EFFECTIVENESS OF INORGANIC NITROGEN AS A REPLACEMENT FOR LEGUMES GROWN IN ASSOCIATION WITH FORAGE GRASSES

by

Lark Poland Carter

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Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
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INTRODUCTION

Of all the essential nutrient elements required for plant growth, other than carbon, hydrogen and oxygen, nitrogen is needed in greatest quantity. Protein found in plant tissue is approximately 16 percent nitrogen. In addition, nitrogen is a constituent in other organic compounds. Since herbage of high quality may contain over 3 percent nitrogen, large quantities of this element are needed for optimum plant growth.

Essentially, there are two ways of supplying supplemental nitrogen needed for growth of forages. One source, inorganic nitrogen fertilizer, may be applied to the soil in such forms as ammonium sulfate, anhydrous ammonia, ammonium nitrate, calcium nitrate, and sodium nitrate. Atmospheric nitrogen fixed by root nodule bacteria of *Rhizobium* spp., growing symbiotically with legumes, is another source.

Since inorganic commercial nitrogen has become relatively inexpensive in recent years the economics of growing legumes solely as a source of nitrogen has been questioned by many farmers and agronomists. Although much information has been accumulated concerning dry matter and nitrogen yield of forage crops, little is known of the comparative relationships and the effectiveness of inorganic nitrogen as a replacement for legumes grown in association with grasses.

This study was initiated to determine the effectiveness of inorganic nitrogen as a replacement for legumes grown in association with grasses. The forages utilized in this study were Vernal alfalfa (Medicago sativa), Dollard red clover (Trifolium pratense), Ladino white clover (Trifolium repens), Empire birdsfoot trefoil (Lotus corniculatus), Fisher bromegrass (Bromus inermis), and Commercial orchardgrass (Dactylis glomerata), grown alone and in association. Specific objectives were to determine dry matter production of these grasses when fertilized at different rates with ammonium nitrate and harvested frequently to simulate rotational grazing. The seasonal distribution of dry matter production of the above species grown alone and in association was investigated. The botanical composition and the nitrogen content of the forage were determined.

The amount of residual nitrogen in the soil from the year of application to the following year could affect the results of such a study. A treatment was included to determine the effect of residual nitrogen under the conditions of these experiments.

Small amounts of nitrate are found in most growing plants. However, under certain soil and environmental conditions nitrate appears to accumulate. Since high rates of nitrogen were applied in this experiment, there was a possibility that nitrate accumulation in the forage might reach a level that would be toxic or even lethal to livestock. For this reason nitrate content of the forage was determined.

REVIEW OF PERTINENT LITERATURE

The concept that legumes appear to enrich the soil and thus contribute to non-legumes grown in association is now new. Although Greek and Roman writers recognized the value of alfalfa (medic), lupines and other legumes for soil improvement (86), it was not until the nineteenth century that the studies and experiments of Boussingault, Liebig, Ville and later, Lawes and Gilbert at Rothamsted, gradually led to a better understanding of the nature of the soil improving quality of legumes. The question of how legumes accomplished this phenomenon was finally solved by Hellriegel and Wilfarth in 1887. A detailed historical review with respect to this topic has been written by Waksman (80) and Wilson (91).

Since 1945, the relatively high value of forage and low cost of nitrogen fertilizer have induced trends for both research workers and farmers to use inorganic sources of nitrogen for forage grasses. It is generally accepted that successively greater amounts of nitrogen fertilizer increase yield of grasses grown in pure stand (13, 28, 35, 39, 45, 82). In addition to higher yields of dry matter, nitrogen fertilization, especially at heavier rates, may increase the nitrogen percentage of the forage. Consequently, the per acre yield of crude protein may be increased by supplying this element. Vandecaveye (76) in 1940, after an extensive review of the literature stated that sixty investigations indicated that applications of nitrogen fertilizer for pasture grass and hay could be expected to increase the percentage of nitrogen in the herbage. In most of ten exceptions noted, the cause for reduction in percent of nitrogen (or apparent ineffectiveness of nitrogen fertilizer) was traceable to small applications or an unbalanced nutrient condition in the soil.

Anderson _et al_. (3) noted that seed and forage yields of bromegrass increased with increasing amounts of nitrogen to approximately the 100 pound level of application per acre. Beyond that rate, the fertilizer became relatively less effective in stimulating yields. Protein percentages in the fairly mature forage sampled in this study were not appreciably increased by amounts of nitrogen under 100 pounds per acre, but rates of 140 and 200 pounds per acre increased protein percentages, thus the yield of protein per acre was increased at higher rates of fertilization.

Ramage _et al_. (56) observed that annual dry matter yields per acre ranged from 2 tons with 50 pounds of nitrogen to 4.5 tons with 400 pounds of nitrogen. The protein percentage of the dry matter ranged from 12 to 20 percent as the nitrogen application was increased from 50 to 400 pounds per acre. The authors attempted to show the economics of fertilization of forages and concluded that 100 pounds of nitrogen per acre gave the greatest yield of dry matter and protein per pound of nitrogen applied. This rate also gave the most efficient return in yield on a cost basis.

An investigation by Harrington and Washko (25) indicated that desirable levels of forage and protein production could be obtained from grasses grown alone with nitrogen fertilization if harvested at a height of six to eight inches. They found in this Pennsylvania study that applications of 100 pounds of nitrogen per acre after each harvest produced the highest yield of dry matter and protein, but in terms of efficiency this treatment was the lowest. The application of 50 and 100 pounds of nitrogen per acre in the spring produced more forage and protein per pound of nitrogen than split applications in 25, 50 or 100 pound increments. However, they noted that seasonal distribution of production was better with split applications.

Wagner (77, 78) found that orchardgrass and tall fescue responded markedly both in protein percentage and yield to application of nitrogen. Both grasses utilized high proportions of the applied nitrogen. More total protein was produced in mixtures with legumes than by grasses in pure stand fertilized with 160 pounds of nitrogen per acre. Distribution of production throughout the season also was superior in the mixtures.

On grazed permanent pastures, Brown and Munsell (11) reported that spring applied nitrogen gave a 30 percent increase in total yields. Most of the additional growth occurred before mid-June. Nitrogen applied in spring and summer resulted in less spring feed but more summer feed than from applying all of the nitrogen in April. The most uniform seasonal distribution of pasturage was obtained by adding nitrogen only in the summer, but the returns per unit of nitrogen were about half those from spring applications.

Burton (12), Kimbrough et al. (32), Prine and Burton (55) and Wallace et al. (81) in the southeastern United States have shown phenomenal increases in yield from very high rates of nitrogen fertilization on some of the warm-season grasses, particularly bermudagrass.

In summarizing pasture research conducted in Indiana, Mott (45) stated that nitrogen was the most important factor limiting the growth of grasses, particularly where no legumes were present. Inadequate nitrogen was the first limiting factor for growth of grasses in permanent pastures. Nitrogen increased production, particularly during years of adequate spring rainfall and where botanical composition was dominantly grass.

Mulder (47), from research conducted in Netherlands, noted that yields of 10,000 kilograms of forage per hectare were not uncommon. This much forage would require approximately 300 kilograms of nitrogen. The author commented that nitrogen fixation by legumes was approximately 300 kilograms per hectare in a pure legume stand. However, he stated that this level of fixation would not hold true in mixtures of 50 percent or less of legume. He considered the use of nitrogen fertilizers on grass-legume mixtures as detrimental through suppression of legumes by grasses.

Lipman (36) in commenting on the growth of legumes and non-legumes in association stated:

> Under favorable conditions non-legumes associated with legumes may secure large amounts of nitrogen from the latter, even though this may not be indicated by an increased proportion of nitrogen in the dry matter of the non-legume. The presence of the non-legume in the mixed growth need not decrease the yields of dry matter and nitrogen in the legumes.
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> When sodium nitrate is applied to such crop mixtures, the non-legumes gain an advantage in the competition for light, moisture, and plant food and the growth of the legume is depressed. It seems probable that nitrogen compounds pass out of the root of at least some legumes and that such nitrogen compounds may become available to the non-legumes in mixed growths. . . . Legumes seem to differ as to their capability to supply nitrogen compounds to non-legumes associated with them.

Blackman (7), in reporting on the effect of light intensity and nitrogen supply on the clover content of a sward stated that the decrease in clover content brought about by shading was a direct effect, and was not related to competition with the grasses. The reduction in clover associated with the addition of nitrogen primarily was due to such competition, the nature of which was obscure. In this relationship competition for nitrogen played a part, since with a high external concentration of inorganic nitrogen, nitrogen fixation would be at a minimum. The author commented further that where defoliation was infrequent, competition for light would predominate. Under these conditions added nitrogen would further depress the clover because of increased height and density of the grass.

In investigating some of the above and below ground relationships of an alfalfa-orchardgrass mixture, Chamblee (15) noted that the above-ground direct benefits to orchardgrass by alfalfa included the effects of shading on air and soil temperature. Below-ground benefits included sloughing nodules and possibly excretion of nitrogen. Alfalfa benefited in that orchardgrass offered less competition for various growth factors.

Aberg et al. (1) found that nitrogen percentages were not significantly different for grasses when grown in association with legumes than when grown alone. However, these results were obtained from new seedlings on a highly productive soil.

Greaves and Jones (22) found that after growing alfalfa for 16 years and removing the top growth for hay, the crop did not measurably increase the total nitrogen of the soil. This was true for both inoculated and uninoculated plants. A highly significant gain in soil nitrogen was obtained when the crop was returned to the soil. These authors noted that even properly inoculated legumes appear to feed first on the nitrogen of the soil and utilize atmospheric nitrogen only if the soil nitrogen is insufficient for optimum growth. There may be a nitrogen balance in some soils below which legumes could increase the soil nitrogen even where the complete crop was removed, according to these authors.

Free and Engdahl (19, 20), Robinson and Sprague (58, 59), Robinson et al. (60), Levine et al. (34), Bennett et al. (6), Brown and Munsell (9), Eby et al. (17), McAuliffe (41), Nelson and Robins (49), and Parsons (50, 51), report increased yields of grass-legume mixtures from nitrogen fertilization. The nitrogen fertilizer stimulated grass growth and resulted in greater competition to the legume, which may be completely lost from the mixture.

Much of the increased yield from nitrogen fertilization of mixtures has been in the spring when production is ordinarily at a high level. All workers have reported a lower percent of white clover in nitrogen fertilized mixtures, though in some cases good stands of white clover were retained where drastic clipping or grazing management was utilized to retard the grasses, especially in the spring. By fertilizing orchardgrass-Ladino clover and bromegrass-Ladino clover mixtures in the spring with 60 pounds of nitrogen, Sprague and Garber (67) noted some increase in yield. Significant increases in yield occurred when the first crop was removed late and also when the clover stands were poor. Nitrogen fertilizer stimulated the grass to the point that Ladino clover was almost entirely crowded out in later cuttings. It was observed that the bromegrass-Ladino clover mixture yielded more in the early harvests but the orchardgrass-Ladino clover mixture gave one more midseason cutting and slightly more herbage when cut throughout the season under an eight-inch back to two-inch harvesting system.

Levine et al. (34) found with a good bromegrass-fair alfalfa mixture that nitrogen fertilization up to 200 pounds per acre resulted in significant increases in yield. However, on a good alfalfa-fair bromegrass mixture, nitrogen fertilizer did not result in a significant increase in yield. Irrigation significantly increased yields of both mixtures. The interaction between fertilization and irrigation was not significant.

Bear (5) stated that often more than 50 pounds of nitrogen per acre can be applied to advantage on permanent pastures, but higher rates tend to increase grass at the expense of the clovers. Dodd (16) in Ohio reported that addition of 50 pounds of nitrogen annually, to either phosphate or phosphate and potash treated areas of permanent pasture, gave very good returns. The advantage of nitrogen fertilization was small on areas with heavy clover stands.

Wagner (77, 78) fertilized a pure stand of Ladino clover with up to 160 pounds of nitrogen per acre and found that nitrogen fertilizer lowered the yield of protein and dry matter. Hughes and MacDonald (29) report no increase in yield of birdsfoot trefoil when nitrogen fertilizer alone was applied.

It is generally accepted by agronomists that well-established and inoculated legumes will give little or no response to nitrogen fertilization.

In evaluating nitrogen fertilized grasses as substitutes for legume-grass mixtures on southern Indiana soils, Teel et al. (70) reported that 200 pounds of nitrogen per acre was needed to produce grass growth comparable to the yield of grass-legume mixtures. This amount applied in split applications was used more efficiently by the grass. These authors recommended that 50 to 75 pounds of nitrogen per acre be applied in early fall to stimulate growth, followed by an application of 50 to 75 pounds per acre in March to support spring growth and finally 50 to 100 pounds per acre after the flush spring growth if moisture conditions were favorable.

Bromegrass, timothy, Kentucky bluegrass, alfalfa, birdsfoot trefoil and Ladino clover were grown by McCloud and Mott (42) for three years in all combinations of two species. The performance of different mixtures varied from mutually depressive, depressive, no interaction, beneficial and mutually beneficial, thus indicating the multiplicity of reactions involved.

The influence of inorganic nitrogen on nitrogen fixation by legumes was investigated by McAuliffe _et al_. (41). They used N^{15} labeled ammonium sulfate on a Ladino clover-tall fescue mixture. Total nitrogen and atom percent excess N^{15} were determined for both the grass and the legume. The percent nitrogen in the legume from non-fixation sources was then determined. The authors noted that the percent nitrogen fixed in Ladino clover decreased from 65 percent when 25 pounds of nitrogen was applied to 10 percent when 200 pounds of nitrogen was added. These determinations were made three weeks after application of the nitrogen fertilizer. The same effect lasted throughout the season but decreased with time. This same relationship also held true for alfalfa.

The management of legume-grass mixtures to maintain a balance of components varies with the legume in the mixture. Clipping or grazing frequently may be detrimental to alfalfa stands. Willard (87), in a review of alfalfa management, explained that the number of cuttings of alfalfa that can be made for hay depends upon the length of growing season. Along parallel 40° east of the 95th meridian, the length of growing season permits three cuttings a year and provides abundant fall growth to build up root reserves for overwintering and early spring growth. More cuttings result not only in decreased yields but also increase the hazard of losing the stand of alfalfa over winter. North of this zone, beginning about parallel 43°, the season is too short for three cuttings. South of this zone the longer season permits more cuttings. Though these conclusions were made for hay production, they may well be applied to pasturing alfalfa since new growth generally starts from crown buds, regardless of how the top growth is removed.

Alfalfa tends to build up root reserves more rapidly under dry conditions (88). Thus in the semi-arid and arid West, it is possible to graze or cut alfalfa more frequently without detrimental effects than in the humid East, except in dry years.

Wagner (79), Tysdal (73), and Van Horn _et al_. (75) report loss of alfalfa under frequent grazing or cutting of alfalfa mixtures. However, allowing early spring growth to go to the hay stage was effective in favoring the alfalfa portion of orchardgrass-Ladino clover-alfalfa pastures under rotational grazing in Tennessee (74).

Lush _et al_. (38) suggested that stands of both alfalfa and orchardgrass could be maintained at least four years with comparatively high yields all season when rotational grazing was practiced. Wagner (79) maintained best stands of alfalfa-orchardgrass and alfalfa-bromegrass in Maryland when first harvesting was deferred until June 1 and clipped for the rest of the season when 12 inches high. Clipping at 6 inches in height rapidly reduced vigor and stand of alfalfa in the mixtures.

Scholl (63) emphasized the importance of good management in forage production. The grazing schedules should be geared to using the forage when quality was high, with care not to overgraze. Early spring grazing of perennial pastures should be avoided, according to this author, if good stands are to be maintained.

The competitive effect of tall grass on low growing white clover, especially in early spring, was recognized by Sprague et al. (68). Brown and Munsell (10) recognized the morphological differences between the clover and grass as a major reason why close mowing is beneficial to white clover. Ladino clover stems are at or just below the surface of the ground and regardless of height when the leaves are cut, new leaves must start from buds on prostrate stems. When grass blades are mowed, the leaf blades continue to grow and the higher the cut, the longer are the leaves at any time after cutting. This would be true only when the assumption is made that the meristematic tissue at the base of the grass leaf blades is not removed by mowing. Thus, higher mowing means more shading of the legume by the accompanying grasses.

In California, Peterson and Hagan (53) harvested irrigated birdsfoot trefoil-, alfalfa- and Ladino-grass mixtures at 2-, 3-, 4-, and 5-week intervals. As the length of clipping interval increased the yield of all mixtures increased.

Generally speaking, longer clipping intervals contained highest percentages of legume in the harvested forage. Ladino clover-grass was the most productive mixture when cut at 2-week intervals and alfalfa-grass mixtures least productive. Alfalfa became most productive at the 5-week clipping interval with birdsfoot-trefoil least productive of the three mixtures. Increasing clipping interval from 2 to 5 weeks increased the production of Ladino clover-grass, birdsfoot trefoil-grass and alfalfa-grass mixtures 43, 90, and 177 percent respectively.

The removal of water from soil at various depths under alfalfa, birdsfoot trefoil and Ladino clover plots was studied by Hagan and Peterson (23). Alfalfa removed the most water from the greater depths, followed by birdsfoot trefoil and then by Ladino clover. The clipping interval apparently affected the removal pattern only as it affected the botanical composition.

Supplemental irrigation generally has resulted in increasing the yields of forage crops in drought years (48, 74). However, there are many years when irrigation gives little or no increase in yield in the mid-western and eastern states. A review of the effect of irrigation on forage production has been presented by Prine (54).

In early studies at Wisconsin, Mortimer and Ahlgren (44) and Ahlgren (2) found that irrigation of Kentucky bluegrass increased forage yields more on fertilized plots than on unfertilized plots.

In Michigan a three-year study on an alfalfa-Ladino clover-bromegrass pasture indicated that the yield of forage was increased from 4.0 tons to 4.7 tons by irrigation (27). Illinois workers (30, 43, 83) reported an increased production of meat and forage on irrigated pastures plus increased length of grazing season and animal carrying capacity.

In a report on pasture irrigation in New Jersey, Willits (90) stated that an alfalfa-Ladino clover-orchardgrass-bromegrass mixture gave an average increase of only seven percent in forage production for a three-year period. He reported that in some sections of New Jersey irrigation had little effect on summer grazing of bluegrass.

In experiments on bluegrass-white clover sod, Robinson and Sprague (58) and Robinson _et al_. (57, 60) obtained yield increases from irrigation and nitrogen fertilizer. For a four-year period, irrigation increased yields both with no nitrogen and high nitrogen treatments about 1300 pounds per acre. Under irrigation yields of 40 to 50 pounds of dry matter per acre per day were obtained during July and August from Kentucky bluegrass in pure stand with fertilizer or with white clover mixture and adequate fertilizer other than nitrogen. The range of increase due to irrigation was from practically none in wet years to 40 to 50 pounds per acre per day in dry years.

Levine *et al.* (34) reported very little difference in performance of treatments under three different rates of irrigation: (1) calculated water need to bring highest irrigated treatment to field capacity, (2) 80 percent of field capacity, and (3) 60 percent of field capacity.

At Stoneville, Mississippi the green weight yields of millet and Sudangrass were doubled by irrigation (6). In Tennessee, an irrigated alfalfa-Ladino clover-orchardgrass pasture produced 61 percent more grazing (calculated from yields of total digestible nutrients) and 58 percent more milk than an unirrigated pasture (74). However, grazing results and observations indicated that the non-irrigated pasture produced more grazing than the irrigated pasture during periods of sufficient rainfall.

In Michigan, Tesar *et al.* (71) studied irrigation of an alfalfa-Ladino clover-bromegrass dairy pasture and noted that the alfalfa in the mixture was reduced from 53 percent to 35 percent from 1952 to 1954 as a result of irrigation. When irrigation was not applied the reduction was from 55 percent to 45 percent.

