THE EFFECT OF ALFALFA AND LADINO CLOVER ON THE GROWTH AND NITROGEN CONTENT OF TIMOTHY IN SAND CULTURE

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THESIS

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INTRODUCTION

The use of legumes for soil-improving purposes was practiced thousands of years ago in China. The Chinese farmers at that time did not know that legumes through the action of nodule bacteria could fix nitrogen from the air. They did, however, realize that the inclusion of leguminous crops in a rotation or used as green manures was helpful and that crops which followed were benefited. Legumes growing in association with non-legumes was also a very common practice. Usually soybeans were grown in rotation with sorghum or millet. Similar observations were made by the farmers of Western Asia and Northern Africa. Even ancient Greek and Roman farmers knew that legumes were soil-building crops. Later in the 17th century oats and peas were one of the common mixtures in Europe. The practice of growing clover persisted in 18th and 19th centuries, but became more general in the 20th century.

These practical experiences and observations were confirmed later when more scientific methods of research were developed. In 1837 Boussingault initiated the famous experiments on fixation of atmospheric nitrogen. He found that vegetables took from the atmosphere a material which he called "azote" which was necessary to their constitution and that "azote" may enter the living frame of the plant directly. Liebig did not agree with this view. He explained that the beneficial effect of cultivating legumes was due to their large leaf surface and consequently greater area for absorption of ammonia in the atmosphere. Ville rejected the ammonia hypothesis. He explained that the quantity of ammonia in the atmosphere was very small, only 2-3 parts per hundred million. The only logical hypothesis, he said, was that the source of nitrogen was the free element in the atmosphere. He demonstrated this idea by growing wheat, rape, rye, cress, lupines and maize in a greenhouse. All plants showed a large gain in nitrogen. In 1857 Lawes, Gilbert and Pugh at Rothamsted Experiment Station set up experiments and tried to settle this problem. Their results showed that plots continuously cropped to non-legumes without addition of manure soon declined to and remained at a low yield, whereas plots cropped to legumes maintained almost the original normal values. Moreover, if a non-legume followed a legume in rotation, the yield was as high as though the field had been fallowed for a year. They rejected Leibig's hypothesis and explained the phenomena by suggesting that legumes fixed free atmospheric nitrogen but they did not show that the fixation was due to the symbiotic nodule bacteria in the roots of legumes.

The first scientist who recognized that the occurrence of nodule bacteria in legumes was the primary cause for nitrogen fixation was Hellriegel. In 1886 he confirmed by experiment that bacteria from the air fell into sand cultures.

and infected the peas, forming nodules that enabled the plant to use free nitrogen. The bacteria had no effect on cereal crops. Later Beijerinck isolated from nodules pure cultures of legumes bacteria. Lawes and Gilbert agreed to the results and this explanation was then accepted as a true solution.

The problem of nitrogen fixation by symbiotic legume bacteria is a complicated one. Many scientists during the past fifty years have attacked different phases of the problem. Fred, Baldwin, McCoy, (6) and later, Wilson (22) gave excellent reviews on this subject. A lot of work was done but wide gaps still exist in our Knowledge.

As the biochemical studies on symbiotic nitrogen fixation made progress, the practical application of these studies from an agronomic standpoint became very important. Probably the first scientific work on legumes and non-legumes was done by Lyon and Bizell in 1911 (11). They used timothy grown with alfalfa or red clover and oats grown with peas in soil. The results showed timothy and oats grown with legumes contained greater percentages of protein than did they grown alone. Soils on which alfalfa had grown for five years contained more nitrate than did soil which had grown timothy for the same length of time. The nitrifying power of a soil which grew alfalfa for five years and kept bare for a summer was greater than soil which grew timothy for the same length of time and likewise kept bare for summer.

Later (1912) Lipman (10) set up a series of experiment to prove the beneficial effect of non-legumes in association with legumes. He concluded under favorable conditions (moisture, light and plant-food) non-legumes grown with legumes may secure a large amount of nitrogen from the latter and the yields of dry matter and nitrogen content in legumes were not decreased.

The result of Fergus (5) in field experiment with Kentucky bluegrass grown alone or in association with white clover also favored the mixed cropping. He said the legumes improved the pasture by (a) directly and indirectly increasing the total dry matter production, (b) improving the vigor of the grass sods and preventing the weed growth, (c) increasing the protein and mineral content of the pasture herbage.

McConkey (14) obtained similar results in the pasture of western Ontario, Canada. He said not only the yield and protein content of the pasture was greater when one to two pounds of wild white clover seeds were included in the grass field but also calcium and phosphorus content were higher. The nutritive value of the pasture was increased.

