Abstract:
This study was part of a broader, longer term investigation into the nutritional responses of sainfoin and alfalfa. A strip plot, randomized complete block experiment was designed with sainfoin and alfalfa strips as the main plots. Each of four replications included 25 fertility treatments. Treatments were composed of one factorial set of four levels of potassium over three levels of phosphorus, and another set of four levels of nitrogen over selected combinations of phosphorus and potassium. Additional treatments included additions of sulfur and sulfur with selected micronutrients. Dependent variables studied were yield, water use efficiency, and soil nitrate. Also studied were the forage percentages and uptakes of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium.

Alfalfa yielded more than sainfoin in both years, had a greater water use efficiency, and was higher in protein and concentrations of potassium, calcium, magnesium, and sodium. Sainfoin was much higher in percent phosphorus. Yields and concentrations of most elements were higher the second year for both species. Soil nitrate was in- creased the spring following nitrogen fertilization, but nitrate levels were low by fall of that year (1977), and the effect of applied nitrogen was no longer evident. Nitrate levels were below levels of detection on all units when sampled in the summer and fall of 1978, In 1977, the year of seeding, alfalfa yield was increased by nitrogen and phosphorus, but a negative interaction existed between the effects of these two nutrients. Phosphorus also increased the uptakes of nitrogen, potassium, and calcium. Nitrogen increased sainfoin yield, potassium concentration and uptake, and phosphorus concentration in 1977, but caused percent protein to decline. Phosphorus increased the concentration and uptake of that element in both species both years.

In 1978, moderate applications of phosphorus appeared to stimulate the growth of sainfoin and uptake of most elements, but larger applications reversed this trend.

In 1977, potassium reduced soil nitrate levels on alfalfa units and improved the water use efficiency of sainfoin. The following year, potassium decreased yield, potassium concentration, and the uptakes of nitrogen, phosphorus, potassium, calcium, and magnesium by alfalfa. Calcium concentration of alfalfa was increased to some extent by phosphorus in 1978 and nitrogen in 1977, but not by potassium.

Sulfur sharply reduced alfalfa yield and the uptakes of most elements in both years. Micronutrients usually decreased sainfoin yield and the uptakes of most elements.

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Signature Alan R. Foss

Date 1 April 1979

YIELD AND ELEMENTAL COMPOSITION OF SAINFOIN AND ALFALFA AS AFFECTED BY FERTILIZER VARIABLES

by

ALAN ROY FOOS

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Soil Science

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ABSTRACT

This study was part of a broader, longer term investigation into the nutritional responses of sainfoin and alfalfa. A strip plot, randomized complete block experiment was designed with sainfoin and alfalfa strips as the main plots. Each of four replications included 25 fertility treatments. Treatments were composed of one factorial set of four levels of potassium over three levels of phosphorus, and another set of four levels of nitrogen over selected combinations of phosphorus and potassium. Additional treatments included additions of sulfur and sulfur with selected micronutrients. Dependent variables studied were yield, water use efficiency, and soil nitrate. Also studied were the forage percentages and uptakes of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium.

Alfalfa yielded more than sainfoin in both years, had a greater water use efficiency, and was higher in protein and concentrations of potassium, calcium, magnesium, and sodium. Sainfoin was much higher in percent phosphorus. Yields and concentrations of most elements were higher the second year for both species. Soil nitrate was increased the spring following nitrogen fertilization, but nitrate levels were low by fall of that year (1977), and the effect of applied nitrogen was no longer evident. Nitrate levels were below levels of detection on all units when sampled in the summer and fall of 1978.

In 1977, the year of seeding, alfalfa yield was increased by nitrogen and phosphorus, but a negative interaction existed between the effects of these two nutrients. Phosphorus also increased the uptakes of nitrogen, potassium, and calcium. Nitrogen increased sainfoin yield, potassium concentration and uptake, and phosphorus concentration in 1977, but caused percent protein to decline. Phosphorus increased the concentration and uptake of that element in both species both years. In 1978, moderate applications of phosphorus appeared to stimulate the growth of sainfoin and uptake of most elements, but larger applications reversed this trend.

In 1977, potassium reduced soil nitrate levels on alfalfa units and improved the water use efficiency of sainfoin. The following year, potassium decreased yield, potassium concentration, and the uptakes of nitrogen, phosphorus, potassium, calcium, and magnesium by alfalfa. Calcium concentration of alfalfa was increased to some extent by phosphorus in 1978 and nitrogen in 1977, but not by potassium.

Sulfur sharply reduced alfalfa yield and the uptakes of most elements in both years. Micronutrients usually decreased sainfoin yield and the uptakes of most elements.

