GERMINATION OF ARROWLEAF CLOVER SEED IN BOVINE FECES AND CHARACTERISTICS OF PHOSPHORUS FERTILIZED ARROWLEAF CLOVER IN A TALLGRASS PRAIRIE

By

NANCY KATHLEEN MAHER

Bachelor of Science in Agriculture

Oklahoma State University

Stillwater, Oklahoma

1983

Submitted to the Faculty of the
Graduate college of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1986

GERMINATION OF ARROWLEAF CLOVER SEED IN
BOVINE FECES AND CHARACTERISTICS OF
PHOSPHORUS FERTILIZED ARROWLEAF
CLOVER IN A TALLGRASS PRAIRIE

Thesis Approved:

Donald G. Wagner
Thesis Adviser

David M. Engle

Ted McCollum III

John J. Guenther

Norman N. Durham
Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to express her appreciation and gratitude to the members of her thesis committee, Drs. Don Wagner, Dave Engle, J.J. Guenther and Ted McCollum for their advice and guidance during preparation of this manuscript. A special thanks must also be extended to Steve Welty, Drs. R. Ahring, W.E. McMurphy, R. McNew, and D. Buchanan for their contributions and to everyone in the Ag Division of the Noble Foundation who who generously offered their time, knowledge and resources throughout this study. Additionally thanks are extended to the OSU Animal Science and Agronomy Departments and to the USDA-ARS for providing the cattle, pasture, facilities and equipment used in this study.

A deep appreciation is due to Stephanie Martin, Sharon Norton, Larry Burditt, Joe Ward, Mark Richards and Eugene Tinker for their friendship and ecouragement.

Finally, the author wishes to acknowledge her parents, John and Nancy Maher for teaching her the importance of education. For the loving support that they, along with her sisters Mary, Peggy, and Patricia and her brother Sean provided, she will always be grateful.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

Introduction

Stand establishment of arrowleaf clover (*Trifolium vesiculosum* Savi) may be difficult, with good stand development sometimes requiring several years. In pastures, arrowleaf clover has been observed germinating from cattle feces. By adding arrowleaf clover seed to winter fed protein meals or feeding arrowleaf clover hay which contains seed cattle may be used to disseminate seed in a pasture (a practice which is occasionally used today).

Arrowleaf clover is a winter annual legume that can be effectively combined with warm-season grasses to produce high quality forage that lasts from early spring well into the hot summer months. A limited amount of fall grazing is also possible. Unfortunately, neither the nutritive characteristics of arrowleaf clover in a mixed tall grass prairie, nor the effect of phosphorus on the nutritive composition of arrowleaf clover have been well documented.

The following studies were conducted in an attempt to determine the 1) germination characteristics of arrowleaf clover seed when fed to cattle and 2) nutritive characteristics and yield of arrowleaf clover under various levels of P fertilization.

CHAPTER II

REVIEW OF LITERATURE

Germination and Recovery of Ingested Seeds

Several studies have documented the role of animals in the dispersal of plant seeds through the feces. A variety of studies with types of seeds and disseminators have been conducted. McCully (1951) fed mature Macartney Rose (*Rosa bracteata* Wendl.) buds to cattle (*Bos taurus*) and compared germination of the seeds that were obtained directly from the plant to that of seeds recovered from feces to establish the importance of livestock in the propagation and spread of this noxious species. No germination studies were conducted, but fifty percent of the seeds fed to cattle were recovered and approximately 90% of those recovered seeds suffered no apparent visual damage.

Heady (1954) conducted studies to identify the feeding habits and preferences of sheep (*Ovis aries*) and deer and to assess their importance in the spread of noxious plants and desirable forage species to cultivated fields. Some seeds found in rumen and fecal samples of grazing sheep and deer were viable. The results indicated that sheep and deer contribute to the spread of plants on California ranges.

Results from most studies are inconsistent. For example, most results indicate improved germination following digestion (McCully, 1951; Heady, 1954; Livingston, 1972). The mechanism for this improvement is partly due to increased permeability of the seedcoat resulting from scarification. There appears to be a direct relationship between a reduction of weed seed (several varieties) viability and seed retention time in the digestive tract in dairy cows (Atkeson, Hubert and Warren, 1934). Johnsen (1962) reported no significant improvement in percent germination of juniper (*Juniperus monosperma*) seeds after ingestion, however, the rate at which seeds germinated was more rapid following digestion. Germination periods required for juniper seeds found in various animal feces ranged from 7 weeks for birds, 5 weeks for coyotes (*Canis latrans*), sheep, and 4 weeks for jackrabbits (*Lepus californicus* M.) compared to 10 weeks for the control (no digestive action) seed. Burton and Andrews (1948) reported that viability of mesquite (*Prosopis juliflora*) seeds was 45 and 12% when fed to steer calves and sheep, respectively. The greater reduction noted with sheep may be related to more extensive comminution or longer retention times in the digestive tract. Similarly, mesquite seed (*Prosopis glandulosa* Torr.) viability was reduced from 57% for unfed seed to 48% for seed ingested by peccaries (*Pecari tajacu*) (Everitt and Gonzalez 1983). These data suggest that the seedcoat of mesquite is damaged, but not completely removed, when exposed to the digestive tract of the peccary.

Recovery of seed in fecal material appears highly variable (Burton and Andrews 1948; Glendening and Paulsen 1950; and McCully 1951), but no work has shown greater that 50% recovery.

Scarification

Little is known about the the effects of digestive action on arrowleaf clover seed (Trifolium vesiculosum Savi). Engle (unpublished data) compared the germination of unscarified arrowleaf clover seed to scarified seed (rubbed with an emory cloth). The average germination of unscarified seed was only 23% compared to 96% for scarified seed. An alternative to mechanical scarification is chemical treatment of hard seeds, such as acid scarification where seed is soaked in a sulfuric acid (H_2SO_4) solution (Blankenship and Smith, 1967). McDonald and Khan (1983), recommended that seeds be allowed to soak in the H_2SO_4 at 25-C followed by washing and air-drying. The low pH in the abomasum of cattle may act similarly to acid scarifying seeds. In addition, seeds that are scratched, but not crushed, during mastication may display improved germination. On the other hand, seeds that are scarified before or during passage through the digestive tract may eventually be digested or destroyed.

Nutritive Characteristics

Arrowleaf clover can be overseeded in perennial grass pastures either mechanically or via cattle feces. Seeding of arrowleaf clover in warm-season pastures offers the potential of providing high production and excellent quality forage over a long period since it begins to grow early in the spring and since it can be grazed the subsequent fall and winter (Hoveland 1974). Combining warm-season grasses and cool-season annual legumes may provide almost year round forage production in some areas (Van Keuren and Hoveland 1985). In the majority of studies involving mixed stands of arrowleaf clover and a warm-season grass, the grass is bahia (*Paspalum notatum* Flugge) or bermuda grass (*Cyndon dactylon* L.) rather than native tallgrass prairie (Watson 1975; Knight 1963; Beaty and Powell 1961; Hoveland, et al. 1981). Furthermore, most data concerning arrowleaf clover and warm-season grasses have been concerned with cattle performance or forage production of arrowleaf clover and grass, with little or no emphasis on nutritive characteristics of the forage stand.

Neither the National Research Council's (NRC) nutrient requirements for beef cattle (1984) nor the joint United States - Canadian feed Composition Tables (1982) report any nutritive data for arrowleaf clover.

Yield of Arrowleaf Clover

Yuchi arrowleaf clover appears to be higher yielding than many other clovers under most conditions. A longer growing season may account, in part, for the relatively high yields. Evers (1979), reported an arrowleaf clover yield of 4630 kg dry matter (DM) ha^{-1} in the first year of establishment and 5145 kg DM ha^{-1} the second year. Yield data from established arrowleaf clover pastures have also been measured. Maximum forage production, collected from arrowleaf clover (Yuchi) cut bi-weekly until April then once more, for hay, in late May (Hoveland et al. 1972) was 7300 kg DM ha^{-1}. Bokhari (1982) measured arrowleaf clover (Yuchi) yields of 9475 kg ha^{-1}. In another study, arrowleaf clover (Amclo) cut weekly until it reached mature stage, in early June, yielded 8632 kg ha^{-1} with a peak forage production of 100 kg ha^{-1} d^{-1} (Stanley et al. 1968). A 5 year average of only 3773 kg ha^{-1} of oven dried arrowleaf clover (Yuchi) clipped, with a rotary mower to simulate rotation grazing was reported by Bates (1986). The clover in the Bates study was not clipped after early April, however, leaving several weeks of forage production unmeasured. Yield of arrowleaf clover appears to vary extensively from year to year (Stanley et al. 1968; Evers 1979; and Bates 1986). Removing clover, by clipping or grazing, in late April or May appears to decrease clover yields or eliminate the stand completely. This problem may be avoided if grazing or bi-weekly clipping of the clover is administered (Hoveland et al. 1972).

Dry Matter Digestibility

Arrowleaf clover appears to be a high quality forage and is highly palatable in that cattle have been observed selecting it over young coastal bermuda grass even when it blooms and becomes stemmy (Hoveland et al. 1970). One probable explanation for this behavior is that arrowleaf clover appears to have a relatively low cell wall content even when the percentage of stem is high (Stanley et al. 1968). Stanley et al. (1968) assessed that the cell wall content of arrowleaf clover during the vegetative stage was 40%, increasing to 47% during flowering, and at maturity was greater than 50%. Also, there appears to be a significant variation of cell wall content between years. The in vivo digestible dry matter (DDM) of bermuda grass (44%) and alfalfa (63%) was determined by Hoveland et al. (1970) and compared to the DDM of arrowleaf clover at a similar stage of maturity. Arrowleaf clover was much higher than both reference forages (bermuda grass and alfalfa), ranging from 70-79% DDM. Akin and Robinson (1982) separated clover into individual morphological parts then compared the in vitro dry matter digestibility (IVDMD) of those parts. Samples were obtained at prebloom, early bloom, full bloom and 95% seed set. Digestibility did not decrease in the leaf of arrowleaf clover until the seed set stage, but the digestibility of the stem declined with stage of maturity.