The greenhouse experiments, in general, were in agreement with the results obtained from the field. Thornton and Nicol (18) used Italian rye grass alone or in mixture with lucerne in sand culture where no nitrogen was added. After 18 weeks the grass in the mixture showed 2½ times as much nitrogen as the grass growing alone. Nowotnowna (16) set up the similar experiment by using different plants. The total yield, percentage of nitrogen and total nitrogen of rye grass grown with peas, red clover or serradella in sand without nitrogen fertilizer were all higher than rye grass grown alone. Barley grown with peas gave similar results.

Wagner and Wilkins (20) in Maryland used orchard grass and brome grass growing alone or in association with ladino clover or alfalfa. Their results also indicated that grasses in mixture had higher protein content than those grown alone. Recently an experiment in Florida showed the same result.(4) When lespedeza or white clover were used with the carpet grass mixture, the crude protein, phosphorus, potassium and calcium content of herbage were much higher than for the carpet grass pasture. The increase in nutritive value of the herbage was shown in terms of increase in the daily gains of steers.

On the other hand mixed cropping was not always successful. Westgate and Oakley (21) in 1914 took samples of pure non-legumes and legumes and non-legumes mixture from fields of normal fertility in different locations. The protein content of the plants was analysed. The results did not show all non-legumes in the mixture were high in protein content.

Madhok (12) in Punjab, India set up the experiments by using the native legumes, chickpea, senji and guara, growing with wheat, oats and chari respectively. His results agreed with Westgate and Oakley. Of the associated non-legumes, wheat and oats showed very little gain in crop weight or nitrogen content but chari showed considerable gain. He concluded that in certain combinations the non-legume did benefit by its association with legumes, while sometimes it did not. He said this was probably due to the roots of the legumes exerted some deleterious effect on the uptake of fixed nitrogen the non-legumes.

The failure of mixed cropping may be due to many causes. Ahlgren and Aamodt (2) made a preliminary study of the possible existence of harmful root interaction between various species of pasture grasses and legumes. They had sown two strains of brome grass, timothy and Kentucky bluegrass in the field plats. One of the grasses was grown with the mixture of red, white and alsike clovers. Next year a very marked differential interaction had taken place between the clovers and one of the grasses. The stands of the two strains of brome grass and timothy were uniformly good on all plats. The Kentucky bluegrass was practically eliminated from that portion of each plat on which the clovers were growing in association with the grass. At the same time they set up another experiment in the greenhouse. Kentucky bluegrass, red top, timothy and Canadian bluegrass were growing alone or two in mixture. They found the root and top growth in pure culture were all greater than in mixture. Although no legumes were used the data indicated the harmful root interaction may occur.

Later on Roberts and Olson (17) studied the root interrelationship between legumes and non-legumes. They used equal number of bluegrass plants grown in association with lespedeza or white clover. They found bluegrass had greater weight when growing alone. In association with sweetclover and alfalfa, the weight of the bluegrass was less than in pure stand. Red clover and alsike clover appeared not to have appreciably influenced the weight of bluegrass produced. The behavior of red top in the various association was similar to bluegrass. They said different legumes have different capacities to compete with grasses for nutrients or different capacities to supply the grasses with additional nitrogen. Lespedeza and white clover seemed to have less competition with grass and the greater yields of grass were obtained.

Aberg, Johnson and Wilsie (1) confirmed the results of Roberts and Olson in field and greenhouse experiment. Although no evidence foreither an antagonistic or beneficial effect was obtained as measure in yield of top and root per plant, they found that significant gains or losses in yield for one crop usually resulted in significant losses or gains respectively for the other crop in association and the significant gains in a crop combination usually were made by the least vigorous crop. The competition between legumes and non-legumes was obvious though the response of crops in mixtures was of compensating type and no loss of yields could be measured. They concluded the environmental conditions were important factor in studies of crop associations.

The competition between legumes and non-legumes also occurs in nature. As we all know in the worn-out soil or soil low in fertility legumes may predominate because they can use the atmospheric nitrogen by the action of root nodule bacteria and enrich their cells. The grasses cannot make a stand without combined nitrogen. When the legumes die the fixed nitrogen is returned to the soil. Soil bacteria then attack these residues and mineralize the organic nitrogen of the plants to ammonia and nitrates. After a time the soil acquires a moderate content of organic matter and a suitable supply of fixed nitrogen. Then the non-legumes or grasses move in and in a short time they may smother the legumes in the competitive race for nutrients, water and sunlight. They thrive for a time and then once again nitrogen limits their development. Under a more favorable competitive condition the legumes return again. From these natural phenomena scientists thought that legumes are unable to compete successfully with grasses in the presence of an ample supply of fixed nitrogen. Virtanen (19) and Wilson (22) have discussed this in detail in their reviews. A few example may be given. Lipman (10) in his early studies obtained the result that the addition of large amount of sodium nitrate tends to depress nitrogen fixation by legumes in mixed or unmixed growth. Thornton and Nicol (18) confirmed this result in their experiments. Johnstone-Wallace (8) studied the fertilizer treatments in mixed cropping. He indicated the use of phosphate and lime without nitrogen stimulated the growth of clover and subsequently resulted more forage obtained. The use of nitrogen together with phosphate, lime and potash resulted in less clover.

