A COMPARISON OF THE RELATIVE NITROGEN FIXING ABILITY OF FIVE LEGUMES

By

RAYMOND W. VAN CAMP

Bachelor of Science
Oklahoma Agricultural and Mechanical College
Stillwater, Oklahoma
1953

Submitted to the faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
1954

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Thesis Approved:

J. C. Lynd
Thesis Adviser

L. W. Reed

D. F. Murphy

Robert Henderson
Dean of the Graduate School

ACKNOWLEDGEMENT

The writer wishes to give his earnest appreciation to the staff of the Agronomy Department of Oklahoma Agricultural and Mechanical College for the advice and help that was so freely given. I wish to thank Dr. J. Q. Lynd for his kind and always helpful advice in this study and in the preparation of this thesis.

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I. INTRODUCTION

Species of the plant family, Leguminosae, have long been recognized as "soil improvers." Even before reasons for this natural phenomena were known it was observed that the growing of clovers and other legumes tended to maintain and increase soil productivity. Likewise, it was known that the continued growing of non-legumes led to soil depletion. It is now generally recognized that the beneficial effects of legumes upon succeeding crops is due largely to three factors; organic matter is added in their crop residues, various plant nutrients are made available in the soil by decomposition of the additional plant materials, and the fixation of atmospheric nitrogen brought about by the activities of the Rhizobium group of nodule organisms.

The objective of this study was to compare the relative nitrogen fixing ability of five legumes under greenhouse conditions. These five legumes, commonly grown in Oklahoma, were sweet clover, Melilotus officinalis L. Lam.; big hop clover, Trifolium procumbens L.; hairy vetch, Vicia villosa Roth.; white clover, Trifolium repens L.; and common lespedeza, Lespedeza stipulacea Max. The nitrogen fixation of these legumes was measured on one soil type, Norge sandy loam. The amount and rates of ammonification and nitrification were measured by soil incubation studies following growth of the legume crops.

II. REVIEW OF LITERATURE

Natural biological fixation of atmospheric nitrogen has long been a fascinating subject of study. This subject received critical study by some of the early agricultural chemists and they realized the complexities involved in this phenomenon. "The subject is one of the most delicate imaginable, and he who enters it requires indulgence," according to Boussingault (Fred et al (5)).*

Priestley 1774-1779, (5) was the first man to propose that the soil improving ability of legumes was due to nitrogen fixed by the legume plant from the air. It remained a very controversial subject until Hellriegel and Wilfarth in 1886 proved that the bacteria in the root nodules of the legume plant were necessary for nitrogen fixation.

Complete and detailed reviews of the voluminous literature on the subject of symbiotic nitrogen fixation are presented by Fred et al (5), Wilson (17) and Waksman (16). The review in this thesis will be concerned only with a number of papers directly applicable to the type of study undertaken.

The actual process of nitrogen fixation is still not known. Jensen (8) states that it is not known whether it is the bacteria that fix nitrogen or that the presence of the bacteria enables the plant to fix nitrogen. According to Jensen the fixation process appears to be the reduction of elemental nitrogen to ammonia, or to hydroxylamine, which then reacts with oxalacetic and or a-ketoglutaric acid. These form the corresponding amino acids and are used by the host plant in its

*Figures in parenthesis refer to Literature Cited pp. 34-35.

protein complex.

Apparently nitrogen compounds may be secreted from the nodules under certain conditions. Jensen (8) further states that the fixation process is hindered by free hydrogen and carbon monoxide gases and that oxygen is essential for the process. Nitrogen fixation is related to photosynthesis, when photosynthesis ceases nitrogen fixation stops. When photosynthesis is normal, nitrogen fixation sometimes becomes so rapid that the host plant cannot incorporate all the nitrogen compounds into its protein complex and, according to Jensen, may be secreted from the nodule.

Fred et al (5) and Wilson (17) relate the nitrogen fixation process to the amounts of carbohydrate produced by the host plant. The greater the amount of carbodydrate in relation to the amino acids that are produced, the larger the amounts of nitrogen that will be fixed.

Waksman (16) classified symbiotic bacteria into seven groups. 1. Alfalfa group, Rhizobium melitoti; 2. Clover group, Rh. trifolii; 3. Pea group, Rh. leguminosarium; 4. Bean group, Rh. phaseoli; 5. Lupine group, Rh. lupini; 6. Soybean group, Rh. japonicum; 7. Cowpea group, Rh. species. Erdman (4) presents a complete cross inoculation grouping for legumes common in the United States. Allen and Baldwin (1) made a rather complete study of strains within several groups. They reported that there is a very marked difference between different strains in their ability to fix nitrogen. There is a change in the amount of nitrogen fixed by pure strains after the successive passage through plant generations.

Fred et al (5) and Wilson (17) point out that the large clump nodules near the tap roots are an indication of effective modulation. Small

nodules well distributed on the root hairs are probably inefficient nitrogen fixers. Erdman (4) maintains that the best method of checking the effectiveness of the nodulation is in the presence or absence of adequate nitrogen in the host plant.