In general, relatively small yield increases have been reported from well adapted and fertilized forage crops as a result of irrigation in the humid regions (30). In drought years, spectacular responses have been reported due to irrigation. However, one year or several successive years may occur when little or no irrigation would be needed. Often the increased production due to irrigation was large, but the total production still may be too low for profit when one considers the high cost of irrigation. Carreker and Lillard (14) and Jones and Wakeland (30) point out that further investigation, especially in the management phase of irrigation, may point the way to profitable irrigation.

Thornthwaite and Mather (72) give data for a 25-year period which indicate that in 4 of 25 years at Wooster, Ohio, and in 5 of 25 years at Charles City, Iowa, rainfall was adequate and distributed well enough that no water deficiency occurred. Moreover, since the median annual water deficiency for the period was 2.72 inches at Charles City and 2.91 at Wooster, the supplemental irrigation water needed for half of the 25 years was less than 3 inches.

The possibility that irrigation of pastures may maintain milk flow of dairy cows during dry seasons has been suggested by the work of Rumery _et al_. (61), Van Horn _et al_. (75) and Hoglund _et al_. (27). Here again the value of irrigation would depend upon the amount and distribution of rainfall during the growing season.

In a recent study by White _et al_. (84), the effects of residual soil nitrogen on oats following fertilized corn were estimated to be equivalent to quantities as large as 49 percent of application to corn the preceding year. The residual nitrogen in the soil one year after application to corn appeared to be in the form of nitrate and most of it was found in the 6 to 21 inch depth of soil.

The investigations of Kay et al. (31) in California indicate that nitrogen fertilizers applied one year may produce increased feed on a range the following year or in areas of low rainfall even two years later, where initial applications of nitrogen were high. Willhite et al. (89) also noted that high rates of nitrogen gave the greatest residual effects. Significant residual effects were obtained only at rates of 320 pounds of nitrogen per acre or more.

There are small amounts of nitrate found in most growing plants. Under certain environmental conditions, however, nitrates have been known to accumulate in plants in quantities sufficient to be toxic to livestock. Accumulations of relatively large amounts of nitrate have been reported in corn, sorghum, oats, and other cereals, weeds and other plants (8, 13, 21, 40, 46, 47). Forage grasses and legumes seldom contain large amounts of nitrate (18, 33), but nitrate also has been found to accumulate in these plants, especially where large applications of nitrogen fertilizer have been applied (62, 85). Whitehead and Moxon (85) have reviewed the general aspects of the subject of nitrate accumulation in plants.

In 1895, Mayo (40) reported that in Kansas many cases of nitrate poisoning had been reported. In one case, seven head of cattle in a herd of twelve died within a few hours after eating dried corn stalks grown on a rich soil formerly used as a hog lot. Upon examination of the corn stalks, crystals of potassium nitrate could be observed and easily recognized by taste. A chemical examination of the stalks gave 18.8 percent potassium nitrate on a dry weight basis.

It was reported by Bradley *et al*. (8) that in Wyoming many cases of poisoning of cattle due to high concentrations of potassium nitrate resulted from ingestion of oat hay and straw. According to these authors, the ingestion of one-fourth of a gram or more of potassium nitrate per pound of body weight is enough to cause cattle to develop sufficient methemoglobinemia (lack of oxygen) to cause their death. This methemoglobinemia is probably produced by nitrite which is formed from the nitrate in the gastrointestinal tract. They further reported that cases of sheep and horses being poisoned by high nitrate have been noted, but primarily cattle are affected. They noted also that the concentration of nitrate in the soil is one factor which determines the amount of nitrate in the plants. On the basis of their experiments they arbitrarily set the equivalent of 1.5 percent potassium nitrate in forages eaten by livestock as the lower toxic limit that may result in fatal poisoning.

Hanway and Englehorn (24) noted that when nitrate accumulates, it is concentrated in the stems or stalks. The nitrates also were found to be concentrated more in the lower portion of the stalks of corn and sorghum than in the upper portion. Leaves and mature grain contained very little nitrate. These authors also found that the nitrate content decreased as the plants matured. They concluded that any practice that increases nitrogen availability in the soil, such as growing legumes in the rotation or applying manure or nitrogen fertilization, will increase the nitrate content of plants.

Scharrer and Seibel (62) investigated the effects of nutrition and light intensity on the nitrate content of forage crops in Germany. Sand culture experiments on ryegrass, rape, fodder rye and white mustard were established. They found that levels of phosphorous influenced the nitrate contents only at nitrate levels lower than 8-10 milligrams per gram of dry weight. Doubling the potassium supply increased the accumulation of nitrate. Experiments on ryegrass and rape demonstrated the dependence of their nitrate content on the sun's energy, according to these authors. Despite similar crop yields, the nitrate contents of plants grown in the shade were considerably higher than those of plants grown in full sunlight. The authors commented that this lends weight to the hypothesis that a photochemical reaction enhances nitrate reduction in aerial plant parts.

Sund and Wright (69) indicate that nitrates in certain weeds cause, or are related to the cause, of abortion in cattle grazing on unimproved lowland areas where soils are high in available nitrogen. They noted accumulation of nitrates on soils shown to be high in nitrogen and low in P and K. Symptoms present in internal organs and on the placentas of aborted fetuses indicated that tissue anoxia was caused by nitrate ingestion by pregnant heifers.

Commenting on research done at Missouri, Longwell (37) stated that conclusive proof was available that nitrate levels of 0.6 percent potassium nitrate equivalent or above can cause loss in milk production and abortion, even if it does not kill the cow. They further stated that nitrate will accumulate in forage plants, especially the cereal grasses on soil high in nitrate, under adverse weather conditions. Severe drought and high temperatures were conducive to nitrate accumulation. One gram of potassium nitrate equivalent per pound of an animal's weight was considered a fatal dose.

METHODS AND MATERIALS

Field experiments having similar basic objectives were conducted at two locations in Iowa during the period 1957-1959. In addition, a third experiment was undertaken to determine nitrate content of the forages. The forages studied were evaluated as pasture crops and therefore were harvested to simulate an alternate grazing system of management. The procedures used in each experiment will be described under the appropriate heading.

Ames Experiment

A field experiment was established in the spring of 1957 at Ames, Iowa, which is located in the Clarion-Webster soil association area as designated by Simonson _et al_. (65). This experiment was conducted to measure (1) dry matter and nitrogen yields of two grasses and two legumes grown alone and in association, (2) the seasonal distribution of dry matter production, (3) the botanical composition of the forage mixtures and (4) the residual carry-over of nitrogen from one year to the next. From this experiment estimates of the effectiveness of inorganic nitrogen as a replacement for legumes grown in association with forage grasses can be made.

General agronomic information

This experiment was located at the Agronomy Farm near Ames. The site was so located that replications one, two and three were on a Nicollet loam - Webster Silty clay loam intergrade, and replication four on Webster silty clay loam. This site had been planted to oats the previous year and the seeding turned under in the fall. It was assumed that the location was in a high state of fertility insofar as phosphorous and potassium were concerned since they had been applied the previous fall.

Germination tests were made on the seed by the Iowa State College Seed Testing Laboratory. The species, varieties, and seeding rates used in this experiment are presented in Table 1. These rates were corrected by using the adjusted seeding rate formula.

Table 1. Seeding rates utilized in the Ames, Iowa experiment

Species	Variety	Seeding rate alone (lbs. per acre)	Seeding rate in mixture (lbs. per acre)
Alfalfa	Vernal	12	8
Red clover	Dollard	10	7
Orchardgrass	Commercial	8	5
Bromegrass	Fisher	12	8

$$\text{Adjusted Seeding Rate} = \frac{\text{Recommended Seeding Rate} \times \text{Expected quality}}{\text{Percent Purity} \times \text{Percent Germination}}$$

The seedbed was disked and harrowed on April 9, 1957. After the legumes were inoculated with the proper strain of Rhizobia the plots were seeded and rolled on April 12, 1957. Excellent stands resulted in every plot. During the seeding year, weeds were controlled by clipping. In the fall any plants found growing out of place were removed. The weeds were kept under complete control throughout the experiment. Alleys were cut between the plots with a wheel hoe to delineate the plots and to keep the bromegrass from spreading into adjacent plots. These alleys were maintained throughout the experiment by repeated wheel hoeing.

There were twelve nitrogen treatments applied to each of the grasses. Where the grass was grown alone, nitrogen was applied at 0, 30, 60, 120 or 240 pounds per acre. The effect of applying nitrogen in split applications also was studied. In this treatment, 60 pounds of nitrogen were applied in early spring and another 60 pound increment was applied after the first harvest each year. Another treatment consisted of applying 240 pounds of nitrogen in 1958, and no nitrogen in 1959, to evaluate the effect of residual nitrogen.

Each of the grasses also was grown in association with alfalfa and with red clover. The mixtures also were grown without added nitrogen and with a 60 pound application. The final two treatments were alfalfa and red clover grown alone. Volunteering grass and weeds were removed from these plots by hand.

Nitrogen was applied as ammonium nitrate on March 25, 1958, and on March 28, 1959. The additional 60 pound increments in split-application plots were applied on May 28, 1958, and May 29, 1959, soon after the first harvests had been taken each year.

Poison bait was used on plots that appeared to be harboring field mice. Minor damage to a few plots occurred before rodents were controlled.

Since soil moisture affects the results of field experiments, Table 2 has been included as an aid in evaluating the data.

Table 2. Monthly precipitation in inches during the 1958 and 1959 growing seasons at Ames, Iowa

Month	Normal[a] precipitation	1958	1959
March	1.94	0.48	3.62
April	2.53	1.63	2.27
May	4.03	1.66	8.08
June	5.51	4.29	3.26
July	3.19	9.59	2.15
August	3.92	1.79	2.65
September	3.31	4.07	4.32
October	2.05	.18	2.40

[a]Average for period 1931-1955 at Ames, Iowa.

Experimental design and statistical analysis

A split plot design with four replications was employed in this experiment. The two grasses comprised the whole plots and each split consisted of the twelve treatments previously described in plots 8 ft. by 18 ft. in size. The data were subjected to standard analysis of variance procedures as outlined by Snedecor (66).

Methods of harvesting and processing

Plots were harvested four times each year to simulate a system of alternate grazing management. All residue was removed from the plots in the spring before growth started and at each harvest.

In 1958, the first harvest was on May 21 when orchardgrass was in the late boot stage and bromegrass was in the early boot stage. The alfalfa and red clover were in the early bud stage. Subsequent harvests were taken at approximately five-week intervals. There was not enough growth in late October to warrant harvesting a fifth time each year as was planned.

Plots were cut at a one and one-half inch height with a National mower. A 38-inch swath, ten feet long, was harvested from the center of each plot. The clipped plant material was placed in cloth bags in a forage drier at 135° F. until it was dried to apparent constant weight (26). On the basis of this dry weight, yields were calculated in pounds per acre.

All samples that contained mixtures were thoroughly mixed and divided to provide a subsample about one-fourth the size of the total (52). This subsample was hand separated into the grass and legume components, dried and used to calculate the botanical composition on a dry weight basis.

The grass and legume components were ground separately for subsequent chemical analysis. All samples were ground to pass through a 60 mesh screen.

Chemical analyses

All samples from two replications were analyzed for nitrogen percentage according to the official Kjeldahl method approved by the Association of Official Agricultural Chemists (4). Duplicate determinations were made on samples from the first harvest. Blanks or duplicates were included in each group of 12 while analyzing samples from the second, third, and fourth harvest, as a check on the technique. A 0.5 gram sample was used. Copper selinite was used as a digestion catalyst and a mixture of 50 percent methyl red and 50 percent methylene blue was used as the indicator. Periodic analyses of secondary dephenylguaridine, an organic nitrogen compound, revealed approximately 100 percent recovery of nitrogen.

Albia Experiment

The field experiment established at the College Pasture Improvement Farm at Albia, Iowa, was similar to the Ames experiment with modifications described in the subsequent sections.

General agronomic information

This experiment was established on a Belinda silt loam - Pershing silt loam intergrade. The soil conditions over the experimental area were quite uniform. The entire experiment was situated near the crest of a slightly rounded to nearly level ridge on a one percent slope. The soil consists of a light grayish brown silt loam surface which is underlaid at a depth of about eight inches by a light gray silt loam subsurface horizon. This subsurface horizon extends to a depth of about 20 to 22 inches and is underlaid by a very plastic silty clay subsoil.

Since this experiment was designed to compare performance of these forages under normal rainfall conditions and under conditions where lack of moisture would not limit production, it was located in close proximity to a watershed reservoir which furnished water for irrigation. It is recognized that in most years growth of forages is limited at some time by a moisture deficiency. The water was piped from the reservoir where a pump furnished pressure for the perforated-pipe sprinkler system. A measure of the amount and distribution of irrigation water applied was obtained from data gathered from tin cans, with vertical sides, spaced at several locations in the irrigated plot.

The irrigation equipment was not available for use until June, 1958. Some moisture deficit did occur in early spring, but as soon as the irrigation equipment was available, the rains began and there was an abundance of moisture throughout the remainder of the season. Consequently, the irrigation equipment was not put into use until June, 1959.

Table 3 shows the rainfall at Albia for 1958 and 1959 and indicates the additional water applied in 1959 by irrigation. It can be noted that a moisture deficit did not occur often in the 1959 season.

There is no proven rule for use at the present time to determine when irrigation water should be applied. Shaw *et al.* (64) give data showing that about .15 inch of water is lost per day in recovering forage and about .20 inch is lost as the forage matures under Iowa conditions. To determine how much water was available at field capacity, soil samples were taken on May 15, 1958. Samples were taken at four depths, 0-6, 6-12,12-18 and 18-24 inches. Wilting point and field capacity were then determined and the results showed that at field capacity there was about two inches of water available in each foot of soil. When the loss by transpira-

Table 3. Monthly precipitation and supplemental irrigation in inches during the 1958 and 1959 growing seasons at the College Pasture Improvement Farm at Albia, Iowa

Month	Normal precip.[a]	1958		1959	
		Precip.	Irrigation[b]	Precip.	Irrigation
March	2.38	.48	-	5.18	0
April	2.95	1.22	-	5.12	0
May	3.68	1.70	-	5.70	0
June	5.21	3.51	0	3.40	2.50
July	3.15	10.90	0	4.49	1.00
August	3.97	5.06	0	3.30	1.50
September	3.25	3.90	0	5.69	0
October	2.04	1.34	0	3.67	0

[a]Averages for period 1931-1955 at Albia, Iowa.

[b]Irrigation equipment was not available for use until June, 1958.

tion and evaporation reached about 40 to 50 percent of the available water, the plots were irrigated. Because of the nature of the perforated pipe irrigation equipment, uniform distribution of water could be accomplished only when air currents were very slight. Consequently, irrigation was done in the evening or early morning on calm days.

Prior to seeding, the experimental area was fertilized with 400 pounds of 0-20-10 per acre. Lime also was applied at the rate of 1.5 tons per acre. The seedings were made in the spring of 1957 and excellent stands resulted.

The treatments were essentially the same as at the Ames experiment except that Ladino clover (seeded at 2 pounds per acre) and birdsfoot trefoil (seeded at 6 pounds per acre) were included in mixtures with the grasses. The split application of nitrogen and the legumes-grown alone treatments were omitted. Harvesting and chemical analyses were made in the same manner as in the Ames experiment.

Experimental design and statistical analysis

In this experiment a split-split plot design with three replications was used. The first split consisted of irrigation and non-irrigation. The second was the split between bromegrass and orchardgrass. There were twelve treatments in each of the split-split plots, each six feet by twenty feet in size. The data were subjected to standard analysis of variance procedures.

Nitrate Accumulation Experiment

The objective of this phase of the study was to determine the effect of nitrogen fertilization on the accumulation of nitrate in the resulting forage. Samples were taken from the Ames grass plots fertilized in late March with 0, 60, 120, and 240 pounds of nitrogen per acre. Two replications from each of the first two harvests were analyzed for nitrate content. Samples from the third and fourth harvests were analyzed only from plots receiving 240 pounds of nitrogen in the spring. These nitrate analyses were made on samples taken in both 1958 and 1959.

The ground plant samples were dried at 60° centigrade and a one-gram sample used for analysis. The nitrate was extracted by adding 100 ml. of boiling water to the plant material and shaking it for 30 minutes. The extract was then filtered. A 25 ml. aliquot of the extract from each sample was added to a solution containing magnesium oxide and Devarda's alloy. Another 25 ml. aliquot of the extract from each sample was added to a solution containing only magnesium oxide. These solutions were then distilled and the ammonia released was caught in a standard acid solution. The distillate was then made up to 250 ml. in a volumetric flask. The ammonia was then determined by Nessler's method (4) used for total nitrogen determination. The correct percentage of nitrate nitrogen for each sample was then determined by subtracting the percent nitrogen of the blank from that of the sample reduced by Devarda's alloy.

EXPERIMENTAL RESULTS

The results of these investigations will be presented in three sections: Ames experiment, Albia experiment and nitrate accumulation experiment.

