A COMPARISON OF THE AMOUNT OF NITROGEN FIXED BY COMMON, RHIZOMATOUS, AND CREEPING ALFALFAS

by

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INTRODUCTION

In the United States there are approximately 74 million acres of hay, 48 million acres of crop land, and 61 million acres of plowable pasture land. Plowable pastures comprise about 25 percent of all the farm land in the United States. From pastures dairy cattle obtain approximately 38 percent of their feed requirements, beef cattle 60 percent, sheep 80 percent, and horses 32 percent. From the above it can be seen that the pasture is an important source of income to the American farmer. Nevertheless, many pastures in use today are in poor condition because of infestations of weeds and brush. As a result the return per acre per year is low compared to what these pastures could produce.

Alfalfa (Medicago sativa L.) and smooth brome (Bromus inermis L.) are adapted to Kansas conditions and in combination provide a highly palatable, nutritious forage for animals. When smooth brome is grown alone it soon becomes weakened from lack of nitrogen and the stands become thin, allowing the encroachment of weeds. Alfalfa, when grown alone for pasture, if poorly managed, supplies a constant source of danger to ruminants from bloat. However, when alfalfa and smooth brome are grown together the life of the grass is extended and the danger of bloat reduced. This mixture usually will maintain a high level of production for approximately 3 years. In some areas of the United States, broadleaf birdsfoot trefoil (Lotus corniculatus L.) and orchardgrass (Dactylis glomerata L.) are grown in combination and may persist 20 years. This reduces the per acre per year cost of the pasture to $3.92 as compared to \$7.09 for alfalfa-smooth brome combinations that have to be reseeded every three or four years, (Heady and Scholl, 11).

Alfalfa is a perennial legume that may live 15 to 20 years or even longer in dry climates. However, under grazing and in competition with grass, common alfalfa often fails to be productive after three or four years. The spreading alfalfas with their prostrate growth habit and spreading characteristics, may be able to compete with grasses under grazing.

The two kinds of spreading alfalfas are the creeping and rhizomatous types. The creeping alfalfas spread by aerial shoots from lateral roots several inches below the surface of the ground. The rhizomatous type of spreading occurs from the initiation of roots at the point of emergence of the unusually long crown buds. In either case the new plant is capable of survival independent of the mother plant.

It has been found that when nitrogen is applied to grass pastures the protein content of the forage is increased for a short time only. When legumes are grown with grasses a constant source of nitrogen is supplied to the grass by the sloughing of nodules, the decay of the roots of the legume, and from the grazing animals. This tends to maintain a higher level of protein in the grass plant during the entire grazing period. It is estimated that immature smooth brome in a good pasture contains as high a percentage of digestible protein as alfalfa on a fresh weight basis.

Therefore, the legume in itself probably is not necessary in the animal diet on a grass-legume pasture, but is necessary to supply the grass with nitrogen. A prostrate type alfalfa, less subject to close removal by grazing, possessing high competitive characteristics, and capable of supplying a large amount of nitrogen would be of great value in improving Kansas tame pasture mixtures. The purpose of this study is to compare the relative ability of common, rhizomatous, and creeping alfalfas to fix nitrogen.

REVIEW OF LITERATURE

According to Wilson (39), La Flize in 1889 probably was the first to observe that a grass, barley, when grown in association with a legume, peas and vetches, on a soil deficient in nitrogen, grew vigorously without developing nitrogen deficiencies. Later in 1911, Lyon and Bizzell (16) conducted field experiments which showed that the percentage of protein was greater in timothy (Phleum pratense L.) grown in association with alfalfa than when the timothy was grown alone. These early observations and studies opened the field to extensive investigations on the nitrogen fixation of legumes and a modification of farming practices.

For many years it was generally assumed that nodule formation was a property common to all leguminous species. Allen and Baldwin (3) found that the actual occurrence of nodules on leguminous species is not nearly so common as is often implied. Approximately 10 to 12 percent of the species of the Leguminosae have been examined for nodule formation and of these only about 88 percent are known to bear nodules. Allen and Allen (2) presented three reasons why rhizobia attack only legumes: (1) the compatibility of the bacterial and plant protein, (2) the production by the leguminous plant of an enzyme which enables it to select, entrap, and use the particular form of organic nitrogen contained in its symbiont, and (3) a high level of calcium in legumes.

Allen and Allen (2) described the nitrogen fixation organisms as being aerobic, heterotrophic, gram negative rods with two distinct properties: (1) the ability to invade the roots of leguminous plants and stimulate the production of nodules, and (2) the ability to enter into a symbiotic relationship with the proper host plant as evidenced by the fixation of atmospheric nitrogen. Starkey (30) found that there were 12 times as many bacteria on the roots of non-leguminous species and 50 times as many on the roots of legumes as in adjacent soil. West (36) suggested that the selective action of the plant may depend upon the excretion of specific growth factors. It was found that bacteria existing farther from flax and tobacco roots in the soil required less thiamin and biotin than those isolated from the rhizosphere of the plants. These growth stimulants were liberated by the roots of flax and tobacco. This was referred to as the reaction of the bacteria to the plant. The reaction of the plant to the bacteria was explained by Wilson (39) as the curling of the root hairs around the bacteria. He also found that extractions from the organisms caused the root hairs to curl. The actual manner in which the organism enters the host plant is not known.

According to Wipf (42) the basic chromosome number in the genus _Medicago_ is 8(2n=16). Cells of the nodules from _Medicago_ species (2n=16) contain 32(4n) chromosomes. _Medicago_ _sativa_ L. is a natural tetraploid. Its normal root tip cells have 32(4n) chromosomes, while in the infected cells 64(8n) chromosomes are found.