Nitrogen fixation is dependent somewhat on the amount of nitrogen available to the plant from the soil. The greater the supply of available soil nitrogen the less will be the amount of nitrogen fixed by the symbiotic nitrogen fixing bacteria (16). If other conditions for nitrogen fixation are favorable, the amount of nitrogen available to the plant from the soil becomes less important. Greaves and Jones reported that alfalfa fixed up to 50 pounds of nitrogen per acre on soils that were high in organic nitrogen.

Waksman (16) declared that the soil nitrogen was increased from 0 to 400 pounds per acre from plowing under legumes, the average amount being from 50 to 100 pounds per acre. He proposes that about two-thirds of the nitrogen found in legumes normally is fixed by the symbiotic organisms. Erdman (4) proposes the following as averages for the amount of nitrogen fixed per acre by some legumes; sweet clover - 119 pounds, white clover - 103 pounds, vetch - 80 pounds, bur clover - 78 pounds and the annual lespedezas - 85 pounds of nitrogen per acre.

Legumes have high requirements for plant nutrient elements supplied from the soil other than nitrogen. Reynolds (13) demonstrated that a soil that was high in other plant nutrients also produced higher rates of nitrogen fixation by legumes.

Greaves and Jones found that there was no appreciable difference in the soil nitrogen after growing alfalfa if the tops were removed. Miles (10) found the nitrogen content of legume roots was far less than the

Pounds of nitrogen per acre in tops and roots of Hairy Vetch and percentage of total plant nitrogen found in roots and tops. 1947 (13).

Fertilizer	Pounds of nitrogen per per acre			Percentage of total plant nitrogen	
	Tops	Roots	Tops and Roots	Tops	Roots
None	55	14	69	80	20
0-40-0	86	25	110	77	23
20-40-0	97	38	135	72	28
0-40-20	85	36	121	70	30

nitrogen content of the legume tops. He found few legumes that contained as much as 20 percent of the total plant nitrogen in the plant roots. Pieters (12), Miles (10), Fred et al (5) have reported that there is a higher percent of total plant nitrogen in roots of perennial legumes than in roots of annual legumes. These men are also in agreement that when the tops were removed there may be a decrease in the total soil nitrogen.

Nielsen et al (11) found that legumes release fixed phosphorus when they were plowed under. Other benefits reported were an increase of total soil organic matter and improvement of physical structure and an increase in soil pH.

Laurie and Kiplinger (9) found that 40 percent of the roots of alfalfa and of other legumes are below 30 inches in the soil. Grasses and small grains had 50 percent of the roots in the top ten inches of the soil. It was concluded that among the beneficial effects of these deep rooted legumes is the translocation of plant nutrients in the subsoil to the surface soil. These are subsequently released by decomposition of the legume residues. These nutrients are thus made available for the shallow rooted crops.

III. EXPERIMENTAL PROCEDURES

Soil Description

The soil used in these experiments is classified as a Norge sandy loam. Galloway et al (6) describe a Norge soil as a deep granular prairie soil with a permeable subsoil, developed under bluestem grasses on uplands and high dissected terraces from alluvial and/or wind blown materials. This soil type has a surface of dark brown sandy loam 8-12 inches, a subsoil of reddish brown sandy loam 18-22 inches and a substratum of red sandy clays.

The soil used in these experiments was collected from the surface four inches of the experimental series 1800 of the Agronomy Experimental Farm located at Perkins, Oklahoma.

The soil particle size distribution included 75 percent sand, 13.75 percent silt and 11.25 percent clay as determined by the method of Bouyoucos (2).

The untreated soil had a pH 5.8 an cation exchange capacity of 13.6 millequivalents per 100 grams of soil .06 percent total nitrogen, extractable phosphorus equivalent to 32 pounds of P_2O_5 per acre and extractable potassium equivalent to 300 pounds of K_2O per acre. After liming, the soil reaction was pH 6.8.

Soil pH, exchange capacity, total nitrogen and extractable potassium were determined essentially by the methods of Russel (14). Extractable phosphorus was determined by the method of Bray and Kurtz (3).

Greenhouse Studies

The soil was air dried and 8,000 grams of soil were placed in two gallon glazed pots. Each pot received soluble phosphorus and potassium at the rate of 1,000 pounds of 0-10-10 per acre. Solutions of mono-calcium phosphate and potassium chloride were used to supply these fertilizer treatments. Calcium carbonate was supplied to all pots at the rate equivalent to three tons of lime per acre.

The legumes were planted October 3, 1953. Six pots of each legume were inoculated with a commercial inoculant and six pots were left uninoculated. Twelve pots of oats, Cherokee variety, Avena sativa L., were also planted at that time for comparative purposes in later studies.

Both roots and tops were removed from two pots of each legume series, inoculated and uninoculated. Only the above ground portions of the plants were removed from another two pots in each legume treatment series. The plant tops were harvested, weighed, then returned as green manure in the two remaining pots in each legume series.

The oats were treated in the same manner as the legumes, four pots of oats were harvested roots and tops, four pots were harvested tops only and four pots were harvested above ground parts only, weighed and then returned to the soil.