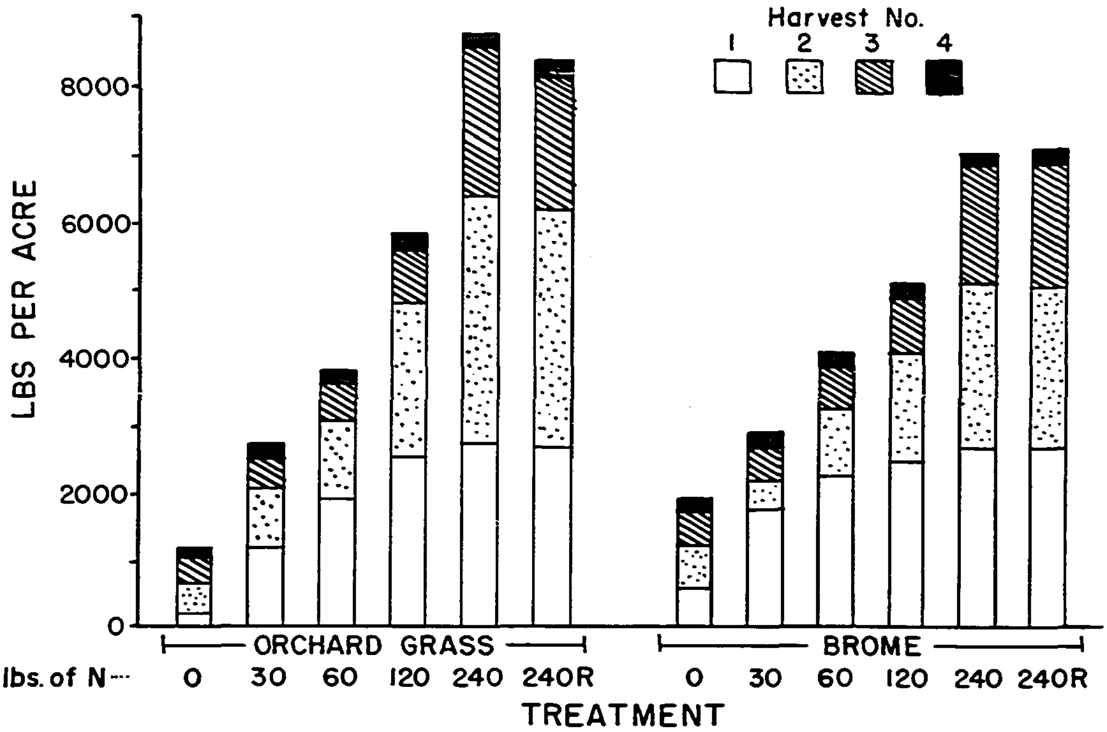
Ames Experiment

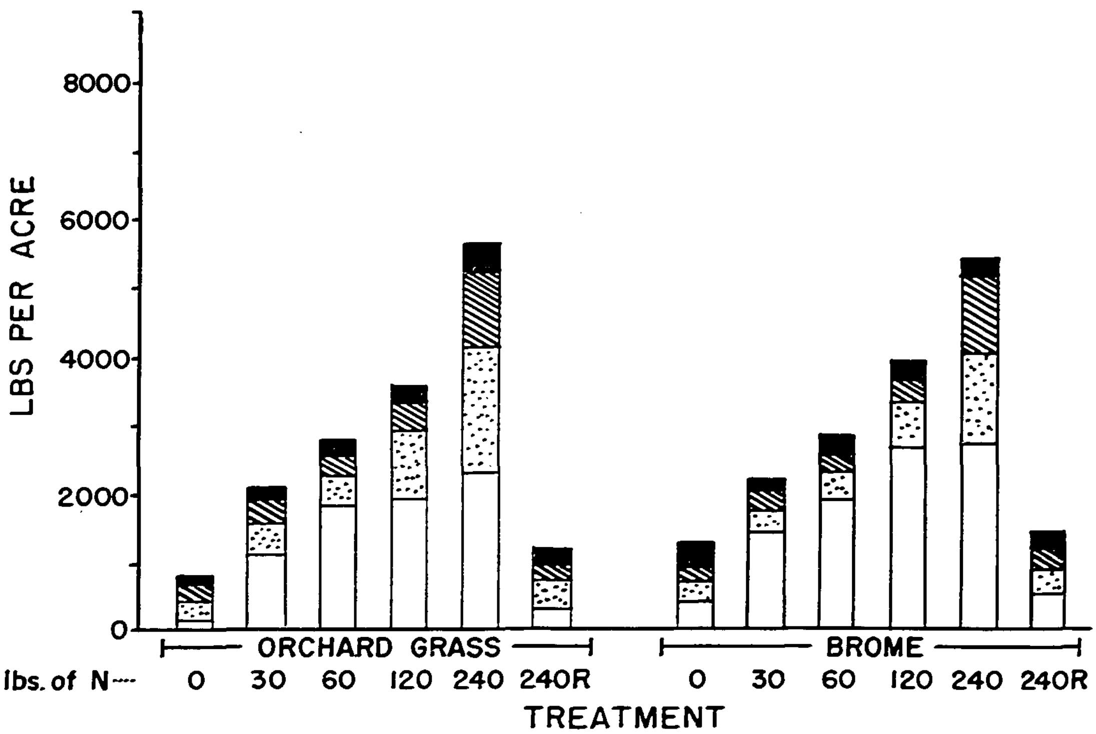
The dry matter yields of the grasses grown alone in 1958 and 1959 with increasing rates of nitrogen fertilizer are presented in Table 4. These results are shown graphically in Figures 1 and 2 in which the proportion of the total yield which came from each harvest is shown. A good response was obtained from the added increments of nitrogen fertilizer. Most of the differences in total dry matter yield can be accounted for in the first and second harvest. The low seasonal yield of the check, 1260 pounds with orchardgrass and 1905 pounds with bromegrass, indicated that the available nitrogen in this soil was very low and demonstrated the need for nitrogen by these forage grasses to more fully express their yield potential. It is evident that the total dry matter yields in 1959 were not as high as in 1958 and that most of the reduction came in the second and third harvests.

Visual differences due to added nitrogen were quite evident. Figure 3 shows the added vigor, density, and growth

Figure 1. Dry matter yield of orchardgrass and bromegrass with and without nitrogen fertilizer at Ames, 1958

Figure 2. Dry matter yield of orchardgrass and bromegrass with and without nitrogen fertilizer at Ames, 1959


Harvest No.
1 2 3 4
LBS PER ACRE
8000
6000
4000
2000
0
ORCHARD GRASS
BROME
lbs. of N
0 30 60 120 240 240R
0 30 60 120 240 240R
TREATMENT


LBS PER ACRE
8000
6000
4000
2000
0
ORCHARD GRASS
BROME
lbs. of N
0 30 60 120 240 240R
0 30 60 120 240 240R
TREATMENT

of orchardgrass when 120 pounds of nitrogen was applied. A similar response for bromegrass is shown in Figure 4.

Analysis of variance (Table 55, Appendix) revealed no significant difference between the dry matter yields of

Table 4. Dry matter yield of orchardgrass and bromegrass fertilized with nitrogen, Ames

Species	Nitrogen (lbs./A.)	Mean yields of dry matter (lbs./A.) 1958	1959
Orchardgrass	0	1260	827
	30	2864	2113
	60	3867	2755
	60 + 60[a]	6906	3975
	120	5890	3617
	240	8877	5640
	240R[b]	8459	1239
Bromegrass	0	1905	1345
	30	3420	2275
	60	4133	2800
	60 + 60[a]	5524	3835
	120	5133	3941
	240	7140	5516
	240R[b]	7149	1492

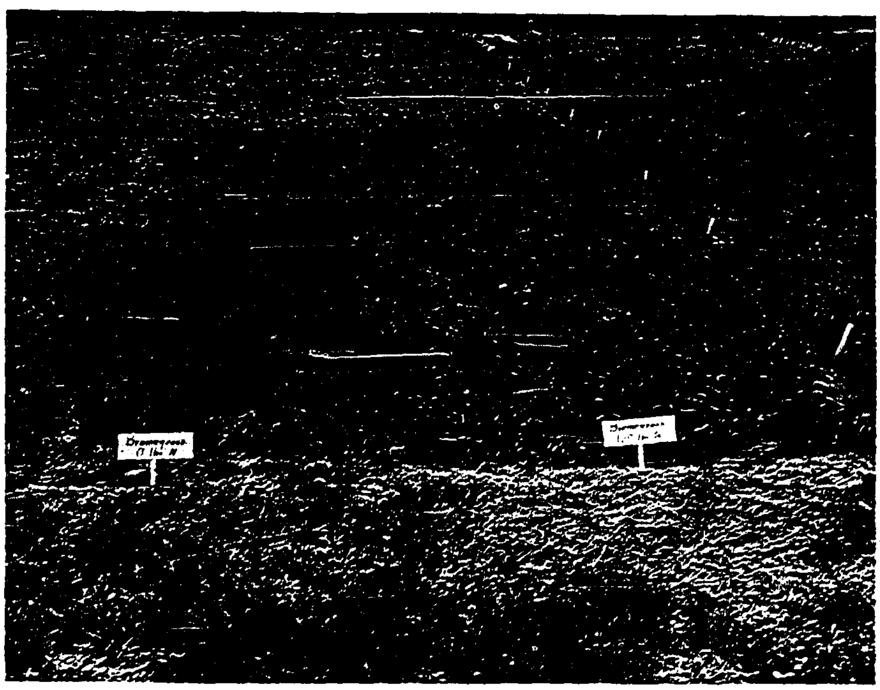
[a]Split application of 120 pounds of nitrogen, 60 pounds applied in early spring and 60 pounds applied after the first harvest.

[b]240 pounds of nitrogen were applied in 1958 and no nitrogen in 1959.

orchardgrass and bromegrass. However, as shown in Figures 1 and 2, orchardgrass yields tended to be lower at low rates of nitrogen fertilization and higher at the high rates of nitrogen fertilization than bromegrass. Since the response due to

Figure 3. Orchardgrass growth with no nitrogen fertilizer (left) and with 120 pounds of nitrogen (right), taken May 6, 1958

Figure 4. Bromegrass growth with no nitrogen fertilizer (left) and with 120 pounds of nitrogen (right), taken May 6, 1958



each added increment of nitrogen fertilizer appeared to be uniform, a regression comparison was made. To have equal intervals between treatments (which was necessary for this comparison) the log of the ratio of rates 1, 2, 4, 8 and 16 was utilized. This comparison shown in Table 5 revealed that most of the variance was due to the linear component. The quadratic component, although its mean square was much smaller, also was significant. With the analysis of variance statistical technique, homogeneous variance is assumed. Therefore, the error term from the overall dry matter yield analysis of variance (Table 55, Appendix) was used. The split application and the 240 pound residual treatments were not included in this comparison.

Table 5. Regression comparison among rates of nitrogen on dry matter yield of grasses, Ames

Source of variation	Df	Mean squares	
		1958	1959
Treatments	4	12,320,517**	5,595,790**
Linear	1	47,898,762**	21,711,216**
Quadratic	1	1,105,617**	370,944**
Remainder	2	138,845	150,455
Error B	60	88,153	51,982

**Exceeds the 1% level of significance.

It can be noted from Table 4 that in 1958 and 1959 both orchardgrass and bromegrass gave an added dry matter yield response when 120 pounds of nitrogen was applied in split increments as compared to a single application. Table 6 shows that the increase in 1958 was significant, but in 1959 the difference was not large enough to be significant at the five percent level.

Table 6. Dry matter yields per harvest of plots receiving single and split application of nitrogen, Ames

N applied	Pounds of dry matter 1958	Pounds of dry matter 1959
120	1378.0	949.8
60 + 60	1553.8	976.2
Difference	175.8	26.4
L.S.D. 5% level	148.5	114.0

It is evident in Figure 2 that when 240 pounds of nitrogen was applied in 1958 and no nitrogen applied in 1959, the dry matter yield was only slightly greater than the yield of the check. Table 7 shows that the difference between the mean of these two treatments was not significant at the five percent level.

Dry matter yields of grass-legume mixtures with and without additional nitrogen and legumes grown alone in 1958 and 1959 are presented in Table 8. The contribution of each

Table 7. Dry matter yields per harvest for check and residual nitrogen treatments, Ames

Treatment	Pounds of dry matter 1959
Check (0 lbs. N)	271.6
240R	341.4
Difference	69.8
L.S.D.	
5% level	114.0

harvest to the total dry matter yield also is shown. A grass and legume component breakdown of the total dry matter yield is presented graphically in Figures 5 and 6. It can be observed that the total yields of the orchardgrass-legume mixtures without added nitrogen were greater than the yields of the grasses grown alone with 120 pounds of nitrogen (see Table 8). In some cases the mixtures with the addition of 60 pounds of nitrogen yielded more dry matter than the grasses fertilized at 240 pounds of nitrogen. Again, as with the grasses, it is evident that the 1959 yields were lower than in 1958, but they do not appear to be reduced as much as were the grass yields. This may be related to the deep-rooted nature of the legumes.

The dry matter yield of legumes grown alone was comparable to the yield of the respective grass-legume mixtures in 1958. The alfalfa yield in 1959 was nearly as high as the grass-alfalfa associations. The drastic reduction of red

Table 8. Dry matter yields of grass-legume mixtures, Ames

Mixtures	Nitrogen (lbs./A.)	Pounds of dry matter per acre at each harvest 1	2	3	4	Total
		1958				
Orchardgrass-						
alfalfa	0	1817	2235	2207	1269	7528
alfalfa	60	3198	2273	1877	977	8325
red clover	0	2283	2810	1991	766	7580
red clover	60	3195	2531	1715	686	8127
Bromegrass-						
alfalfa	0	2584	2434	2229	1107	8354
alfalfa	60	3215	2638	2185	1123	9161
red clover	0	2466	2602	1817	704	7589
red clover	60	3487	2785	1689	651	8612
Alfalfa alone	0	2656	2785	2047	1039	8527
Red clover alone	0	2251	3078	1492	602	7423
		1959				
Orchardgrass-						
alfalfa	0	2176	1932	1556	1336	7000
alfalfa	60	2792	2011	1397	1151	7351
red clover	0	1039	1388	1040	470	3937
red clover	60	1779	1326	870	386	4361
Bromegrass-						
alfalfa	0	2150	2078	1529	1346	7103
alfalfa	60	2633	2050	1241	1362	7286
red clover	0	1120	1392	1050	533	4095
red clover	60	1493	1339	959	506	4297
Alfalfa alone	0	2163	2233	1342	969	6707
Red clover alone	0	637	1504	754	142	3037

Figure 5. Dry matter yield of grass-legume mixtures and legumes grown alone at Ames, 1958

Figure 6. Dry matter yield of grass-legume mixtures and legumes grown alone at Ames, 1959

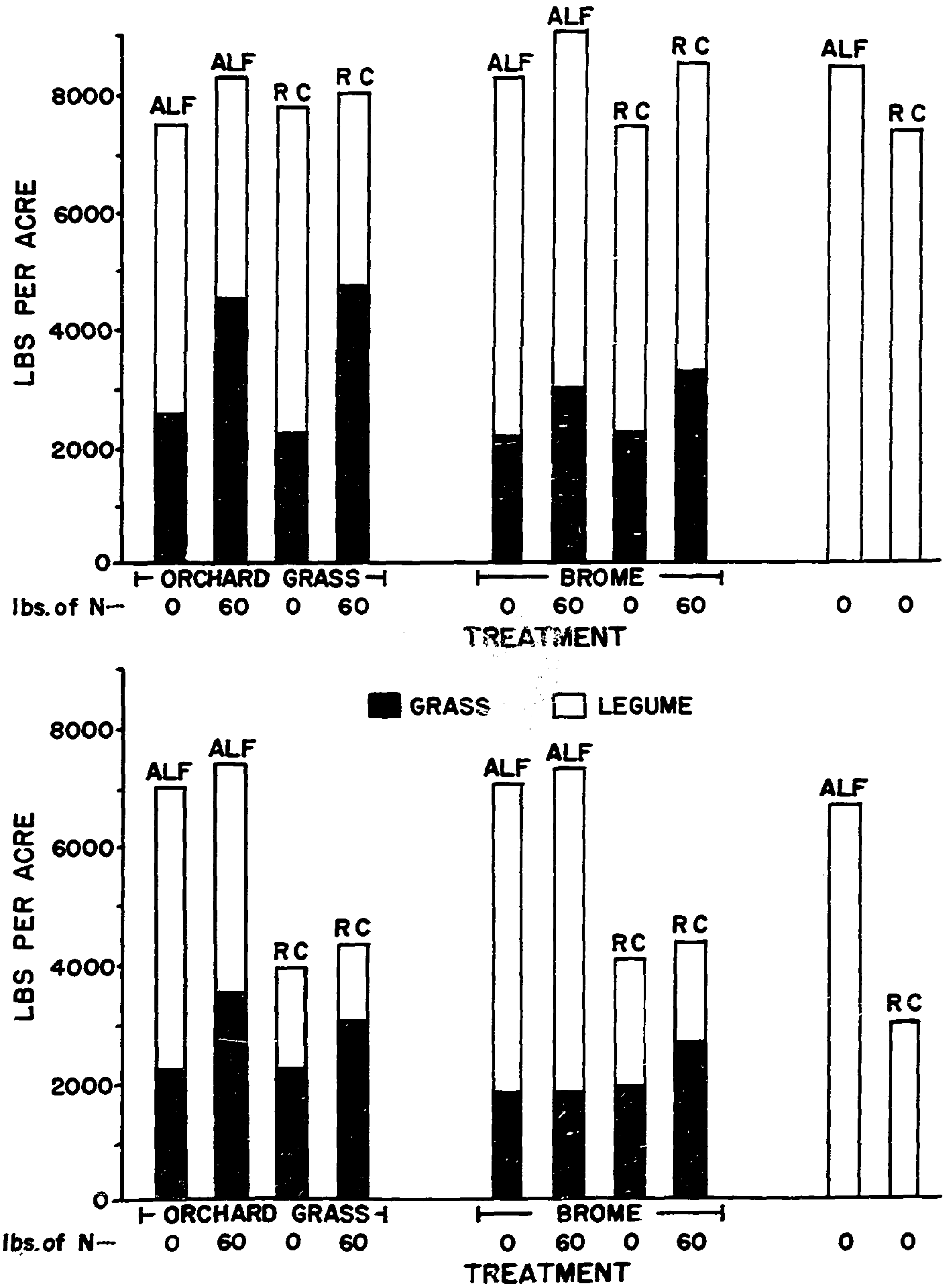

ALF
ALF
R C
R C
ALF
ALF
R C
R C
ALF
R C
LBS PER ACRE
8000
6000
4000
2000
0
ORCHARD GRASS
BROME
lbs. of N—
0 60 0 60
0 60 0 60
0 0
TREATMENT
GRASS
LEGUME
ALF
ALF
R C
R C
ALF
ALF
R C
R C
ALF
R C
LBS PER ACRE
8000
6000
4000
2000
0
ORCHARD GRASS
BROME
lbs. of N—
0 60 0 60
0 60 0 60
0 0
TREATMENT

Table 9. Treatment totals of the dry matter yield of the grass-legume mixtures, Ames

Mixture	Levels of nitrogen 0	Levels of nitrogen 60	Total
	1958		
Grass-alfalfa	63,489.3	69,941.3	133,430.6
Grass-red clover	62,749.7	66,958.0	129,707.7
Total	126,239.0	136,899.3	263,138.3
	1959		
Grass-alfalfa	56,419.6	59,829.4	116,249.0
Grass-red clover	32,059.4	35,459.8	67,519.2
	88,479.0	95,289.2	183,768.2

Table 10. Analysis of variance of the dry matter yields of the grass-legume mixtures, Ames

Source of variation	Df	Mean squares 1958	Mean squares 1959
Treatments	3	345,146*	63,046
Between nitrogen levels	1	887,827**	3,623
Between mixtures	1	108,281	185,515
Interaction	1	39,331	0
Error B	60	88,153	51,982

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

clover yields in 1959 both alone and in association with grass is accounted for by the fact that this was the second harvest year.

Figures 5 and 6 show that there was an increase in dry matter yield in each case when nitrogen was applied to the mixture. This increase appeared to be greater in 1958 than in 1959. Table 9 gives the treatment totals of dry matter yield of grass-legume mixtures and Table 10 the analysis of these data. The error terms were derived from Table 55 in the Appendix. These data indicate that the increase in yield due to addition of nitrogen were significant in 1958 but non-significant in 1959.

Since the forage from the grass-legume associations were hand-separated into their component parts, it was possible to analyze the dry matter yields of each of these components separately. Only two replications were hand-separated in 1959 because of the time and labor involved. Figures 5 and 6 show that the addition of nitrogen increased the grass component dry matter yield of these mixtures in every case and in some cases it appeared to reduce the dry matter yield of the legumes. Table 11 shows the treatment totals of the grass component dry matter yield from the grass-legume mixtures in 1958 and 1959. By examining the analysis of variance as shown in Table 12, it can be noted that there was a significant increase in dry matter yield of the grass component of the mixtures when 60 pounds of nitrogen was applied. There did not appear to be a significant difference between dry matter yields of grasses grown with red clover and alfalfa. In both years the harvests by treatment interaction was significant,

Table 11. Treatment totals of the grass component of mixtures with two levels of nitrogen, Ames

Grass association	Levels of nitrogen 0	Levels of nitrogen 60	Total
		1958	
Grass-alfalfa	19,354.3	30,797.8	50,152.1
Grass-red clover	18,255.2	32,373.9	50,629.1
Total	37,609.5	63,171.7	100,781.2
		1959	
Grass-alfalfa	8,281.4	10,883.8	19,165.2
Grass-red clover	8,386.6	11,961.1	20,347.7
Total	16,668.0	22,844.9	39,512.9

suggesting that the response in later harvests was significantly less than in the earlier harvests. Table 13 gives the treatment totals of the legume component dry matter yields of grass-legume mixtures in 1958 and 1959. The analysis of variance of these data as shown in Table 14 indicates that in 1958 there was a significant reduction and in 1959 there was no significant difference in the dry matter yield of the legume component of the mixtures when nitrogen fertilizer was applied. The fact that 1959 was the second harvest year for the red clover association resulted in a significantly lower dry matter yield of the red clover component in 1959.