In comparing the digestibility of (Amclo) arrowleaf clover and (Dixie) crimson clover (*Trifolium incarnatum* L.) from prebloom to full bloom Akins and Robinson (1982) found both clovers were similar and stem decreased more rapidly than leaf. The arrowleaf clover, however, had a consistantly higher IVDMD than crimson clover.

Percent and Quality of Protein

As might be expected from the digestibility values stated above, arrowleaf clover appears to be a high quality forage. Stanley et al. (1968) reported an average of 11.8% crude protein (CP) for arrowleaf clover harvested from early March to late May. In one study, arrowleaf clover contained 23% total crude protein (CP) in mid-April and 13% CP in late May (Hoveland et al. 1972). Although CP of arrowleaf clover tends to decrease more rapidly in stems than leaves, the pattern is similar to the decrease in CP noted in tropical legumes. Over a 26-week period, CP of a tropical legume declined from 26 to 15% in the leaves, 12 to 10% in the stems and from 19 to 13% in the whole plant (Siewerdt and Holt 1975). Arrowleaf clover seems to be high in total CP, though its protein value is due not only to the apparently high amounts of CP but also to the quality of the protein. Hoveland et al. (1970) assessed the amino acid profile of arrowleaf clover to be similar to the amino acid profile of alfalfa (*Medicago sativa* L.).

Chemical Composition of Native Grasses

In a mixed stand, the chemical composition of the companion grasses should also be considered. big bluestem (*Andropogon gerardii*), little bluestem (*Andropogon scoparius*), indiangrass (*Sorghastrum nutans*) and switchgrass (*Panicum virgatum*) are the predominate grasses found in native tallgrass pastures in the eastern and central portion of Oklahoma. Yield and chemical composition of these four grasses were measured for 15 years in Stillwater, Oklahoma (Waller et al. 1972). Average CP (averages for all grasses, all years) ranged from 2.0% in the winter months to a high of 10% in May.

Phosphorus Fertilization

Survival and distribution of a plant outside its natural ecosystem may depend, in part, on the availability of minerals as well as the reaction of the plant to mineral elements in the soil. Many workers have reported deficiencies of P on permanent grass-clover pastures (Mays 1974). Mengel and Kirkby (1978) indicate that the P requirement of legumes may be initiated by *Rhizobium* activity. On fine, sandy loam soils, arrowleaf clover, like many other legumes, has been shown to increase forage production when P fertilizer is added (Van Keuren and Hoveland 1985).

On a grass/legume pasture, the level of P fertilization and initial soil P can influence the ratio of grass to legume. When substrate P levels are low grass has a greater response than legume to added P, however, at high substrate P levels the legume is more responsive (Andrew and Johansen 1978). Therefore, as the level of P in the soil changes, competition between the grass and legume increases for most nutrients. It may be necessary to add P to a mixed sward to maintain the legume species (Mengel and Kirkby 1978) yet reduction of clover in the sward when a large amount of P is added has been reported in a few instances, possibly due to competition from grass (Mays 1974).

CHAPTER III

GERMINATION OF ARROWLEAF CLOVER SEED

Abstract

Arrowleaf clover seed *Trifolium vesiculosum* Savi usually contain a high percentage of hard or dormant seeds. Mechanical or chemical scarification of the seedcoat can improve germination and stand establishment. Passage of seeds through animal digestive tracts may have similar results. Our objective was to determine the effect of the ruminant digestive process on (1) the proportion of fed seed which was recovered in the feces and (2) the percent of germination for recovered seed. Known amounts of seed were fed to four rumen cannulated 3-yr old heifers in two studies. In the preliminary study, two animals were given seed via the rumen cannula while three animals received seed in their daily feed rations. Seed was enclosed in gelatin capsules and administered via the rumen cannula for all animals, in the second study. Fecal material was collected every 8 h after dosage for a period of 88 h in the first study and 96 h in the second study. A 25 g aliquot of mixed fecal material from each collection was washed and sieved to remove seed. Recovered seeds were dried, counted and

examined for differences in germination among discrete excretion times. Two unfed treatments, unscarified and scarified, were used for comparison.

Only 21% of the unfed, unscarified seed germinated while unfed, scarified seed had a 92.% germination. Germination of recovered seed was only 1%, but was increased to 90% after mechanical scarification. These data indicate that only hard seed survive digestion and additional scarification or weathering may be required for the seed to germinate. Eleven percent of the fed seed was recovered in the rumen by 96 h after dosing and peak of seed recovery appeared to be 80 h post dosing. It seems, however, that seed excretion may continue past 96 h.

Arrowleaf clover (Trifolium vesiculosum Savi) is an annual that can be effectively used as a companion legume in perennial grass forage systems. Traditional means of establishing legumes can either damage or destroy pasture and rangeland vegetation. Moreover, the terrain of many ranges may not permit conventional seeding. An alternate method of arrowleaf clover establishment may be to feed livestock arrowleaf clover seed, separately or as mature hay, to permit seed dispersal via feces.

Seeds of arrowleaf clover have an indurate (i.e. hard) seedcoat (Hoveland 1967; Kendall and Stringer 1985). Weathering or commercial scarification (Hoveland 1974) is usually required to improve both the rate and percent of germination. One alternative to mechanically scarifying the seedcoat is exposure of the seed to H_2SO_4 (Blankenship and Smith 1967). low pH of the animal abomasum seems to play a similar role in scarification of other kinds of seeds. In addition, seeds may be scarified during mastication.

Several workers have shown that certain seeds when ingested by animals such as mesquite (*Prospopis juliflora*) (Fisher 1947); a variety of weed species (Burton and Andrews 1948) and McCartney rose (*Rosa bracteata* Wendl) (McCully 1951) can be recovered in feces and germinated. Most studies indicate that only a part of the total seed intake is recoverable and only a part of the seed excreted or recovered is viable.

Although many studies have been conducted regarding the effects of animal digestion on seed, none have involved seed of arrowleaf clover. Our objective was to determine the effect of bovine digestion on arrowleaf clover seed germination, since the use of cattle as an overseeding practice may involve two phenomena: i) dispersal and ii) preconditioning of seed for rapid germination seedling emergence and survival.

Materials and Methods

Seed size and/or density may be related to fecal recovery. Thus, the seed lots used in these studies were blown with the aid of a South Dakota Seed Blower model B to standardize seed weight by removing and discarding light seed. Five random 5-g aliquots of blown seed were counted on an Old Mill Company, Electronic Counter, Model 850-2. There were an average of 830,000 seeds kg-1, which was similar to estimations of Hoveland (1974). Five, 226 g units were weighed, one for each animal in the study and one to serve as a control (unfed). Thus, approximate numbers of seed fed to each of four animals in the study were known.

In the first study, the seed samples were administered by mixing each 226 g of seed in the daily ration of two 3-year old heifers or by placing seed directly into the rumen fistula of two heifers of the same age. The fistulated animals used had been maintained on a daily diet consisting of 5 kg medium quality prairie hay (cut annually from a prairie meadow early in July) and 0.9kg animal^{-1} of 40% crude protein (CP) supplement containing vitamins and minerals. No dietary changes were made with the exception of the added arrowleaf clover seed. Loss of seed was observed from both methods of administration. After dosing, total fecal material excreted from each animal was collected, weighed, and recorded at 8-h interval for a period of 88 h. Each interval fecal sample was thoroughly mixed and subsampled twice. One fecal subsample was dried in a forced-air oven at 50 C for 36 h and stored indefinitely and the other was placed into plastic container and refrigerated.

After two weeks of refrigeration, subsamples were mixed and three 10 g (as-is) fecal aliquots were weighed and placed on paper towel blotters in 15 X 100 mm petri dishes. Unfed seed was divided into two groups, unscarified seed and scarified seed (scarified with a emory cloth). Three replications of 50 seeds from each unfed group were prepared for germination according to the Rules of Seed Testing (Association of Official Seed Analysts 1965). Eight ml of tap water was added to both the fecal and control seed samples in petri dishes which were sealed with masking tape to retain the moisture. One petri dish was randomly placed on each of three trays in a Stults germinator set at a constant 17 C, dark for 10 d. This experiment consisted of 11 fecal sample collection times and 2 controls (one scarified and one unscarified) with 3 replications of each in a randomized complete block design. Germination (protrusion and elongation of the root radicle) was determined at 4 and 10 d. In the unfed seed, the number of seedlings considered abnormal and firm seed (seed that remains hard but neither germinates nor decays) were counted at the end of the 10 d germination period.

In the second study, twenty g of blown seed (approximately 16,530 seeds) were enclosed in a gelatin capsule to reduce seed losses encountered in the preliminary study. Capsules containing seed were deposited directly into the rumen via rumen fistula. Animals were kept in metabolism stalls and were fed a 0.9 kg CP supplement, vitamin and mineral mix and 5 kg good quality prairie hay (cut annually from a prairie meadow in early July) daily.

Total feces were collected every 8 h from metal trays positioned behind the metabolism stalls. Fecal samples were collected for 96 h as was done by Burton and Andrews (1948); Glendening and Paulsen 1950; and McCully (1951), to allow time for complete passage of all seeds.