The lespedeza was harvested on December 15, 1953. All other legumes and the oats were harvested on January 28, 1954. All pots were planted to oats, Cherokee variety, February 23, 1954 and harvested April 24, 1954.

Total nitrogen determinations were made on all plant materials

harvested. Total nitrogen, soil pH, extractable phosphorus and potassium determinations were made on soil samples obtained before and following the growth of the legume crops.

Incubation Studies

Seven hundred grams of soil were taken from each pot following the legume and first oat crop harvest to be used in soil incubation studies. These incubation studies were designed to determine the rate and the amounts of ammonia, nitrite and nitrate nitrogen produced in the soil following the various crops and treatments.

Pint mason jars containing 400 grams of soil were used. These jars were covered with lids which had a three-quarter inch hole cut in the lid. These holes were stoppered with gauze and cotton to exclude foreign material, decrease evaporation and permit free movement of gases. Soil moisture content was carefully checked by periodic weighings and maintained as nearly as possible to a soil moisture content about twice the soil moisture equivalent.

Concentrations of extractable ammonia, nitrites and nitrates were determined periodically during the incubation period. Extractions were made by shaking the soil for 30 seconds with a 4 percent KCl solution. Procedures of Spurway and Lawton (15) were used to determine concentrations of ammonia, nitrite and nitrate in the soil extractant.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

Greenhouse Studies

All crops were harvested January 28, 1954 except lespedeza. The lespedeza was harvested December 15, 1953 because it had set a very heavy seed crop and the foliage had started to drop off. Plant growth and total nitrogen content of both tops and roots of the first crops grown are presented in Table 1. Total weights of tops and roots of all crops are found in Figure 1.

All legume crops grown had higher nitrogen content than did the oat crop grown at the same time. The composition of all inoculated legumes, excepting that of the inoculated white clover, was higher in percent nitrogen than that of the non-inoculated legumes.

The dry weights of the vetch and white clover forage were higher than the dry weights of the oat forage produced. The dry weights of the big hop clover, sweet clover and lespedeza were less than that of the oats.

The nitrogen composition of the inoculated legume roots was in all cases higher than the nitrogen percentage of the non-inoculated legume roots. The nitrogen percentage of the oat roots was much lower than that of any legume roots inoculated or not inoculated.

The number and size of nodules found on legume roots, Table 2, indicated that there were some symbiotic nitrogen fixing bacteria in the soil that were able to invade all legumes grown in this experiment. In all cases there was some nodulation of the non-inoculated legumes.

Table 1. Yield and nitrogen content of tops and roots of legumes, planted with and without inoculation, and oats grown in greenhouse experiments, on Norge sandy loam.

Treatment	Plant tops* Dry Wt. grams	%H_2O	%N	Plant roots Dry Wt. grams	%H_2O	%N
			Sweet Clover			
Inoculated	4.35	79.7	3.12	3.55	49.0	1.73
Not inoculated	4.95	81.0	3.05	2.30	54.6	1.55
			White Clover			
Inoculated	9.73	83.8	2.42	4.35	58.6	1.74
Not inoculated	9.10	81.0	2.80	5.45	47.8	1.61
			Big Hop Clover			
Inoculated	3.60	78.0	2.73	1.00	55.1	2.25
Not inoculated	2.40	75.4	2.53	.25	77.9	1.87
			Vetch			
Inoculated	8.60	82.5	2.83	3.95	48.4	1.88
Not inoculated	7.50	77.7	2.41	3.60	65.7	1.75
			Korean Lespedeza			
Inoculated	1.17	58.8	4.91	.17	37.0	1.91
Not inoculated	1.42	42.0	4.78	.11	47.0	1.55
			Oats**			
	7.50	74.7	1.91	.60	66.0	1.10

*Average of six pots used for plant top weights, mean of four pots used for plant root weights and nitrogen percentage of both tops and roots.

**Mean of twelve pots used for oat weights and eight pots used for nitrogen percentage.

The extent of nodulation was greatly increased where the legumes had been inoculated. Nodules were located generally near the tap roots on all inoculated legumes. On the white clover and big hop clover, nodules were also present on the root hairs and on the small branch roots. On the non-inoculated legume roots, nodules were located on the small branch roots some distance from the tap roots, except in the case of the uninoculated big hop clover. The non-inoculated big hop clover had a few nodules near the tap roots.

The total nitrogen composition of the soil following growth of the first crop of legumes and oats are shown in Table 3. Nitrogen content of the soil following legume growth was higher than that following the oat crop except for the inoculated sweet clover. Differences in the nitrogen composition of the soil were small and not considered reliable indicators of the nitrogen fixing ability of the legumes.

The inoculated vetch was dark green and was not lacking in nitrogen, Figure 2. The non-inoculated vetch was pale green and was lacking in nitrogen.

Waksman (16) proposes that two-thirds of the total nitrogen present in legumes may be fixed from atmospheric nitrogen by the symbiotic relation of the plant and the adapted strain of Rhizobium. Assuming that one-third of the total nitrogen in the legume plants in this experiment was fixed, the following figures are the calculated pounds of nitrogen fixed by legumes when inoculated at planting.