Wipf and Cooper (43) found cells with double the 2n number of chromosomes in root hairs of peas and vetch. Cytological evidence suggests that the invading bacteria enter only these natural disomatic cells. This would explain why bacteria are found in cells having the double chromosome number. Upon entering the root hair bacteria, according to Allen and Allen (2), produce nodules which in widely different plant species have a histological pattern remarkably similar. Four areas are conspicuous in longitudinal sections of all nodules. The exterior consists of a spongy layer of loosely packed cortical parenchyma cells generally devoid of prominent contents. Within this nodule cortex is contained a peripheral vascular system which unites with the primary xylem groups of the root stele. Innermost is the pacteroid area consisting of plant cells packed with rhizobia. A meristematic zone of compact, small, actively dividing, non-invaded cells is found between the distal ends of the vascular branches and the outer boundary of the bacteria filled region.

Nutman (23) stated that the infection appears to be restricted to those root zones where there is meristematic activity. The rate of infection is, however, controlled in part by the presence or absence of effective or ineffective nodules already existing on the host plant. The first formed ineffective nodules do not exhibit any inhibitory activity and thus allow the initial rate of infection to continue. Effective nodules, on the other hand, which are persistent, tend to inhibit further infection. Nodule formation is also inhibited by the presence of some other legumes or non-legumes when grown in association, Nutman (24). Nodule formation on clover was depressed significantly by two lettuce plants per culture, the inhibition here being the same as that produced by a single clover plant. He concluded that inhibition in paired cultures is caused by some modification of the root medium not connected with the normal nutrition of the plant. However, the possibility of mutual shading of the very young seedlings being responsible for inhibition was not excluded.

Nutman (24) found that clover plants differ among themselves in relative susceptibility and earliness of infection. These differences are determined by hereditary factors in the plants which also effect the number of lateral roots formed. Clover seedlings are not normally susceptible to infection until about the third week of growth. Some plants, however, formed their first nodules as early as 10 days and others as late as five or six weeks. He found that the differences in nodulation time are hereditarily determined and that early and late nodulating lines may be produced by simple selection. The early nodulating plants, which produced more nodules and roots, grew to a greater size. However, this may be a result of the longer time available for nitrogen fixation. Also clover plants exhibited great variability in relative susceptibility with regard to active number of nodules formed. Nutman (21) postulated that the number of nodules produced seemed to be a function of heritable host factors as well as that of the rhizobial strain. Progeny of sparsely or of abundantly nodulated pure lines of red clover (Trifolium pratense L.) bred true in this respect, whereas crossings produced intermediate numbers of nodules. In addition clover may be completely resistant to infection to its own strain of bacteria. According to Nutman (21) resistance to nodulation among genetically selected red clover plants was attributed to a recessive gene (r), which acts in conjunction with a maternally transmitted cytoplasmic component (p). These resistant red clover plants were described as generally less vigorous, more chlorotic, and hairier than those susceptible to nodulation. In the presence of rhizobia the root hairs of these resistant mutants were curled but were not penetrated. By grafting susceptible red clover root scions on resistant stock and vice versa, Nutman (22) showed that plant factors which may be responsible for resistance or susceptibility were not translocated across the graft union. Nodules were produced only on the roots of the susceptible stock or on adventitious roots of the susceptible scions. Wilson (38) postulated that a relationship exists between the pollinating character of a leguminous plant and the number of rhizobial strains with which it could symbiose. His hypothesis embodied two principles: (a) self-pollinating leguminous plants tend to produce pure lines in which the inherent character permitting symbiosis is absent or carried as a recessive, whereas (b) cross-pollinating species have either maintained, or developed, in a dominant state those characters which make possible promiscuity with diverse rhizobia.

A comparison was made of the carotene and nitrogen content of the forage from 11 selected alfalfa clonal lines as well as from the variety Grimm by Staker and Crandall (29). Significant differences were found in nitrogen content of the selected lines. Agronomic characteristics, with the exception of wilt resistance, were not related to either nitrogen or carotene content. The clones from the variegated variety which were low in wilt resistance were high in nitrogen and carotene content as compared to clones with Turkestan in their pedigrees which were high in wilt resistance. It was suggested that a breeding program could be designed in alfalfa to increase the nitrogen and carotene content. Significant correlations were found between the nitrogen and carotene content.

Lyon and Bizzell (16) suggested that organic matter either excreted or sloughed off by the roots of the legume promoted nitrification in the soil which aided the non-legume. Lipman (15) of the New Jersey Station found that not only was the percentage nitrogen of the non-legume increased, but the total yield of dry matter and nitrogen per acre was greater than when either of the crops was grown alone. Lipman went on to say that the legume excreted nitrogenous compounds that were utilized by the non-legumes. However, the increase in yield of nitrogen per acre was not explained. Wilson (39) explained this by assuming that the non-legume utilized more than its share of the soil nitrogen thereby making the conditions more favorable for nitrogen fixation by the legume. Lipman's (15) theory that nitrogenous compounds were excreted by legumes brought on further controversies concerning the manner in which the legume benefited the non-legume grown in association. Many investigators felt that it was the sloughing of nodules and the decay of roots. Wilson and Burton (40) made numerous tests by using various species of non-legumes and varieties of peas. The conditions such as type of sand in which they were grown, amount of available moisture, time of seeding, time of harvest, ratio of plants, and aeration were varied, but in these tests the legumes were not found to excrete nitrogenous compounds. Virtanen et al. (32) of the Biochemical Institute of Helsinki, Finland, conducted numerous experiments and found as much as 50 percent of the total nitrogen produced was excreted. The average range was usually from 10 to 20 percent. The legumes excreted these nitrogenous compounds both in association with non-legumes and when grown alone. It was found that the excretion began early before the sloughing of the nodules or roots. Wilson and Burton (40) in further tests at lower temperatures found that in a few cases the legume did excrete nitrogenous compounds, but this was the exception rather than the rule. Under these conditions it was observed that legumes grew at a slower rate than in previous experiments. Wilson and Wyss (41) conducted an experiment in open sunlight. Under these conditions, which favored photosynthesis, little nitrogen was fixed by the legume and no nitrogenous compounds were excreted. When the same experiment was conducted under shaded conditions nitrogen fixation and growth were increased, but carbohydrate synthesis was reduced. Under these conditions it was found that the legumes did excrete nitrogenous compounds. Wilson (39) from this and other experiments concluded that excretion was dependent on low temperatures and low light intensities which in turn determined the amount of carbohydrates produced. With high levels of carbohydrates the nitrogen was tied up and was not excreted.