The nitrogen yield of grasses grown alone with increasing rates of nitrogen in 1958 and 1959 are presented in Table 15 and the results are shown graphically in Figures 7 and 8. The

Table 12. Analysis of variance of grass component dry matter yields from grass-legume mixtures, Ames

Source of variation	Df	Mean squares 1958	Df	1959
Replications	3	428,629	1	3,426
Species	1	1,520,986	1	614,872
Error A	3	630,380	1	63,460
Treatments	3	1,720,860**	3	210,924**
Between nitrogen levels	1	5,104,891**	1	596,158**
Between grasses in association	1	1,778	1	21,849
Interaction	1	55,911	1	14,765
Treatment X species	3	310,031	3	95,762**
Error B	18	103,978	6	14,282
Harvests	3	6,797,136**	3	1,819,162**
Harvests X species	3	97,216	3	174,893**
Harvests X treatment	9	626,955**	9	164,778**
Harvests X species X treatment	9	117,334*	9	52,746**
Error C	72	58,027	24	15,123

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

nitrogen yield responded similarly to the dry matter yield, as might be expected, since nitrogen yield is a function of dry matter yield as well as percent nitrogen. Most of the difference in total nitrogen yield can be accounted for in the first and second harvest. It is evident that nitrogen yields in 1959 were not as high as in 1958 and that most of the reduction was due to lower yields in the second and third harvest.

To test for uniformity of response from each added

Table 13. Treatment totals of dry matter yield of legume component of mixtures with two levels of nitrogen, Ames

Legume	Levels of nitrogen 0	60	Total
	1958		
Alfalfa	44,135.0	39,143.5	83,278.5
Red clover	44,494.5	34,584.1	79,078.6
Total	88,629.5	73,727.6	162,357.1
	1959		
Alfalfa	20,478.4	19,348.3	39,826.7
Red clover	7,386.8	5,974.5	13,361.3
Total	27,865.2	25,322.8	53,188.0

increment of nitrogen fertilizer a regression comparison was made. The logs of the nitrogen rates were used as previously explained to satisfy the requirements for using this comparison. This regression comparison shown in Table 16 indicates that most of the variance was due to the linear component. The variance due to the quadratic component was large enough to be significant, although in both years it was considerably smaller than the linear component. The error term from the overall nitrogen yield analysis of variance (Table 56, Appendix)was used in this comparison.

It can be noted from Table 17 that the nitrogen yield of the grass was increased slightly by the application of 60 pounds of nitrogen in early spring and 60 pounds applied after

Table 14. Analysis of variance of legume component dry matter yields from grass-legume mixtures, Ames

Source of variation	Df	Mean squares 1958	Df	1959
Replications	3	723,362	1	605,206
Species	1	3,939,379	1	533,448
Error A	3	1,266,107	1	12,438
Treatments	3	687,243	3	3,682,088**
Between nitrogen levels	1	1,734,895**	1	100,997
Between legumes	1	137,805	1	10,944,022**
Interaction	1	189,029	1	1,244
Treatment X species	3	570,800	3	190,146
Error B	18	227,957	6	215,183
Harvests	3	6,454,014**	3	852,277**
Harvests X species	3	193,184	3	102,225**
Harvests X treatment	9	632,282**	9	56,082
Harvests X species X treatment	9	56,313	9	22,312
Error C	72	77,499	24	29,293

**Exceeds the 1% level of significance.

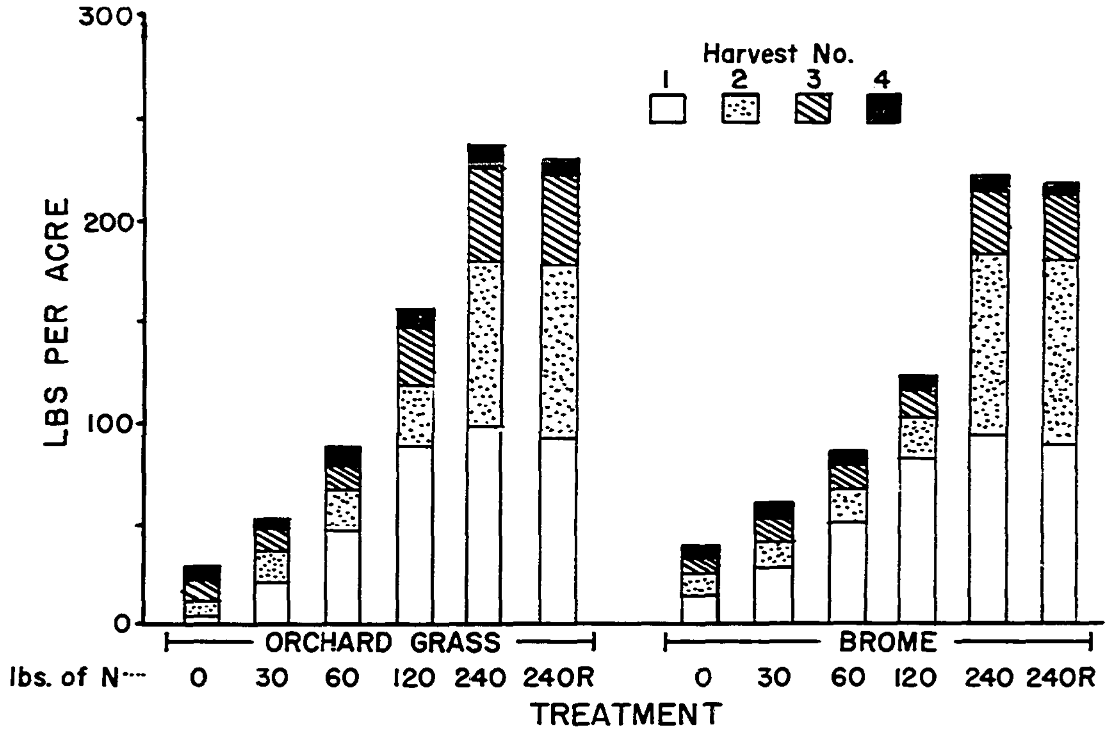
the first harvest, as compared to applying 120 pounds in early spring. However, Table 17 shows that in neither year was the increase enough to be significant at the five percent level.

Where 240 pounds of fertilizer nitrogen was applied in 1958 and none in 1959, the nitrogen yield in 1959 was only slightly higher than where no nitrogen was applied, as shown in Table 18.

Since nitrogen yield is a function of nitrogen percentage as well as dry matter yield, the data on nitrogen percentages

Figure 7. Nitrogen yield of orchardgrass and bromegrass with and without nitrogen fertilizer at Ames, 1958

Figure 8. Nitrogen yield of orchardgrass and bromegrass with and without nitrogen fertilizer at Ames, 1959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1 2 3 4
LBS PER ACRE
300
200
100
0
ORCHARD GRASS
BROME
lbs. of N
0 30 60 120 240 240R
0 30 60 120 240 240R
TREATMENT

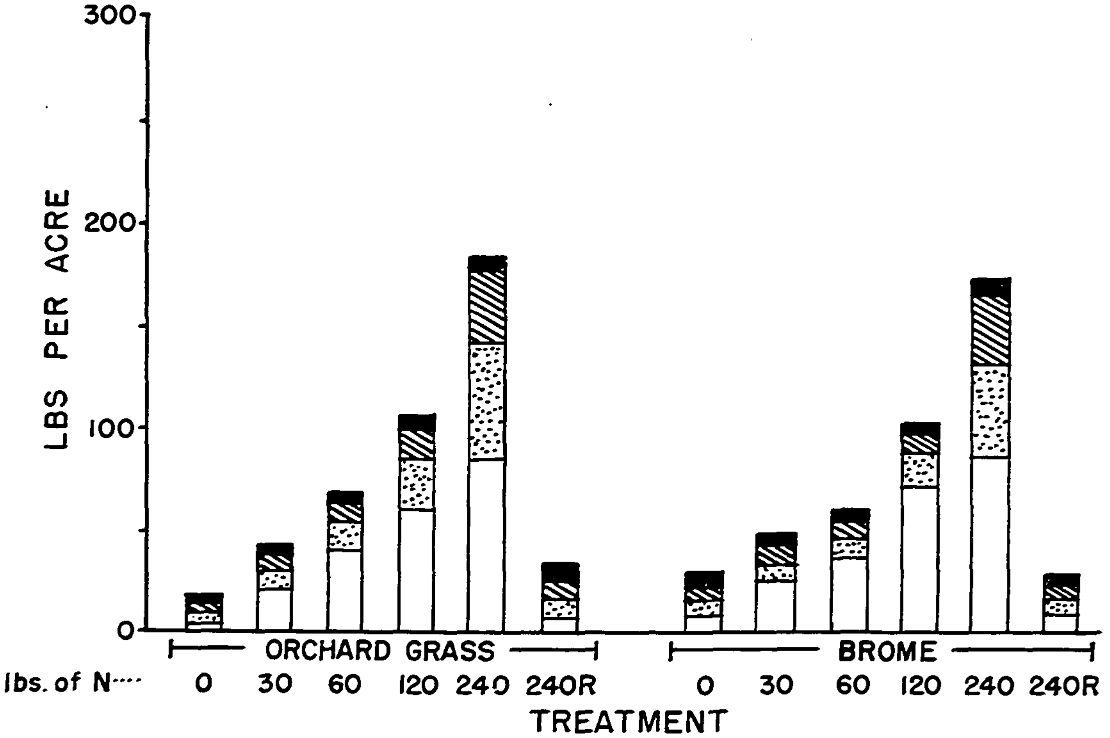

LBS PER ACRE
300
200
100
0
ORCHARD GRASS
BROME
lbs. of N
0 30 60 120 240 240R
0 30 60 120 240 240R
TREATMENT

Table 15. Nitrogen yield of orchardgrass and bromegrass fertilized with nitrogen at Ames, 1958 and 1959

Species	Nitrogen (lbs./A.)	Pounds of nitrogen per acre 1958	1959
Orchardgrass	0	27	21
	30	54	44
	60	88	68
	60 + 60[a]	147	103
	120	143	104
	240	238	185
	240R[b]	230	32
Bromegrass	0	36	30
	30	62	49
	60	87	61
	60 + 60[a]	131	94
	120	123	100
	240	222	171
	240R[b]	217	31

[a]Split application of 120 pounds of nitrogen, 60 pounds applied in early spring and 60 pounds after the first harvest.

[b]240 pounds of nitrogen were applied in 1958 and no nitrogen in 1959

Table 16. Regression comparison among rates of nitrogen on nitrogen yield of grasses, Ames

Source of variation	Df	Mean squares 1958	1959
Treatments	4	6,030**	3,701**
Linear	1	22,128**	13,373**
Quadratic	1	1,756**	1,234**
Remainder	2	117	99
Error B	20	108	68

**Exceeds the 1% level of significance.

Table 17. Nitrogen yield treatment means per harvest for single vs. split-application of 120 pounds of nitrogen fertilizer, Ames

Treatment	Pounds of nitrogen per acre	
	1958	1959
120	34.9	25.5
60 + 60	33.2	24.6
Difference	1.7	.9
L. S. D. 5% level	7.7	6.1

Table 18. Nitrogen yield treatment means per harvest for residual nitrogen vs. the check on grasses at Ames, 1959

Treatment	Pounds of nitrogen per acre
Check (0 lbs. N)	6.1
240R	7.9
Difference	1.8
L. S. D. 5% level	6.1

of the check and fertilized grasses were analyzed as shown in Table 19. Variances for treatments were significant in both years. There did not appear to be a significant difference between the two grass species. Harvests also were significant, indicating that the percent nitrogen was not the same from harvest to harvest. Mean nitrogen percentages from each harvest for 1958 and 1959 are shown in Table 20. The percent

Table 19. Analysis of variance of the nitrogen percentages of grasses fertilized with increasing rates of nitrogen at Ames

Source of variation	Df	Mean squares	
		1958	1959
Replications	1	.00004	.00189
Species	1	2.22042	.09258
Error A	1	.01653	.02460
Treatments	5	1.35654**	1.96516**
Treatment X species	5	.02083	.03915
Error B	10	.01368	.02329
Harvests	3	.85115**	.24536**
Harvests X species	3	.08147**	.21305**
Harvests X treatments	15	.32481**	.19530**
Harvests X species X treatments	15	.02406	.01767
Error C	36	.02342	.03627

**Exceeds the 1% level of significance.

nitrogen in the first harvest both years had the greatest range. Each successive harvest showed less difference among the treatments until in the fourth harvest there is little difference among treatments receiving 0 and 240 pounds of nitrogen fertilizer. It can be observed also that with each successive harvest the percent nitrogen in the treatments was increased, for the lower rates. This indicates that the forage was more mature when the early harvests were taken than in the subsequent harvests.

Nitrogen yields of the grass-legume mixtures with and without additional nitrogen fertilizer, and legumes grown alone, in 1958 and 1959, are presented in Table 21. The yield

Table 20. Percent nitrogen of grass at each harvest when fertilized with increasing rates of nitrogen at Ames

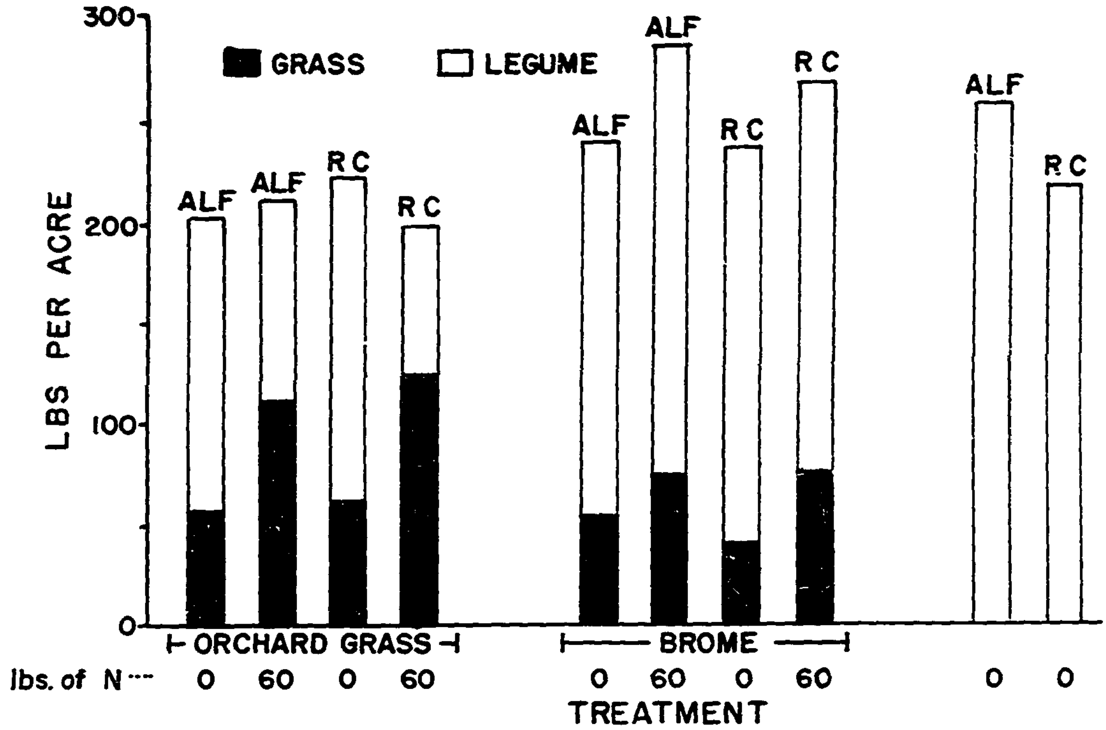
Grass	Nitrogen (lbs./A.)	Percent nitrogen at each harvest 1	2	3	4
		1958			
Orchardgrass	0	1.53	1.58	2.09	2.31
	30	1.62	1.59	2.04	2.34
	60	1.97	1.56	1.86	2.20
	60 + 60	2.11	1.89	2.02	2.50
	120	2.59	1.80	1.80	2.16
	240	3.30	2.35	2.02	2.61
Bromegrass	0	1.78	1.87	2.40	2.50
	30	1.90	1.95	2.23	2.33
	60	2.32	1.85	2.23	2.53
	60 + 60	2.25	2.60	2.23	2.69
	120	2.78	2.01	2.30	2.41
	240	3.37	3.04	2.61	2.89
		1959			
Orchardgrass	0	1.90	1.97	2.17	2.45
	30	1.77	1.88	2.17	2.39
	60	2.19	1.77	2.14	2.45
	60 + 60	2.46	2.20	2.40	2.71
	120	2.90	2.27	2.34	2.71
	240	3.61	2.91	2.97	2.65
Bromegrass	0	1.99	1.91	2.15	2.22
	30	1.90	2.23	2.37	2.21
	60	2.13	2.20	2.68	2.42
	60 + 60	2.51	2.54	2.43	2.62
	120	2.69	2.45	2.63	2.48
	240	3.49	3.21	3.18	2.57

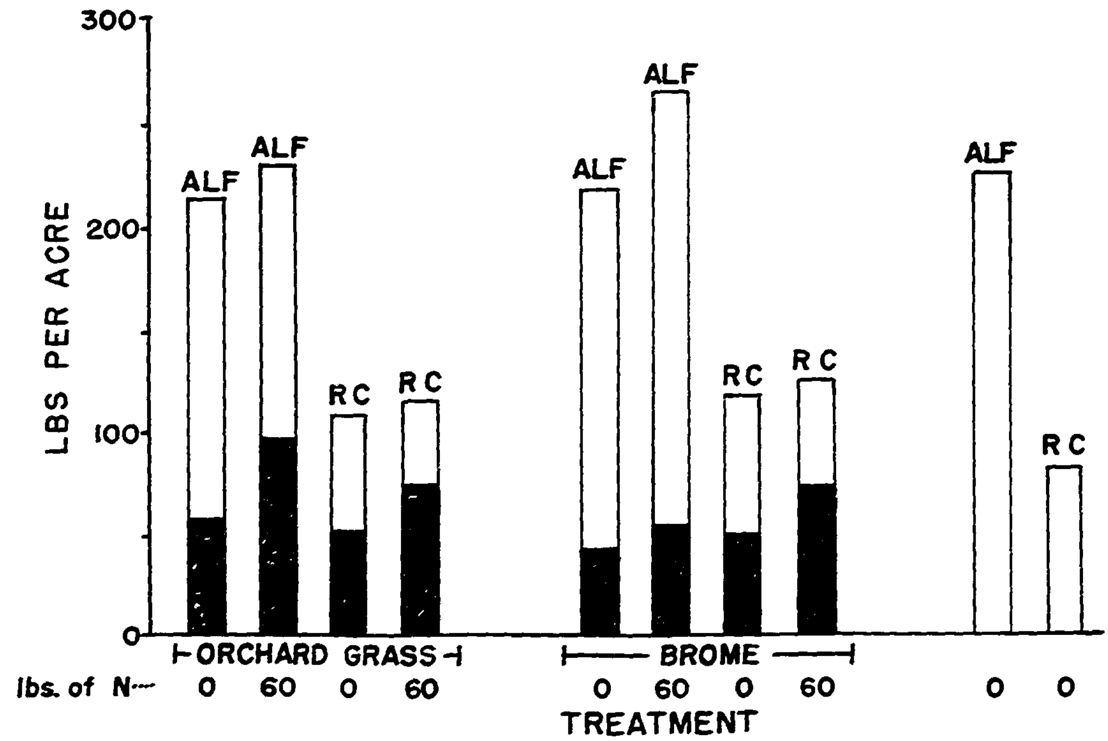
Table 21. Nitrogen yields of grass-legume mixtures fertilized with nitrogen at Ames

Mixture	Nitrogen (lbs./A.)	Pounds of nitrogen per acre at each harvest 1	2	3	4	Total
		1958				
Orchardgrass						
alfalfa	0	48	54	61	40	203
alfalfa	60	77	45	56	34	212
red clover	0	70	67	57	26	220
red clover	60	88	47	41	19	195
Bromegrass						
alfalfa	0	61	71	71	39	242
alfalfa	60	93	74	76	44	287
red clover	0	67	81	65	26	239
red clover	60	106	78	60	24	267
Alfalfa alone	0	72	80	66	42	260
Red clover alone	0	65	87	46	19	217
		1959				
Orchardgrass						
alfalfa	0	60	53	51	49	213
alfalfa	60	91	56	42	42	232
red clover	0	33	28	33	14	107
red clover	60	60	26	20	11	118
Bromegrass						
alfalfa	0	71	57	46	44	218
alfalfa	60	107	58	50	50	265
red clover	0	38	39	31	17	119
red clover	60	48	36	27	14	126
Alfalfa alone	0	78	68	51	28	225
Red clover alone	0	17	44	18	3	82

Figure 9. Nitrogen yield of grass-legume mixtures and legumes grown alone at Ames, 1958

Figure 10. Nitrogen yield of grass-legume mixtures and legumes grown alone at Ames, 1959


300
200
100
0
LBS PER ACRE
GRASS
LEGUME
ALF
ALF
R C
R C
ALF
ALF
R C
R C
ALF
R C
ORCHARD GRASS
BROME
lbs. of N
0
60
0
60
0
60
0
60
0
0
TREATMENT


300
200
100
0
LBS PER ACRE
ALF
ALF
R C
R C
ALF
ALF
R C
R C
ALF
R C
ORCHARD GRASS
BROME
lbs. of N
0
60
0
60
0
60
0
60
0
0
TREATMENT

of each harvest also is given. The contribution of the grass and legume component to the total yield is presented in Figures 9 and 10. Although the complete analysis of variance (Table 56, Appendix) did not show a significant difference between the grass species it can be noted that when the mixtures are considered alone the bromegrass-legume mixtures appeared to yield higher than did the orchardgrass-legume mixtures. In 1958 the nitrogen yield of the grass-legume mixtures appeared to be comparable to the nitrogen yield of the grasses alone with 240 pounds of nitrogen fertilizer.