From the analysis of many legumes and non-legumes we find that legumes are not only higher in protein content but also higher in calcium, potassium, magnesium and phosphorus than most non-legumes. It is logical that they require adequate supplies of these nutrients. Albrecht (3) noted in his experiment that nitrogen fixation depended on the supplies of calcium, magnesium, potassium and phosphorus. McCalla (13) emphasized the influence of available calcium on the reproduction of root nodule bacteria and the ability of nitrogen fixation in the host plant. He concluded if the bacteria were deprived of calcium they changed to an abnormal form which could not invade the plant. If calcium was applied the abnormal bacteria could change to normal form and infected the host. But, unless more available calcium was added the bacteria in the infected host could not fix nitrogen.

It has become a very common practice now to apply lime, phosphate and potash in order to obtain a good stand of legumes. Nitrogenous fertilizer is not required or only is applied in a small amount to prevent the excess accumulation of carbohydrates in plants in the early stages of growth. and to induce nitrogen fixation.

From the above literature, the problem of mixed cropping is shown to be by no means simple. More research should be done on this line before we have a clear conception of all relationships. The purpose of this experiment is to get more information about whether the growth and nitrogen content of the grass, timothy, is materially increased when grown with legumes, alfalfa and ladino clover and whether the competition between legumes and non-legumes is severe enough to check the growth of either one under the controlled nitrogen-free-sand-culture experiment in the greenhouse.

MATERIAL AND METHODS

Timothy (Phleum pratense) and legumes, alfalfa (Medicago sativa), ladino clover (Trifolium repens) were used for study. In October 17, 1948 all seeds were sown in the 10-inch earthenware pots which were filled with a calcareous No. 2 plasterer's sand in the greenhouse. The sand was washed several times with tap water before using. Legume seeds were mixed with small amount of soil in which the proper legumes had grown for the purpose of inoculating with the effective bacteria. The experiment was divided into two groups each containing thirty pots. Shive's nutrient solution was used in Group I and Knop's solution in Group II. Both solutions (15) which contained nitrate-nitrogen were added to the group until December 20, 1948 in order to stimulate the growth of legumes before nodules were formed and to permit good growth of grass. Later on nitrates were substituted with chlorides. Following are the solutions used:

Shive's Solution

KH_2PO_4	0.0180 M i.e.	2.45 grams/liter
$Ca(NO_3)_2$	0.0052 M	0.53 grams/liter
$MgSO_4 \cdot 7H_2O$	0.0150 M	3.69 grams/liter

Knop's Solution

$Ca(NO_3)_2 \cdot 4H_2O$	1.15 grams/liter
KNO_3	0.20 grams/liter
KH_2PO_4	0.20 grams/liter
$MgSO_4 \cdot 7H_2O$	0.41 grams/liter

After December 20, 1948 Shive's and Knop's solution were changed to :

Shive's Solution

KH_2PO_4	2.45 grams/liter
$CaCl_2$	0.58 grams/liter
$MgSO_4 \cdot 7H_2O$	3.69 grams/liter

Knop's Solution

$CaCl_2$	0.80 grams/liter
KCl	0.20 grams/liter
KH_2PO_4	0.20 grams/liter
$MgSO_4 \cdot 7H_2O$	0.41 grams/liter

A stock solution of each chemical was prepared separately at a concentration one hundred times that of the final nutrient solution. When it was used it was diluted with tap water to the desired concentration. In the first two months solution was added to the pots every five days. Afterward it was added every three days. Each pot was supplied with 1000 c.c. every time. In the first few months the temperature in the greenhouse was about 65° F. in day time and 55°F. at night. After April the temperature was gradually increased to 80°F.-90°F. in day time and 70°F. at night. The temperature was very high at the end of the experiment (sometimes over 110°F.). During the cold winter days tap water was added to each pot every other day, but in hot summer days water was added twice a day.

Each group was divided into four series, each series having ten pots. Alfalfa seeds were sown with timothy seeds in the first series; ladino clover seeds with timothy seeds in the second; and timothy seeds alone in the third. All pots were set side by side in a long bench as follows:

Shive's Solution						Knop's Solution					
Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.	Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.
Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.	Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.
Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.	Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.
Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.	Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.
Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.	Alf. Tim.	Alf. Tim.	Lad. Tim.	Lad. Tim.	Tim.	Tim.

Two days after sowing alfalfa and ladino clover seeds germinated. Timothy seeds germinated three days later. The nutrients were applied when seeds were sown. On November 8 plants were thinned in each pot. On November 19 they were thinned again. In the pots of grass and legume mixtures each pot contained ten legume plants and ten grass plants. In the pots of grass growing alone each pot contained twenty.

