standard error of the mean.
‡ (-) Could not be calculated as only one defoliation regime was sampled.
a-b, Means within a row followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

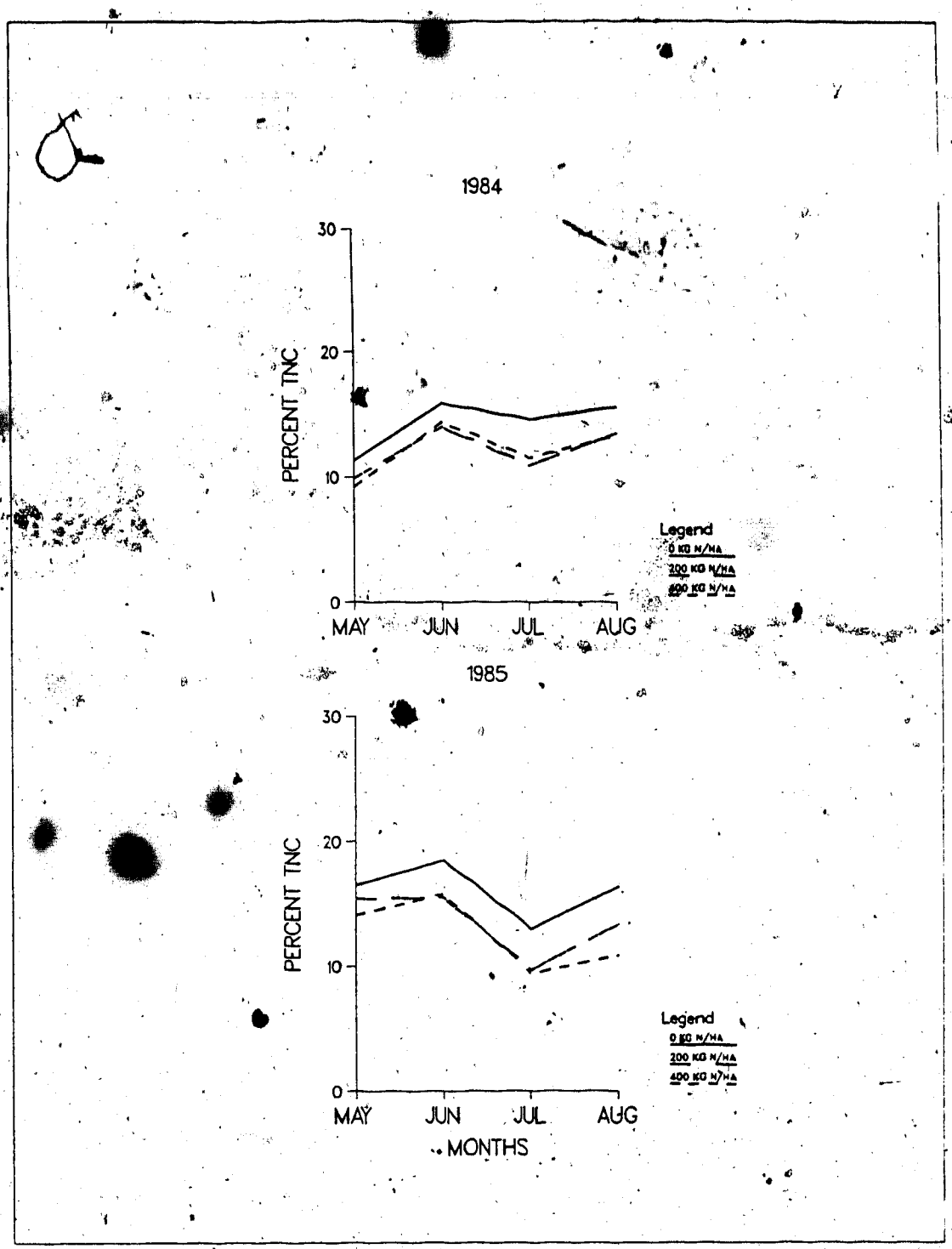


Figure 4.5 Root total nonstructural carbohydrate over the growing season for smooth bromegrass under three nitrogen regimes in two years in trial A.

Table 4.16 Total nonstructural carbohydrate reserve percentage (and least significant difference) in smooth bromegrass roots for the growing season† of 1984 and 1985 under three nitrogen regimes in trial A.

Year	Month	Nitrogen (Kg/ha/year) 0	200	400	SE‡
1984	May	11.3a	9.9b	9.2c	(0.4)
	June	15.9a	14.0b	14.4b	(0.6)
	July	14.6a	10.9b	11.5b	(0.5)
	August	15.6a	13.5b	13.3b	(0.9)
1985	May	16.5a	15.4b	14.1c	(0.3)
	June	18.5a	15.5b	15.8b	(1.1)
	July	13.0a	9.7b	9.4b	(1.4)
	August	16.4a	13.4b	10.9c	(1.9)

† Growing season = May to August.
‡ Standard error of the mean.
a-c Means within a row followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

Table 4.17 Correlation data between TNC reserves and yield in trial A.

Species	Year	r		Intercept	Slope
Alfalfa	1984	0.58	*	-71.8	22.5
	1985	0.64	*	-118.6	18.3
	Combined	0.63	*	-139.9	23.4
Bromegrass	1984	0.14		110.6	-23.7
	1985	0.10		934.1	-25.9
	Combined	0.10		570.6	-4.2

* Significant at p=0.10.

in 1984 ($r = 0.58$), 1985 ($r = 0.64$) and a combined analysis ($r = 0.63$). All correlations of bromegrass TNC reserves and yield were negatively correlated but not significant, Table 4.17.

4.3 Discussion

4.3.1 Nitrogen

4.3.1.1 Field data

Applications of nitrogenous fertilizer to grasses and grass-legume mixtures are used to increase herbage production of the grass and thereby manipulate sward composition. However nitrogen applications will not result in favorable yield increases if the other growing conditions are unsatisfactory. The total response of a grass-legume mixture to nitrogen applications are complex; comprehensive reviews on the subject reveal highly variable results and interpretations. The components of a mixture respond and interact not only in response to the type and amount of the nitrogen application but also to other management practices and the prevailing climatic conditions.

Air temperatures were near normal for May to August in 1984 and 1985 and as such were condusive to good forage growth. However, precipitation for the same period was substantially lower in 1984 than 1985, with both years being well below normal compared to the long term average (Appendix 3). The differences in precipitation between the two study years elicited extreme responses in yield, plant phenology and species composition that illustrate the dynamic nature of a grass-legume sward. In addition to the need for adequate soil moisture and temperature, it has been illustrated that high carbohydrate reserve levels during the fall and winter are essential to maximize the response to fertilizer applied the following year (Graber *et al.*, 1927).

The grass and legume components of the mixture responded differently to nitrogen applications in 1984 and 1985 in sward composition and yield (Table 4.3). The bromegrass yield and percentage grass of the sward increased substantially in 1985, whereas under the drier conditions of 1984 legume yield and sward composition were higher. The change in botanical composition and yield of alfalfa in 1984 reflect adaptations to dry conditions. Van Riper *et al.* (1964) found that alfalfa has the ability to extract water from greater

depths than bromegrass and is able to use soil moisture more efficiently thus providing adequate yields during exceptionally dry years such as 1984. Due to the higher moisture levels throughout the growing season of 1985 bromegrass responded by a dramatic increase in its contribution to the sward and dry matter yield. Smith (1960) reported that bromegrass has 56% of its root system in the upper 7cm of the soil profile. Hence bromegrass can exploit the soil surface layers and take up moisture more efficient than alfalfa. The data presented here shows an increase in grass percent and yield in 1985.

Total herbage yields of the sward in both years were significantly increased by use of nitrogenous fertilizer, with yield increases being more pronounced in 1985 than (Figure 4.1). The greater response in 1985 appeared to be related to two effects. Firstly, a rapid decline in productivity of the plots receiving no nitrogen compared to those being fertilized and secondly, the overall higher moisture levels throughout the growing season with the exception of May (Appendix 3). Total herbage production decreased in the second experiment year for the plots receiving no nitrogen, whereas yield differences between the 200 and 400Kg N/ha treatments increased.

4.3.1.2 Herbage quality

Herbage quality was not influenced by nitrogen fertilizer applications in 1984. Acid-pepsin dry matter disappearance (APDMD) was also not affected by nitrogen application in 1985. Crude protein (CP) and phosphorus (P) values increased with higher nitrogen applications, while acid detergent fibre (ADF) values decreased. These results are consistent with the study of Folkins *et al.*(1983). The lack of response to applied nitrogen in herbage quality in August 1984 is in part due to the below average rainfall received during the latter half of the growing season. Thus the forage species were not able to fully utilize the available soil nitrogen. Carter and Sheaffer (1983) found that digestibility (IVDMD) was increased under prolonged dry conditions similar to the growing season of 1984. The increase in APDMD values was probably in part a result of a decrease in the amount of structural components laid down, with the corresponding increase in the labile

pool of carbohydrates. It would appear that since no increase in APDMD occurred in August 1984 as a result of applied nitrogen, structural tissues were not affected.

Fertilizers are normally used to increase forage quality of pure grass stands by delaying maturity. When grass and legume mixtures are involved the overall response to nitrogenous fertilizers varies. In 1985 the seasonal degradeble dry matter (DDM) and crude protein (CP) yields were increased with nitrogen applications of 0 to 400 Kg/ha/yr. This effect was not elucidated through seasonal changes in the phenology of the grass and legume as nitrogen did not significantly delay maturity. However nitrogen is expected to increased the CP content of the grass as well as its growth rate and vigor (reflected by increased yield and percentage grass in the sward). These events would in turn suppress the legume fraction. Any uptake of nitrogen from the soil by alfalfa would be offset by a reduction in the fixation of atmospheric nitrogen. At the high rates of nitrogen applied to the soil in this trial, nitrogen fixation by alfalfa may have been eliminated. Therefore the alfalfa plants may have changed little in their CP and DDM content. Hence the increases in DDM and CP content with nitrogen applications of 200 and 400 Kg/ha may be explained by increases in (1) the CP content of the grass (2) the percentage grass of the sward and (3) greater dry matter yield. These results are comparable to the studies of Kust and Smith (1961) and Sharman (1971).

4.3.1.3 Root total nonstructural carbohydrates

Although no significant differences in alfalfa root carbohydrate reserve levels at any sampling date were observed among the nitrogen treatments during 1984 and 1985, with the exception of 400 Kg N/ha in 1985 increased applications of nitrogen fertilizer enhanced root TNC accumulation. The effect of heavy dressings of nitrogen applied at 200 and 400 Kg/ha combined with a lack of moisture throughout the growing season in both years, probably resulted in minimal leaching of the applied nitrogen to lower soil layers. Therefore, despite alfalfa's capability to withdraw moisture from depths of up to 5m the applied nitrogen was probably not available at such depths and increases in plant growth as

a response to the applied nitrogen were not possible. This is supported by the lack of significant differences in legume dry matter yield at 200 and 400 KgN/ha in 1984 and 1985 (Figure 4.2 and 4.3). Another possible explanation is that the energy expended in extracting moisture from such depths for tissue maintenance and plant growth, prevented the accumulation of TNC reserves. In this study the relatively low level of carbohydrate reserves during July 1985 under the 2 cut system may have been a response to warm temperatures (Robinson and Messangale, 1968).

The trend in root TNC in bromegrass plants which received nitrogen fertilizer were similar to the trends in the plants that received no nitrogen. However the percentages were generally lower and the range between the upper and lower concentration values were greater in the fertilized than in the unfertilized. The trend in available carbohydrates showed nitrogen fertilization stimulated the utilization of photosynthates for the production of new top growth thereby preventing the accumulation of reserves. The response patterns obtained in this study resemble those reported by Paulsen and Smith (1969) for smooth bromegrass and Okajima and Smith (1964) for timothy and bromegrass.

Root reserves of bromegrass were at their lowest level in May for the 1984 growing season in contrast to May 1985 where levels were at a high. The difference in May root TNC levels between 1984 and 1985 may be attributed to (1) a mid winter thaw in January and February 1985 when temperatures were 9°C above normal. Snowmelt occurred and the stand was exposed to the sun. During this time bromegrass crown buds may have matured in preparation for spring growth. (2) The excellent growing conditions (ie. high moisture and warm temperatures) caused bromegrass to begin spring growth earlier in 1985 than 1984 (Appendix 3 and 6). Hence the conditions in 1985 were condusive to the accumulation of reserves prior to the sampling date in late May. Despite the higher respiration rate that would have occurred during this period, photosynthates may have been stored in excess of respiration and bud development resulting in a net accumulation of reserves.

In general root reserves of bromegrass increased throughout the growing season of 1984 in contrast to a decrease in 1985. This change in storage pattern is related to the amount of rainfall in the two study years (Appendix 3). 1984 was a dry year which resulted in low grass yields (ie. poor growth) and hence carbohydrates from photosynthesis were stored as reserves. The 1985 growing season had higher rainfall levels than 1984 with the result that substantial yield increases were recorded (Figure 4.3). Carbohydrates from concurrent photosynthesis were not stored as reserves as in 1984 but were utilized for new growth (structural components), hence the decrease in TNC levels in 1985.

