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Digestibility, perloline content, and year-round production of tall fescue (*Festuca arundinacea schreb*) as influenced by soil fertility and stock-piling schemes

Richard Warren Loveland

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To the Graduate Council:

I am submitting herewith a dissertation written by Richard Warren Loveland entitled "Digestibility, perloline content, and year-round production of tall fescue (*Festuca arundinacea* schreb) as influenced by soil fertility and stock-piling schemes." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Henry A. Fribourg, Major Professor

We have read this dissertation and recommend its acceptance:

John H. Reynolds, James B. McLaren, Karl M. Barth

Accepted for the Council:

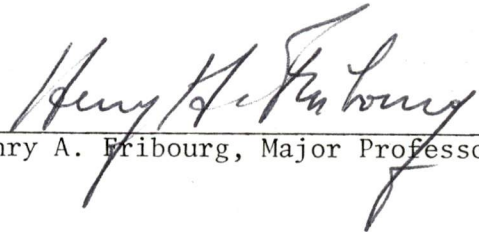
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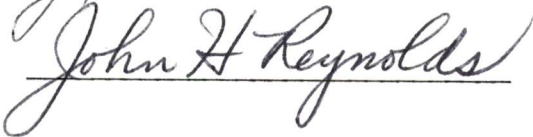
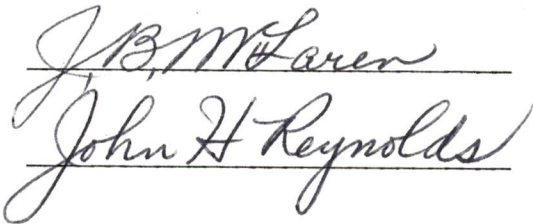
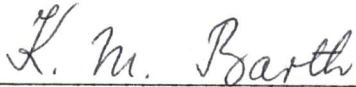
To the Graduate Council:

I am submitting herewith a dissertation written by Richard Warren Loveland entitled "Digestibility, Perloline Content, and Year-Round Production of Tall Fescue (Festuca arundinacea Schreb.) as Influenced by Soil Fertility and Stockpiling Schemes." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Science.

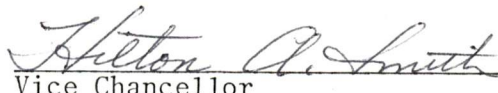


Henry A. Fribourg, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



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Graduate Studies and Research

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DIGESTIBILITY, PERLOLINE CONTENT, AND YEAR-ROUND
PRODUCTION OF TALL FESCUE (FESTUCA ARUNDINACEA
SCHREB.) AS INFLUENCED BY SOIL FERTILITY
AND STOCKPILING SCHEMES

A Dissertation
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Doctor of Philosophy
Degree
The University of Tennessee

Richard Warren Loveland

March 1976

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ABSTRACT

The in vitro digestible dry matter (DDM), perloline content, yield, and chemical composition of Kentucky 31 tall fescue (Festuca arundinacea Schreb.), grown on an Etowah clay loam soil, were determined in two experiments involving various fertility and stockpiling management practices. The chemical composition included analyses for $\text{NO}_3\text{-N}$, N, P, K, Mg, and Ca. Samples also were taken to determine the percent dry matter. Nitrogen fertilization rates were 0, 50, 63, 125, and 250 kg of N/ha, applied from one to seven times per year, depending on the rate. Phosphorus and K rates, when applied, were 30 kg of P and 60 kg of K/ha. Stockpiling schemes consisted of spring, summer, late summer, fall, and spring/fall stockpiling periods.

Nitrogen fertilization increased the yield and the contents of perloline, $\text{NO}_3\text{-N}$, N, and K of tall fescue forage. High rates of N fertilization increased the DDM of tall fescue. Nitrogen fertilization decreased the percent dry matter and had variable effects on the P, Ca, and Mg contents. Phosphorus and K fertilization had little effect on any of the variables analyzed.

Spring stockpiling resulted in the highest yields of the stockpiling schemes. The summer regrowth after spring stockpiling was higher in DDM and content of perloline, N, and K than the continuously clipped fescue. Fall stockpiling resulted in lower yields than the other schemes. Stockpiled forage was lower in DDM and content of N and P, but variable in contents of K, Mg, and Ca.

Nitrogen fertilization was the only variable that increased the perloline content other than the increase observed in the regrowth after spring stockpiling. The higher levels of perloline did not affect DDM.

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CHAPTER I

INTRODUCTION

Tall fescue (Festuca arundinacea Schreb.) is a forage grass that is easy to establish and maintain in Tennessee and surrounding states. It is estimated that there are about three million acres of tall fescue pasture in Tennessee. This is about three fifths of the total acres of pasture in Tennessee. Tall fescue has a high-yield capacity when properly managed. Investigators have found that the digestibility of tall fescue and its chemical composition indicate that tall fescue should be a high quality forage. However, poor performance by animals eating tall fescue, especially during summer months, has caused tall fescue to be considered a low quality forage. Quality and production of tall fescue are low in summer and winter and high in spring and fall. Increasing the yield and quality of fescue, especially during the summer and winter, would be of great importance in improving forage production.

The objectives of this investigation were to evaluate the effects of several rates and times of fertilizer applications, particularly nitrogen, and the effects of several stockpiling schemes on tall fescue quality and quantity. Digestibility as an estimate of forage quality and perloline content as an adverse factor affecting forage quality were of primary concern. Dry matter yield, percent dry matter, and content of nitrate-nitrogen, phosphorus, potassium, magnesium, calcium, and nitrogen were determined.

Stockpiling, i.e., letting growth accumulate in the field during spring and fall, could provide additional forage for summer and winter, respectively. The stockpiling schemes were designed to determine the time periods when stockpiling would be appropriate for use in Tennessee.

Small amounts of nitrogen fertilizer applied at frequent intervals during the summer months have increased summer productivity of tall fescue without significant damage to the stand. Fall fertilization has produced earlier spring growth without stand damage during the winter. Fall fertilization also may help avoid the spring "grass tetany" problem.

The alkaloid perloine and nitrate-nitrogen are thought to be among the factors that result in poor animal performance when animals are eating fescue. Finding management practices that would decrease the forage content of these materials may increase the forage quality.

CHAPTER II

LITERATURE REVIEW

Management practices such as stockpiling and fertilizer applications are known to influence the quantity and quality of tall fescue. Measurements of dry matter yield; digestibility; plant content of N, P, K, Ca, Mg, $\text{NO}_3\text{-N}$, and perloline; and other measurements have been used to estimate the influence of various management practices on the quality and quantity of tall fescue forage. Fertilization and content of N, K, Ca, Mg, and P; stockpiling; in vitro digestion; perloline; and $\text{NO}_3\text{-N}$ are areas in which much research with tall fescue has been done.

I. FERTILIZATION AND CONTENT OF N, K, CA, MG, AND P

Woodhouse and Griffith (1973) and Rhykerd and Noller (1973) reviewed the fertilization of forages and the relationships of N, P, K, Mg, and Ca to forage production. Some aspects of their reviews which are common knowledge follow.

Phosphorus is an important nutrient in growth and cell division and tends to be concentrated in young, actively growing tissues. In P fertilization, residual P is an important consideration since the P in the forage is often derived from fertilizers applied in previous years.

Potassium is required for protein synthesis, the formation and translocation of sugars and starches, stomatal action, and neutralization of organic acids within the plant. Levels of 2 to 3 percent K in the plant are adequate under most conditions, while levels above 3.5 percent are considered "luxury consumption" and wasteful absorption by the plant, except in cases of very high yields.

The Mg in tall fescue is essential for chlorophyll formation and is important in forage growth and animal health.

Nitrogen is a constituent of proteins and chlorophyll of green plants, and is essential for photosynthesis, growth, and reproduction. Productive grasses respond to applied N unless other nutrients, moisture, temperature, exceptionally high soil supply of N, or other factors sharply limit growth. Increases in productivity of fescue may not occur when N is applied during its summer dormant period. Heavy N applications at this period may weaken the sod and make plants more susceptible to drought or disease injury, or to cold injury in the winter. With the higher rates of applied N, the N content in fescue usually increases even beyond the point where yields cease to increase. The N content may be quite high for two or three weeks after application. It then declines rather rapidly over the next several weeks, thus making the time interval between application and harvesting (cutting or grazing) an influence on the N content.

Increased percentages of alkaloids and total organic acids, greater carrying capacity, and more livestock products per ha generally result from N fertilization of grasses, while individual animal performance usually is not improved and may not be as good as on comparable

grass receiving no N. The percent dry matter of forage crops tends to be decreased and the percentage of water-soluble carbohydrates in plants generally is decreased by N fertilization, while crude protein is increased. According to Rhykerd and Noller (1973) this may result in an imbalance between protein and energy, especially in cool-season grasses, and may be responsible for the low animal performance and the health problems frequently associated with the application of fertilizer N.

Nitrogen fertilization of tall fescue at rates of 448 kg/ha/yr, of which 200 kg/ha were applied in three equal summer applications, caused severe stand depletion (Hallock et al., 1965). Excellent stands of fescue were maintained when annual rates up to 224 kg/ha were used, of which 90 kg/ha were applied in three equal summer applications. Hallock et al. (1973) postulated that tall fescue might tolerate and respond favorably to high rates of N if the N was applied frequently in small increments. Annual rates up to 549 kg/ha, of which 381 kg/ha were applied in 17 equal summer applications, resulted in improvement of stands of tall fescue. Annual rates of 834 and 1,120 kg/ha, of which 666 kg and 952 kg were applied in the summer, caused a 30 percent stand depletion. Alexander and McCloud (1962) found that 134 kg/ha applied to tall fescue in April, in June, and in September resulted in stand reductions of about 25 percent.

Chamblee (1972) indicated that when 224 kg/ha of N were applied between June 1 and August 15, stands of tall fescue were completely eliminated. McKee et al. (1967) reported that stands of tall fescue were reduced by high N levels applied in spring when grass was cut at

flowering stage, but stands were not reduced when grass was cut frequently. Stands and regrowth during summer were reduced only slightly by high rates of N applied in spring. Woodhouse and Chamblee (1953) found that increasing the N applied to fescue increased yield and N content. Regardless of the rate of N applied, plants made very little growth during periods of extreme cold or drought. Improved forage yields in the fall as well as a carry-over of added N into the following spring when large amounts of N were applied in late July have been noted by Ryan et al. (1972).

Powell et al. (1967) fertilized tall fescue in the fall and winter with N. When N was applied in October and followed by monthly or bimonthly N applications, foliage color was maintained throughout the winter, and spring growth rates were rapid. Although no measurable top growth occurred during winter, turf fertilized with high rates of N was very dense. Ledebor and Skogley (1973) reported that late November N fertilization of cool-season grasses resulted in more uniform turf quality. Grasses maintained better winter color and greened up earlier in spring. Lane et al. (1975) have studied the relationship of plant color to the nutritive value and yield of tall fescue within season. Significant correlations were found between color and crude protein percentage and between color and dry matter yield.

The National Research Council (1970) suggested nutrient requirements for beef cattle. The following requirements were expressed as percent dry matter of diet: (1) a dry, pregnant beef cow requires at least 0.18 Ca and P, 0.60 K, 0.95 N; and 0.04 Mg; (2) a lactating beef cow

requires a minimum of 0.29 Ca, 0.23 P, 0.80 K, 1.47 N, and 0.10 Mg. The percent N was calculated by dividing 6.25 into the total protein requirement. It is noted that 0.20 percent Mg has been considered by Kemp (1960) as a safe level in forages in relation to grass tetany.

Reid et al. (1970) noted that increasing the level of N fertilizer applied to forages increased the content of K in the plant. There was also a tendency for N to increase the levels of Ca and Mg, and to depress the content of P. Phosphorus content of the herbage tended to decline with advancing maturity. Deficiency of K was not a problem, but an excess of K was more likely to create nutritional problems through interference with the utilization of other elements. Increasing levels of N markedly increased the content of K in the grass over that in either the unfertilized fescue, or in the fescue treated with K or P fertilizer alone. When compared with the control treatment, the tall fescue had little or no increase in K content in response to the application of a high level of K fertilizer.

The Mg content of pasture had a marked seasonal variation, being lowest in spring and increasing until autumn (Todd, 1961). Todd reported that K is known to have a depressing effect on Mg uptake and that responses to fertilizer applications of N and P have been inconsistent.

Potassium content in tall fescue was found to be highest during March and April (Hannaway, 1975). Calcium and Mg contents were lowest during the cooler months and increased during the summer months. Phosphorus content was markedly affected by season, with the highest contents occurring during the warmer summer months. The Mg content was

significantly depressed by K fertilization from November through April. Hannaway suggested that N fertilization be divided into three applications to reduce the negative effects of N fertilization on the K:(Ca+Mg) ratio in early spring and to provide for a more uniform distribution of dry matter. He suggested that K fertilization should be delayed until after the spring growth period (grass tetany period) to reduce the possible danger to animals grazing the forage produced.

Phosphorus content in tall fescue was 0.6 percent in April (Fleming and Murphy, 1968) and declined steadily to below 0.3 percent in late July; thereafter, it increased and varied between 0.5 and 0.4 percent from September through December. Levels of K varied from 2.5 to 4.7 percent, being highest in May and June, and lowest in December. Levels of Ca generally increased as the season advanced and ranged from 0.6 to 1.0 percent. Levels of Mg increased as the season progressed until mid-September, and then declined, ranging between 0.2 and 0.7 percent.

Contents of P and Ca in fescue decreased with age while that of Mg increased (Sabbe and Hileman, 1974). As soils became drier, N and K contents decreased. At the young growth stage and with a slightly dry soil moisture level, decreases in plant Mg were correlated with increases in plant N. Correlations of Mg with K indicated a decrease in plant Mg as plant K increased.

II. STOCKPILING

In Tennessee, tall fescue stockpiled from May to September or from September to May contained from 6 to 9 percent crude protein or from 6 to

10 percent crude protein, respectively (Reynolds, 1975). These results were compared to the nutritional requirements of a 500 kg lactating beef cow (National Research Council, 1970) and it was found that this type of animal would require supplemental protein. The crude protein content of the forage was found to be adequate for a dry, pregnant 500 kg beef cow.

White (1975) described tall fescue stockpiling in Virginia. Nitrogen fertilizer was applied at rates of 73 to 112 kg/ha between August 2 and September 4. Average crude protein content decreased from 14.1 percent in October to 11.6 percent in January. TDN levels decreased from 63 to 60 percent. The average hay-equivalent yields were 2.2 kg/ha throughout the winter.

Accumulating growth for the entire growing season gave the highest dry matter yields of stockpiled tall fescue in Missouri (Matches et al., 1973). The yields of the stockpiled fescue accumulated until September were above 4 tons/ha. Yields of the fescue accumulated beyond September decreased with time. Stockpiling after May 10, June 21, or August 2, yielded less dry matter in proportion to the period of stockpiling when harvested in January, but the forage available for grazing was of higher quality. Dry matter yields of stockpiled fescue harvested on October 25, from starting dates of May 10, June 21, and August 2, were 2,614, 1,211, and 774 kg/ha, respectively. Yields harvested on January 18 for the same starting dates were 2,005, 849, and 510 kg/ha, respectively. The authors concluded that losses in dry matter and forage quality following the onset of freezing weather may

not be serious if the producer is interested only in a maintenance ration for dry beef cows. If the goal is to obtain maximum efficiency per acre, it seemed desirable to graze the stockpiled growth by early winter.

Spring-stockpiled tall fescue pasture yielded from 4.7 to 5.6 tons of dry matter/ha when harvested during the month of June (Bryan et al., 1970). Tall fescue stockpiled in spring and summer yielded 4.6 to 6.2 tons/ha when harvested in late July. Fall stockpiling yielded 4.7 to 5.0 tons/ha when harvested in early November and 2.9 to 3.9 tons/ha when harvested in late November. Fall-stockpiled fescue could extend the grazing season for up to 2 months (Wedin et al., 1967). They recommended applying at least 67 to 124 kg/ha of N in late July, stockpiling until mid- or late October, and then grazing in November and December.

III. DIGESTIBILITY

In vitro measurements of digestibility have been found to be highly correlated with in vivo measurements. The most widely accepted in vitro procedure being used routinely in many laboratories is the Tilley and Terry two-stage in vitro rumen fermentation system, or a modified version of that procedure (Mott, 1973). The modified two-stage Tilley and Terry method was the most reliable predictor of in vivo digestibility in studies by Scales et al. (1974).

There are differences of opinion concerning the effects of the diet of the donor animal on in vitro digestibility measurements. Nelson et

al. (1972) reported a highly significant difference in the in vitro digestible dry matters of the substrates when digested with inocula from different diets and donor animals without urea and glucose. With the addition of these nutrients, no significant differences were found in the in vitro digestible dry matters among donor animals. The data of Monson and Utley (1974) indicate that a diet change need not interrupt a forage screening program aimed at relative ranking of substrate, but that it could jeopardize nutritive evaluations. The authors referred to others who considered the effect of diet to be small and to some who consider careful diet control to be essential. Scales et al. (1974), found that in vitro studies employing inoculum from grazing animals did not provide as reliable a prediction of in vitro digestibility as did studies where inoculum from an animal fed a standard diet of grass hay had been used.

After in vitro incubation with rumen microorganisms for 48 hours, 48 percent of tall fescue leaf tissue dry matter had disappeared (Akin et al., 1973). Within this time, mesophyll and outer bundle sheath tissues were degraded. The epidermis, inner bundle sheath, and small vascular bundle tissues were in the initial stage of degradation. After 72 hours, the epidermal tissue had been degraded.

Terry and Tilley (1964) found that all parts of tall fescue and other forages have a high digestibility at early stages of growth. As maturity progresses, the digestibility of the stem decreases at a much faster rate than that of the leaf. They associated the decline of digestibility with a reduction in the content of water-soluble constituents and protein in the plant, and with a reduction in the digestibility of fiber.

According to Lassiter et al. (1956) a number of workers have shown that the digestibility of pasture grasses is highest during the early part of the pasture season and declines as the season advances, with a further decline or increase in late summer or early fall depending on moisture and temperature conditions. In comparing tall fescue with other grasses, they found that dry matter digestibility was high when the dry matter content of the grasses was low. However, Buckner et al. (1967) reported that digestibility of tall fescue and other cool-season grasses was higher in fall than in summer or spring. Means of all grasses were approximately 69 percent digestibility in the spring, 66 percent in the summer, and 73 percent in the fall. The grasses were clipped throughout the growing season.

Results from Matches et al. (1973) involved the total accumulation of tall fescue through the entire growing season. Harvests were taken throughout the growing season to determine what influence the period and duration over which growth was accumulated had on the quantity and quality of stockpiled tall fescue. Accumulated yields of dry matter were above 4 tons/ha in September. In mid-April, digestibility was above 70 percent. By May 20 (early to full heading stage) digestibility had decreased to 65 percent. From mid-June through March, digestibility averaged between 45 and 55 percent. In a second experiment, growth accumulated from May 10 and harvested on October 25 had approximately 58 percent digestible dry matter (DDM) (Matches et al., 1973). It had decreased to 50 percent DDM when harvest was delayed until January 18. Fescue stockpiled from June 21 to October 25 had 66 percent DDM, but

digestibility had decreased to 54 percent DDM when harvested on January 18. Growth accumulated from August 2 to October 25 had 68 percent DDM. When it was stockpiled until January 18, the DDM decreased to 59 percent. Stockpiled tall fescue harvested on May 27 when the fescue was still in a vegetative stage had 68 percent DDM (Pritchard et al., 1963). The June 5 harvest had 65 percent DDM and was still in the vegetative stage. The June 14 harvest, when 10 percent of the fescue was headed, had 59 percent DDM. The DDM continued to drop as the fescue matured and was 38 percent on the July 24 harvest at full maturity. The authors stated that it is well recognized that digestibility of forages decreases as plants mature, and they suggested that this decrease depends on type of herbage and stage of morphological development.

According to Rhykerd and Noller (1973) nitrogen fertilization of grasses does not have a pronounced effect on digestibility. Reid and Jung (1965) found no differences in DDM of tall fescue hay from N fertilized and nonfertilized plots. However, N fertilization resulted in increases of DDM of aftermath cuttings. In vitro fermentation was faster in fescue hay fertilized with P than in hay fertilized with N.

Tall fescue has compared favorably with other cool-season grasses in digestibility, but intake has been lower and more variable than those of other grasses. Low correlations between digestibility and voluntary intake were obtained in feeding trials (Rhykerd and Noller, 1973). Some workers (Buckner et al., 1973; Bryan et al., 1970; Warren et al., 1974) have suggested that although digestibility is an effective

measure of forage quality in many cool-season grasses, it does not seem to be so for tall fescue.

Buckner et al. (1973) suggested that undesirable constituents are responsible for the erratic nutritional quality of tall fescue and subsequent poor animal performance during late spring and summer. One undesirable constituent is the alkaloid perloine. Boling et al. (1975) reported that apparent crude protein digestibility and cellulose digestibility coefficients were lower in the group of lambs with perloine added to their diet, than in the control group of lambs.

IV. PERLOINE

Introduction

Tall fescue has been associated with poor animal performance, which usually occurs during the summer months, and as a result it has been classified often as a low quality forage. Bush and Buckner (1973) defined a high quality forage as one high in usable nutrients and acceptable to animals. They concluded that the chemical composition of tall fescue would rate it as a high quality forage, but that its ability to provide the nutrient requirements of an animal would be a better measure of quality. They also referred to several investigations that illustrate the extreme variability of animal performance on tall fescue pastures.

In trials using orchardgrass, smooth bromegrass, and Kentucky bluegrass, animal performance was consistent (Jacobson et al., 1970). The tall fescues in these trials led to variable animal performance, ranging from excellent to very poor.

Tall fescue compared favorably with other cool-season grasses in digestibility but was not consumed as well, and intake by cattle was more variable than that of other grasses (Buckner et al., 1973). The authors felt that undesirable constituents were responsible for the poor animal performance during late spring and summer. Poor animal performance paralleled the accumulation of alkaloids in tall fescue during the growing season (Bush and Buckner, 1973).

Alkaloids usually have the following properties: they are chemically basic and have a complex molecular structure; they contain nitrogen, are of plant origin, and have significant pharmacological activity (Pelletier, 1970). Many alkaloids are related to amino acids (Robinson, 1974). Indole alkaloids come from tryptophan, benzyloquinoline alkaloids from tyrosine, pyrrolidine alkaloids come from glutamic acid, and some piperidine alkaloids come from lysine. In the past, alkaloids have been thought to be inert end products, but in many cases they are in a dynamic state, fluctuating in both total concentration and in rate of turnover. The observed variations in content often correlate well with developmental stages. It is important to note that the presence of an alkaloid in a plant indicates that the rate of degradation is slow enough to permit some accumulation, and also that the plant can tolerate the alkaloid. Some plants that lack alkaloids may, in fact, degrade them faster than they synthesize them.

Pelletier (1970) also stated that plants are known to synthesize many alkaloids from amino acids. According to him, the functions of alkaloids are still largely unknown. It has been speculated that they

may be (1) by-products of plant metabolism; (2) reservoirs for protein synthesis; (3) protective materials discouraging animal or insect attacks; (4) plant stimulants or regulators in such activities as growth, metabolism, and reproduction; or (5) detoxicating agents, which render harmless, by processes such as methylation, condensation, and ring closure, substances which by their accumulation might otherwise damage the plant.

Properties of Perloline

In 1941, Melville and Grimmett reported the presence of a yellow, fluorescent alkaloid in perennial ryegrass (Lolium perenne L.) and called this base perloline. The structure of perloline and related compounds is shown in the Appendix, Figure 41. Reifer and Bathurst's (1942) preliminary data on the amounts of total alkaloids and perloline in ryegrass showed considerable variation. In certain periods of the year and at different stages of growth, the alkaloids varied between less than .05 mg/g to more than 1.00 mg/g on a dry weight basis. In addition to ryegrass, the only species found to contain comparable amounts was tall fescue, and these amounts varied from 0.02 mg/g to 0.40 mg of perloline/g of dry matter (Grimmett and Waters, 1943). The same authors separated fractions containing alkaloids designated A, B, and C. Fraction A was perloline. Fraction C was colorless with a bright blue fluorescence in ultraviolet light. Fraction B was amber, pink, or red, and fluorescent.

The most spectacular property of perloline was the marked fluorescence of solutions of the base in chloroform and alcohol

(Reifer and Bathurst, 1943). Such solutions were golden yellow to almost orange with transmitted light, depending on concentration. Perloline was found to be soluble in chloroform, alcohol, and acetone, and very slightly soluble in ether, benzene, and water. Oxidation of perloline with permanganate in sulfuric-acetic acid yielded a colorless material in alkaline solution and exhibited a blue fluorescence in acid. The authors found no single environmental factor that could be correlated with a high or low content of perloline. They generally concluded that at times of poor growth the alkaloid content was negligible, and that when growth was rapid, particularly when the N supply to the plant was adequate, the content was high. They noted large variations in content over short periods of time, and observed that a sudden change in weather conditions sometimes resulted in a rapid and marked decrease in alkaloid content.

Cunningham and Clare (1943) concluded that the amounts of perloline ingested by grazing animals were unlikely to cause harmful effects. They found no cumulative effect from continued oral intake of perloline in sheep. It was observed that rapid destruction of perloline occurred after oral administration, or after intravenous or intraperitoneal injection. From observations made in vitro, however, it was concluded that perloline was not destroyed by the contents of the alimentary canal. The alternative was rapid absorption and destruction within other organs of the body. Evidence indicated that perloline movement was rapid along the alimentary tract, and that one site of destruction of perloline was the liver.

Yates (1963) detected nine alkaloids by paper chromatography in extracts of tall fescue hay. Three of these alkaloids exhibited fluorescence. Perloline appeared at R_F 0.46. One colorless alkaloid that had a light-blue fluorescence in ultraviolet light appeared at R_F 0.60, similar to that emitted by fraction C of Grimmett and Waters (1943). A third alkaloid had a violet color, fluoresced, and appeared at R_F 0.65. Although the fluorescent color was not given, this alkaloid seemed similar to fraction B found by Grimmett and Waters in 1943. Yates also detected a nonfluorescent alkaloid at R_F 0.10; this compound accounted for about one half of the alkaloidal material separated from tall fescue. Yates and Tookey (1965) later identified this alkaloid as festucine, $(C_8H_{14}N_2O)$, a positional isomer of loline. Structure formulas for festucine and loline are displayed in the Appendix, Figure 41.

Jeffreys (1964) separated 10 alkaloids from perennial ryegrass and classified them in zones. Zone 8 was identified as perloline, zone 3 was later identified as perlolidine, and zone 4 was later identified as perlolyrine. Structural formulas for the above are in the Appendix, Figure 41.

Perloline and various methods of degrading it were discussed by Jeffreys and Sim (1963). They showed that perloline degraded to yield perlolidine $(C_{12}H_8N_2O)$. Ferguson et al. (1966) suggested that the precursors of perloline $(C_{20}H_{17}N_2O_3)$ may be tryptophan and 3,4-dihydroxyphenylalanine. Akhtar et al. (1967) discussed the synthesis of perlolidine and described it in chromatography as a colorless spot

with a brilliant violet-blue fluorescence under ultraviolet light at R_F 0.67. This is similar to those alkaloids noted by Yates (1963) and Grimmett and Waters (1943). Jeffreys (1970) synthesized perlolyrine ($C_{16}H_{12}N_2O_2$) from tryptophan and 5-acetoxymethyl-2-formylfuran. Perlolyrine was identified as the zone 4 alkaloid isolated by Jeffreys in 1964.

Perloline in Plants and Plant Parts

Perloline was found by Manske (1955) in only five out of 85 species of Gramineae, Cyperaceae, and Juncaceae that were examined: Lolium perenne L., L. temulentum, L. multiflorum Lam., Festuca arundinaceae Schreb., and Setaria lutescens (Weigel) Hubb. Jeffreys (1964) stated that perloline was found also in Dactylis glomerata L. and Phleum pratense L.

Gentry et al. (1969) measured perloline content at different stages during growth from seed to maturity. Perloline was not measurable in dormant seed, but appeared during formation of primary roots before shoot formation. In rapidly growing vegetative pasture plants, the highest content of perloline occurred in the roots. As the fescue plant approached maturity, the perloline content was reduced. At the dough stage, the stems contained more perloline than did the leaves, roots or heads.

Hagman et al. (1975) showed that leaf blades of reed canarygrass contained more than twice as much alkaloid as leaf sheaths or stems. This seems to be opposite to what Gentry et al. (1969) found in fescue.

They further stated that the upper third of the total herbage had higher and more uniform alkaloid contents than the middle and lower thirds. Marten (1973) suggested that highest alkaloid contents occur in leaf sheaths, stems, roots, rhizomes, and inflorescences. He stated that alkaloid content was enhanced in reed canarygrass by moisture stress, high N fertilization, and low light intensities, and was highest in immature grass. Marten also found no consistent relationship between alkaloid content and in vitro digestibility.

According to James (1950) the alkaloids in living tissues are invariably found as water soluble salts dissolved in the cell vacuole, while in dried tissues the alkaloids may be found impregnating the cell walls. The accumulation of alkaloids also occurs during the active growth of juvenile tissues. It follows that any factor tending to increase growth of an individual plant will be likely to increase the total formation of alkaloids.

Perloline Content in Plants

Perloline, festucine, and three other alkaloids of the pyrrolizidine class were identified by Robbins et al. (1972) in tall fescue seed and forage. The three pyrrolizidine alkaloids are named N-acetyl loline, N-formyl loline, and dimethyl N-acetyl loline. Tall fescue fertilized with NH_4NO_3 in 1969 and 1970 was analyzed for perloline. In 1969, the measured perloline level varied from less than 0.05 to 0.50 mg/g dry weight. In 1970, it ranged from less than 0.05 to 1.0 mg/g dry weight.