Seed recovery from feces was accomplished by placing 25 g of each fecal sample into a slant edge bowl and adding water and a water softener to make a slurry (H. Jordan, personal communication). Arrowleaf clover seeds are heavier than much of the particulate matter in the fecal slurry and settle to the bottom of the dish. The light particles were skimmed off the top of the mixture and the remaining slurry, which contained the seed, was allowed to separate. Both the light particles and the heavier slurry were filtered through the screen of a tea strainer. More water was added to the slurry and the process repeated until all fecal material had been filtered. When all feces had been removed, seeds were counted and percent recovery and excretion curves determined (Balch 1950). Germination was determined simultaneously with seed recovery. Arrowleaf clover seed found in each fecal sample were germinated at 20 C for 10 d immediately following the 96 h fecal collection.

Since few seeds were found in the feces before the 48 h collection only seeds recovered from the 64, 72 and 80 h fecal samples were used to determine germination of recovered seed. Samples from these collection periods were chosen as intermediate between the 48 h and 96 h collection periods. Recovered seed from each of the three fecal samples were divided into two groups. One group of seeds was scarified with an emory cloth prior to germination and the other placed directly into the germinator without scarification. Germination of unfed arrowleaf clover seed, both scarified and unscarified, was also tested for comparison.

Results and Discussion

No recovery of seed was conducted in the preliminary study. In the second study, less than 0.1% of the total fed seed was recovered in the first three 8 h interval fecal samples (Table 1). The presence of seed in the feces collected at 8 h post-dosing was not entirely unexpected. The reticulo-omasal orifice is more than 2 cm in diameter in the cow (Poppi 1980, Welch 1981). Arrowleaf clover seeds are certainly small enough to pass immediately through the reticulo-omasal orifice if the seed escapes the rumen mat and falls close enough to the orifice following ingestion. Seed recovery had increased to 0.5% at the 32 h collection. The peak of recovery was at 80 h post-dosing. At 96 h post-dosing the amount of recovered seed had decreased to 0.6%. The majority of arrowleaf clover seed was excreted between 56 and 96 h (Table 1). Overall, an average of 11% of the total seed given to the heifers was recovered in the feces, a recovery rate lower than previously reported for ingested seed. McCully (1951) recovered 50% of ingested mesquite seed from the feces of mature cows, while Fisher (1947)

Table 1. Percent of total arrowleaf clover seed fed which were recovered in bovine feces in the second study.

HOUR	% Recovery
8	0.02
16	0
24	0
32	0.52
40	0.07
48	1.58
56	1.36
64	2.15
72	0.75
80	2.80
88	0.98
96	0.61

Table 2. Germination percentages of unscarified and scarified seed for three treatments.

SEED TREATMENT	% GERMINATION	
	UNSCARIFIED	SCARIFIED
UNFED	21	92
RETAINED IN FECES	0	0
RECOVERED FROM FECES	1	90

recovered 45% and Harmon and Keim (1934) recovered 23% of mesquite seed fed to calves.

In the preliminary study, none of the arrowleaf clover seed in feces germinated, whereas, unfed, unscarified seed resulted in 21% germination (Table 2). In both studies, no germination occurred for arrowleaf clover seed without first washing it free of feces. No statistical analysis of seed germination was conducted. Germination of seed in feces, for species other than arrowleaf clover, has been described in previous literature, however. Fisher (1947), Glendening and Paulsen (1950), Lehrer and Tisdale (1956), and Everitt (1983) observed limited germination of a variety of seeds after passage through the digestive tract of cattle, sheep, mules, rabbits, and peccaries. Timmons (1942), reported 62% of the pricklypear cactus (Opuntia spp.) seed in jackrabbit feces would germinate compared to 44% for hand picked seed. Some studies, however, suggest that digestive action may decrease seed viability. Livingston, 1972, found germination of both pasture juniper (Juniperus communis) and eastern redcedar (Juniperus virginiana L.) was reduced after passage through the digestive tract of birds.

A small amount of arrowleaf clover seed passed intact through the digestive tract of the heifers. Since seed, contained in feces, did not germinate either the seed which survived digestion was not viable or only hard seed survived the digestive process. Germination of seed recovered from feces was only 1%, much lower than the 90% germination noted for the recovered seed that was hand-scarified (Table 2). Unfed arrowleaf clover seed displayed a 21% germination without scarification and 92% when scarified. The percent germination of scarified seed was similar for the control and fed seed. There was, however, a large difference between the the unscarified, unfed seed and the unscarified, fed seed. From these data, it seems probable that only hard seed survived digestion. Seeds with a softened seedcoat were apparently destroyed or the embryo damaged during the digestive process.

Only one fed, unscarified seed germinated. We could not determine at what point in the digestion or recovery processes the seedcoat was broken. Improved seedcoat permeability due to weathering rather than digestive action may account for arrowleaf clover seed observed germinating from cattle feces in the pasture.

Additional experiments are needed to establish length of seed retention and viability of seeds which remain in the digestive tract longer than 96 h. An in vitro digestion study could be conducted to determine the rate of seed disappearance. Studies considering how different diets may indirectly influence arrowleaf clover dissemination and stand establishment by livestock might also be appropriate. However, the effect of animal diet on establishment of arrowleaf clover, through cattle, may be related more to rate of seed passage than to increased germination. According to Atkeson, Hubert, and Warren (1934), time of seed retention may be directly related to germination reduction in several weed species. Furthermore, length of time viable seed is excreted could be important in pasture management.

There is an apparent difference in the germination of mesquite seed recovered in the feces of calves and mature cows (Glendening and Paulsen 1950; Lehrer and Tisdale 1956). A study dealing with the effect of animal age on embryo viability and germination of arrowleaf clover seed, to determine if a difference exists between stocker calves and mature cows in clover establishment would be worthwhile.

Management Implications

If establishment of arrowleaf clover in a pasture is not desired, cattle that have eaten arrowleaf clover containing seed should not be left on the pasture more than 24 h or they should be moved to the pasture no earlier than 96 h after feeding. If, however, the producer wishes to establish an arrowleaf clover stand using cattle to disperse the seed, he should allow the cattle to remain on the field to be established for at least 4 days. The producer should also be aware that when using cattle for seed dispersal more than one year may be required for good stand development.

CHAPTER IV

CHARACTERISTICS OF ARROWLEAF CLOVER IN A NATIVE TALLGRASS PRAIRIE

Abstract

Incorporating a legume into a perennial grass sod can increase yield, quality and length of grazing season. Arrowleaf clover can be effectively combined with some warm season grasses, but no studies have reported production and forage quality of arrowleaf clover in native tallgrass prairie. Our objectives were to determine the 1) nutritive characteristics and yield of arrowleaf clover when grown in native tallgrass and 2) effect of phophorus fertilization on the nutritive characteristics and yield of arrowleaf clover.

Six treatments of various combinations of arrowleaf clover seed and P_2O_5 and a control were established in a native tallgrass prairie. Four replications of each treatment were used in a randomized block design. Yield data were obtained in June and August. Samples for nutritive analysis were taken at vegetative and bloom stage year 1 (Y1) and year 2 (Y2), then hand separated into clover, grass, and forbs. Clover was further divided into leaf and stem which were analyzed for crude protein (CP), neutral detergent fiber (NDF), cellulose (C) permanganate

(PL), and ash. These determinations were made for each morphological part at each sampling period. Crude protein of grass in bloom stage of Y2 was measured.

In the leaf, CP ranged from 19 to 28% and remained relatively constant with advancing maturity. Both % PL and % cell wall (CW) of the leaf decreased as plants matured. The stem portion of arrowleaf clover declined in quality with maturity, as measured by % CP ($p<.001$), % CW, % cellulose (C), and % PL ($p<.01$). Forage from the high P treatments were higher ($p<.05$) in CP, CW, PL, and C. There was a significant decrease ($p<.05$) in leaf:stem ratio for forage with high levels of P, however, the yield from the high P treatments was significantly higher ($p<.05$) than the no P, low P or medium P treatments.

CP of native tallgrass forage was analyzed the second year of collection and ranged from 6 to 9%. The high P treatment was significantly lower ($p<.05$) than all but the control treatments. Mean CP in June was $11\pm.6$% and did not differ among treatments or between years.

In recent years, arrowleaf clover (Trifolium vesiculosum Savi) has received considerable attention in the southeastern United States where production is not limited by insufficient rainfall. Arrowleaf clover is used primarily as a companion legume which can be incorporated into many types of existing pastures. It has many features which make it more valuable as a companion legume than some other clovers, such as white clover (Trifolium repens L.) or crimson clover (Trifolium incarnatum L.). Arrowleaf clover has earlier production in the spring and is later maturing than most clovers allowing more actual grazing days. It is also more drought tolerant and seems to have a lower bloat potential than many of the other varieties of clover (Hoveland 1974).

Presently, no nutritive data is reported for arrowleaf clover in the National Research Council's nutrient requirements (NRC) for beef cattle (1984) or in the joint United States - Canadian Feed Composition Tables (1982). Cattle have been observed selecting arrowleaf clover over young coastal bermuda grass even when it blooms and becomes stemmy (Hoveland et al. 1970). Akin and Robinson (1982), found the digestibility of arrowleaf clover leaves did not decrease until seed set, but digestibility of the stem declined as phenological development progressed. Arrowleaf clover has a combination of high digestibility and high crude protein (Hoveland et al. 1972, 1974). Levels of CP range from 23% in mid-April to 13% in late May (Hoveland et al. 1972). Although various combinations of clovers and grasses have been used to improve pastures (Van Keuren and Hoveland 1985), there is little or no information available regarding the nutritive characteristics of arrowleaf clover in a mixed species pasture.

The objectives were to determine 1) the nutritive characteristics of arrowleaf clover (including crude protein, fiber components, and leaf to stem ratio) when grown in a native tallgrass prairie and 2) the effect of phosphorus fertilization on the nutritive characteristics and yield of arrowleaf clover.