Legume	Pounds of Nitrogen Fixed per Acre
Sweet Clover	113
White Clover	193
Big Hop Clover	83
Vetch	204
Lespedeza	48

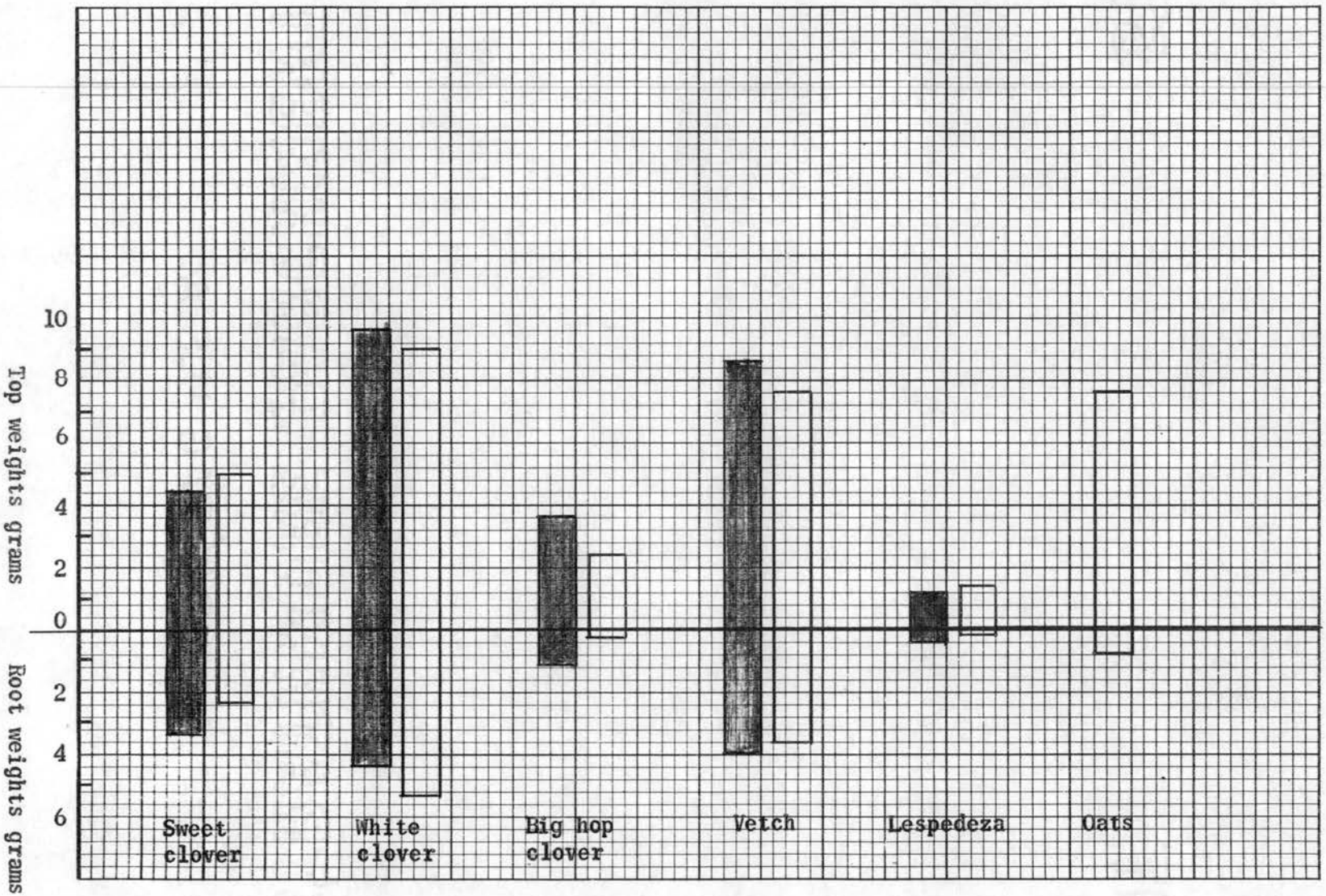

Figure 1. Yield of tops and roots of legumes, planted with and without inoculation, and oats grown in greenhouse experiments, Norge sandy loam.

Table 2. Size and number of nodules found on roots of various legumes, inoculated and not inoculated at planting, Norge sandy loam.

Crop	Numbers of nodules*	Remarks
	Inoculated at planting	
Sweet clover	26, 11, 9, 9, 4, 0, 0, 11,	Large, pink color, located mainly near tap root.
White clover	30, 35, 37, 40, 12, 17, 20, 10,	Large, white color, uniformly distributed.
Big hop clover	40, 50, 46, 44, 50, 30, 40, 43,	Medium to small, white color, uniformly distributed.
Vetch	9, 17, 15, 16,	Large, brownish, located mainly near tap root.
	Not inoculated at planting	
Sweet clover	6, 4, 6, 0, 0, 0, 0, 0,	Large, white, located on root hairs.
White clover	6, 4, 4, 5, 4, 6, 0, 0,	Clumps, white, located on root hairs.
Big hop clover	0, 0, 0, 0, 0, 0, 6, 4,	Large, white, near tap root.
Vetch	8, 9, 12, 11,	Small, brownish, well distributed.