4.3.2 Defoliation

4.3.2.1 Field data

Frequency of defoliation influenced the growth stage of grass and legume plants by affecting the degree of maturity reached at the time of cutting. The individual clippings of the frequent defoliation schedule occurred during the periods of rapid growth with the consequence that herbage yields (for total production, grass and legume) were reduced markedly as compared to less frequent defoliation. These results are similar to those of Baese and Decker (1966), Paulsen and Smith (1968) and Knievel *et al.* (1971). Highest yields were recorded for the 2 cut system followed by the 1 cut in 1984 and 1985. The frequent defoliation schedule (4 cuts) gave the lowest yields of dry matter (Table 4.3). Total production of the sward was greatly influenced by the defoliation treatments in both study years. Frequent defoliation resulted in reduced yield as found by Langille and Warren (1962) in Ontario, Canada and Counce *et al.* (1984) in Georgia, USA. The differential yield response to defoliation found in this study may be attributed to the grass fraction. A pronounced grass yield increase in 1985 for all defoliation treatments was a reflection of higher soil moisture levels throughout the growing season. Under frequent defoliation the amount of rainfall affected whether the grass or legume dominated the sward and gave the highest yield. The dry conditions of 1984 favoured alfalfas with its deep

root system, whereas increased precipitation in 1985 favoured bromegrass. Comstock and Law (1948) also found the grass component constituted a greater proportion of the sward under frequent defoliation if moisture was high. This type of response is shown in Figure 4.3. Legume yield was favored by a 2 cut system, where at each cutting the legume was at the 50-100% bloom stage. A significant reduction in yield with infrequent cutting (1 cut/year) was mainly a result of the stand's growth cycle. With no defoliation until August both species showed a rapid senscence from mid July until harvest, whereas the 2 and 4 cut systems exhibited rapid growth during this period. Legume dry matter production and sward composition decreased for all defoliation schedules in the second year, in contrast to the sharp increase in the grass fraction for yield and sward composition. The factors contributing to the change in the dominant species are numerous. Light, temperature, canopy structure, nutrient status and soil moisture all interact to determine which species will predominate a stand. The most important factor is soil moisture. In 1984 low soil moisture limited grass growth to a greater extent than alfalfa as reflected by dry matter yields and botanical composition. Grasses commonly grown with alfalfa have a higher proportion of their root system in the upper 30cm of the soil horizon compared to alfalfa. This supports the evidence of Wolf *et al.* (1964) and Van Riper *et al.* (1964) who found that grasses utilized more moisture in the first 30cm of the soil profile than alfalfa. However, Van Riper *et al.* (1964) also found that because the roots of alfalfa penetrate the soil to greater depths than grasses, alfalfa was more efficient at using the moisture from deeper levels especially during times of limited rainfall. In 1985 the grass fraction of the sward responded to the higher moisture levels during the growing season and increased its contribution to total yield and its percent composition by weight of the sward. An increase in the grass fraction of the mixture was the result of an increase in plant height and leaf area causing shading of the lower growing alfalfa canopy, reduced light intensity which in turn reduced legume growth. Another factor contributing to the decline in legume yield in 1985 was the reduction in plant population density under the frequent defoliation schedules

(Table 4.2). In general frequent cutting of alfalfa has been shown to result in a rapid decline of the stand in both plant density and yield (Silket *et al.*, 1937). In May 1985 TNC levels of alfalfa were the same irrespective of the defoliation treatment imposed the previous year (Table 4.15). However, the stress imposed by frequent defoliation in 1985 is clearly evident as TNC levels remained constant throughout the season. Those plants less frequently defoliated increased their reserve levels. Another factor to be considered is that frequent defoliation resulted in plants entering the winter with 7% less TNC reserves than those cut less frequently. Hence the plants had less carbohydrates for hardening, resisting diseases and becoming winter dormant. This combined with the high stress imposed by the 1985 30 day cutting regime resulted in a gradual decrease in the alfalfa plant density.

The timing and height of cutting of smooth bromegrass (especially in the spring) can greatly affect persistence and productivity. Grass yields were lower for all defoliation regimes in 1984 compared to 1985 mainly due to the limited rainfall in the first year. Under frequent defoliation bromegrass was cut at the jointing stage (equivelent to the growth score 2, Table 3.2). At this time TNC reserves may be low and cutting will weaken the plants. Also basal activity of the crown buds may not be developed sufficiently to initiate rapid regrowth, so plants recover slowly. Subsequent defoliations at similar stages of growth will continue the withdrawl of reserves as in 1985. This situation did not arise in 1984 as regrowth was limited due to insufficient rainfall; thus TNC levels increased as carbohydrates were stored and were not utilized for growth. Under the dry conditions in 1984 the 1 cut had the highest yield compared to the 2 cut in 1985 when moisture was not limiting regrowth. The first cut of the 2 cut regime occurred when bromegrass was between head emergence and anthesis. Other studies also indicate that the best time to cut bromegrass for high seasonal yields and stand persistence is between full-head and anthesis (Smith, 1962; McElgun *et al.*, 1972).

4.3.2.2 Herbage quality

The effect of defoliation regimes on herbage quality were similar in both 1984 and 1985. The results showed that herbage degradebility (acid pepsin dry matter disappearance, APDMD), crude protein and phosphorus contents increased with frequent defoliation. No differences in herbage quality values for the 1 cut system were observed for the August harvest in 1984 and 1985. The grass and legume plants were senescent at this time and were given a maturity score of 6. As the frequency of defoliation increased, morphological development was retarded. The 2 cut system had a maturity score of 3.5 which corresponds to the 10% bloom stage for alfalfa and head emergence for bromegrass. A further retardation in the growth of both species was evident under the 4 cut system as scores averaged 2.7 (relating to stem elongation with bud development in alfalfa and stem elongation with head formation in bromegrass). Variations to the phenology of grass and legumes are primarily the result of management practices, climate and varietal differences of the species used (Kalu and Fick, 1983). As the maturity of the grass and legume increased there was a corresponding reduction in herbage quality. In a review of management and forage quality Marten (1985a) noted that as forages mature they are less digestible, have less protein and minerals and contain more fibre.

The 1 and 2 cut systems reflect the higher moisture levels through the growing season of 1985 by having increased APDMD, protein, minerals and less fibre as compared to 1984. The increased herbage quality under the higher moisture conditions in 1985 can possibly be explained by a higher leaf : stem ratio for both the grass and legume as plants remained in the vegetative state for a longer period of time than in 1984.

The defoliation treatments affected the seasonal forage degradeble dry matter (DDM) yield of the sward in 1985 (Table 4.9). The DDM yield for the 2, 1 and 4 cut systems ranked in the order listed. For the 2 cut system yield was almost double that of the 1 and 4 cut regimes.

Seasonal crude protein yield was influenced to a lesser extent by defoliation than by nitrogen (Table 4.10). The effect of cutting schedules on the seasonal crude protein yield of the sward is comparable to previous studies by Smith (1962) who reported increased CP content with more frequent defoliation. The lower CP content of herbage harvested under the frequent defoliation (4 cut system) may be explained as follows. The grass and legume components of a mixed sward harvested in the vegetative growth stage are high in CP, however, dry matter yield is usually correspondingly low. Hence the increase in CP content of the herbage is at the expense of reduced yield, thus the resultant CP yield for the season may be low. This was found to be true for the 4 cut system (0.6 Mg CP/ha) compared to the 2 cut system (1.1 Mg CP/ha). The trend in seasonal CP content is similar to the grass dry matter yield (Figure 2.3). The increase in protein can be attributed to the grass fraction having a higher nitrogen content as fertilizer applications increased. No differences were found for the legume or grass growth stage scores among nitrogen treatments, therefore it can be assumed that the legume and grass CP content of the sward was the same. Hence the reasoning that the increased CP yield was a result of the greater grass yields observed.

4.3.2.3 Root total nonstructural carbohydrate

The trends of root total nonstructural carbohydrate (TNC) reserves of alfalfa and bromegrass were similar in their response to defoliation but the range and fluctuation of reserves differed between years and species. The greatest fluctuation occurred in alfalfa and the least in bromegrass. Root carbohydrates of both species were depleted by frequent defoliation. Carbohydrate reserves were at a maximum when harvested once at the end of the growing season (August).

The seasonal pattern of TNC in the roots of alfalfa under the various defoliation regimes resemble that reported by Smith (1962), Nelson and Smith (1968) and Cooper and Watson (1972). The percent root TNC of uncut alfalfa (Figure 4.5) increased from May to August in both years. With the initiation of new shoots in May reserves will have

declined for a period and then increased steadily until August. The lower reserve levels in June and July 1984 compared to the same months in 1985 can be explained by the early maturing of alfalfa as a result of water stress and high temperatures. Alfalfa had reached maturity (ie. seedset) in July 1984 and August 1985, hence the increase in food levels in August of both years was primarily the result of excess photosynthates being accumulated.

The pattern of TNC root reserves of alfalfa under the 2 cut system in both years is similar to those reported previously by Graber *et al.* (1927), Granfield (1935) and Nelson and Smith (1968). Root carbohydrates were at the lowest level in May and increased until the first cutting in June (at which time alfalfa was at the 10% bloom stage). A similar trend was reported by Alberda (1966) and Cooper and Watson (1972). In July 1985 a greater depletion of reserves occurred than in 1984 and may be explained by a slower accumulation of reserves after the June defoliation. This is supported by the observation that the grass canopy in July 1985 was above that of the alfalfa. This in-turn was a response to higher precipitation in June- August 1985 than 1984 thus favouring bromegrass regrowth over alfalfa. Shading by the grass would have caused a reduction in leaf expansion and photosynthetic rate of alfalfa thereby decreasing the amount of carbohydrates available for storage. Similar results were reported by Silva (1968) and Greub and Wedin (1971). High temperatures in July 1985 may have reduced the accumulation of reserves even further. As the season progressed and the grass matured alfalfa was able to compete favourably for light and combined with the response to shorter days and cooler nights TNC levels increased.

The TNC in alfalfa roots cut 4 times per season responded differentially over the two years (Figure 2.4). The year of 1984 was characterised by a hotter and drier than normal growing season, reserves were initially depleted after the May harvest and thereafter remained constant at this fairly low level in June and July and only increased in August. This is similar to the results of Feltner and Massengale (1965) who found a decrease in TNC levels of alfalfa under high temperatures. In 1985 carbohydrate reserves

were usually replenished to the level of the previous harvest by the time of the next cutting. A slight increase in TNC content occurred from July to August. The difference in the rate of TNC accumulation from July to August in the two years may be explained as follows. At the August root harvest in 1984 there were no difference in bud numbers or their stage of development between the various cutting treatments; however, in 1985 substantial differences existed between the defoliation treatments. Under the 4 cut system buds were present in large numbers and were of varying lengths. Bud production occurred at the expense of stored TNC reserves or by the utilization of concurrent photosynthates, the net result being little or no accumulation of reserves.

4.3.3 Models for yield prediction

In the past plant biomass yields have most frequently been estimated by the use of clipping techniques. Non-destructive methods for yield prediction are being recognized by researchers, extention workers and argicultural consultants as quick and accurate measures of plant yields insitu. Plant height has been the most frequently used criterion for the estimation of yields for numerous forage crops although the degree of success has varied. One of the problems identified while reviewing the literature on this subject was that plant height can be interpreted and measured in many ways. Several instruments have been developed to measure plant biomass that combined plant height and sward density (Shrivastava *et al.*, 1969; Baker *et al.*, 1981). Correlation coefficients greater than 0.90 are commonly reported for measuring forage yield using disc meters (Sharrow, 1984).

A ruler was employed to measure mean sward height in this study. Sward height was calculated by measuring the height of the grass and legume plants and taking the mean value. Hussey *et al.* (1985) estimated forage production of a pure stand of alfalfa using a ruler and reported a correlation coefficient of 0.92. In some cases height has been combined with other variables. Michalk and Herbert (1977) used plant height x percent ground cover for estimating dry matter yield. Methods for predicting forage yield in pasture by measuring plant density

and/or height have been studied previously (Tan *et al.*, 1977).

In this study the mean sward height was combined with a growth stage score of the grass (Table 3.2). The growth stage scores found in Table 3.2 for alfalfa and bromegrass were comparable to those used by Nelson (1925), Albert (1927) and Metcalf (1973). The six score system used in this study was chosen for its simplicity in identification of plant stages. As plant height is related to plant maturity, it was reasoned that combining a height measurement with a growth stage score would enhance the prediction of a grass-legume sward.

This was found to be the case in 1984 and 1985 and in the combined analysis. Adding the growth stage component increased the prediction of dry matter yield from the grass-legume pasture. The models presented in Table 4.5 were developed using data collected from both a hay and grazing management system and are therefore reliable for both systems. The simple model for yield prediction under various nitrogen applications and management regimes presented here could be a valuable aid to extension workers and farmers who require a rapid and accurate method to estimate the yield potential of pastures and hay crops.

4.3.4 Correlation of TNC reserves and dry matter yield

A close relationship of herbage yield with the level of TNC reserves has been demonstrated in alfalfa by Graber *et al.* (1927), Smith (1962) and bromegrass by Reynolds and Smith (1962). However the correlation coefficients have varied considerably which indicates that TNC reserves do not always reflect the amount of regrowth after a defoliation. The low correlation coefficients (r) obtained in this study (Table 4.17) account for between 1 and 40% of the variability in yield. It is therefore evident that factors other than TNC reserves are determining the amount of regrowth after a defoliation. This adds weight to the conclusions of May (1960), Baker (1963) and more recently Richards *et al.* (1987) who state that the level of TNC reserves has little bearing in determining regrowth yield. The lack of significance for bromegrass correlations in 1984 and 1985 plus a combined analysis casts doubt on the role of TNC in regrowth yield as stated above. Possible causes for the lack of association between TNC

and regrowth yield in smooth bromegrass are (1) that the contribution of concurrent photosynthesis to regrowth is large, (2) morphological or meristematic features limit regrowth ie. the large number of growing points in brome for roots and tillers compared to alfalfa, and (3) possibily the higher energy costs associated with (2) above. The association of TNC reserves (measured as a concentration) and the amount of herbage prodction has been demonstrated in alfalfa by Graber *et al.* (1927), Granfield (1935), Smith (1962) and others. This study showed that alfalfa yield was correlated with the level of TNC reserves in 1984 (a dry year), 1985 (a wet year) and a combined correlation (Table 4.17). Despite the correlations being significant they accounted for only 38% of the variability on average. Hence factors other than TNC reserves are involved in regrowth. This lends support to the findings of Richards *et al.* (1987), who stated that the contribution of TNC reserves to regrowth was small. The level of association between TNC reserves and yield in alfalfa and bromegrass may the resuly of fundamental differences between the two species. These differnces may be the result of the species ability to reallocate its TNC reserves for new foliage growth, and ultimately future yield.