INTRODUCTION

Alfalfa (Medicago sativa L.) is the major forage legume in Montana. Because of its wide use and long-term popularity it has been the subject of more research than any other forage legume. However, little research has been done to investigate soil fertility requirements of alfalfa in Montana. Recent studies have shown that alfalfa in Montana is frequently deficient in P and that both P and K may increase yields. That alfalfa yields statewide are very low emphasizes the need to clarify nutritional requirements of alfalfa on Montana soils.

Sainfoin (Onobrychis viciifolia Scop.) has attracted recent attention as a substitute for alfalfa. It is ideally suited to many conditions in the Northern Rocky Mountains and Canada that are less favorable to alfalfa production. Its nutritional quality is comparable to that of alfalfa. Its potential for improvement is excellent. It has not been subjected to the long history of intensive breeding that alfalfa has. Nutritional requirements and physiological status are by comparison nearly unknown, but it is certainly very different from alfalfa. In the United States, sainfoin has proven to have an outstanding ability to extract soil P and usually shows no response to additions of highly available forms. One problem with sainfoin is that it often shows symptoms of N deficiency and poor N fixation. It is also susceptible to a crown and root rot complex that frequently reduces yields and stands, especially on heavy, irrigated soils.

The importance of studying the effects of soil nutrients on yield is obvious. Knowledge of soil nutrient effects on chemical composition is also desirable. Such information reveals forage quality and physiological status of the plant. It also characterizes in depth the manner in which each species responds to soil nutrients. Protein is the most valuable indicator of forage quality. Concentrations of other elements are indicators of general health, physiological strengths and weaknesses, and differences in such characteristics among species.

The objective of this experiment was to clarify and compare yield and compositional responses of irrigated sainfoin and alfalfa to soil fertility factors. Specific items of investigation were: 1) soil NO_3^- levels; 2) water use efficiency; 3) yield; and 4) plant concentrations and uptakes of N, P, K, Ca, Mg, and Na. Major treatments were factorial combinations of N, P, and K. Additional treatments included an annual application of 0-45-112, an annual application of 11-55-0 and KCl (24-45-28), an application of S, and an application of S combined with B, Co, and Mo. Effects were analyzed for each species and harvest for the year of seeding (1977) and the year following.

These experimental objectives were, however, only part of a larger experimental plan. Final conclusions will not be reached until results are obtained over a period of 5 or more years. Other investigators will be studying effects of soil nutrients on stand longevity, nodulation, and disease.

LITERATURE REVIEW

General Characteristics of Sainfoin and Alfalfa

Sainfoin (*Onobrychis viciifolia* Scop.) is usually taller than alfalfa (*Medicago sativa* L.), growing to a height of 3 feet or more (37), while the height of alfalfa is usually under 3 feet. The leaves are pinnately compound like those of alfalfa, but vetch-like, having a large number of leaflets (37,63). Alfalfa is trifoliolate. Stems are decumbent (26) instead of upright like those of alfalfa. Sainfoin seeds are larger than those of alfalfa. The flowers are bright pink in color until pod stage (26,37), while those of alfalfa are usually purple but may be yellow or variegated. The long tap root of sainfoin, resembling that of alfalfa, may be 5 cm in diameter and 1 to 10 m deep (26), with about twice as many laterals (56).

The popularity of sainfoin has been increasing for a number of reasons. Depending on environmental conditions and location, sainfoin may yield less than, the same as, or more than alfalfa (14,26,37,79,81). Usually, sainfoin yields more than alfalfa at the first cutting and less at the second cutting (16,26,60) due to a slow rate of recovery. Sainfoin is therfore particularly likely to perform well in areas where springs are warm and moist and climate limits harvest to a single cutting (60). It is recommended for dryland production in Montana and Canada only where annual precipitation is greater than 12-13 inches

(26,37). The variety Remont is a multicut variety, as opposed to the single-cut Eski, and is recommended for irrigated pastures in Montana where a more even seasonal distribution of growth is desired (14,25).

Sainfoin is highly resistant to serious pests, in particular the alfalfa weevil (26,37,78) which has been a serious threat to alfalfa production in Montana. It is highly resistant to drought and is very winterhardy (20). Koch et al. (46) found sainfoin yields to be high in years of low moisture with little decline in forage quality from early to late bloom stages of maturity. Sainfoin, like alfalfa, is easily established (26), and at least in the early stages of growth is salt tolerant (87). A large variation in protein content among species gives promise of improvements through breeding programs (22). Sainfoin, unlike alfalfa, has never been known to cause bloat (26,28,37,63, 67). Seedlings have a wider temperature optimum for growth than alfalfa or cicer milkvetch (*Astragalus cicer* L.) which permits earlier seeding (82).