The first formed product of fixation by symbiotic bacteria is unknown, however, Wilson (39) has suggested that regardless of what the first formed product is, soon after it enters the metabolic stream of the plant it is transformed into aspartic acid and possibly to asparagine. The latter compound may act as a storehouse for nitrogen and as a means of transfer. The carbon skeleton for the aspartic acid is believed to originate from respiratory reactions. The plant protein is formed through the combination of preformed amino acids, the primary source of aspartic acid. Through deamination or by direct transfer of the amino group to a keto-acid, aspartic acid furnishes nitrogen to the plant's metabolic activities.

For some time the controversy as to whether or not nitrogen should be applied to a stand of legumes existed. To study the effect of inhibition to nodulation by nitrates, Wilson (37) adopted the divided-root method. He divided the roots of a plant and placed them in different concentrations of nitrates. By using this method he was able to observe that the depression of nodule formation by nitrates was local in character, since each half of the root system reacted independently of the other. On soil depleted of nitrogen, Giobel (10) found that gains in soil nitrogen resulted from the growth of six crops of inoculated alfalfa, when the tops were removed. On the other hand, on soils well provided with this element in available form, losses in soil nitrogen occurred. He also observed no evidence of any increased resistance of soybean (Glycine Max L.) plants to the entrance of the nodule forming bacteria into the roots from increased nitrogen fertilization of the plants. However, Thornton (31) observed a depression of nodulation on soybean when nitrogen was applied at planting time. When the plants were harvested five weeks after emergence it was found that 50 percent of the total nitrogen in the plant was from fixation even though a near ample supply of combined nitrogen was present. He also found that red clover and sweetclover (Melilotus L.) yields and nitrogen content were increased by nitrogen fertilizers. Red clover plants receiving 66 p.p.m. of nitrogen at planting time or ammonium sulfate with N^{15} enrichment and harvested six weeks after emergence, fixed more nitrogen than plants receiving no nitrogen. Demolon and Dunez (8) concluded that host plants benefited more from symbiosis than from nitrogenous fertilization. In pot trials inoculated alfalfa plants supplied with potassium nitrate or ammonium sulfate (10 to 20 percent of the total requirements) grew better at first than plants entirely dependent on fixation, but the latter produced a superior final yield and nitrogen content.

The physical advantages of planting a legume with a grass over that of fertilizing has been demonstrated by many investigators. Total dry matter and protein production were found by Wagner (33) to be more uniformly distributed throughout the season by mixed seeding than by nitrogen-fertilized grasses. Mixed seedings also contained less weeds than the pure seedings. The mixtures produced nearly one-half of their annual yield of protein after the July harvest, whereas production from nitrogen-fertilized orchardgrass and tall fescue after the July cutting was only about one-fourth of the annual yield, (Wagner, 35). It was concluded by Wagner (34) that the nitrogen produced by legumes had the advantage of being available over a longer period of time. However, Johnstone-Wallace (13) observed that the maximum soil temperature between July 3 and October 12 at a depth of one inch, averaged eight degrees lower (fahrenheit) in a plot of wild white clover (Trifolium repens L.) and grass than in plots of grass alone. This he concluded would tend to permit active growth of the grass over a longer period when in a mixture. When ladino clover (Trifolium repens L.) was seeded with either Kentucky bluegrass (Poa pratensis L.) or Rhode Island bentgrass (Agrostis tenuis Sibth.), closely mowed eight times per season for seven years, Brown and Munsell (6) obtained slightly larger total and better distributed yields than when nitrogen was applied in each of the months of April, June, and August to the grasses alone. McCloud and Mott (19) observed an increase in yield of smooth brome in mixtures over that when grown alone irrespective of whether the associate was a legume or another grass. In several associations the beneficial effect was produced by a non-legume, indicating that nitrogen fixed by the legume was not the only factor involved. In a greenhouse study, Aberg et al. (1) found that grasses when grown in association with alfalfa or sweetclover had a significantly higher yield of roots, indicating that the fibrous-rooted grasses utilized the soil area not occupied by these tap-rooted legumes. Roberts and Olson (27) from several grass-legume mixtures got greater yields of dry weight and nitrogen from mixtures on a unit area of soil than the total yield of pure stands of grass and a legume each on one-half the area of the mixtures. The largest gains from association occurred when a legume of vigorous growth habits was grown with a grass with weak growth habits. They concluded that gains in dry weight and total nitrogen due to the associated growth of a grass and legume resulted from spreading the plants with vigorous growth habits over a greater soil area.

Along with the physical advantages of having a grass-legume mixture described earlier, there are chemical advantages such as increased protein, carotene, and mineral content in the grass. Orchardgrass when grown in combination with wild white clover contained 5.8 percent more protein than when grown alone (Johnstone-Wallace, 14). When ladino clover was grown in association with orchardgrass, it was found by Bressani and Johnson (5) that the carotene content of the legume was not changed, but the carotene content of the orchardgrass was considerably increased. The nitrogen content of orchardgrass in the third cutting was increased 24 percent by association with ladino clover. Hodgson (12) obtained a lower total yield of protein from ladino clover when grown in association with grasses, however, a very marked increase in the yield of the grasses resulted in a higher total protein yield per acre from the grass-clover mixtures. Fergus (9) found that clippings from bluegrass-white clover plots contained 19.75 percent protein while the legume-free bluegrass plots contained only 14.95 percent protein, or an increase of 32 percent in favor of the herbage containing white clover. It was also noted that the mineral content of the grass was increased.