4.4 Summary and Conclusion

(1)The application of nitrogen fertilizer to a mixed grass-legume sward was found to increase dry matter production and herbage quality but lower root carbohydrate reserves of the grass and legume.

(2)With frequent defoliation (4 cuts per season) quality of the herbage (digestibility, crude protein and phoshorus) was increased but at the cost of reduced dry matter yield.

(3)A two cut system gave optimum yields, good quality herbage, and in addition carbohydrate root reserves were maintained at fairly high levels for both species.

(4)Combining a growth stage score with height increased the prediction of dry matter yield for the bromegrasss-alfalfa sward compared to a height correlation alone. Such a simple model has potential for use in extension work and by forage managers for the estimation of herbage production and thus may assist in predicting grazing capacity or hay yield.

5. PART III: EFFECTS OF FALL DEFOLIATION AND APPLIED NITROGEN ON FORAGE PRODUCTION

5.1 Materials and methods

5.1.1 Field trial B location and climate

Field trial location, soil type, climatological information for the area and site history are presented in Part I (section 3.1.1, page 15).

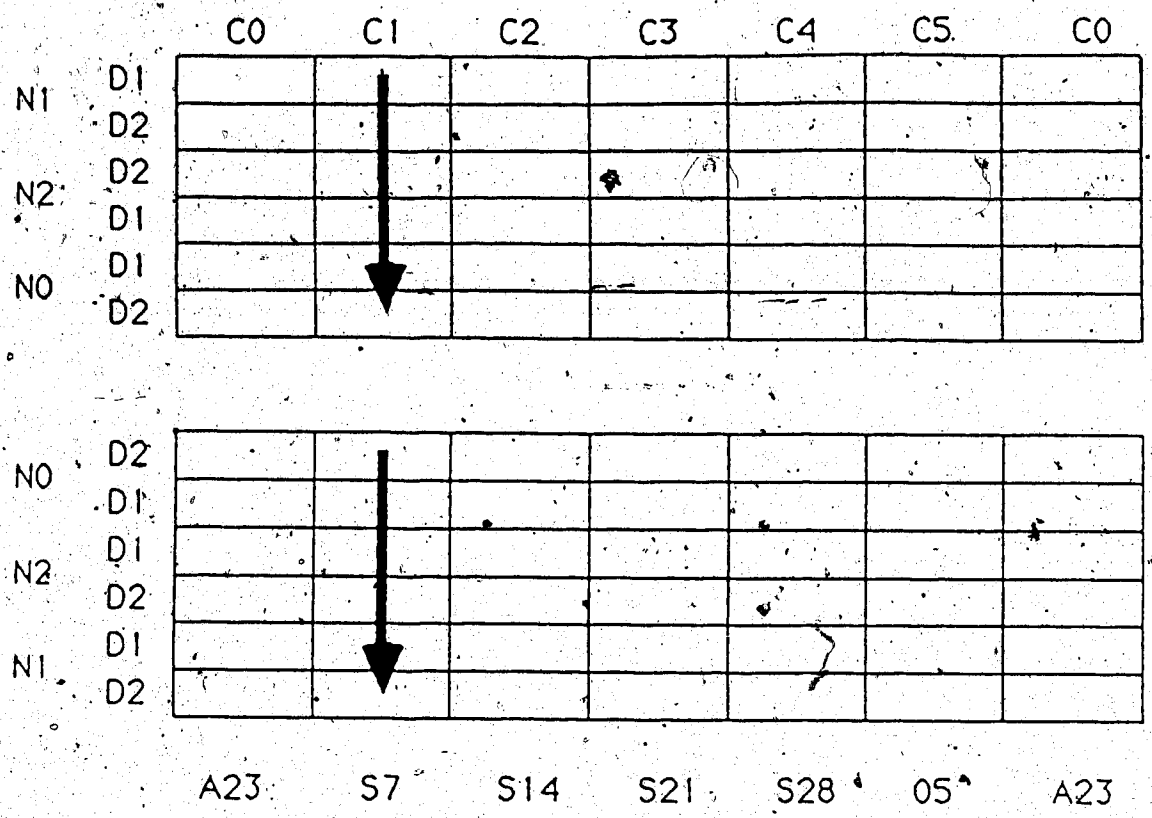
5.1.2 Experimental design and treatments

Trial B was a strip split plot test (Table 5.1). This design is similar to a split split plot test but with differently constructed second level experimental units. The defoliation (D) treatment factor was randomized within each of nitrogen (N) treatment factor resulting in a normal split plot design (as trial A). However, the third treatment factor "fallcutting (C)" was not randomized within each first experimental unit (DxN) but was applied as strips across replicates for each DxN combination; each strip pertaining to a different fallcutting treatment (Figure 5.1). To enable access to the sub-sub-plots to mechanically harvest the herbage it was not feasible to use any other design.

The main plots consisted of three fertilizer applications (as trial A) with sub-plots consisting of two summer defoliation treatments (two cuts, D1, as for commercial hay production and four cuts, D2, to simulate rotational grazing). Each sub-sub-plot was harvested once in the fall starting on September 7, 1984. There were thus six sub-sub-plots harvested consecutively at weekly intervals from September 7 to October 5, 1984. Fifteen replicates were used, five for destructive root sampling in the fall and ten for other data collected at each harvest in the following year. At each summer harvest in 1985 the growth stage of the grass and legume was scored, the yield of dry matter and sward height was recorded as in trial A.

Table 5.1 Partition of degrees of freedom (df) for trial B.

Source		df
Replication(R)	(r-1)	9
Nitrogen(N)	(a-1)	2
Error 1 (E1)	(r-1)(a-1)	18
Defoliation(D)	(b-1)	1
DxN	(a-1)(b-1)	2
Error 2 (E2)	a(r-1)(b-1)	27
Fallcutting(C)	(c-1)	5
Error 3 (E3)	(r-1)(c-1)	45
CxN	(a-1)(c-1)	10
Error 4 (E4)	(r-1)(a-1)(c-1)	90
CxD	(b-1)(c-1)	5
CxDxN	(a-1)(b-1)(c-1)	10
Error 5 (E5)	a(b-1)(c-1)(r-1)	135



Legend:

R = Replicates (10 in total- only 2 are illustrated above, R1 and R2).
N = Nitrogen treatments, Kg/ha/yr (N0=0, N1=200, N2=400).
D = Defoliation treatments, applied as summer cuts (D1=2, D2=4).
C = Fallcutting treatments, (C0= control- no fall cut, August 23 (A23), with 1 fall cut applied to the remaining treatments (C1-C5) at weekly intervals starting on September 7 (S7) until October 5 (05), 1984.

N and D treatments were randomized as a split plot design. Fallcutting (C) was applied as a strip effect (↓) across the N x D combinations, as illustrated above.

Figure 5.1 Field layout showing two replicates of the strip split plot design of trial B.

5.1.3 Field observations

In trial B dry matter yield was recorded at each summer harvest in 1985 (as trial A, Appendix 5). Sub plots measured 39.5m x 2.5m. Forage was harvested from each sub-sub-plot using a modified Mott harvester by clipping an area 4.2m² (two strips 3.45m x 0.61m). The clipping height, dry matter yield, growth stage of the grass and legume and mean sward height were determined as in trial A (section 4.1.3, page 42).

5.1.4 Field sampling of root material

To study fall variation in TNC reserve levels, alfalfa and smooth bromegrass root samples were collected from trial B in the fall of 1984. The fallcutting treatments consisted of a single defoliation applied on the harvest dates set out in Figure 5.1. Each sub-sub-plot was harvested for root material of both species on the fall harvest date and at weekly intervals until October 12, 1984. Thus an early fall cut treatment (September 7, 1984; code 1) had six root sampling dates whereas a late fall cut treatment (October 5, 1984; code 5) had only two sampling dates. Two replications were used for root sampling in 1984. The same methods of sampling, grinding and storage were employed as described in Part I (section 3.1.4).

5.1.5 Chemical analysis

5.1.5.1 Herbage quality analysis

Due to insufficient biomass at the time of the applied fall cut treatments in 1984, quality analysis of herbage material was not possible. Samples were collected from three replicates (randomly selected) at all summer harvests in 1985. Sub-samples for quality analysis were collected from the harvested forage (used for dry matter yield determination). All samples were assayed for acid pepsin dry matter disappearance, crude protein, acid detergent fibre and phosphorus using the NIR prediction equations developed in trial A 1984. Part I contains details on the validity of using the calibration equations from trial A 1984 on samples from trial B 1985.

5.1.5.2 Total nonstructural carbohydrates (TNC)

Root samples for TNC reserve determinations of alfalfa and smooth bromegrass were collected in the fall of 1984 and 1985 as described above. The NIR prediction equations of trial A, 1984 were used to assay root TNC concentration in both species. A detailed outline of the methods is presented in Part I.

5.1.6 Data analysis

Analysis of variance was used to test if there were significant effects for nitrogen, defoliation, fallcutting and year for the variables measured. Means for all variables were tested for significance using Duncan's New Multiple Range test at $p=0.05$ (Steel and Torrie, 1980).

Root TNC reserves of alfalfa and bromegrass were correlated with dry matter yield and mean sward height. As yield and sward height were collected from 10 replicates as compared to 2 for TNC determination, correlation analyses were performed using two methods. First, treatment means ($N=6$) were used to correlate TNC with yield and height. Second, the replicates used for field data collection adjacent to the TNC root sampling replicates were paired ($N=12$) and correlated. Root reserves on October 12, 1984 were used for the correlation of TNC reserves with yield and height in 1985 for both methods.

5.2 Results

This section examines the effects of nitrogen, defoliation, fallcutting and their interactions for the parameters recorded under three sub-sections. Results are set out for field data, herbage quality and root TNC reserves. The first table in each sub-section lists the parameters recorded; each parameter is taken through the various statistical analyses used, once completed the next parameter follows in the same pattern. The same format is retained for the remaining sub-sections.

5.2.1 Field data

The mean square values and significance levels are presented in Table 5.2 for total forage dry matter yield. Highly significant differences ($p=0.001$) were found for both nitrogen fertilization and defoliation, whereas their interaction, fallcut by nitrogen and defoliation, were significant at the $p=0.05$ level. The third order interaction of fallcut by nitrogen by defoliation was also significant ($p=0.01$). However, the fallcut treatments of 1984 produced no significant differences in total dry matter production in the summer of 1985. Fall cutting in 1984 had no effect on total dry matter yields in 1985 (Table 5.3). Mean sward height was influenced to varying degrees by fall cutting, generally a fall cut increased sward height. The nitrogen treatment effects showed applications of 200 and 400Kg/ha yielded significantly higher dry matter compared to the nil treatment. The same trend was found across nitrogen treatments within each defoliation regime. The maximum yield was attained under the 2 cut system with either 200 or 400Kg N/ha.

The fallcut by nitrogen interaction (Table 5.4) showed an increase in forage production with later fallcut treatments for all nitrogen fertilization treatments. The main nitrogen treatment effect showed no difference between 200 and 400Kg/ha rates. Dry matter yield also differed due to fallcut by defoliation interactions(Table 5.5). The 2 cut system produced substantially higher dry matter than the 4 cut system. No differences in the total dry matter yield under the 4 cut system were noted.

Table 5.2 Mean square values from analysis of variance for total dry-matter (TDM) yield, growth stages of grass (GSG) and legume (GSL), and mean sward height (Ht) in trial B for the summer of 1985.

Source	df	TDM Mg/ha	GSG score‡	GSL score	Ht cm
Replication(R)	9	32.5	0.008	0.024	22
Nitrogen(N)	2	5693.0 ***	0.677 ***	0.251 **	40
Error a†	18	63.4	0.008	0.033	13
Defoliation(D)	1	10471.0 ***	72.003 ***	979.770 ***	587 ***
DN	2	121.6 *	0.8553 *	0.123 *	10950
Error b	27	35.9	0.007	0.034	14
Fallcut(C)	5	6.6	0.006	0.005	38 ***
Error c	45	6.6	0.005	0.029	4
CN	10	64 *	0.005	0.065	5
Error d	39	0.009	0.005	0.029	2
CD	5	5.8 *	0.012	0.014	2
CDN	10	58 **	0.012	0.052	2
Error e	135	2.0	0.009	0.028	3

*,**,*** Significant at 0.05, 0.01 and 0.001, respectively.
† Error a=RxN; Error b=DR/N; Error c=CR/N; Error d=CRN; Error e=CRD/N.
‡Scored, 1= vegetative to 6= seed set.

Table 5.3 A comparison of fall cut treatments on the total herbage dry matter yield and mean sward height in the summer of 1985 in trial B.

Fall harvest Date	Fall code number	Total herbage dry matter yield Mg/ha	Mean sward height cm
Aug23	0	4.60a	27.8c
Sept. 7	1	4.43a	27.5c
Sept. 14	2	4.64a	28.3bc
Sept. 21	3	4.66a	29.1a
Sept. 28	4	4.66a	29.5a
Oct. 5	5	4.68a	28.9ab

a-c, Means within columns followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

Table 5.4 The effect of nitrogen fertilizer applied at three levels and six fall cutting treatments on total dry matter yield in the summer of 1985 in trial B.