The nitrogen yield of the alfalfa-grass mixtures did not appear to differ greatly from 1958 to 1959 as did the nitrogen yields of the grasses grown alone. The second harvest year for the red clover in 1959 accounted for its reduced nitrogen yield.

When alfalfa was grown alone its nitrogen yield was comparable to the nitrogen yield of the bromegrass-alfalfa mixture without additional nitrogen fertilizer. However, the orchardgrass-alfalfa association yielded somewhat lower. The nitrogen yield of red clover grown alone and the red clover-grass mixtures without nitrogen fertilizer were about the same in 1958. Red clover grown alone in 1959 yielded somewhat lower than the associations.

Table 22 gives the treatment totals of the nitrogen yield of the grass-legume mixtures. It can be noted that there was

Table 22. Treatment totals of nitrogen yield of grass-legume mixtures at two levels of nitrogen at Ames

Mixture	Levels of nitrogen 0	60	Total
	1958		
Grass-alfalfa	887.3	994.1	1881.4
Grass-red clover	915.3	925.6	1840.9
Total	1820.6	1919.7	3722.3
	1959		
Grass-alfalfa	865.1	953.2	1818.3
Grass-red clover	453.5	489.2	942.7
Total	1318.6	1442.4	2761.0

Table 23. Analysis of variance of the nitrogen yield of grass-legume mixtures, Ames

Source of variation	Df	Mean squares 1958	1959
Treatments	3	128	4,078**
Between nitrogen levels	1	214	239
Between mixtures	1	26	11,974**
Interaction	1	145	21
Error	60	108	68

only a slight increase in nitrogen yield due to the added increment of nitrogen fertilizer. The difference between alfalfa and red clover associations is again evident in the 1959 data. The analysis of variance of these results is presented in Table 23. It revealed that there was no significant increase in nitrogen yield due to the added nitrogen ferti-

Table 24. Treatment totals of the nitrogen yield of the grass component of mixtures at two levels of nitrogen, Ames

Grass association	Levels of nitrogen 0	60	Total
	1958		
Grass-alfalfa	221.7	374.5	596.2
Grass-red clover	207.7	401.8	609.5
Total	429.4	776.3	1205.7
	1959		
Grass-alfalfa	201.7	299.1	500.8
Grass-red clover	202.7	287.9	490.6
Total	404.4	587.0	991.4

lizer and that the reduction in nitrogen yield of the red clover associations in 1959 was significant.

Since the grass-legume associations were separated into their component parts and nitrogen yields determined for each portion, it was possible to separately analyze these components. From the nitrogen yield shown in Figures 9 and 10 it is apparent that in each case the added nitrogen fertilizer increased the nitrogen yield of the grass component. In most cases there appeared to be a reduction in the legume component when nitrogen fertilizer was added, this being more evident in the orchardgrass than in the bromegrass. From the treatment totals for the grass component nitrogen yields shown in Table 24, the nitrogen yield was increased with the addition

Table 25. Analysis of variance of the grass component nitrogen yields from grass-legume mixtures, Ames

Source of variation	Df	Mean squares 1958	1959
Replications	1	153	1
Species	1	678	238
Error A	1	114	54
Treatments	3	636**	175**
Between nitrogen levels	1	1,880**	521**
Between grasses in association	1	3	2
Interaction	1	27	2
Treatment X species	3	104	103
Error B	6	27	28
Harvests	3	1,396**	1,252**
Harvests X species	3	20	64*
Harvests X treatment	9	333**	148**
Harvests X species X treatment	9	35*	38*
Error C	24	12	14

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

of nitrogen fertilizer. There appeared to be little difference between the grass-alfalfa and grass-red clover association yields. The analysis of variance of the grass component nitrogen yield as presented in Table 25 revealed that the variance due to treatments was significant in both 1958 and 1959. A breakdown of the treatment variance showed that there was a significant difference between the nitrogen yield of the associations with and without nitrogen fertilizer. No significant difference between alfalfa and red clover associa-

Table 26. Treatment totals of the nitrogen yield of the legume component of mixtures at two levels of nitrogen, Ames

Legume	Levels of nitrogen 0	60	Total
		1958	
Alfalfa	665.6	619.6	1,285.2
Red clover	707.6	523.8	1,231.4
Total	1,373.2	1,143.4	2,516.6
		1959	
Alfalfa	663.4	654.0	1,317.4
Red clover	250.8	201.3	452.1
Total	914.2	855.3	1,769.5

tions was revealed in either 1958 or 1959.

The treatment totals for the legume component nitrogen yield is given in Table 26. The nitrogen yield of the legume component was reduced slightly in both 1958 and 1959. There appeared to be little difference in nitrogen yield of the legume component of the alfalfa and red clover associations in 1958, but the red clover yield was much reduced in 1959. The analysis of variance of these data as shown in Table 27 revealed no significant difference in nitrogen yield of the legume component when nitrogen fertilizer was applied as compared to no nitrogen fertilizer. The reduced yield of the red clover component in 1959 was found to be significant.

Table 27. Analysis of variance of the legume component nitrogen yields from grass-legume mixtures, Ames

Source of variation	Df	Mean squares 1958	1959
Replications	1	515	419
Species	1	5,996	599
Error A	1	1,416	50
Treatments	3	389	3,927**
Between nitrogen levels	1	825	54
Between legumes	1	45	11,700**
Interaction	1	297	25
Treatments X species	3	480	127
Error B	6	330	124
Harvests	3	2,006**	433**
Harvests X species	3	266	62
Harvests X treatments	9	462**	57
Harvests X species X treatments	9	47	33
Error C	24	110	40

**Exceeds the 1% level of significance.

Albia Experiment

The dry matter yields of grasses grown alone with increasing rates of nitrogen in 1958 and 1959 are shown in Table 28. These yields are presented graphically in Figures 11 and 12 where the proportion contributed by each harvest also is shown. It should be pointed out that Figure 12 represents the results of the irrigated plots. Since moisture did not appear to be a limiting factor in 1958, comparisons can be made with the plots irrigated in 1959. The response

Table 28. Dry matter yields of orchardgrass and bromegrass fertilized with nitrogen at Albia, 1958 and 1959

Species	Nitrogen (lbs./A.)	Pounds of dry matter per acre 1958 Not irrigated	1959 Irrigated	1959 Not Irrigated
Orchardgrass	0	3346	2443	2372
	30	4318	3536	3409
	60	5347	4237	3923
	120	6550	5504	5057
	240	8258	7117	7002
	240R[a]	8109	2470	2752
Bromegrass	0	3269	2825	2293
	30	4261	3999	2773
	60	4848	4312	3724
	120	5564	5366	4610
	240	7133	6380	5758
	240R[a]	7084	3207	2477

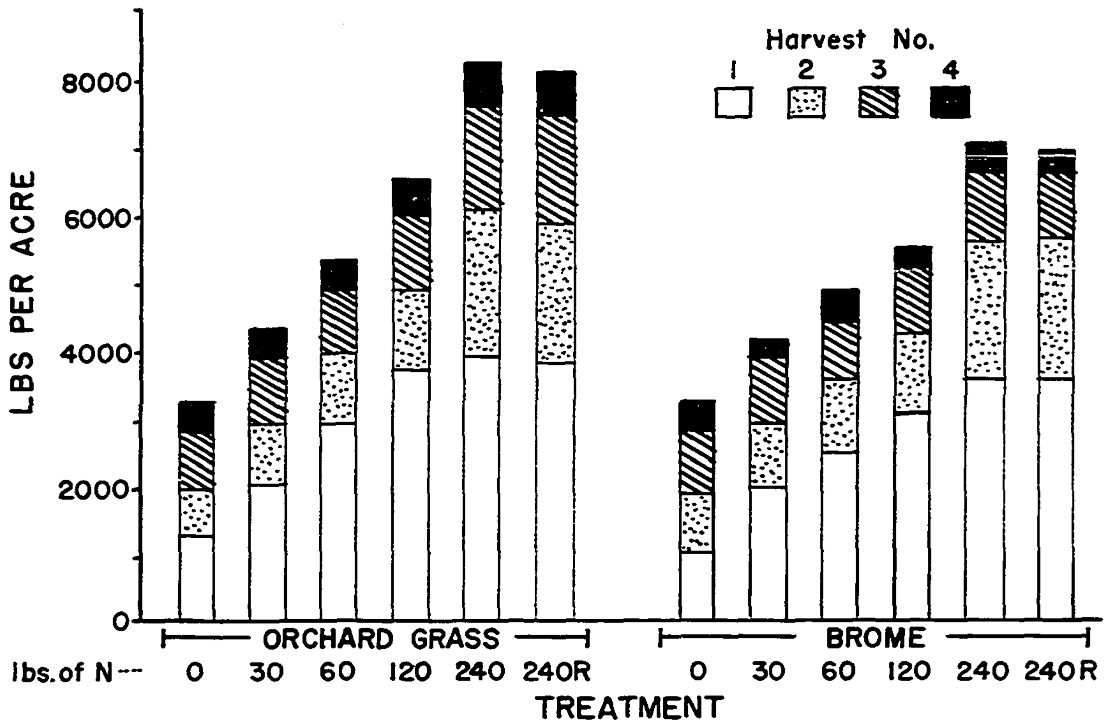
[a]240 pounds of nitrogen were applied in 1958 and no nitrogen applied in 1959.

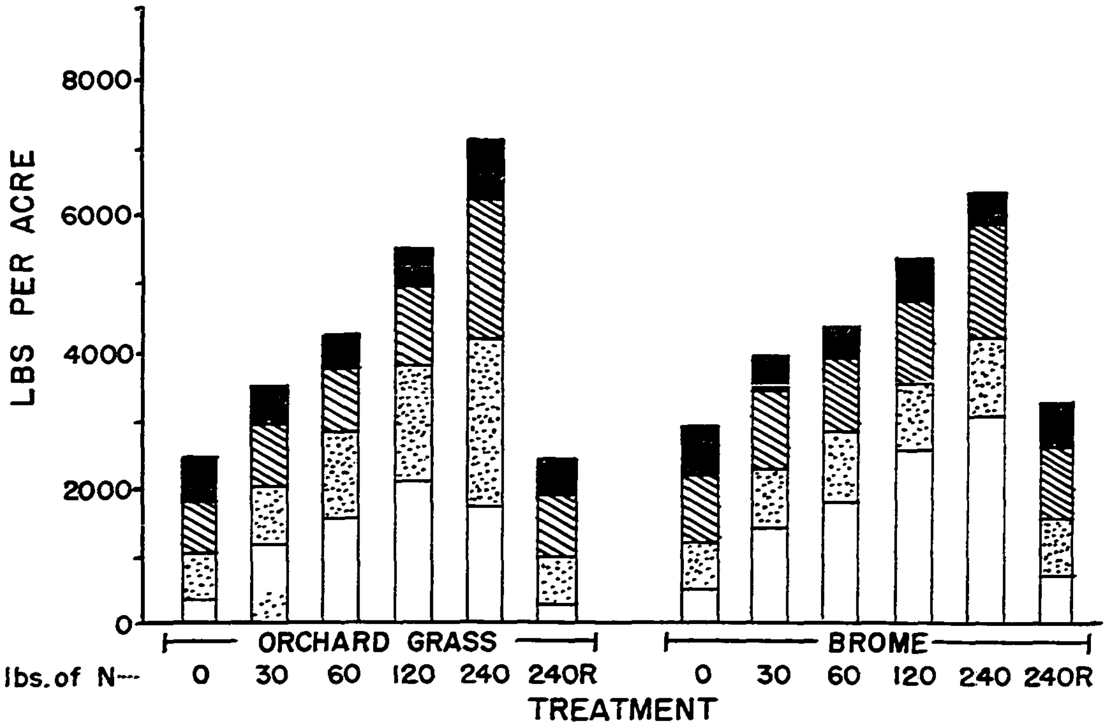
from the added nitrogen was similar to the response obtained in the Ames experiment. It can be noted, however, that the dry matter yield of the check (0 pounds of nitrogen) in all cases was considerably higher than it was in the Ames experiment. It can be noted again that most of the difference in the total dry matter yield was due to the first and second harvest.

Analysis of variance of the Albia dry matter yields in 1958 (Table 57, Appendix) revealed a significant difference between the orchardgrass and bromegrass dry matter yields.

Figure 11. Dry matter yield of orchardgrass and bromegrass with and without nitrogen fertilizer at Albia, 1958

Figure 12. Dry matter yield of orchardgrass and bromegrass with and without nitrogen fertilizer under irrigation at Albia, 1959


Harvest No.
1 2 3 4
8000
6000
4000
2000
0
LBS PER ACRE
ORCHARD GRASS
BROME
lbs. of N---
0 30 60 120 240 240R
0 30 60 120 240 240R
TREATMENT


8000
6000
4000
2000
0
LBS PER ACRE
ORCHARD GRASS
BROME
lbs. of N---
0 30 60 120 240 240R
0 30 60 120 240 240R
TREATMENT

From Figure 11 it can be noted that the orchardgrass yielded higher than bromegrass. Analysis of variance of the 1959 dry matter yields (Table 58, Appendix) revealed no significant difference between orchardgrass and bromegrass yields.

Irrigation was applied in 1959 and the analysis of variance showed there was a significant increase in yield of dry matter due to irrigation. However, since no irrigation water was applied until after the second harvest, it is possible that this difference was due to variation within the experiment and not to irrigation. Bromegrass appeared to respond more to irrigation than did orchardgrass, but even with this added response bromegrass did not yield as much dry matter as did orchardgrass.

The dry matter yield response at Albia appeared to be relatively uniform with each added increment of nitrogen fertilizer, as was found in the Ames experiment. A regression comparison among the rates of nitrogen on the dry matter yields of grasses is presented in Table 29. The error terms were taken from the complete analysis of variance (Tables 57 and 58, Appendix). These analyses show that most of the variance was contributed by the linear component but, as in the Ames experiment, the quadratic component also was significant.

A treatment to determine the effect of residual nitrogen also was included in the Albia study. The 1959 dry matter

Table 29. Regression comparison among rates of nitrogen on dry matter yields of grasses, Ames

Source of variation	Df	Mean squares 1958	Mean squares 1959
Treatments	4	8,461,430**	7,638,193**
Linear	1	33,075,945**	28,910,918**
Quadratic	1	486,718*	409,551**
Remainder	2	141,529	116,151
Error	110 and 88	79,941	53,791

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

Table 30. Treatment means per harvest for residual nitrogen vs. the check on grasses at Albia, 1959

Treatment	Pounds of dry matter per acre
Check (0 lbs. N)	620.8
240R	667.5
Difference	46.7
L.S.D.	
5% level	94.1

yields for the check treatment and the treatment receiving 240 pounds of nitrogen fertilizer in 1958 and none in 1959 are presented in Table 30. Since the difference was only 46.7 and the L.S.D. was 94.1, it appears that the residual plots did not yield significantly greater than the check in the second year.

Table 31. Dry matter yields of grass-legume mixtures fertilized with nitrogen at Albia, 1958

Mixtures	Nitrogen (lbs./A.)	Pounds of dry matter per acre at each harvest 1	2	3	4	Total
Orchardgrass						
alfalfa	0	1522	1133	1883	1015	5553
alfalfa	60	2929	1231	1619	869	6648
Ladino	0	1891	1499	2057	1260	6707
Ladino	60	2824	1396	1744	959	6923
trefoil	0	1281	1749	1600	600	5230
trefoil	60	2721	1504	1441	604	6270
Bromegrass						
alfalfa	0	1457	1543	2087	1157	6244
alfalfa	60	2610	1649	1859	937	7055
Ladino	0	1776	1547	1868	1012	6203
Ladino	60	2654	1591	1742	920	6907
trefoil	0	1227	1777	1484	323	4811
trefoil	60	2622	1534	1440	307	5903

Dry matter yields of the grass-legume mixtures with and without additional nitrogen fertilizer at each of the four harvests in 1958 and 1959 are presented in Tables 31 and 32. A graphic presentation of these data given in Figures 13 and 14 show total yields and the contribution of each component of the mixture to the total. These data show that total dry matter yields of bromegrass and orchardgrass mixtures in 1958 were about the same. In 1959, the alfalfa and trefoil mixtures yielded higher and Ladino clover mixtures yielded lower

Table 32. Dry matter yields at each harvest of grass-legume mixtures fertilized with nitrogen at Albia, 1959

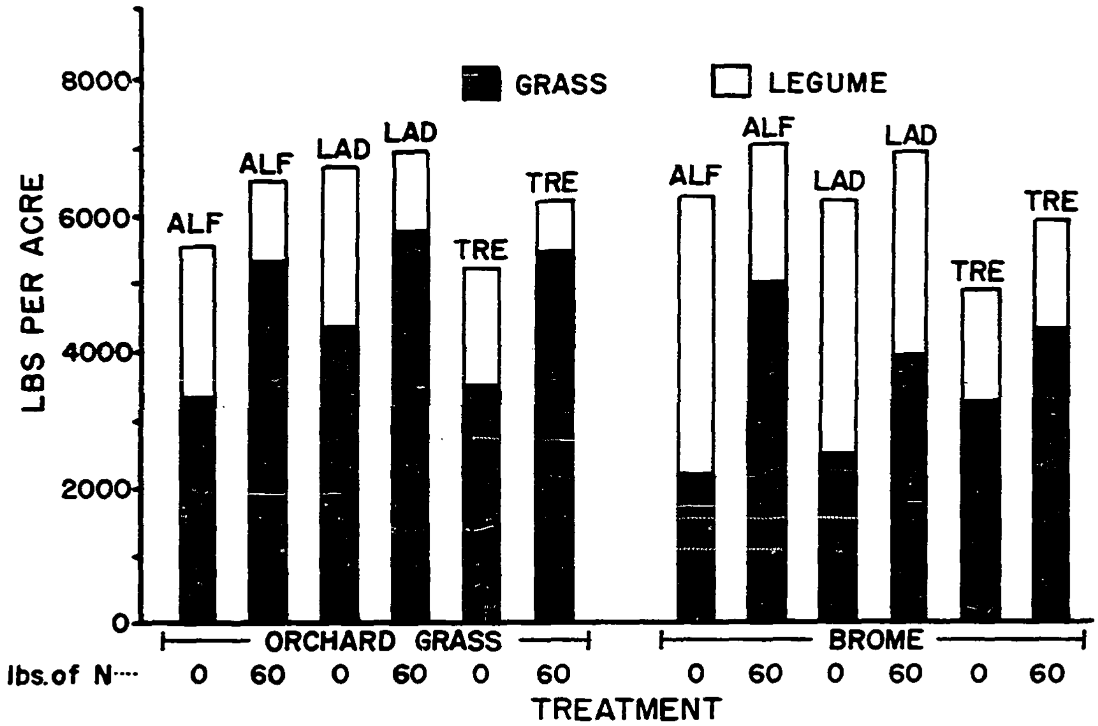
Species	Nitrogen (lbs./A.)	Pounds of dry matter per acre at each harvest									
		1		2		3		4		Totals	
		I[a]	NI[b]	I	NI	I	NI	I	NI	I	NI
Orchardgrass											
alfalfa	0	1682	964	1799	1446	1926	1528	1333	1178	6740	5116
alfalfa	60	2005	2093	1476	1373	1733	1393	1078	977	6292	5836
Ladino	0	1256	969	2432	2272	1924	1692	854	1306	6918	5787
Ladino	60	1821	1706	2212	1970	1928	1451	1158	745	7119	5872
trefoil	0	358	482	1428	1310	1600	1613	798	770	4184	4175
trefoil	60	1748	1914	1269	1297	1490	1307	708	732	5215	5250
Bromegrass											
alfalfa	0	1956	1601	2197	1793	2207	1972	1691	1089	8051	6455
alfalfa	60	2314	2312	1557	1671	2047	1949	1456	1225	7374	7157
Ladino	0	987	838	2238	2160	1099	1129	1452	574	5776	4701
Ladino	60	1420	1332	2072	2116	1197	1310	984	523	5673	5281
trefoil	0	1005	1010	1755	1727	1568	1640	802	508	5130	4885
trefoil	60	2315	1923	1413	1323	1706	1455	954	577	6388	5278

[a]I = Irrigated: Total of five inches applied after the second harvest.