Feeding trials in Montana have indicated the nutritional value of sainfoin hay to be equal to or superior to that of alfalfa (14,43). It is highly palatable to livestock. Sheep will graze sainfoin first, alfalfa second, and cicer milkvetch last, although the total consumption of sainfoin and alfalfa is about the same (81). Sainfoin is more easily pollinated and much more heavily worked by bees than alfalfa, the honey yield being large and of excellent quality (27,34,43).

Sainfoin possesses some undesirable characteristics in comparison to alfalfa. Due to poor recovery it may not be able to withstand grazing (49), a problem aggravated by its high palatability. Stand longevity has been seriously reduced in many cases due to a root rot caused by a complex of organisms including _Fusarium solani_ (2,76) and _Pseudomonas_ and _Erwinia_ (29). Unlike alfalfa, sainfoin often seems to be ineffective at N fixation (2,26,79), even when abundantly nodulated (10). It is a poor competitor with most weeds and grasses (38,84), especially aggressive, rhizomatous types like bromegrass (_Bromus inermis_ L.) (37), as well as most cereal crops (26). Hanna et al. (38) found alfalfa in Canada to give better yields than sainfoin either alone or in grass mixtures. However, Cooper (19) found sainfoin in grass mixtures in Montana to have greater persistence and vigor than birdsfoot trefoil (_Lotus corniculatus_ L.) under hay-stockpile management, and additions of sainfoin to birdsfoot trefoil-grass mixtures have consistently increased yields in Montana (26).

Growth and Forage Quality

For alfalfa, maximum dry matter accumulation occurs at 2% to 45% bloom, but at 100% bloom for sainfoin. These occur at about the same date on dryland but sainfoin may be much later on irrigated land (15). At maximum yield the crude fiber of sainfoin is less than or about the same as alfalfa, TDN (total digestible nutrients) is about the same, but alfalfa has 3% to 6% more protein and 9% to 10% less nitrogen-free extract (15,25,41). The protein content of sainfoin is sufficient for the needs of beef cattle (15). Steers grazing sainfoin have gained more than those grazing other legumes and legume-grass mixtures (26). Swine have gained as much with 3% ground sainfoin as with 3% ground alfalfa (61).

Baker et al. (4) summarized previous literature and data of their own on the composition of sainfoin. Protein content, lower than that of alfalfa, was found to vary from about 16% to 19% at preflowering to about 12% at full bloom. At full bloom, Ca was about 1.4%, P 0.6%, K 1.6%, Mg 0.35%, and Na 0.6%. These figures varied considerably with climate and soil fertility status. They found large differences between leaf and stem composition for most nutrients, protein being concentrated in the leaf, which is also true of alfalfa (70). Large differences in the mineral composition of sainfoin were attributed by Baker et al. (4) to differences in the leaf-stem ratio. This ratio is higher in sainfoin than in alfalfa (41). In Montana, alfalfa has been higher in N-free extract, TDN, and P (15,26). Varga (89) rated the nutritional value of sainfoin in Romania 26% higher than that of alfalfa.

Nitrogen Fixation

Nodulation is generally decreased in legumes by N applications, but large amounts of N seem to be required for complete suppression

(55). However, Hallsworth (36) presented evidence that N fixation itself is unaffected or even sharply increased over a range of 0.5 to 60 ppm soil N.

The literature dealing with N fixation of sainfoin is inconsistent. Burton and Curley (10) found that moderate additions of N had little or no effect on nodulation or symbiotic fixation by sainfoin in Wisconsin. On the other hand, Radomirov et al. (65) found N to decrease the number of nodules on sainfoin in Bulgaria.

Panosian and Kirakosian (62) reported that, under sainfoin in Russia, soil N increased each year up until the third or fourth year and then declined. In Russian the nodules of sainfoin are reported to be more numerous than those of alfalfa and 5 to 8 times larger (44,83). Steergeva (83) maintained that the quality of sainfoin nodules was also greater than those of alfalfa in that yields of wheat were greater following sainfoin than those following alfalfa. He stated that sainfoin stored 10 times more N for release into the soil than alfalfa. Davidovskii (21) also reported that sainfoin accumulated N in the soil. However, Rovshanov (73) found alfalfa to be superior to sainfoin in increasing seed cotton yields the following year. Koter (48) found that while application of N as ammonia or small amounts of ammonium nitrate stimulated nodulation and N fixation in Poland, other forms of N (urea, nitric acid, ammonium nitrate) had a negative effect.