On December 18 all pots were washed with tap water ten times in order to remove all nitrogen from the sand. From that time all nutrient solution used were free of nitrogen. Two weeks later the sand in each pot was tested to determine the nitrogen content. A Spurway soil testing kit was used. After that all pots were washed with tap water ten times again. Later on January 22 and February 19 second and third sand tests were done. All pots were washed as before.

Within the period of actual experiment plants were cut four times and the percentages of nitrogen were determined in the chemical laboratory. The first cutting was on January 14, second cutting on February 20, third cutting on March 4, and fourth cutting on May 9. Pictures were taken from the first and fourth cuttings. (Fig. 1,2,3, and 4).

RESULT

The growth of plants in Shive's solution seemed to be more vigorous than that of plants in Knop's solution although the difference may not be observed in the pictures. The sand test showed the average nitrogen content of the latter was higher (4.5 p.p.m.) but the former still contained some nitrogen (2.4 p.p.m.).

On January 14, about three months after the seeds were sown, the top growth of the plants was cut. They were weighed, dried in the oven drier and the percentage of nitrogen were determined. The results are shown in Table I.

At the same time on pot was chosen from each series and the plants in each pot were carefully washed with tap water to free from sand. These pots were chosen as to represent their own series. Pictures were taken before and after washing as shown in Fig. 1 and Fig. 2.

The analyses showed that the percentages of nitrogen of the timothy growing in association with alfalfa or ladino clover were all higher than those in timothy growing alone. In calculating the dry weight and total nitrogen of timothy we must remember the number of timothy in the timothy alone pots was double (20 plants) that in the legume-grass pots (10 plants). In order to compare them on an equal basis, the dry weight of timothy alone must be divided by two. From the table we can see the dry weight and total nitrogen of timothy growing with legumes were lower than timothy growing alone. (Timothy grown with ladino clover in Shive's solution was the only exception).

During the first three months, while nitrogen was available from the nutrient solutions, timothy was not very much benefited when growing in association with legumes. In examining the roots of legumes we noticed that nodules were formed but small and few in number. Presumably the fixation of nitrogen by the legumes was not great and little such nitrogen would be utilized by the grass. In comparing the timothy growing with alfalfa and ladino clover, although the percentages of nitrogen of timothy in alfalfa was a little higher than in ladino, the dry weight was less, so the total nitrogen calculated was lower.

On January 22, a second sand test was made the results showed the nitrogen content of the pots in Group II (Knop's solution) dropped down to average 2.4 p.p.m., but the nitrogen content of the pots in Group I (Shive's solution) remained unchanged (2.3 p.p.m.). This indicated that some, although a very small amount, of nitrate-nitrogen was left in the sand. All pots were washed ten times again. After ten days timothy plants growing alone in several pots were not as green as timothy growing with legumes. When growing without legumes, their lower leaves were yellow and their growth was stunted. That definitely showed nitrogen deficiency. Almost a month later (February 19) a third sand test was made, all pots showed only a trace of nitrogen.

That is, all nitrogen left in the sand was washed out or utilized by the plants. The pots were washed again to insure better control. At that time the deficiency of nitrogen of timothy growing alone was very severe, the lower leaves died and the growth was completely stopped, where-as the timothy plants growing with legumes were growing faster than before and the leaves showed a healthy green color.

A second cutting was made on February 20. This was to study the percentages of nitrogen in the timothy. Legumes were untouched. In each pot three mature timothy leaves which were not dead and three young but fully developed timothy leaves were taken from each plant. They were analyzed. The results are shown in Table II.

From the Table II, one may observe that all young leaves were higher in percentage of nitrogen as would be expected. The young and old leaves of timothy grown with legumes were higher in percentages of nitrogen than timothy grown alone. This showed timothy was definitely benefited in growing with legumes. In the old and young leaves the percentages of nitrogen were lower in ladino clover than in alfalfa. The result coincided with the first cutting.

After the timothy plants which were growing alone were cut, they drew on their organic reserve to produce new leaves. This resulted in a better balance of carbohydrate-nitrogen for a while. The new leaves were green in color.

After a time nitrogen was limited and the carbohydrate-nitrogen relationship was again out of balance. The growth of plant was stopped and leaves turned yellow once more. In the case of timothy growing with legumes, after they were cut they not only grew rapidly but also grew longer and leaves were dark green color. This indicated they could obtain nitrogen from the legumes and carbohydrate-nitrogen relationship was in good balance.

On March 4, all the top growth of timothy was cut again. At that time ladino clover was setting flowers. The results were shown in Table III.

The data indicated clearly that the dry weight, the percentage of nitrogen and total nitrogen of timothy growing with alfalfa or ladino clover were all higher than timothy growing alone. This again showed the former was benefited by the nitrogen fixed by the legumes. In considering the efficiency of supplying fixed nitrogen to timothy between alfalfa and ladino clover the result was different in two nutrient solutions. The dry weight, percentages of nitrogen and total nitrogen of timothy growing in association with alfalfa were higher than with ladino clover in Knop's solution while the reverse was true in Shive's solution. We cannot definitely say which legume was more efficient.