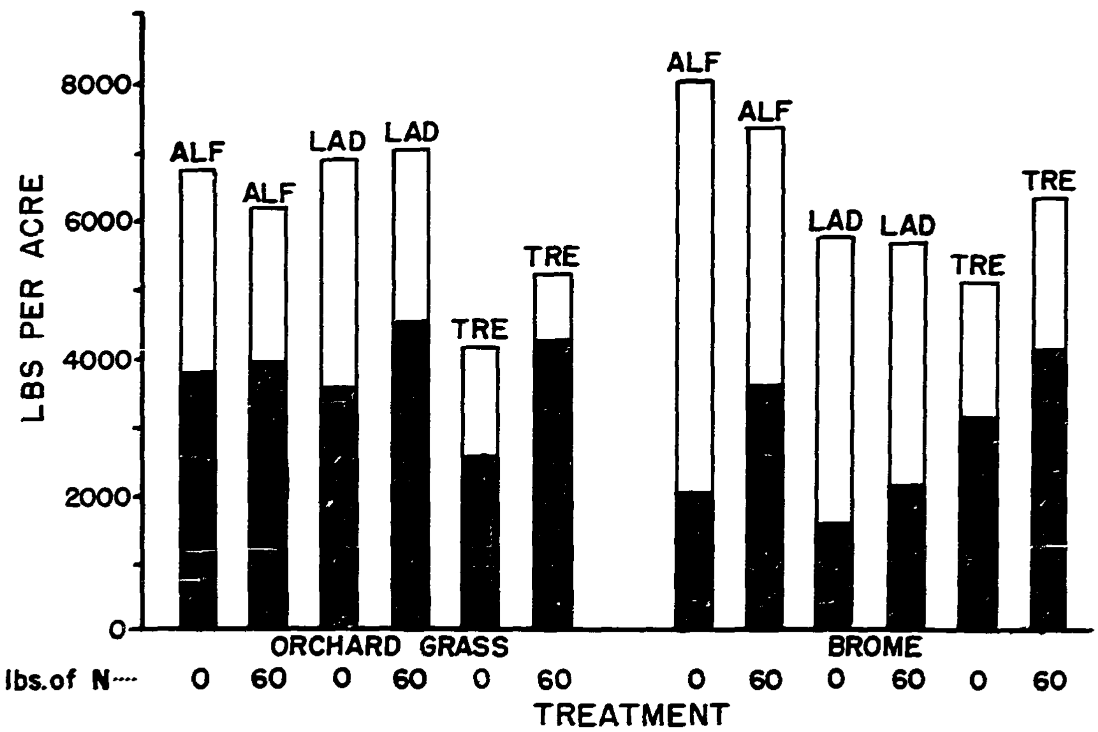
[b]NI = Not irrigated.

Figure 13. Dry matter yield of grass-legume mixtures with and without additional nitrogen fertilizer at Albia, 1958

Figure 14. Dry matter yield of irrigated grass-legume mixtures with and without additional nitrogen fertilizer at Albia, 1959



GRASS
LEGUME
8000
6000
4000
2000
0
LBS PER ACRE
ALF
ALF
LAD
LAD
TRE
TRE
ALF
ALF
LAD
LAD
TRE
TRE
ORCHARD GRASS
BROME
lbs. of N
0 60 0 60 0 60
0 60 0 60 0 60
TREATMENT



8000
6000
4000
2000
0
LBS PER ACRE
ALF
ALF
LAD
LAD
TRE
TRE
ALF
ALF
LAD
LAD
TRE
TRE
ORCHARD GRASS
BROME
lbs. of N
0 60 0 60 0 60
0 60 0 60 0 60
TREATMENT

Table 33. Treatment totals of the dry matter yield of grass-legume mixtures, Albia (pounds per acre)

Mixture	Levels of nitrogen 0	60
	1958	
Grass-alfalfa	70,833.7	82,227.3
Grass-Ladino	77,462.8	82,974.2
Grass-trefoil	60,251.1	72,793.1
Total	208,547.6	237,994.6
	1959	
Grass-alfalfa	79,012.5	79,984.7
Grass-Ladino	68,045.4	72,063.5
Grass-trefoil	55,130.8	66,390.1
Total	202,188.7	218,438.3

with bromegrass than with orchardgrass. The grass component of the orchardgrass mixtures appeared to comprise more of the total than in the bromegrass mixtures. The dry matter yield of the mixtures in general without added nitrogen fertilizer appeared to fall between the 120 and 240 pound nitrogen fertilization treatment of grasses grown alone.

Table 33 gives the treatment totals of the dry matter yield of the grass-legume mixtures for 1958 and 1959. Dry matter yields of alfalfa and of Ladino clover mixtures were higher than the birdsfoot trefoil mixtures in both years. There also was an increase in total dry matter yield in the fertilized treatment as compared to the treatment receiving no nitrogen fertilizer. The analysis of variance in Table 34

Table 34. Analysis of variance of the dry matter yield of the grass-legume mixtures, Albia

Source of variation	Df	Mean squares	
		1958	1959
Treatment	5	1,298,554**	1,762,737**
Between nitrogen levels	1	3,010,853**	916,839**
Among mixtures	2	2,092,783**	3,657,560**
Interaction	2	148,175	290,863**
Error B and C	110 and 88	79,941	53,791

**Exceeds the 1% level of significance.

indicates that both the difference between nitrogen levels and the difference among the grass-legume associations were significant in both 1958 and 1959.

The forage from the grass-legume mixtures in three replications in 1958 and two replications in 1959 were hand-separated into their component parts, making it possible to separately analyze the dry matter yields of each component. The treatment totals of dry matter yields of the grass component of mixtures at two levels of nitrogen fertilizer for 1958 and 1959 are given in Table 35. In both years there was an increase in dry matter yield of grass when nitrogen fertilizer was applied. In 1958 the grass component of the three associations yielded about the same, but in 1959 the grass in the trefoil association was considerably above the others. Analysis of variance of these data, as shown in Tables 36 and

Table 35. Treatment totals of the dry matter yield of the grass component of mixtures at two levels of nitrogen, Albia

Grass association	Levels of nitrogen 0	60
	1958	
Grass-alfalfa	18,307.0	30,254.3
Grass-Ladino	20,588.2	30,027.5
Grass-trefoil	19,445.4	29,962.5
Total	58,340.6	90,244.3
	1959	
Grass-alfalfa	20,794.3	29,132.5
Grass-Ladino	19,955.9	26,212.2
Grass-trefoil	22,180.1	34,101.6
	62,930.3	89,446.3

37, revealed that the increase in dry matter yield of the grass with the addition of nitrogen fertilizer was significant in both years. The difference in dry matter yield of the grass component among the associations was significant only in 1959. It can also be observed that the dry matter yield of orchardgrass was significantly higher than bromegrass in both 1958 and 1959 when considering the grass component of mixtures only. The variance due to irrigation in 1959 was not significant.

The dry matter yield (treatment totals) of the legume component of mixtures at two levels of nitrogen fertilizer for 1958 and 1959 are presented in Table 38. The dry matter yields

Table 36. Analysis of variance of the grass component dry matter yields from grass-legume mixtures at Albia, 1958

Source of variation	Df		Mean square
Replications	2		108,410
Species	1		2,653,777*
Error A	2		33,462
Treatments	5		1,435,749**
Between nitrogen levels		1	7,068,375**
Among grasses in association		2	22,209
Interaction		2	32,977
Treatment X species	5		238,499**
Error B	20		38,887
Harvests	3		14,280,928**
Harvests X species	3		269,023**
Harvests X treatments	15		708,209**
Harvests X species X treatments	15		11,185
Error C	72		28,282

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

of the legume components were reduced considerably in both years when nitrogen fertilizer was applied to these mixtures. Alfalfa and Ladino clover yields were comparable but the trefoil yield was considerably lower in both years. Analysis of variance of these results as presented in Tables 39 and 40 show the variance due to nitrogen levels and the variance among legumes was significant in both years. The variance due to irrigation was not significant.

The nitrogen yield of the grasses grown alone with increasing rates of nitrogen fertilizer is shown in Table 41. A

Table 37. Analysis of variance of the grass component dry matter yields from grass-legume mixtures at Albia, 1959

Source of variation	Df	Mean square
Replications	1	305,634
Irrigation	1	15,077
Error A	1	213,760
Species	1	2,462,327*
Species X irrigation	1	84,152
Error B	2	47,234
Treatments	5	947,047**
Between nitrogen levels	1	3,661,970**
Among grasses in association	2	408,327**
Interaction	2	128,305
Treatments X irrigation	5	12,634
Treatments X species	5	299,117**
Treatments X irrigation X species	5	139,774
Error C	20	43,658
Harvests	3	1,655,908**
Harvests X irrigation	3	269,449**
Harvests X species	3	452,426**
Harvests X treatments	15	555,366**
Harvests X irrigation X species	3	103,934*
Harvests X irrigation X treatments	15	31,782
Harvests X species X treatments	15	49,178
Harvests X irrigation X species X treatments	15	23,904
Error D	72	30,746

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

graphic presentation of these results showing the proportion of the total derived from each harvest is presented in Figures 15 and 16. Most of the difference in total nitrogen yield can be accounted for in the first and second harvest. The inconsistent result in Figure 15 where bromegrass was fertilized

Table 38. Treatment totals of dry matter yields of the legume components of mixtures at two nitrogen levels, Albia

Legume	Levels of nitrogen 0	60
	1958	
Alfalfa	19,884.5	9,907.1
Ladino	17,697.9	12,181.8
Trefoil	9,462.5	6,372.5
Total	47,045.2	28,461.4
	1959	
Alfalfa	29,994.4	21,411.4
Ladino	23,798.8	20,436.1
Trefoil	14,725.1	9,904.7
Total	68,518.3	51,752.2

Table 39. Analysis of variance of the legume component dry matter yields from grass-legume mixtures at Albia, 1958

Source of variation	Df	Mean square
Replications	2	645,464
Species	1	2,728,884*
Error A	2	127,976
Treatments	5	1,125,898**
Between nitrogen levels	1	2,398,317**
Among legumes	2	1,361,307**
Interaction	2	254,279
Treatments X species	5	291,233*
Error B	20	107,441
Harvests	3	1,117,130**
Harvests X species	3	98,679**
Harvests X treatments	15	317,647**
Harvests X species X treatments	15	27,002
Error C	72	24,913

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

Table 40. Analysis of variance of the legume component dry matter yields from grass-legume mixtures at Albia, 1959

Source of variation	Df	Mean square
Replications	1	565,209
Irrigation	1	1,053,628
Error A	1	185,002
Species	1	3,801,236
Species X irrigation	1	32,742
Error B	2	395,817
Treatments	5	1,538,929**
Between nitrogen levels	1	1,464,073**
Among legumes	2	3,001,916**
Interaction	2	113,369
Treatments X irrigation	5	62,009
Treatments X species	5	306,060**
Treatments X irrigation X species	5	58,269
Error C	20	64,795
Harvests	3	2,099,424**
Harvests X irrigation	3	35,886
Harvests X species	3	281,270**
Harvests X treatment	15	372,649**
Harvests X irrigation X species	3	51,955
Harvests X irrigation X treatments	15	48,431
Harvests X species X treatments	15	68,137
Harvests X irrigation X species X treatments	15	14,134
Error D	72	46,451

**Exceeds the 1% level of significance.

Table 41. Nitrogen yield of orchardgrass and bromegrass fertilized with nitrogen at Albia, 1958 and 1959

Species	Nitrogen (lbs./A.)	Pounds of nitrogen per acre 1958	1959
Orchardgrass	0	55	49
	30	72	73
	60	97	99
	120	142	138
	240	215	186
	240R[a]	217	54
Bromegrass	0	64	65
	30	96	95
	60	93	105
	120	129	143
	240	215	191
	240R[a]	216	79

[a]240 pounds of nitrogen were applied in 1958 and no nitrogen in 1959.

Table 42. Regression comparison among rates of nitrogen on nitrogen yield of grasses, Albia

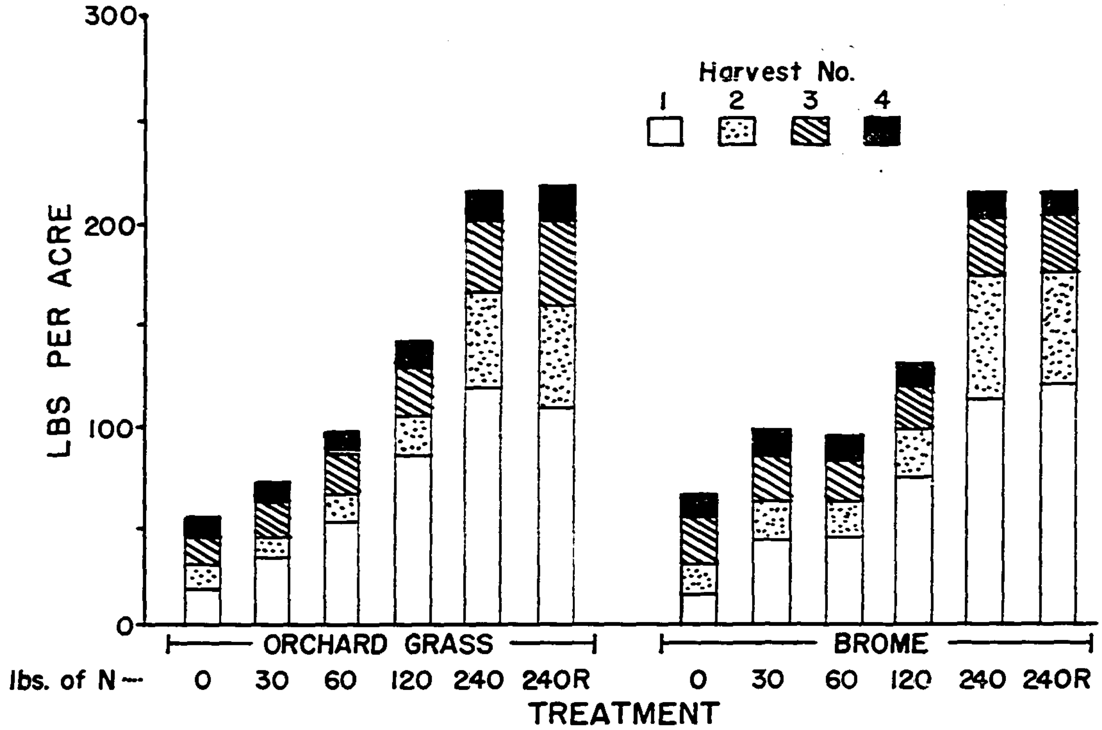
Source of variation	Df	Mean squares 1958	1959
Treatments	4	3,715**	2,650**
Linear	1	13,142**	10,261**
Quadratic	1	1,420**	280*
Remainder	2	149	30
Error	22	103	43

*Exceeds the 5% level of significance.

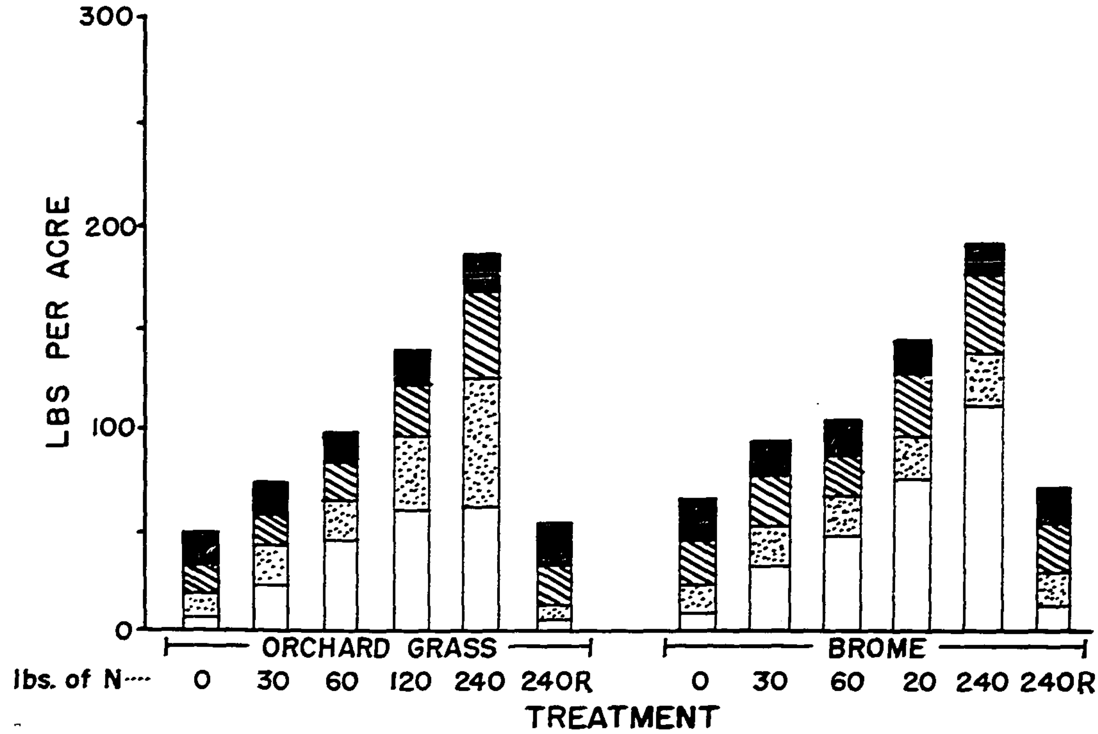
**Exceeds the 1% level of significance.