Applications of N to sainfoin have consistently given greater yields, the responses being more marked than those of alfalfa (3,11,48). In Montana, symptoms of N deficiency have been noted in the first 5 to 6 weeks of growth and in some cases resulted in depressed yields but not in others (79). Sims et al. (79) concluded that soils with less than 80 pounds NO_3^--N to 4 feet were N deficient for sainfoin.

The inconsistent behavior of infection leading to root rot could be responsible for some of the disagreement in the literature on N fixation by sainfoin. It is known that Fusarium reduces the effectiveness of Rhizobium (2,76). The effectiveness of various Rhizobium strains is also variable, and there are many capable of nodulating sainfoin (88).

There may be other organisms of unknown identity which are important to N fixation in sainfoin. Sainfoin plants dipped in Rhizobium inoculum and transferred to sterile soil develop a massive accumulation of $CaCO_3$ in the roots and lower levels of Ca in the forage (72). This is accompanied by root distortion, decreased numbers of nodules, and decreased capacity to fix N. These effects were not observed in plants grown on non-sterile soil.

Meyer's data typify sainfoin responses to N (58). Nitrogen increased yield, vigor, and regrowth at all rates of application up to 448 kg/ha. Stand persistence was poor except in the 448 kg/ha treatment. Alfalfa usually shows little response and sometimes shows a negative response to N in both yield and stand persistence (30). Brown (9) found N to decrease alfalfa stands and facilitate weed invasion.

Potassium

The critical plant level of K for alfalfa was found to be somewhere between 1.75% and 2.00% by Kresge and Younts (51). Adams and Sheard (1) considered it to be about 1.75% and Gerwig and Ahlgren (31) between 1.42% and 1.84%.

Kresge and Younts (51) found maximum yield of alfalfa was obtained with a single application of 165 lb K/acre, but this was reduced to 83 lb if the application was evenly split between one spring application and another after the first cutting. This was attributed to a higher recovery of K through more even seasonal distribution. Reports of vigorous yield responses of alfalfa to K are common in the literature (5, 6,30,31,33,45,77,85).

Potassium also increases stand longevity (9,31,85) of alfalfa, and according to Seay (77) uptake of P is increased by K. Gervais found K requirements to increase sharply with continued cropping, and that K decreased the Ca and P contents of alfalfa (30). Kimbrough (45) found the growth rate and leaf area of alfalfa to increase faster with applications of K and suggested that timely use of K could make possible an increase in the number of seasonal cuttings.

Butseroga (11) found that both P and K or the two combined decreased sainfoin yields in Russia without applications of N. This does not seem congruent with other reports (54,55,68) that K in all cases and P in most greatly enhance the ability of Rhizobium to fix N. Phosphorus in particular increases total root growth and nodule density (55,65).

Phosphorus

Blair and Prince (5) in 1939 found no significant response to P by alfalfa, even on soil previously cropped for ten years without any addition of P. Gerwig and Ahlgren (31) found no benefit of P to yield or stand persistence of alfalfa. Stivers and Ohlrogge (85) found no yield response of alfalfa on one soil type but a large response on another that was associated with a low soil test for available P. In most cases, however, marked yield responses of alfalfa to P are observed (30,34,47,52,80). Larson et al. (52) found large yield increases of alfalfa still occurring 3 years after an application of 240 lb P/acre on a P deficient soil. Halstead et al. (35) observed 100% to 300% yield increases of alfalfa in the first year following applications of P_2O_5 up to 2000 lb/acre. Cary et al. (17) found P and S effective in increasing alfalfa yields.

Stivers and Ohlrogge (85) and Gerwig and Ahlgren (31) found P not to affect stand persistence of alfalfa; yield responses were not observed either.

On an acidic soil, Singh (80) found additions of P not to affect the P content of alfalfa, but % P is usually increased (5,30,31,35,47, 52). Halstead et al. (35) raised the P content of alfalfa from an average of 0.18% to 0.54% with a high rate of P. Seay and Weeks (77) found alfalfa to take up P even when dormant in the winter. Adams and

Sheard (1) found P to decrease the N content of alfalfa, although Blair and Prince (5) found N content not to be significantly affected by P. Koehler et al. (47) found P not to affect the concentration of N or any cations. Gervais et al. (30) found P to decrease % K, but % Ca was unaffected.