The amount of nitrogen fixed by various legumes under actual growing conditions is difficult to determine and at best is only an estimate. Cheney and Anderson (7) reported the addition to the soil of 80 to 100 pounds of nitrogen per acre by biennial sweetclover when plowed under by the end of the year it is seeded. Alfalfa produced about 60 to 80 percent as much while red clover, Hubam sweetclover (Melilotus alba var. annua L.) and alsike clover (Trifolium hybridum L.) produced 50 to 70 percent as much in one year's time. It was also reported that the number of pounds of nitrogen per ton of dry hay produced was: alfalfa, 50 to 60; red clover, 45 to 55; alsike clover, 60 to 70; and soybeans, 45 to 55. Recent work by Wagner (35) showed that ladino clover in mixtures with orchardgrass or tall fescue (Festuca arundinacea L.) fixed on the average more than 150 pounds of utilizable nitrogen per acre each year, or, for example, the equivalent of 750 pounds of ammonium sulfate per acre. With 160 pounds of nitrogen per acre added to the grasses, the mixed seedings still produced more total protein than did the grasses in pure stands.

The transfer of nitrogenous products from the legume to the grass is by the decay of legume roots and the excretion by the nodules as discussed earlier. However, the animal in grazed pastures serves as a means of conveying the nitrogen to the grass. Melville (20) from investigations on the consumption and return of both milk production and excreta of dairy animals presented the following information. An animal consuming about 6000 pounds of herbage in dry weight, containing about 180 pounds of nitrogen, will produce milk containing about 40 pounds of nitrogen. The other 140 pounds is excreted by the animal in dung and urine and in this manner returned to the grass. Approximately 75 percent of the nitrogen is in the urine and is readily available. The other

25 percent appears in the dung. In a grass-legume plot with no return of animal excreta the grass to legume ratio was 45:55, however, when the animal excretions were applied in relation to forage yield, but not under grazing the grass to legume ratio was 65:35. The increased forage yield by returning the animal excretions was 3000 pounds per acre. He believed that the major reason for the shift in percent legumes was the increased nitrogen. These results were substantiated by Sears (38). By using sheep specially harnessed and equipped to collect the excreta he found that the return of urine encouraged the growth of the grass, while the dung initially encouraged the growth of clovers. The end result was a marked increase in the grass-clover ratio. In one trial where both the dung and urine were returned under normal grazing, a good distribution of annual production and a well balanced grass-clover association were obtained. The total production of the plot was lowered and less well distributed when the dung and urine were not returned. However, in another trial on a mixed pasture the returned dung and urine from the animals did not increase the total production. In this case where no excreta were returned the clover increased to such an extent that it increased the yield of the grass. This equalled in yield the production of the plots where the animal excreta was returned to the plots.

The major benefit derived by the grass growing in association with a legume has been proven by many investigators to result from the nitrogen fixed by the symbiotic bacteria. Those legumes that have the ability to produce larger amounts of nitrogen per acre are capable of benefiting the grass more than those producing smaller amounts. The work presented in this paper is an evaluation of the comparative amounts of nitrogen produced by common, rhizomatous, and creeping alfalfas.

MATERIALS AND METHODS

The three types of alfalfa used in the greenhouse study were common, rhizomatous, and creeping alfalfa. Representing the types were Buffalo, the common type; Nomad and Rhizoma, the rhizomatous type; and the lines Sc34919F, ScMa501, Sc3513F, Sc3484F, Sc34916F, ScMa531, Sc34922F, ScMa532, and S.D. 1108 x 1076, the creeping type. The creeping lines, with the exception of S.D. 1108 x 1076, were developed by Dr. D. H. Heinricks of the Forage Plants Division of the Canadian Department of Agriculture, Swift Current, Saskatchewan. The parentage of these plants trace back to Ladak and a Siberian alfalfa. The line S.D. 1108 x 1076 was developed by Dr. M. W. Adams of the South Dakota Agricultural Experiment Station, College Station, South Dakota.

Vermiculite was used to provide a nitrogen free growing medium for these plants. The nutrient solution, devoid of nitrogen, was basically a Crone's solution in which potassium nitrate was replaced by potassium chloride. To counteract the high cation holding capacity of the vermiculite, those elements containing cations in the Crone's solution were doubled. This proved to be a good nutrient solution to be used with vermiculite as abundant growth was obtained during the course of the experiment.

The containers were one gallon metal cans without holes in the bottoms to minimize the loss of roots and possible contamination resulting from the roots obtaining nitrogen from the soil filled greenhouse tables.

To reduce the amount of substances containing nitrogen that would be placed in the containers, the seed was germinated in petri dishes, and twelve germinated seeds were placed in each pot. Following the planting, the plants were inoculated by adding a commercial inoculant to the vermiculite. The complete plants in excess of nine were removed after the danger of damping off had passed.

Eight replications in a randomized block design were used because of the heterozygous nature of the plants and the small differences to be detected. The alfalfas described earlier in this section plus Buffalo fertilized with nitrogen comprised one replication.

When the plants were 2 1/2 months old they began to form buds. Flowering began on all alfalfas within a period of a few days. The plants were removed at the 1/4 bloom stage. The tops were separated from the roots at the crown and placed in separate envelopes. The samples were then dried, weighed, and analyzed for total nitrogen by the Kjeldahl method.

The field study was conducted on a sandy loam bottom land soil of the Ashland Agronomy Farm located near Manhattan, Kansas. The same alfalfa strains used in the greenhouse study, with the exception of S.D. 1108 x 1076 for which no seed was available at the time of planting, were planted in the field plots September 9, 1954.

Four replications in a randomized block design were used.

Each plot consisted of two rows 10 inches apart and 17 feet long. The seed was not inoculated as alfalfa had been grown on the area two years prior to the planting. One month after planting a number of plants from each replication were dug. Good inoculation was noted in all replications.

The following spring, when the plants were in the prebud stage, composite samples were taken consisting of top growth only pulled at random locations within each plot. These samples were then dried and analyzed for total nitrogen.

EXPERIMENTAL RESULTS

Yield of Dry Matter, Greenhouse Study

The yield in grams of dry weight per pot of tops, roots, and total plants are shown in Table 1 as the average of eight replications.

The creepers, ScMa532, Sc34922F, and Sc34916F, shown in Plates I, II, and III, produced a significantly larger amount of top growth than either Buffalo unfertilized or Buffalo fertilized with nitrogen. On the average the creepers yielded more than the rhizomatous or common types as indicated by significant *t*-values at the 5 percent level.