Fall harvest Date	Fallcut code	Nitrogen applied (Kg/ha) 0	200	400
No cut	0	z2.70 ab	y5.30 a	x5.80 a
Sept. 7	1	y2.50 b	x5.10 ab	x5.60 b
Sept. 14	2	y2.70 ab	x5.40 a	x5.75 b
Sept. 21	3	y2.80 a	x5.40 a	x5.65 b
Sept. 28	4	y2.90 a	x5.45 a	x5.90 a
Oct. 5	5	z2.80 a	y5.40 a	x5.90 a
Nitrogen mean		y 2.70	x 5.45	x 5.80

x-z, Means within a row preceded by the same letter are not siginificantly different p=0.05 according to Duncan's New Multiple Range test.

a-b, Means within a coloumn followed by the same letter are not siginificanely different p=0.05 according to Duncan's New Multiple Range test.

Table 5.5 Herbage dry matter yields in the summer of 1985 following fall cutting treatments for trial B in 1984.

Fall harvest Date	Fallcut code	Summer defoliation regime			
		2 cuts/yr		4 cuts/yr	
		Mg/ha			
Aug. 23	0	5.81	bc	3.38	a
Sept. 7	1	5.66	d	3.27	a
Sept. 14	2	5.89	b	3.30	a
Sept. 21	3	6.03	a	3.38	a
Sept. 28	4	5.95	ab	3.33	a
Oct. 5	5	6.04	a	3.32	a
Defoliation mean		x	6.01	y	3.36

a-d, Means within columns followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

x-y, Means within a row preceded by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

An analysis of variance by months was performed to investigate differences in total dry matter yield for the growing season of 1985 (Table 5.6). A significant difference in total yield for the nitrogen, month and their interaction for both defoliation systems. It was noted that fallcutting was not significant, however significant differences were found for the interaction of fallcut by month and fallcut by nitrogen for the 4 and 2 cut system respectively. Further investigation of the fallcut by nitrogen interaction revealed that for the 4 cut system the major proportion of the total yield was produced in May (Table 5.7). Fallcutting produced no significant differences in yield for the last 3 harvests (June, July and August 1985). The fallcut treatment on September 21, 1984 and the control resulted in the highest dry matter yield in May 1985. For the 2 cut system (Table 5.8) yields in June and August 1985 were vastly different. June contributed the most towards total production. The fallcut treatments of September 21 and October 5, 1984 were the highest yielding in June 1985. The August 1985 harvest yields were significantly increased by fall cuttings on or after September 21, 1984.

An analysis of variance for the growth stage of alfalfa and bromegrass for nitrogen, defoliation and their interaction was significantly different at various levels of probability (Table 5.2). Fallcutting and other interactions were not significant. Main treatment effects differed for growth stage of the grass (GSG) and legume (GSL) with the GSG showing significant increases in maturation for all nitrogen treatments. No maturation increases for treatments 0 and 200Kg N/ha were observed for GSL (Table 5.9). The main defoliation means were the same for the legume and grass score with the 2 cut system being the more mature. The interaction to note is that of 4 cuts/season and nitrogen for the GSG where the grass maturity was increased to a greater extent under 200Kg N/ha than 400Kg N/ha or zero treatment.

The mean square values and significance levels for mean sward height in the summer of 1985 for trial B are presented in Table 5.2. Significant differences ($p=0.001$) for defoliation and fallcut treatment effects were found. The height of the sward in 1985 was greater in the fallcut treatments on or after September 21, 1984 (Table 5.3).

Table 5.6 Mean square values from an analysis of variance by months for total dry matter (TDM) yield under four and two cuts per year in trial B 1985.

Source	df	4 cuts/year TDM		2 cuts/year TDM	
Replication(R)	9	2.2		20.5	
Nitrogen(N)	2	534.0	***	1839.3	***
Error a‡	18	1.6		5.1	
Month(M)	3	3160.1	***	1149.0	***
MN	6	181.1	***	335.1	***
Error b	81	1.5		29.3	
Fallcut(C)	5	0.3		5.6	
Error c	45	0.5		3.3	
CN	10	0.5		5.1	**
Error d	90	0.4		1.6	
CM	15	0.81	***	1.5	
CMN	30	0.4		2.5	
Error e	405	0.4		1.4	

, * Significant at 0.01 and 0.001, respectively.
‡ Error a=RxN; Error b=MR/N; Error c=CR/N; Error d=CRN; Error e=CRM/N.

Table 5.7 Total dry matter yield for individual months for 4 cuts per year at each fall harvest treatment for trial B in the summer of 1985.

Fall harvest Date	Fall Harvest code	Month			
		May	June	July	August
		Mg/ha			
Aug. 23	0	x2.41a	y0.22a	y0.40a	y0.35a
Sept. 7	1	x2.27b	y0.25a	y0.41a	y0.34a
Sept. 14	2	x2.29b	y0.21a	y0.44a	0.35a
Sept. 21	3	x2.38a	y0.19a	y0.47a	y0.34a
Sept. 28	4	x2.28b	y0.21a	y0.48a	y0.35a
Oct. 5	5	x2.30b	y0.20a	y0.47a	y0.36a
Month mean		z2.32	y0.21	y0.44	y0.35

x-y, Means within a row preceded by the same letter are not significantly different p=0.05 according to Duncan's New Multilpe Range test.

a-b, Means within a column followed by the same letter are not significantly different p=0.05 according to Duncan's New Multilpe Range test.

Table 5.8 Total dry matter herbage yield over the growing season under two cuts per year for each fallcut treatment for trial B in the summer of 1985.

Fall harvest date	Fall code number	Month			
		June		August	
		Mg/ha			
Aug 23	0	4.25	c	1.55	b
Sept 7	1	4.10	d	1.55	b
Sept 14	2	4.31	b	1.53	b
Sept 21	3	4.40	a	1.63	a
Sept 28	4	4.31	b	1.64	a
Oct 5	5	4.37	a	1.67	a
Month mean		4.32	x	1.60	y

a-d, Means within a column followed by the same letter are not significantly different at $p=0.05$ according to Duncan's New Multiple Range test.

x-y, Means within a row followed by the same letter are not significantly different at $p=0.05$ according to Duncan's New Multiple Range test.

Table 5.9 Main nitrogen and defoliation treatment means and their interaction for the growth stage score of the grass (GSG) and legume (GSL) for trial B in the summer of 1985.

Nitrogen treatment Kg/ha	Defoliation regime (cuts/yr)		Nitrogen mean
	2	4	
GSL			
0	x4.28b	y1.03b	2.65b
200	x4.29b	y1.02b	2.65b
400	x4.42a	y1.05a	2.73a
Defoln mean	x4.33	y1.03	
GSG			
0	x2.00b	y1.00c	1.50c
200	x2.00b	y1.30b	1.65a
400	x2.06a	y1.08b	1.57b
Defoln mean	x2.02	y1.13	

a-c, Means within a column for the grass or legume followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.
x-y, Means within a row followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

5.2.2 Herbage quality

An analysis of variance of herbage for percent forage degradebility (measured as acid pepsin dry matter disappearance, APDMD) showed nitrogen and defoliation were significant at various levels of probability (Table 5.10). Fallcutting did not significantly affect APDMD in the following year. A closer examination of the nitrogen and defoliation effects on forage degradebility (Table 5.11) showed that nitrogen application and frequent defoliation increased APDMD compared to the control treatment.

Although the effect of fall cutting in 1984 had no significant effect on the forage quality parameters tested in 1985 forage growth, an analysis of variance by months for each defoliation regime was performed. The mean square values for the 4 cut system revealed that nitrogen fertilization and months, plus their interaction were significant for all quality parameters (Table 5.12). The interaction means for fallcut by months showed APDMD varied considerably from May to August 1985 (Table 5.12). After May, 1984 APDMD increased for all fallcutting treatments. The mean squares and significance levels obtained under the 2 cut system (Table 5.13) are similar to the 4 cut response (Table 5.14). Nitrogen, months, their interaction and that of fallcut by month were significantly different. As found for the 4 cut regime, August 1985 produced herbage with a higher APDMD than June across fallcuts and for the monthly mean value (Table 5.15). The later fall harvest in 1984 generally resulted in higher APDMD in June 1985, with the exception of August 1985 when a lower digestibility was recorded. The 1984 fallcut control treatment produced the lowest APDMD in June1985, but was the highest in August of that year. Herbage APDMD for the 2 cuts per season differed substantially from the June and August harvests (Table 5.15). In June the later fallcutting gave highest yields; yet by August, yields were the lowest. The converse was found for the early fallcuttings.

The mean square values for nitrogen and defoliation were significantly different for crude protein (CP) in 1985 (Table 5.10). Crude protein content of the herbage increased with the application of 200 Kg N/ha or 400 Kg N/ha when compared to the zero nitrogen treatment

Table 5.10 Mean square values from analysis of variance for percent acid pepsin dry matter disappearance (APDMD), crude protein (CP), acid detergent fibre (ADF) and phosphorus (P) of herbage material in trial B for the summer of 1985.

Source	df	APDMD %	CP %	ADF %	P %
Replication(R)	2	12.5	30.5	6.5	0.67
Nitrogen(N)	2	455.5 **	409.9 **	171.6 **	40.55 **
Error a‡	4	15.8	18.0	5.2	0.75
Defoliation(D)	1	952.3 ***	593.6 ***	1347.6 ***	82.77
DN	2	0.0	9.5	30.6	0.24
Error b	6	3.6	12.4	5.9	0.27
Fallcut(C)	5	7.3	8.3	3.3	0.28
Error c	10	3.8	6.8	1.7	0.18
CN	10	2.4	11.4	1.7	0.18
Error d	39	0.009	0.005	0.029	2
CD	5	2.3	13.1	1.6	0.17
CDN	10	2.1	7.6	1.6	0.17
Error e	30	1.9	8.6	2.0	0.07

*, **, *** Significant at 0.05, 0.01 and 0.001, respectively.
‡ Error a=RxN; Error b=DR/N; Error c=CR/N; Error d=CRN; Error e=CRD/N.

Table 5.11 The main treatment effects of nitrogen application and defoliation regime on herbage quality constituents† measured for trial B in the summer of 1985.

Treatment		APDMD	CP	ADF	P
		%			
Nitrogen	0	42.97b	17.34b	23.38a	0.1883b
Kg/ha	200	47.56a	22.48a	20.55b	0.234a
	400	49.96a	23.69a	19.09b	0.254a
Defoliation	2cuts/yr	43.86b	18.83b	24.54a	0.198b
regime	4cuts/yr	49.80a	23.52a	17.48b	0.253a

† APDMD = Acid-pepsin dry matter disappearance; CP = crude protein; ADF = acid detergent fibre; P = phosphorus.

a-b, Means within a column followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

Table 5.12 Mean square values from analysis of variance by months for four cuts of acid pepsin dry matter disappearance (APDMD), crude protein (CP), acid detergent fibre (ADF) and phosphorus (P) for trial B 1985.

Source	df	APDMD %	CP %	ADF %.	P %
Replication(R)	2	9.3	20.5	11.4	0.009
Nitrogen(N)	2	875.4 **	924.5 **	609.0 ***	0.097 ***
Error a‡	4	20.3	17.4	10.6	0.001
Month(M)	3	879.7 ***	576.4 ***	1008.2 ***	0.072 ***
MN	6	39.9 ***	29.3 **	126.2 ***	0.003 ***
Error b	18	2.6	6.5	3.4	0.000
Fallcut(C)	5	3.95	4.9	3.3	0.000
Error c	10	2.7	3.4	1.4	0.000
CN	10	2.9	6.1	2.5	0.000
Error d	20	2.9	7.4	1.5	0.000
CM	15	5.2 ***	8.4	2.4	0.030
CMN	30	4.5 ***	8.7 *	4.2 ***	0.005 ***
Error e	90	1.6	4.9	1.4	0.000

*,**,*** Significant at 0.05, 0.01 and 0.001, respectively.
‡ Error a=RxN; Error b=MR/N; Error c=CR; Error d=RCN; Error e=CRM/N.

Table 5.13 Acid pepsin dry matter disappearance (APDMD) for individual months for four cuts per year for trial B in 1985 across fall harvest treatments.

Fall harvest Date	Fall Harvest	Month			
		May	June	July	August
		%			
Aug. 23	0	z42.4c	y49.4c	y49.4a	x54.3a
Sept. 7	1	z43.8b	y49.0b	y49.3a	x54.1a
Sept. 14	2	z43.8b	y49.8bc	y49.1a	x54.4a
Sept. 21	3	z44.7a	y50.6a	y49.6a	x54.4a
Sept. 28	4	z43.8b	y49.7bc	y49.5a	x52.1b
Oct. 5	5	z45.1a	y50.3ab	y49.6a	x52.6b
Month mean		z43.9	y49.8	y49.4	x53.7

x-z, Means within rows preceded by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

a-c, Means within a column followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

Table 5.14 Mean square values from analysis of variance by months for the two cut system for acid pepsin dry matter disappearance (APDMD), crude protein (CP), acid detergent fibre (ADF) and phosphorus (P) for trial B 1985.

Source	df.	APDMD %	CP %	ADF %	P %
Replication(R)	2	6.1	6.0	4.5	0.0007
Nitrogen(N)	2	577.5 **	801.3 ***	651.0 ***	0.0851 ***
Error a‡	4	14.0	8.7	9.6	0.009
Month(M)	1	1832.8 ***	1158.3 ***	1127.5 ***	0.1289 ***
MN	2	3.8 ***	36.3 **	99.2 ***	0.0042 ***
Error b	2	1.3	0.6	2.1	0.0001
Fallcut(C)	5	3.6	1.8	1.5	0.0002
Error c	10	2.4	1.5	2.2	0.0001
CN	10	4.0	4.1	4.9	0.0005
Error d	20	1.1	0.9	1.2	0.0001
CM	5	6.9 ***	4.5	0.5	0005
CMN	10	3.8 ***	3.5 ***	2.9	0.0004
Error e	30	1.3	0.6	2.1	0.0001

, * Significant at 0.01 and 0.001, respectively.
‡ Error a=RxN; Error b=MR/N; Error c=CR; Error d=RCN; Error e=CRM/N.