In most instances, sainfoin does not respond to applications of P. Butseroga (11), as previously noted, found P to decrease sainfoin yield unless N was also applied. Babian and Karagulian (3) found the same on a humus soil but on a basic soil ($CaCO_3$ = 5.9%) P increased the yield of sainfoin. Sainfoin was believed to have a strong ability to change unavailable forms of P to available forms. Ukrainskii (86) found the P content of sainfoin roots to be high. Roath and Graham (67) suggested that sainfoin might be grown to advantage on soils low in available P. Where alfalfa and other legumes have responded to P in Montana, sainfoin has not (50). Phosphorus does not appear to influence recovery or stand persistence of sainfoin (58), but may favor late development of both sainfoin and alfalfa (66). However, Rorison (71) and Sariceva (75) both found higher concentrations of soil P to reduce initial dry weight loss of sainfoin seedlings and to accelerate later weight gain. In most legumes, additions of P overcome the suppression of nodulation brought about by high levels of Mo, N, or Ca (40). The uptake of Mo may also be increased by as much as 5 to 30 times by P additions (40). Phosphorus greatly stimulates the growth, number, and density of nodules if adequate K is present, and P also increases total root growth (55).

Sulfur and Micronutrients

The effects of S deficiency are similar to those of N in that S is required for the conversion of N into protein. Sulfur usually decreases the uptake of Mo by above ground portions (40). A deficiency of S leads to small, greenish nodules instead of large, branched, pink ones (55).

Adams and Sheard (1) found that a deficiency of S reduced alfalfa yields more than a deficiency of K. Pumphrey and Moore (64) found S to increase alfalfa yields. The S requirement of alfalfa is high: about 0.3% S content is required if the plant is adequately supplied (12). An adequate supply of S considerably increases N content (12,13, 63), the S content (13,64), and the Mn content (13) of alfalfa. Sulfur reduces the P and B contents of alfalfa, other elements being unaffected (13). Inorganic S is nearly absent in plants deficient in S (23). Dijkshoorn and Lampe (23,24) showed that only when the S content of protein falls below about 2.7% is S deficient, more exact values being 2.5% for legumes and 3.2% for grasses (24).

Much of the importance of micronutrients relates to N fixation. Molybdenum increases nodule size if S is adequate but nodule numbers are decreased (40). On a soil of neutral pH, Radomirov et al. (66) found that Mo applied at 300 g/ha increased seed yields of sainfoin 54%. With the addition of P, Mo increased yields more than NH_4NO_3

applied at 120 kg/ha. Since nitrogenase contains Mo, most of the benefit obtained from Mo by legumes is probably through its effect on N fixation (36). Molybdenum is also required in the host plant for reduction of nitrates in protein synthesis (40,55). Liming increases the availability of Mo (55).

Both the legume plant and the N fixing process require B (40). A deficiency results in poor root and nodule development (55). Nodulation requirements are probably less than those of the plant, but effects of B on nodulation and N fixation are dramatic. Small amounts of B, if deficient, will increase nodule numbers up to 9 times and the amount of N fixed per nodule 5 times with small increases of soil B up to 10 ppm (40). Radomirov et al. (66) found B to stimulate nodule development of sainfoin but not to affect yields. Hasler et al. (39) also found little response of sainfoin to B. Cobalt is also probably required for the fixation process (74).

MATERIALS AND METHODS

Plot Description

On 12 and 13 October 1976, fertility treatments as listed in Table 1 were applied to a 128 by 200 foot plot at the Montana State University Agricultural Experiment Station Farm west of Bozeman, Montana. Soil on the experimental units was then worked lightly with a duckfoot cultivator. The units were arranged in a strip plot, randomized complete block design with four replications. Strips were alfalfa and sainfoin. Soil was the Bozeman silt loam, a fine-silty, mixed Argic Pachic Cryoboroll. Blocks were oriented north and south such that a soil moisture gradient intersected at a right angle. Sources of fertilizer were ammonium nitrate (34-0-0), superphosphate (0-45-0), potassium chloride (0-0-60), calcium sulfate, cobalt chloride, molybdic acid, and sodium borate. Table 2 shows prefertilizer soil test results for soil nutrients and other characteristics.

Soil Sampling

On 25 April 1977 soil samples were taken from eleven selected treatment units in each replication at depths of 0-1, 1-2, 2-4, and 4-6 feet. Samples were weighed and then dried for 50 hours at $60^{o}C$, then reweighed to determine percent moisture and then converted to centimeters of moisture to the full 6-foot depth. This procedure was repeated on 27 October 1977. The difference in moisture values was added to total precipitation over this period and to the total water appli-

Table 1. Treatment numbers and levels of applied fertilizer in kg/ha.

Treatment Number	N	P	K		Treatment Number	N	P	K
1	0	0	0		7	0	45	112
2	0	0	28		8	0	45	336
3	0	0	112		9	0	224	0
4	0	0	336		10	0	224	28
5	0	45	0		11	0	224	112
6	0	45	28		12	0	224	336
1	0	0	0		16	112	0	0
11	0	224	112		17	112	224	112
12	0	224	336		18	112	224	336
13	56	0	0		19	224	0	0
14	56	224	112		20	224	224	112
15	56	224	336		21	224	224	336
22	0	224	112	+	45S			
23	0	45	112	-	Annually			
24	21	45	28	-	(11-55-0 + KCl, Annually)			
25	0	224	112	+	45S + 2.2B + 2.2Co + 0.28Mo			

Table 2. Prefertilizer soil test results.