*Number of plants checked are indicated by total number of figures given. Plants were taken from pots at random.

Table 3. Total nitrogen of Norge sandy loam following growth of several legumes and oats.*

Previous crop	Percent total nitrogen in soil	
	Inoculated	Not inoculated
Sweet clover	.045	.052
White clover	.056	.064
Big hop clover	.062	.064
Vetch	.063	.064
Lespedeza	.053	.052
Oats		.049

*Total nitrogen content of soil before crop growth was .060 percent. Each value presented represents the mean of six replicates except that of the soil after oats and that value represents the mean of twelve replicates.

Following the first crop of legumes and oats an oat crop was planted in all pots February 23, 1954. This oat crop was harvested April 24, 1954, and the results are presented in Table 4.

During the first month differences in the oat growth following a previous oat crop were very pronounced. These differences are illustrated in Figure 3. The best growth of oats in this series were produced initially in the pots where the tops and roots of the previous oat crop had been removed. Growth was depressed during this same period in those pots in which all previous crop materials had been returned as green manure. Following the first six weeks of growth this trend was reversed and the oat crop following the previous oat green manure crop produced the highest yield, in this series. Symptoms of nitrogen starvation were very prominent on the oat growth following the green manure treatment for about 4 to 5 weeks. In the second month these symptoms were more prominent in the pots where the previous plant growth had been removed. For the first month the growth of oats following the oat crop was not as good as the growth of oats following any of the legumes, for any of the treatments, Figure 4 and Figure 5. This difference was not so prominent and in some cases reversed toward the end of the experiment, Figure 9 and Figure 11.

The oat crop following inoculated sweet clover, Table 4, had more total growth where the legume forage was turned under. The next highest growth was obtained where the tops had been removed and the least growth was where both tops and roots had been removed. This trend was evident throughout the growth period, Figure 6 and Figure 10. The growth of oats following white clover and vetch followed this same pattern.

On the soil where big hop clover had been grown, the highest oat

Table 4. Dry weight and nitrogen content of oats following previous growth of various legume and oat crops having different dispositions of plant materials.*

Previous crop	Tops and roots removed		Tops only removed		No plant parts removed	
	Dry Wt. grams	% N	Dry Wt. grams	% N	Dry Wt. grams	% N
Previous crop inoculated at planting						
Sweet clover	11.8	.99	14.1	.94	14.7	0.98
White clover	12.1	.94	13.7	.97	14.8	0.92
Big hop clover	14.2	.99	11.2	.99	13.1	1.00
Vetch	12.3	.99	14.7	.90	16.9	0.98
Korean lespedeza	22.7	.90	21.4	.94	22.7	0.98
Previous crop not inoculated at planting						
Sweet clover	16.2	.95	14.4	.98	14.6	0.97
White clover	16.4	.93	11.0	.92	13.2	0.93
Big hop clover	14.1	.89	13.2	.91	14.5	1.00
Vetch	16.6	.92	12.0	.93	12.0	0.95
Korean lespedeza	23.9	.94	20.9	.92	23.1	1.00
Oats	15.8	.90	15.7	.94	17.8	0.94

*Each value represents the mean of replicate pots.

yield was obtained where the clover tops and roots had been removed. The next best growth was produced where the tops and roots had been turned under. The least growth was where the clover tops had been removed.

There was little difference in the oat crop following the lespedeza crop with regard to the disposition of the legume crop. The oat growth in the lespedeza series was higher than that of any other treatment, Figure 7. This trend continued throughout the entire experiment, Figure 8. This soil was fallowed for six weeks longer than the soil supporting the other legume crops because of the early lespedeza harvest.

The oat growth following the non-inoculated legumes was better where the legume tops and roots had been removed. The oat growth was less where the tops had been removed and slightly less where the tops and roots had been returned to the soil, except for the big hop clover where the tops and roots had been returned to the soil.

The nitrogen percentage of the oat forage varied less than .11 percent on all soil treatments. The amount of oat forage varied considerably indicating differences in total nitrogen utilized by the oat crop.

Incubation Studies

Tables 5 - 9 show the results of incubation studies on the soil following the growth and disposition of the legume and oat crops as indicated for each treatment. Ammonia, nitrite and nitrate production following growth of sweet clover is presented in Table 5. Nitrate production in the soil following growth of sweet clover, inoculated at

Table 5. Extractable ammonia, nitrites and nitrates produced during eight weeks of incubation following growth of sweet clover Melilotus officinalis, on Norge sandy loam (parts per million).*