All plants were growing rapidly after this third cutting except the timothy growing alone. This was because the temperature was more favorable and daylight was longer and stronger. The effect of cutting on the timothy was obvious. In the case of timothy growing alone, complete defoliation resulted a better balance of carbohydrate-nitrogen relationship and induced a rapid growth of new leaves. The stop growth remained green in color for about two weeks then the starvation of nitrogen checked further development and leaves finally turned to yellow color again. On the other hand the growth of timothy in association with legumes lasted longer and no symptom of nitrogen deficiency was found. This again confirmed the beneficial effect of legumes to the grass.

As the condition were more favorable the legumes grew vigorously. A severe competition between the legumes and timothy can be seen even by mere observation. This was especially true with ladino clover. Because the ladino clover has creeping stolons timothy growing in association with it was crowded out. In the fourth cutting, (Table IV) the dry weight of timothy growing with ladino clover in both solutions was lower than that of timothy growing alone even when compared on an equal basis, though the percentage of nitrogen and total nitrogen were higher. Timothy in alfalfa in Shive's solution grew more vigorously than in pure culture, but in Knop's solution the dry weight of timothy grown with alfalfa was inferior to timothy grown alone. The poor growth of timothy in legume

mixture was not only due to the harmful root interaction but probably also to the competition for sunlight and nutrients. The difference of growth between two nutrient solutions was also noted. Legumes in Shive's solution were all growing better and yielded more dry weight than in Knop's solution. This was not surprising because the former contained more potash and phosphate and the legumes in this group obtained more nutrients for their needs. In examining the data, the above explanation is confirmed. As ladino clover grew abundantly the growth of timothy was suppressed. In several pots timothy was entirely disappeared as shown in Fig. 3 and 4.

In all cases ladino clover was growing better than alfalfa. It yielded more dry weight and total nitrogen although the percentage of nitrogen was not consistently higher but due to the harmful interaction of the roots of ladino clover, timothy growing with alfalfa had more dry weight and total nitrogen.

One pot was chosen from each series and plants were washed with tap water to free them from sand. Pictures were taken and roots were analysed. An interesting fact was noted that the roots from timothy alone were all growing around the circumference of the pot. It seemed that roots of the timothy plants had been extending to secure nitrogen. In the pots of alfalfa or ladino clover growing with timothy their roots mingled together and could not be separated. The results are shown in Table V.

It is very clear that the roots of timothy alone were low in dry weight, percentage of nitrogen, and total nitrogen while the legume and timothy roots were considerably high. The beneficial effect of legumes on the nitrogen composition of timothy was unquestionable. Ladino and timothy roots contained a higher percentage of nitrogen and total nitrogen than alfalfa-timothy roots, especially in Shive's solution. The result coincided with the top growth.

DISCUSSION

The effects of mixed cropping of legumes and non-legumes have been studied extensively. Wilson (22) has summarized the results obtained from many scientists and discussed them in detail. He said the beneficial effects are due (1) to excretion of nitrogen by legumes, (2) to the fact that mixed cropping may substitute for crop rotation and (3) that efficient agronomic management may thereby be used. The greatest emphasis in his review was concentrated on the excretion of nitrogen. Probably the earliest investigators who clearly suggested this idea were Lyon and Bizzell (11). They found that the roots of legumes excreted or sloughed off organic matter and this organic matter promoted the activities of soil microorganisms especially the nitrifying organisms, thereby making nitrogen available for the non-legumes. Lipman (10) demonstrated the occurrence of nitrogen excretion in sand cultures by using large glazed earthenware pots with small glazed and unglazed pots placed in them. Both legumes and non-legumes were grown in the small pots, He found when the non-legumes grew in the inner unglazed pots, that would allow the diffusion of substances in solution, they developed normally and on analysis showed considerable quantities of nitrogen. If the non-legumes were in the inner glazed pots, they grew very poorly and contained little nitrogen. He concluded that non-legumes benefit from association with legumes as a result of the excretion of nitrogen by the latter.

Virtanen (19) and his collaborators further investigated this subject. In 1938 he gave a complete summary of their work. He confirmed that several legumes could excrete nitrogen from the nodules. The quantity and the nature of nitrogen excreted could not be ascribed to the sloughed-off nodules and portions of the roots. The factors influencing the excretion, as he suggested, are:

1. Effect of culture medium--- the rate of excretion is higher if the absorption ability of the medium is high. Fine quartz sand. kaolin or soil are good media. No excretion was found in water culture.