Material and Methods

Yuchi arrowleaf clover was overseeded onto a native tallgrass prairie which consisted mostly of big bluestem (Andropogon gerardii), little bluestem (Andropogon scoparius), indiangrass (Sorghastrum nutans) and switchgrass (Panicum virgatum) at the O.S.U. Agronomy Research Range, west of Stillwater, Oklahoma. The soils are of a Grainola-Lucien complex with sandy loam topsoil, characterized as a shallow prairie range site. Preliminary soil testing showed a soil pH of 6.6. The P in the soil was eighty percent (80%) sufficient for optimum clover production with 23 kg ha^{-1}. Precipitation for 1984 and 1985 was 78.3 cm and 113.1 cm, respectively; mean annual precipitation in the study area is 81.7 cm.

Different seeding and phosphorus application rates were used to determine their potential influence on the quality and yield of an arrowleaf clover stand in an existing native tallgrass prairie. There were seven treatments with four replications per treatment. Each experimental plot was 6 x 15 meters with treatments being randomized within each of 4 blocks. Seeding and phosphorus rates are given in Table 3. Fertilizer was applied in August, 1981, at rates of 0, 45 or

Table 3. Amount and date of application of arrowleaf clover and phosphorus to native tallgrass prairie.

TREATMENTS	ARROWLEAF CLOVER kg seed/hectare[a]	PHOSPHORUS kg/hectare
CONTROL	0	0
0P	11.2	0
135P	11.2	45b+90c
180P	11.2	90b+90c
0P	22.4	0
45P	22.4	45b
90P	22.4	90b

a clover seeded October 1981
b P_2O_5 applied August 27, 1981
c P_2O_5 applied September 16, 1983

90 kg of actual P from P_2O_5 ha^{-1}. The plots were then seeded in October, 1981, with 0, 11.2 or 22.4 kg seed ha^{-1}. After the first year (1982) of establishment, no visual difference could be distinguished between clover stands from the low and high seeding rates (W. McMurphy unpublished data). Plots were not mowed until September of 1982 to allow reseeding of clover. In 1983, yield data from other trials indicated that 90 kg P ha^{-1} did not permit maximum yield, so an additional 90 kg P ha^{-1} from P_2O_5 was added to the high P treatments to permit an evaluation of the effect of even higher P levels (Westerman et al. 1984). Yield data were collected by the O.S.U. Agronomy Department. On approximately June 1 (clover yield) and August 15 (grass, clover and forbs yield) the entire plots were mowed to stubble height of 13 cm, using a Carter Self-Propelled Harvester. Treatment 1 (control) was mowed only in August since there was no arrowleaf clover in the plots of that treatment. The forage was weighed to determine wet weight then a subsample was obtained and used for DM determination. Forage samples from each plot were hand collected from randomly chosen areas of the plot on May 4 (vegetative stage) and June 4 (mid-bloom) of year 1 (1984) and June 7 (mid-bloom) of year 2 (1985).

Forage samples were hand separated by species. Leaf to stem ratio of the clover was determined by taking dry weight of the separated leaf and stem. Samples were dried at 50-C in a forced air oven for 36 hours and ground using a Wiley

Mill equipped with a 1 mm screen. Chemical analyses of the clover included CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), permanganate lignin (PL) and ash (Goering and Van Soest, 1970). Cellulose was determined by difference of PL and ash. Only in year 2 was the residual native grass from the mixed stand retained and crude protein measured by the Kjeldahl procedure (A.O.A.C. 1980).

Statistical Analysis

Clover and grass quality data were analyzed as a randomized block using Least Squares analysis of variance procedures from the Statistical Analysis System (SAS Institute Inc. 1986). The model included treatment, date, replication and appropriate interactions for each variable (dry matter, CP, NDF, ADF, PL, C, and leaf:stem ratio). Yield data was analyzed using Least Squares Difference.

Results and Discussion

Nutritive Characteristics

Throughout this paper the terms higher and lower are used to refer to significance at the $p<.05$ level, unless otherwise stated. Nutritive values of clover from the high P treatments (135P and 180P) tended to be either significantly above or below values of the no P (0P), medium P (90P) and low P (45P) treatments. Crude protein of the leaf ranged from 19-28% which was similar to CP values of the whole arrowleaf clover plant (23% in mid-April and 13%

in late May) reported by Hoveland et al. (1972). In May, the CP was 25-40% higher in the 135P and 190P leaves than in the 0P, 45P or 90P (p<.001). As the clover matured, CP values of the leaf declined (p<.01) in the 135P and 180P treatments. The CP content of the 135P and 180P tended to remain slightly above CP values for all other treatments in June, however, there were no significant differences among treatments (Figure 1).

The NDF (Figure 2) content of the leaves will be referred to as cell wall (Van Soest et al. 1967). Cell wall (CW) content at the vegetative stage, was similar for clover in most treatments, only the forage from 45P, with 39%, was significantly lower than forage from 135P with 48% (p<.05). At bloom stage CW content was lower in 40P than in either the 135P or 180P treatments. As the clover progressed in maturity, CW tended to decrease, though only significantly so for unfertilized clover.

Percent PL of the leaf (Figure 3) was greater (p<.01) for 135P and 180P (12%) than 0P (4%) or 45P (4) and 90P (7%) in May. Also in May, PL was higher for forage in the 90P treatment than for 45P and one of the 0P treatments. Lignin content was nearly 50% higher for clover leaves in the 135P than 0P or 40P treatments. The most suprising aspect of the PL (leaf) data was the marked decline (p<.01) which was noted in the 135P and 180P treatment as the plant matured. There was a tendency for PL to decrease in all treatments, except one 0P treatment. Again, this decrease in CW and PL

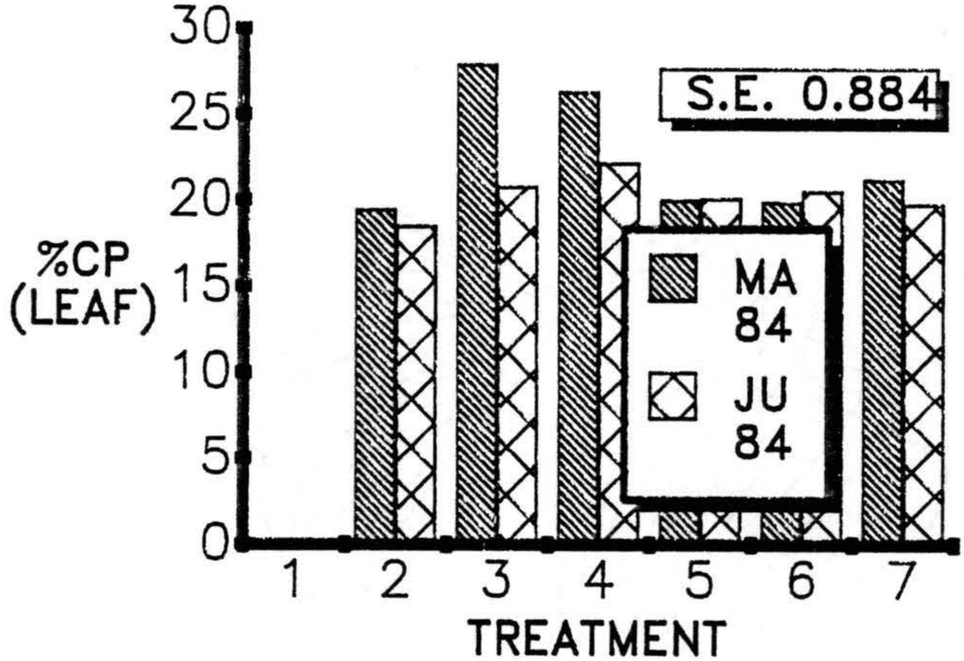

Figure 1. Comparison of Mean Crude Protein Content in the Leaf of Arrowleaf Clover Among Phosphorus Treatments at Two Stages of Maturity

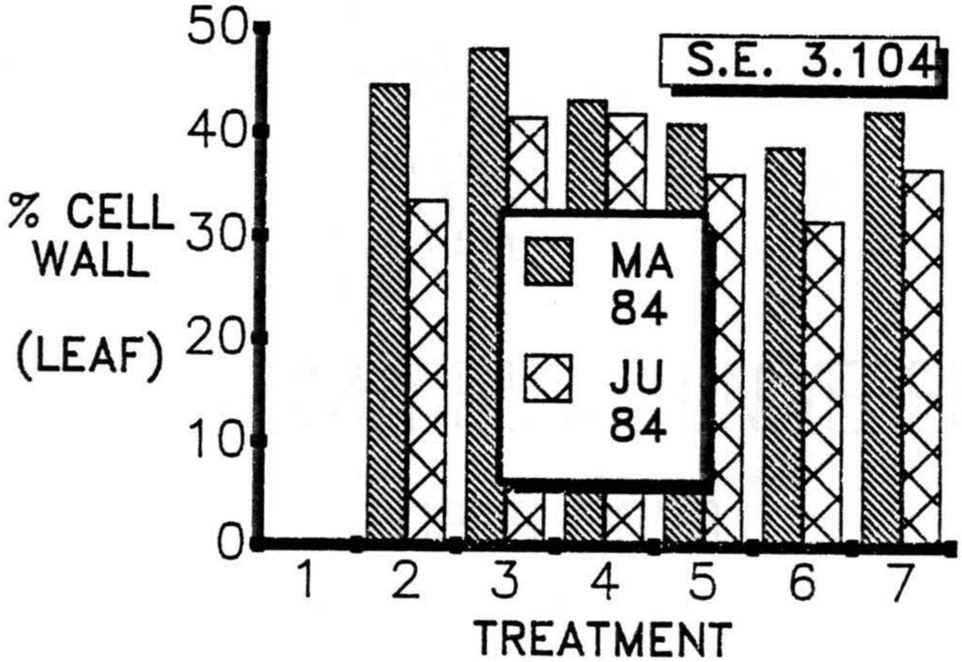

Figure 2. Comparison of Mean Cell Wall Content in the Leaf of Arrowleaf Clover Among Phosphorus Treatments at Two Stages of Maturity