Gentry et al. (1969) found eleven alkaloids in tall fescue by paper and thin layer chromatography. Among these were perloline at R_F 0.32

and festucine at R_F 0.10. Levels of perloline in tall fescue fertilized at high rates of N, P and K ranged from 0.17 to 1.59 mg/g dry weight. Levels of perloline in the unfertilized fescue varied from 0.10 to 1.04 mg/g dry weight. Plants that were fertilized with K and P contained less perloline than plants that were not fertilized. Perloline contents in the fescue fertilized with P and K varied from 0.03 to 0.70 mg/g dry weight. These results are similar to those of Bennett (1963) in which the perloline content was significantly higher in perennial ryegrass plants receiving applied N. However, the application of P either alone or with N had no significant effect on the perloline content.

In a study of the influence of nutrient supply on reed canarygrass alkaloids, it was concluded that P and K fertilization of peat soils deficient in these elements significantly reduced alkaloid content, provided applied N levels did not exceed 240 kg/ha (Marten et al., 1974). They hypothesized that the lower alkaloid contents were primarily due to increased K availability since addition of P to a P-deficient peat soil did not affect alkaloid content. They found that ammonium sources of N (NH_4Cl and urea) resulted in greater alkaloid contents than did a nitrate source (NaNO_3), while grass supplied with both sources (NH_4NO_3) had an intermediate alkaloid content.

Perloline and Digestion

The alkaloid perloline has been shown to inhibit in vitro ruminal cellulose digestion (Bush et al., 1970), fatty acid production, and growth of rumen cellulolytic bacteria (Bush et al., 1972). The authors hypothesized that the alkaloids inhibited the rate of digestion in the

rumen; subsequently, the rate of passage through the animal decreased, thereby decreasing energy and nutrient availability to the animal.

Bush and Buckner (1973) reported on the effect of rumen inoculum from animals grazing high- or low-perloline tall fescue pastures on in vitro cellulose digestion. Digestion of the dried pasture grass with the high perloline content was 27 percent greater with rumen inoculum from the animal on the high-perloline grass than with inoculum from the animal on the low-perloline grass. No difference was observed in the digestion of the low-perloline grass with the two rumen inocula. These observations suggest an alteration in the microflora of the rumen caused by the alkaloid components which influence the rate of digestion. Bush et al. (1972) suggest that the concentration of perloline and length of time the rumen microorganisms are exposed to perloline dictate the extent of toxicity of the alkaloid. The toxicity also may be selective—at some concentrations and times of exposure, some organisms may be killed and others not affected.

V. NITRATE-N

Wright and Davison (1964) and Hanway et al. (1963) have published comprehensive reviews on nitrate accumulation in plants and nitrate poisoning in animals. These two conditions are more likely to become problems in forage production when high rates of commercial nitrogen fertilizers are used.

Nitrate poisoning can be lethal or sublethal depending on the bacterial action in the digestive tract of an animal. When nitrates are

ingested in sufficient quantity, they may cause gastroenteritis and diarrhea, and reduce the performance of animals. When bacterial action converts nitrate to nitrite and nitrite is absorbed into the bloodstream, death may occur. Absorption of the nitrite by the hemoglobin results in the formation of methemoglobin, which does not release oxygen to the cells of the body tissues. Nitrites in the rumen also are converted by microorganisms to NH_4^+ and subsequently to amino acids and proteins. If this process is rapid enough, ingested nitrates may have little apparent effect on ruminant animals. Nitrate poisoning usually occurs when animals graze or are fed forages that have high contents of nitrates. Nitrate contents in plants are influenced by root uptake of NO_3^- in the soil, by nitrate reductase activity, and possibly by oxidation of some other nitrogenous compound.

There is much uncertainty as to the critical level of $\text{NO}_3\text{-N}$ accumulation in forages being fed to animals. Ryan et al. (1972) considered $\text{NO}_3\text{-N}$ percentages greater than 0.15 percent as potentially unsafe. George et al. (1972) found a maximum $\text{NO}_3\text{-N}$ content in tall fescue of approximately 0.18 percent obtained from a 168 kg/ha N fertilizer application. Average daily gain of grazing steers was not influenced by increasing increments of N fertilizer up to 168 kg/ha. Reid and Jung (1973) suggest that levels of 0.34-0.45 percent $\text{NO}_3\text{-N}$ may be regarded as potentially toxic. Much lower levels may affect growth performance or metabolism in the animal. They also pointed out that the total quantity of NO_3^- consumed is important rather than the content in the forage.

According to Hanway et al. (1963) light is essential for plant growth and for reduction of nitrates in plants. Nitrate contents of plants may be higher in the morning than in the evening, and may be higher on cloudy days than on sunny days. Factors that restrict the normal growth and development of a plant, such as drought, hail, frost, or disease, may result in increased NO_3^- contents of plants if nitrates are available in the soil.

High $\text{NO}_3\text{-N}$ contents have been associated with drought conditions. According to Wright and Davison (1964) a long sustained drought is not as likely to bring about NO_3^- accumulation as a brief drought. After a state of dormancy due to drought, plants may accumulate a dangerous amount of NO_3^- within a few days after a rain, once microbial release and processes of transport are stimulated. Nitrate accumulation may be rapid also when a plant experiences a moisture shortage. Nitrate reduction by nitrate reductase may be slowed while the soil may be wet enough to sustain mineralization and delivery of nitrogen, especially if the plant is losing turgor only during the hours of maximum evaporative load and regaining it at night. Plant roots must have both nitrates and moisture present for an accumulation of $\text{NO}_3\text{-N}$ to take place in plants.

Nitrate-N contents of three cool-season grasses were significantly lower in material harvested during periods of cooler temperature and favorable moisture with rapid growth and high yield. Contents reached a maximum during July and August cutting dates when temperature and moisture stress reduced yields (George et al., 1973).

Crawford et al. (1961) concluded that decreases in the contents of $\text{NO}_3\text{-N}$ as oat plants matured were due in part to a decrease in the NO_3^-

concentration that was available from the soil, and in part to a dilution in the content of $\text{NO}_3\text{-N}$ from increased yield. Experimenters have found that repeated or delayed applications of N through the season will overcome in part the usual downward trend in $\text{NO}_3\text{-N}$ content (Wright and Davison, 1964).

Hojjati et al. (1973) showed that NO_3^- accumulation peaks almost always were reached within 14 days following fertilization of tall fescue, after which the values gradually declined. The length of time required for dissipation of the effect of N application on NO_3^- levels was related directly to quantity of fertilizer used. Tall fescue consistently contained 0.2 percent $\text{NO}_3\text{-N}$ or more six weeks after N fertilization at the 200 kg/ha rate. Fertilization rates of 50 kg/ha had $\text{NO}_3\text{-N}$ contents that reached a maximum within one week. For this rate the highest level of $\text{NO}_3\text{-N}$ was less than 0.1 percent when fertilized April 9 but was over 0.2 percent when fertilized May 21. This investigation showed that $\text{NO}_3\text{-N}$ levels also varied with growth periods or time of year. The authors suggested that such changes could be related to one or more aspects of the environment and/or developmental physiology of the plants.

Vanderlip and Pesek (1970) reported that early in the season the applied nutrients N, P, and K increased the $\text{NO}_3\text{-N}$ content of smooth brome grass (Bromus inermis Leyss.). As the season progressed, only applied N had an effect. The $\text{NO}_3\text{-N}$ content decreased sharply with time after application. Murphy and Smith (1967) found that increased N fertilization had little if any effect upon the $\text{NO}_3\text{-N}$ content of various

species. Phosphorus fertilization of soils has raised the $\text{NO}_3\text{-N}$ content of plants in some experiments, lowered it in others, and had no effect or variable effects in others (Wright and Davison, 1964). Some investigators have found that applied K increased the $\text{NO}_3\text{-N}$ accumulation in corn and forages (Vanderlip and Pesek, 1970), and in some species there is a strong positive relationship between $\text{NO}_3\text{-N}$ content and K content (Wright and Davison, 1964).

Sabbe and Hileman (1974) reported that plant $\text{NO}_3\text{-N}$ was correlated with plant N and with soil and plant P. The correlation with P was only in fescue samples intermediate in age and in those selected from plants grown on soils with adequate moisture. Plant K was correlated with plant $\text{NO}_3\text{-N}$ except in mature samples and samples from droughty sites. The highest $\text{NO}_3\text{-N}$ level reported was 0.068 percent.

VI. SUMMARY

The literature indicates that moisture and temperature conditions greatly influence the response of tall fescue to N fertilizer applications. Some research workers have claimed that fescue may not respond productively to N applied during the summer. Other workers have shown that frequent fertilization in smaller amounts will avoid the harmful effects of single high N applications in the summer. Evidence indicates that late fall N applications to tall fescue may give earlier and higher spring yields and may be beneficial to stand maintenance. Applied N has decreased the percent dry matter of tall fescue, and a lower percent dry matter has been associated with increased digestibility of tall fescue.

The contents of perloline, $\text{NO}_3\text{-N}$ and N in tall fescue have been increased by N fertilization.

Potassium content of tall fescue is increased by N fertilization, but K fertilization does not increase the K content.

Magnesium content of tall fescue may be depressed by K fertilization. Nitrogen fertilizer applications have increased Mg content in some cases and have decreased Mg content in others. A seasonal variation of the Mg content in tall fescue has been observed, with the lowest content occurring in the early spring months.

The P content of tall fescue is variable. It has been reported as lowest in the summer by some workers and as highest in the summer by others. Applied N may decrease the P content. Phosphorus content may decrease with plant maturity.

The Ca content of tall fescue increases during the growing season and with applied N, and decreases with maturity.

Stockpiling has extended the grazing season into the late fall and winter months, and into the summer months. Many different times of stockpiling and lengths of stockpiling periods have been studied. The maturity of the tall fescue and the length of the stockpiling period have been found to influence greatly the quantity and the quality of the fescue forage. In spring-stockpiled tall fescue, quality decreases rapidly after heading is initiated, but quantity increases. In fall-stockpiled tall fescue, both quality and quantity rapidly decrease after October and early November.

Digestibility of tall fescue has been influenced the most by seasons. It is high in spring and fall, and low in summer. Moisture

and temperature stresses may lower the digestibility in the fall. Some workers have reported higher digestibilities in the fall than in the spring. Nitrogen applications are reported not to influence greatly the digestibility of tall fescue.

Three fluorescent alkaloids have been found in tall fescue. They are perloline, perlolidine, and one which has not been named. The perloline content increases with applications of N fertilizer. It seems that K fertilizer may decrease perloline content. The upper one third of the plant may have a higher alkaloid content than the lower two thirds. Perloline in forages has not been toxic to grazing animals, but it has affected rumen bacteria, and possibly has had an effect on the performance and productivity of these animals. The effect of the perloline on digestibility seems to depend on concentration and time of exposure. It seems that the alkaloids of dried plant material impregnate the cell walls, while in fresh or living tissues the alkaloids are found in the cell vacuole. These effects, coupled with the time factor, may account for failure of in vitro studies to reflect influences of alkaloids on digestibility.

Cool temperatures and favorable moisture resulting in rapid growth and high yields are associated with low $\text{NO}_3\text{-N}$ content in tall fescue. The amount of $\text{NO}_3\text{-N}$ and its time of persistence are related to the amount of N fertilizer applied. It has been noted that with a N rate of 50 kg/ha, the $\text{NO}_3\text{-N}$ content reaches a maximum within one week after fertilization. This appears to be more likely in the early spring than at other times. The $\text{NO}_3\text{-N}$ content has been observed to increase after brief drought periods.

CHAPTER III

MATERIALS AND METHODS

I. FIELD EXPERIMENTS

Kentucky 31 tall fescue was established on an Etowah clay loam (fine-loamy, siliceous, thermic Typic Paleudults) at the Knoxville Plant Science Farm on September 25, 1972. A preplant application of 0-20-20 fertilizer at 900 kg/ha was made in late summer. The fescue was seeded at a rate of 84 kg/ha of viable seed.

Two experiments were initiated on March 1, 1973: a "fertility" experiment and a "stockpile" experiment. Each experiment had 18 treatments arranged in a randomized complete block design replicated 4 times. Individual plots measured 2.13 x 9.14 meters.

The treatments used in the fertility and in the stockpile experiments are listed in Table I and in Table II, respectively. In the fertility experiment an area of 4.87 m² (.533 x 9.14 m) was harvested from the center of each plot with a rotary mower at a 5-cm stubble. This was done throughout the year whenever growth reached 8 to 10 cm in height.

The harvesting procedure was the same for the stockpile experiments except for the periods of stockpiling. Harvesting at the end of the stockpile periods consisted of two methods: a hay cut and a gradual clipping. The hay cut was made using a sickle-bar mower at a 5-cm stubble. The area harvested from each plot was 9.75 m² (1.07 x 9.14 m). The gradual clipping, to simulate grazing, was done with a rotary mower

TABLE I

FERTILITY TREATMENTS ARRANGED IN 17 CONTRASTS USED IN THE
 ORTHOGONAL COMPARISONS FOR YIELD, % DM, NO₃-N, N,
 P, MG, CA, AND K

Treatment ¹ Number	Months and Amounts of Fertilizer Applications ²							Figure ³ Number
	Mar	May	Jun	Jul	Sep	Oct	Nov	
3	250PK	-	-	-	-	-	-	1
14	125PK	-	-	-	125PK	-	-	
9	63PK	63	-	63	63PK	-	-	2
14	125PK	-	-	-	125PK	-	-	
13	50PK	-	-	-	50PK	-	-	3
14	125PK	-	-	-	125PK	-	-	
14	125PK	-	-	-	125PK	-	-	4
17	63PK	-	-	125	-	63PK	-	
5a	50PK	-	50	50	50	50	50	5
5b	250PK	50	125	50	125	50	50	
17	63PK	-	-	125	-	63PK	-	
16	50PK	-	-	50	-	50PK	-	6
17	63PK	-	-	125	-	63PK	-	
1	-	-	-	-	-	-	-	7
5a	50PK	-	50	50	50	50	50	
5b	250PK	50	125	50	125	50	50	
1	-	-	-	-	-	-	-	8
15	50PK	-	-	50	-	50	-	
6	50PK	50	-	50	50	-	-	9
15	50PK	-	-	50	-	50	-	
15	50PK	-	-	50	-	50	-	10
18	-	-	-	50	-	50	50PK	
6	50PK	50	-	50	50	-	-	11
7	50PK	50	-	50	50	-	50	
2	50PK	-	-	-	-	-	-	12
3	250PK	-	-	-	-	-	-	
2	50PK	-	-	-	-	-	-	13
4	-	-	-	-	50PK	-	-	

TABLE I (continued)

Treatment ¹ Number	Months and Amounts of Fertilizer Applications ²							Figure ³ Number
	Mar	May	Jun	Jul	Sep	Oct	Nov	
11	50PK	-	-	-	50	-	-	15
12	50	-	-	-	50PK	-	-	
12	50	-	-	-	50PK	-	-	16
13	50PK	-	-	-	50PK	-	-	
8	50PK	50	-	50	50PK	-	-	17
10	50PK	50	PK	50	50PK	-	-	
8	50PK	50	-	50	50PK	-	-	14
9	63PK	63	-	63	63PK	-	-	

¹a = 1973 application
b = 1974 application

²Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha.

³Figures where the comparison data are presented in the Results and Discussion.

TABLE II

STOCKPILED TREATMENTS ARRANGED IN THE 17 CONTRASTS USED
IN THE ORTHOGONAL COMPARISONS

Treatment		Months of Stockpile and N Fertilization ¹									Figure
No.	Stockpile Dates ⁵	Mar	Apr	May	Jun	Jul ²	Aug	Sep	Oct	Nov	Number ³
5	Spring(3,4,5)	S	S	S	-	-	-	N-	-	-	28 34a ⁴
14	Fall(9,10,11)	-	-	-	-	-	-	NS	S	S	
5	Spring(3,4,5)	S	S	S	-	-	-	N-	-	-	27 33a
17	Spring & Fall H-74s	S	S	S	-	-	-	NS	S	S	
2	Control	-	-	-	-	-	-	N-	-	-	26 32a
5	Spring(3,4,5)	S	S	S	-	-	-	N-	-	-	
11	Summer(7,8,9)	-	-	-	-	NS	S	S	-	-	30 35a
14	Fall(9,10,11)	-	-	-	-	-	-	NS	S	S	
8	Summer(6,7,8)	-	-	-	NS	S	S	-	-	-	29 36a
11	Summer(7,8,9)	-	-	-	-	NS	S	S	-	-	
1	Control	-	-	-	N-	-	-	-	-	-	31 37a
8	Summer(6,7,8)	-	-	-	NS	S	S	-	-	-	
2	Control	-	-	-	-	-	-	N-	-	-	- -
18	Trt 17 H-74f	S	S	S	-	-	-	NS	S	S	
3	Trt 5 H-73	S	S	S	-	-	-	N-	-	-	- -
5	Spring(3,4,5)	S	S	S	-	-	-	N-	-	-	
4	Trt 5 H-74	S	S	S	-	-	-	N-	-	-	- -
5	Spring(3,4,5)	S	S	S	-	-	-	N-	-	-	
6	Trt 8 H-73	-	-	-	NS	S	S	-	-	-	- -
8	Summer(6,7,8)	-	-	-	NS	S	S	-	-	-	
7	Trt 8 H-74	-	-	-	NS	S	S	-	-	-	- -
8	Summer(6,7,8)	-	-	-	NS	S	S	-	-	-	
9	Trt 11 H-73	-	-	-	-	NS	S	S	-	-	- -
11	Summer(7,8,9)	-	-	-	-	NS	S	S	-	-	
10	Trt 11 H-74	-	-	-	-	NS	S	S	-	-	- -
11	Summer(7,8,9)	-	-	-	-	NS	S	S	-	-	

TABLE II (continued)

Treatment		Months of Stockpile and N Fertilization ¹										Figure	
No.	Stockpile Dates ⁵	Mar	Apr	May	Jun	Jul ²	Aug	Sep	Oct	Nov	Number ³		
12	Trt 14 H-73	-	-	-	-	-	-	NS	S	S	-	-	
14	Fall(9,10,11)	-	-	-	-	-	-	NS	S	S	-	-	
13	Trt 14 H-74	-	-	-	-	-	-	NS	S	S	-	-	
14	Fall(9,10,11)	-	-	-	-	-	-	NS	S	S	-	-	
15	Trt 17 H-73s	S	S	S	-	-	-	NS	S	S	-	-	
17	Spring & Fall H-74s	S	S	S	-	-	-	NS	S	S	-	-	
16	Trt 17 H-73f	S	S	S	-	-	-	NS	S	S	-	-	
18	Trt,17 H-74f	S	S	S	-	-	-	NS	S	S	-	-	

¹N = 50 kg of N/ha; S = stockpile period; 50 kg of N/ha were applied at the beginning of each stockpile period, or in September if the stockpile period started in March; all treatments received 100 kg of N, 60 kg of P, and 120 kg of K/ha; 50-30-60 was applied to every treatment in March; 0-30-60 was applied to every treatment in September.

²Treatments 6, 7, and 8 were stockpiled from July 15 to September 15.

³Figures where the comparison data are presented in the Results and Discussion.

⁴a = treatments selected for the digestibility and perloline analyses.

⁵Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt (number) H-73 or H-74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the designated year; s = spring; f = fall.

mounted on a mobile-steel frame with which the cutting height could be adjusted from 5 cm up to 26 cm. The plot area harvested was the same as that mentioned earlier for the rotary mower. The plan for gradual clipping was to cut the stockpiled grass first down to a 15-cm height, a week later cut it to a 10-cm height, and a week after that to cut to a 5-cm height. Thereafter, it was maintained in a manner similar to that described for the fertility experiment. The height obtained by the grass at the end of the stockpile period determined whether the initial cut was at 15 or at 10 cm.

Both fresh and dry weights were obtained from the material harvested from each plot. Drying was done in a forced air oven at 65°C. A composite sample from the four replications was obtained for each treatment from the yield samples and ground in a Wiley mill to pass a 2-mm screen. These ground samples were used for digestibility and other laboratory analyses.

II. LABORATORY PROCEDURES

The ground samples were analyzed for digestibility, and for contents of perloline, $\text{NO}_3\text{-N}$, total N, K, Ca, Mg, and P. Because of the large number of samples, only selected treatments were used for the digestibility and perloline analyses. These selected treatments are delineated in Table III for the fertility experiment and in Table II for the stockpile experiment.

Digestibility was determined using the Tilley-Terry in vitro digestion procedure (Tilley and Terry, 1963). A timothy-crimson clover

TABLE III

FERTILITY TREATMENTS ARRANGED IN 8 CONTRASTS USED IN THE
ORTHOGONAL COMPARISONS FOR PERLOLINE AND % DDM

Treatment ¹ Number	Months and Amounts of Fertilizer Applications ²							Figure ³ Number
	Mar	May	Jun	Jul	Sep	Oct	Nov	
1	-	-	-	-	-	-	-	
4	-	-	-	-	50PK	-	-	18
1	-	-	-	-	-	-	-	
5a	50PK	-	50	50	50	50	50	
5b	250PK	50	125	50	125	50	50	19
5a	50PK	-	50	50	50	50	50	
5b	250PK	50	125	50	125	50	50	
17	63PK	-	-	125	-	63PK	-	20
3	250PK	-	-	-	-	-	-	
17	63PK	-	-	125	-	63PK	-	21
8	50PK	50	-	50	50PK	-	-	
16	50PK	-	-	50	-	50PK	-	22
13	50PK	-	-	-	50PK	-	-	
16	50PK	-	-	50	-	50PK	-	23
16	50PK	-	-	50	-	50PK	-	
17	63PK	-	-	125	-	63PK	-	24
16	50PK	-	-	50	-	50PK	-	
18	-	-	-	50	-	50	50PK	25

¹
a = 1973 application
b = 1974 application

² Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha.

³ Figures where the comparison data are presented in the Results and Discussion.

hay was fed to two rumen-fistulated steers used as the source of rumen fluid. Samples harvested in 1973 were analyzed during August 1974 and samples harvested in 1974 were analyzed during February 1975. Results are reported as percent digestible dry matter (DDM).

The alkaloid perloine was analyzed according to the procedure of Bush (1973) which is a modification of the procedure reported by Gentry et al. (1969). The procedure is listed in the Appendix, Table IV. Perloine for use in standards was obtained from Bush (Department of Agronomy, University of Kentucky, Lexington). Procedures for purification of perloine are reported by Bush et al. (1970). The procedure for preparing the TLC plates (Stahl, 1965) is described in the Appendix, Table V. The TLC plates were read using a Spectrodensitometer Model SD 3000 with a SD 300 Density Computer. The results are reported as mg/g on a dry weight basis. A visual check was made on the TLC plates for the presence of perloine, using a long-wave ultraviolet light machine. Photographs were taken of selected plates.

Samples from the fertility experiment were analyzed for $\text{NO}_3\text{-N}$ concentration, which was determined using a modification of the nitrate electrode method described by Paul and Carlson (1968). Aluminum resin and silver resin were not added. Results are reported as percent $\text{NO}_3\text{-N}$ on a dry weight basis.

A 0.2 g portion of each ground sample was digested in concentrated sulfuric acid and in 35 percent hydrogen peroxide and then analyzed with a Technicon Autoanalyzer. Nitrogen was determined using the phenolhypochlorite color reaction in the Autoanalyzer procedure described by

Thomas et al. (1967). The K and Ca were determined using a Technicon III dual-channel flame photometer. The Mg was determined colorimetrically by the magnesium blue reaction, and P was determined by the ammonium vanadate reaction. These procedures were described by Steckel and Flannery (1971). Results are reported as percent N, K, P, Mg, or Ca on a dry weight basis.

Maximum and minimum air temperatures (Appendix Figure 38) and precipitation values (Appendix Figures 39 and 40) are provided for the years 1973 and 1974.

III. STATISTICAL ANALYSES

Since it was biologically desirable to compare a single treatment with two or more treatments, nonorthogonal contrasts were used to analyze the data. Each experiment had a set of 17 contrasts. Partial sums of squares were obtained for each contrast from several sets of orthogonal contrasts to avoid confounding, and were individually tested with the appropriate error term. The data were analyzed within seasons for each year: Spring, March 1—May 31; Summer, June 1—August 31; and Fall, September 1—December 31.

Since selected treatments were used for the digestibility and perloline analyses, nonorthogonal sets with 9 contrasts for the fertility experiment and 7 contrasts for the stockpile experiment were used.

Data analyses were performed on the IBM 360/65 at the University of Tennessee Computer Center. Procedures of the Statistical Analysis

System (Barr and Goodnight, 1972) were employed. Correlations were used to verify the orthogonal sets and the regression procedure was used to estimate the nonorthogonal and orthogonal contrasts.

CHAPTER IV

RESULTS AND DISCUSSION

I. FIELD OBSERVATIONS

Five days after the March 2, 1973, fertilizer application, those plots receiving N fertilizer were distinctly greener than plots not receiving N. Those plots receiving 250 kg of N/ha applied on March 2 had uniform color and growth until about May 14. On May 14, areas receiving 250kg of N/ha were losing their dark green color. This contrast in color was more striking since the dark green areas were also taller. These combined effects made the growth in these plots appear very jagged. Almost all the dark green color of the growth in the fertilized plots had faded and resembled the color of the growth in the nonfertilized plots by May 23.

Plots that were spring-stockpiled and harvested at the end of May had considerable bare earth exposed regardless of whether they had been harvested as a hay cut or with gradual clipping; however, the gradual clipping plots, when finally clipped to 5 cm, were greener than the hay cut plots.

Moisture became limiting in July and large brown splotchy areas were observed in both experiments on July 20. Growth had resumed on July 30, after a three-day rain on July 24, 25, and 26. Moisture became limiting again in August. The fertility plots were brown on September 4 and the leaves had shriveled. These symptoms were not

apparent in the stockpile plots. In plots that had received N applied on September 4, a visible color change was noted on September 18, five days after a rain on September 13.

On October 16, in the stockpile experiment, brown, weedy summer grasses contrasted sharply with the green fescue. Based on visual estimates, those plots that were stockpiled in the spring or fall had 25 percent or more brown color. Spring-stockpiled plots had more weeds than the fall-stockpiled plots. Those plots that had been stockpiled in the summer or continuously clipped had less than 25 percent brown color.

Those fertility plots that had been fertilized with N on March 1, 1974 were yellow on April 10. This color contrasted with the greener nonfertilized plots. The color change was apparently due to a frost the night before, since the minimum air temperature had been recorded at -1°C . The yellow color was no longer visible on April 15.

It was noted on May 3 that almost all the fertility plots had headed out. This had not occurred in the fertility plots the preceding year. The apparent difference in stage of maturity during this period for the two years may have some bearing on the results.

When the 1974 spring stockpiled plots were harvested, the 5 cm stubble of the hay plots cut on May 29 was greener than the 5 cm stubble of the gradual clip plots cut on June 13. These observations were exactly the reverse of those made in 1973. Earlier drought conditions in 1974 may explain this difference. Regardless of whether the spring stockpile was harvested as a hay cut or as gradual clipping, seedlings

of foxtail (Alopecurus sp.) were coming up just under the fescue stubble. The presence of a considerable number of volunteer orchardgrass plants was noted in spring stockpiled plots. This also was observed in the spring of 1975.

On August 19, 1974, the fertility treatment which had received 250 kg of N/ha in March, 50 kg/ha in May, 125 kg/ha in June, and 50 kg/ha in July had large areas of dead grass. Approximately 20 to 30 percent of the stand was killed. However, the plots had recovered in the spring of 1975, but some weedy grasses also had become established. A treatment that had received 63 kg/ha of N in March and 125 kg/ha in July had no apparent reduction in stand. This seemed to indicate that the high summer N application alone did not reduce the tall fescue stand, but in conjunction with the heavy spring N application, the high summer N application did contribute to tall fescue stand reduction.

A long drought and early cold weather severely limited the fall growth of tall fescue in 1974.

II. FERTILITY EXPERIMENT

The 17 contrasts are discussed in relation to yield, percent dry matter, and content of P, Ca, K, Mg, $\text{NO}_3\text{-N}$, and N. Eight of the contrasts also are discussed in relation to digestibility and perloline content. Differences discussed are significant at the .05 level of probability unless stated otherwise. Increases or decreases in measurements that occurred near the end of one season and the beginning of another season may not be significant because their total effect is divided between the two seasons.

Yield

Dry matter yields of tall fescue in 1973 and 1974 are presented in Appendix Table VI. Seasonal yields ranged from a low of 160 kg/ha in the fall of 1974 to a high of 5,831 kg/ha in the spring of 1974.

The application of 250 kg of N/ha in March (treatment 3) did not increase the spring yields of tall fescue above the yields derived from the application of 125 kg of N/ha in March (treatment 14, Figure 1). The 1973 fall yield was increased by the application of 125 kg of N/ha plus 30 kg of P and 60 kg of K/ha in September. Although the treatments were not significantly different for the season, treatment 3 had higher yields in late spring and early summer than did treatment 14. Applying N in September may increase the fall yield. Applying N in March at rates above 125 kg/ha may be advantageous for higher late spring and early summer yields.

The 125 kg of N/ha applied in March increased the 1974 spring yield above that derived from the application of 63 kg of N/ha in March (treatment 9, Figure 2). The 63 kg of N/ha applied in May and in July increased summer yields. Applying 125 kg of N/ha in September did not increase yields above those derived from 63 kg of N/ha in September. Applying N in September may increase the fall yield but applications in excess of 63 kg/ha appear to have no additional benefit.

The comparison of treatments 14 and 13 (Figure 3) verifies the yield results of the March and September nitrogen applications from the comparison of treatment 14 with 9.