Table 5.15 Herbage degradebility over the growing season under two cuts per year for each fallcut treatment for trial B in the summer of 1985.

Fall harvest date	Fall code number	Month			
		June		August	
		%			
Aug 23	0	43.4	c	50.0	a
Sept 7	1	43.5	c	50.1	a
Sept 14	2	44.0	b	50.3	a
Sept 21	3	44.6	a	50.3	a
Sept 28	4	44.4	a	49.2	b
Oct 5	5	44.6	a	49.1	b
Month mean		44.0	y	49.8	x

a-c, Means within a column followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

x-y, Means within a row followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.

and under the 4 cut system compared to the 2 cut regime (Table 5.11). The analysis of variance by months for the 4 cuts/year regime (Table 5.12) showed significant differences for nitrogen, month, the interaction of month by nitrogen and the second order interaction of month by nitrogen by fallcut. Significant values were found under the 2 cut system (Table 5.14) for the anova for the same variables as for the 4 cut regime, with the exception of the fallcut by month interaction which was not significant.

An analysis of variance of herbage for percent acid detergent fibre (ADF) showed nitrogen and defoliation were significant at $p=0.01$ and $p=0.001$, respectively (Table 5.10). Examination of the main nitrogen treatment effects revealed that higher nitrogen applications lowered the herbage fibre content. An increase in the frequency of defoliation decreased ADF percent of the sward (Table 5.11). Results tabulated in Tables 5.12 to 5.14 indicate month, fallcutting and their interaction were significant for herbage ADF content under the 2 and 4 cut defoliation regimes. The response for both regimes was similar in that nitrogen, months and their interaction were significantly different at $p=0.001$. The second order interaction, months by nitrogen by defoliation produced a significant difference under the 4 cut system only.

The mean square values in Table 5.10 for herbage quality showed phosphorus (P) was affected by nitrogen and defoliation treatments as were digestibility, crude protein and fibre content. The second order interaction fallcut by nitrogen by defoliation was significantly different. The application of nitrogen substantially increased the phosphorus content of the herbage compared to the control (Table 5.11). As observed with APDMD and CP greater frequency of defoliation significantly increased the P content compared to the 2 cut system. An analysis of variance by months for the defoliation treatments were similar with nitrogen, defoliation and their interaction highly significant at $p=0.001$ (Tables 5.12 to 5.14). With the 4 cut system the fallcut by month by nitrogen interaction was also significant.

5.2.3 Root total nonstructural carbohydrate

Root total nonstructural carbohydrate (TNC) of alfalfa and smooth bromegrass (Table 5.16) were significantly different for the three nitrogen treatments. The two species responded differently to the applied treatments. The alfalfa root carbohydrate levels were significantly affected by defoliation, fallcutting, their interaction and the second order interaction of fallcut by nitrogen by defoliation (Table 5.16). The interaction means of root TNC alfalfa for fallcutting date by nitrogen level are presented in Table 5.17. There was a general trend of increasing root TNC reserves by delaying the fallcutting. Fallcutting treatments 4 and 5 yielded the highest root sugar levels for the fallcut mean value and each fallcut by nitrogen interaction. The exception was the October 5, 1984 cutting date with zero nitrogen, which had a substantially lower root reserves level compared to the 200 and 400 nitrogen treatments.

Root TNC levels of alfalfa samples decreased with the number of harvests per year. A significant difference for root sugar content was found between 2 cut (mean 19.0%) and 4 cut (mean 15.5%) defoliation treatments.

Figure 5.2 was compiled to illustrate the differences in the 2 defoliation regimes for TNC reserves of alfalfa in the fall of 1984. The fallcut treatments under the 4 cut regime reduced root sugar levels below the control which did not recover to the August 23, 1984 level. The trend for other fall harvests (not shown in Figure 5.2) were similar under both defoliation regimes (Tables 5.18 and 5.19). There was an overall decrease in root reserves of the control from August 23 to October 12, 1984. After September 7, 1984 a decrease in TNC reserves for all treatments was followed by a corresponding increase after a killing frost (considered as -5°C), that occurred on September 27, 1984. A further decrease was observed in root sugar levels after the October 5th harvest under both regimes. Under the 2 cut system, fall cutting significantly increased TNC levels (Table 5.18) by October 12, 1984 compared to the control. Root TNC of the fallcut treatments for the 2 and 4 cut regimes were compared for differences between levels attained on August 23 and October 12, 1984 and those on October 12,1984 (Tables 5.18 and 5.19). The two sets of comparisons gave the same results.

Table 5.16 Mean square values from an analysis of variance of percent total nonstructural carbohydrate in the root of alfalfa and smooth bromegrass for trial B in the fall of 1984.

Source	df	Alfalfa	Bromegrass
Replication(R)	1	0.78	0.36
Nitrogen(N)	2	2.96	38.89 *
Error a†	2	1.69	1.33
Defoliation(D)	1	222.25 **	0.86
DN	2	15.26	1.39
Error b	3	1.79	1.75
Fallcut (C)	5	21.72 **	3.10
Error c	5	2.99	2.27
CN	10	2.99 *	1.34
Error d	10	0.94	0.96
CD	5	1.73	1.01
CND	10	4.69 *	1.52 *
Error e	15	1.52	0.46

*,** Significant at 0.05 and 0.01, respectively.
† Error a=RxN; Error b=DR/N; Error c=CR/N; Error d=CRN; Error e=CRD/N.

Table 5.17 Root total nonstructural carbohydrate reserves in alfalfa at three levels of nitrogen fertilizer and six fall harvest treatments for trial B in the fall of 1984.

Fall harvest Date	Fall Harvest code	Nitrogen applied (Kg/ha)			Fallcut mean
		0	200	400	
		%			
Aug. 23	0	17.5b	15.2c	17.0b	16.6b
Sept. 7	1	14.5c	15.3c	16.4c	15.4c
Sept. 14	2	17.1b	16.9b	17.1b	17.0b
Sept. 21	3	17.8b	16.1b	17.0b	16.9b
Sept. 28	4	20.0a	18.6a	18.5a	19.0a
Oct. 5	5	17.7b	19.1a	19.1a	18.6a

a-c, Means within a column followed by the same letter are not significantly different at $p=0.05$ according to Duncan's New Multiple Range test.

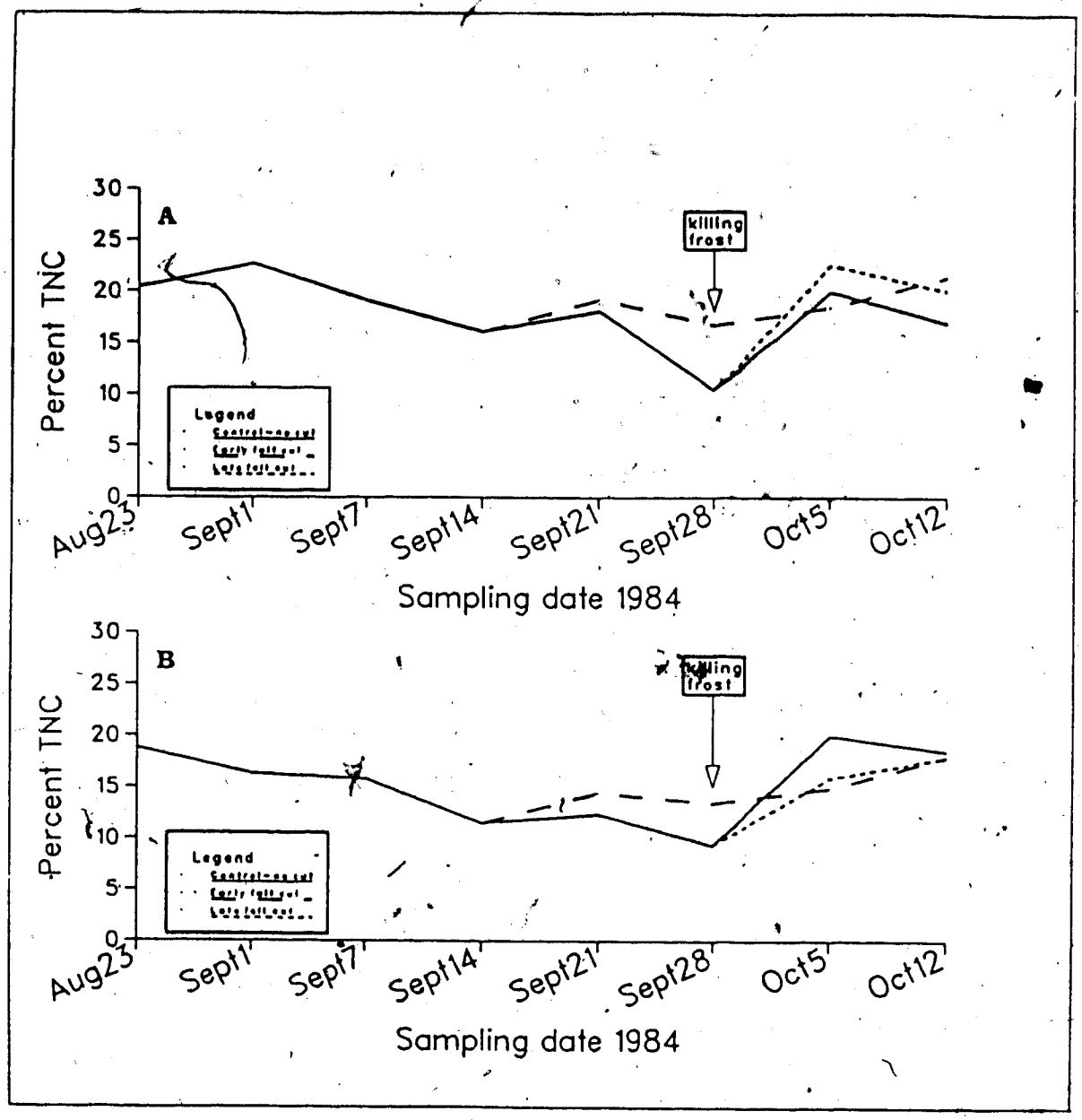


Figure 5.2 Trends in alfalfa root total nonstructural carbohydrate in the fall of 1984 under two defoliation regimes for trial B. (A = two summer cuts plus a fall cut, B = four summer cuts plus a fall cut.)

Table 5.18 Mean values for percent total nonstructural carbohydrate in the roots of alfalfa under the two cuts per year regime at each sample date and fallcutting treatment for trial B in the fall of 1984.

Fallcut code number	Sampling date							
	Aug 23	Sept 1	Sept 7	Sept 14	Sept 21	Sept 28	Oct 5	Oct 12
	%							
0	20.4	22.7	19.1	16.1	18.1	10.4	19.9	16.9b
1			19.1†	18.5	17.2	14.7	20.3	19.3a
2				16.1†	19.2	16.7	18.4	21.3a
3					18.1†	13.9	19.7	20.9a
4						10.4†	22.5	20.0a
5							19.9†	20.5a

a-b, Means within the October 12 coloumn followed by the same letter are not singificantly different at
p=0.05 according to Duncan's New Multiple Range test.
† Indicates the time of fall cutting plus root sampling. Subsequent root samples were collected at weekly intervals.

Table 5.19 Mean values for percent total nonstructural carbohydrate in the roots of alfalfa under the four cuts per year regime at each sample date and fallcutting treatment for trial B in the fall of 1984.

Fallcut code number	Sample date							
	Aug 23	Sept 1	Sept 7	Sept 14	Sept 21	Sept 28	Oct 5	Oct 12
	%							
0	17.8	16.3	15.8	11.5	12.3	9.3	20.0	18.5a
1			15.8†	12.0	13.8	9.8	14.1	15.9b
2				11.5†	14.4	13.4	14.9	17.9a
3					12.3†	12.2	17.0	18.0a
4						9.3†	15.9	17.9a
5							20.0†	17.7a

a-b, Means within the October 12 coloumn followed by the same letter are not significantly different at p=0.05 according to Duncan's New Multiple Range test.
†, Indicates the time of fall cutting and root sampling. Subsequent root samples were collected at weekly intervals.

An analysis of variance of smooth bromegrass root TNC levels (Table 5.16) showed significant differences for nitrogen and the second order interaction of fallcutting by nitrogen by defoliation. An increase in the nitrogen application per year lowered bromegrass root reserves (0 N- 17.7%; 200 Kg N/ha- 16.4%; and 400 Kg N/ha- 15.2%). A significant reduction ($p=0.05$) in reserves was found between the 0 and 400 Kg N/ha treatments but not for 0 and 200 or 200 and 400Kg N/ha treatment comparisons.

Figure 5.3 and Tables 5.20 to 5.22 show the trends in bromegrass root sugar reserves during the fall of 1984 for each nitrogen treatment. In the fall of 1984 root carbohydrates in bromegrass showed less variation compared to those of alfalfa (Figure 5.2). Application of nitrogenous fertilizer reduced TNC levels at all sampling dates, irrespective of the fallcutting treatment. TNC reserves increased after the killing frost on September 27, 1984 but decreased after October 5, 1984 (as was observed for alfalfa root TNC, Figure 5.2).

5.2.4 Correlation of fall TNC levels with spring yield and height

Regression analyses were conducted on dry matter yield (extrapolating botanical composition values from trial A to trial B 1985) for alfalfa and bromegrass. All correlations performed using either means or paired values were not significant at $P = 0.10$. TNC concentration measured on October 12, 1984 was correlated with mean sward height in the spring of 1985. Correlations were performed using height measured in May for the 4 cut system and height in June for the 2 cut regime. None of the analyses performed were significant at $P = 0.10$.