Treatment		Weeks of incubation						
		Start	1	2	3	4	6	8
				Inoculated at planting				
Tops removed	NH_3	6	2	2	4	2	15	2
	NO_2	0	0	0	0	0	0	0
	NO_3	15	18	25	23	35	23	18
Tops and roots removed	NH_3	8	2	2	2	2	10	2
	NO_2	0	0	0	0	0	0	0
	NO_3	11	20	25	20	35	20	20
No plant materials removed	NH_3	8	2	4	2	2	2	2
	NO_2	0	0	0	0	0	0	0
	NO_3	6	20	18	23	35	23	25
				Not inoculated at planting				
Tops removed	NH_3	5	1	2	3	2	5	7
	NO_2	1	0	0	0	0	0	0
	NO_3	7	18	23	23	37	23	20
Tops and roots removed	NH_3	3	5	6	3	2	5	2
	NO_2	0	0	0	0	0	0	0
	NO_3	5	18	23	20	43	18	27
No plant materials removed	NH_3	6	2	2	3	3	8	5
	NO_2	0	0	0	0	0	0	0
	NO_3	16	23	25	25	25	23	25

*Each value represents mean of two replicate incubations.

planting was somewhat erratic during the first three weeks of incubation then sharply increased during the fourth week for all treatments. The ammonia production raised and lowered inversely to the nitrate production in most cases.

The non-inoculated sweet clover followed the same pattern as the inoculated sweet clover with the exception of the fourth week increase in nitrate production. This increase did not occur where all plant materials were returned to the soil.

There was no nitrite indicated in the soil extracts during the incubation of the sweet clover series.

The soil that had grown white clover, Table 6, produced increasing amounts of nitrate until the third and fourth weeks. This increase was followed by a decrease on the sixth week and a rise on the eighth week. The increase in nitrate production in the soil that had grown the inoculated white clover was more pronounced in the eighth week than the increase in nitrate production in the soil that had grown the non-inoculated white clover.

There was a trace of nitrites produced during the first two weeks of incubation where the inoculated white clover forage had been turned under.

The soil on which big hop clover had been grown, Table 7, showed the same pattern as the soil that had grown white clover. There was a slow rise of nitrate production with a peak in the third or fourth week. This was followed by a drop in nitrate production on the sixth week and a rise on the eighth week.

The ammonia production, in general, followed a trend of being inverse to the amount of nitrates present in the soil extract. No

Table 6. Extractable ammonia, nitrites and nitrates produced during eight weeks of incubation following growth of white clover Trifolium repens, on Norge sandy loam (parts per million).*

Treatment		Weeks of incubation						
		Start	1	2	3	4	6	8
				Inoculated at planting				
Tops removed	NH_3	5	5	5	3	2	3	2
	NO_2	Trace	0	0	0	0	0	0
	NO_3	10	13	18	18	18	13	35
Tops and roots removed	NH_3	3	4	2	3	2	3	2
	NO_2	Trace	0	0	0	0	0	0
	NO_3	8	18	25	23	50	23	25
No plant materials removed	NH_3	5	5	5	6	4	4	2
	NO_2	0	Trace	Trace	0	0	0	0
	NO_3	16	17	25	33	25	23	35
				Not inoculated at planting				
Tops removed	NH_3	5	3	2	10	2	3	2
	NO_2	0	Tr.	0	0	0	0	0
	NO_3	13	18	18	23	25	18	25
Tops and roots removed	NH_3	6	2	2	8	6	4	2
	NO_2	0	0	0	0	0	0	0
	NO_3	8	18	23	15	20	15	27
No plant materials removed	NH_3	6	4	2	4	2	2	2
	NO_2	Trace	0	0	0	0	0	0
	NO_3	10	15	18	20	23	20	35

*Each value represents mean of two replicate incubations.

Table 7. Extractable ammonia, nitrites and nitrates produced during eight weeks of incubation following growth of big hop clover Trifolium procumbens, on Norge sandy loam (parts per million).*

Treatment		Weeks of incubation						
		Start	1	2	3	4	6	8
				Inoculated at planting				
Tops removed	NH_3	3	2	3	2	2	3	3
	NO_2	Trace	0	0	0	0	0	0
	NO_3	17	50	25	50	20	25	23
Tops and roots removed	NH_3	4	5	2	5	2	6	2
	NO_2	Trace	0	0	0	0	0	0
	NO_2	20	25	18	50	23	15	27
No plant Materials removed	NH_3	2	2	3	10	2	2	2
	NO_2	Trace	0	Trace	0	Trace	0	0
	NO_3	25	25	20	50	25	25	25
				Not inoculated at planting				
Tops removed	NH_3	4	3	3	3	2	3	10
	NO_2	1	0	0	0	0	0	0
	NO_3	18	25	25	25	23	18	23
Tops and roots removed	NH_3	2	2	2	2	2	5	10
	NO_2	0	0	0	0	0	0	0
	NO_3	20	25	25	23	23	20	23
No plant materials removed	NH_3	1	3	5	3	4	6	8
	NO_2	Trace	0	0	0	4	6	8
	NO_2	25	35	25	50	25	25	20

*Each value represents mean of two replicate incubations.

nitrites were extractable from this soil following growth of hop clover.

Nitrate production in the soil that had produced vetch, Table 8, in general, followed the trends of the other legumes except that less nitrate could be extracted from this soil than from soils that had supported the other legumes.