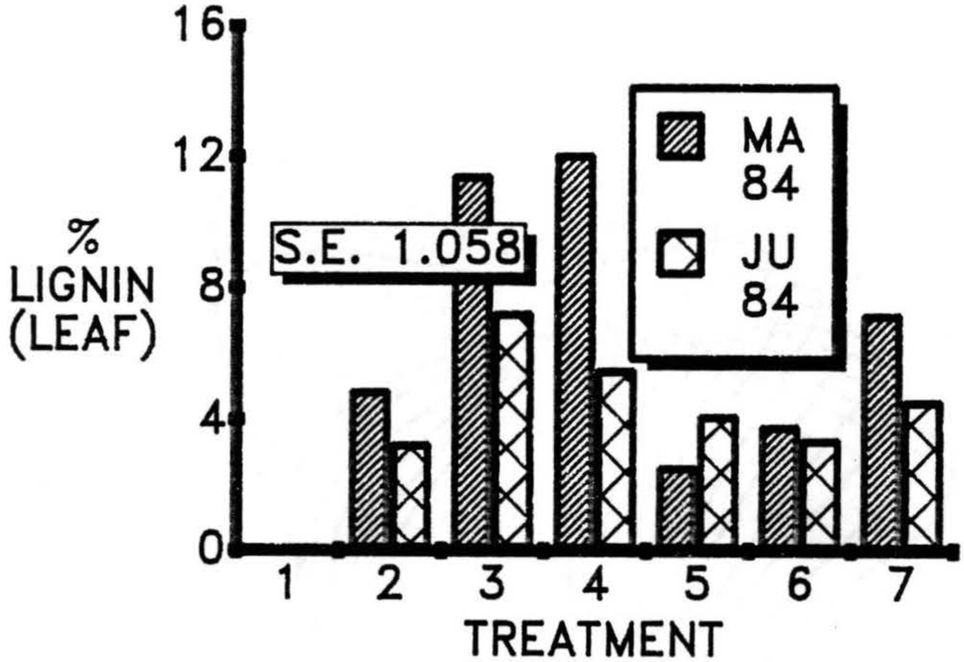

Figure 3. Comparison of Mean Lignin Content in the Leaf of Arrowleaf Clover Among Phosphorus Treatments at Two Stages of Maturity

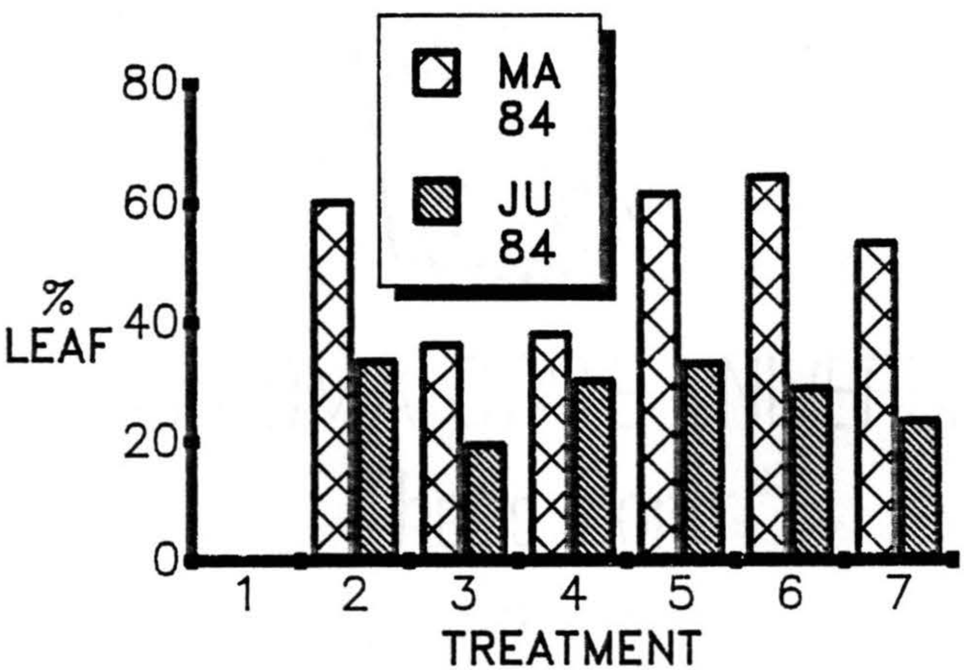

Figure 4. Comparison of Leaf:Stem Ratio, Reported as Mean Percent Leaf, Among Phosphorus Treatments at Two Stages of Maturity

might account, in part, for the increase in dry matter digestibility (DDM) of arrowleaf clover leaves with increasing plant maturity, until the seed set stage which was reported by Akin and Robinson (1982).

The C portion of the leaf was greater ($p<.01$) in 180P than in 0P, when clover was cut while still in the vegetative stage. Younger forage had less C in the leaf ($p<.01$) than older forage. No data concerning C content of arrowleaf clover was found in the literature. In other leguminous species, though, C content has been measured and decreases with increasing plant maturity, similar to the percent C decrease found in this trial for arrowleaf clover leaves .

The stems of arrowleaf clover expressed the normal decline ($p<.01$) of quality usually associated with increasing maturity. There was a notable reduction ($p<.001$) of CP from vegetative to bloom stage for all treatments. The CP of the stem ranged form 8-13% but did not vary significantly among treatments prior to blooming. At bloom stage, CP content of the stems in 180P was lower than the 45P treatment. CW increased ($p<.01$) as the clover matured in 135P and 180P and one of the 0P treatments. In May, CW content was 45% in the 135P and 180P which was higher than the 38% noted for 90P. In June, more of the stem was contained in the cell wall fraction of 180P than of the stem of one 0P treatment($p<.05$).

As with the CW fraction, the C and PL fractions of the stem increased as the plants matured ($p<.01$). In May, the 22% C in the stem with 135P and 180P was greater ($p<.05$) than the 16% C in the stem of the 0P, 45P, and 90P treatments. The percent C from the 135P and 180P was higher than 0P only, in June. Percent PL ranged from 5-12%. The PL content of leaves from 135P treatment was higher than from 0P, 45P, and 90P treatments, while 180P was greater than 80P, in May. From clover at bloom stage, 180P was significantly higher than 0P, 45P, and 90P.

In most instances, the variations between years were slight yet there were some significant differences. In the stem, lignin content of 180P was lower in Y2 ($p<.01$). The percent of CP was lower ($p<.05$) in Y2 for stems from NP and 90P and also for the 135P and 180P treatments ($p<.001$). CP was the only fraction of the leaf that changed between years; CP from treatments 135P and 0P was higher in the second year of forage collection. The leaf:stem ratio (Figure 4) increased ($p<.05$) from Y1 to Y2 for clover of 0P, 90P, and 180P. The percent leaf of 0P, 45P, 90P and 135P and 180P was 61, 56, and 37%, respectively.

Since precipitation was the only weather factor which was recorded and weather conditions change from year to year, it is probable that some of the variation between years was due to changes in weather conditions. It was not possible, therefore, to determine the percent of variation that was due to differences in weather.

The CP content of the total forage harvested in June was

11$\pm$.6 in Y1 and in Y2. No significance could be determined between years or among treatments. This corresponds well with the 11.8% CP reported by Stanley et al. (1968) from arrowleaf clover harvested from early March to late May. The CP content of the total forage was similar to the CP content of the grass (at bloom stage) which ranged from 6-9%. If allowances were made for selective grazing by cattle, however, the differences would likely be significant. The control did not differ in CP content from any other treatments. 135P was lower than all treatments, except the control. Waller et al. (1972) found that native grass contained insufficient levels of CP for maintenance of beef cows for 9 months of the year (no allowance for selective grazing). Establishing arrowleaf clover in the native range improves the nutrient potential of the pasture as well as decreasing the nuber of days when supplemental protein must be fed.

Yield

Application of P at high levels (135 or 180 kg ha^{-1}) had ($p<.05$) significant influence on both quality and yield of arrowleaf clover in native range. The yield of 45P was not different from the 0P. The plots with 90P treatments in Y1 (Figure 5) yielded nearly twice as much clover as the 0P plots and the yield of clover from the 135P and 180P plots were three times greater ($p<.05$) than from the 0P plots.