Area Sampled	Depth (cm)	Texture	pH	O.M. (%)	P (ppm)	K (ppm)	Ca (meq)	Mg (meq)	Na (meq)	EC mmhos
South	0-15	Si L	6.9	2.4	21	344	13.5	3.7	Tr.	0.40
	15-30	Si L	7.0	2.1	16	296	13.5	4.0	0.1	0.40
Middle	0-15	Si L	7.0	2.2	24	363	13.9	4.3	0.1	0.40
	15-30	Si L	7.1	1.8	24	334	14.7	4.6	0.1	0.40
North	0-15	Si L	6.9	2.1	21	334	13.1	4.4	0.1	0.40
	15-30	Si L	7.1	1.6	19	296	14.7	5.1	0.2	0.30

cation from 4 irrigations. This was then used to obtain water use efficiency (WUE), defined as kilograms forage at 12% moisture produced per centimeter of water evapotranspired.

Nitrate was determined from the soil samples so that the influence of applied N or other nutrients on soil nitrate levels could be determined. Soil samples were taken again in 1978 on 10-11 July and 2-3 October. Soil nitrate was determined using the phenoldisulfonic acid method (7).

Seeding and Harvesting

The plot was seeded on 1 May 1977 at the rate of 50 kg/ha sainfoin seed (variety Remont) and 13 kg/ha alfalfa seed (variety Thor). Seedlings were counted on selected treatments on 9-11 June 1977 in sets of two one-meter counts per unit. Counts were made to confirm uniform germination over the plot. Seedling height was determined on 30 June 1977 on each experimental unit by taking eight measurements per unit. This was done in case yields in the year of seeding were insufficient to warrant harvesting.

The first harvest was taken in the year of seeding on 3 August 1977. Sainfoin was at 100% bloom and alfalfa at 10% bloom. Second harvest was obtained on 17 October while sainfoin was in the early pod stage and alfalfa at 80% bloom. Samples for yield determination were taken by harvesting with a Mott forage harvester which chops the material to facilitate subsampling. A 4-foot middle section of each 8 foot wide unit was harvested. Subsamples of forage were then weighed and dried at $65^{o}C$ for 4 to 5 days and then reweighed. In this way percent moisture was determined for calculating yields in metric tons per hectare at 12% moisture. The dried subsamples were then analyzed for protein, phosphorus, potassium, magnesium, and sodium. Total uptake of these minerals were then determined by multiplying the plant fraction times yield in kilograms per hectare.

The year after seeding, harvesting was performed on 29 June and 5 September 1978. Maturities for both harvests were 10% and 100% bloom for alfalfa and sainfoin, respectively.

Chemical Analysis

Forage nitrogen content was determined using the semimicro-Kjeldahl method as described by Bremner (8).

Other elements were determined by ashing samples of 2 g each at $550^{o}C$ to $600^{o}C$ for 10 to 15 hours in a muffle furnace. Concentrated HCl (1.5 ml) was added to each crucible after cooling. Distilled-deionized water (2.5 ml) was added and the mixture was evaporated at $145^{o}C$. Finally, 1.0 ml of 2.5N HCl and 5.0 ml H_2O were added and the solution was poured into 100 ml volumetric flasks, and brought to volume. An aliquot was taken for analysis for P by the vanadomolybdic acid method (42). A separate aliquot was taken for analysis by atomic absorption spectrophotometry to determine K, Ca, Mg, and Na. Readings as percent transmission or absorbance were converted to percent of plant forage. This value was multiplied by dry forage yield in each unit to obtain values of total uptake in kg/ha.

Statistical Analysis

Thirteen nutritional characteristics were studied for each plant species for each cut in each year: yield, percent protein, uptake of N, and percents and uptakes of P, K, Ca, Mg, and Na. Data for each of these characteristics fit a randomized complete block design with 25 treatments for each plant species. The treatments can be arranged to fit a complete factorial with four rates of applied K over three rates of applied P. Another complete factorial exists with four rates of applied N over three rates of P and K combinations. Table 1 groups the treatments into the two factorials. In addition are four other treatments from which various paired comparisons can be made. These treatments are an annual application of the 0-45-112 treatment, the 0-224-112 treatment but with S added, the same but with B, Co, and Mo added as well, and an annual application of 24-45-28(11-55-0 + KCl). The latter treatment was included to determine whether or not any benefit was realized from the application of 11-55-0. The manufacture of 11-55-0 results in a product where the N portion contributes less to the cost than if N were applied separately. Therefore, any resulting benefit is relatively cost-free.