The ammonia produced on the vetch soil was greater in amount than from the soils that had supported the other crops. The first week showed a rather high accumulation of ammonia, this was followed by a slight drop on the second week and an increase on the third week. The ammonia production then leveled off and decreased towards the end of the incubation period.

Nitrate production in the soil that had supported lespedeza, Table 9, was higher than the nitrate production following the other legumes and the oats. It was much higher than the nitrate produced in the soils where the other legumes had been grown from the end of the second week on. There was a small amount of ammonia and no nitrites extracted from the soil where the lespedeza had been grown.

Nitrate production in the soil that had supported a previous crop of oats, Table 10, was somewhat less where plant tops had been removed, with a large drop in nitrate production during the second week of incubation. This drop was followed by a slow rise in nitrates during the rest of the incubation period.

The nitrate production of the oat soil where tops and roots had been returned to the soil dropped very sharply on the second week of the incubation period and was followed by a rise in nitrate production.

Table 8. Extractable ammonia, nitrites and nitrates produced during eight weeks of incubation following growth of vetch *Vicia villosa*, on Norge sandy loam (Parts per million).*

Treatment		Weeks of incubation						
		Start	1	2	3	4	6	8
				Inoculated at planting				
Tops removed	NH_3	2	8	2	8	7	2	2
	NO_2	0	1	0	0	0	0	0
	NO_2	6	20	9	23	20	20	23
Tops and roots removed	NH_3	2	8	2	8	7	2	2
	NO_2	0	Trace	0	0	0	0	0
	NO_3	10	25	17	23	20	25	25
No plant materials removed	NH_3	8	9	5	5	5	1	2
	NO_2	0	1	0	0	0	0	0
	NO_3	6	17	7	15	20	25	25
				Not inoculated at planting				
Tops removed	NH_3	5	10	2	5	3	3	6
	NO_2	Trace	0	0	0	0	0	0
	NO_3	12	17	13	16	14	15	23
Tops and roots removed	NH_3	2	8	5	3	3	1	3
	NO_2	1	0	0	0	0	0	0
	NO_3	3	8	13	15	10	18	25
No plant Materials removed	NH_3	6	9	2	2	2	1	5
	NO_2	Trace	0	0	0	0	0	0
	NO_3	5	10	6	13	15	13	25

*Each value represents mean of two replicate incubations.

Table 9. Extractable ammonia, nitrites and nitrates produced during eight weeks of incubation following growth of lespedeza Lespedeza stipulacea, on Norge sandy loam (parts per million).*

Treatment		Weeks of incubation						
		Start	1	2	3	4	6	8
				Inoculated at planting				
Tops removed	NH_3	2	0	2	3	3	3	4
	NO_2	Trace	0	0	0	0	0	0
	NO_3	25	50	35	50	50	50	50
Tops and roots removed	NH_3	3	3	2	5	3	2	3
	NO_2	0	0	0	0	0	0	0
	NO_3	25	50	50	50	50	50	50
No plant materials removed	NH_3	3	0	2	2	4	2	6
	NO_2	Trace	0	0	0	0	0	0
	NO_3	25	50	35	50	50	50	50
				Not inoculated at planting				
Tops removed	NH_3	2	0	2	2	7	1	4
	NO_2	Trace	0	0	0	0	0	0
	NO_3	25	35	35	50	50	35	50
Tops and roots removed	NH_3	4	0	2	2	6	1	3
	NO_2	Trace	0	0	0	0	0	0
	NO_2	25	18	50	35	35	50	35
No plant materials removed	NH_3	3	0	6	2	5	1	6
	NO_2	Trace	0	0	0	0	0	0
	NO_3	25	35	35	35	50	35	50

*Each value represents mean of two replicate incubations.

Table 10. Extractable ammonia, nitrites and nitrates produced during eight weeks of incubation following growth of oats Avena sativa, on Norge sandy loam (parts per million).*

Treatment		Weeks of incubation						
		Start	1	2	3	4	6	8
Tops removed	NH_3	5	1	4	3	2	2	2
	NO_2	Trace	0	0	0	0	0	0
	NO_3	15	12	9	16	13	22	25
Tops and roots removed	NH_3	7	1	2	2	7	3	2
	NO_2	0	0	0	0	0	0	0
	NO_3	16	18	15	21	17	23	25
No plant materials removed	NH_3	6	1	2	2	2	2	5
	NO_2	Trace	0	0	0	0	0	0
	NO_3	14	11	2	15	21	23	25

*Each value represents mean of four replicate incubations.

The rate of nitrate production was much slower from the oat series that from the legume series. In all treatments the nitrate production of the oat series reached a peak late in the incubation period. Ammonia production was high in the first few weeks of the incubation. There was no extractable nitrites produced after the start of this incubation series.

V. SUMMARY AND CONCLUSIONS

Five legumes commonly grown in Oklahoma; sweet clover, white clover, vetch and lespedeza were grown in greenhouse studies on Norge sandy loam. One half of each legume series was inoculated at planting time, the other half was not inoculated when planted. Oats were grown to serve as an indicator crop relative to nitrogen fixation by the legumes. Crop yields and nitrogen contents were determined for both tops and roots of all crops grown.