In root yield Sc34919F, ScMa531, Sc34922F, Sc3484F, ScMa532, Sc3513F and Buffalo fertilized with nitrogen yielded significantly less than Buffalo unfertilized. Rhizoma, Nomad and Buffalo unfertilized yielded significantly more than Buffalo with nitrogen fertilizer. As indicated by significant *t*-values at the 1 percent

EXPLANATION OF PLATE I

ScMa532 and Buffalo alfalfa showing their comparative growth at the end of $2\frac{1}{2}$ months.

PLATE I

EXPLANATION OF PLATE II

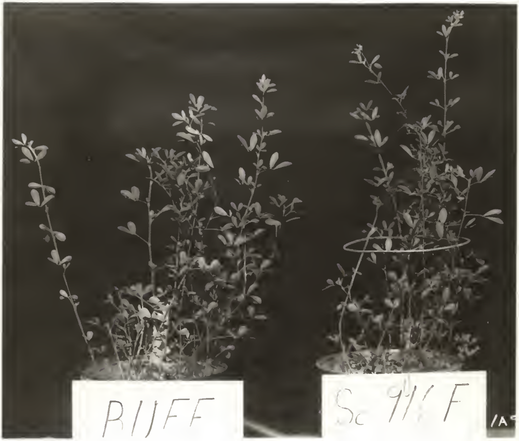
The comparative growth of Sc34922F and Buffalo alfalfa at the end of $2\frac{1}{2}$ months.

PLATE II

EXPLANATION OF PLATE III

Buffalo and Sc34916F alfalfa showing their comparative growth at the end of 2 1/2 months.

PLATE III



level, the common and rhizomatous types on the average yielded more than the creepers.

Those alfalfas that ranked high in yield of top growth ranked low in root growth. As a result, when the individual total plant weights were analyzed statistically there were no significant differences by the analysis of variance or by t-tests when testing for average differences among types.

Percent Nitrogen, Greenhouse Study

The percent nitrogen determined on a dry weight basis in the tops, roots, and total plants is shown in Table 2 as a mean of eight replications.

The creepers, Sc34922F and Sc34919F, contained a significantly lower percent nitrogen in the tops than Buffalo unfertilized, while no alfalfas ranked significantly higher. Rhizoma, Nomad, ScMa532, Sc34916F, Sc3484F, ScMa501, Sc34919F, and Sc34922F ranked significantly lower than Buffalo fertilized with nitrogen. There were no significant differences between the average percent nitrogen in the tops of the creepers and that in the common or rhizomatous types.

In percent nitrogen in the roots, Buffalo fertilized with nitrogen ranked significantly higher than all of the others. Only Buffalo fertilized with nitrogen ranked significantly higher than Buffalo unfertilized, while Nomad ranked significantly lower. On the average the creepers contained a higher percent nitrogen in the roots than the rhizomatous types as indicated by a t-test significant at the 1 percent level. No differences existed between

Table 1. Alfalfa yield in grams of dry matter expressed as a total of nine plants per pot and as a mean of eight replications, grown in the greenhouse.

Top weight		Root weight		Total plant weight	
Alfalfas	grams of dry matter	Alfalfas	grams of dry matter	Alfalfas	grams of dry matter
ScMa532	3.34	Rhizoma	5.90	Rhizoma	8.68
Sc34922F	3.28	Buffalo, unfertilized	5.63	Buffalo, unfertilized	8.33
Sc34916F	3.23	Nomad	5.58	Sc34916F	8.28
ScMa531	3.04	S.D. 1108 x 1076	5.29	Sc34922F	8.21
Sc34919F	2.91	ScMa501	5.13	Nomad	8.08
ScMa501	2.84	Sc34916F	5.05	ScMa532	8.05
Sc3484F	2.80	Sc34919F	4.96	S.D. 1108 x 1076	8.04
Rhizoma	2.78	ScMa531	4.94	ScMa531	7.98
Buffalo, fertilized	2.76	Sc34922F	4.93	ScMa501	7.97
S.D. 1108 x 1076	2.75	Buffalo, fertilized	4.84	Sc34919F	7.86
Buffalo, unfertilized	2.70	Sc3484F	4.73	Buffalo, fertilized	7.60
Sc3513F	2.65	ScMa532	4.72	Sc3484F	7.53
Nomad	2.51	Sc3513F	4.62	Sc3513F	7.27

Top weight: $F = 2.77^{**}$; $L.S.D._{.05} = .42$

Root weight: $F = 2.96^{**}$; $L.S.D._{.05} = .62$

Total plant weight: $F = 1.12$ ns

Interval of non-significance (top weight)
Buffalo, fertilized ± L.S.D.
2.34 - 3.18
Buffalo, unfertilized ± L.S.D.
2.28 - 3.12

Interval of non-significance (root weight)
Buffalo, fertilized ± L.S.D.
4.22 - 5.46
Buffalo, unfertilized ± L.S.D.
5.01 - 6.25

Table 2. Percent nitrogen, on a dry weight basis, in the tops, roots and total plants of alfalfa grown in the greenhouse. The data given is a mean of eight replications.

Percent nitrogen tops		Percent nitrogen roots		Percent nitrogen plant	
Alfalfas	Percent	Alfalfas	Percent	Alfalfas	Percent
Buffalo, fertilized	2.76	Buffalo, fertilized	2.16	Buffalo, fertilized	2.38
S.D. 1108 x 1076	2.68	ScMa531	1.73	ScMa531	2.07
Sc3513F	2.67	S.D. 1108 x 1076	1.73	S.D. 1108 x 1076	2.06
ScMa531	2.61	Sc34922F	1.71	Sc34916F	2.04
Buffalo, unfertilized	2.60	Sc34916F	1.70	Sc3513F	2.00
Rhizoma	2.57	ScMa532	1.67	ScMa532	1.98
ScMa532	2.56	Rhizoma	1.67	Buffalo, unfertilized	1.96
Sc34916F	2.55	Buffalo, unfertilized	1.66	Rhizoma	1.96
Sc3484F	2.48	ScMa501	1.64	Sc34922F	1.93
Nomad	2.45	Sc3513F	1.62	ScMa501	1.93
ScMa501	2.44	Sc3484F	1.60	Sc3484F	1.93
Sc34922F	2.42	Sc34919F	1.60	Sc34919F	1.90
Sc34919F	2.40	Nomad	1.47	Nomad	1.77