A standard analysis of variance (ANOVA) for each characteristic for each harvest, year, and species to evaluate the significance of the above factors. Appendix II contains treatment means and selected statistical parameters from these analyses. In addition, the percent composition values were averaged over both harvests, yields and uptakes summed for each year, and these data analyzed in the same way. Each of these analyses included tests for eight selected paired comparisons using the 4 odd treatments just described. See Table 3 for a list of these comparisons. Thus, each ANOVA includes information on applied P and K in a factorial design at 0 N, and N and P+K as another set. For each set the same error term is used from the overall ANOVA. Note that the analysis with respect to a single factor (such as N) is performed at the mean level application of the others in each factorial set. Thus, the analysis for K at three levels of P implies that any conclusions drawn about the effect of K are under the assumption that mean levels of N and P exist from the first 12 treatments. This is no problem except in cases where an interaction is present. If the interaction is significant, then one is committed to accepting both factors as significant and describing the behavior of one factor at each separate level of the other, or vice versa.

For each standard ANOVA a regression analysis was also performed using all treatments from 1 through 21. Note that in using all of these treatments, orthogonality as in the previous ANOVA is lost. That is, the same levels of non-respective factors do not exist for each

Table 3. List of paired comparisons performed for each forage characteristic.

Treatment numbers	Treatments
11 vs. 25	0-224-112 vs. 0-224-112+S+B+Co+Mo
11 vs. 22	0-224-112 vs. 0-224-112+S
22 vs. 25	0-224-112+S vs. 0-224-112+S+B+Co+Mo
7 vs. 23	0-45-112 vs. 0-45-112(Annually)
12 vs. 23	0-224-336 vs. 0-45-112(Annually)
6 vs. 24	0-45-28 vs. 21-45-28(Annually)
14 vs. 24	56-224-112 vs. 21-45-28(Annually)
23 vs. 24	0-45-112(Annually) vs. 21-45-28(Annually)

level of the other. However, if only one factor (e.g. N, P, or K) has an effect, this is of no consequence. If the effects of more than one factor are significant, then the inclusion of both in one equation is still valid. Note that this can cause a significant effect for one factor which is the result of another, but to eliminate these one can: 1) check the significance of both factors when combined in one regression, and 2) check the significance of the factor in the standard ANOVA which is orthogonal. It was felt that the increased precision gained by using all 21 treatments for regression analysis overcame the orthogonality question given these ways to resolve it. Regression analysis is a higher-powered test (i.e., conclusions are more likely to be correct) than the standard ANOVA, so the results of the regression analysis were preferred and form the basis of conclusions drawn in this thesis. In some cases regression analysis indicates significance not substantiated by the other, and vice versa. With few exceptions, regressions that were chosen as significant were so at the 1% level or less. Appendix I contains a list of all regression equations and probabilities which were chosen as significant.

A series of single and combined factors were run as regressions for each characteristic in both linear and quadratic form. From these and from the standard ANOVA, significant effects were selected and the appropriate factors run as one regression to give one equation. Because the EMS (error mean square) from the standard ANOVA (based on treatment means) contained a greater degree of precision, the standard ANOVA EMS was corrected for number of observations and substituted into the regression analysis to give a new error term.

As for the standard ANOVA, the assumption is implicit that values predicted by an equation are at the mean level of omitted factors among the first 21 treatments. Factors which are combined into one equation are graphed separately in this thesis at the mean level of the others. If an interaction exists, the graph necessarily portrays a family of lines. Most of the selected equations are quadratic models, not because the quadratic is significant over the linear, but because nonlinearity is generally assumed and the best fit is non-linear.

All probability values refer to Type I errors so that smaller probabilities indicate greater significance. Also, rather than establishing significance by F values associated with certain levels of probability, e.g. 10% or 5%, actual probability levels associated with respective F ratios are reported. These values were rounded to three decimal places and except in Appendix III reported only for significant factors. Also, probabilities are listed under the heading "p(F)" to indicate probability as a function of F.

A separate ANOVA was used to evaluate the overall differences between years, species, treatments, and their interactions. Tables in the text report only significant effects from this analysis. These effects are drawn from the following sources and degrees of freedom:

Source	Degrees of freedom
Replications	3
Species	1
Error a	3
Years	1
Treatments	24
Species x Years	1
Species x Treatments	24
Years x Treatments	24
Error b	318

RESULTS AND DISCUSSION

It was not practical to discuss in detail all of the significant effects for each harvest and year. Only total and average effects for each year are discussed unless a special reason warrants attention to a particular harvest. However, the appendices list information for each harvest separately in addition to the total or average effect in one year. Appendix I lists all regression equations and probabilities, if significant. Appendix II lists significant paried comparisons. Appendix III lists all treatment means and selected statistical information. Each EMS is also included so that additional tests may be performed. Appendix III also allows examination of data before conversion into predictive models.