The July application of 125 kg of N/ha (treatment 17) increased the 1973 summer yields (Figure 4). This yield increase occurred almost a

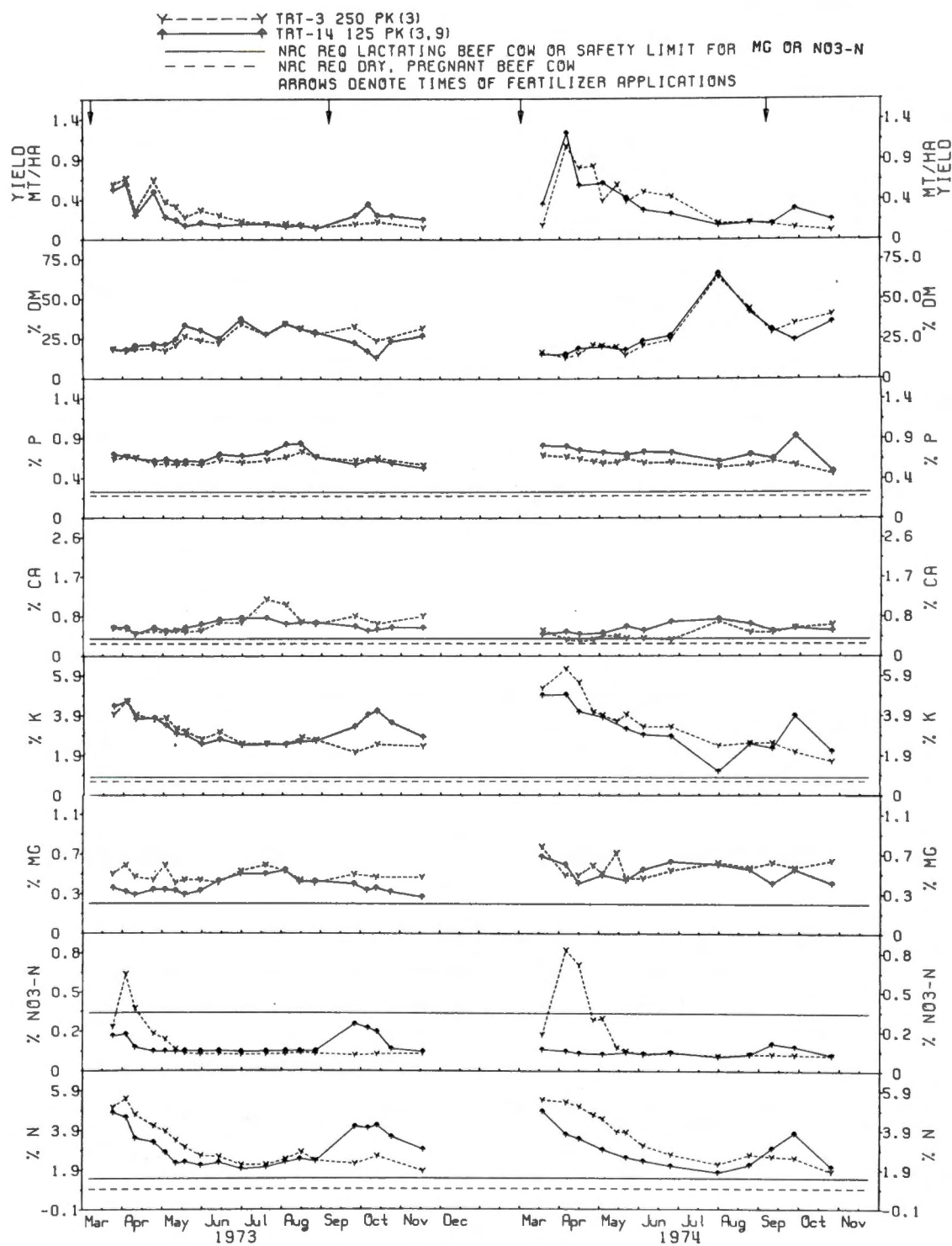


Figure 1. Comparison of the effects of a single fertilizer application and a split application on tall fescue productivity and composition in 1973-74.

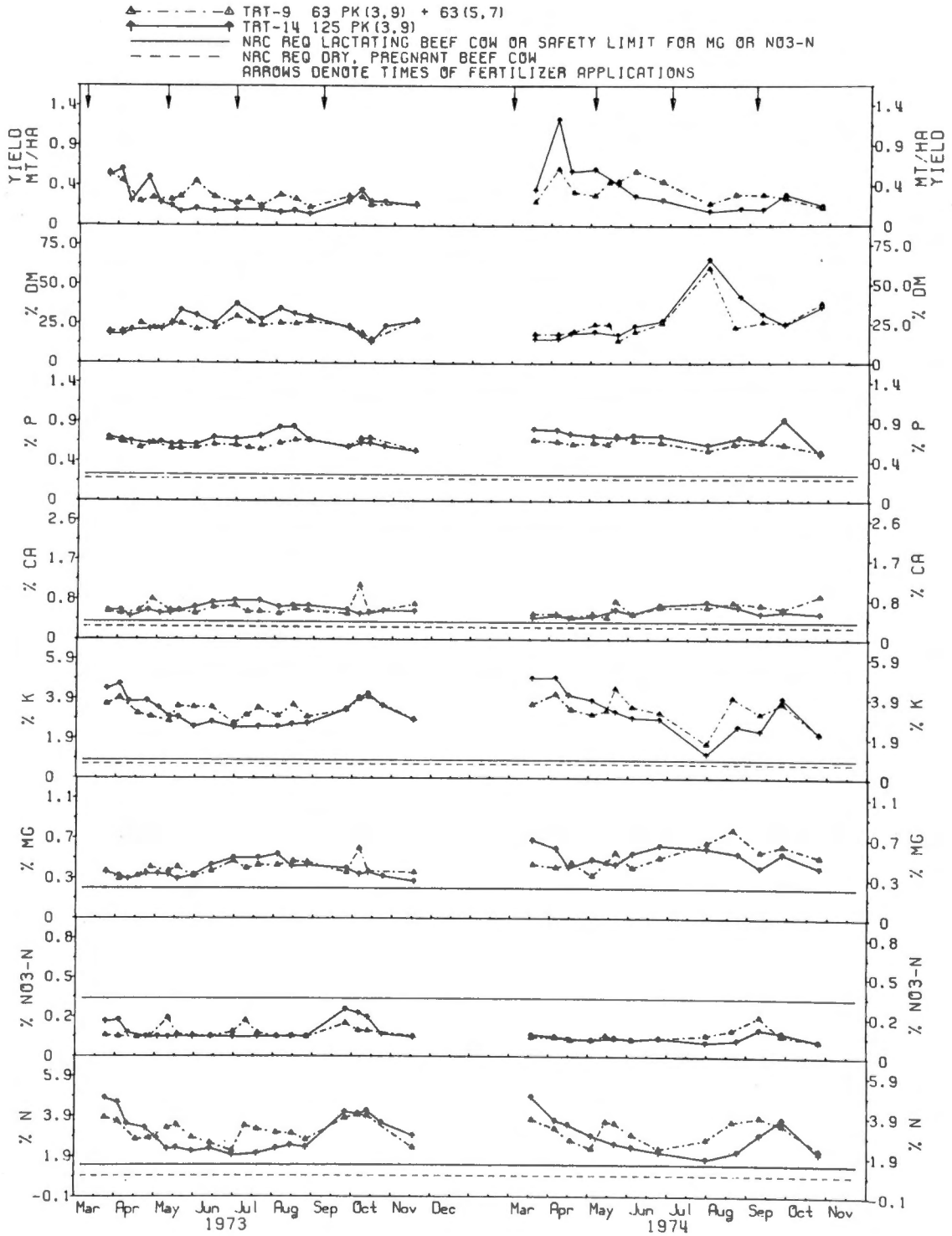


Figure 2. Comparison of the effects of four fertilizer applications and two applications on tall fescue productivity and composition in 1973-74.

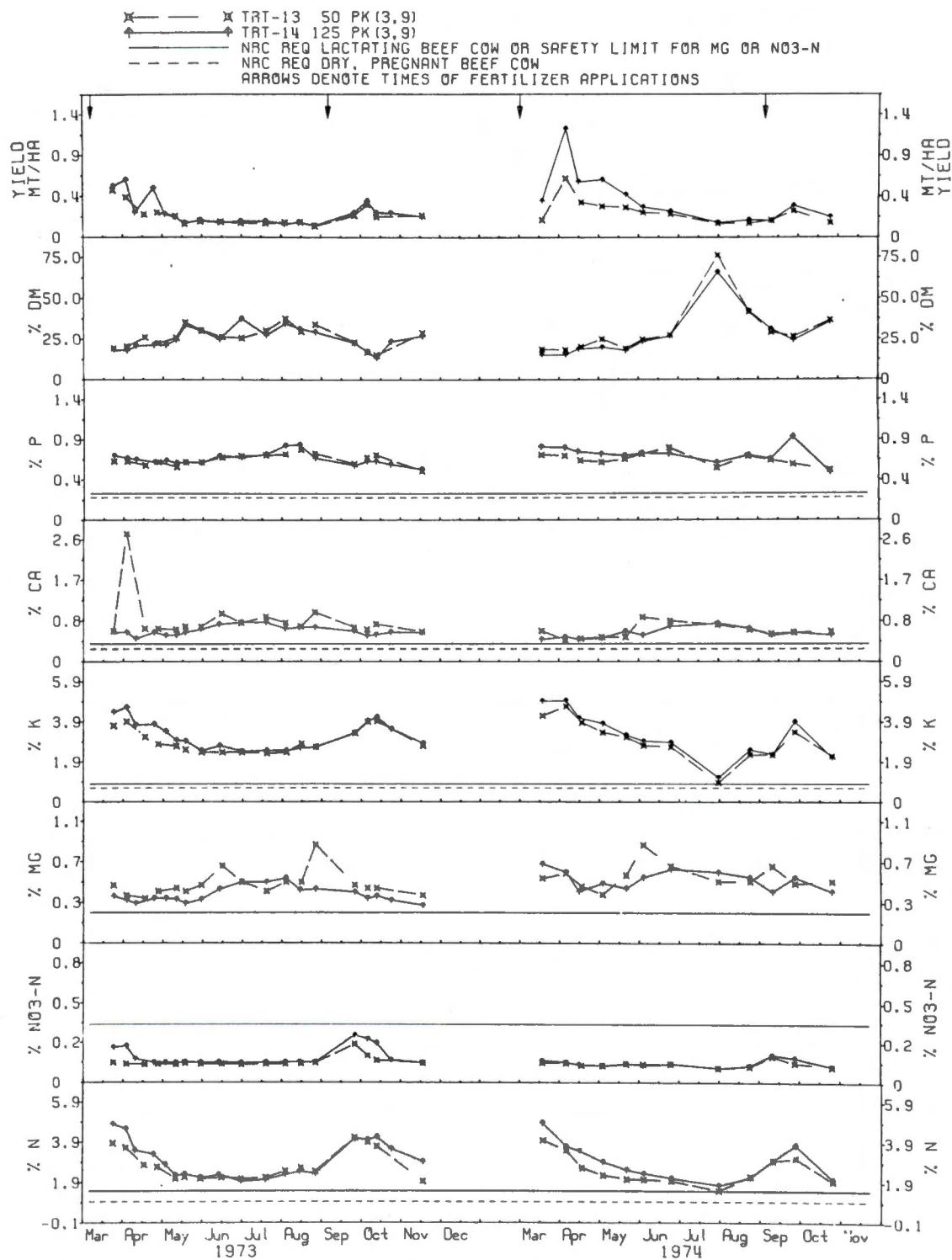


Figure 3. Comparison of the effects of increasing the amounts of nitrogen fertilizer applied on tall fescue productivity and composition in 1973-74.

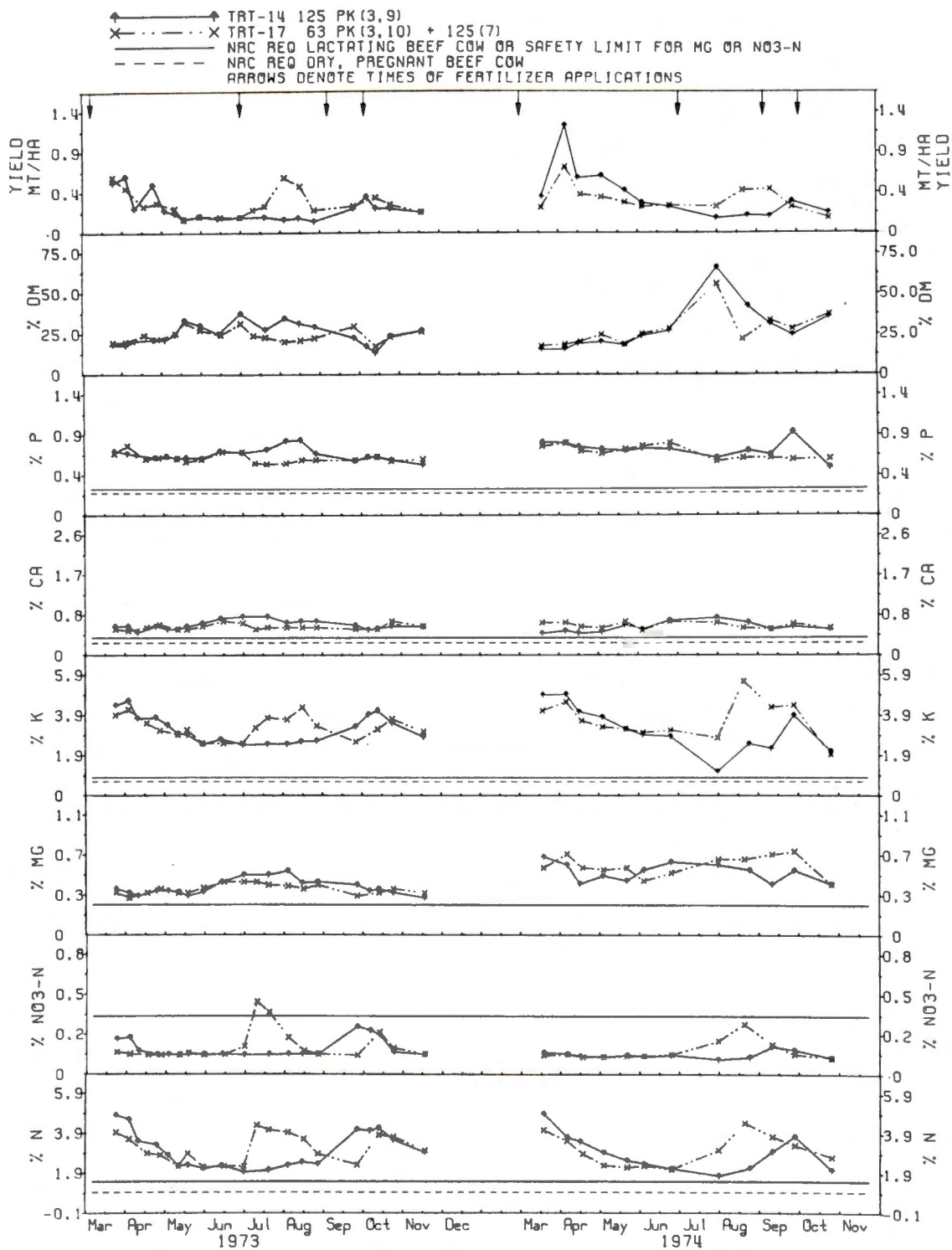


Figure 4. Comparison of the effects of two and three fertilizer applications on tall fescue productivity and composition in 1973-74.

month after the fertilizer application. Drought conditions appear to have influenced the delayed yield response. In 1974, increases in yield were delayed to the extent that a portion of the increases was observed in September (fall season). This may account for the nonsignificant results in the summer. The fall yields resulting from application of 63 kg of N/ha in October, were not different from those obtained when 125 kg of N/ha were applied in September.

Applications of 50 kg of N/ha in September, October, and November (treatment 5a) increased the 1973 fall yield above that obtained from 63 kg of N/ha applied in October (Figure 5), while the single application of 125 kg of N/ha in September (Figure 4) did not. The smaller, more frequent N applications in the summer did not increase yields above those from the large single July application, in contrast to the information in the literature. The November N application apparently influenced the earlier 1974 spring harvest of the fescue in treatment 5b.

Nitrogen applied in March at the rate of 250 kg/ha and in May at 50 kg/ha (treatment 5b) increased the 1974 spring yield above the yield of treatment 17. The 125 kg of N/ha applied in June and the 50 kg/ha applied in July increased the summer yields above those obtained from applying 125 kg of N/ha in July. However, the added nitrogen in July in treatment 5b damaged the tall fescue stand, reducing it by about 20 to 30 percent. This stand reduction and the effects of the droughty fall are factors which may have prevented fall yield increases from the nitrogen applied in September, October, and November at rates of 125 kg/ha, 50 kg/ha, and 50 kg/ha, respectively.

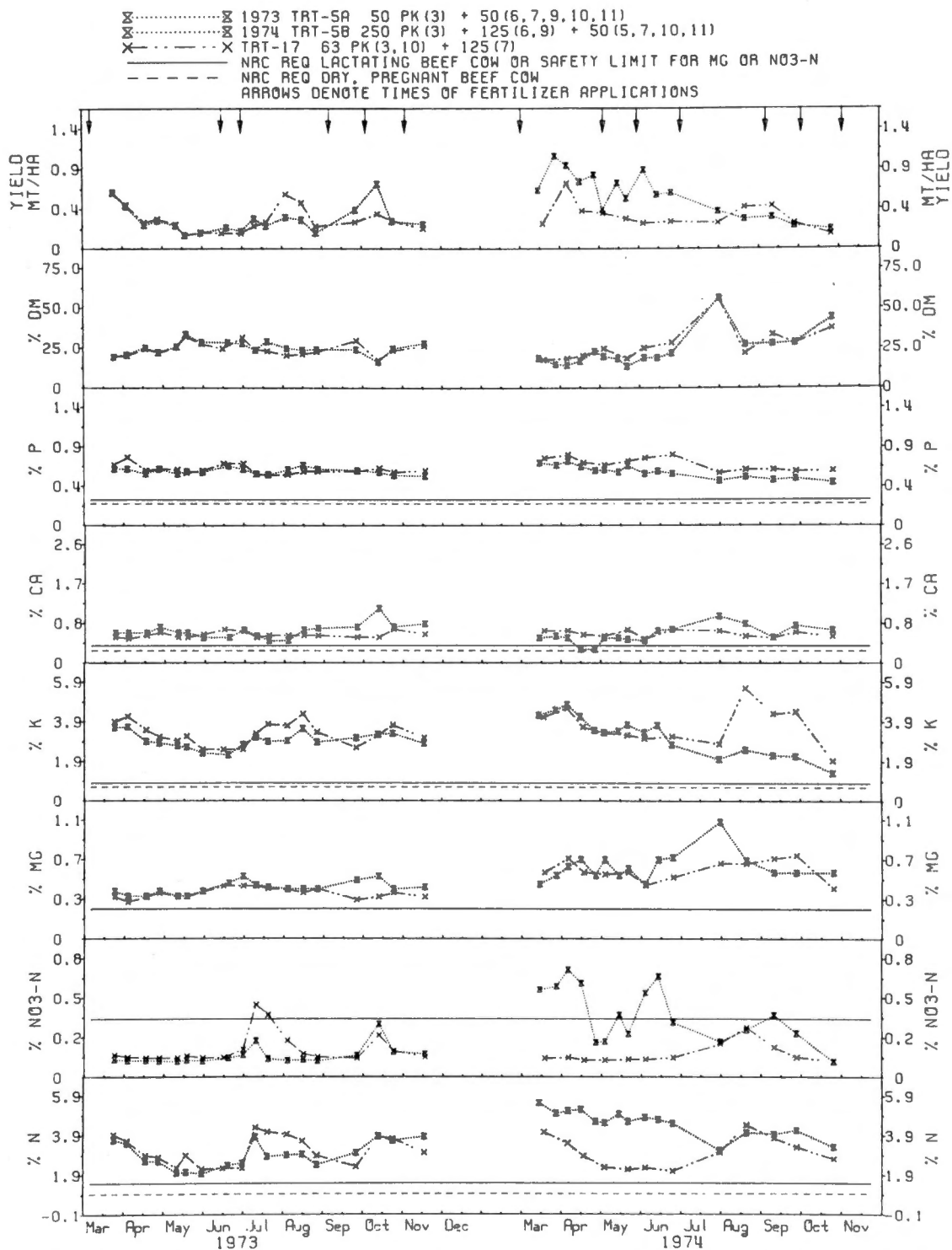


Figure 5. Comparison of the effects of three, six, and seven fertilizer applications on tall fescue productivity and composition in 1973-74.

Treatments 16 and 17 compared summer rates of applied N (Figure 6). July applications of 125 kg of N/ha and 50 kg of N/ha were made. The higher N rate increased summer yields in 1973 and increased fall yields in 1974.

Nitrogen applied at 50 kg/ha in March, June, July, September, October, and November (treatment 5a) increased yields in all seasons above those yields obtained where nitrogen was not applied (treatment 1, Figure 7). The higher N rates of treatment 5b also increased the yields in all seasons.

Nitrogen applied at 50 kg/ha in March and July (treatment 15) increased the spring and summer yields, but fall yields were not increased by 50 kg of N/ha applied in October (Figure 8). The first spring harvest of the tall fescue receiving N fertilizer was one month earlier than the first spring harvest of tall fescue that was not fertilized.

Although the effect was not significant, 50 kg of N/ha applied in May (treatment 6) tended to increase late spring and early summer yields (Figure 9). October-applied N (50 kg/ha) neither increased the spring yield nor caused an earlier spring harvest.

The application of 50 kg of N/ha in March of 1973 (treatment 15) increased yields above those yields obtained where N was not applied (treatment 18, Figure 10). The November application of 50 kg of N/ha did not increase fall yields, but did initiate an earlier spring harvest and resulted in spring yields that were comparable to those yields obtained from the March N application of treatment 15. The effect of a

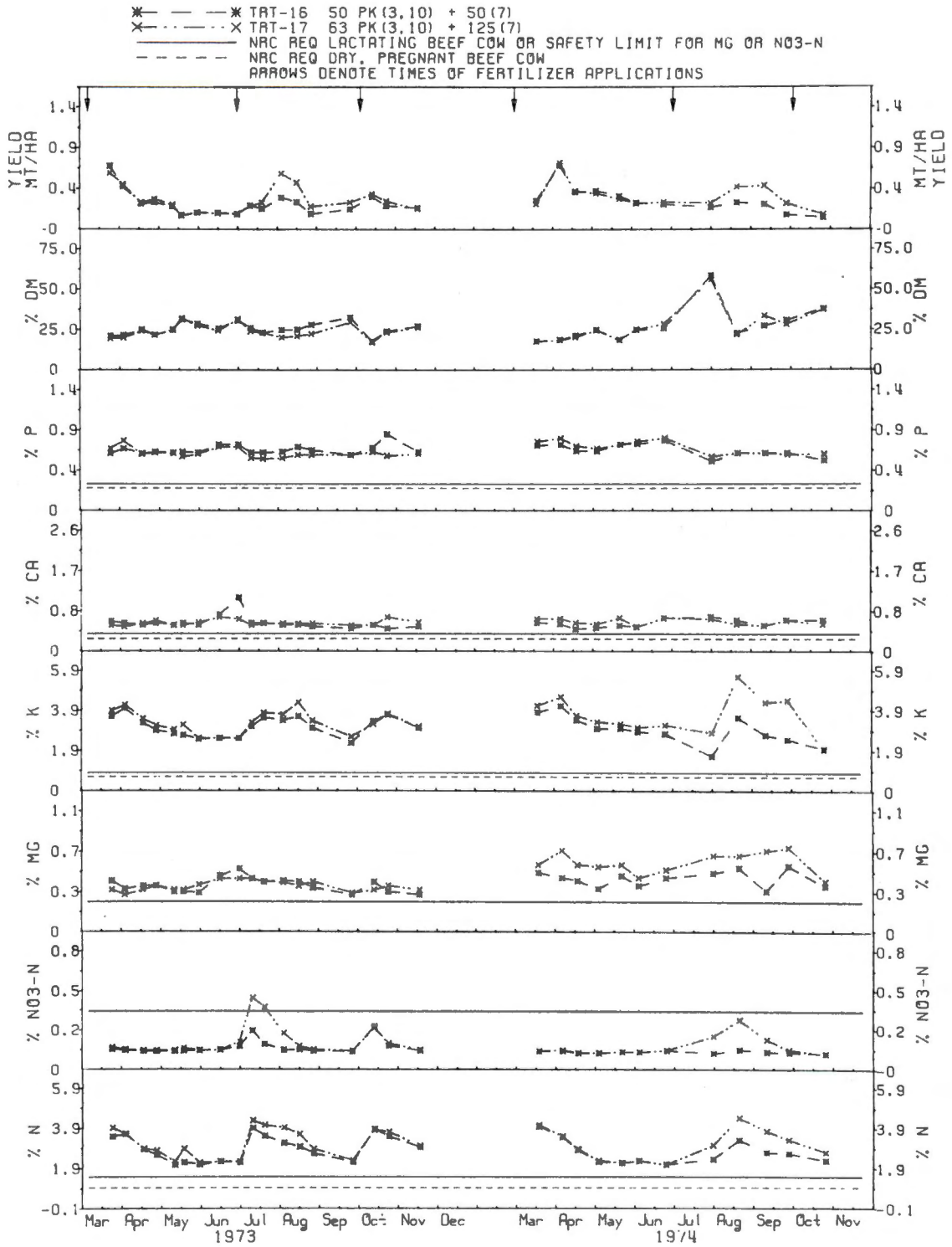


Figure 6. Comparison of the effects of a high and a low summer N application on tall fescue productivity and composition in 1973-74.

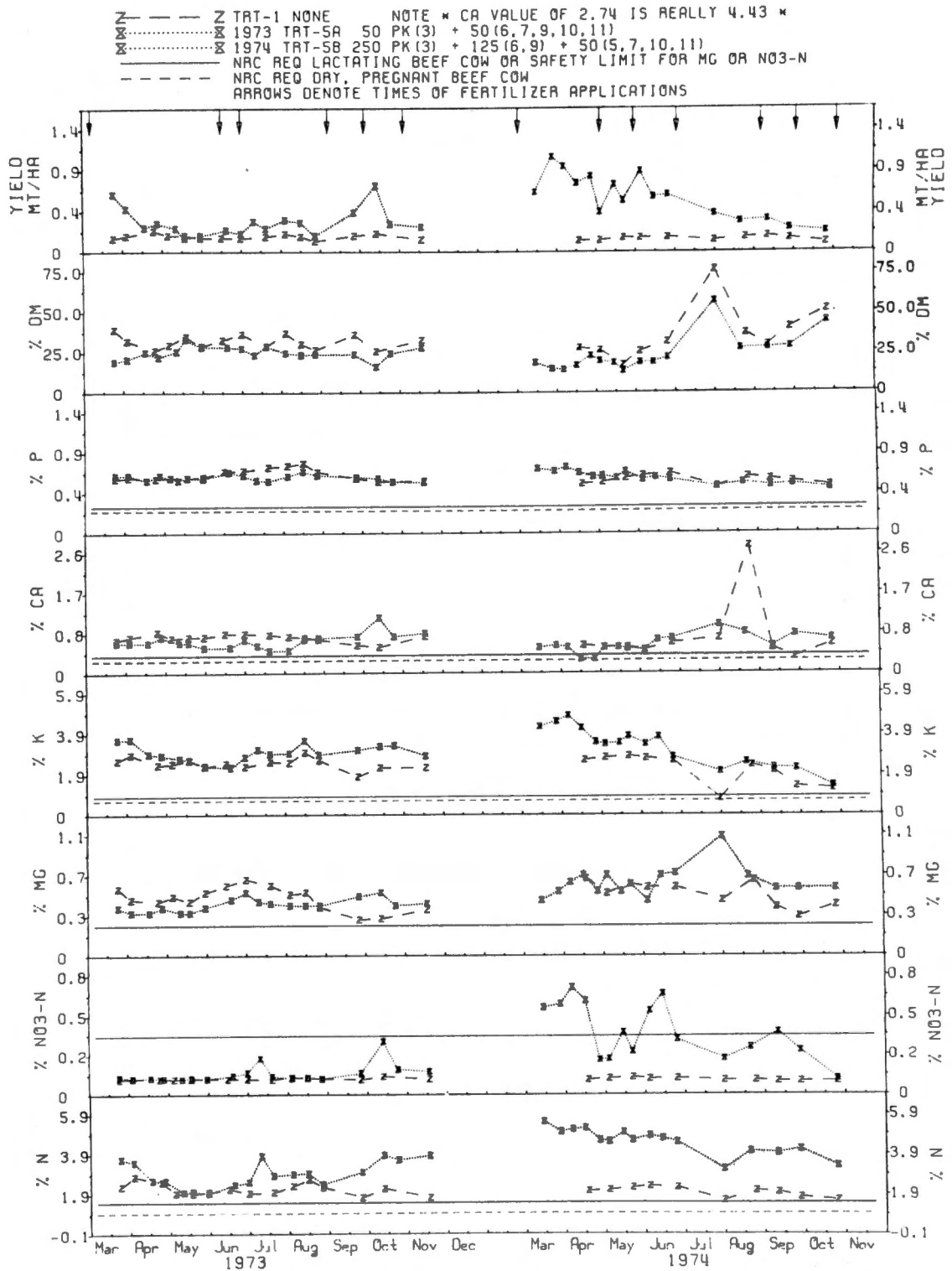


Figure 7. Comparison of the effects of zero, six, and seven fertilizer applications on tall fescue productivity and composition in 1973-74.