Tops	Roots	Plant
F = 3.07**	F = 3.60**	F = 5.49**
$L.S.D._{.05}$ = .17	$L.S.D._{.05}$ = .13	$L.S.D._{.05}$ = .16
Interval of non-significance	Interval of non-significance	Interval of non-significance
Buffalo, fertilized $\pm$ L.S.D.	Buffalo, fertilized $\pm$ L.S.D.	Buffalo, fertilized $\pm$ L.S.D.
2.59 - 2.93	2.03 - 2.29	2.22 - 2.54
Buffalo, unfertilized $\pm$ L.S.D.	Buffalo, unfertilized $\pm$ L.S.D.	Buffalo, unfertilized $\pm$ L.S.D.
2.43 - 2.77	1.53 - 1.79	1.80 - 2.12

the averages of the creeping and common types.

Buffalo fertilized with nitrogen contained a significantly higher percent nitrogen in the total plant than any of the alfalfas tested. Nomad ranked significantly lower than Buffalo unfertilized. The creepers on the average contained a higher percent nitrogen than the rhizomatous type as indicated by a _t_-value significant at the 1 percent level. There was no significant difference between the creepers and the common type.

Total Nitrogen, Greenhouse Study

The yield of total nitrogen in milligrams in the tops, roots, and total plants as a mean of eight replications is shown in Table 3.

In the top growth, only Nomad contained significantly less nitrogen than Buffalo fertilized with nitrogen. There were none that ranked significantly higher than Buffalo fertilized with nitrogen. ScMa532 and Sc34916F contained significantly more nitrogen than Buffalo unfertilized and none ranked significantly lower. The creepers on the average contained more nitrogen in the tops than the common or rhizomatous alfalfas, as indicated by _t_-values significant at the 5 percent level.

All of the creepers and Nomad contained significantly less nitrogen in the roots than Buffalo with nitrogen fertilizer. Sc34919F, ScMa532, Sc3484F, and Sc3513F contained significantly less nitrogen in the roots than Buffalo unfertilized, while none ranked significantly higher. The rhizomatous and common types on the average contained more nitrogen in the roots than the creepers

Table 3. Total nitrogen in the tops, roots, and total plant of alfalfa grown in the greenhouse expressed as a mean of eight replications.

Total nitrogen tops		Total nitrogen roots		Total nitrogen plants	
Alfalfas	Milligrams	Alfalfas	Milligrams	Alfalfas	Milligrams
ScMa532	85.260	Buffalo, fertilized	104.647	Buffalo, fertilized	180.880
Sc34916F	82.892	Rhizoma	99.371	Rhizoma	171.395
Sc34922F	79.122	Buffalo, unfertilized	92.773	Sc34916F	169.733
ScMa531	79.036	S.D. 1108 x 1076	92.053	S.D. 1108 x 1076	166.628
Buffalo, fertilized	76.239	Sc34916F	86.840	ScMa531	164.228
S.D. 1108 x 1076	74.574	ScMa531	85.191	ScMa532	164.127
Rhizoma	72.024	Sc34922F	84.308	Sc34922F	163.431
Sc3513F	70.968	ScMa501	84.218	Buffalo, unfertilized	162.760
Sc34919F	70.177	Nomad	82.349	ScMa501	153.696
Buffalo, unfertilized	69.987	Sc34919F	79.611	Sc34919F	149.788
ScMa501	69.478	ScMa532	78.869	Sc3513F	145.919
Sc3484F	69.405	Sc3484F	75.084	Sc3484F	144.488
Nomad	61.871	Sc3513F	74.950	Nomad	144.220

F = 2.19*	F = 3.80**	F = 1.99*
$L.S.D._{.05}$ = 12.180	$L.S.D._{.05}$ = 12.397	$L.S.D._{.05}$ = 22.242
Interval of non-significance	Interval of non-significance	Interval of non-significance
Buffalo, fertilized ± L.S.D.	Buffalo, fertilized ± L.S.D.	Buffalo, fertilized ± L.S.D.
64.059 - 88.419	92.250 - 117.044	158.644 - 203.120
Buffalo, unfertilized ± L.S.D.	Buffalo, unfertilized ± L.S.D.	Buffalo, unfertilized ± L.S.D.
57.807 - 82.167	80.376 - 105.170	140.518 - 185.002

as shown by t-values significant at the 5 percent level.

Those plants containing significantly less nitrogen in the total plant than Buffalo fertilized with nitrogen were ScMa501, Sc3513F, Sc3484F, and Nomad, while none ranked significantly higher. There was no significant difference between unfertilized Buffalo and the other alfalfas. On the average there were no differences among the creepers, common or rhizomatous types as shown by non-significant t-values.

There was no significant difference in total milligrams of nitrogen in the plant between Buffalo unfertilized and Buffalo fertilized with nitrogen, even though nodulation was almost completely inhibited by the addition of nitrogen into the growing medium as shown in Plate IV.

Distribution of Nitrogen Within the Plant, Greenhouse Study

The percentage of the total nitrogen in the top growth is shown in Table 4 as a mean of eight replications.

All of the creepers except S. D. 1108 x 1076 had a significantly higher percentage of their total nitrogen in the tops than Buffalo fertilized with nitrogen. With the exception of ScMa501 and S. D. 1108 x 1076, all of the creepers ranked significantly higher in this respect than Buffalo unfertilized. A significantly higher percentage of the total nitrogen was contained in the tops of the creepers than of the common or rhizomatous types as indicated by t-values significant at the 1 percent level.

The fact that the creepers had a higher percentage of their

EXPLANATION OF PLATE IV

Buffalo alfalfa roots dug when they were 2 1/2 months old. Both plants were inoculated after planting. Nitrogen was added to the growing medium in which the plant to the right was grown, depressing nodulation.