Numbers given in the test are carried to a certain number of places. This was not meant to imply accuracy to that point. The number of digits reported is based on the size of significant intervals. For example, if a significant interval is less than 0.01 then digits are reported to the third place beyond the decimal, indicating precision, if not accuracy, to that point. Appendix data carry an extra number of digits to avoid rounding errors in the event further analyses or transformations are to be performed. Probability levels are designated by "p(F)".

Emergence and Plant Height

Seedling counts taken 9-11 June 1977 showed no significant differences except between species. This was expected. Mean seedling number was 25.4/meter for sainfoin (s = 6.7) and 72.6/meter for alfalfa (s = 12.5)

Plant height measurements made on 30 June 1977 did not show significant differences among sainfoin treatments. For alfalfa, N and P both increased seedling height (see Table 4). Mean heights were 40 cm and 35 cm for sainfoin and alfalfa, respectively.

Table 4. Plant height of alfalfa in the year of seeding as affected by applications of N and P.

Effect of N		Effect of P	
Applied N, kg/ha	Plant height, cm	Applied P, kg/ha	Plant height, cm
0	33.7	0	30.4
56	37.1	45	33.9
112	36.5	224	36.0
224	37.2		
	CV=5.9% p(F) 0.001		

Soil Nitrate

Soil NO_3^- was determined primarily to see if applied N significantly affected levels of this soil nutrient. By 1978 there was no measurable NO_3^- remaining in the 0 to 6 foot depth sampled. Samples taken in the spring of 1977 showed a significant effect due to applied N. The mean level of soil NO_3^- among treatments with N applied at 224 kg/ha was 306 kg/ha. But random variation was extremely high (CV = 41%). Significance due to applied N was present among alfalfa treatments but not sainfoin (see Table 5). However, mean levels of soil NO_3^- did not differ significantly between the two. Mean soil NO_3^- among sainfoin treatments was 207 and, among alfalfa, 194 kg/ha. As expected, other fertilizer treatments did not affect NO_3^- levels in the spring of 1977.

Table 5. Effects on soil nitrate levels to 6 feet under alfalfa.

Date of sampling 25 April 1977		Date of sampling 25 October 1977	
Applied N, kg/ha	Soil NO_3^- kg/ha	Applied K, kg/ha	Soil NO_3^- kg/ha
0	139	0	54
112	174	112	32
CV=40% p(F)=0.001		CV=62% p(F)=0.037	

Soil NO_3^- taken in the fall of 1977 showed a mean value of only 33 compared to the spring mean of 201 kg/ha. Also, the coefficient of variation for fall 1977 was 79%. This high coefficient was apparently due in part to many units having no measureable NO_3^- remaining. Applied N no longer showed any significant effect; however, among alfalfa units, applied K decreased soil NO_3^- as seen in Table 5.

Potassium had a detrimental effect on both yield and chemical composition of alfalfa in 1978. If these effects and reduced soil NO_3^- by K in 1977 were related, the relationship was not understood. Since soil NO_3^- reserves were completely depleted by 1978, and the response of alfalfa to N was not large, it is unlikely that NO_3^- reduction by K could directly cause reduced yield and forage quality. The lack of effect by K on soil NO_3^- confirms that its effects were exerted through action on the plant rather than the soil. The effects on yield and soil NO_3^- suggest a possible weakening of the plant-Rhizobium relationship. However, the additional effects on plant composition suggest that either root growth or function was disturbed by high levels of K.

Yield and Water Use Efficiency

Table 6 shows that alfalfa yield was considerably greater than that of sainfoin although this difference narrowed in the year after seeding. Only the probability levels of significant factors are shown.

Table 6. Mean yields for species and years in metric tons/hectare.

Species	Yield, metric tons/ha 1977	1978	Probability levels for significant differences
Sainfoin	5.52	11.1	$p(F, species) < 0.001$
Alfalfa	7.48	12.6	$p(F, years) < 0.001$
			$p(F, species \times years) = 0.042$

Yields from the two harvests of 1977 showed similar patterns. Sainfoin showed a response to N demonstrated by the regression curve in Figure 1. Sainfoin yield was raised slightly more than 1 metric ton/ha with 224 kg applied N/ha. This response was not evident in 1978, nor