2. Young nodules excreted more than old ones.

3. Excretion is inversely proportional to the utilization by the host plant. If the host plant utilizes for its protein synthesis the aspartic acid excreted from the root nodule bacteria at the rate at which it is being formed , no excretion can be expected.

4. Nitrate or ammonium salts present in the medium would inhibit or decrease the excretion.

5. More excretion would be resulted if non-legumes are growing in association with legumes.

6. Different bacterial strains have different abilities of nitrogen fixation and excretion was highest in the heaviest nodules.

7. Excretion is affected by the host plant. Even in closely related legume varieties in which the same bacterial

strains were effective, the amount of excretion was different.

8. Excretion is stopped after the blooming stage.

Wilson (22) and his associates at the University of Wisconsin, duplicated exactly the experiments of Virtanen (Finland). They failed to find any evidence of excretion. Other investigators also have obtained negative results. Yet Wilson, working in Virtanen's laboratory, found excretion under his conditions, and confirmed Virtanen's results. It has been concluded that environmental conditions such as temperature, length and intensity of sunlight etc. are the determining factors and that excretion of nitrogen may play an important role in mixed cropping in certain regions, such as Finland, but on occasion may be limited in other regions. In a permanent pasture mixture the occurrence of excretion may be relatively unimportant because the associated grass will eventually obtain nitrogen as a result of the decomposition of roots and nodules.

In this experiment although the excretion of nitrogen was not studied, there are evidences that non-legumes growing in association with legumes could obtain the fixed nitrogen from the latter and their growth was stimulated. The percentages of nitrogen were higher than non-legumes growing alone in all cuttings. The nitrogen obtained by non-legumes might be both from excretion and decomposition of roots and nodules.

Many scientiests believe that different legumes vary in supplying fixed nitrogen to the associated grasses. Wagner and Wilkins (20) found ladino clover to be more effective than alfalfa in increasing the protein content of associated orchard grass and brome grass. Roberts and Olson (17) noted that bluegrass grown with alfalfa yielded less dry weight than with white clover and lespedeza. However from the result of the present experiment no conclusion can be drawn that ladino clover was better than alfa lfa in supplying nitrogen to timothy. But there were evodences that owing to the growth behavior of ladino clover, the competition between timothy and clover was severe enough to suppress the growth of the former. The competition seemed mainly due to shading and to the harmful root interaction. Although alfalfa showed the same tendency, the result was not in such an entreme. A conclusion can be drawn that under the conditions of this experiment the growth of timothy growing with alfalfa was superior to that of timothy growing with ladino clover or alone.

SUMMARY

The growth and nitrogen content of timothy growing in association with alfalfa and ladino clover and timothy growing alone in sand culture were studied in the greenhouse. Shive's and Knop's solution were used as separate groups. Each group contained three series, each series having ten pots. Nitrates were supplied during the early growth period to stimulate the growth of legumes and timothy. After two months pots were washed to remove nitrates and the nutrients were also nitrogen-free.

About three months after the seeds were sown the top growth of the plants was cut and the dry weight, percentages of nitrogen and total nitrogen were determined. The percentages of nitrogen of the timothy growing with legumes were higher than timothy alone but the dry weight and total nitrogen were lower. This showed when nitrogen was still available from the nutrient solutions and that timothy was not very much benefited in growing with legumes.

The second cutting of timothy (on February 20) indicated the percentages of nitrogen in the young and old leaves of timothy grown with legumes were all higher than those of timothy grown alone. The effect of legumes on the nitrogen content of non-legumes was obvious, both in appearance and in chemical analysis.

In the later period all timothy plants growing alone showed definite nitrogen deficiency and growth was stunted

while timothy plants growing with legumes grew rapidly and leaves were dark green in color, The third cutting confirmed the results from previous cuttings. The dry weight, percentages of nitrogen and total nitrogen of timothy in legumes were all higher than timothy alone.

As the conditions for the growth of plants improved, the legumes grew vigorously. A severe competition between the legumes and timothy occurred. This was especially true with ladino clover. The result of fourth cutting (May 9) showed the dry weight of timothy growing with ladino clover was considerably lower than timothy growing alone, though the percentages of nitrogen and total nitrogen were higher in the grass grown with the legumes. The growth of timothy in alfalfa was not so much depressed as in ladino clover cultures but in Knop's solution it was inferior to timothy grown alone. The poor growth of timothy in legume mixtures was probably both due to the harmful root interaction and the competition for sunlight and nutrients.

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Fig. 1.---- Pots showing timothy growing alone and timothy growing with legumes in Shive's and Knop's solution.

SL---Ladino and timothy in Shive's solution.

ST---Timothy alone in Shive's solution.

SA---Alfalfa and timothy in Shive's solution.

KL---Ladino and timothy in Knop's solution.

KT---Timothy alone in Knop's solution.

KA---Alfalfa and timothy in Knop's solution.