PLATE IV

Table 4. Data on the distribution of nitrogen within alfalfa plants grown in the greenhouse and presented as the percentage of the total nitrogen appearing in the tops as a mean of eight replications.

Unadjusted percent of total nitrogen appearing in the tops		Adjusted percent of total nitrogen appearing in the tops	
Alfalfas	Percent	Alfalfas	Percent
ScMa532	51.80	Sc3513F	48.53
Sc34916F	49.33	ScMa532	47.66
Sc3513F	48.77	Sc3484F	47.42
Sc34922F	48.54	ScMa531	46.70
ScMa531	48.44	Sc34916F	46.69
Sc3484F	48.26	Nomad	46.51
Sc34919F	46.65	Sc34919F	46.41
ScMa501	45.46	S.D. 1108 x 1076	45.97
S.D. 1108 x 1076	44.71	ScMa501	45.82
Buffalo, unfertilized	42.98	Buffalo, unfertilized	45.74
Nomad	42.85	Sc34922F	45.60
Buffalo, fertilized	42.24	Rhizoma	45.06
Rhizoma	42.00	Buffalo, fertilized	42.30

Unadjusted:

F = 7.44**
$L.S.D._{.05} = 3.21$

Interval of non-significance
Buffalo, fertilized ± L.S.D.
39.03 - 45.45
Buffalo, unfertilized ± L.S.D.
39.77 - 46.19

Adjusted:

F = 2.89**
$L.S.D._{.05} = 1.85$

Interval of non-significance
Buffalo, fertilized ± L.S.D.
40.45 - 44.15
Buffalo, unfertilized ± L.S.D.
43.89 - 47.59

total nitrogen in the tops could be explained by the significantly higher top-root ratio of the creepers compared to the rhizomatous and common types as indicated by significant t-values at the 1 percent level. The top-root ratio and percentage of total nitrogen in the tops had a high positive correlation of 0.82. When the percentage nitrogen appearing in the tops was adjusted to a common top-root ratio by an analysis of covariance there was a tendency to decrease the differences as shown in Table 4. However, a significant F indicated that differences still existed which were not attributable to the top-root ratio. After adjusting to a common top-root ratio, Buffalo fertilized with nitrogen contained a significantly lower percentage of its total nitrogen in the tops than the other alfalfas tested. In this respect only Sc3513F and ScMa532 ranked significantly higher than Buffalo unfertilized. On the average the creepers contained a higher percentage of their total nitrogen in the top growth compared to the common type as indicated by a significant t-test at the 5 percent level. When the average of the creepers was compared to that of the rhizomatous types, significance was approached at the 5 percent level. From the significant F value when testing for differences in percentage of total nitrogen in the top growth after adjusting to a common top-root ratio and the positive correlation between the top-root ratio and percentage of nitrogen in the tops, it can be concluded that the factors controlling the distribution of nitrogen in the plant are dependent upon, (1) a high top-root ratio on a dry weight relationship and (2) a high percentage of nitrogen, on a dry weight basis, in the top growth as compared to a low percentage of nitrogen, on a dry weight basis, in the roots. No correlation existed between the percent nitrogen in the tops, on a dry weight basis, and the percentage of the total nitrogen appearing in the tops, thereby indicating that the second factor was dependent upon a relationship between the tops and roots in percent nitrogen, on a dry weight basis.

Field Data

Under field conditions there were no significant differences among the alfalfas being tested, or among the averages of the types tested in percent nitrogen in the top growth. However, the correlation between the percent nitrogen in the tops in the greenhouse and field study was a positive 0.64. This would show that the greenhouse study gave a good indication of what might be expected of these plants growing under field conditions.

DISCUSSION AND CONCLUSIONS

Any alfalfa variety in a grass-legume pasture mixture, whether it be the common, rhizomatous or creeping type, that produces a large amount of nitrogen which ultimately will be available to the grass, should be capable of producing the largest yields. However, the alfalfa that excels in this respect must also have the ability to withstand grass competition under grazing pressure.

In the greenhouse study, even though there were no differences among the alfalfas in total dry matter produced, it is significant to note that ScMa532, Sc34922F and Sc34916F yielded significantly more top growth than Buffalo even with nitrogen fertilizer. Also the average yield of the creepers was significantly higher than that of the common and rhizomatous types under the stress of the lack of nitrogen in the growing medium.

In percent nitrogen on a dry matter basis in the top growth, Sc34922F and Sc34919F ranked significantly lower than Buffalo unfertilized. However, in milligrams of nitrogen in the top growth there was no difference between them and Buffalo unfertilized. ScMa532 and Sc34916F, even though they did not contain a higher percent nitrogen in the top, contained significantly more milligrams of nitrogen than Buffalo unfertilized.

There were no differences in percent nitrogen in the plant among the alfalfas except that Nomad ranked lower than Buffalo unfertilized. On the average, however, the creepers had a higher percent nitrogen than the rhizomatous type. This was largely due to the low nitrogen percentage in Nomad.

In so far as there were no differences among the alfalfas in total milligrams of nitrogen produced, it is important to note where the nitrogen was located. The creepers on the average contained a higher percentage of their total nitrogen in the top growth. Only two of the creepers, ScMa502 and S.D. 1108 x 1076, did not differ significantly from Buffalo unfertilized. This factor was attributed to the high top-root ratio of the creepers and a higher percent nitrogen, on a dry matter basis, in the tops in in relation to a low percent nitrogen in the roots.

No differences were found among the alfalfas in the field study; however, the percent nitrogen in the top growth, on a dry weight basis, in the field study was highly correlated with the greenhouse findings. This indicates that the greenhouse study gave a good indication of what may be expected under field conditions.

It seems feasible to assume that the transfer of nitrogen from the legume to the grass would be more rapid by the grazing animal than by the excretion of nitrogenous products by nodules and the decay of legume roots. If this is so, it is reasonable to conclude that the creepers with a higher percentage of their total nitrogen in the tops would benefit the grass more than the common or rhizomatous types.