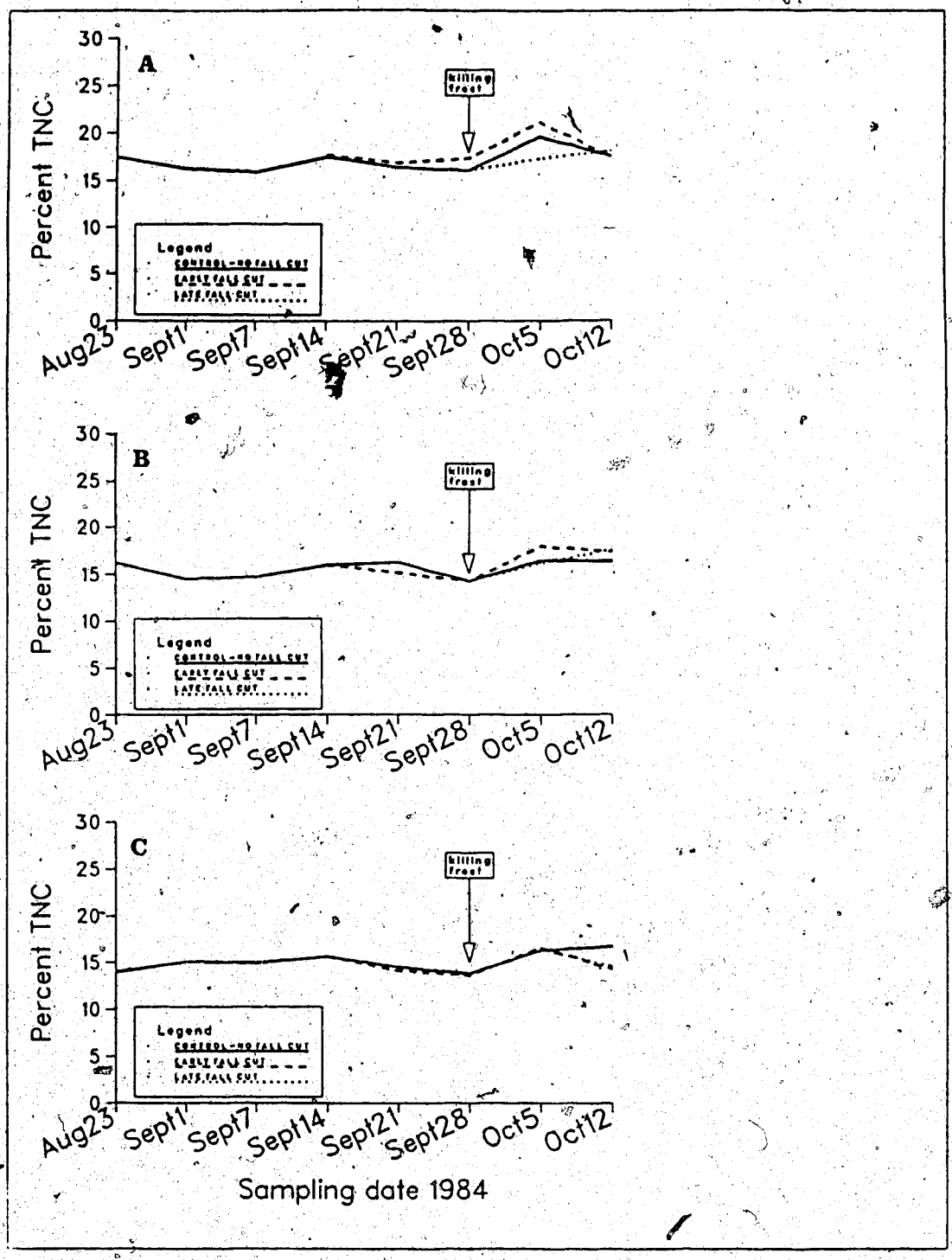


Figure 5.3 Trends in smooth bromegrass root total nonstructural carbohydrate in the fall of 1984 under three nitrogen regimes for trial B.(A= 0 Kg N/ha, B= 200 Kg N/ha, C= 400 Kg N/ha.)

Table 5.20 Mean values for percent total nonstructural carbohydrate in the roots of bromegrass under zero nitrogen at each sample date and fallcutting treatment for trial B in the fall of 1984.

Fallcut code number	Sample date							
	Aug 23	Sept 1	Sept 7	Sept 14	Sept 21	Sept 28	Oct 5	Oct 12
	%							
0	17.4	16.2	15.8	17.4	16.3	16.1	19.7	17.6c
1			15.8†	17.6	16.8	17.4	21.3	17.6c
2				17.4†	15.4	14.9	18.0	18.1b
3					16.3†	16.3	20.4	19.4a
4						16.1†	17.4	18.3b
5							19.7†	17.6c

a-c, Means within the October 12 coloumn followed by the same letter are not singificantly different at
p=0.05 according to Duncan's New Multiple Range test.
†, Indicates the time of fall cutting and root sampling. Subsequent root samples were collected at weekly intervals.

Table 5.21 Mean values for percent total nonstructural carbohydrate in the roots of bromegrass under nitrogen at 200Kg/ha/year at each sample date and fallcutting treatment for trial B in the fall of 1984.

Fallcut code number	Sample date							
	Aug 23	Sept 1	Sept 7	Sept 14	Sept 21	Sept 28	Oct 5	Oct 12
	%							
0	16.2	14.5	14.7	15.9	16.2	14.2	16.4	16.3c
1			14.7†	15.3	15.1	13.9	16.2	16.8b
2				15.9†	15.1	14.2	17.9	17.3a
3					16.2†	16.0	18.9	17.7a
4						14.2†	16.1	17.5a
5							16.4†	17.0b

a-c, Means within the October 12 coloumn followed by the same letter are not significantly different at
p=0.05 according to Duncan's New Multiple Range test.
†, Indicates the time of fall cutting and root sampling. Subsequent root samples were collected at weekly intervals.

Table 5.22 Mean values for percent total nonstructural carbohydrate in the roots of bromegrass under nitrogen at 400Kg/ha/year at each sample date and fallcutting treatment for trial B in the fall of 1984.

Fallcut code number	Sample date							
	Aug 23	Sept 1	Sept 7	Sept 14	Sept 21	Sept 28	Oct 5	Oct 12
	%							
0	14.0	15.1	15.0	15.6	14.5	13.8	16.3	16.7a
1			15.0†	14.9	14.4	14.8	16.7	15.9b
2				15.6†	14.1	13.6	16.6	14.3c
3					14.5†	13.8	15.4	15.4b
4						13.8†	16.4	14.6c
5							16.3†	15.5b

a-c, Means within the October 12 coloumn followed by the same letter are not significantly different at
p=0.05 according to Duncan's New Multiple Range test.
†, Indicates the time of fall cutting and root sampling. Subsequent root samples were collected at weekly intervals.

5.3 Discussion

This discussion is divided in the same manner as the results section (field data, herbage quality and root carbohydrate).

5.3.1 Field data.

Grass fertilized with nitrogen have been utilized to extend the grazing season into the early fall and winter (Archer and Decker, 1971; Taylor and Templeton, 1976). Little data, however, is available concerning changes in chemical composition of grass-legume mixtures during the year subsequent to a fall harvest. Fall cutting date, nitrogen application and frequency of defoliation had variable effects in this study on the herbage yield, sward height, morphological development, quality and root total nonstructural carbohydrate (TNC) reserves of alfalfa and bromegrass.

Yield and plant height are highly correlated (Tan *et al.*, 1977); however in this study mean sward height increased for harvests on or after September 21 1984, whereas the total seasonal forage yield in 1985 was not affected. These findings are contrary to those of Fulkerson (1970) in Ontario, Canada where alfalfa cut in the late summer and early autumn reduced the height of the following spring crop compared to the unharvested plots. However, Collins and Taylor (1980) studied an alfalfa- orchard grass stand and found plant height of the spring crop was highly variable for autumn and winter harvests imposed the previous year. No direct comparison *per se* with the results of this study can be made as the calculation of plant (=sward) height was not outlined in either paper. The results of this study may be explained as follows. Sward height was calculated as the mid-point between the grass and legume heights. Field observations in 1985 noted the grass component was taller than the legume. A reduction in alfalfa plant population density occurred in trial A 1985 as a result of the summer defoliation regimes. Extrapolating the winter kill of alfalfa to trial B helps explain, in part, the domination of the sward by the grass in 1985. With the legume reduced the measure of sward height increased as a result of the domination of the grass component. However, yield of the fall cut

treatments were not affected as the grass and legume components showed compensatory growth. Similar reductions in alfalfa plant density were found by Sholar *et al.* (1983) as a result of fall cutting. Smith (1961) found a high correlation between alfalfa plant height in the fall and winter injury the following year. The data indicated that winter injury was progressively more sever as plant height increased. A total of 106mm of precipitation (more than double the normal) was measured during September 1984 while temperatures recorded were normal. These conditions are known to predispose alfalfa to winter kill (McKenzie, 1984) and may help explain the reduction in alfalfa plant density observed in 1985.

Dry matter yields in the following year were significantly increased for all fall cut treatments by a nitrogen application of 200 Kg/ha with little or no subsequent increase with higher applications. The fall harvest at all nitrogen treatments did not increase yield compared to the control. Total dry matter production for the haying regime (2 cuts) was almost double the simulated grazing treatment (4 cuts) irrespective of fall treatment. Forage yields were not significantly affected by fall cutting treatments with five harvests the previous year. There were, however, substantial yield increases for fall treatments on or after September 14, 1984. The highest was recorded for the October fall harvest. This is in agreement with studies by Folkins *et al.* (1961) in Ontario, Canada and Sholar *et al.* (1983) in Oklahoma, USA. The effect of fall cutting on yield was apparent for the spring harvest in 1985 where significantly higher yields were recorded for all treatments. Remaining summer defoliations were unaffected (Table 5.7 and 5.8). Accepting the traditional concept of a 4-6 week critical harvest period (Smith, 1972) before a killing frost, the Edmonton area would have a critical period from early September to mid October. When first cut yield is used to compare the effects of fall cutting, results are inconsistent for the above concept. Yield should have decreased if the above holds true, however yield increased with fallcutting. This may mean that the critical fall harvest period is not related to interference with cold acclimation and winter injury. Evidence to support this is provided by Tesar and Yager (1985) who found that fall cutting in Michigan did not decrease stand persistence provided hardy cultivars were grown.

The dry growing conditions in the summer of 1984 and 1985 (Appendix 3) produced little regrowth at the time the last cut treatments were made, so no attempt was made to determine forage yields. The effects of a first harvest in late May compared to late June in reducing subsequent regrowth yields are clearly shown in Tables 5.7 and 5.8. The reason differences between the fall treatments are evident only in the first harvest, irrespective whether it be in May or June is a consequense of the level of TNC reserves accumilated the previous fall. The level of TNC reserves effect not on the health of plants but also the regrowth vigor the following spring. The higher reserves accumilated by those treatments that occurred after September 21, 1984 are reflected in the subsequent yield the next spring. Hence, the September 21, 1984 fall cutting gave the highest yield in May and tended to be better for other summer harvests in 1985, despite the fall cutting date treatments in the second, third and fourth harvests not being statistically different. With the 2 cut system, September 21 and October 5, 1984 fall harvests gave highest yields in June and August 1985, whereas the control plots were the lowest yielding for the same months.

5.3.2 Herbage quality

Numerous studies by Collins and Taylor (1980), Fleming *et al.* (1983) and Collins (1983) showed changes in herbage quality during the fall and related these changes to yield, stand persistence and root TNC levels the following spring. However, no studies have been conducted that have examined the effects of fall harvest on the herbage quality the following year.

Fall cut treatments did not affect the total seasonal herbage quality in the following year (Table 5.10). The effects of nitrogen application and defoliation followed well known trends. Higher nitrogen dressings and defoliation frequency subsequently produced higher APDMD, protein and phosphorus values and lower fibre content. A closer scrutiny of fall cutting for individual defoliation regimes revealed fallcut by months was highly significant for forage degradebility (acid pepsin dry matter disappearance, APDMD). Under both summer

harvest regimes the fall cut treatment on September 21, 1984 gave consistently higher APDMD values throughout the growing season of 1985 (Tables 5.13 and 5.15). A substantial reduction in digestibility of August harvested herbage occurred for the September 28 and October 5, 1984 autumn harvest under both defoliation regimes. A possible explanation is the weather pattern during the growing season of 1985 and sward composition. Temperatures for May to July were near normal while rainfall was well below the 30 year average. In August 1985 a higher than normal precipitation was recorded. With the reduction in alfalfa stand density due to summer defoliations combined with the below average rainfall from May to July 1985 the contribution of both the grass and legume to sward APDMD remained constant. With the higher rainfall received in August 1985, the grass exhibited rapid growth thus increasing its contribution to the sward. A corresponding decrease in APDMD was evident since grasses are less digestible than legumes at similar growth stages.

5.3.3 Root total nonstructural carbohydrate reserves

Alfalfa is the most commonly grown legume in western Canada where climate differs from one region to another, one might also expect the critical time for carbohydrate storage to vary. A knowledge of this critical storage period is a prerequisite for optimum management of alfalfa.

Most studies conducted by researchers in determining whether an autumn harvest on alfalfa is detrimental have been conducted on pure alfalfa stands. The general recommendation in northern Alberta (McKenzie, 1984) has been that alfalfa should not be harvested from mid August to mid September, although the top growth could be removed safely after the first killing frost in late September or early October. This is because research has shown that the early fall is considered a critical time for storage of food reserves in alfalfa (Johnston, 1966; Willard *et al.*, 1934). This recommendation ignores the influence of environmental factors and management practices prior to the fall harvest.