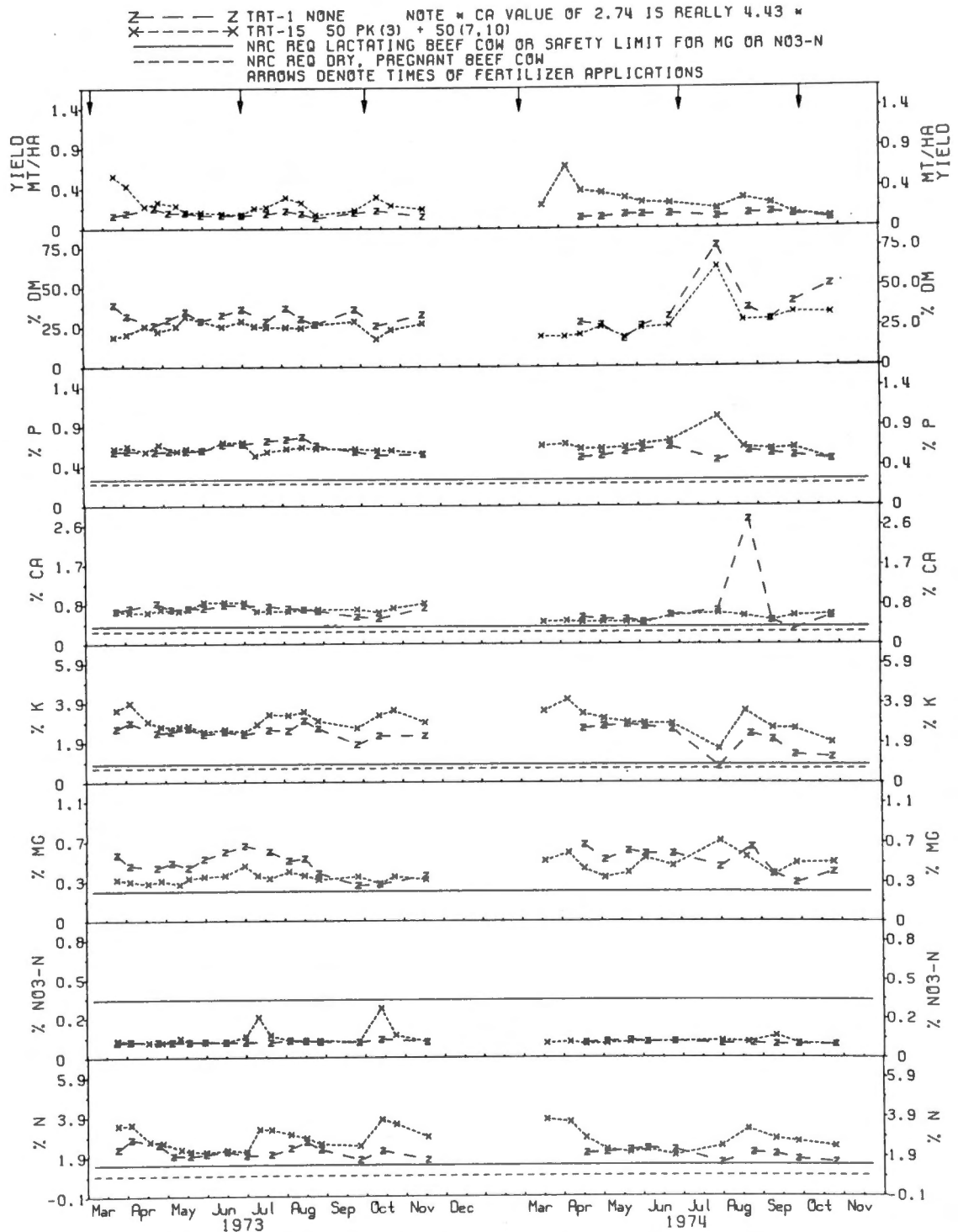


Figure 8. Comparison of the effects of zero and three fertilizer applications on tall fescue productivity and composition in 1973-74.

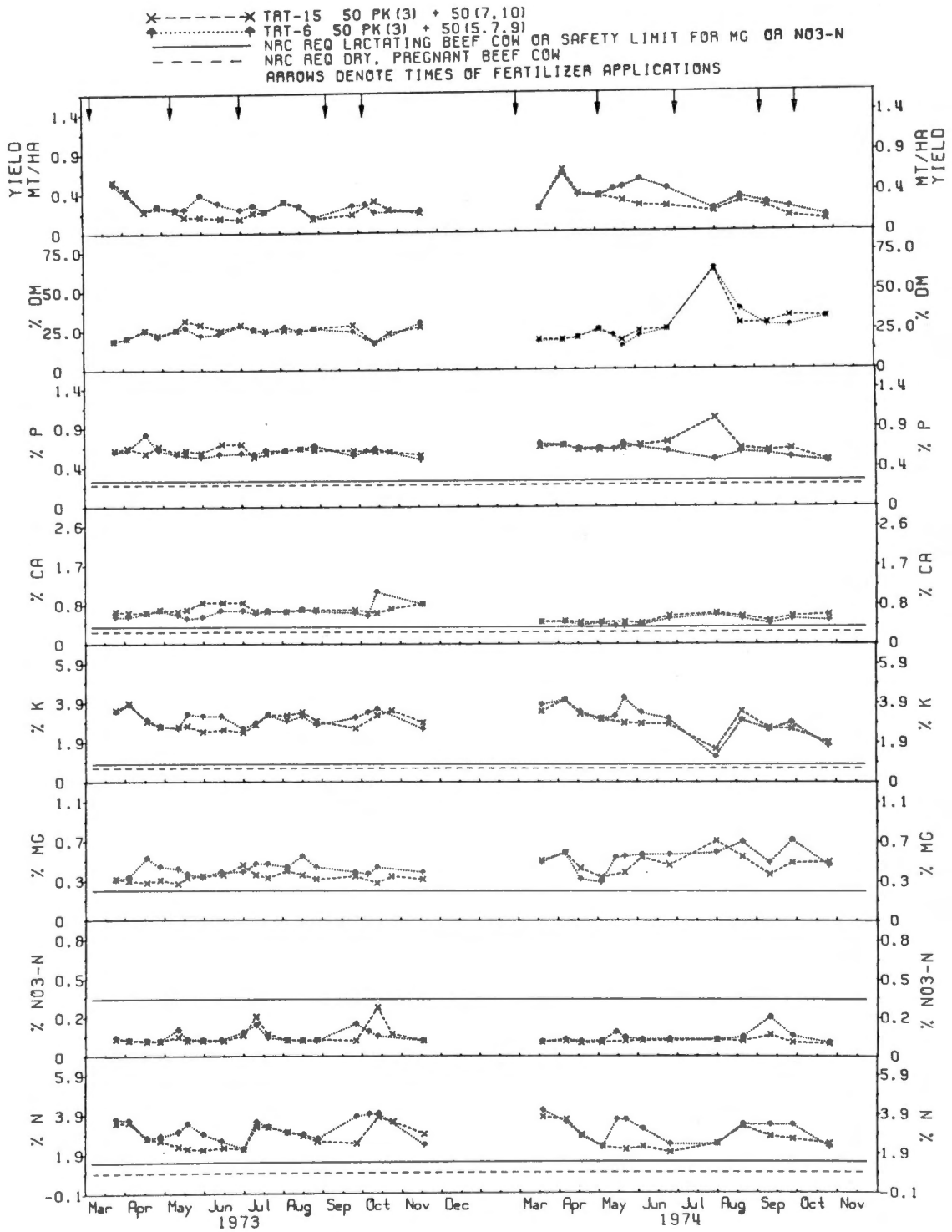


Figure 9. Comparison of the effects of three and four fertilizer applications on tall fescue productivity and composition in 1973-74.

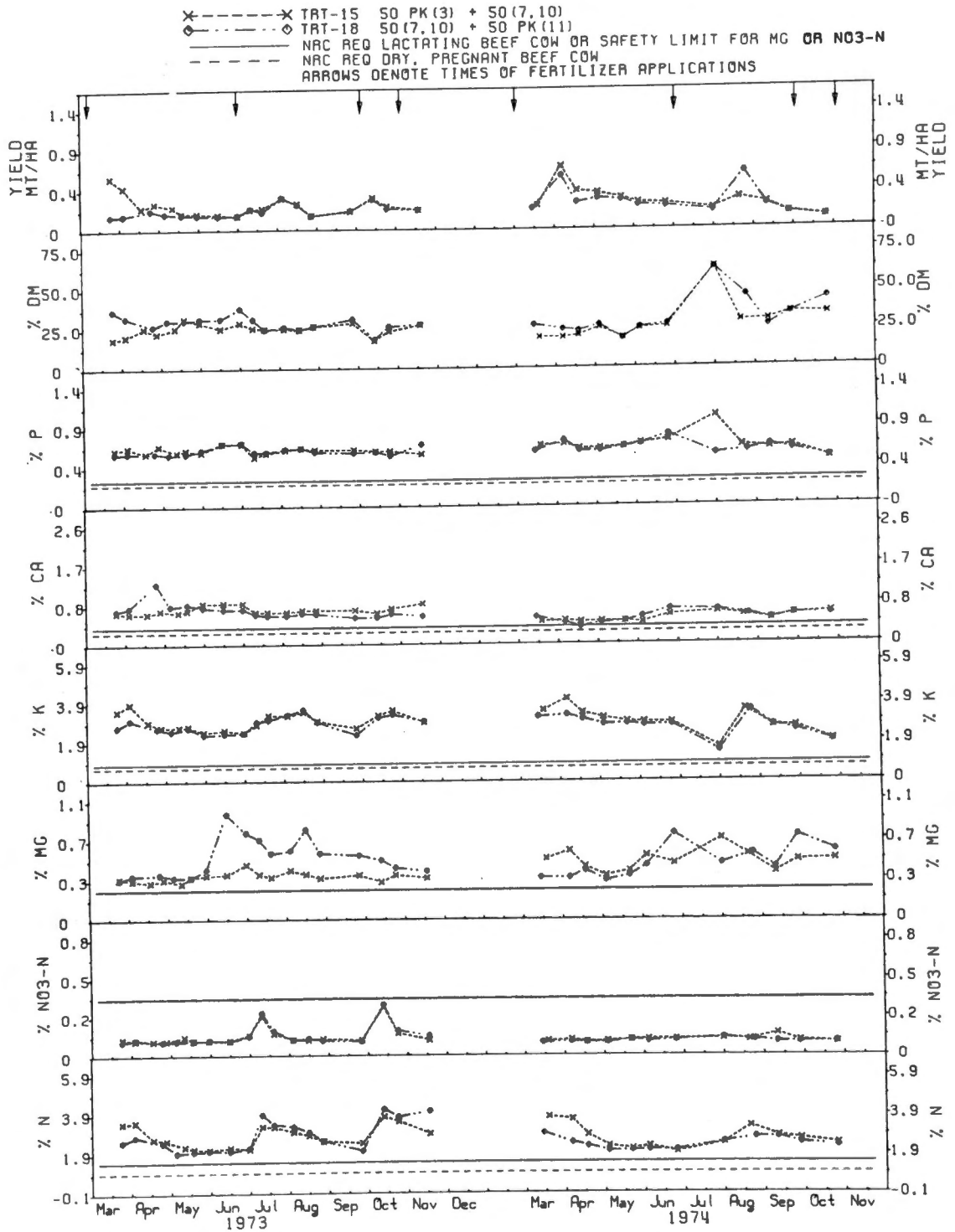


Figure 10. Comparison of the effects of March and November fertilizer applications on tall fescue productivity and composition in 1973-74.

November N application also was observed in the comparison of treatments 6 and 7 (Figure 11). Both treatments are identical except for the November N application in treatment 7. In the 1974 spring, the tall fescue of treatment 7 was harvested earlier, and the yields were higher although not significantly so. These results are consistent with reports in the literature which indicate that fall N fertilization may result in earlier and larger spring yields.

The application of 250 kg of N/ha in March (treatment 3) increased the spring yields of tall fescue above the yields derived from the application of 50 kg of N/ha in March (treatment 2, Figure 12). Although not significant, early summer yields from treatment 3 were higher than those from treatment 2.

In comparing the treatments in Figures 8, page 52, and 10, page 54, the March application of 50 kg of N/ha increased yields above those obtained from treatments where no N was applied in March. However, the 50 kg of N/ha of treatment 2 applied in March did not increase the spring yields (Figure 13). The September N application (50 kg/ha, treatment 4) increased yields in the 1973 fall season above the yields of the tall fescue that had not received fall N. There were no apparent spring yield responses to the September N application of treatment 4. Increasing the N applied from 50 kg/ha to 63 kg/ha did not increase yields (Figure 14).

Applying 60 kg of K/ha in March and/or September, or in July, did not increase yields (Figures 15, 16, and 17).

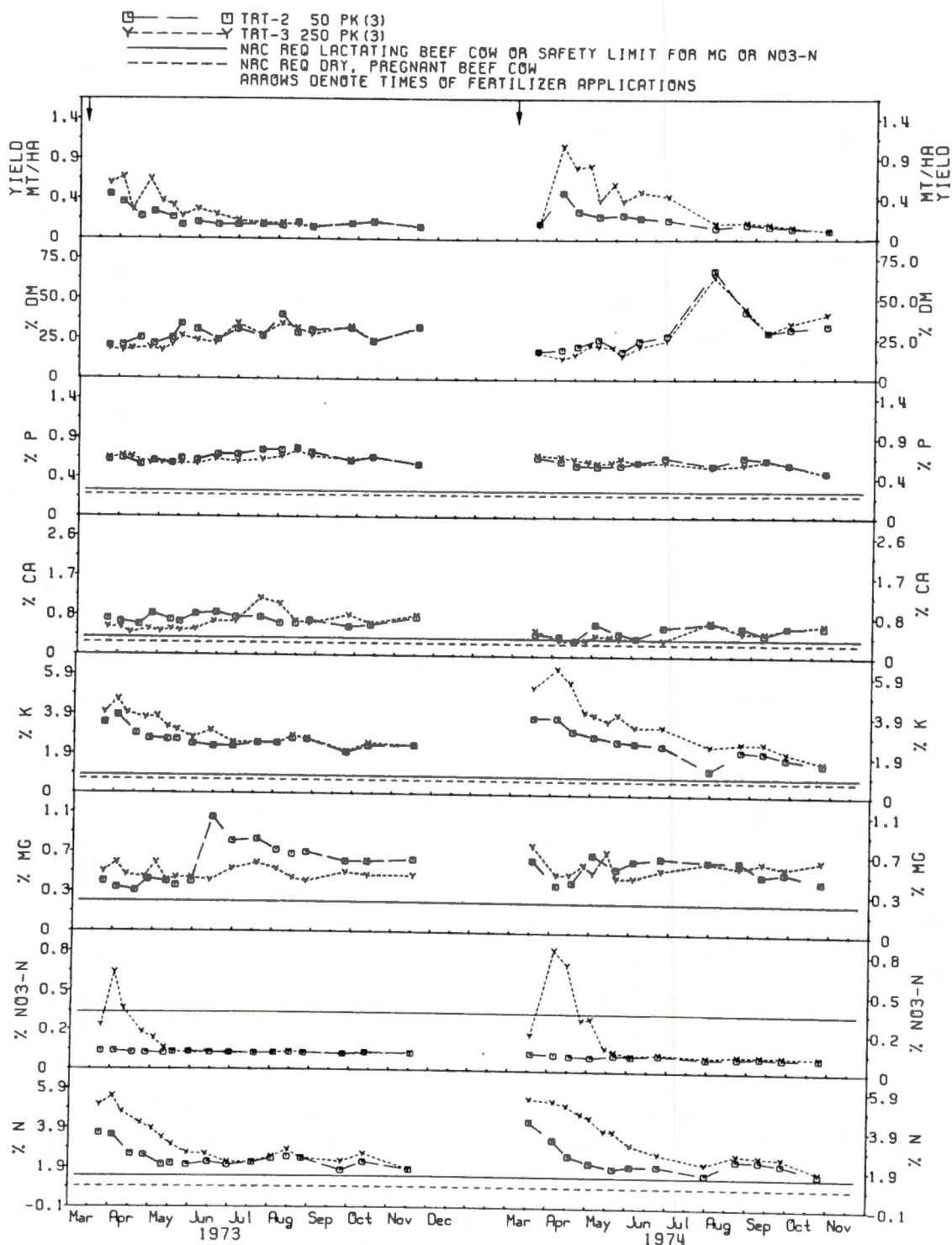


Figure 12. Comparison of the effects of high and low March fertilizer applications on tall fescue productivity and composition in 1973-74.

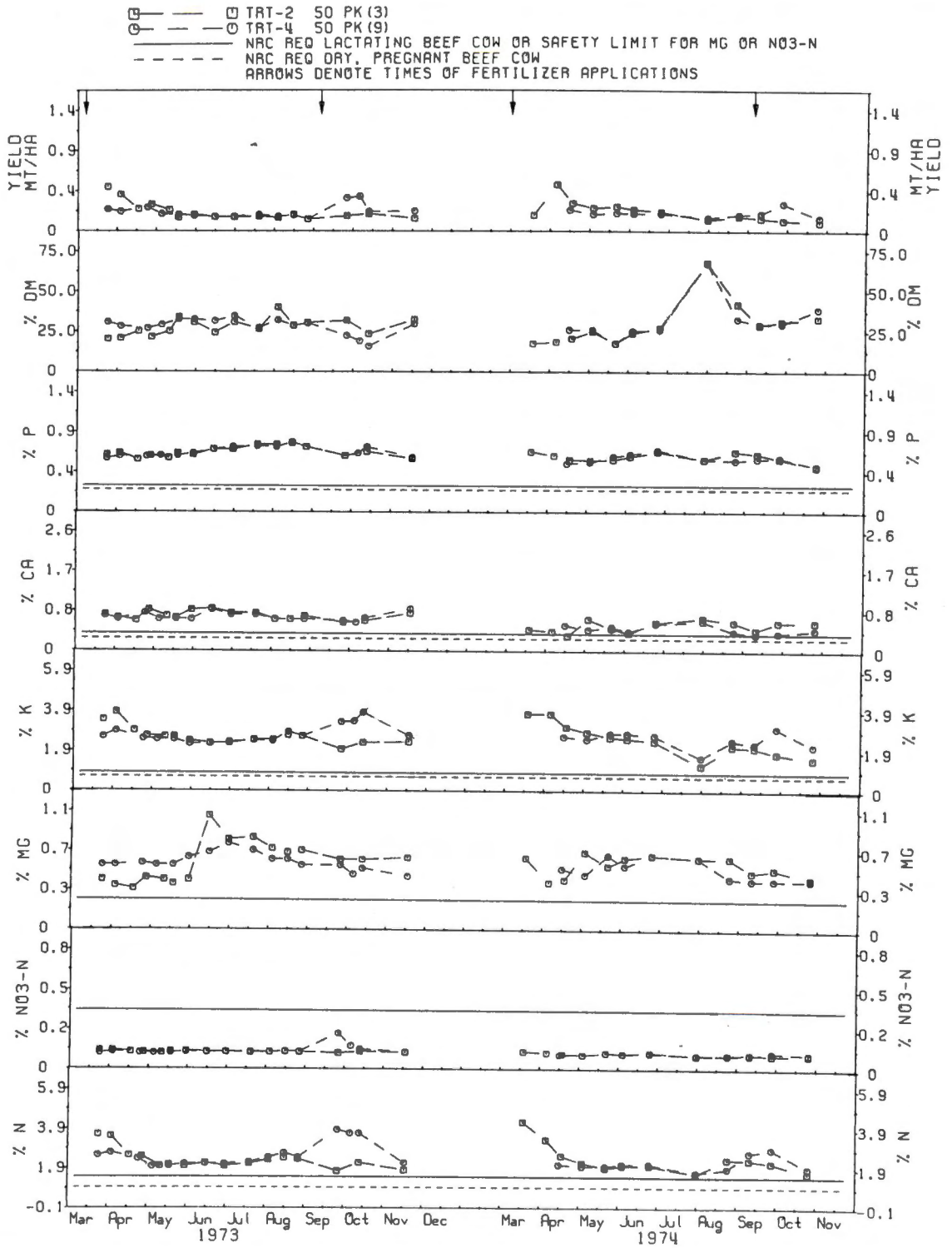


Figure 13. Comparison of the effects of March and September fertilizer applications on tall fescue productivity and composition in 1973-74.

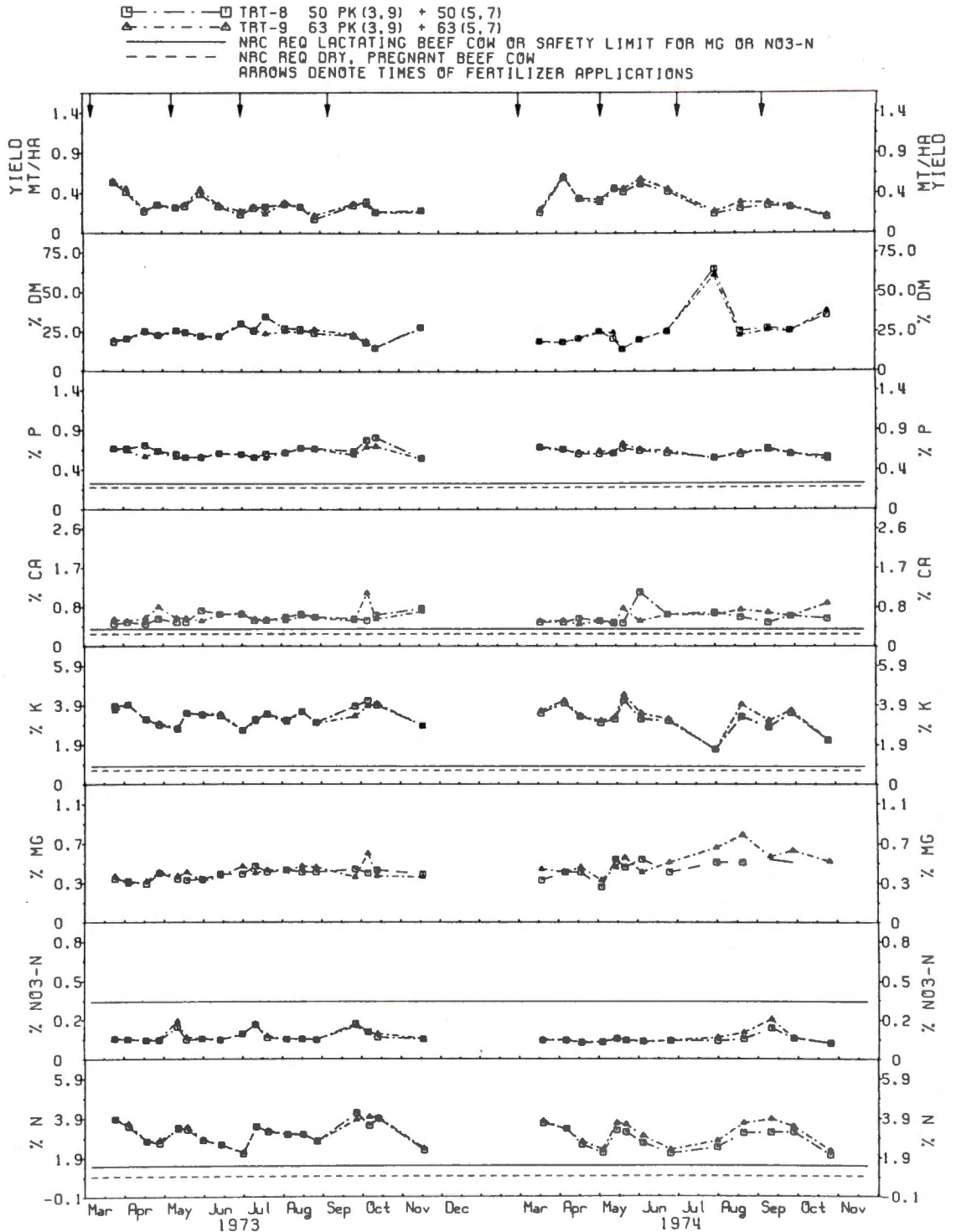


Figure 14. Comparison of the effects of two low N fertilizer applications on tall fescue productivity and composition in 1973-74.

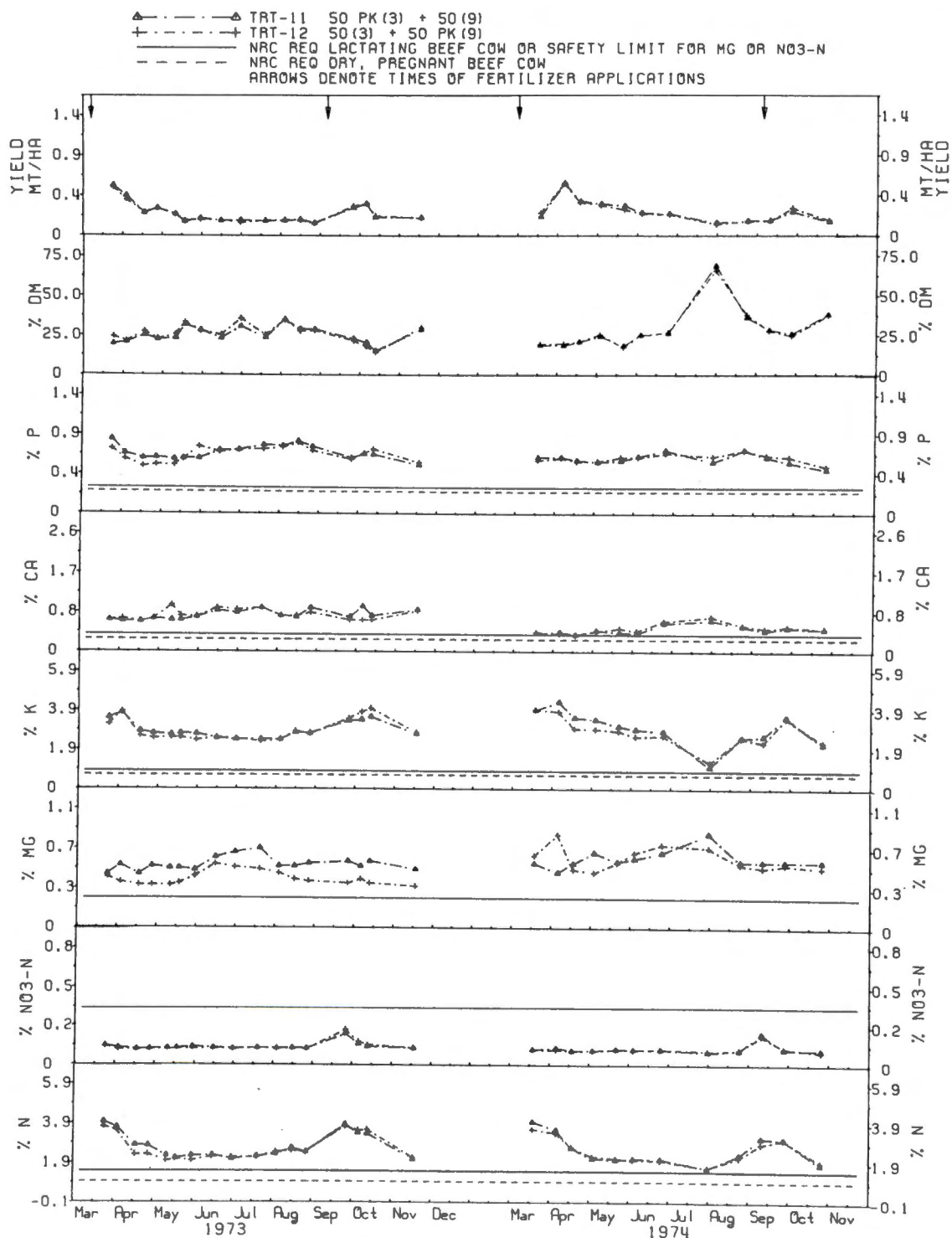


Figure 15. Comparison of the effects of March and September K applications on tall fescue productivity and composition in 1973-74.

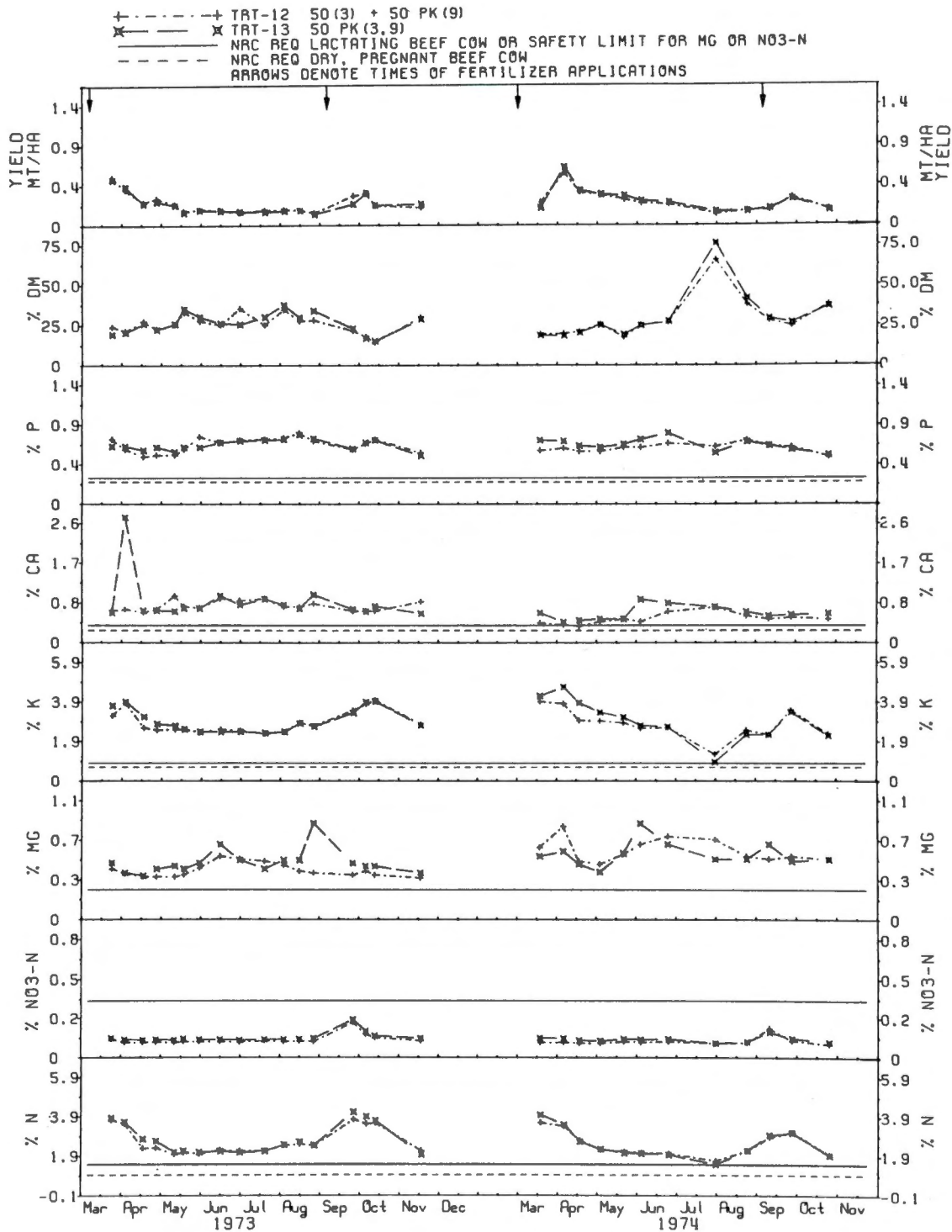


Figure 16. Comparison of the effects of March-September and September K applications on tall fescue productivity and composition in 1973-74.