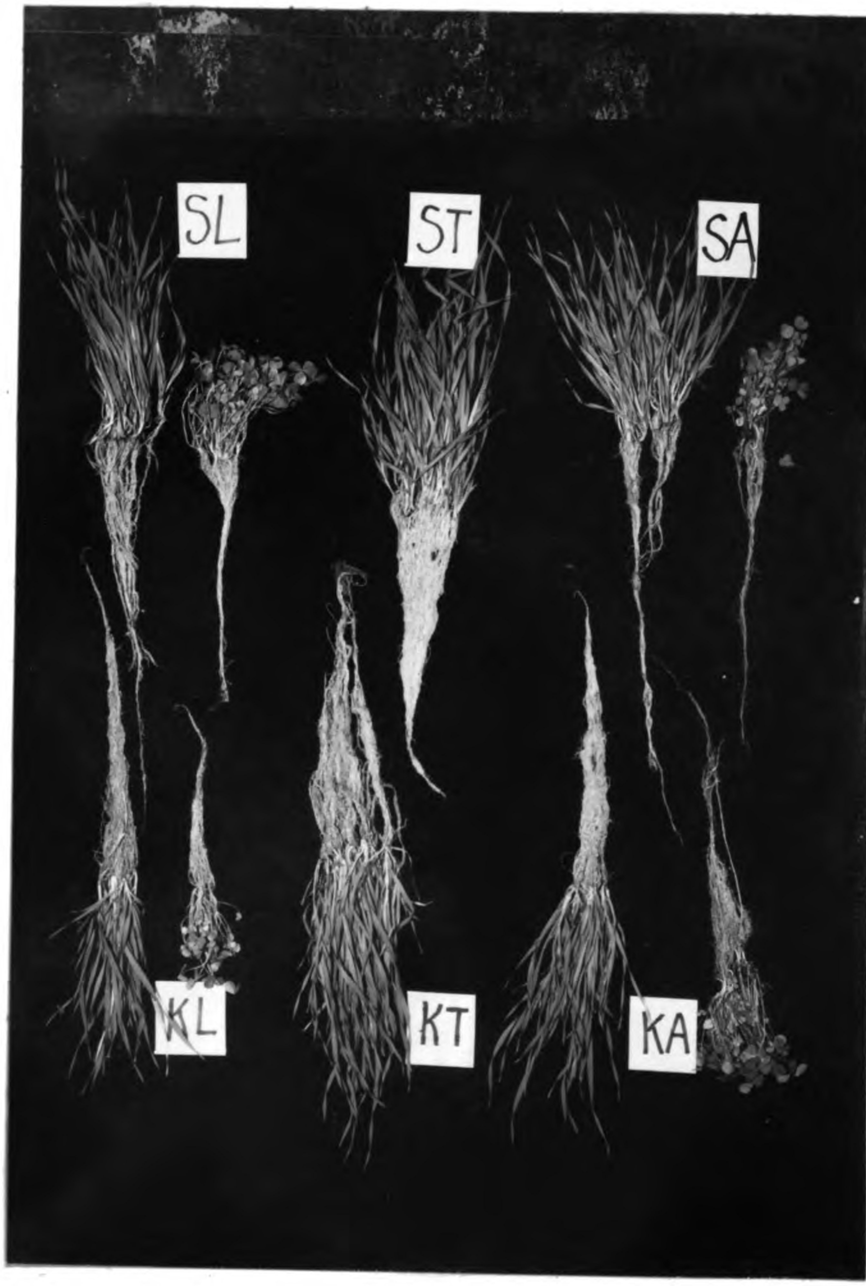

Fig. 2.---The early growth of alfalfa-timothy, ladino-timothy, and timothy in Shive's and Knop's solution.

The symbols are same as indicated in Fig. 1.

Fig. 3.--- The growth of alfalfa-timothy, ladino-timothy and timothy alone in Shive's solution on the fourth cutting.

SAT--- alfalfa-timothy

SLT--- ladino-timothy

ST --- timothy alone


SAT
SLT
ST

Fig. 4 --- The growth of alfalfa-timothy, ladino-timothy and timothy alone in Knop's solution on the fourth cutting.

KAT--- alfalfa-timothy

KLT--- ladino-timothy

KT--- timothy alone


KAT
KLT
KT

Table I

THE GREEN WEIGHT, DRY WEIGHT, TOTAL NITROGEN AND PERCENTAGE OF NITROGEN OF THE TOP GROWTH OF ALFALFA, LADINO CLOVER AND TIMOTHY ON THE FIRST CUTTING. THESE SAND CULTURES RECEIVED A COMPLETE NUTRIENT SOLUTION UP UNTIL DECEMBER 20. CUT ON JANUARY 14.