Figure 15. Nitrogen yield of orchardgrass and bromegrass with and without nitrogen fertilizer at Albia, 1958

Figure 16. Nitrogen yield of orchardgrass and bromegrass with and without nitrogen fertilizer at Albia, 1959



300
200
100
0
LBS PER ACRE
Harvest No.
1 2 3 4
ORCHARD GRASS
BROME
lbs. of N
0 30 60 120 240 240R
0 30 60 120 240 240R
TREATMENT



300
200
100
0
LBS PER ACRE
ORCHARD GRASS
BROME
lbs. of N
0 30 60 120 240 240R
0 30 60 20 240 240R
TREATMENT

Table 43. Pounds of nitrogen per acre per harvest for check and residual nitrogen treatments at Albia, 1959

Treatment	Pounds of nitrogen per acre
Check (0 lbs. N.)	14.2
240R	15.8
Difference	1.6
L.S.D.	
5% level	4.8

with 60 pounds of nitrogen may be due to taking a non-representative sample for nitrogen analysis in the first harvest. It can be observed that the nitrogen yields in general were higher in 1958 than in 1959.

The response in nitrogen yield was relatively uniform with each added increment of nitrogen fertilizer. A regression comparison among rates of nitrogen on nitrogen yield of grasses is presented in Table 42. It can be observed that the linear component accounted for most of the variance. The quadratic component was significant also. The error term for this comparison comes from the overall analysis of variance (Table 59, Appendix).

When 240 pounds of nitrogen fertilizer was applied in 1958 and none applied in 1959, the 1959 nitrogen yield, as shown in Table 43, was about the same as the check treatment (0 pounds nitrogen).

The analysis of variance of the nitrogen percentage of

Table 44. Analysis of variance of the nitrogen percentages of grasses fertilized with increasing rates of nitrogen at Albia

Source of variation	Df	Mean squares 1958	Mean squares 1959
Replication	1	.00128	.00202
Species	1	2.36672**	.23010
Error A	1	.00144	.01307
Treatments	4	1.00352**	.89898**
Treatments X species	4	.05575	.01616
Error B	8	.03781	.00820
Harvests	3	1.66993**	1.28203**
Harvests X species	3	.05091	.08889**
Harvests X treatments	12	.33654**	.41006**
Harvests X species X treatments	12	.02942	.01834
Error C	30	.04667	.01429

**Exceeds the 1% level of significance.

the grasses fertilized with increasing rates of nitrogen is presented in Table 44. The variance due to treatments was significant in both 1958 and 1959. Variance due to the difference between orchardgrass and bromegrass was significant only in 1958 but the variance due to harvests was significant in both years. Table 45 gives the mean nitrogen percentages from each of the four harvests for 1958 and 1959. The range in percent nitrogen of the grasses fertilized with 0 to 240 pounds of nitrogen was greatest in the first harvest in both years. This range became narrower in the second and third harvest and there was little range in percentage by the fourth harvest. The forage at each subsequent harvest appeared to be

Table 45. Percent nitrogen of grass at each harvest when fertilized with increasing rates of nitrogen, Albia

Grass	Nitrogen (lbs./A.)	Percent nitrogen at each harvest			
		1	2	3	4
			1958		
Orchardgrass	0	1.49	1.67	1.74	2.38
	30	1.59	1.61	1.78	2.29
	60	1.72	1.57	1.80	2.55
	120	2.31	1.61	2.22	2.41
	240	2.89	2.15	2.20	2.43
Bromegrass	0	1.82	2.03	2.11	2.60
	30	2.17	2.09	2.31	2.77
	60	2.66	2.15	2.23	2.60
	120	2.89	2.42	2.34	2.39
	240	3.77	2.61	2.37	2.51
			1959		
Orchardgrass	0	1.76	1.79	1.99	2.48
	30	2.04	1.98	1.93	2.74
	60	2.71	2.07	2.04	2.56
	120	3.02	2.21	2.15	2.59
	240	3.37	2.44	2.16	2.63
Bromegrass	0	1.77	1.93	2.39	2.68
	30	2.04	1.85	2.62	2.83
	60	2.05	1.69	2.33	2.54
	120	2.59	1.87	2.40	2.82
	240	3.41	2.58	2.55	2.64

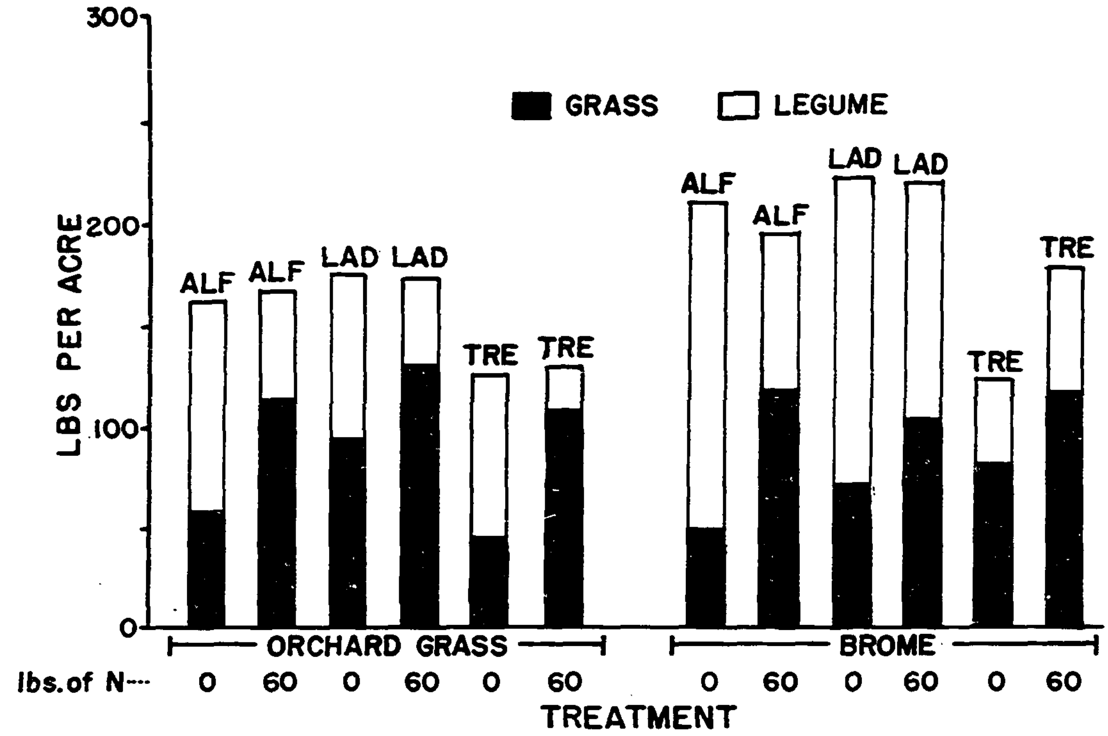
less mature than the previous harvest as indicated by the higher overall nitrogen percentage from the first to the fourth harvest at the lower nitrogen rates. The nitrogen percentages of the bromegrass were in general higher than those of the orchardgrass.

Nitrogen yields for each of the four harvests and the yearly totals of the grass-legume mixtures with and without nitrogen fertilizer are presented in Table 46. Graphic presentations of these results showing the proportion of the total contributed by each component are given in Figures 17 and 18. In general, these nitrogen yields appeared to fall between the nitrogen yields of the grasses alone with 120 and 240 pounds of nitrogen fertilizer. There did not appear to be a reduction in the nitrogen yield of the grass-legume mixtures in general from 1958 to 1959, as was noticed with the grasses grown alone. The exception, as seen in Figure 18, is Ladino clover which yielded about 35 pounds less in 1959 than in 1958. With the exception of birdsfoot trefoil, the nitrogen yields did not appear to be increased by added nitrogen and in some cases were reduced. The nitrogen yield of birdsfoot trefoil associations appeared to be increased, especially when in association with bromegrass.

The treatment totals of the nitrogen yield of the grass-legume mixtures are shown in Table 47. The difference in nitrogen yield with the addition of 60 pounds of nitrogen was very small. The grass-alfalfa and grass-Ladino treatments had comparable yields but the yield of grass-trefoil was considerably lower than these. Analysis of variance of these data, as shown in Table 48, revealed that the variance between nitrogen levels was not significant. The low yield of the grass-trefoil mixture was sufficient to make the variance among mixtures

Table 46. Nitrogen yields of grass legume mixtures fertilized with nitrogen, Albia

Mixture	Nitrogen (lbs./A.)	Pounds of nitrogen per acre at each harvest 1	2	3	4	Total
			1958			
Orchardgrass-						
alfalfa	0	31	32	63	35	161
alfalfa	60	66	26	43	31	166
Ladino	0	42	29	55	49	175
Ladino	60	80	24	40	30	174
trefoil	0	25	44	40	16	125
trefoil	60	57	24	34	14	129
Bromegrass-						
alfalfa	0	33	56	70	51	210
alfalfa	60	70	42	53	31	196
Ladino	0	59	51	66	44	220
Ladino	60	81	44	56	37	218
trefoil	0	24	46	42	10	122
trefoil	60	76	50	42	10	178
			1959			
Orchardgrass-						
alfalfa	0	50	46	37	40	173
alfalfa	60	58	41	38	30	167
Ladino	0	38	70	49	43	200
Ladino	60	53	65	52	37	207
trefoil	0	9	40	40	23	112
trefoil	60	42	34	37	20	133
Bromegrass-						
alfalfa	0	59	60	69	61	249
alfalfa	60	66	47	66	48	227
Ladino	0	26	68	37	34	165
Ladino	60	24	68	40	34	166
trefoil	0	27	45	39	26	137
trefoil	60	58	35	48	34	175


GRASS
LEGUME
LBS PER ACRE
300
200
100
0
ALF
ALF
LAD
LAD
TRE
TRE
ALF
ALF
LAD
LAD
TRE
TRE
ORCHARD GRASS
BROME
lbs.of N---
0 60 0 60 0 60
0 60 0 60 0 60
TREATMENT

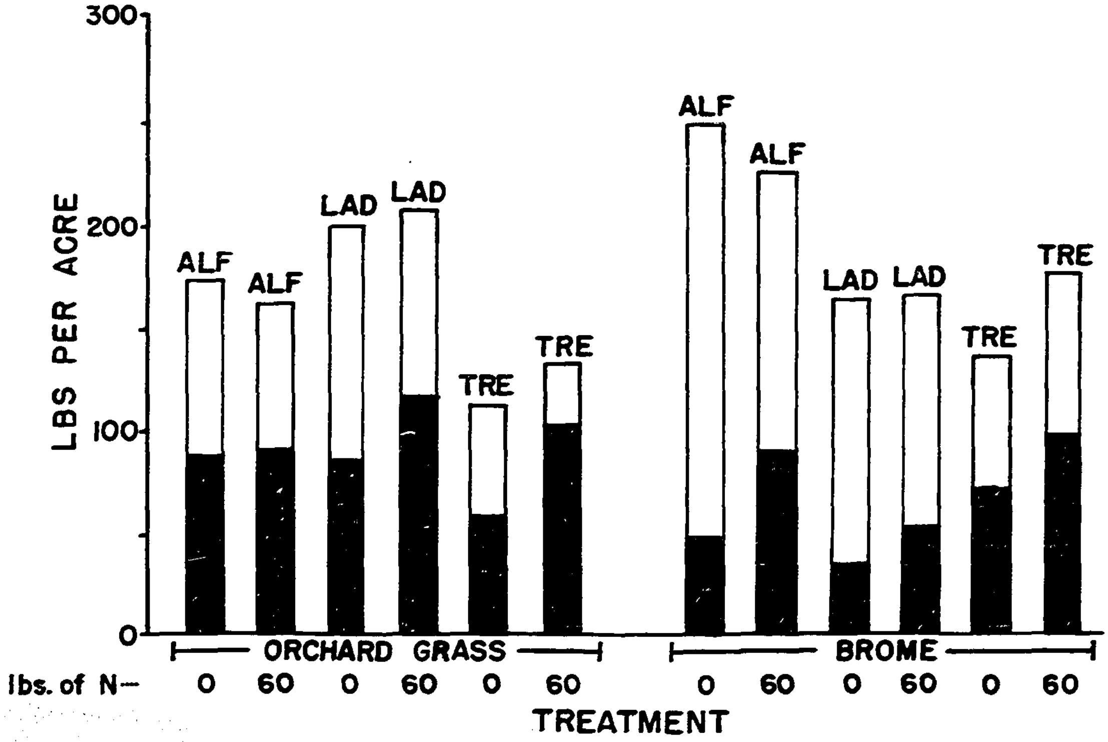

LBS PER ACRE
300
200
100
0
ALF
ALF
LAD
LAD
TRE
TRE
ALF
ALF
LAD
LAD
TRE
TRE
ORCHARD GRASS
BROME
lbs. of N—
0 60 0 60 0 60
0 60 0 60 0 60
TREATMENT

Table 47. Treatment totals of the nitrogen yield of the grass-legume mixtures, Albia

Mixture	Levels of nitrogen 0	60
	1958	
Grass-alfalfa	741	721
Grass-Ladino	788	782
Grass-trefoil	495	609
Total	2,024	2,112
	1959	
Grass-alfalfa	869	790
Grass-Ladino	728	764
Grass-trefoil	498	622
Total	2,095	2,176

Table 48. Analysis of variance of the nitrogen yield of the grass-legume mixtures, Albia

Source of variation	Df	Mean squares 1958	1959
Treatments	5	830**	1,092**
Between nitrogen levels	1	80	67
Among mixtures	2	3,726**	4,743**
Interaction	2	340	648**
Error B	22	103	43

**Exceeds the 1% level of significance.

Table 49. Treatment totals of the nitrogen yield of the grass component of mixtures at two levels of nitrogen, Albia

Grass association	Levels of nitrogen 0	60
	1958	
Grass-alfalfa	232.6	458.8
Grass-Ladino	329.2	465.5
Grass-trefoil	276.1	444.7
Total	837.9	1,369.0
	1959	
Grass-alfalfa	276.6	366.6
Grass-Ladino	245.4	339.0
Grass-trefoil	256.2	402.0
Total	778.2	1,107.6

significant. The error term was derived from Table 59 of the Appendix.

As was noted in the Ames experiment, the nitrogen yield of the grass component was increased in each case when 60 pounds of nitrogen was applied to the mixture (Figures 17 and 18). The grass and legume components were analyzed separately for nitrogen content. The treatment totals for the grass component nitrogen yields are presented in Table 49. In both 1958 and 1959 there was a considerable increase in the nitrogen yield when nitrogen was applied to the mixtures. The analysis of variance, Table 50, revealed that the variance due to nitrogen levels was significant. It also can be noted that in

Table 50. Analysis of variance of the grass component nitrogen yield from grass-legume mixtures at Albia

Source of variation	Df	Mean squares 1958	1959
Replications	1	11	350
Species	1	149	922
Error A	1	27	55
Treatments	5	649**	257**
Between nitrogen levels	1	2,939**	1,131**
Among grasses in association	2	89*	48
Interaction	2	65	30
Treatment X species	5	60*	242**
Error B	10	16	39
Harvests	3	4,318**	249**
Harvests X species	3	222**	254**
Harvests X treatments	15	423**	161**
Harvests X species X treatments	15	10	44
Error C	36	24	29

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

1958, the variance due to grass-legume associations was significant at the five percent level. There did not appear to be a significant difference between orchardgrass and bromegrass component nitrogen yields.

The treatment totals for the nitrogen yield of the legume component of mixtures is given in Table 51. It can be seen that the nitrogen yield of the legume component was reduced by the addition of nitrogen fertilizer to the mixtures. It

Table 51. Treatment totals of the nitrogen yield of the legume component of mixtures at two levels of nitrogen at Albia

Legume	Levels of nitrogen 0	60
	1958	
Alfalfa	509.0	261.9
Ladino	458.4	316.9
Trefoil	218.7	164.1
Total	1,186.1	742.9
	1959	
Alfalfa	592.5	422.9
Ladino	482.4	425.1
Trefoil	242.2	219.9
Total	1,317.1	1,067.9

also is apparent that the nitrogen yield of birdsfoot trefoil was much lower than that of the alfalfa and Ladino clover components. Analysis of variance of the legume component nitrogen yield, as shown in Table 52, revealed that the variance due to levels of nitrogen was significant in both 1958 and 1959. The variance of nitrogen yield among the legumes also was significant in both years.

Nitrate Accumulation Experiment

Since nitrates have been found to accumulate in forage grasses which have been fertilized with nitrogen, a laboratory study was undertaken to determine if nitrates were accumulating with fertilization rates used in this study. The percent

Table 52. Analysis of variance of the legume component nitrogen yield from grass legume mixtures, Albia

Source of variation	Df	Mean squares 1958	1959
Replications	1	304	932
Species	1	3,064	3,170
Error A	1	319	210
Treatments	5	1,160**	1,278**
Between nitrogen levels	1	2,045**	647*
Among legumes	2	1,586**	2,687**
Interaction	2	290	185
Treatments X species	5	221	284*
Error B	10	126	80
Harvests	3	912**	1,050**
Harvests X species	3	127	337*
Harvests X treatments	15	328**	246*
Harvests X species X treatments	15	48	76
Error C	36	50	101

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

nitrate-nitrogen in orchardgrass and bromegrass fertilized at 0, 60, 120 and 240 pounds of nitrogen are presented in Table 53. It is apparent from these results that nitrates did accumulate in the forage at the high levels of applied nitrogen. There was no appreciable nitrate-nitrogen present at 0, 60, and 120 pounds of nitrogen fertilization rates, but a sharp increase occurred between the 120 and 240 pound rates. It can be noted that in 1959 accumulation of nitrate in orchardgrass occurred at the 120 pound rate of fertilization.

Table 53. Percent nitrate-nitrogen in orchardgrass and bromegrass fertilized with nitrogen

Grass	Nitrogen (lbs./A.)	1958 harvest 1	1958 harvest 2	1959 harvest 1	1959 harvest 2
Orchardgrass	0	.01	.02	.02	.01
	60	.01	.01	.04	.02
	120	.02	.02	.19	.10
	240	.30	.27	.54	.61
Bromegrass	0	.00	.00	.02	.00
	60	.01	.00	.02	.02
	120	.05	.01	.02	.02
	240	.25	.29	.36	.34

Analysis of variance of these data, as shown in Table 54, reveals that treatments were significant at the one percent level in 1958 and at the five percent level in 1959. A further breakdown of the variance due to treatments showed that the 240 pounds of nitrogen fertilizer treatment compared to the other treatments pooled was significant in both 1958 and 1959. Variance among the other treatments was not significant. The nitrate-nitrogen content of the forage from plots receiving 240 pounds of nitrogen fertilizer in the third and fourth harvest was very low, indicating that the nitrogen content of the soil had been reduced sufficiently to prevent accumulation of nitrate in the forage.