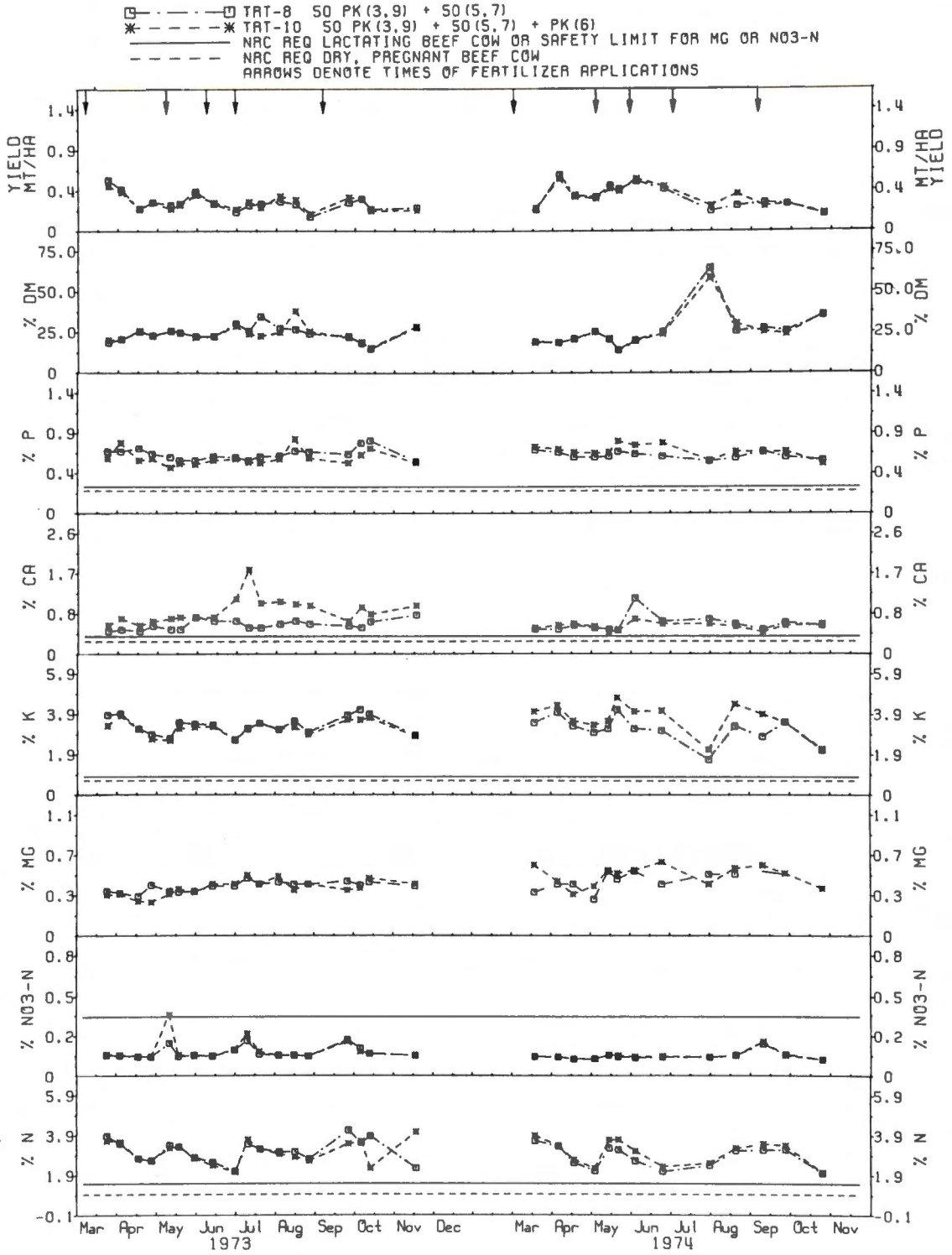


Figure 17. Comparison of the effects of June and no June K applications on tall fescue productivity and composition in 1973-74.

Percent Dry Matter

Some investigators are interested in percent DM data because a high or low percent DM may decrease or increase the intake of forage by animals, respectively. Reports in the literature indicate that a high percent DM may lower digestibility and a low percent DM may increase digestibility of tall fescue.

The percent DM generally decreased when N fertilizer was applied, and increased during the summer season or droughty periods, such as the fall of 1974. Although differences among seasons were not analyzed statistically, the percent DM was generally higher during summer than during spring and fall (Appendix Table VII).

The September application of 125 kg of N/ha decreased the percent DM for the fall season (Figure 1, page 43). Increasing the amount of N applied in the spring did not decrease the percent DM.

Applying 63 kg of N/ha in May and July decreased percent DM for the summer season (Figure 2, page 44). The drought in the 1974 summer may have increased the percent DM, thus masking the effect of the N fertilizer. Increasing the amount of N applied in the spring and fall did not decrease the percent DM (Figure 3, page 45). The application of 125 kg of N/ha in July decreased the percent DM for the summer season (Figure 4, page 46). Frequent applications of N during a season did not decrease the percent DM below that obtained when at least one N application was made during that season (Figure 5, page 48).

The nonfertilized tall fescue of treatment 1 had a higher percent DM in all seasons than did fertilized fescue (Figure 7, page 51).

July N application was higher than that obtained from the smaller, more frequent applications of 50 kg/ha in June and July (Figure 5, page 48).

The 250 kg of N/ha applied in March and the 125 kg of N/ha applied in June and in September increased the $\text{NO}_3\text{-N}$ content in all seasons (Figure 7, page 51). The $\text{NO}_3\text{-N}$ content was near or above the potentially toxic range in all seasons with these rates.

Nitrogen

The total N contents of tall fescue for 1973 and 1974 are presented in Appendix Table IX. Observed values for percent N in all treatments were above the calculated NRC requirement of 1.47 percent for a lactating beef cow.

The application of 250 kg of N/ha in March increased the N content of tall fescue above that obtained from applications of 125 kg of N/ha (Figure 1, page 43). The N content was increased in the fall season by the September application of 125 kg of N/ha.

The N contents for the spring and fall seasons resulting from the applications of 125 kg of N/ha in March and September were not higher than those from the applications of 50 kg of N/ha in March and September (Figures 2, page 44 and 3, page 45). The 63 kg of N/ha applied in May and July did increase the summer N content of tall fescue.

Applying 125 kg of N/ha in July increased the N content of tall fescue for the summer (Figure 4, page 46). This N content obtained from applying 125 kg of N/ha in July was higher than the N content obtained from applying 50 kg of N/ha in June and July, but it was lower than the N content obtained from applying 125 kg of N/ha in June and 50 kg/ha in July (Figure 5, page 48).

The seasonal nitrogen content was generally increased by N fertilization; however, some increases obtained from the 50 kg of N/ha rate were not significant (Figures 7, page 51 and 8, page 52). The persistence of the increased N content appears related to the amount of N applied. In some instances where single, low N applications were the only ones applied during a season, the increases in N content were not sustained enough to influence the N content for the season. In other instances, as in the 1973 fall, a single application of 50 kg of N/ha in September increased the N content of tall fescue (Figure 13, page 58).

Fertilization with K and P did not increase the N content of tall fescue (Figures 15, page 60; 16, page 61; and 17, page 62).

Potassium

The percent K was well above the 0.80 percent NRC requirement for a lactating beef cow in all treatments for both years except for two observations on July 30, 1974. Treatment 1 had 0.70 percent K and treatment 13 had 0.86 percent K. The K contents of tall fescue for 1973 and 1974 are presented in Appendix Table X.

The fertilizer application in September (125 kg of N, 30 kg of P, and 60 kg of K/ha) increased the K content of tall fescue in the fall (Figure 1, page 43). The application of 63 kg of N/ha in May and July also increased the K content (Figure 2, page 44). The 125-30-60 (125 kg of N, 30 kg of P, and 60 kg of K/ha) fertilizer application in March increased the K content of tall fescue above that obtained from the 50-30-60 fertilizer application (Figure 3, page 45). There were no differences in K content during the fall when the same rates of fertilizers were applied in September.

There were no differences in spring K content of tall fescue when 125-30-60 and 63-30-60 fertilizer rates were applied in March (Figure 4, page 46). The K content of tall fescue during the summer months was increased by the July application of 125 kg of N/ha. However, the K content was not increased by the 125 kg of N/ha applied in June and September (Figure 5, page 48). This was apparently influenced in some way by the factors that caused the stand reduction which accompanied the fertilizer applications of treatment 5b. The percent K in treatment 5b for the summer and fall was not different from the percent K in the nonfertilized treatment 1 (Figure 7, page 51).

Fertilization with 60 kg of K/ha did not increase the K content of tall fescue in this experiment (Figures 15, page 60; 16, page 61; and 17, page 62). The June application of 60 kg of K/ha may have increased the K content; however, this increase was not significant.

These results support reports in the literature that K content of tall fescue is increased by N fertilization, but K fertilization does not increase the K content.

Calcium

In the analysis for Ca, most of the observations were near the lower end of the standard curve. This leaves some question as to the reliability of the results. Some of the observations were below the 0.29 percent NRC requirement for a lactating beef cow. Appendix Table XI contains the percent Ca of tall fescue in 1973 and 1974.

Reports in the literature indicate that Ca content of tall fescue increases during the growing season and with N fertilizer applications,

and decreases as the fescue forage matures. A seasonal pattern related to the Ca content of tall fescue was not found. The Ca values were very variable, and occasionally some were extremely high. Significant differences existed even in treatments that were almost identical (Figure 11, page 56). Although the results were variable, N fertilization appeared to decrease the Ca content of tall fescue (Figures 7, page 51 and 8, page 52).

Applying P and K in June may increase the summer Ca content of tall fescue (Figure 17, page 62).

Magnesium

Most of the observations of percent Mg were near the lower end of the standard curve, and their results may be questionable. All observations of percent Mg were above the 0.20 percent level, below which grass tetany problems may occur. Appendix Table XII contains the percent Mg of tall fescue in 1973 and 1974.

The Mg content of tall fescue was variable. No apparent seasonal pattern of the Mg content in tall fescue was observed in the fescue that was fertilized; however, the nonfertilized fescue had the lowest Mg content in the fall (Figures 7, page 51 and 8, page 52). In 1973, applied N decreased the Mg content in the tall fescue, especially in the spring (Figures 1, page 43; 7, page 51; and others). This decrease in Mg content after N application did not occur in all cases, especially in 1974 and when 250 kg of N/ha was applied. This is consistent with reports in the literature, where it is reported that N fertilizer applications have increased Mg content in some cases and have decreased

it in others. The reported seasonal variation of the Mg content in tall fescue with the lowest content occurring in the early spring months may be valid in some occasions with N-fertilized fescue, but apparently is not valid with fescue that has not been fertilized.

Potassium fertilization (60 kg of K/ha) applied in March did not decrease the Mg content (Figures 15, page 60; and 16, page 61). The literature indicated that K fertilization may decrease the Mg content of tall fescue. This may have happened in 1973 when the Mg content was lower after applying 60 kg of K/ha in September. However, the results in other treatments (Figures 11, page 56; and 16, page 61) were so variable that reliable conclusions cannot be made about the influence of fertilizer applications on the Mg content of tall fescue.

Phosphorus

All observations for percent P were above the NRC requirement of 0.29 percent P for a lactating beef cow. The P contents of tall fescue for 1973 and 1974 are presented in Appendix Table XIII.

The P content of tall fescue as reported in the literature is variable. Some workers report it as lowest in the summer, while others report it highest at that time. It has been reported that applied N and plant maturity may decrease the P content in tall fescue. An apparent pattern or reason for the observed differences in P content in these results was not found.

Perloline

The perloline content increased only in response to N fertilization. It is indicated in the literature that perloline content increases with

applications of N fertilizer, and that the perloline content is highest in the summer months. Consistent seasonal patterns of fluctuation in the perloline content were not found, except where influenced by N fertilization. Increases in perloline content due to N fertilization were larger in the summer and fall than in the spring.

Perloline increases were of short duration within seasons, and the averaging of values in seasons appeared to dilute the true significance of the increases, since the increases were found to be in response to N fertilization. It was decided that the values of the perloline content would be considered significant at $P \leq 10$.

The perloline content of tall fescue ranged from a low seasonal average of 0.007 mg/g to a high seasonal average of 0.757 mg/g. The 1973 and 1974 seasonal results are presented in Appendix Table XIV.

Applying 50 kg of N/ha in September increased the fall perloline content of tall fescue (Figure 18). Nitrogen fertilization in March did not increase the spring perloline content, except in 1974, when the 250 kg/ha rate did increase it (Figures 19, 20, and 21). However, 250 kg of N/ha applied in March and 50 kg of N/ha applied in May of 1974 did not increase the spring perloline content (Figure 19). The above becomes difficult to explain since 50 kg of N/ha applied in March and 50 kg of N/ha applied in May increased the perloline content of tall fescue in the spring of 1973 and 1974 (Figures 22 and 23). The high fall perloline content and the 1973 November application of 50 kg of N/ha may have influenced the low spring perloline content in the tall fescue of treatment 5b.

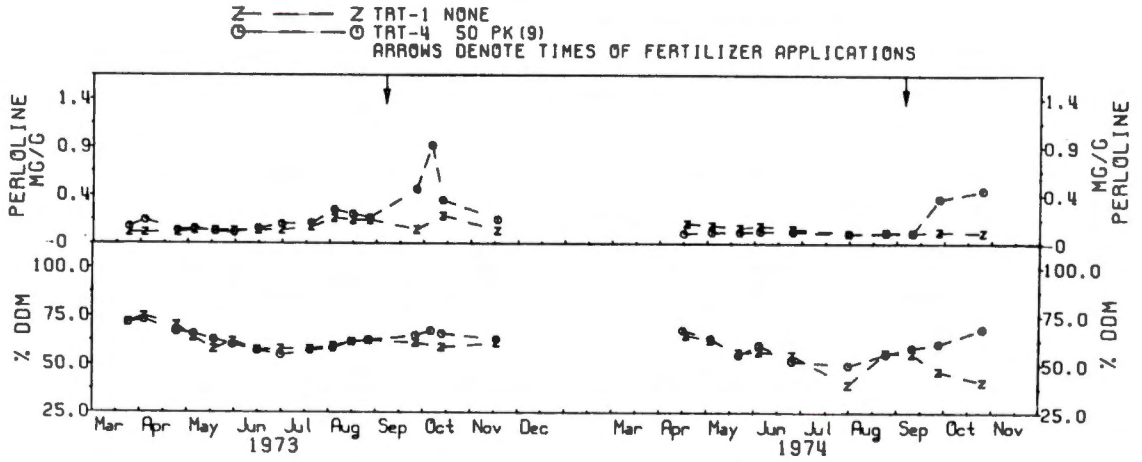


Figure 18. Comparison of the effects of no fertilization and September fertilization on tall fescue digestibility and perloline content in 1973-74.

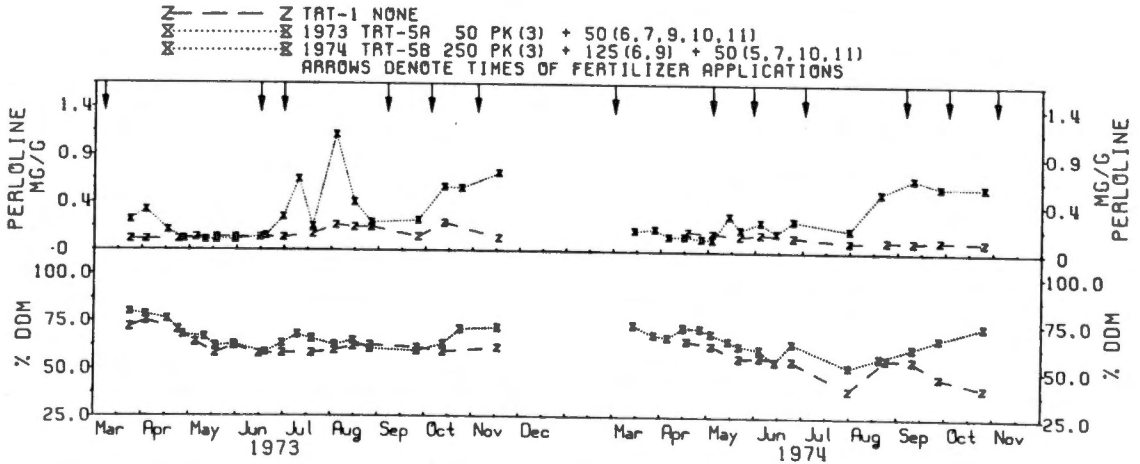


Figure 19. Comparison of the effects of no fertilizer and six and seven fertilizer applications on tall fescue digestibility and perloline content in 1973-74.

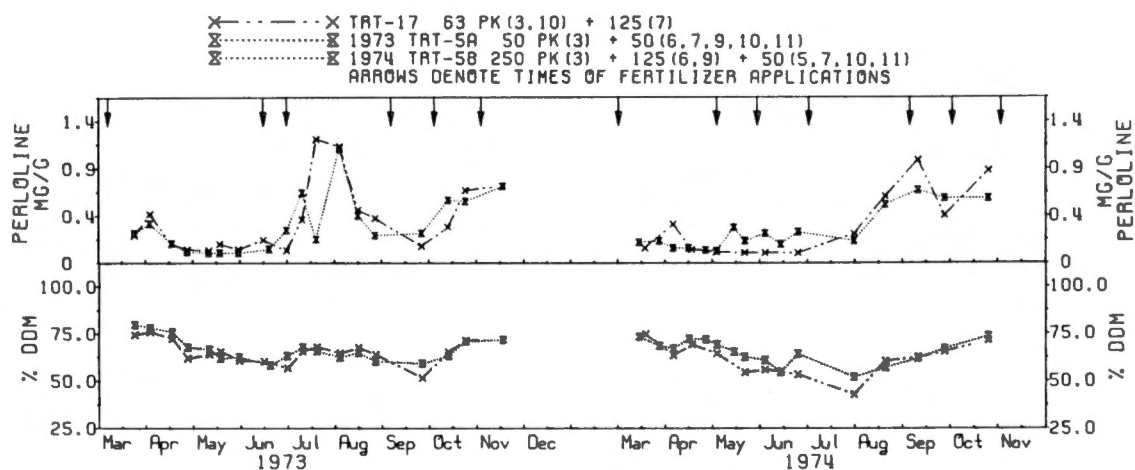


Figure 20. Comparison of the effects of three, six, and seven fertilizer applications on tall fescue digestibility and perloine content in 1973-74.

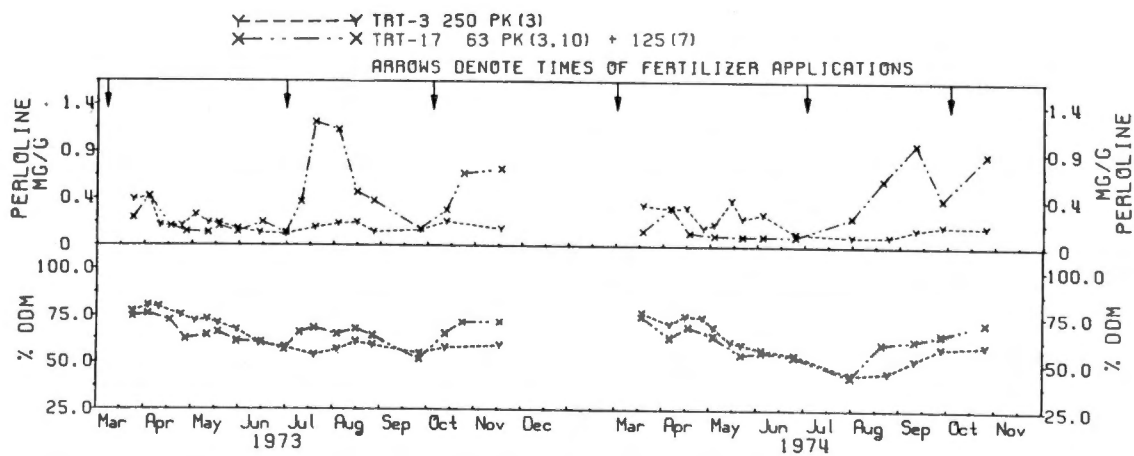


Figure 21. Comparison of the effects of one and three fertilizer applications on tall fescue digestibility and perloine content in 1973-74.

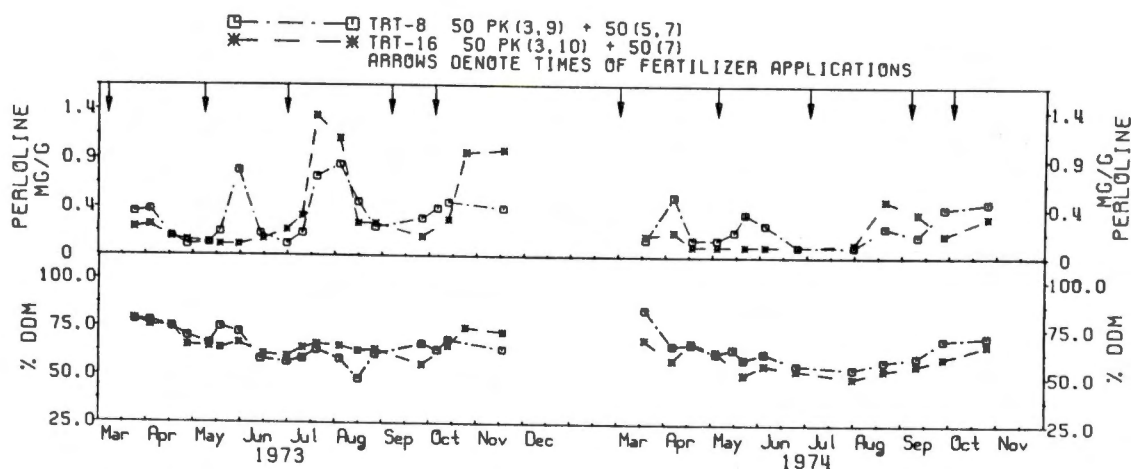


Figure 22. Comparison of the effects of three and four fertilizer applications on tall fescue digestibility and perloline content in 1973-74.

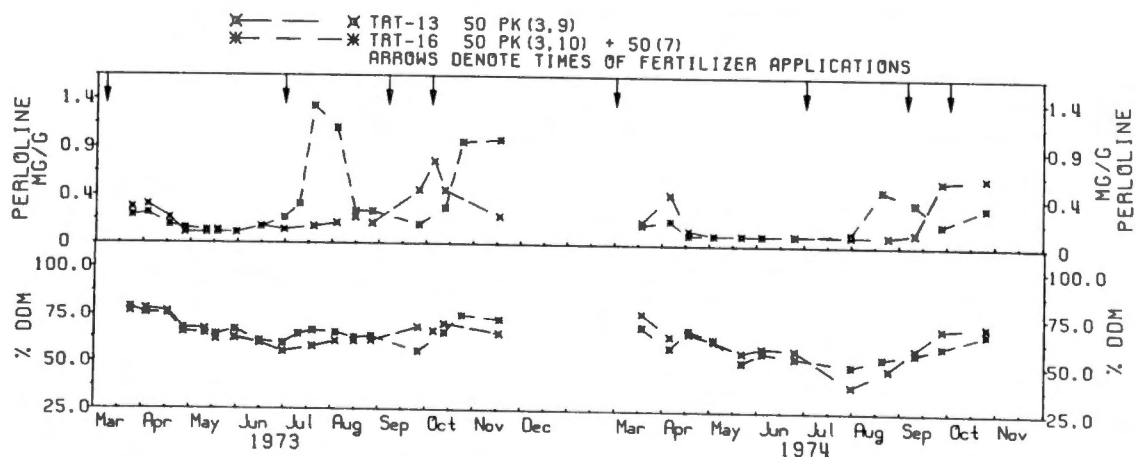


Figure 23. Comparison of the effects of two and three fertilizer applications on tall fescue digestibility and perloline content in 1973-74.

Applying either 50 kg of N/ha or 125 kg/ha in July increased the perloline content (Figures 23, 24, and 25), but the two increases were not different.

When the TLC plates were observed under long-wave ultraviolet light, three fluorescent compounds were observed. The closest one to the base line had a dull yellow fluorescence and was the same as the perloline standard. The other two were near the top of the TLC plates. The one nearer the bottom had a bright blue fluorescence; judging from the literature description, this probably was perlolidine. The top one had a bright red fluorescence and may be the third alkaloid reported by Yates (1963). The R_f 's were not recorded but positions of the fluorescent compounds were very similar to those shown by Yates. In room light the perloline had a yellow color. There was no visible color where the blue fluorescent compound was located. The brilliant red fluorescent compound was within a green area which may have had chlorophyll and other compounds, so the color associated with this red fluorescent compound is not certain.

Based on visual estimates of the amount of fluorescence, the blue compound generally increased as the perloline increased, although there were instances when large amounts of blue were present with small amounts of perloline. There appeared to be always large amounts of the red compound, although its brilliant color could easily have been deceiving.

Digestible Dry Matter

The percent digestible dry matter (DDM) had a consistent pattern of decreasing from spring to summer and increasing from summer to fall.

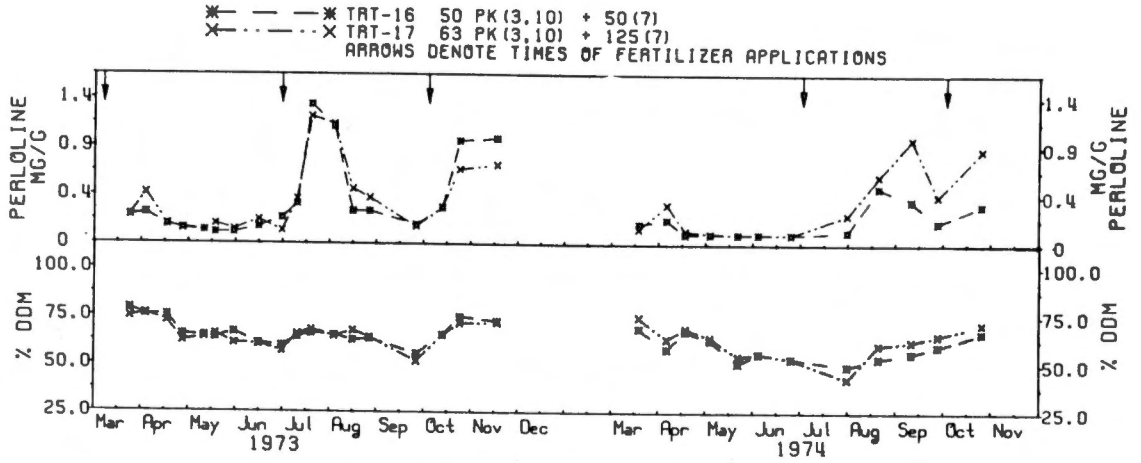


Figure 24. Comparison of the effects of high and low summer applications on tall fescue digestibility and perloline content in 1973-74.

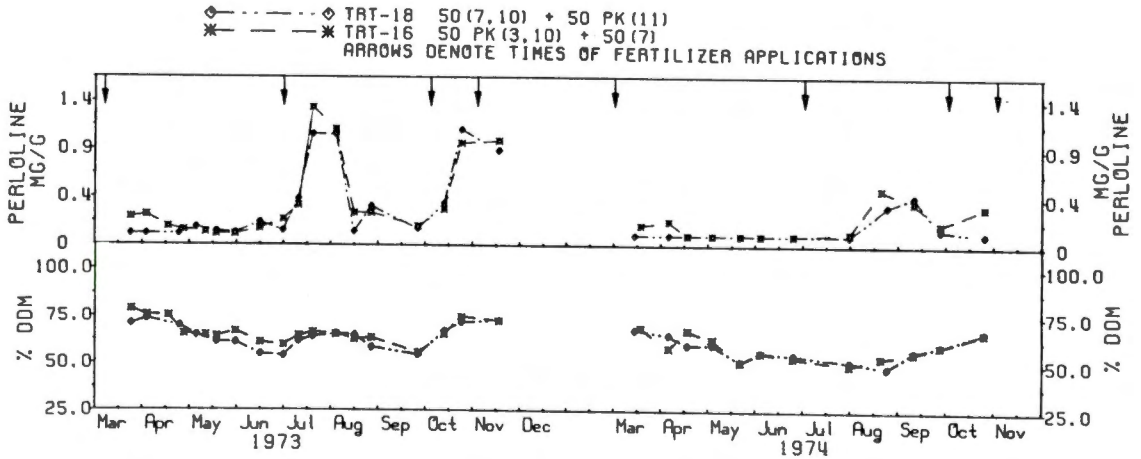


Figure 25. Comparison of the effects of March and November fertilizer applications on tall fescue digestibility and perloline content in 1973-74.

Digestibility generally was not influenced by N fertilization. Information in the literature indicates that the digestibility of tall fescue has been influenced the most by seasons. It is high in spring and fall, and low in summer. Nitrogen applications are reported not to influence greatly the digestibility of tall fescue. The percent DDM ranged from a low seasonal average of 47.7 to a high seasonal average of 74.4 (Appendix Table XV).

In the fall of 1974, the percent DDM obtained after the application of 50 kg of N/ha in September was higher than that obtained from tall fescue that had not received nitrogen (Figure 18, page 71). It has been reported in the literature that moisture and temperature stresses may lower the digestibility of tall fescue in the fall. This may have caused the lower percent DDM in the nonfertilized fescue.

Applying 50 kg of N/ha in June and July increased the percent DDM in tall fescue in the summer of 1973 (Figure 19, page 71). Nitrogen applied at 125 kg/ha in July increased the percent DDM in the summer (Figure 21, page 72). In the spring of 1973, the percent DDM of tall fescue was increased by the application of 250 kg of N/ha.

In the summer of 1973, 50 kg of N/ha applied in July increased the percent DDM of tall fescue (Figure 23, page 73). Why the percent DDM of treatment 16 (Figure 22, page 73) was higher than that of treatment 8 in the summer of 1973 is not apparent. Both treatments received 50 kg of N/ha in July. The application of 125 kg of N/ha in July did not increase the summer percent DDM in tall fescue above that obtained from the application of 50 kg of N/ha.