The timing of the final harvest affected alfalfa root carbohydrate levels (significant fallcut $p=0.01$) in 1984 (Table 5.16) with maximum root reserves for the September 28 and October 5 1984 cutting treatments. Delaying the fall harvest after September 7 1984 tended to increase reserve levels. Such differences may be related to the regrowth period between the last two harvests in 1984 and its effects on tiller production and development. A defoliation on August 23, 1984 was applied to all plots prior to the fall harvest treatment. Hence, the regrowth period before the final harvest varied from 17 to 49 days. This same period allowed for -8 to 35 days of regrowth before the first killing frost (the negative value indicates the fall harvest occurred after the killing frost, Table 5.23). In this study -5°C was observed to be the temperature at which the upright top growth of alfalfa was severely damaged or killed. Similar observations in Oklahoma were made by Sholar *et al.* (1983). All the succeeding growth arose from the residual biomass remaining after the August 23 1984 defoliation, terminal buds of unelongated rhizomes or from other crown. Thus regrowth was produced under the shortening day lengths and cooler nights of autumn. It was observed that early fall cutting resulted in increased bud formation compared to the control. Silkett *et al.* (1937) reported similar responses in bud formation of alfalfa. The increase in root reserves of alfalfa for fallcutting after September 7, 1984 may be explained as follows. The cyclic pattern of root reserves in Wisconsin and Ontario (Reynolds and Smith, 1962; Johnston, 1966) was a 3 week decline followed by a 2 week relenishment period. Consequently in this study the late summer harvest permitted the reserve cycle to be completed, the early fallcuts of September 7 and 14 allowed sufficient draw-down of reserves for regrowth while the late fallcuts September 28 and October 5 allowed photosynthates to be accumulated (as temperature and moisture were above normal (Appendix 2 and 5) but did not allow sufficient regrowth to reduce stored reserves.

The interaction of nitrogen by fall cutting was significant (Table 5.16). The response of alfalfa root TNC reserves (Table 5.17) to the nitrogen applications of 200 and 400 Kg/ha was such that an increase in root reserves was observed (1) between nitrogen treatments and (2) with lateness of the fall cut. On average maximum root reserves occurred with the September

Table 5.23 Recovery time from the last harvest to the first hard frost (-5°C) for alfalfa and bromegrass root samples for trial B in 1984.

Fall harvest date	Fall code number	Recovery time† in days
Aug 23	0	35
Sept 7	1	20
Sept 14	2	13
Sept 21	3	6
Sept 28	4	-1
Oct 5	5	-8

†Negative values show the last cutting treatment occurred after the first hard frost.

28, 1984 cutting treatment. Root reserves were depleted by the September 7, 1984 harvest date irrespective of nitrogen treatment (Table 5.17).

It became apparent during the course of this study that management practices prior to the fall (in particular the number of defoliations) had a significant influence on root reserves of alfalfa and yield in the following year. The results from this study tend to disagree with the traditional recommendation that a fall cut 4-6 weeks prior to a killing frost would reduce TNC reserves and consequently reduce production in the following year. The possible reasons why the results of this study do not hold to the above concept are discussed below.

When fall cutting was imposed on a 2 and 4 summer defoliation regime, root TNC levels of alfalfa were altered (Figure 5.2). On October 12, 1984 with 2 summer defoliations TNC levels were significantly increased by a fall cutting. This is an advantage as plants entered the winter with higher root reserves which enabled them to develop and maintain winter hardiness and increased vigor in the following spring. This was reflected in the total dry matter yield where all fall cuts were found to have had increased yield compared to the control with the exception of September 7, 1984 (Table 5.5). It therefore appears to be advantageous to harvest an alfalfa-bromegrass stand under a 3 cut system in central Alberta. Two cuts taken as hay in June and August with a third defoliation either as hay or grazing in late September or early October. Frequently the second hay cut cannot be made as scheduled in late August because of unfavourable weather conditions, or the pasture is required for winter grazing. The results of this study indicate that harvesting or grazing in September or October would not reduce herbage yield or quality in the following year. From this study grazing appears to be the more feasible as plant growth remained prostrate in the fall. Many farmers do utilize forage in the fall for grazing in western Canada (Walton, 1987 personal communication). When a 4 cut system had an additional cut in the fall the reserve levels attained by October 12, 1984 were no different from the uncut fall control. These results are similar to those of Collins and Taylor (1980) in Kentucky where fall TNC levels were not consistently reduced by fall harvest treatments. The fluctuations in reserve levels are consistent with those reported by Sholar *et al.*

(1983) and Folkins *et al.* (1961) for the first year's fall cutting effects. In 1984 cool and sunny weather in September and October combined with the slowly regrowing alfalfa produced good photosynthetic conditions. This may be partially responsible for the fairly stable TNC levels observed for the fall cut treatments under the 4 cut system where plant vigor had been previously reduced as a result of summer defoliations.

When alfalfa plants were harvested in the late summer (ie. the control) root reserves were similar under both 2 and 4 summer defoliation regimes (Figure 5.2). Carbohydrate levels steadily decreased after September 1 until the killing frost on September 28, 1984. A greater reduction in root reserves of alfalfa were noted for plants previously harvested for hay (2 cuts) compared to simulated grazing (4 cuts). The greater reduction under the hay regime was a consequence of the higher shoot numbers and leaf production observed. The increase in carbohydrate reserves after the killing frost can possibly by explained by (1) photosynthates being accumulated under the favourable climatic conditions that prevailed that fall, (2) reduced respiration due to lower night temperatures and leaf loss by frost damage and (3) a remobilization of carbohydrates from the herbage into the roots. Root reserves decreased 2 weeks after the killing frost as plants entered winter and became dormant; depletion of reserves would continue as they are utilized for respiration over the winter months. The decrease in TNC reserves was similar to that found by Bula and Smith (1954) in Wisconsin and Reynolds (1971) in Tennessee.

An early fall defoliation (eg. September 14, 1984, Figure 5.2) increased root reserves which continued to rise until early October. When TNC levels were measured on October 12, 1984 all fall cut treatments imposed on the summer hay regime had significantly higher levels than the control.(Table 5.18). However, under the simulated grazing regime only the September 7, 1984 treatment had significantly reduced its TNC levels compared to the control (Table 5.19) As previously stated, sufficient regrowth had occurred to decrease reserves prior to the killing frost. Accumulation after this time did not restore reserves to the level of the control when sampled on October 12, 1984.

The response in TNC reserves of alfalfa subjected to a late fall cutting were similar under both summer defoliation regimes (Figure 5.2). As the killing frost occurred before the late fall harvests (Table 5.23) little or no regrowth (as stem production or elongation) was observed. A consequence was that root reserves increased thereafter until October 12, 1984.

The timing of the fall harvest did not alter the root TNC reserve levels of bromegrass as fallcutting was not significant (Table 5.16). These results confirmed the research findings that smooth bromegrass is hardy and does not winter kill as easily as alfalfa (Walton, 1983). The root sugar levels of bromegrass were affected by nitrogen application and fall cutting date. Nitrogen applications of 200 and 400 Kg/ha reduced root carbohydrate levels compared to the unfertilized treatment. A reduction in root sugar levels in the fertilized plots may be explained by the high number of tillers and basal leaves observed, ie. better growth. Early or late fall cutting (Figure 3.3) had variable effects on root carbohydrate reserves when nitrogen was applied. The fall cut on September 14, 1984 accumulated the highest sugar reserves for the unfertilized and 200 Kg/ha treatments whereas the control had the lowest carbohydrate reserves. Reserves were the highest for the uncut plants in the 400 Kg N/ha treatment.

Controlled temperature research with bromegrass indicated that growth under cool temperatures results in higher TNC reserves than levels under warm temperatures. This is due to partitioning of carbohydrates into the reserve pool rather than into structural components. This was shown by the increase in TNC reserves between trial A and trial B. Substantial increases in reserves for the unfertilized and 200 Kg N/ha plots and slight increases for the 400 Kg N/ha treatment were observed in 1984. TNC reserve levels after September 1 were relatively constant until the first hard frost on September 27, 1984. Thereafter levels increased to a maximum on October 5 and had declined by October 12, 1984. A further decline in reserves was likely after October 12, 1984 because sugars were utilized during winter dormancy for respiration and tissue maintenance. This has been documented by Bula and Smith (1954). The lower TNC reserves of the nitrogen treated plants followed the field observation that the grass plants had higher basal cover compared to the unfertilized plants. When the plants entered the

fall, photosynthetic conditions were good. However with the small amount of leaf tissue remaining after the August 23, 1984 defoliation photosynthetic accumulation of carbohydrates probably did not exceed the depletion by respiration, hence TNC reserves were not replenished and levels remained low. The balance between photosynthesis and respiration during the sampling period varied little. This is reflected by the fairly constant level of carbohydrates from August 23 to September 28 irrespective of the fall cut. The severity of tissue damage observed for bromegrass increased in the zero, 200 and 400 nitrogen treatments by the first hard frost (considered to be -5°C) in the order listed. This may explain the decrease in root carbohydrate levels found between October 5 and 12 1984 fall cutting treatments and the control. A stable TNC level was observed for the 200 Kg nitrogen treatment while an increase was noted for the 400 Kg treatment.

The response at each nitrogen level was examined to explain the differences in TNC levels to an early or late fall cut. An early fall defoliation [illegible]d to unfertilized plants reduced respiration and was found to lessen the depletion of root reserves. This may be due in part to a decrease in leaf area as a result of the fall cut combined with a cessation in rhizome production and root growth. Therefore when compared to unfertilized, uncut plants the TNC levels were high. The plants that received 200 and 400 Kg N/ha had a higher mass of photosynthetic tissue entering the fall. When defoliated early in the fall, the presence of available moisture and soil nitrogen initiated regrowth thus reducing root TNC levels below those of the fertilized uncut plants. The unfertilized plants sampled on October 12, 1984 for root TNC reserves showed significant increases for fall cut treatments on September 7 (18.1%), 14 (19.4%) and 21 (18.3%) as compared to the control (17.6%). A similar response was obtained for the 200 Kg N/ha treatment where increases for all fall cut treatments were observed compared to the control. The September 14, 1984 harvest had the highest reserve level at 17.7%. The 400 Kg N/ha treatment for all fall cuts reduced TNC levels below that of the uncut control.

The results of this study of fall cutting on root TNC reserves in an alfalfa bromegrass mixture for yield and quality in the following year should be viewed with caution as only one

year's data is presented. Research studies conducted by numerous workers indicate that fall cutting effects are more pronounced during subsequent years.

5.3.4 Correlation of fall TNC levels with spring yield and height

To correlate alfalfa and smooth bromegrass TNC reserve level with their respective dry matter yields the following spring the percentage composition obtained in trial A (May and June 1985) were extrapolated to trial B to estimate legume and grass yields. Cook (1971) from various studies concluded that the level of TNC in late fall was a good index of treatment severity imposed that year. Hence, the TNC reserve values for samples collected on October 12, 1984 were correlated with yield in 1985. All correlations performed using either treatment means or paired values (as explained in the method and materials) were not significant even at the $P = 0.10$ level.

Collins and Taylor (1980) recorded high positive correlations ($r = 0.90^{**}$ or greater.) between spring height of alfalfa and root TNC in mid-November. Similar correlations in this study revealed there to be no association between TNC levels in mid-October 1984 and height in May or June 1985.

Three reasons may be responsible for the lack of significance for correlations between TNC and yield or height found in this study; (1) TNC levels in mid-October are not as good an indicator of spring vigor as those in mid-November, (2) mean sward height was the used as the independent variable as compared to alfalfa height used by Collins and Taylor (1980), and (3) Height was recorded at the end of May and June and not in the early spring(April). The effects of plant vigor resulting from TNC levels would have been masked by photosynthetic contributions to growth.

5.4 Summary and Conclusion

(1) The results from this one year study indicated that a potential exists for autumn utilization of established alfalfa-bromegrass stands when previously utilized for hay or grazing.

(2) When the sward was harvested for hay (2 cuts), a fall harvest in late September increased dry matter yield and herbage quality in the following year.

(3) When the sward was harvested to simulate rotational grazing (4 cuts), an additional fall harvest caused no reduction in yield or herbage quality the following year.

(4) No response to fall cutting was measured for herbage crude protein, acid detergent fibre or phosphorus content. Herbage degradebility (measured as acid pepsin dry matter disappearance) was increased by a fall harvest after September 21, 1984 for both the hay and grazing systems.

(5) Alfalfa TNC levels were not affected by nitrogen application but were increased by fallcutting after September 21, 1984. When a fall cutting was imposed on a hay regime reserves were accumulated to a level greater than the uncut control. Under simulated grazing a fall cutting did not alter reserve levels.

(6) Smooth bromegrass root total nonstructural carbohydrate (TNC) reserves were depleted by nitrogen fertilizer applications of 200 and 400 Kg/ha.

(7) An autumn harvest generally increased bromegrass and alfalfa root TNC when it occurred on or after September 21, 1984.

6. GENERAL DISCUSSION

An important aspect of forage management, especially in research is the determination of forage quality. The particular quality constituents assayed will determine the cost of analyses. Laboratory chemical analysis by which quality constituents are routinely determined is time consuming and expensive. Near infrared reflectance (NIR) spectroscopy has been used to rapidly predict chemical constituents, digestibility and animal intake of forages (Norris *et al.*, 1976; Shenk *et al.*, 1979; Mathison *et al.*; 1985). Recently Brink and Marten (1986) used NIR to predict root total nonstructural carbohydrates of alfalfa. When large numbers of samples are involved that require numerous quality determinations NIR technology has the ability of simultaneous multiconstituent sample analysis, thus reducing analysis time and costs substantially. NIR spectrophotometers and their application to forage analysis will enable accurate and rapid assay at a cost considerably less than conventional laboratory determinations. Analytical chemists recognize that new instrumentation (with cheaper and more powerful computers) will further enhance the use of NIR technology. As a result of the low expense involved, samples to be assayed may be collected more frequently thereby enhancing the research data base creating a better understanding of the changes in forage quality which ultimately benefits the farmer.

The research studied the effects of summer and fall management of alfalfa and bromegrass root reserves and the way they influence yield and quality. NIR spectroscopy was used to determine herbage quality and root TNC reserves of alfalfa and bromegrass. The thesis consisted of two trials. Trial A studied the effect of summer defoliation and nitrogen fertilizer on yield, herbage quality and root TNC reserves. Trial B investigated the effect of fall cut imposed on trial A treatments on TNC root reserves in the fall with yield and herbage quality measured the following year.