Table 54. Analysis of variance of the percent nitrate-nitrogen in orchardgrass and bromegrass fertilized with nitrogen in 1958 and 1959

Source of variation	Df	Mean squares 1958	1959
Replications	1	.0003	.2813
Species	1	.0003	.0685
Error A	1	.0071	.0312
Treatments	3	.1331**	.3661*
240 vs. others	1	.3963**	1.0760**
Among others	2	.0016	.0112
Treatments X species	3	.0006	.0238
Error B	6	.0019	.0752
Harvests	1	.0003	.0010
Harvests X species	1	.0001	.0000
Harvests X treatments	3	.0002	.0017
Harvests X species X treatments	3	.0008	.0027
Error C	8	.0022	.0007

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

DISCUSSION

The findings of this study on dry matter and nitrogen yields of grasses in pure stand fertilized with nitrogen reaffirm the need for nitrogen fertilizer to increase production of grasses. In 1958 and 1959, at both Ames and Albia, the yields of dry matter and nitrogen in the forage were increased with every additional increment of nitrogen fertilizer up to 240 pounds of elemental nitrogen per acre. The available nitrogen in the soil at Ames apparently was at a very low level which would account for the extreme range in yield from the check plot (0 pounds of added nitrogen) to the plots fertilized with 240 pounds of nitrogen. Although the check plots gave very low yields, the potential of this Webster-Nicolet soil was demonstrated to be superior to the Belinda soil at Albia when comparable levels of a limiting factor, nitrogen, was supplied.

In every case the response to the increasing increments of nitrogen fertilizer on the grasses grown alone was found to be predominantly linear within this range of fertilizer. Since this linear relationship was based on the logarithm of the rates, one could plot these dry matter and nitrogen yields using the actual intervals and a typical Mitscherlich response curve would result. However, the 240-pound rate of nitrogen did not appear to be high enough to cause the response to reach a peak. The quadratic component in these regression comparisons, although much smaller than the linear component also was significant and by examining Figures 1, 2, 7, 8, 11, 12, 15 and 16, the curve appears to be concave. This would indicate that the response curve had not reached its peak and that an addition of more than 240 pounds of nitrogen would have resulted in further increases in dry matter and nitrogen yields.

The results for two years at both locations showed that most of the difference in yield of dry matter and nitrogen on the grasses came from the first harvest. In each successive harvest the yield of the differentially fertilized grasses became more uniform until in the fourth harvest there was very little difference in yield between unfertilized plots and those receiving up to 240 pounds per acre. There probably were several factors causing this relationship. Related factors would include: (1) the nature of these cool-season grasses is such that they give their greatest yield in the spring and early summer and (2) the nitrogen available for use was being depleted rapidly early in the summer. Lack of water was not a serious problem during the two years of this experiment, but this also could have contributed in some cases to this relationship.

The dry matter and nitrogen yields of the grasses grown alone was, in general, lower in 1959 than in 1958 at both locations. The lower dry matter and nitrogen yield in the second and third harvest of 1959 accounted for most of this reduction. Even when irrigation water was applied at Albia in 1959, the yield did not reach the same level as in 1958. This possibly could be due to the management of the stands. The forage was harvested to a height of one and one-half inches at approximately five-week intervals. This management was more drastic than hay management and probably reduced the vigor of the plants in the second harvest year. It is possible that phosphorous or potassium may have been limiting in the second year. The stand also may have been weakened by winter injury although there was no evidence of population losses except in red clover plots.

The results of the treatments, involving the split application of 120 pounds of nitrogen fertilizer as compared to 120 pounds in one application, were not conclusive. In 1958 there was a significant increase with the split application increasing the yield 1200 to 1400 pounds per acre over the single application. [Teel (70) obtained similar results in his studies on split application of nitrogen at Purdue in 1957.] However, in 1959, the increase was only 150 to 250 pounds per acre and was not significant. Since the second and third harvests in 1959 were much less than in 1958, at all rates of nitrogen, this may account for the lack of response to the second application of 60 pounds of nitrogen fertilizer in late May of that year.

There did not appear to be a significant difference in the dry matter and nitrogen yields between orchardgrass and bromegrass. However, there was a tendency for the orchardgrass to yield lower in the check plots and at low rates of nitrogen fertilizer and to be higher in the plots receiving high rates of nitrogen fertilizer in the Ames experiment. In the Albia experiment orchardgrass tended to produce more at all levels of fertilizer nitrogen. The ability of orchardgrass to produce superior aftermath growth may account in part for its higher yield when large amounts of nitrogen were available.

The part of this study on the effect of residual nitrogen on the yields the following year gave evidence that there was very little carry-over of nitrogen from the 1958 season to the 1959 season. In every case, with both the Ames and the Albia experiments, the 1959 dry matter and nitrogen yields of plots receiving 240 pounds of nitrogen in 1958 and no nitrogen in 1959 were not significantly greater than the check (0 pounds of added nitrogen). It should be noted that in every case both the dry matter and nitrogen yields of the 240 pound residual nitrogen treatments were greater than the check and less than the yield of plots receiving 30 pounds of nitrogen fertilizer. This would substantiate the results of Willhite et al. (89) who found the significant residual effects were obtained only at rates of 320 pounds of nitrogen per acre or more.

The nitrogen content of the forage is indicative of its quality and feeding value. An estimate of the crude protein content of forage can be derived from the nitrogen percentage. The increase in nitrogen percentage of the fertilized grasses was greatest in the first harvest, and the range in subsequent harvests became less and less until in the fourth harvest there was little difference between plots receiving 0 and 240 pounds of nitrogen. This is in agreement with the results in the residual nitrogen treatment, giving evidence that most of the applied nitrogen was utilized during the first season after application. Between 70 and 80 percent of the 240 pounds of nitrogen applied was recovered in the harvested forage in 1958. Some of the remaining nitrogen was undoubtedly included in the root and unharvested portion of the plant as well as a small amount of undecomposed residue from fallen leaves. Some loss from leaching and volatilization probably occurred.

In only one case, the 1958 Albia results, was the difference between orchardgrass and bromegrass nitrogen percentages significant. In this case the percent nitrogen in bromegrass was significantly higher than in orchardgrass. The general trend followed this pattern, even though it was not significant. It is possible that the stage of maturity when harvested would account in part for this difference. It was evident that the grasses were less mature in the later harvests than in the first since the percent nitrogen of the experiment as a whole was higher with each subsequent harvest.

The dry matter yields of legumes grown alone in the Ames experiment were comparable to the respective unfertilized grass-legume associations in 1958. Since 1959 was the second harvest year, the red clover yield was much reduced. However, the alfalfa alone in 1959 yielded nearly as much as it did in association with grass. The nitrogen yields of alfalfa alone was higher than the yield from the unfertilized alfalfa-grass associations. In general, the fertilized grass-legume associations yielded more dry matter and nitrogen than the legumes grown alone without added nitrogen. The competitive relationship here is very complex since there are many factors involved.

The performance of each of the grass-legume mixtures at Albia was different. In general, grass-alfalfa mixtures appeared to give the greatest yield of both dry matter and nitrogen. The grass-Ladino clover yields in 1958 were comparable to the alfalfa associations, but in 1959 they were considerably lower. Birdsfoot trefoil was the lowest in yield both years. A study by McCloud and Mott (42) showed that the performance of different mixtures varied from mutually depressive to mutually beneficial, thus indicating the multiplicity of reactions involved in these associations.

The ability of a species to compete successfully in its environment depends on its morphological characteristics, the type of management, its adaptation, the immediate climatic conditions, the characteristics of the soil, the existence of a disease or insect infestation and on other factors. Since there are so many factors involved it is difficult to definitely state that any one alone is the cause of a particular phenomenon. It can be noted, however, that the morphological characteristics of the species utilized in this study differed greatly and this factor certainly was instrumental in the differences that resulted. Since the same management was applied to all species one may mistakenly assume that this variable was held constant. However, the interaction between the morphological characteristics and the type of management must be considered. The plots were harvested at a height of one and one-half inches. The interval between harvests was about five weeks. This type of management was more favorable to some associations than to others. The characteristics of the soil and climatic conditions were relatively uniform within each of the experiments.

When the grass-legume mixtures were fertilized with 60 pounds of nitrogen the yield of the association as a whole was significantly increased. Here the competitive relationship was changed because of a change in the soil factor. Several investigations have reported increased yields when associations were fertilized with nitrogen (9, 17, 19, 49, 51).

In this study, the magnitude of each component of the association was determined and the relative contribution of each component calculated. It was found that the addition of nitrogen fertilizer significantly increased dry matter and nitrogen yield of the grass component in every case. It was interesting to note that there was no significant difference in the dry matter and nitrogen yields of the grass component of alfalfa and red clover associations in either 1958 or 1959 at Ames. The same relationship was noted at Albia, in general, with one exception. The grass component yield of the birdsfoot trefoil association was significantly higher than the others in 1959.

When the legume component of these mixtures was analyzed, the results, in general, showed that when nitrogen was applied to the mixtures the dry matter and nitrogen yields were reduced. There was a significant reduction in both years with the Albia experiment and although the difference was not significant in the Ames experiment, the trend appeared to be similar. The lower total yield of the grass-birdsfoot trefoil association appeared to be the result of the lower yield of the birdsfoot trefoil component. The grass component of this mixture did not yield lower and in some cases yielded higher than when it was grown in association with the other legumes.

A severe water deficit did not occur at any time during this experiment at either Ames or Albia. The irrigation factor in the Albia experiment did not attain the importance in this study that was originally planned. No irrigation water was applied in 1958 and only five inches in 1959. The 1959 applications were not needed until after the second harvest had been removed. Although the analysis showed a significant increase in dry matter yield in the irrigated plots, it is possible that this was due to some source other than the variance between the irrigated and unirrigated treatments. By examining 1959 data, it was noted that the yield of the plots designated for irrigation yielded more dry matter in the first and second harvest than the unirrigated plots even though no irrigation water had yet been applied.

The results of these two years emphasize the findings of Thornthwaite and Mather (72) who found that in five of 25 years at Charles City, Iowa, rainfall was adequate and distributed well enough that no water deficiency occurred and in half of the 25 years less than three inches of water was needed in the form of supplemental irrigation water.

In considering the results of the Ames and Albia experiment as a whole it was noted that the dry matter and nitrogen yield of the grasses grown in pure stand did not compare favorably with the yield of grass-legume mixtures. In general, it required between 120 and 240 pounds of fertilizer nitrogen on the grasses to equal the yields of the grass-legume mixtures. From the results of the Ames study in 1959, it might be concluded that it would take more than 240 pounds of fertilizer nitrogen on pure stands of grass to equal the yields of the grass-alfalfa mixtures. However, from the 1958 Ames results and from the 1958 and 1959 results at Albia, one could estimate that under the conditions of these experiments it would take approximately 150-200 pounds of fertilizer nitrogen on these grasses to equal the dry matter and nitrogen yield of the unfertilized grass-alfalfa mixtures. The grass-Ladino clover mixtures at Albia reacted similarly to alfalfa with the exception of the bromegrass-Ladino clover association in 1959 which yielded considerably less. Birdsfoot trefoil did not, in general, appear to be as efficient at supplying nitrogen to the grass grown in association as did alfalfa and Ladino clover.

The grasses that were fertilized with 240 pounds of nitrogen in general contained about the same percent nitrogen in the first harvest as the legumes. This demonstrated that the grasses had the capacity to product dry matter with a protein content comparable with that of grass-legume mixtures when adequate nitrogen was supplied. When the legumes were grown alone their dry matter and nitrogen yields, in general, were comparable to the yields of the grasses fertilized with 240 pounds of nitrogen.

The accumulation of nitrate-nitrogen in forage and its toxic effect on livestock has been found to be a problem in some locations. Since nitrates tend to accumulate in some cases when forages are grown on soils high in nitrogen, this part of the study was considered applicable. The results from the analysis for nitrate-nitrogen in these forage samples indicated that there were only small amounts of nitrate present in the unfertilized grasses and that little accumulation occurred when nitrogen fertilizer was applied up to the rate of 120 pounds per acre. With orchardgrass in 1959 there did appear to be some accumulation at the 120 pound rate of nitrogen fertilizer. However, the forage from plots receiving 240 pounds of nitrogen from fertilizer had accumulated nitrate-nitrogen in sufficient quantities in both the first and second harvest to be considered lethal levels according to some investigations (8 and 37).

There appears to be some controversy as to what concentration of nitrate-nitrogen is toxic and lethal to livestock. It apparently does not depend directly on the percent nitrate nitrogen in the forage but on the total amount of nitrate-nitrogen consumed by the animal in a given length of time in relation to the body weight of the animal. Any factor affecting the amount of forage consumed also would affect the percent nitrate-nitrogen that would be considered toxic or lethal. This probably accounts for the variability of reports from the various investigators.

SUMMARY AND CONCLUSIONS

Investigations having similar basic objectives were conducted at two locations in Iowa during the period 1957-1959. The major objective of these experiments was to determine the effectiveness of inorganic nitrogen as a replacement for legumes grown in association with forage grasses. To accomplish this objective experiments were established at Ames and Albia, Iowa, to measure (1) dry matter and nitrogen yields of orchardgrass and bromegrass grown alone with rates of nitrogen ranging from 0 to 240 pounds per acre, (2) dry matter and nitrogen yields of orchardgrass and bromegrass in association with legumes without and with 60 pounds of nitrogen fertilizer, (3) the seasonal distribution of dry matter production, (4) the botanical composition of the forage mixtures and (5) the carry-over of nitrogen in the soil from one year to the next. An additional experiment was initiated to determine the nitrate content of the grasses.

The results of the investigation are summarized as follows:

1. Nitrogen fertilization increased the dry matter and nitrogen (protein) yield of pure grass stands. The magnitude of this increase depended not merely upon the amount of nitrogen applied, but upon the inherent ability of these grasses to use nitrogen fertilizer efficiently.

2. There was not sufficient evidence in this study to conclude that either orchardgrass or bromegrass was superior in performance, but orchardgrass tended to produce more than bromegrass in most cases.

3. The regression comparisons of both the dry matter and nitrogen yields showed the response to nitrogen fertilizer on the pure grass stands had not reached a maximum at 240 pounds per acre. An additional increment of nitrogen fertilizer probably would have resulted in a further increase in yield.

4. Both dry matter and nitrogen yields appeared to be lower in 1959 than in 1958. The reduced yield of the second and third harvest in 1959 accounted for most of this difference.

5. When a split application of 60 pounds in early spring plus 60 pounds after the first harvest was compared to a single application of 120 pounds of nitrogen applied in early spring, the results were too variable to make any definite conclusion. There appeared to be a good dry matter yield response to the split application in 1958 and little response in 1959. In neither year was the increase in nitrogen yield of the forage sufficient to be significant.

6. The part of this study devoted to investigating the effect of residual nitrogen on the dry matter and nitrogen yield of the grasses gave evidence that there was little

carry-over of the 240 pounds of nitrogen applied in 1958 to the 1959 season. Growing conditions were favorable for using nearly all the nitrogen in increased growth in 1958.

7. The percent nitrogen in the forage was increased by nitrogen fertilization. The increase was greatest in the first harvest and the range in subsequent harvests became less and less until in the fourth harvest there was little difference between plots receiving 0 and 240 pounds of nitrogen per acre.

8. The competitive ability of the legumes used in this study showed that each grass-legume association reacted somewhat differently. The complexity of the factors involved in competition make it difficult to pinpoint any one factor as causing the associations to react as they did.

9. The application of nitrogen fertilizer to grass-legume mixtures resulted in increased yield of dry matter and nitrogen. However, this was usually due to increased growth of the grass component. The increased growth of grass resulted in a corresponding decrease in legume growth.

10. The management of grass-legume mixtures to maintain a desirable balance of components is difficult. The manager must attempt to balance the factors favoring growth of grass with those favoring the legume. Allowing the balance to shift in either direction may result in loss or serious reduction in one or another of the components.

11. There was very little difference, in general, between the yields of the grasses receiving 240 pounds of nitrogen and the unfertilized grass-legume mixtures. It appears that under the conditions of this experiment it would take about 200 pounds of nitrogen fertilizer on pure grass stands to produce as much dry matter and nitrogen as can be produced by an unfertilized grass-alfalfa mixture. Ladino clover compared favorably with alfalfa the first harvest year, but its production declined in 1959. Birdsfoot trefoil was less productive on this land and it did not appear to benefit the grasses grown in association as much as alfalfa and Ladino clover.

12. A severe water deficit did not occur at either location during this experiment. Therefore, the irrigation factor in the Albia experiment did not attain the importance that was originally expected. The fact that little supplemental water was needed during this two-year period does show that treatment responses were probably the result of factors other than soil moisture.

13. Small amounts of nitrate-nitrogen have been found in most plants. Large amounts of nitrate-nitrogen have been found to accumulate in these forage grasses when they have been heavily fertilized with nitrogen. Significant accumula-

tion began to occur in this experiment somewhere between the rates of 120 and 240 pounds of nitrogen per acre. The amount of accumulation at 240 pounds per acre possibly was sufficient to be considered lethal to animals consuming this forage in large quantities.

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APPENDIX

Table 55. Analysis of variance of dry matter yields, Ames

Source of variation	Df	Mean squares 1958	1959
Replications	3	346,868	368,638
Species	1	200,808	94,186
Error A	3	252,211	155,709
Treatments	10	12,057,590**	8,811,264**
Treatments X species	10	445,209**	22,259
Error B	60	88,153	51,982
Harvests	3	62,123,245**	22,695,662**
Harvests X species	3	991,366**	382,262**
Harvests X treatments	30	1,767,634**	1,000,809**
Harvests X species X treatments	30	123,015**	96,710**
Error C	198	59,174	26,866

**Exceeds the 1% level of significance.

Table 56. Analysis of variance of the nitrogen yields, Ames.

Source of variation	Df	Mean squares 1958	1959
Replications	1	80	221
Species	1	547	8
Error A	1	223	9
Treatments	10	6,317**	52,447**
Treatments X species	10	294*	171
Error B	20	108	1,358
Harvests	3	18,855**	25,639**
Harvests X species	3	144*	110
Harvests X treatments	30	1,025**	14,298**
Harvests X species X treatments	30	43	1,088
Error C	66	40	1,986

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

Table 57. Analysis of variance of the dry matter yields at Albia, 1958

Source of variation	Df	Mean square
Replications	5	192,944
Species	1	918,426**
Error A	5	34,667
Treatments	11	5,216,285**
Treatments X species	11	226,892**
Error B	110	79,941
Harvests	3	80,549,409**
Harvests X species	3	663,402**
Harvests X treatments	33	3,052,488**
Harvests X species X treatments	33	54,400
Error C	360	52,866

**Exceeds the 1% level of significance.

Table 58. Analysis of variance of the dry matter yields at Albia, 1959

Source of variation	Df	Mean square
Replications	2	104,646
Irrigations	1	2,955,419*
Error A	2	40,327
Species	1	65,540
Species X irrigation	1	100,410
Error B	4	29,275
Treatments	11	6,712,225**
Treatments X irrigation	11	92,271
Treatments X species	11	623,399**
Treatments X irrigation X species	11	67,554
Error C	88	53,791

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.

Table 58. (Continued)

Source of variation	Df	Mean square
Harvests	3	15,737,573**
Harvests X irrigation	3	133,883*
Harvests X species	3	1,406,810**
Harvests X treatments	33	1,763,153**
Harvests X irrigation X species	3	39,888
Harvests X irrigation X treatments	33	29,336
Harvests X species X treatments	33	319,264**
Harvests X irrigation X species X treatments	33	28,254
Error D	288	38,711

Table 59. Analysis of variance of the nitrogen yields, Albia

Source of variation	Df	Mean squares	
		1958	1959
Replications	1	157	53
Species	1	1,091	835*
Error A	1	10	1
Treatments	11	2,850**	3,076**
Treatments X species	11	137	232**
Error B	22	103	43
Harvests	3	13,718**	2,270**
Harvests X species	3	331**	244**
Harvests X treatments	33	1,287**	687**
Harvests X species X treatments	33	47	143**
Error C	72	44	53

*Exceeds the 5% level of significance.

**Exceeds the 1% level of significance.