SUMMARY

The study to determine the comparative ability of common, rhizomatous, and creeping alfalfas to fix nitrogen was conducted in the greenhouse and under field conditions.

The greenhouse study included 9 creepers (Sc34919F, ScMa501, Sc3513F, Sc3484F, Sc34916F, ScMa531, Sc34922F, ScMa532, and S.D. 1108 x 1076), 2 rhizomatous types (Rhizoma and Nomad), 1 common type (Buffalo), and Buffalo with nitrogen fertilizer planted in a randomized block design. These plants were grown in nitrogen free vermiculite and fed by a Crone's solution devoid of nitrogen. At the 1/4 bloom stage the plants were removed from the pots. The tops were separated from the roots at the crown and analyzed separately for nitrogen by the Kjeldahl method.

The field study, conducted at the Ashland Agronomy Farm

located near Manhattan, Kansas, included the same alfalfas as the greenhouse study, with the exception of S.D. 1108 x 1076. The planting was made September 9, 1954, in a randomized block design, and the following spring composite samples of the top growth were taken from each plot. These samples were then dried and analyzed for total nitrogen.

Under the stress of a low nitrogen level in the growing medium the creepers on the average produced a larger amount of top growth than the common or rhizomatous types. ScMa532, Sc34922F, and Sc34916F produced more top growth than Buffalo unfertilized or Buffalo fertilized with nitrogen. Also from the standpoint of top growth, ScMa532 and Sc34916F contained significantly more milligrams of nitrogen than Buffalo unfertilized. There were no significant differences between any of the alfalfas and Buffalo unfertilized in total milligrams of nitrogen in the entire plant. However, the creepers on the average contained a significantly higher percentage of their total nitrogen in their top growth. This was attributed to a high top-root ratio and a high percent nitrogen, on a dry weight basis, in the tops compared to a low percent nitrogen in the roots.

No statistically significant differences were detected among the alfalfas in the field study. This was probably a result of an insufficient number of replications. However, the percent nitrogen in the tops, on a dry weight basis, of the field study was highly correlated with the greenhouse study, which indicated high validity in the greenhouse experiment.

The grazing animal provides a means of rapid transfer of the nitrogen in a readily available form from the legume to the grass. The characteristic of having a higher percentage of their total nitrogen in the tops should make the creepers more valuable in a pasture mixture than the common or rhizomatous types.

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A COMPARISON OF THE AMOUNT OF NITROGEN FIXED BY COMMON, RHIZOMATOUS, AND CREEPING ALFALFAS

by

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In recent years considerable attention has been given to tame pasture mixtures. Alfalfa (Medicago sativa L.) and smooth brome (Bromus inermis L.) in combination provide a palatable and productive pasture. However, under grazing pressure the alfalfa usually dies out allowing the grass to become weakened from lack of nitrogen. When this occurs weeds encroach reducing the value of the forage. Broadleaf birdsfoot trefoil (Lotus corniculatus L.) and orchardgrass (Dactylis glomerata L.) when grown in combination may persist twenty years in some areas of the United States. Unfortunately, broadleaf birdsfoot trefoil is not adapted to all areas.

The search for a legume that will withstand grazing and compete with grass in a pasture under grazing has led to the development of creeping and rhizomatous alfalfas. The creeping alfalfas spread by the development of aerial shoots from lateral roots several inches below the surface of the ground. The rhizomatous type of spreading occurs from the initiation of roots at the point of emergence of the unusually long crown buds. In either case the new plant is capable of survival independent of the mother plant.

Several characteristics of these new types of alfalfas are unknown. In so far as they are being developed as pasture alfalfas the amount of nitrogen they are capable of fixing is an important factor. For this reason a study was conducted to compare the relative ability of common, rhizomatous and creeping alfalfas to fix nitrogen.

Nine creepers, two rhizomatous, and one common type were grown in the greenhouse in nitrogen-free vermiculite. Crone's solution devoid of nitrogen was used as a nutrient solution. The tops and roots were harvested at the 1/4 bloom stage, separated at the crown, and analyzed separately for total nitrogen by the Kjeldahl method.

Included in the field study were eight creepers, two rhizomatous types, and one common type planted at the Ashland Agronomy Farm located near Manhattan, Kansas. Composite samples from each plot were taken when the alfalfas were in the prebud stage and analyzed for percent nitrogen by the Kjeldahl method.

In the greenhouse study the creepers ScMa532, Sc34922F and Sc34916F yielded significantly more top growth than Buffalo unfertilized or fertilized with nitrogen, even though there were no differences between Buffalo and these alfalfas in total dry matter produced. In grams of top growth the creepers on the average yielded significantly higher than the common or rhizomatous types under the stress of a lack of nitrogen in the growing medium.

ScMa532 and Sc34916F contained significantly more nitrogen in their top growth than Buffalo unfertilized, even though there were no significant differences between those alfalfas and Buffalo unfertilized in percent nitrogen in the top growth, on a dry weight basis.

The creepers on the average contained a higher percent nitrogen, on a dry weight basis, in the plant than the rhizomatous types; however, there was no difference between the creepers and the common type. In total milligrams of nitrogen produced in the plant, on the average there were no significant differences among the creeping, rhizomatous or common types. However, the creepers had a higher percentage of their total nitrogen in the top growth. This was attributed to a high top-root ratio and a high percent nitrogen, on a dry weight basis, in the tops as compared to a low percent nitrogen, on a dry weight basis, in the roots.

From the field study no differences were observed among the alfalfas in percent nitrogen in the top growth on a dry weight basis. This was probably due to a lack of a sufficient number of replications. However, the percent nitrogen in the tops of those alfalfas grown in the field correlate highly with those grown in the greenhouse. This would indicate that the greenhouse study could be used to predict the manner in which these alfalfas would behave when grown under field conditions.

It seems feasible to assume that the transfer of nitrogen from the legume to the grass would be more rapid by the grazing animal than by the excretion of nitrogenous products by the nodules and the decay of legume roots. If this is so, it is reasonable to conclude that the creepers with a higher percentage of their total nitrogen in the tops would benefit the grass more than the common or rhizomatous types.