	Shive's Solution					Knop's Solution				
	Alfalfa-Timothy Culture		Ladino-Timothy Culture		***Timothy Culture	Alfalfa-Timothy Culture		Ladino-Timothy Culture		*** Timothy Culture
	Alf.	Tim.	Lad.	Tim.	Tim.	Alf.	Tim.	Lad.	Tim.	Timothy
Green Weight (gram)	4.00	7.70	5.40	9.90	20.70 (10.35)	6.40	13.70	2.60	13.00	28.70 (14.35)
Dry Weight	0.30	0.40	0.50	1.50	3.00 (1.50)	0.70	1.50	0.20	2.00	5.00 (2.50)
*% Nitrogen	4.02	3.40	3.70	3.15	2.72	3.49	2.50	3.27	1.98	1.80
** Total Nitrogen (gram)	1.21	1.36	1.85	4.73	8.16 (4.08)	2.43	3.75	0.65	3.96	9.00 (4.50)

* Expressed on dry weight basis.

** In ten pots.

*** Figures in parenthesis are the actual weight divided by two.

Table II

THE PERCENTAGE OF NITROGEN OF THE YOUNG AND OLD LEAVES OF TIMOTHY GROWING ALONE AND WITH LEGUMES ON THE SECOND CUTTING (FEBRUARY 20). NO NITROGEN IN NUTRIENT SOLUTION SINCE DECEMBER 20.

		Shive's Solution	Knop's Solution
Young Leaves	Timothy in alfalfa	2.94%	3.00%
	Timothy in ladino	2.65%	2.93%
	Timothy alone	2.33%	2.54%
Old Leaves	Timothy in alfalfa	2.61%	2.50%
	Timothy in ladino	2.31%	2.45%
	Timothy alone	2.07%	2.22%

Table III

THE GREEN WEIGHT, DRY WEIGHT, TOTAL NITROGEN AND PERCENTAGE OF NITROGEN OF THE TOP GROWTH OF TIMOTHY GROWING ALONE AND WITH LEGUMES ON THE THIRD CUTTING (MARCH 4) NO NITROGEN IN NUTRIENT SOLUTION SINCE DECEMBER 20.

	Shive's Solution			Knop's Solution		
	Timothy in Alfalfa	Timothy in Ladino	**Timothy Alone	Timothy in Alfalfa	Timothy in Ladino	**Timothy Alone
Green Weight (gram)	193.00	204.00	260.00 (130.00)	215.00	187.00	250.00 (125.00)
Dry Weight (gram)	37.90	37.40	58.30 (29.65)	38.20	37.60	52.20 (26.10)
*% Nitrogen	1.92	2.20	1.74	2.39	2.24	1.49
*** Total Nitrogen (gram)	72.77	82.28	101.44 (50.72)	91.30	84.22	77.78 (38.89)

* Expressed on Moisture-free basis.

** Figures in parenthesis are the actual weight divided by two.

*** In nine pots.

Table IV

THE GREEN WEIGHT, DRY WEIGHT, TOTAL NITROGEN AND PERCENTAGE OF NITROGEN OF THE TOP GROWTH OF ALFALFA, LADINO CLOVER AND TIMOTHY ON THE FOURTH CUTTING (MAY 9)

	Shive's Solution					Knop's Solution				
	Alfalfa-Timothy Culture		Ladino-Timothy Culture		**Timothy Culture	Alfalfa-Timothy Culture		Ladino-Timothy Culture		**Timothy Culture
	Alf.	Tim.	Lad.	Tim.	Timothy	Alf.	Tim.	Lad.	Tim.	Timothy
Green Weight (gram)	958.00	109.00	2988.00	41.00	89.00 (44.50)	434.00	73.00	1078.00	72.00	210.00 (105.00)
Dry Weight (gram)	250.00	29.00	470.00	10.00	28.70 (14.35)	114.50	23.00	209.00	17.70	53.50 (26.75)
*% Nitrogen	2.93	1.66	3.26	2.63	1.19	3.29	1.65	3.20	2.13	1.31
*** Total Nitrogen	7.33	0.48	15.32	0.26	0.34 (0.17)	3.77	0.38	6.31	0.38	0.70 (0.35)

* Expressed on moisture-free basis.
** Figures in parenthesis are the actual weight divided by two.
*** In nine pots.

Table V

THE DRY WEIGHT, PERCENTAGE OF NITROGEN AND TOTAL NITROGEN OF THE ROOTS IN THE VARIOUS CULTURES. (May 9)

	Shires Solution			Knop's Solution		
	Alfalfa and Timothy	(1)Ladino and Timothy	Timothy Alone	Alfalfa and Timothy	Ladino and Timothy	Timothy Alone
Dry Weight (gram)	35.00	70.50	21.70	29.00	22.50	11.40
*% Nitrogen	1.71	1.75	0.34	1.52	2.21	0.53
** Total Nitrogen (gram)	0.60	1.23	0.07	0.44	0.50	0.06

* Expressed on moisture - free basis.

** In one pot.

(1) Includes ladino clover stolons.