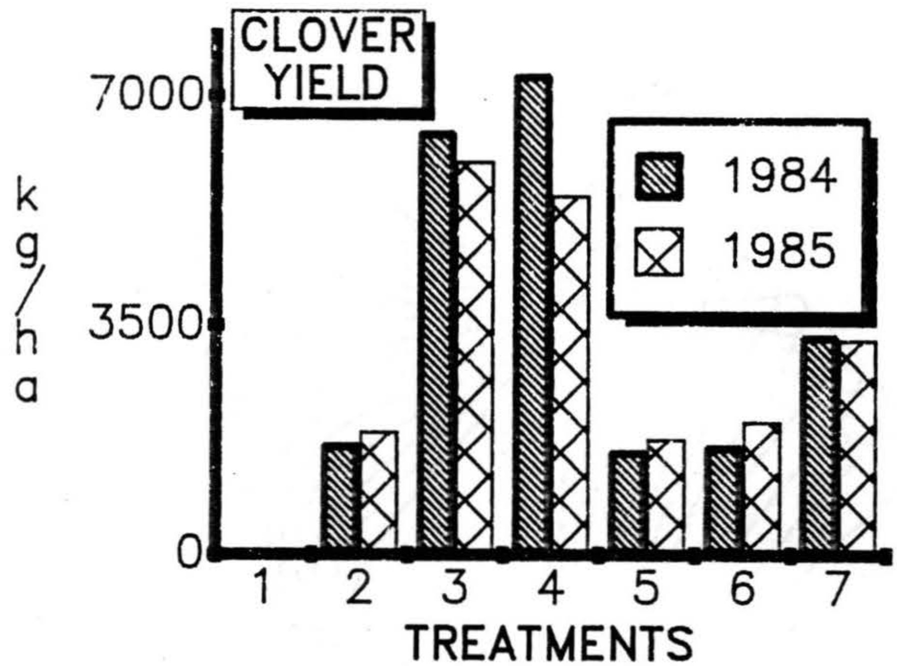

Figure 5. Comparison of Mean Arrowleaf Clover Yields Among Phosphorus Treatments for Two Consecutive Years

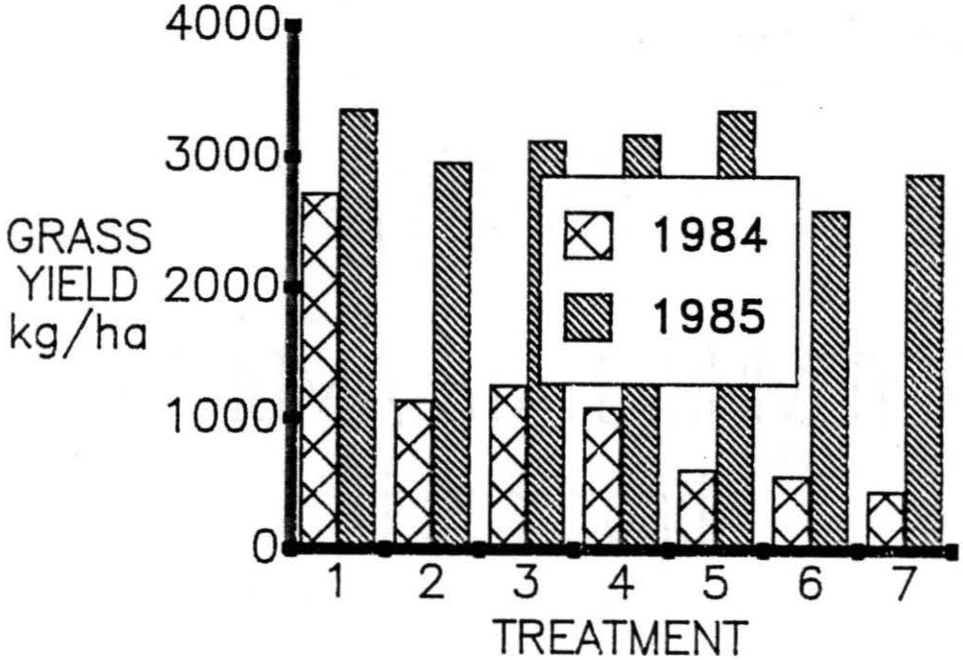

Figure 6. Comparison of Mean Grass Yields Among Phosphorus Treatments for Two Consecutive Years

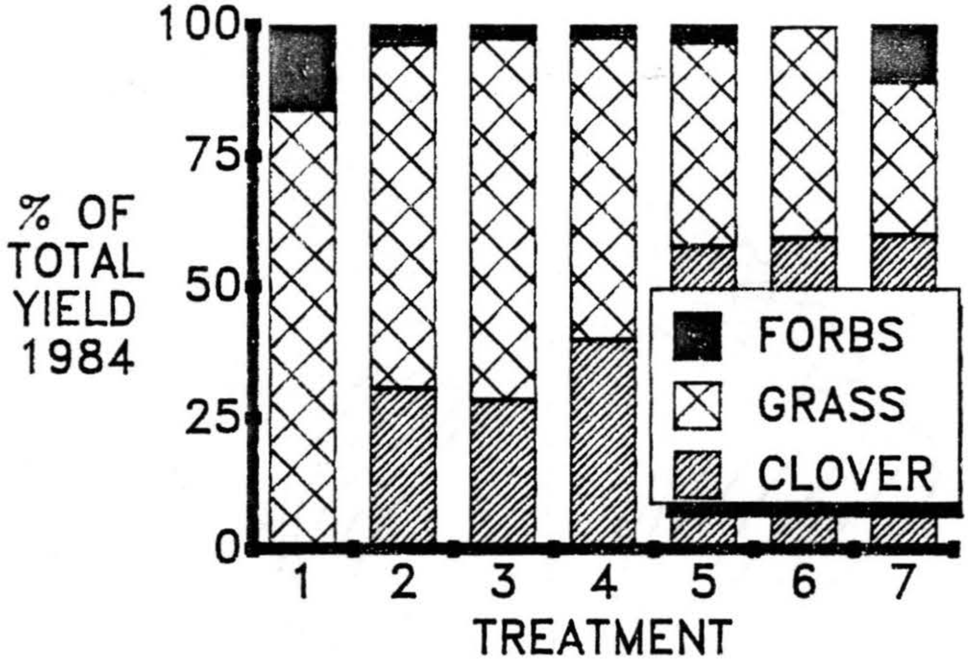

Figure 7. Yield of Arrolweaf Clover, Grass, and Forbs Presented as a Percent of Total Biomass (1984)

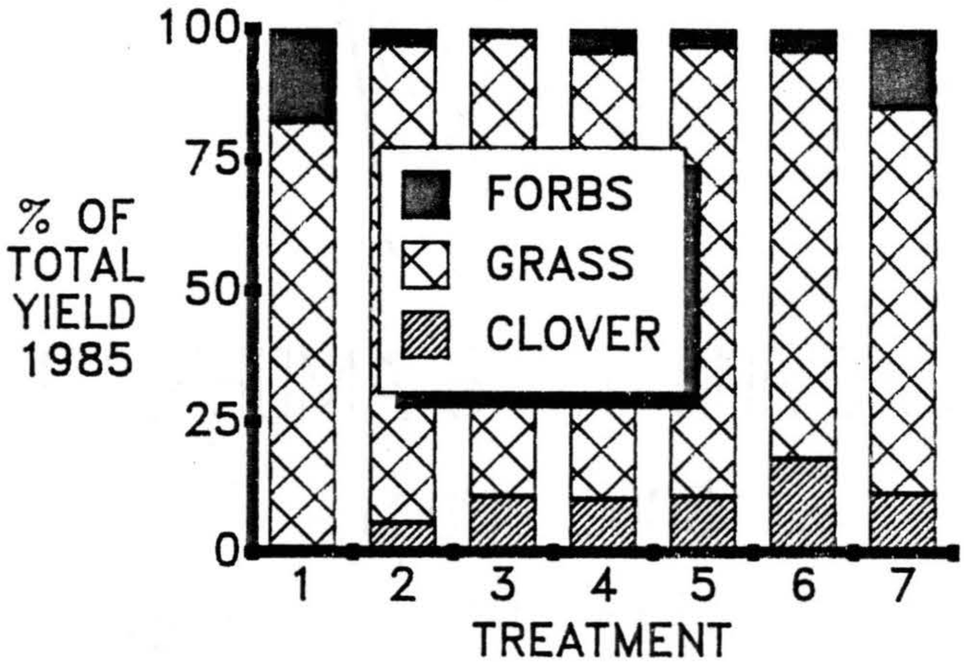

Figure 8. Yield of Arrowleaf Clover, Grass, and Forbs Presented as a Percent of Total Biomass (1985).

The pattern was similar in Y2 although there was an unexplainable increase in yield of clover for 0P and 45P while yields from 90P and 135P and 180P treatments decreased.

There may be some concern that when arrowleaf clover is incorporated into native tallgrass prairie it will compete for nutrients, light, and water and eventually eliminate the grass from the stand. It appears, however, from yield data taken in August (Figure 6), when clover is normally dormant, that even at high levels of P, the majority of the increase in clover is offset by a decrease in forbs (Figures 7 and 8). Evers (1979) reported arrowleaf clover in the first year of establishment was as effective as some chemicals and more effective than those chemicals in following years. Arrowleaf clover also seems to be effective against a wider range of weed species than many chemicals. It may be possible that incorporating arrowleaf clover into native range actually protects the grass from invading weeds rather than inhibiting grass growth. The high clover yields in 1984 were due to early fall regrowth rather than a replacement of the grass stand.

Conclusions

The quality of arrowleaf clover leaves decreased ($p<.05$) with increasing maturity. This was true for % CP, % CW and % PL for all of the P treatments yet the only significant increase in quality was in the 135P and 180P.

The increase in quality might have been due to the loss of old leaves from the plant due to shading from the canopy, created by arrowleaf clover plants, when adequate P is available. However, these data are similar to those of Akin and Robinson (1982), where arrowleaf clover leaves increased in digestibility from prebloom to full bloom then rapidly declined at the seed set stage. It is probable that a similar pattern would have been established in this trial, if clover had been sampled at the seed set stage of clover maturity. The differences among treatments seemed to be more pronounced in the leaves than stems. Quality of the stem decreased as the plant matured which also supports the data reported previously (Akin and Robinson 1982 and Hoveland et al. 1970).

Data generated from the detergent fiber analysis system could also be used to predict digestibility and net energy of the forage. In recent years, many forage testing laboratories have included a predicted net energy of lactation (NE_l) and/or predicted dry matter digestibility (DDM) in their forage analyses (Coppock 1981). Comparison of the DDM predicted values with In Vitro Dry Matter Digestion (IVDMD) values (Tilley and Terry 1963) might be considered for future studies.