The following discussion combines the results of both trials. Hay and simulated grazing regimes in the summer and the effects of an additional fall harvest are discussed in relation to yield, quality and TNC reserves.

6.1 Harvesting for Hay

When forage is grown for hay, quantity and quality are the two most important considerations influencing the time to harvest. The decision when to harvest is a compromise between the highest quality and the greatest quantity, however quantity concideration usually predominats. In central Alberta hay is usually taken as two cuts/year, depending on the growing season (McElgun *et al.*, 1972). Harvests are normally taken at the beginning of June and the end of August, hence the dates used in this study.

The results of this study follow well documented trends (Walton, 1983), showing high total dry matter yields combined with high yields of degradeble dry matter and crude protein (Tables 4.11 and 4.12). When compared to harvesting for pasture, carbohydrate reserves were maintained at levels adequate to support such high yields. When harvested alfalfa and bromegrass were between 50-100% bloom stage and stem elongation and head emergence, respectively. This corresponds well to the optimum time predicted for harvesting alfalfa and bromegrass from other studies (Smith, 1962; McElgun *et al.*, 1972). Under this harvest regime the crown buds of alfalfa and auxillary buds of bromegrass are sufficiently developed to enable rapid regrowth (Smith, 1981a). This ensures the crop canopy is able to intercept most of the available light. As the leaf area of the sward increased and attained a ceiling level photosynthates were channeled from structural components into storage reserves, hence root TNC levels remained high.

6.1.1 Effects of a fall defoliation

Fall management of forage crops, especially alfalfa is probably the most important aspect of management affecting the health, vigor, persistence and production of the stand. This is due to a critical period in the fall when TNC reserves are stored to enable the attainment of winter hardiness and to provide adequate reserves to sustain winter dormancy and allow rapid growth the following spring. A cut during this period causes TNC reserves in the root to be used to initiate new plant growth, as a consequence plants enter the winter with low TNC

reserves. Such plants are most likely to be injured or killed and therefore reduce yields (Graber *et al.*, 1927). Cutting after a killing frost is less hazardous than cutting before. However, late fall cutting removes the stubble that would catch the snow and act as an insulator during the winter and early spring. This critical period extends from mid August to early October in central Alberta (McKenzie, 1980). Harvesting 4-6 weeks prior to the first killing frost usually reduces yield and TNC levels. This was not found to be the case in this study. Fall cutting after September 15, 1984 increased yield and herbage quality in the following year. As foliage in the fall remained prostrate it would appear to be more feasible to graze the stand rather than take a hay. This is a management practice used by some farmers in this region (Walton, 1987 personal communication). Due to the interaction of weather and management practices the exact reasons as to why herbage quality was increased by fallcutting is not known. Irrespective of the fallcutting date in 1984 carbohydrate reserves were increased compared to the uncut control. This is an advantage as plants entered the winter with higher root reserves which enabled them to develop and maintain winter hardiness and increased vigor the following spring. This was reflected in higher total dry matter yields in 1985. Frequently the second hay cut can not be taken as scheduled in late August because of unfavourable weather conditions or the pasture is required for grazing in late September or early October. Extrapolating from the results of this study combined with other research (Fulkerson, 1966; Reynolds, 1971) it appears that this practice would not be detrimental to yield or stand longevity.

6.2 Harvesting as simulated grazing

Research has shown that rotational grazing gives high forage yields with little or no trampling and permits even grazing. This is the recommended practice in Alberta. Bromegrass-alfalfa mixtures are recommended for grazing in central Alberta. Grazing should not commence before June 1 as grazing prior to this may reduce total yield and hasten the loss of alfalfa from the stand. The rest period should be 3-4 weeks if the legume is to be retained. Hence the use of a simulated rotational grazing regime in this study.

When the sward was harvested at monthly intervals starting at the end of May until August herbage quality was higher than the two cut treatment but was obtained at the cost of reduced dry matter yield. These effects combined produced degradeble dry matter and crude protein yields that were low when compared to hay. The effect of frequent defoliation was to reduce plant height and maintain plants in a vegetative state (Table 4.3). The effects of reduced production appears to be related to low levels of TNC reserves. With frequent cutting alfalfa and bromegrass were harvested while reserves were being utilized for regrowth. For both species buds had not developed sufficiently to enable rapid regrowth, therefore stored carbohydrates were subsequently utilized for bud and leaf development. Despite the higher photosynthetic capacity of young leaves the time interval between harvests did not permit reserves to be replenished to their original level. Hence alfalfa plants were sufficiently weakened that a reduction in alfalfa population density was recorded in 1985. The increased quality compared to hay is because leaf tissue predominates in vegetative material and is of higher quality than stem tissue. As herbage matures the stem:leaf ratio increases thereby reducing overall quality. Hence the trends observed in this study (Table 4.7).

6.2.1 Effects of fall defoliation

As outlined earlier fall cutting is an important aspect of management often underestimated. When winter hay shortages predominate, grazing a pasture into the fall can supply the forage needed to maintain the particular type of animal being fed. However the consequences of such a decision can be disastrous as outlined previously. This study revealed that a fall harvest from early September to early October when imposed on a pasture previously used for rotational grazing neither reduced yield or quality the following year. Carbohydrate root reserves of alfalfa and bromegrass were not significantly altered by a fallcutting when applied during the early fall. This indicates an alfalfa -bromegrass stand can be grazed until early October with no detrimental effects yield in the following year. Caution is required however as other published research shows the effects of fall utilization of pasture only

becomes evident after 2 or 3 years use.

6.3 Applied nitrogen to a mixed sward

Applications of nitrogenous fertilizer to alfalfa -bromegrass mixtures are used to increase herbage production, by increasing the percentage grass composition and dry matter yield. Such responses are only possible if other factors (eg. moisture, temperature and light) are not limiting growth.

The effects of moisture in limiting the response of a grass -legume stand to applied nitrogen were observed in this study. Although total herbage yields were increased by the use of nitrogenous fertilizer, yield responses were more pronounced in 1985 than 1984 (Figure 4.1). The higher herbage yields recorded in 1985 were an effect of high soil moisture levels throughout the growing season as compared to 1984 (Appendix 3). Grass yield and total herbage yield had identical responses to applied nitrogen in the two study years. Yield increased correspondingly with the amount of nitrogen applied. Due to the dry soil conditions in 1984 alfalfa with its long tap root was able to exploit the soil profile to greater depths than bromegrass, and hence obtain more water for growth causing substantially higher yields. However no increase in legume yield was recorded therefore we can postulate that alfalfa requires adequate soil moisture to efficiently utilize applied nitrogen. At each sampling date alfalfa had reached the 50% bloom stage, thus root TNC reserve were being replenished by sugars from photosynthesis. The result being that carbohydrate reserve levels of alfalfa were not affected by the nitrogen treatments. Applied nitrogen has variable effects on TNC reserve levels ranging from an increase or decrease to no effect (Paulsen and Smith, 1969). The response elucidated is dependent upon the species, the level of TNC at the time of nitrogen application and prevailing abiotic factors. This type of variability in TNC levels were obtained in the two study years.

As a result of applied nitrogen and available soil moisture in 1985 the grass fraction of the sward increased its percentage composition, height and yield. The consequence was the grass

shaded the alfalfa plants causing reduced photosynthesis which was accounted for in stable TNC reserve levels. This was also reflected by reduced legume percentage composition. Carbohydrate reserve levels in bromegrass were reduced in response to the increased plant growth that occurred in 1985 (Table 4.3). Fertilizers are normally used to increase forage quantity but can greatly influence quality. When a grass -legume stand is involved the overall response to applied nitrogen varies. This study showed no changes in the digestibility indicator APDMD in either year when quality of the August herbage was measured. The reasons are related to the habit at that time of year. As temperatures are cooler and moisture is less plants are not vigorously growing, but instead are advanced in maturity. Hence the addition of nitrogen in increased plant growth is not evident at this time of year and the reason APDMD values were constant in both years. The dry soil conditions in 1984 also negated the increases in crude protein and phosphorus by nitrogen application to the stand. Higher quality as measured by the above were recorded in 1985 as a response to high soil moisture levels.

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8. APPENDICES

8.1 Appendix 1 Description of Soil type at Ellerslie Research Station.

Soil: Eluviated Black Chernozem

Location: Ellerslie Research Station, Edmonton, Alberta, SE 1/4 Section 24, Tp 51, r 25, W 4.

Parent Material: Lacustrine with interbedded till.

Landform and Site Position: Undulating, upper slope position, very gently sloping to the West.

Soil Drainage: Well drained.

Present Landuse: Active cropland.

Vegetation: Grasses, legumes and common weeds.

8.2 Appendix 2 Mean monthly maximum and minimum temperatures at Ellerslie Research Station in 1984, 1985 and 1951-80 average.

Month	1984		1985		1951-80 Ave.	
	min	max	min	max	min	max
	°C					
January	-13.3	-1.6	-16.4	-4.2	-21.7	-11.5
February	-9.1	2.5	-19.0	-6.5	-17.0	-5.9
March	-8.2	1.9	-8.3	2.2	-12.4	-1.7
April	-1.1	13.3	-1.1	9.9	-2.8	8.9
May	3.7	14.6	5.1	18.9	3.4	19.2
June	7.9	20.2	5.8	19.6	7.6	20.3
July	8.8	23.7	9.3	24.1	9.6	22.4
August	9.2	24.1	7.3	20.3	8.5	21.3
September	2.8	12.3	2.3	12.2	3.9	10.2
October	-10.1	-2.3	-2.4	9.2	-1.7	10.8
November	-15.5	-5.2	-19.2	-9.4	-9.7	-0.1
December	-23.0	-11.5	-12.1	-3.5	-17.2	-7.2

8.3 Appendix 3 Monthly precipitation (mm) at Ellerslie Research Station in 1984, 1985 and 1951-80 average.

Month	1984	1985	1951-80 Ave.
	mm		
January	18.0	13.1	25.1
February	7.2	15.2	17.4
March	9.1	5.1	17.2
April	5.6	27.0	20.1
May	60.6	38.7	44.8
June	53.5	58.8	77.7
July	35.6	40.1	84.5
August	28.2	78.6	66.7
September	106.3	47.6	40.6
October	35.9	17.9	16.9
November	13.0	13.3	16.5
December	26.0	21.4	24.7
Annual Total	399.0	376.8	425.2
Growing season† total	177.9	216.2	273.7

† May to August.

8.4 Appendix 4 Ground cover line transect study of the trial site in the fall of 1983.

Objective: To confirm the visual observation that the proposed trial site had a uniform ground cover of alfalfa and smooth bromegrass.

Method: Two line transects 85m long and 40m apart were marked out at the proposed trial site in the fall of 1983. A visual estimate of percentage ground cover was made using a $0.5m^2$ quadrat at 8m intervals along the transect. The percent ground cover was measured for alfalfa, smooth bromegrass, other herbage and bare ground.

Results: A summary of the study revealed there to be no significant difference (Mann-Whitney U-test at 0.05 level of probability) between the two line transects.

Percentage ground cover of classes recorded

Class	Transect 1	Transect 2
	% ground cover	
Alfalfa	15	13
Bromegrass	68	66
Other herbage	3	4
Bare ground	14	17

8.5 Appendix 5 Harvest dates and defoliation regimes† of trial A in 1984 and 1985.

harvest date‡	Defoliation regime		
	1 cut/yr	2 cuts/yr	4 cuts/yr
May 30			x
June 27		x	x
July 24			x
Aug. 22	x	x	x

†The same harvest date and defoliation regime were applied to the three nitrogen treatments.
‡Cutting dates varied 1-3 days from 1984 and 1985.

8.6 Appendix 6 Acid-pepsin dry matter disappearance procedure.

The following chemical procedure was used to determine the digestibility indicator acid-pepsin dry matter disappearance (APDMD) for all herbage material assayed:

1. Samples were uniformly ground (on a Willey mill, 1mm screen thoughly mixed and reground on a Udy cyclone mill, 1mm screen), and dried forced draft oven at 70°C for 48 hours.

2. A 0.5 gram subsample of each sample was placed in a 125ml Erlenmyer flask to which was added 50ml of the acid-pepsin solution. This mixture was agitated at room temperature on a horizontal shaker for 20 minutes to ensure thorough wetting of the forage.

3. When shaking was completed an additional 25ml of the enzyme solution was run over the inside walls of the flask to wash all the herbage to the bottom of the flask and to provide the recommended volume.

4. The Erlenmyer flask was then incubated at 39+1°C for 24 hours without agitation.

5. The acid-pepsin solution was prepared just before use by diluting 6.1ml conc. HCl to 1 litre with distilled water, warming to 40°C, adding 2g pepsin powder (1:10,000 from Fisher Scientific P-53) and stirring until completely dissolved. Several drops of iso-amyl alcohol was added.

6. Folded filter papers (11cm, Whatman #1) were dried overnight at 100°C in a forced draft oven in uncovered aluminium weighing pans. Filter papers and aluminium weighing pans were weighed after cooling in a desiccator.

7. The Erlenmyer flasks were removed from the incubator and samples were filtered immediately. The flasks were washed with 25ml of hot distilled water; an additional 25ml was used to wash the filter paper.

8. The filter papers plus residue were dried at 100°C for 24 hours in their respective aluminium weighing pan, then placed in a desiccator to cool before weighing.

The percent APDMD was calculated as follows:
APDMD= 100 dried sample-(residue of incubated sample+residue blank)/ dried sample

8.7 Appendix 7 A diagram of the optical properties used in the Technicon 500 and 300 model B NIR instruments.

(Adapted from Operation manual for the Technicon InfraAlyzer 500. Technical publication number TA8-2512-000 Technicon Industrial Systems.)