The percent DDM apparently was not influenced adversely by the perloline content since increases in percent DDM and perloline content generally occurred together. This is consistent with reports in the literature. The in vitro digestion time may be too short to adequately break down cell walls where alkaloids of dried plant material may be located. Increasing the time of digestion or using fresh plant material may be possible methods for better ascertaining influences of perloline on digestibility.

General Discussion

The application of nitrogen generally caused a darker green color in tall fescue, and increased the yields and the N, K, $\text{NO}_3\text{-N}$, and perloline contents. The application of N decreased the percent dry matter and may have decreased the Ca and Mg content. The P content did not appear to be influenced by N fertilization. In vitro digestion was influenced most by season and very little by N applications. The higher rates of N application did increase the percent DDM in some instances.

The N, $\text{NO}_3\text{-N}$, and perloline content increased soon after the fertilizer applications; however, yield increases, especially in summer, sometimes were not observed until a month after fertilizer applications. Animals eating tall fescue forage that has received N fertilizer in the summer months may benefit from the increased N content but also may be harmed by the higher content of $\text{NO}_3\text{-N}$ and perloline. Regardless of the effect on animals, these compounds apparently are present in higher

quantities soon after fertilization and not necessarily at the time of observed yield increases.

Increasing the rates of N application did not always increase yields. Applying 250 kg of N/ha in March gave no spring yield increase over that resulting from applying 125 kg of N/ha in March; however, the 250 kg of N/ha did increase the average N content for the spring by 0.84 percent and increased the $\text{NO}_3\text{-N}$ content by 0.129 percent. Individual $\text{NO}_3\text{-N}$ values from the 250 kg of N/ha application were well above the 0.34 value which is considered potentially toxic, but $\text{NO}_3\text{-N}$ levels from the 125 kg of N/ha were well below the danger level. Applying 250 kg of N/ha in March increased the spring percent DDM by about 6.5 percent above that obtained from applying 63 kg of N/ha in March. Increasing the rate of N applied did not increase the perloline content of tall fescue. There was no apparent advantage to applying more than 50 kg of N/ha in a single application in September.

When N was applied at the rate of 250 kg/ha in March, 50 kg/ha in May, 125 kg/ha in June, and 50 kg/ha in July, the tall fescue stand was reduced 20 to 30 percent. Following the stand reduction, the yields decreased from 2,703 kg/ha in the summer to 637 kg/ha in the fall. The K content also decreased from 2.88 percent in summer to 1.91 percent in fall. The N and $\text{NO}_3\text{-N}$ contents also decreased from the summer values, but were still higher than values observed in other treatments. The perloline content and percent DDM apparently were not influenced by the stand reduction.

Timing of N applications did influence tall fescue more than changing the rates of N. The November N applications of 50 kg/ha

resulted in earlier spring harvest and comparable spring yields with those yields obtained from 50 kg of N/ha applied in March. Applying the N in November added about 800 kg of dry matter/ha to the spring yield. Neither September nor October applications of 50 kg of N/ha resulted in earlier spring harvest.

Summer fertilization to induce increased dry matter yields for summer months is questionable unless adequate moisture is present. Increased yields in 1973 occurred a month after the application of 125 kg of N/ha in July. These increases were observed immediately after a three-day rain in late July.

The same type of application in 1974 produced delayed yields that occurred into the month of September. The 125 kg of N/ha applied in July increased the digestibility of tall fescue and the contents of N, perloline, and $\text{NO}_3\text{-N}$. The $\text{NO}_3\text{-N}$ content was near or above the 0.34 percent danger level.

Frequent applications of low rates of fertilizer (50 kg of N/ha) in May, June, and July increased summer yields and the content of N and perloline in tall fescue without increasing the $\text{NO}_3\text{-N}$ content to the 0.34 percent danger level. This may be one advantage to using frequent applications of low rates of N fertilizer. These applications made in March, May, July, and September were the best overall treatment for total yield, yield distribution, and optimum chemical composition.

Potassium content of fescue increased when N fertilizer was applied. Much of this increase was above the 3.5 percent value and may be considered as "luxury consumption."

III. STOCKPILE EXPERIMENT

In the stockpile experiment, treatments 1 and 2 were controls that were continuously clipped, as described in the Materials and Methods section. Treatment 8 received the same fertilizer applications as the control treatment 1, which was also used as the control for treatment 11. The spring-, fall-, and spring-fall-stockpiled treatments received the same fertilizer applications as applied to treatment 2.

Groupings of 3 or 4 treatments were made so that each stockpiling scheme would have a hay cut and a gradual clip, and these could be rotated among the 3 or 4 treatments within a grouping in order to avoid, as much as possible, the effects that a hay cut might have on future results. The groupings of the treatments are identified in Table II, page 32.

There were no differences in the treatments within groups, except some hay dry matter yields from stockpiled fescue were higher than those from gradual clipping. Thus, a treatment from each group was chosen to compare with representative treatments of the other groups. Only the figures for these treatments are included, while the Appendix tables contain all treatments.

Dry matter yield, percent dry matter, content of N, K, Ca, Mg, P, and perloline, and digestibility, are discussed in relationship to the stockpiling treatments.

Gradual clipping in the summer and fall of 1973 and 1974 consisted of less than three cuts, due to the small amount of growth of the fescue.

Yield

As mentioned above, yield was higher for some hay cuts than for gradual clippings of the stockpiled fescue (Appendix Table XVI). The 1973 fall yields reflected this, because the lower yields obtained from periods of continuous clipping masked the differences in the spring- and summer-stockpile yields. In Appendix Table XVI, the mean yields for the season have been replaced with the total yields.

The dry matter available for summer grazing from the spring-stockpiled fescue was about 4,000 kg/ha more than that from the continuously clipped fescue (Figures 26, 27, and 28). The fescue available for fall grazing from the early summer-stockpile was about 2,100 kg/ha more than that from the continuously clipped fescue (Figure 29), while that from the late summer-stockpile was about 1,500 kg/ha more. Fall stockpiling made about 1,500 kg of fescue dry matter/ha available for late fall or early winter grazing (Figure 30).

The yield of the summer regrowth was not influenced by the harvesting of the spring-stockpiled fescue. This is important, because the N, K, and perloline contents of tall fescue and its percent DDM were influenced by the spring-stockpile harvest, as will be discussed later.

Percent Dry Matter

The seasonal averages of percent dry matter of tall fescue in 1973 and 1974 are presented in Appendix Table XVII. The comparison of spring- and fall-stockpile treatments (Figure 28) indicated that stockpiling

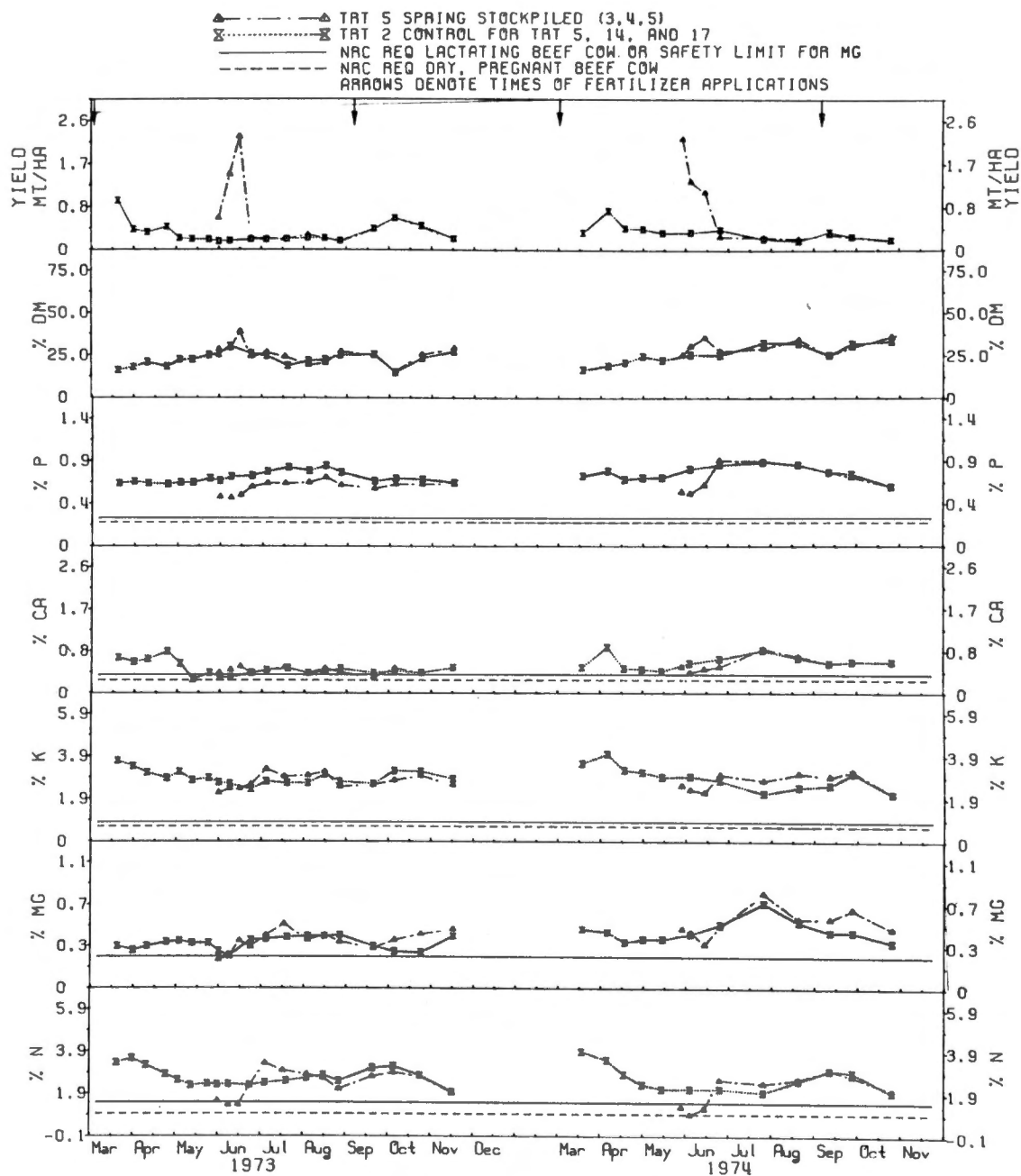


Figure 26. Comparison of the effects of spring stockpiling and continuous clipping on tall fescue productivity and composition in 1973-74.

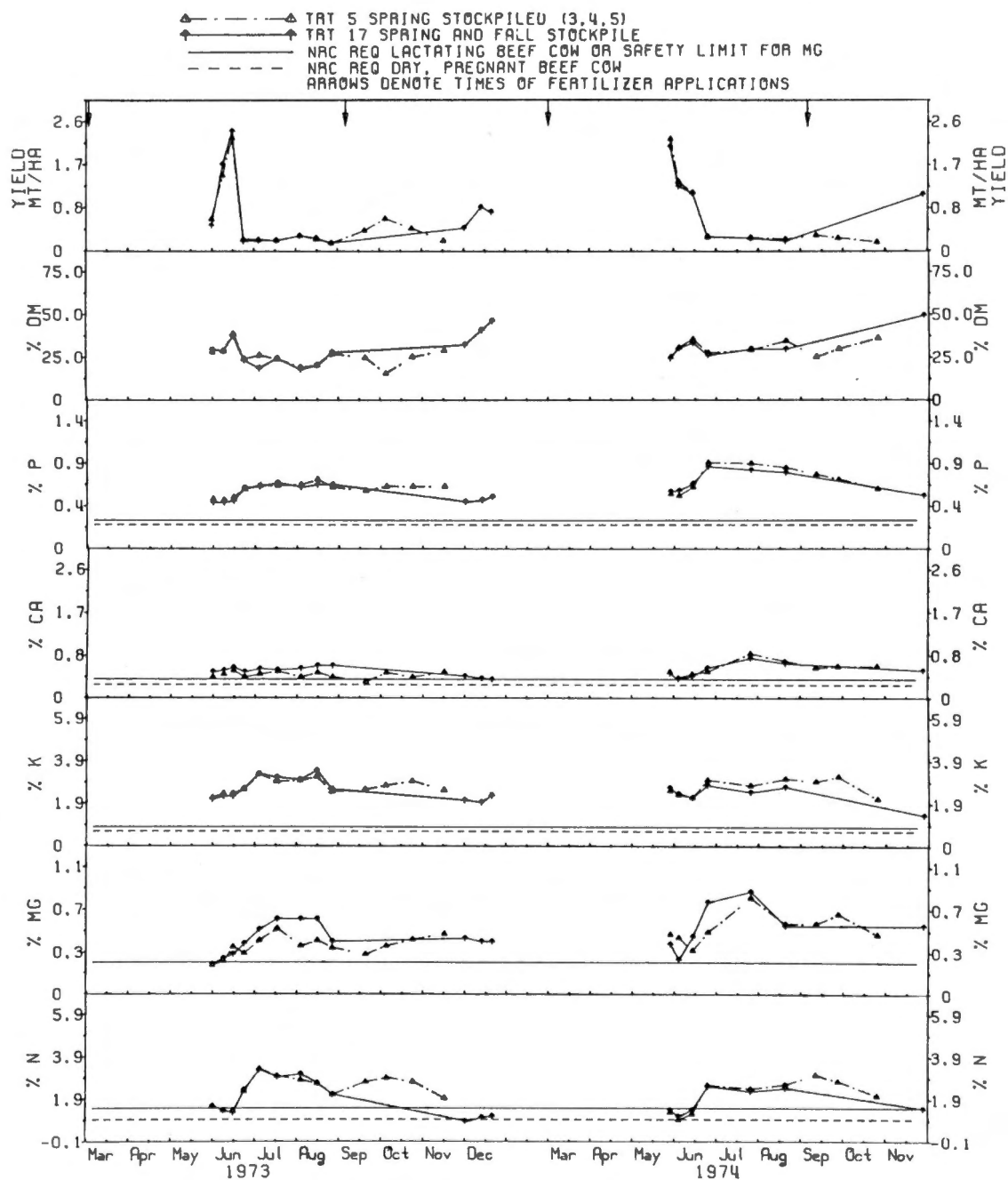


Figure 27. Comparison of the effects of spring and spring-fall stockpiling on tall fescue productivity and composition in 1973-74.

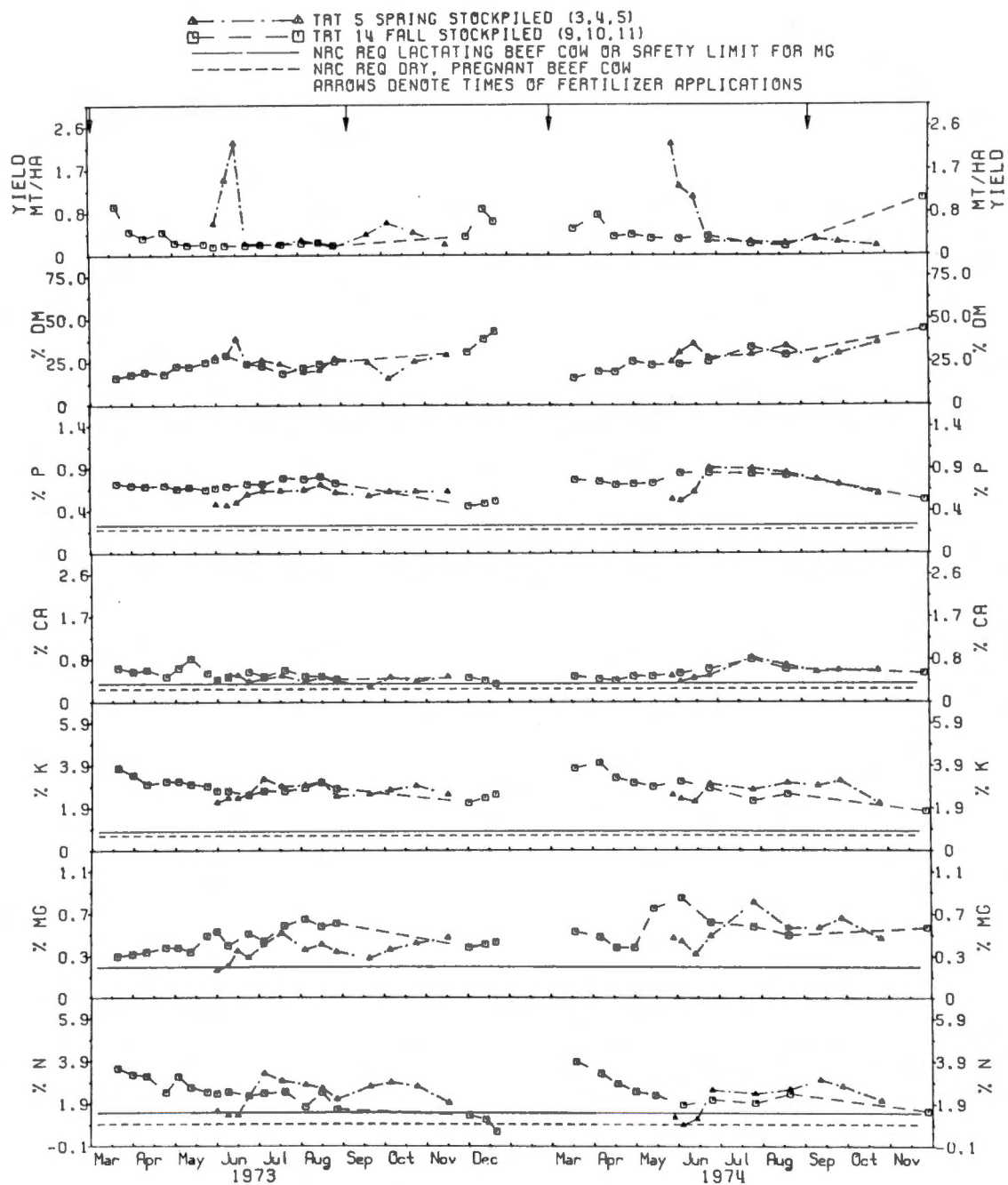


Figure 28. Comparison of the effects of spring and fall stockpiling on tall fescue productivity and composition in 1973-74.

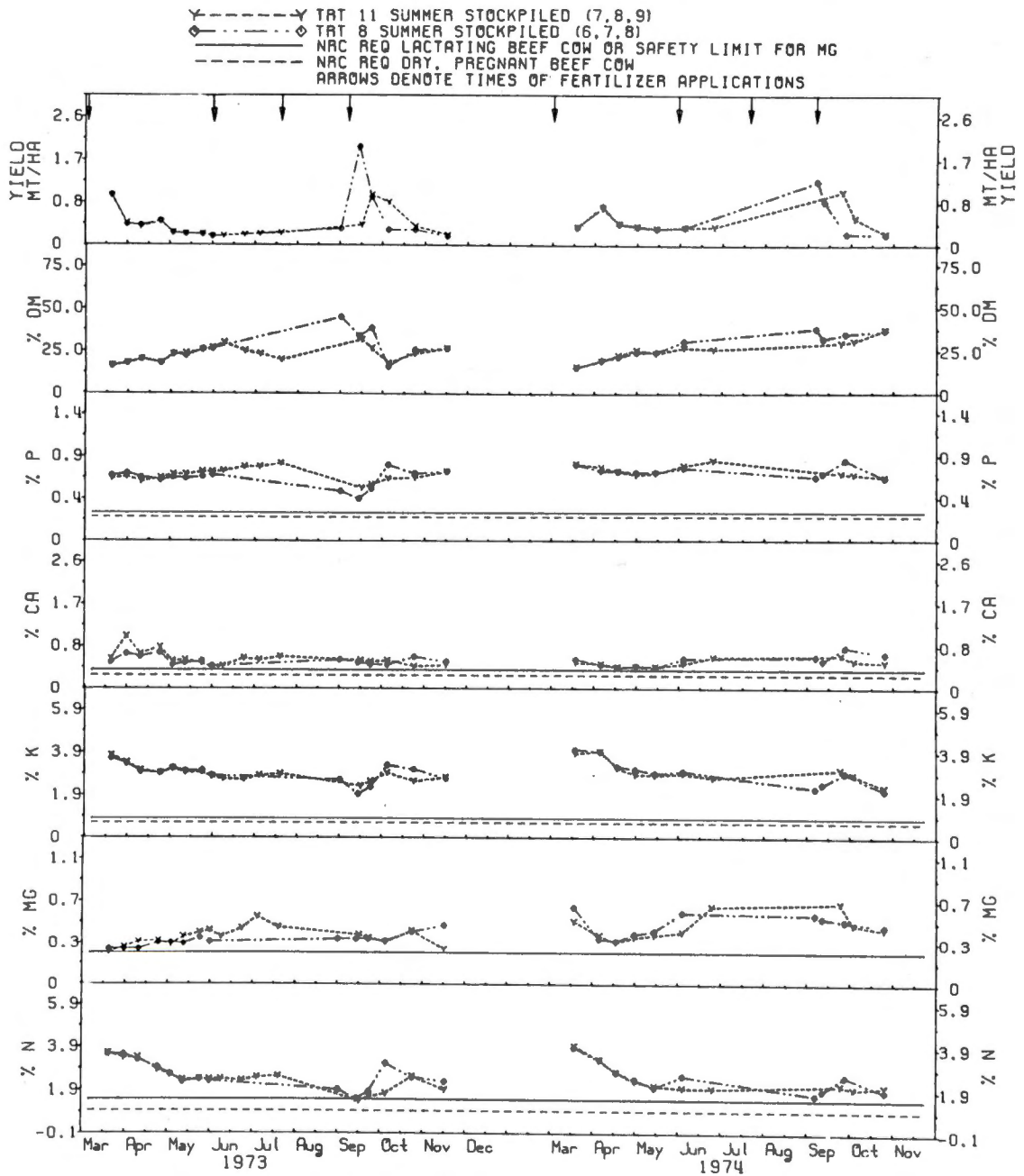


Figure 29. Comparison of the effects of two summer stockpilings on tall fescue productivity and composition in 1973-74.

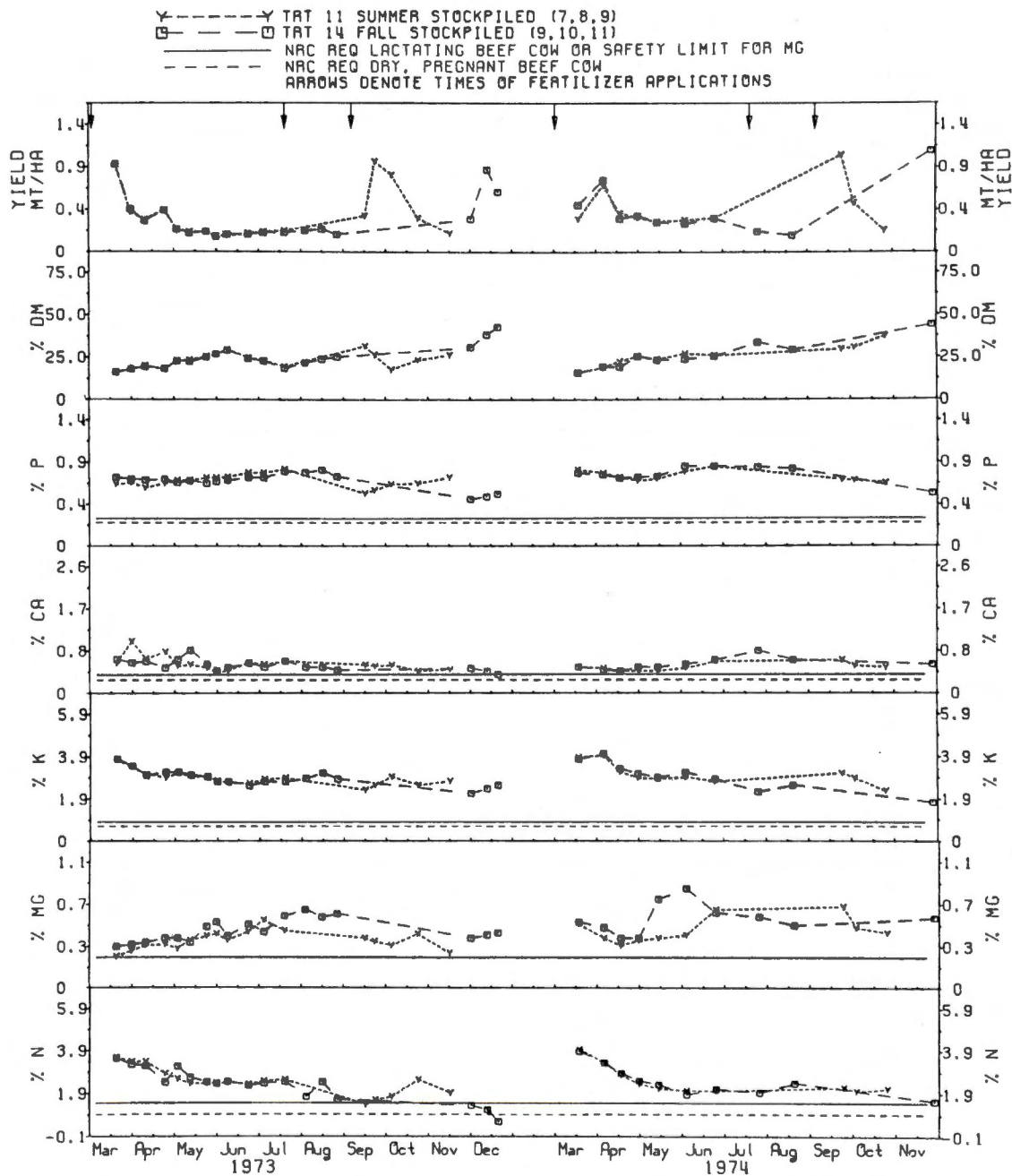


Figure 30. Comparison of the effects of summer and fall stockpiling on tall fescue productivity and composition in 1973-74.

resulted in a higher DM percentage. Gradual clipping, represented by the first three harvests of treatment 5 in 1973 and 1974 and by the last three harvests of treatment 14, indicated that the leafy plant material cut above 15 cm had about the same percent DM as the continuously clipped forage harvested at the same time. The forage harvested at heights of 10 to 15 cm was a week older, and that from 5 to 10 cm was two weeks older than the forage harvested at heights of 15 cm and higher. This forage also was less leafy and had a higher (not significant) percent DM.

The stockpiled fescue was generally higher in percent DM than the continuously clipped fescue. This was expected since more mature plant material has less water content than material from young growing leaves.

Nitrogen

The percent N values for the seasons of 1973 and 1974 are shown in Appendix Table XVIII. Stockpiling resulted in very low N content of tall fescue. The N content of stockpiled fescue was near the 1.47 percent NRC requirement for a lactating beef cow in all seasons, but in the fall of 1973 it was below the 0.96 percent requirement for a dry beef cow. Stockpiling is known to result in lower quality forage; however, the maturity of the forage and the length of the stockpiling period influence greatly the quality of the forage. In fall-stockpiled fescue, crude protein and percent DDM have decreased rapidly after October and early November. In spring-stockpiled fescue, percent DDM has decreased rapidly after heading was initiated. Harvesting the

spring- and fall-stockpiled fescue earlier in the season might have resulted in higher N content of the tall fescue.

The summer regrowth after harvesting the spring-stockpiled tall fescue had a higher (not significant) N content than that of the continuously clipped fescue (Figures 26, page 82; and 28, page 84).

The topmost, leafy stockpiled material in the first cut of the gradual clippings generally had a higher (not significant) N content than the older, stemmy material of the second and third cuts (Figures 26, page 82; 27, page 83; and 28, page 84). Thus, animals consuming stockpiled tall fescue may be getting even lower quality forage as they graze the stockpiled fescue closer to the ground and may need additional supplements of N in the diet to maintain an adequate protein level in the feed. However, decreases in N content of the stockpiled material may be balanced by the increases of N content observed in the regrowth material.

Potassium

Appendix Table XIX contains the percent K of tall fescue for the seasons of 1973 and 1974. The percent K in tall fescue was decreased by spring stockpiling. This was generally true for the other stockpiling periods, but the decrease in percent K was much less and also more variable. The regrowth after harvest of spring-stockpiled fescue contained more K than did fescue from continuously clipped plots (Figures 26 and 28). In the 1974 fall, stockpiling significantly decreased the percent K (Figures 27, 28, and 29).

Calcium

The Ca content of the fescue was variable. Reasons are not apparent for those significant differences which were observed (Appendix Table XX). The Ca content of the fescue in the 1974 summer drought generally increased as the season progressed regardless of stockpiling treatment. This may indicate that drier conditions without fertilizer are favorable to Ca uptake by fescue plants.

Magnesium

The Mg content of the fescue was variable. The results indicate that the Mg content may be higher in the summer seasons than in the spring or fall for most treatments (Appendix Table XXI).

Phosphorus

The P contents of tall fescue are presented in Appendix Table XXII. The results indicate that stockpiling reduced the P content, and this was significant in the 1973 summer (Figures 26, page 82; 27, page 83; 28, page 84; 29, page 85; 30, page 86; and 31).

Perloline

The seasonal results for perloline content in tall fescue are shown in Appendix Table XXIII and in Figures 32 through 37. The perloline content was higher following N fertilization in June (Figure 37). The perloline content increased very little following N applications in March and September.