Further studies, involving the effect of temperature, relative humidity and cloudy verses cloudless days as well as precipitation on quality and yield could be useful. Stanley et al. (1968) and Hoveland (1974) both stressed the importance of a proper clipping interval and height of stubble, for persistence of arrowleaf clover in a stand. Therefore, a study concerning the affect of grazing on grass:legume ratio is needed if the range is to be used for grazing. Finally, determining the nitrogen equivalence of arrowleaf clover in native range and its effect on soil fertility and CP of the native grass similar to the studies by Lynd et al. (1984a and 1984b), could be very beneficial to the pasture manager.

CHAPTER V

SUMMARY AND CONCLUSIONS

Arrowleaf clover is a high quality forage that can be effectively combined with warm-season grasses to increase the quality and grazing potential of a pasture. An alternative method of establishing clover in a grass pasture, that may be both inexpensive and practical, is dissemination of seed, through cattle. To determine the usefulness of an arrowleaf clover/native tallgrass prairie combination, it is necessary to examine both the effect of clover on grass and the influence of phophorus on both clover and grass. Our objectives, in this study, were to determine 1) the recovery and viability of arrowleaf clover seed ingested by cattle 2) the nutritive characteristics of arrowleaf clover when grown in a native tallgrass prairie and 3) the effect of phosphorus fertilization on the nutritive characteristics and yield of arrowleaf clover.

In the first experiment, known amounts of seed were fed to 4 cannulated, 3-year old heifers. Two studies were conducted 1) two heifers were given seed via the rumen cannula while the remaining three heifers were fed seed along with daily feed rations; 2) seed enclosed in gelatin capsules were administered through the rumen cannula in all animals. Total feces were collected every 8 h, after administration of seed, for a period of 88 h in the first study and 96 h in the second. Fecal samples, in the second study were washed and sieved to remove seed. The recovered seeds were dried, counted, divided into two groups (unscarified and scarified)and examined for differences in germination. Three replications of seed from the unfed seed and all collections (seed contained in the feces and seed recovered from the feces) were used in a complete randomized block design. The design consisted of one replication of each collection and control on each of 3 germinator trays.

In the second experiment six treatments and a control were established in a native tallgrass prairie, by the addition of various combinations of seed and P_2O_5. Four replication of each treatment were used in a randomized block design. Samples were harvested at vegetative and bloom stage, Y1 and bloom stage of Y2 and each sample was hand separated into clover, grass, and forbs. Clover was further separated into leaf and stem, then crude protein (CP), neutral detergent fiber, acid detergent fiber, permanganate lignin, and ash were determined for both leaf and stem of clover at each sampling period. Grass, harvested in June Y2, was analyzed for CP.

Arrowleaf clover seed, recovered in the feces, accounted for only 11% of the total number of seeds fed to the animals. Seed was first noticed at 24 h and peaked at 80 h post dosing; some could still be found at 96 h.

Therefore, the producer who wishes to establish arrowleaf clover through this method, should have cattle on the target field within 24 h after seed is fed. Also, cattle should not be removed from the pasture before 96 h post ingestion, for maximum effectiveness.

Germination of unscarified arrowleaf clover seed was 0% for seed contained in the feces, 1% for seed recovered from feces and 21% for unfed seed. When seed from the same source were mechanically scarified, germination jumped to 90% of recovered and 92% of unfed seed. It may be possible that the 1% germination of unscarified seed, recovered seed actually represents the true percentage of seed, post-digestion, that is viable and has a softened seedcoat. If this is true, the arrowleaf clover seed which has been observed germinating from fecal material in pastures may represent 1% of all the clover seed which were ingested. Conversely, since seed in feces failed to germinate, the 1% of recovered seed that germinated may be due to scarification during collection or recovery. If the scarification occurs after passage through the digestive tract, time and weather, rather than digestive action may be the causative factors of seed germinating from dung piles in the pasture. Whatever the mechanism of scarification, one should remember that 90% of recovered seed is viable.

A future study might be conducted which considers how different diets may indirectly influence arrowleaf clover dissemination and stand establishment by livestock. The effect of animal diet, though, may be related more to rate of seed passage than to increased germination percent of seed. Also, an experiment dealing with the effect of animal age on embryo viability and germination of arrowleaf clover seed, to determine if a difference between stocker calves and mature cows in clover establishment, could be worthwhile.

It appears that the quality of arrowleaf clover leaves either remains constant or improves at the plant matures. The leaves from all treatments displayed a tendency to decrease in % cell wall and lignin with age while CP content was unchanged in all but leaves from the high P treatments. Arrowleaf clover stems decreased in CP ($p<.001$), increased in % cell wall, % lignin, and % cellulose ($p<.01$) with increasing maturity.

Arrowleaf clover yields, in May, ranged from 1 to 3 metric tons ha-1; the highest yields occurred in the plots with high P. Grass production measured from the August harvest, increased from Y1 to Y2 in all plots but the greatest growth occurred in the control and high P plots.

Additional studies might be conducted to determine the amount of improvement in quality of arrowleaf clover leaves as the plant matures, and to what stage of maturity this improvement might be observed. Finally, the effect of grazing verses mowing as well as variations in weather conditions on yield and quality of the clover and grass could be investigated.

LITERATURE CITED

Akin, D.E. and E.L. Robinson. 1982. Structure of leaves and stems of arrowleaf clover and crimson clover as related to in vitro digestibility. Crop Sci. 22:24-29.

A.O.A.C. Official methods of analysis (13th ed.) 1980. Association of Official Analytical Chemists. Washington, D.C.

Association of Official Seed Analysts. Rules for seed testing. 1965. Proc. Assoc. Official Seed Anal. Duane Isley (ed.). Graphic Publishing Co., Inc. Lake Mills, Iowa. Vol. 54. No. 2.

Andrews, C.S. and C. Johansen. 1978. In: Plant relations in pastures. p. 119.

Atkeson, F.W., H.W. Hubert, and T.R. Warren. 1934. Effect of bovine digestion and of manure storage on the viability of weed seeds. J. Amer. Soc. Agron. 26:390-397.

Balch, C.C. 1950. Factors affecting the utilization of food by dairy cows. British J. Nutr. 4:361.

Bates, R.P. 1986. Forage yields from legume varieties and strains. R-215. Samuel Roberts Noble Foundation. Ardmore, Oklahoma.

Baylor, J.E. 1974. In: Forage Fertilization. D.A. Mays (ed.). p. 182.

Beaty, E.R. and J.D. Powell. 1969. Forage production of amclo and crimson clover on Pennsecola and Coastal bermudagrass sods. J. Range Manage. 22:36-39.

Blankenship, J.D. and D.R. Smith. 1967. Breaking seed dormancy in Parry's clover by acid treatment.J. Range Manage. 20:50.

Burton, G.W. and J.S. Andrews. 1948. Recovery and viability of seeds of certain southern grasses and Lespedeza passed through the bovine digestive tract. J. Agric. Res. 76:95-103.

Coppock, C.E., C. G. Woelfel, and R.L. Belyea. 1981. Forage and feed testing programs - problems and opportunities.

J. Dairy Sci. 67:1625-1633.

Everitt,J.H. and C.L. Gonzalez.1983. Germination of the honey mesquite (Prosopis glandulosa) seed after passage through the digestive tract of peccaries.

Evers, G.W. 1979. Production and reseeding of cool-season annual clovers in Southeast Texas. Texas Agr. Exp. Sta. PR-3591.

Evers, G.W. 1983. Weed control on warm season grass pastures with clovers. Crop Sci. 23:170-171.

Evers, G.W. 1986. Forage legumes for Texas. Texas Agr. Exp. Sta. MP-1610.

Fisher, C.E. 1947. Present information on the mesquite problem. Texas Agr. Exp. Sta. Prog. Rept. 1056.

Glendening, G.E. and H.A. Paulsen. 1948. Recovery and viability of mesquite seeds fed to sheep receiving 2,4D in drinking water. Bot. Gazette. 111:486-491.

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). USDA-ARS Ag Handb No. 379.

Harmon G.W. and F.D. Keim. 1934. The percentage and viability of weed seeds recovered in the feces of animals and their longevity when buried in manure. J. Amer. Soc. Agron. 26:762-767.

Heady, H.F. 1954. Viable seeds recovered from the fecal pellets of sheep and deer. J. Range Mange. 7:259-261.

Hoveland, C.S. 1967. Registration of Yuchi arrowleaf clover. Crop Sci. 7:80.

Hoveland, C.S., E.L. Carden, W.B. Anthony, and J.P. Cunningham. 1970. Management effects of forage production and digestibility of Yuchi arrowleaf clover. Agron J. 62:115-116.

Hoveland, C.S., R.F. McCormick, and W.B. Anthony. 1972. Productivity and forage quality of Yuchi arrowleaf clover. Agron. J. 64:552-555.

Hoveland, C.S. 1974. Arrowleaf clover (Trifolium vesiculosm SAVI) a valuable clover for the Southeastern USA. Proc. of 12th Internat. Grassl. Congr. (Moscow, USSR) p 192-198.

Hoveland, C.S., J.A. Little, G.W. Granade, and J.G. Starling. 1981. Growth supressant chemicals for establishment of winter annual forages on bahai and bermudagrass sods. Auburn Univ. Agric. Exp. Sta. Bull. 533.

Johnsen, T.M. Jr. 1962. One-seeded juniper invasion of northern Arizona grassland. Ecol. Mono. 32:188-205.

Kendall, W.A. and W.C. Stringer. 1985. Physiological aspects of clover, p.111-117. In: N.L. Taylor (ed.). Clover science and technology. Amer. Soc. Agron. Inc., Crop Sci. Soc. Amer. Inc., Soil Sci. Soc. Amer. Inc. Madison, Wisconsin.