Plant materials for each crop were disposed of in three ways. One third of the pots growing each crop was harvested by removing both tops and roots, one third of the pots was harvested by removing the tops only and one third of the pots was harvested tops only, weighed, than the tops were returned to the soil in the pots in which they were grown.

An oat crop was planted in all pots following the first crop to serve as a biological indicator of the nitrogen status of the soil following the previous crops and treatments.

Incubation studies were made on the soils following growth of the legume and oat crops. These incubation studies were designed to measure the amount and rates of ammonification and nitrification that was taking place in these soils following the various legume and oat crops and dispositions of the plant materials produced.

White clover, big hop clover and vetch produced higher yields of plant tops when inoculated at planting than did these same crops grown without inoculation. Sweet clover, big hop, vetch and lespedeza had higher contents of total nitrogen in plant tops when inoculated at

planting than when not inoculated.

Roots of all inoculated legumes grown in this experiment had higher total nitrogen contents than did the uninoculated legumes. Except for the white clover all inoculated legumes produced higher weights of roots than did the uninoculated legumes.

Total nitrogen contents of roots and tops of legumes, inoculated and uninoculated, were higher than corresponding plant parts of oats grown during the same period.

Differences in total soil nitrogen content were small following growth of the various legume and oat crops and may reflect sampling errors involved in the procedure.

Growth and nitrogen content of oats following the previous legume and oat crops was somewhat erratic. Except for the preceding crop of big hop clover, oat growth following legumes inoculated at planting was highest where all plant materials of the legumes were returned to the soil. Oat growth was higher following lespedeza than following the other crops in this experiment.

Incubation studies indicated a general relation in the amount of nitrates extractable from the soil and the growth of the indicator oat crop.

VI. FIGURES

Figure 2. Effect of Inoculation on Growth of Vetch at 6 weeks. Left, not inoculated; right, inoculated at planting. See Table 1 for yield and nitrogen percentage.

Figure 3. Effect of Crop Disposition on Oats 6 Weeks Growth Following Oats. A, tops removed, B, no plant materials removed, C, tops and roots removed. See Table 4 for yield and nitrogen content.

Figure 4. Effect of Previous Crop, Tops Removed, on Oat Growth of 6 Weeks. Previous crop was A, oats, B, lespedeza, C, vetch, D, sweet clover.

Figure 5. Effect of Previous Crop on Oat Growth at 6 Weeks. Previous crop was A, oats, B, lespedeza, C, vetch, D, sweet clover. No plant parts of previous crop removed.

Figure 6. Effect of Previous Crop Disposition on Oat Growth at 6 Weeks Following Sweet Clover. A, no plant parts removed, B, tops removed, C, tops and roots removed.

Figure 7. Effect of Previous Crop on Growth of Oats at 9 Weeks. Previous crop was A, oats, B, lespedeza, C, vetch, D, sweet clover. All tops of previous crop were removed.

Figure 8. Effect of Previous Crop and Disposition on Oat Growth at 6 Weeks. A, oat tops and roots removed, B, lespedeza tops removed, C, vetch tops and roots removed, D, sweet clover tops and roots removed.

Figure 9. Effect of Previous Crop on Oat Growth at 9 Weeks. No plant materials of previous crop removed. Previous crop was A, oats, B, lespedeza, C, vetch, D, sweet clover.

Figure 10. Effect of Previous Crop Sweet Clover on Oat Growth at 9 Weeks. A, no plant parts removed, B, tops removed, C, tops and roots removed.

Figure 11. Effect of Previous Crop and Disposition on Oat Growth at 9 Weeks. A, oats, tops and roots removed; B, lespedeza, tops removed; C, vetch, tops and roots removed; D, sweet clover, tops and roots removed.

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VITA

Raymond W. Van Camp
Candidate for the degree of
Master of Science

Thesis: A COMPARISON OF THE RELATIVE NITROGEN FIXING ABILITY OF FIVE LEGUMES.

Major: Soils

Biographical and Other Items:

Born: April 19, 1920 at Elkhart, Kansas.

Undergraduate Study: Fort Lewis Agriculture and Mechanical College, Hesperus, Colorado, 1949-1951; Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma, 1951-1953.

Graduate Study: Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma. 1953-1954.

Experiences: Farm reared, irrigated farm in Colorado. United States Army 1940-46. Owned and operated sawmill 1946-1949.

Member of Alpha Zeta, Phi Sigma, Masonic Club, Agronomy Club, International Relations Club, and order of the Red Red Rose.

Date of final Examination: August, 1954.

THESIS TITLE: A COMPARISON OF THE RELATIVE NITROGEN FIXING ABILITY OF FIVE LEGUMES

AUTHOR: Raymond W. Van Camp

THESIS ADVISER: Dr. J. Q. Lynd

The content and form have been checked and approved by the author and thesis adviser. The Graduate School Office assumes no responsibility for errors either in form or content. The copies are sent to the bindery just as they are approved by the author and faculty adviser.

TYPIST: E. Grace Peebles