The perloline content of stockpiled fescue was very low. The values were so low that differences in perloline content of tall fescue

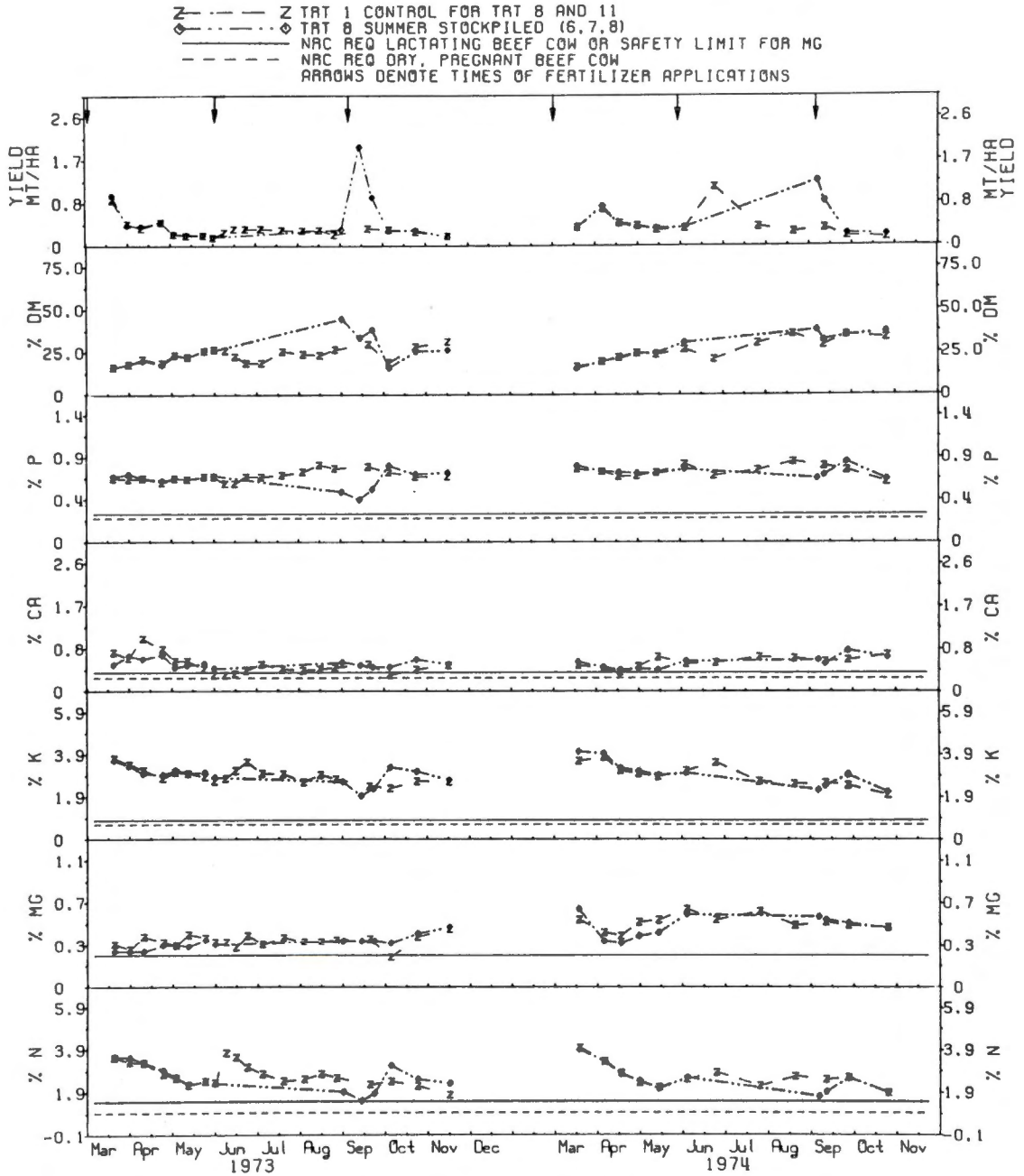


Figure 31. Comparison of the effects of summer stockpiling and continuous clipping on tall fescue productivity and composition in 1973-74.

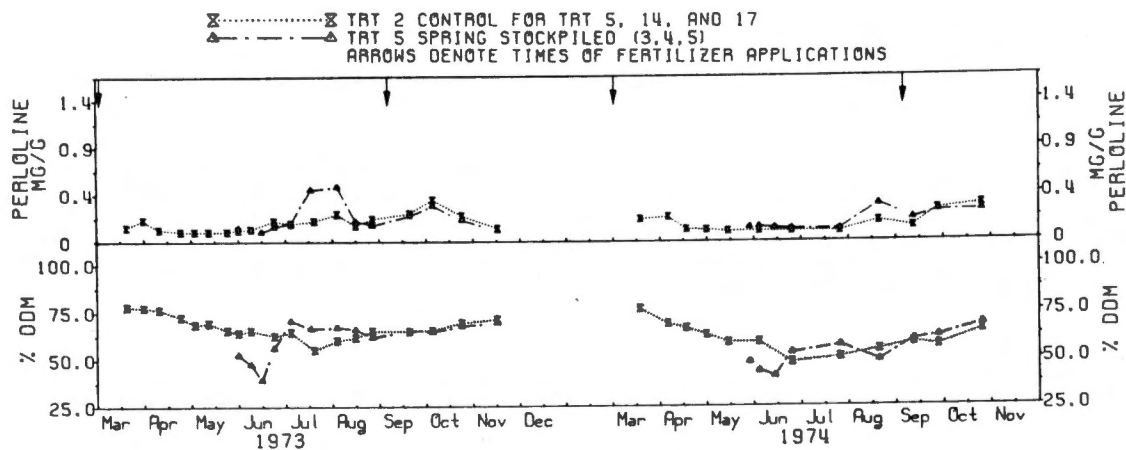


Figure 32. Comparison of the effects of spring stockpiling and continuous clipping on tall fescue digestibility and perloline content in 1973-74.

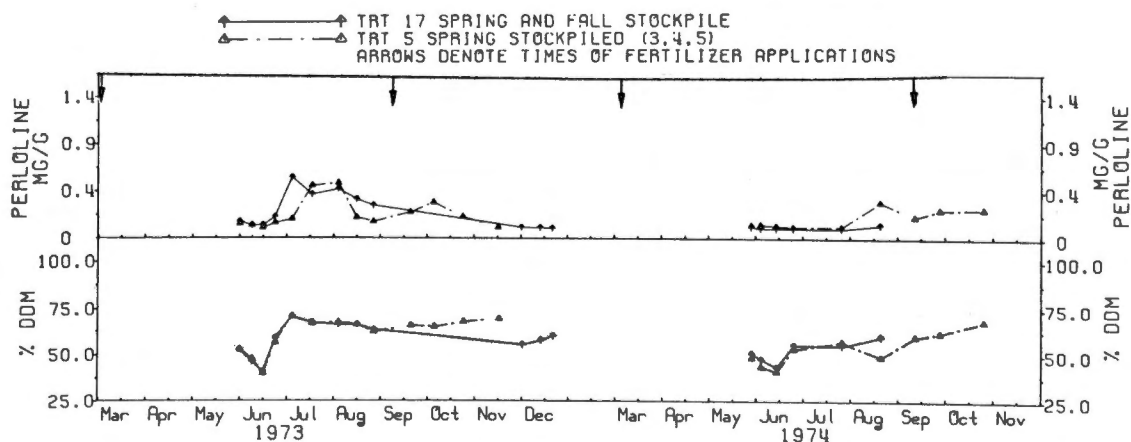


Figure 33. Comparison of the effects of spring and spring-fall stockpiling on tall fescue digestibility and perloline content in 1973-74.

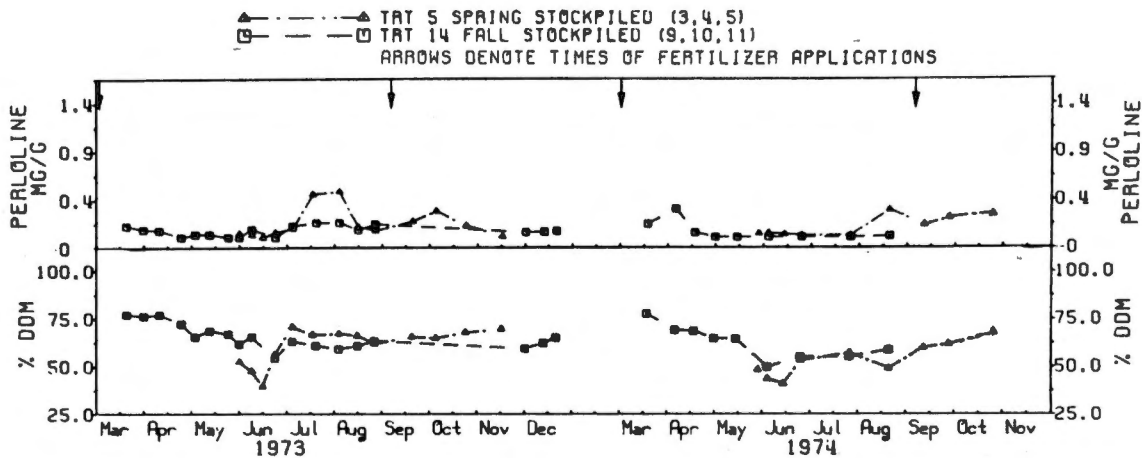


Figure 34. Comparison of the effects of spring and fall stockpiling on tall fescue digestibility and perloine content in 1973-74.

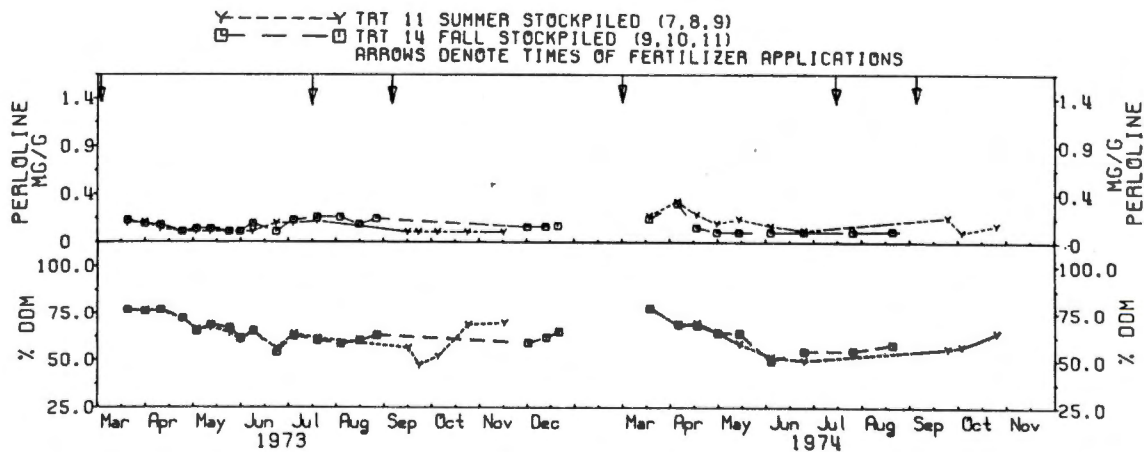


Figure 35. Comparison of the effects of summer and fall stockpiling on tall fescue digestibility and perloine content in 1973-74.

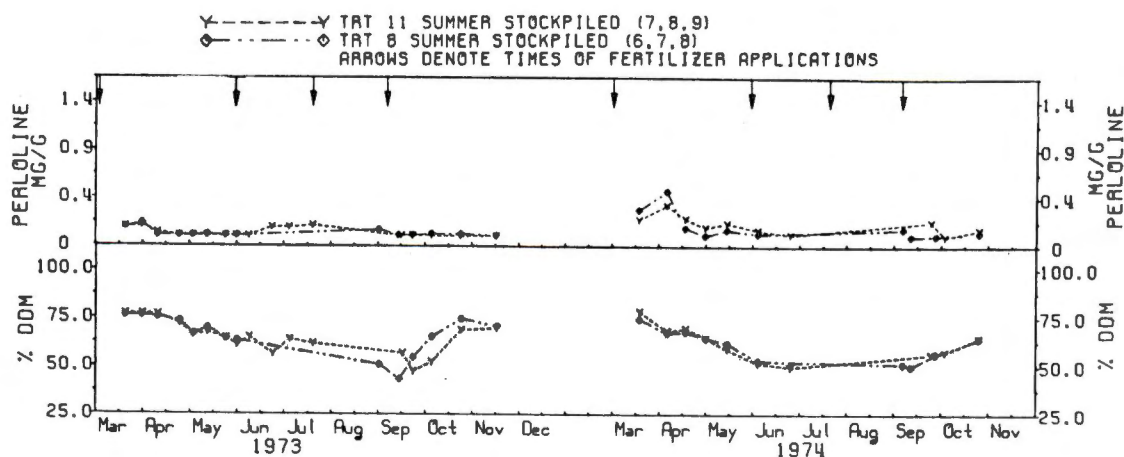


Figure 36. Comparison of the effects of two summer stockpilings on tall fescue digestibility and perloline content in 1973-74.

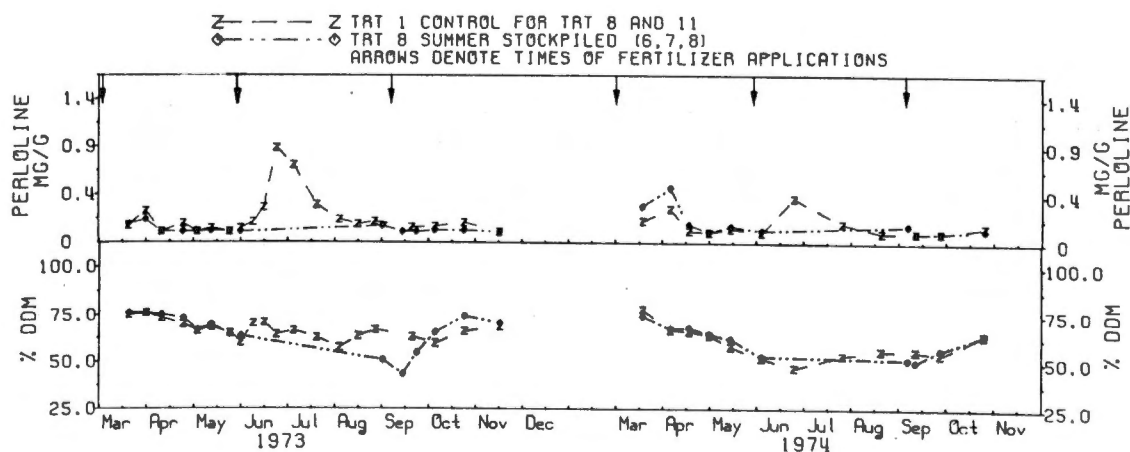


Figure 37. Comparison of the effects of summer stockpiling and continuous clipping on tall fescue digestibility and perloline content in 1973-74.

cut at the gradual clipping heights could not be detected. Thus, the results did not provide support for reports that alkaloid content is higher in the upper one third of the fescue plant than in the lower two thirds of the plant.

Stockpiling may be a management tool that will allow one to avoid periods when high perloline contents occur in tall fescue. Summer stockpiling (Figure 37, page 93) avoided the higher perloline content observed in the nonstockpiled control.

The summer regrowth after the harvest of the spring-stockpiled fescue was higher in perloline content than the continuously clipped fescue (Figures 32, page 91; 33, page 91; and 34, page 92). Bush (1975) indicated that new root growth initiated after the clipping of the stockpiled fescue may be responsible for these results, since perloline production may take place in the roots of tall fescue.

Digestible Dry Matter

The percent DDM data are presented in Appendix Table XXIV. The percent DDM in stockpiled fescue was often below 50 percent. This supports the literature reports that quality of tall fescue is lower in stockpiled material. However, fall-stockpiled fescue had DDM values above 60 percent. This indicates the fescue available for winter grazing may not be as low in quality as previously suspected when the low N contents were considered (page 87).

The summer regrowth after the harvest of the spring-stockpiled fescue was higher in percent DDM than the continuously clipped fescue (Figures 32, 33, and 34).

The upper one third of the fescue plant had a higher percent DDM than the lower two thirds of the plant (Figures 32, page 91; 33, page 91; 34, page 92; 35, page 92; 36, page 93; and 37, page 93). The need for supplemental feeding may increase as animals feeding on stockpiled fescue are consuming the lower plant parts.

General Discussion

Stockpiled tall fescue was lower in percent DDM and in N, P, and K content than was the tall fescue that was continuously clipped. The lower quality is generally expected in stockpiled fescue. However, quality for the spring-stockpiled fescue apparently could have been higher if the fescue had been harvested at first signs of heading. The quality of the fall-stockpiled fescue might have been higher if harvesting had been done earlier in November. The percent DDM of stockpiled fescue appeared to be higher in the fall-stockpiled forage than in the spring- or summer-stockpiled fescue. Thus, fall-stockpiling may result in a forage of better quality than stockpiling at other times.

Stockpiling appears to be a tool for avoiding periods of high perloline content in tall fescue. The summer months have been reported as periods when the perloline content of tall fescue is high. The perloline content of stockpiled tall fescue was very low. Stockpiling in the spring resulted in fescue for summer grazing that was low in perloline content. However, the regrowth of the fescue after harvesting the spring-stockpiled fescue had a higher perloline content than the continuously clipped fescue. This regrowth also was higher in percent DDM and in N and K content.

The N content of stockpiled material was generally below the NRC requirement for a lactating beef cow, and sometimes it was near or below the NRC requirement for a dry, pregnant beef cow. The K and P contents of the stockpiled fescue were always above the NRC requirement. The Ca and Mg content occasionally dropped below the NRC requirement, and this occurred more often in the continuously clipped material than in the stockpiled material.

Spring stockpiling accumulated the highest dry matter yields of any stockpile period. Yields were over two times greater than the yields of continuously clipped fescue during the same time period, and the forage available for summer grazing from these yields was six times greater than the forage available from continuously clipped fescue in the summer months. Summer stockpiling provided dry matter for fall grazing that was twice the amount from continuously clipped fescue in the fall. Fall stockpiling can provide extended grazing for late fall and early winter, but the dry matter yields were the lowest of any stockpile period.

CHAPTER V

SUMMARY AND CONCLUSIONS

Investigations were made to evaluate the effects of several rates and times of fertilizer applications, particularly nitrogen, and of several stockpiling schemes, on tall fescue quality and quantity. The percent digestible dry matter (DDM) of fescue samples was obtained using in vitro methods. The percent DDM was considered an estimate of forage quality, and the perloline content of the fescue samples was considered an adverse factor affecting forage quality. The results indicated that the percent DDM was not influenced by the perloline content of the tall fescue. The variation among seasons appeared to be the major factor influencing the percent DDM of tall fescue.

The dry matter yield, percent dry matter (DM), and the content of nitrate-nitrogen ($\text{NO}_3\text{-N}$), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and nitrogen (N) were determined. Phosphorus, Mg, and Ca contents of tall fescue were variable, and explanations for this variability were not apparent. However, the P content of stockpiled fescue was lower than that of continuously clipped fescue. The percent DDM and the N and K content also were low in the stockpiled fescue.

The $\text{NO}_3\text{-N}$ content was determined to estimate times and conditions which may lead to nitrate poisoning. The $\text{NO}_3\text{-N}$ content was increased by N fertilizer applications. Rates of applied N that were 125 kg/ha or higher increased $\text{NO}_3\text{-N}$ content of tall fescue to near or above the

0.34 percent level which is considered potentially toxic. Increases in the $\text{NO}_3\text{-N}$ content after N application at lower rates than 125 kg/ha were not as great in the spring season as in the summer and fall. Thus, nitrate poisoning may be less likely in spring than in summer or fall if N fertilizer applications are made in these seasons.

Nitrogen fertilization resulted in fescue forage that was higher in N and K content. This might indicate a higher quality forage, but perloline content also was higher after N applications. Reports in the literature indicate that perloline may lower the quality of fescue; however, this research found no evidence to support this. Nitrogen fertilization also resulted in lower percent DM of tall fescue.

Stockpiling during the spring and fall can provide additional forage for summer and winter, respectively. The stockpiling schemes were set up to determine what time periods for stockpiling would be appropriate for use in Tennessee. If increasing dry matter yields and having feed available at given periods of the year is one's objective, then any stockpiling period would appear to be appropriate. The yields of fall-stockpiled fescue were lowest, while the yields of spring-stockpiled fescue were highest. If quality of the stockpiled fescue is important, then the stage of maturity, the length of time the fescue is stockpiled, and weather conditions should be considered for determining a harvest date that will provide a higher quality forage.

Increasing rates of N fertilizer applications resulted in fescue yields that were variable according to seasons. In the spring, increasing N rates from 63 kg/ha to 125 kg/ha applied in March increased spring

yields, but increasing the rates from 125 kg/ha to 250 kg/ha did not increase spring yield. The late spring and early summer yields were higher when 250 kg/ha were applied in March. The 250 kg/ha rate of applied N did not increase the yield above that from the 125 kg/ha rate, but it extended the period during which the higher yield was maintained. In the fall, increasing N rates from 63 kg/ha to 125 kg/ha applied in September did not increase yields. Apparently the 63 kg of N/ha was sufficient for maintaining high yields in the fall.

Times of fertilizer applications were important. Frequent applications of 50 kg of N/ha during the summer season reduced the danger of high $\text{NO}_3\text{-N}$ content and generally provided yields comparable to those obtained from single applications of 125 kg of N/ha. November applications of 50 kg of N/ha provided earlier and larger spring yields. May applications of 50 kg of N/ha increased late spring and early summer yields. Frequent application of high rates of N damaged fescue stands in the late summer of 1974. July applications of 125 kg of N/ha increased the summer yields without damaging fescue stands. Phosphorus and K fertilization had little effect on any of the variables analyzed.

A management practice for increasing yields safely and efficiently might be the application of 125 kg of N/ha in March, 50 kg of N/ha in May, 50 kg of N/ha in September, and 50 kg of N/ha in November. If this would not be economically feasible, at least 125 kg of N/ha in March and 50 kg/ha in September should be applied. Incorporating spring and fall stockpiling schemes with these two rates and times of N applications would provide more summer forage and extend the winter grazing time.

Future stockpiling studies should consider shorter stockpile periods in the spring and fall in an effort to obtain higher quality stockpiled fescue. The higher rates of N applications at the beginning of a stockpile period may influence the quality of the fescue and should be investigated.

In order to have a better understanding of the relationship of N fertilizer application to the content of N, $\text{NO}_3\text{-N}$, and perloline in tall fescue, a one-month or two-month study in which several rates of N are applied, and where daily or every-other-day harvests are made, would be very beneficial.

In vitro digestibility methods which will allow rumen fluid digestion periods of at least 72 hours instead of 48 hours may be instrumental in determining whether or not perloline has an influence on the digestibility of tall fescue. Perhaps a better method would be to predetermine the content of perloline in the fescue sample and add this amount of perloline to digestion samples at the time rumen fluid is added.

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APPENDIX

TABLE IV

PROCEDURE FOR PERLOLINE ANALYSIS

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1. Place 1.0 g dry ground tissue in 50 ml centrifuge tube
 2. Add 0.25 g sodium bicarbonate
 3. Add 2 ml H₂O
 4. Grind for 2 minutes (long screwdriver does fine)
 5. Add 10 ml chloroform: ethanol (95:5) v/v
 6. Grind again for 30 sec.
 7. Allow to stand for 15 minutes in the refrigerator
 8. Centrifuge at 14,500 r.p.m. for 10 min. (refrigerated)
 9. Decant organic phase and filter through kimwipe or rapid filter paper
 10. Spot 25 microliters on Silica Gel G TL plate that has been scored for spectrodensitometer
 11. Develop TLC plates in solvent system of butanol: acetic acid (4:1) v/v saturated with water—this takes 4 to 5 hours. R_f of perloline is .33-.35
 12. Read TLC plates on spectrodensitometer at 390 nm
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TABLE V

PROCEDURE FOR PREPARING TLC PLATES

For 5 plates 20 cm x 20 cm each:

1. Rinse plates thoroughly and place in chromic-sulfuric acid bath for several hours. Remove plates from acid and wash with alconox and warm water. With a tissue, wash plates with alcoholic hydroxide. Wash plates again with alconox and warm water, rinse with distilled water and dry plates in an oven. Avoid fingerprints on the plates, handle plates only by the edges.
 2. Handling plates near the edges, load plates into the leveling device. Use a 5 cm plate on each end for starting and finishing. Place any half plates 10 x 20 cm at the end where spreading will be finished.
 3. Soak a lab tissue with diethyl ether and wash plates in the leveling device.
 4. To obtain a 0.25 mm layer, weigh out 30 g of Silica Gel G and place in a 125 ml glass stoppered erlenmeyer flask. Add 60 ml double distilled water and shake vigorously for 5 seconds. (Alternative: see instruction on bottle.)
 5. Place spreader on end plate (be sure 0.25 mm is in place), pour silica gel into the spreader and spread with a firm positive motion, stopping on the opposite end plate. The time elapsed between adding water to the silica gel and finishing the spreading should not exceed 15 seconds.
 6. Allow plates to dry in the leveling device until the coating is firm. Remove plates to a holder and activate in an oven at 110°C for 30 minutes with the plates in a vertical position. To provide more active layers, plates can be activated at 150°C for 3-4 hours.
 7. Store plates in a desiccator. If plates are not stored in a desiccator, they must be reactivated before use. Plates should be cooled in a desiccator.
 8. Before spotting the plates, remove the coating from the edges of the plate by running a finger or paper towel along the edge. Caution: Do not cut finger. Score plates if necessary. Spot plates such that they are developed at right angles to the direction of spreading.
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Ref: Stahl, Egon. 1965. Thin-Layer Chromatography, a laboratory handbook.

TABLE VI

DRY MATTER YIELDS (KG/HA) OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	601ag ²	472ag	278a	158ag	279a	167a
2	50PK(3)	1,571e	464	265c	1,230e	453	114
3	250PK(3)	3,308e	660	270d	4,117e	1,158	160
4	50PK(9)	831	501	944c	407	430	447
5a	50PK(3) + 50 (6,7,9,10,11)	1,857a	1,412a	1,552hah	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	5,831ah	2,703ah	637a
6	50PK(3) + 50(5,7,9)	2,150	1,511	818	2,409	1,481	525
7	50PK(3) + 50(5,7,9,11)	2,180	1,302	858	3,293	1,272	503
8	50PK(3,9) + 50(5,7)	2,239	1,396	907	2,306	1,300	586
9	63PK(3,9) + 63(5,7)	2,380	1,462f	856	2,313f	1,502f	653
10	50PK(3,9) + 50 (5,7) + PK(6)	2,014	1,597	917	2,180	1,570	553
11	50PK(3) + 50(9)	1,701	514	810	1,581	497	361
12	50PK(9) + 50(3)	1,637	496	840	1,551	466	420
13	50PK(3,9)	1,560	445	789	1,613j	531	411
14	125PK(3,9)	2,326	463fi	1,100d	3,174fij	684f	537
15	50PK(3) + 50(7,10)	1,820gk	1,094g	757	1,869g	850	318
16	50PK(3,10) + 50(7)	1,914	1,164b	812	1,981	845	336b
17	63PK(3,10) + 125(7)	1,923	1,854bi	973h	1,923hi	1,105h	742b
18	50PK(11) + 50(7,10)	600k	1,042	717	1,475	1,076	300

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table 1, page 30) were not significant.

TABLE VII
 PERCENT DRY MATTER OF TALL FESCUE IN 1973-74
 (Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	32af ²	32af	32a	24a	42	40
2	50PK(3)	26be	30	29	20	40	31
3	250PK(3)	20e	30	30d	17	38	35
4	50PK(9)	30b	31	22	23	38	32
5a	50PK(3) + 50 (6,7,9,10,11)	25a	26a	23a	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	17a	29	33
6	50PK(3) + 50(5,7,9)	23	26	23	20	37	29
7	50PK(3) + 50(5,7,9,11)	23	27	23	20	34	32
8	50PK(3,9) + 50(5,7)	23	27	21	20	34	30
9	63PK(3,9) + 63(5,7)	23	26g	21	20	32	30
10	50PK(3,9) + 50(5,7) + PK(6)	23	27	21	20	33	28
11	50PK(3) + 50(9)	24	28	22	20	39	31
12	50PK(9) +50(3)	26	30	21	21	39	30
13	50PK(3,9)	26	31	21	20	43	31
14	125PK(3,9)	24	31gh	21d	18	40	30
15	50PK(3) + 50(7,10)	25cf	26f	24	20	35	32
16	50PK(3,10) + 50(7)	25	26	25	20	33	32
17	63PK(3,10) + 125(7)	24	24h	24	20	33	33
18	50PK(11) + 50(7,10)	31c	29	26	24	40	34

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table 1, page 30) were not significant.

TABLE VIII

PERCENT NITRATE-N IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	.020	.023	.024b	.022b	.014b	.003b
2	50PK(3)	.031d ²	.028	.028	.022d	.015	.011
3	250PK(3)	.210cd	.029	.027c	.347cd	.027	.024
4	50PK(9)	.023	.029	.081	.015	.014	.017
5a	50PK(3) + 50(6,7,9,10,11)	.025	.057e	.132b			
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	.425be	.388be	.206be
6	50PK(3) + 50(5,7,9)	.038	.051	.078	.035	.036	.091
7	50PK(3) + 50(5,7,9,11)	.048	.056	.088	.036	.031	.101
8	50PK(3,9) + 50(5,7)	.061	.072	.097	.038	.041	.073
9	63PK(3,9) + 63(5,7)	.073	.075	.099	.041	.059	.097
10	50PK(3,9) + 50(5,7) + PK(6)	.094	.083	.087	.040	.043	.077
11	50PK(3) + 50(9)	.030	.026	.066	.019	.013	.061
12	50PK(9) + 50(3)	.022	.023	.082	.015	.016	.052
13	50PK(3,9)	.039	.039	.101	.039	.028	.054
14	125PK(3,9)	.081c	.049f	.158c	.043c	.028f	.072
15	50PK(3) + 50(7,10)	.024	.061	.096	.012	.023	.025
16	50PK(3,10) + 50(7)	.042	.078a	.096	.033	.038a	.029
17	63PK(3,10) + 125(7)	.050	.181aef	.100	.036e	.127aef	.066e
18	50PK(11) + 50(7,10)	.017	.065	.111	.005	.019	.003

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table 1, page 30) were not significant.