Kilcher, M.R. 1981. Plant development, stage of maturity and nutrient composition. J. Range Mange. 34:363-364.

Lehrer, W.P. and E.W. Tisdale. 1956. Effect of sheep and rabbit digestion on the viability of some range plant seeds. J. Range Mange. 9:118-122.

Lynd, J.Q., E.A. Hanlon, Jr. and G.V. Odell, Jr. 1984a. Nodulation and nitrogen fixation by arrowleaf clover: effects of phosphorus and potassium. Soil Biol. Biochem. 16:589-594.

Lynd, J.Q., E.A. Hanlon, Jr., and G.V. Odell, Jr. 1984b. Soil fertility and defoliation effects with clover and nitrogen fertilizer equivalence of crimson-arrowleaf clover combinations. Agron. J. 76:13-16.

Knight, W.E. 1970. Productivity of crimson and arrowleaf clover grown in a coastal bermudagrass sod. Agron. J. 62:773-775.

Knight, W.E. 1971. Influence of spring mowing on reseeding and productivity of selected annual clovers in a grass sod. Agron. J. 63:418-420.

Livingston, R.B. 1972. Influence of birds, stones and soil on the establishment of pasture junipers, Juniperus communis and red cedar, J. virginiana in New England pastures. Ecology. 53:1141-1147.

McCully, W.G. 1951. Recovery and viability of rose seeds fed to cattle. J. Range Mange. 4:101-106.

McDonald M.B., Jr. and A.A. Khan. 1983. Acid Scarification and protein synthesis during seed germination. Agron. J. 75:111-114.

Mengel, K. and E. A. Kirkby. 1978. Principle of plant nutrition. Internat. Potash Institute (ed.).Switzerland. p.362.

Mengel, K. and E. A. Kirkby. 1978. Principle of plant nutrition. Internat. Potash Institute (ed.).Switzerland. p.362.

Miller J.D. and H.D. Wells. 1985. Arrowleaf clover. In: Clover science and technology. N.L. Taylor (ed.). p 503-515. Amer. Soc. Agron. Inc. Madison, Wisconsin.

NRC. 1984. Nutrient requirements of domestic animals No. 4. Nutrient requirements of beef cattle (6th ed.). National Academy of Sciences - National Research Council. Washington, D.C.

Poppi, D.D. and B.W. Norton. 1980. The validity of the critical size theory for particles leaving the rumen. J. Agric. Sci. 94:275-280.

Rolston, M.P. 1978. Water impermeable seed dormancy. Bot. Rev. 44:365-396.

Siewert, L. and E.C. Holt. 1975. Changes in nutritive value of siratra forage with age. Agron. J. 67:544-546.

Stanley, R.L., E.R. Beaty, and D.N. Palmer. 1968. Effect of age of harvest on yield and cell wall content of Amclo clover. Agron. J. 60:343-344.

The SAS User's Guide. 1979. Helwig, J.T. and K.A. Council (ed.). SAS institute, Inc. Cary, North Carolina.

Tilley, J.M.A. and R.A. Terry. 1963. A two-stage technique for in in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104-111.

Timmons, R.L. 1942. The dissemination of prickly pear seed by jackrabbits. J. Amer. Soc. Agron. 34:413-520.

United States - Canadian Feed Compostion Tables. 1982. NRC-NAS. Washington, D.C.

Van Keuren, R.W. and C.S. Hoveland. 1985. Clover management and utilization. In: Clover science and technology. N.L. Taylor (ed.). p 325-353. Amer. Soc. Agron. Inc. Madison, Wisconsin.

Van Soest, P. J. and W. C. Marcus. 1967. Method for the determination of cell wall constituents in forages using detergent and the relationship between this fraction and voluntary intake and digestibility. J. Dairy Sci. 47:704.

Waller, G. R., R.D. Morrison, and A.B. Nelson. 1972. Chemical composition of native grasses in central

Oklahoma from 1947-1962. Okla. State Univ. Agr. Exp. Sta. Bull. B-697.

Welch, J.G. 1982. Rumination, particulate size and passage from the rumen. J. Anim. Sci. 54:885-894.

Westermann, R.L., J.C. Silvertooth, H.J. Barreto, and D.L. Minter. 1984. Phosphorus and potassium effects on yield and nutrient uptake in arrowleaf clover. Soil Sci. 48:1292-1296.

APPENDIXES

APPENDIX A

Downey Agronomy Research Range
Monthly Precipitation (cm)

Month	1982	1983	1984	1985	Longtime average
Jan		2.90	1.32	3.94	2.95
Feb		7.44	2.41	8.31	3.43
Mar		3.71	13.94	13.28	4.72
Apr		3.45	6.27	17.09	7.26
May	34.47	12.70	8.13	4.98	11.93
June	7.67	9.32	10.16	19.08	10.77
July	4.97	0	0.41	5.79	8.97
Aug	2.08	1.96	1.52	7.34	8.15
Sept	4.72	4.62	3.12	14.35	8.58
Oct	2.26	18.26	13.72	10.11	7.06
Nov	8.00	3.05	4.70	8.30	4.70
Dec	1.52	1.02	12.60	0.53	3.40
Totals		68.43	78.30	113.11	81.74

APPENDIX B

% CRUDE PROTEIN

TREATMENT	CLOVER						GRASS
	LEAF			STEM			
	M-84	J-84	J-85	M-84	J-84	J-85	J-85
CONTROL	*	*	*	*	*	*	8.0
0P	19.5	18.5	21.5	11.8	7.5	9.1	9.4
135P	27.9	20.8	24.5	12.3	6.6	9.7	9.6
180P	26.3	22.2	24.7	12.6	7.4	10.3	6.4
0P	20.0	20.1	20.9	11.6	7.3	8.6	9.6
45P	19.9	20.5	20.4	12.6	7.8	9.2	9.2
90P	21.2	19.8	22.3	12.6	8.1	9.7	9.3

APPENDIX C

TREATMENT	LEAF			STEM		
	% CELL WALL (CLOVER)					
	M-84	J-84	J-85	M-84	J-84	J-85
0P	44.7	33.5	36.3	45.1	58.3	55.7
135P	48.3	41.6	43.9	45.8	59.2	56.8
180P	43.3	42.0	44.7	42.6	60.8	60.1
0P	41.1	36.2	39.6	45.6	52.2	53.0
45P	38.8	31.7	37.0	39.9	57.2	53.6
90P	42.3	35.7	39.1	37.9	57.2	55.2
	% CELLULOSE (CLOVER)					
0P	10.4	12.4	13.0	17.9	25.5	28.7
135P	9.4	13.4	14.5	22.4	31.8	30.5
180P	9.4	12.3	13.7	22.5	32.4	29.7
0P	9.8	13.5	14.2	16.6	28.9	27.3
45P	11.7	15.5	15.3	16.4	28.4	27.1
90P	12.6	13.7	13.3	16.6	28.8	29.0
	% LIGNIN (CLOVER)					
0P	4.9	3.3	2.8	7.0	8.9	8.5
135P	11.5	7.3	5.3	8.6	10.1	9.4
180P	12.1	5.6	4.1	6.4	11.6	9.1
0P	2.6	4.2	5.3	4.6	9.5	8.7
45P	3.9	3.4	2.9	5.5	8.9	8.6
90P	7.2	4.6	4.0	4.6	8.9	8.5

M-84 - MAY 1984
J-84 - JUNE 1984
J-85 - JUNE 1985

APPENDIX D

% LEAF (CLOVER)

	MAY 1984	JUNE 1984	JUNE 1984
0P	60.8	34.1	37.6
135P	36.9	20.0	28.4
180P	38.7	30.9	29.5
0P	62.2	33.7	45.4
45P	65.0	29.5	29.8
90P	53.9	24.1	31.4

YIELD (JUNE)
(kg/ha)

	CLOVER		GRASS		FORBS	
	1984	1985	1984	1985	1984	1985
CONTROL	*	*	*	*	*	*
0P	1705	1890	*	*	*	*
135P	6470	6007	*	*	*	*
180P	7339	5480	*	*	*	*
0P	1605	1778	*	*	*	*
45P	1687	2046	*	*	*	*
90P	3365	3291	*	*	*	*

YIELD (AUGUST)
(kg/ha)

	CLOVER 1984	CLOVER 1985	GRASS 1984	GRASS 1985	FORBS 1984	FORBS 1985
CONTROL	1	35	2724	3364	525	739
0P	538	180	1148	2964	66	99
135P	523	382	1266	3133	47	51
180P	757	381	1094	3181	47	166
0P	920	417	622	3365	55	130
45P	836	603	573	2607	5	140
90P	931	441	456	2888	172	584

VITA

Nancy Kathleen Maher

Candidate for the Degree of

Master of Science

Thesis: GERMINATION OF ARROWLEAF CLOVER SEED IN BOVINE FECES AND CHARACTERISTICS OF PHOSPHORUS FERTILIZED ARROWLEAF CLOVER IN A TALLGRASS PRAIRIE

Major: Animal Science

Biographical:

Personal Data: Born in Ardmore, Oklahoma September 12, 1961, the daughter of John and Nancy Maher.

Education: Graduated from Dickson High School, Dickson, Oklahoma, May, 1979; received Bachelor of Science Degree in Animal Science from Oklahoma State University, Stillwater, Oklahoma, May 1983; completed requirements for the Master of Science Degree in Animal Science at Oklahoma State University December, 1986.

Experience: Raised on livestock and crop farm in south central Oklahoma; assistant lab technician at the S. R. Noble Foundation; graduate research and teaching assistantships at Oklahoma State University, 1984-1986.

Professional Organizations: American Society of Animal Science.