TABLE IX

PERCENT NITROGEN IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	2.25	2.21ag	1.88ag	2.12a	2.04a	1.78a
2	50PK(3)	2.62d ²	2.25	1.93b	2.86d	2.03	2.07
3	250PK(3)	4.04cd	2.42	2.22c	4.67cd	2.66	2.29
4	50PK(9)	2.30	2.35	3.35b	2.05	1.97	2.52
5a	50PK(3) + 50(6,7,9,10,11)	2.62	2.83ae	3.59a	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	4.95ae	4.24ae	3.81a
6	50PK(3) + 50(5,7,9)	3.17	2.86	3.49	3.31	2.81	2.97
7	50PK(3) + 50(5,7,9,11)	3.28	2.82	3.67	3.43	2.81	3.14
8	50PK(3,9) + 50(5,7)	3.17	2.88	3.44	3.01	2.56	2.79
9	63PK(3,9) + 63(5,7)	3.22	2.89f	3.50	3.20	2.91f	3.19
10	50PK(3,9) + 50(5,7) + PK(6)	3.13	2.82	3.34	3.26	2.81	2.97
11	50PK(3) + 50(9)	2.82	2.32	3.17	2.88	2.04	2.72
12	50PK(9) + 50(3)	2.56	2.31	3.26	2.81	1.99	2.66
13	50PK(3,9)	2.74	2.31	3.42	2.88	1.92	2.68
14	125PK(3,9)	3.20c	2.22fh	3.75c	3.49c	2.09fh	2.93
15	50PK(3) + 50(7,10)	2.68	2.74g	3.15g	2.91	2.47	2.60
16	50PK(3,10) + 50(7)	2.69	2.95	3.13	2.94	2.50	2.52
17	63PK(3,10) + 125(7)	2.93	3.31eh	3.21	2.97e	2.95h	3.25
18	50PK(11) + 50(7,10)	2.30	2.89	3.49	2.32	2.32	2.46

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatment are listed in Table 1, page 30) were not significant.

TABLE X

PERCENT POTASSIUM IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	2.53	2.55bi	2.08bi	2.70b	2.07	1.51
2	50PK(3)	2.93f ²	2.48	2.23c	3.22f	2.07	1.80
3	250PK(3)	3.64f	2.67	2.29e	4.67f	2.91	2.08
4	50PK(9)	2.54	2.49	3.33c	2.64	2.38	2.51
5a	50PK(3) + 50 (6,7,9,10,11)	2.95	2.92bg	3.13b	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	3.97b	2.88	1.91g
6	50PK(3) + 50(5,7,9)	3.17	3.00	3.20	3.64	2.68	2.41
7	50PK(3) + 50(5,7,9,11)	3.28	3.02	3.25	3.65	2.95	2.95
8	50PK(3,9) + 50(5,7)	3.35	3.19	3.69	3.52	2.82	2.82
9	63PK(3,9) + 63(5,7)	3.34	3.16h	3.51	3.65	3.07	2.97
10	50PK(3,9) + 50(5,7) + PK(6)	3.15	3.13	3.44	3.94	3.67	3.21
11	50PK(3) + 50(9)	2.99	2.53	3.26	3.65	2.32	2.77
12	50PK(9) + 50(3)	2.79	2.53	3.51	3.30	2.23	2.67
13	50PK(3,9)	3.02a	2.50	3.48	3.85	2.12	2.61
14	125PK(3,9)	3.54a	2.55hj	3.56e	4.20	2.35j	2.76
15	50PK(3) + 50(7,10)	2.99	2.97i	3.08i	3.38	2.70	2.39
16	50PK(3,10)+ 50(7)	3.09	3.10	3.09	3.47	2.69	2.38d
17	63PK(3,10) + 125(7)	3.34	3.35gj	3.16	3.78	3.65j	3.56dg
18	50PK(11) + 50(7,10)	2.63	2.94	2.93	3.00	2.58	2.33

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table 1, page 30) were not significant.

TABLE XI

PERCENT CALCIUM IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	0.72 ²	0.73c	0.57c	0.48	1.52cn	0.41c
2	50PK(3)	0.71k	0.72	0.63	0.41	0.53	0.50e
3	250PK(3)	0.47k	0.82	0.75i	0.32i	0.43	0.54
4	50PK(9)	0.66	0.69	0.66	0.45	0.46	0.33e
5a	50PK(3) + 50 (6,7,9,10,11) ¹	0.59	0.54c	0.84cl	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	0.41l	0.70c	0.64c
6	50PK(3) + 50(5,7,9)	0.56a	0.66	0.77a	0.36	0.47	0.43
7	50PK(3) + 50(5,7,9,11)	0.78a	0.72	0.53a	0.34	0.50	0.51
8	50PK(3,9) + 50(5,7)	0.49	0.58h	0.60h	0.47	0.76	0.53f
9	63PK(3,9) + 63(5,7)	0.56	0.56	0.71m	0.50	0.62	0.72fm
10	50PK(3,9) + 50(5,7) + PK(6)	0.65	1.11h	0.84h	0.49	0.58	0.50
11	50PK(3) + 50(9)	0.63	0.81	0.80	0.34	0.50	0.42
12	50PK(9) + 50(3)	0.70g	0.82	0.67g	0.35	0.55	0.45
13	50PK(3,9)	0.94bg	0.84	0.64g	0.45	0.75	0.54
14	125PK(3,9)	0.52b	0.70o	0.53im	0.44io	0.63	0.50m
15	50PK(3) + 50(7,10)	0.68	0.72	0.72	0.41	0.52n	0.52
16	50PK(3,10) + 50(7)	0.52	0.62	0.44	0.47d	0.60	0.56
17	63PK(3,10) + 125(7)	0.51	0.55o	0.54l	0.59dl	0.56	0.53
18	50PK(11) + 50(7,10)	0.86	0.63	0.55	0.39	0.60	0.52

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P \leq .05$ within each season. The other contrasts (contrasted treatments are listed in Table 1, page 30) were not significant.

TABLE XII

PERCENT MAGNESIUM IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	0.49ak ²	0.55ak	0.30a	0.60k	0.57a	0.36a
2	50PK(3)	0.38b1	0.80b1	0.62b1	0.53	0.64	0.48
3	250PK(3)	0.49h1	0.49l	0.48h1	0.58	0.55	0.61h
4	50PK(9)	0.57b	0.65b	0.49b	0.55	0.57	0.41
5a	50PK(3) + 50 (6,7,9,10,11)	0.35a	0.44a	0.46am	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	0.59	0.73am	0.57a
6	50PK(3) + 50(5,7,9)	0.39j	0.45f	0.40fj	0.46	0.61	0.56
7	50PK(3) + 50(5,7,9,11)	0.42	0.58f	0.50f	0.46	0.60	0.56
8	50PK(3,9) + 50(5,7)	0.34	0.42	0.42	0.40	0.49	0.49
9	63PK(3,9) + 63(5,7)	0.36	0.43	0.42n	0.44	0.59	0.57
10	50PK(3,9) + 50(5,7) + PK(6)	0.30	0.43	0.40	0.47	0.54	0.50
11	50PK(3) + 50(9)	0.49c	0.59c	0.54c	0.56	0.67	0.57
12	50PK(9) + 50(3)	0.36ci	0.46ci	0.35c	0.60	0.66	0.52
13	50PK(3,9)	0.42di	0.57di	0.43d	0.51	0.64	0.55
14	125PK(3,9)	0.33dh	0.47d	0.34dhn	0.52	0.58	0.46ho
15	50PK(3) + 50(7,10)	0.31jk	0.37ek	0.33ej	0.45k	0.57	0.45
16	50PK(3,10) + 50(7)	0.34	0.42	0.31	0.43g	0.46	0.41g
17	63PK(3,10) + 125(7)	0.33	0.41	0.32m	0.59g	0.57m	0.62go
18	50PK(11) + 50(7,10)	0.35	0.72e	0.47e	0.33	0.56	0.58

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table 1, page 30) were not significant.

TABLE XIII

PERCENT PHOSPHORUS IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	0.59	0.71ak	0.56	0.54ak	0.58k	0.52
2	50PK(3)	0.61	0.73i	0.61	0.59	0.62	0.56
3	250PK(3)	0.61	0.66fi	0.62	0.64f	0.58f	0.55f
4	50PK(9)	0.61	0.73	0.64	0.55	0.61	0.54
5a	50PK(3) + 50 (6,7,9,10,11)	0.59	0.61a	0.56	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	0.65a1	0.541	0.48
6	50PK(3) + 50(5,7,9)	0.62	0.61	0.58	0.66	0.58h	0.52
7	50PK(3) + 50(5,7,9,11)	0.63	0.62	0.58	0.66	0.58	0.52
8	50PK(3,9) + 50(5,7)	0.63e ²	0.62	0.70e	0.64e	0.60	0.61
9	63PK(3,9) + 63(5,7)	0.60	0.61j	0.63	0.66j	0.62	0.59
10	50PK(3,9) + 50(5,7) + PK(6)	0.57e	0.60	0.60e	0.70e	0.69	0.62
11	50PK(3) + 50(9)	0.64	0.75	0.61	0.59	0.65	0.54
12	50PK(9) + 50(3)	0.60	0.73	0.63	0.59g	0.66	0.59
13	50PK(3,9)	0.61	0.72	0.63	0.67cg	0.69	0.58
14	125PK(3,9)	0.64	0.74fjm	0.59	0.76cfj	0.69f	0.69f
15	50PK(3) + 50(7,10)	0.62	0.63k	0.60	0.64k	0.77dhk	0.57
16	50PK(3,10) + 50(7)	0.63	0.66b	0.69	0.69	0.66	0.58
17	63PK(3,10) + 125(7)	0.63	0.60bm	0.60	0.731	0.691	0.60
18	50PK(11) + 50(7,10)	0.58	0.64	0.60	0.63	0.65d	0.57

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or pK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table 1, page 30) were not significant.

TABLE XIV

PERLOLINE (MG/G) IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	.024	.100e	.091ae	.088	.042e	.019e
3	250PK(3)	.199	.078f	.102	.253f	.077	.108f
4	50PK(9)	.057	.149	.471a	.016	.019	.278
5a	50PK(3) + 50 (6,7,9,10,11)	.100	.401e	.514e	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	.107	.240e	.614e
8	50PK(3,9) + 50(5,7)	.254d ²	.355	.383	.207d	.114	.331
13	50PK(3,9)	.116	.100c	.468	.130	.009	.414
16	50PK(3,10) + 50(7)	.087d	.495c	.582	.058d	.134	.266b
17	63PK(3,10) + 125(7)	.134	.532f	.438	.087f	.209	.757bf
18	50PK(11) + 50(7,10)	.032	.432	.600	.007	.086	.173

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P \leq .10$ within each season. The other contrasts (contrasted treatments are listed in Table III, page 35) were not significant.

TABLE XV

PERCENT DIGESTIBLE DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Fertilizer Applied ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	None	66.8	59.7 ^d	60.5	61.3	51.9	47.7 ^{ad}
3	250PK(3)	74.4 ^e	58.2 ^e	57.5	69.7	50.2	56.6 ^e
4	50PK(9)	66.7	58.7	65.4	62.0	54.2	62.6 ^a
5a	50PK(3) + 50 (6,7,9,10,11)	70.5	63.4 ^d	66.4	-	-	-
5b	250PK(3) + 125(6,9) + 50(5,7,10,11)	-	-	-	68.8	57.8	67.6 ^d
8	50PK(3,9) + 50(5,7)	73.5	57.7 ^c	65.4	67.0	57.6	66.9
13	50PK(3,9)	69.9	59.3 ^b	67.5	65.0	50.8	66.0
16	50PK(3,10) + 50(7)	70.0	63.1 ^{bc}	67.1	61.7	53.1	61.0
17	63PK(3,10) + 125(7)	67.9 ^e	63.9 ^e	65.0	65.2	53.2	66.4 ^e
18	50PK(11) + 50(7,10)	66.7	60.3	66.5	60.9	52.7	61.4

¹Numbers 50 or greater refer to kg of N/ha; PK = application of 30 kg of P + 60 kg of K/ha; numbers in parentheses are months of the year when applications of N and/or PK were made.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table III, page 35) were not significant.

TABLE XVI
 DRY MATTER YIELDS (KG/HA) OF TALL FESCUE IN 1973-74
 (Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	2,354	1,787	815	1,907	1,904	431
6	Trt 8 H-73	2,570	-	3,502	2,257	-	2,452
7	Trt 8 H-74	2,513	-	3,677	2,115	-	2,487
8	Summer(6,7,8)	2,420	-	3,240	2,209	-	2,377
9	Trt 11 H-73	2,451	479	2,944	1,909	548	1,563
10	Trt 11 H-74	2,490	499	2,527	1,922	573	1,848
11	Summer(7,8,9)	2,475	459	2,526	1,916	584	1,678
2	Control for trt 5,14, & 17	2,405	876	1,483	2,020	892	608
3	Trt 5 H-73	-	6,812	1,627	-	5,111	438
4	Trt 5 H-74	-	5,147	1,451	-	7,124	695
5	Spring(3,4,5)	-	5,488	1,516	-	5,212	497
12	Trt 14 H-73	2,485	955	2,464a ²	1,994	921	1,171
13	Trt 14 H-74	2,483	899	1,776	2,091	830	1,030
14	Fall(9,10,11)	2,324	854	1,925a	2,089	777	1,092
15	Trt 17 H-73s	-	6,635	1,337	-	4,905	1,079
16	Trt 17 H-73f	-	5,565	2,191b	-	5,036	776
17	Spring & Fall H-74s	-	5,366	1,900	-	7,147	899
18	Trt 17 H-74f	-	5,288	1,653b	-	5,351	1,013

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt (number) H-73/74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring; f = fall.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XVII
 PERCENT DRY MATTER OF TALL FESCUE IN 1973-74
 (Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	22	23	27	21	28	33
6	Trt 8 H-73	21	-	29	22	-	35
7	Trt 8 H-74	21	-	31	22	-	33
8	Summer(6,7,8)	21	-	30	23	-	35
9	Trt 11 H-73	21	24	25	21	26	33
10	Trt 11 H-74	21	24	25	21	27	33
11	Summer(7,8,9)	22	24	26a ²	21	26	32a
2	Control for trt 5,14, & 17	21	24	23c	21	29	33c
3	Trt 5 H-73	-	24	24	-	29	32
4	Trt 5 H-74	-	26	24	-	27	35
5	Spring(3,4,5)	-	25	23bd	-	30b	30bd
12	Trt 14 H-73	21	24	29	21	29	42
13	Trt 14 H-74	21	24	38	21	28	43
14	Fall(9,10,11)	21	24	37ad	21	28	44ad
15	Trt 17 H-73s	-	24	44	-	29	49
16	Trt 17 H-73f	-	26	32	-	30	42
17	Spring & Fall H-74s	-	25	40b	-	26b	52b
18	Trt 17 H-74f	-	26	39c	-	30	49c

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt(number) H-73/74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring; f = fall.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XVIII

PERCENT NITROGEN IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	2.80	2.92	2.15	2.92	2.55	2.33
6	Trt 8 H-73	2.85	-	2.25	2.85	-	2.08
7	Trt 8 H-74	2.86	-	2.20	2.92	-	2.21
8	Summer(6,7,8)	2.77	-	2.19	2.67	-	2.03
9	Trt 11 H-73	2.73	2.45	2.04	2.79	1.90	1.95
10	Trt 11 H-74	2.86	2.44	1.85	2.87	1.96	2.18
11	Summer(7,8,9)	2.82	2.42	1.53	2.91	2.03	2.11
2	Control for trt 5,14, & 17	2.81	2.48	2.77b ²	2.94	2.19	2.46b
3	Trt 5 H-73	-	2.50	2.46	-	1.93	2.73
4	Trt 5 H-74	-	2.26	2.57	-	2.09	2.29
5	Spring(3,4,5)	-	2.26	2.60ac	-	1.82	2.62ac
12	Trt 14 H-73	2.80	2.49	1.73	3.04	2.18	1.44
13	Trt 14 H-74	2.88	2.19	0.83	3.03	2.20	1.41
14	Fall(9,10,11)	2.73	2.34	1.35c	2.97	2.07	1.56c
15	Trt 17 H-73s	-	2.40	1.10	-	1.84	1.46
16	Trt 17 H-73f	-	2.30	1.56	-	1.84	1.41
17	Spring & Fall H-74s	-	2.28	0.97a	-	2.09	1.44a
18	Trt 17 H-74f	-	2.23	1.44b	-	1.82	1.41b

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt (number) H-73/74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring; f = fall.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XIX

PERCENT POTASSIUM IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	3.08	2.97	2.49	3.27	2.95	2.34
6	Trt 8 H-73	3.14	-	2.71	3.40	-	2.44
7	Trt 8 H-74	3.12	-	2.65	3.40	-	2.43
8	Summer (6,7,8)	3.07	-	2.67	3.38	-	2.43
9	Trt 11 H-73	3.03	2.74	2.56	3.30	3.00	2.52
10	Trt 11 H-74	3.12	2.75	2.62	3.32	2.89	2.64
11	Summer (7,8,9)	3.07	2.73	2.67	3.35	2.82	2.76a
2	Control for trt 5,14, & 17	3.07	2.67	2.96c ²	3.37	2.58	2.62c
3	Trt 5 H-73	-	2.68	2.44	-	2.38	2.47
4	Trt 5 H-74	-	2.70	2.71	-	2.57	2.15
5	Spring (3,4,5)	-	2.68	2.71b	-	2.65	2.75bd
12	Trt 14 H-73	3.17	2.82	2.34	3.44	2.64	1.61
13	Trt 14 H-74	3.16	2.78	2.35	3.35	2.44	1.61
14	Fall (9,10,11)	3.18	2.86	2.43	3.42	2.69	1.75ad
15	Trt 17 H-73s	-	2.82	1.76	-	2.52	1.36
16	Trt 17 H-73f	-	2.73	2.21	-	2.52	1.36
17	Spring & Fall H-74s	-	2.73	2.17b	-	2.63	1.18b
18	Trt 17 H-74f	-	2.74	2.15c	-	2.53	1.39c

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt (number) H-73/74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring; f = fall.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XX
 PERCENT CALCIUM IN DRY MATTER OF TALL FESCUE IN 1973-74
 (Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	0.60	0.35	0.38	0.44	0.56	0.60
6	Trt 8 H-73	0.49	-	0.43	0.41	-	0.61
7	Trt 8 H-74	0.51	-	0.47	0.42	-	0.55
8	Summer(6,7,8)	0.56	-	0.43	0.43	-	0.62
9	Trt 11 H-73	0.56	0.51	0.42	0.41	0.60	0.48
10	Trt 11 H-74	0.59	0.51	0.45	0.42	0.53	0.48
11	Summer(7,8,9)	0.53	0.51	0.47	0.40	0.50	0.51
2	Control for trt 5,14, & 17	0.50	0.37a ²	0.38	0.51	0.66	0.57
3	Trt 5 H-73	-	0.37	0.32	-	0.53	0.56
4	Trt 5 H-74	-	0.39	0.37	-	0.56	0.48
5	Spring(3,4,5)	-	0.38bc	0.34	-	0.53	0.56
12	Trt 14 H-73	0.54	0.45	0.36	0.42	0.63	0.51
13	Trt 14 H-74	0.57	0.47	0.36	0.47	0.61	0.48
14	Fall(9,10,11)	0.52	0.49c	0.36	0.42	0.63	0.51
15	Trt 17 H-73s	-	0.49	0.31	-	0.50	0.48
16	Trt 17 H-73f	-	0.47	0.32	-	0.50	0.48
17	Spring & Fall H-74s	-	0.51b	0.33	-	0.60	0.48
18	Trt 17 H-74f	-	0.48a	0.39	-	0.52	0.60

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt (number) H-73/74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring; f = fall.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P \leq .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XXI

PERCENT MAGNESIUM IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	0.34	0.34	0.34	0.48	0.57	0.49
6	Trt 8 H-73	0.30	-	0.39	0.45	-	0.54
7	Trt 8 H-74	0.28	-	0.37	0.43	-	0.52
8	Summer(6,7,8)	0.30	-	0.37	0.45	-	0.52
9	Trt 11 H-73	0.30	0.46	0.32	0.39	0.47	0.33
10	Trt 11 H-74	0.32	0.45	0.34	0.39	0.54	0.37
11	Summer(7,8,9)	0.34	0.44	0.37	0.39a	0.53	0.53
2	Control for trt 5,14, & 17	0.31	0.36	0.30	0.40	0.55	0.39
3	Trt 5 H-73	-	0.36	0.36	-	0.50	0.42
4	Trt 5 H-74	-	0.34	0.38	-	0.56	0.45
5	Spring(3,4,5)	-	0.34b ²	0.40	-	0.52	0.57
12	Trt 14 H-73	0.37	0.56	0.45	0.43	0.73	0.47
13	Trt 14 H-74	0.39	0.54	0.41	0.53	0.54	0.33
14	Fall(9,10,11)	0.36	0.54b	0.42	0.50a	0.64	0.57
15	Trt 17 H-73s	-	0.44	0.40	-	0.54	0.55
16	Trt 17 H-73f	-	0.39	0.38	-	0.49	0.50
17	Spring & Fall H-74s	-	0.42	0.42	-	0.53	0.74
18	Trt 17 H-74f	-	0.42	0.45	-	0.52	0.57

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt (number) H-73/74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring; f = fall.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XXII

PERCENT PHOSPHORUS IN DRY MATTER OF TALL FESCUE IN 1973-74

(Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	0.65	0.69	0.71	0.71	0.78	0.72
6	Trt 8 H-73	0.67	-	0.62	0.74	-	0.69
7	Trt 8 H-74	0.66	-	0.61	0.75	-	0.71
8	Summer(6,7,8)	0.66	-	0.62	0.74	-	0.71
9	Trt 11 H-73	0.66	0.78	0.65	0.71	0.84	0.63
10	Trt 11 H-74	0.67	0.78	0.62	0.73	0.84	0.69
11	Summer(7,8,9)	0.66	0.78	0.63	0.74	0.82	0.67
2	Control for trt 5,14, & 17	0.66	0.78ac ²	0.68	0.73	0.86	0.68
3	Trt 5 H-73	-	0.62	0.61	-	0.74	0.71
4	Trt 5 H-74	-	0.59	0.62	-	0.79	0.68
5	Spring(3,4,5)	-	0.59ab	0.61	-	0.72	0.69
12	Trt 14 H-73	0.69	0.75	0.48	0.74	0.89	0.52
13	Trt 14 H-74	0.68	0.75	0.49	0.73	0.90	0.51
14	Fall(9,10,11)	0.68	0.78b	0.49	0.74	0.84	0.53
15	Trt 17 H-73s	-	0.64	0.47	-	0.71	0.52
16	Trt 17 H-73f	-	0.59	0.50	-	0.73	0.52
17	Spring & Fall H-74s	-	0.57	0.48	-	0.86	0.52
18	Trt 17 H-74f	-	0.58c	0.50	-	0.72	0.53

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; Trt (number) H-73/74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring; f = fall.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XXIII
 PERLOLINE MG/G IN DRY MATTER OF TALL FESCUE IN 1973-74
 (Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	.065	.324	.061	.092	.123	.030
8	Summer(6,7,8)	.036	-	.025	.161	-	.038
11	Summer(7,8,9)	.035	.080	.007	.189	.050	.084
2	Control for trt 5,14, & 17	.037	.113	.192	.070	.032	.189
5	Spring(3,4,5)	-	.154	.150a ²	-	.080	.188
14	Fall(9,10,11)	.051	.111	.066	.104	.011	-
17	Spring & Fall H-74s	-	.238	.014a	-	.019	-

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; H-74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .10$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

TABLE XXIV
 PERCENT DIGESTIBLE DRY MATTER OF TALL FESCUE IN 1973-74
 (Means of All Cuts within Each Season)

Trt. No.	Stockpile Dates ¹	1973			1974		
		Spring	Summer	Fall	Spring	Summer	Fall
1	Control for trt 8 & 11	69.2	65.2	64.3	66.6	52.1	58.1
8	Summer(6,7,8)	70.6	-	59.9	64.9	-	55.6
11	Summer(7,8,9)	70.2	61.8	59.0	68.1	50.9	59.6
2	Control for trt 5,14, & 17	71.7	62.3	67.9	66.8	53.2	60.1
5	Spring(3,4,5)	-	58.8	66.9	-	48.7	63.2
14	Fall(9,10,11)	70.7	61.0	62.2	69.0	54.7	-
17	Spring & Fall H-74s	-	59.0	57.7	-	52.0	-

¹Numbers in parentheses are months of the year in which the fescue was stockpiled; H-74 indicates that the treatment is the same as the treatment denoted by the number except that the stockpiled fescue was cut as a hay for the year designated; s = spring.

²Presence of the letters denotes that treatments with like letters were compared and that the data for those treatments were significantly different at $P < .05$ within each season. The other contrasts (contrasted treatments are listed in Table II, page 32) were not significant.

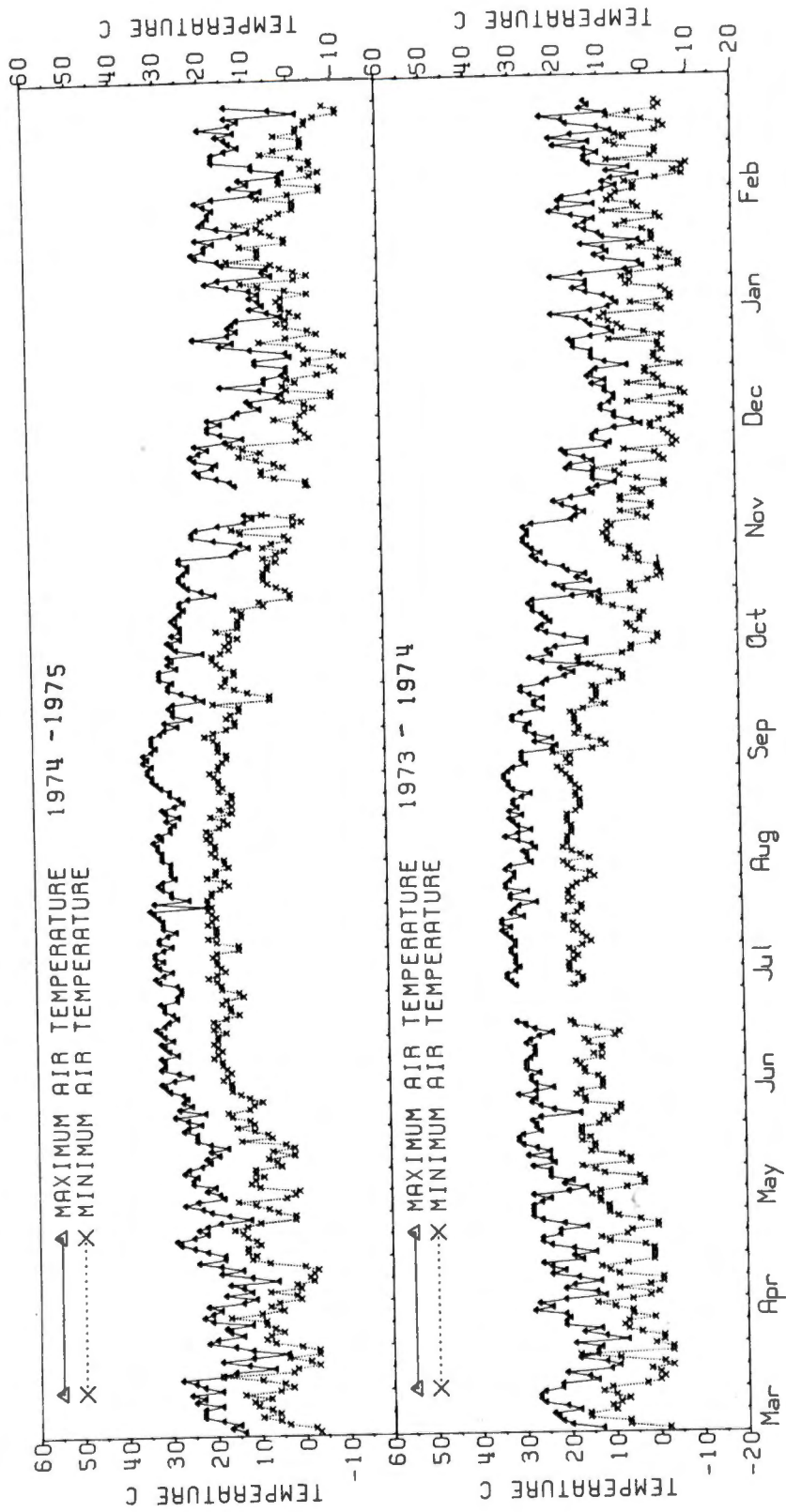
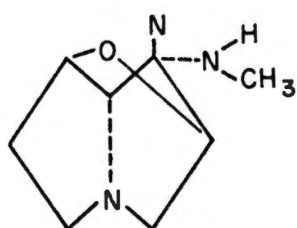
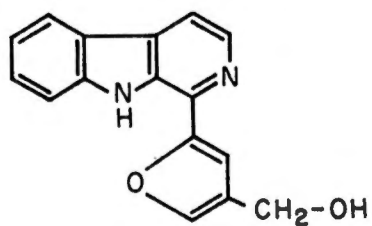


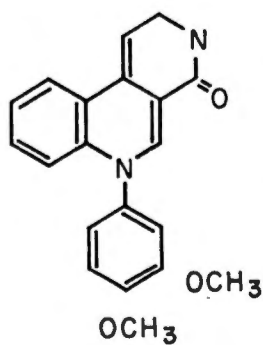
Figure 38. Daily maximum and minimum air temperatures for March 1973-74-March 1975 in degrees Celsius.



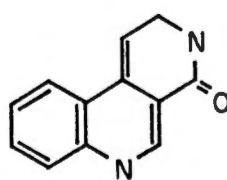
Festucine
(Loline)



Perlolyrine



Perloline



Perlolidine

Figure 41. Structures of the alkaloids festucine (loline), perlolyrine, perloline, and perlolidine.

VITA

Richard Warren Loveland was born April 17, 1946, in Hendersonville, N. C. He is the son of Joseph and Irene Loveland. He graduated from Hendersonville High School in May 1964 and received the Bachelor of Science degree in agronomy in May 1968 from Oklahoma State University. While enrolled, he was employed by the United States Department of Agriculture, Soil Conservation Service as a student trainee in the summers of 1966 and 1967. He was appointed as a Range Conservationist (GS-7) for the Soil Conservation Service in 1968. After graduation he served in the United States Marine Corps as a 2nd and 1st Lieutenant in Artillery from June 1968 to December 1970.

In January 1971 he worked as a graduate research assistant in the Department of Agronomy at Oklahoma State University and received the Master of Science degree with a major in agronomy in July 1972. Starting in June 1972, he worked as a graduate research assistant in the Plant and Soil Science Department of the University of Tennessee. He received the Doctor of Philosophy degree with a major in crop physiology in March 1976.