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BY PLANTS

THESIS

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by

PERRY G. HOLDEN,

Michigan Agricultural College,

August 1, 1895.

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Agriculture as an art is old, as a science it is new. Within the present century it has been elevated to the rank of a science. Perhaps the investigation of no one subject has done so much to place agriculture upon a scientific basis as that of the source of nitrogen of vegetation. It is certain that no subject has enlisted the efforts of so many eminent men, both chemists and botanists.

The importance of the question is apparent. Nitrogen is essential to all life, the nitrogen of animal life coming from the nitrogen stored up by plants. Three-fourths of the weight of the atmosphere is nitrogen. On every square inch of the earth's surface rests 12-1/2 pounds of nitrogen. It surrounds us on every side, we breathe it in at every breath, yet we are unable to use it in its free state. If plants in their growth can use the nitrogen of the air, there is an abundance of it always "on hand" without importing it from the nitre beds, or the guano fields of the far south. If on the other hand, they must depend on the nitrogen of the soil there must sometime come an end to all vegetable, and consequently to all animal life.

The nitrogen of the soil is gradually but constantly being exhausted. The various processes of putrefaction are slowly turning the complex nitrogen compounds into simple ones. Some of it is given off in the form of free nitrogen, but more of it as ammonia. Again when substances are burned the nitrogen is given up to the air as free nitrogen. A large part of the nitrogen of our food plants is carried by drainage water and the sewer to the river and finally to the sea. Lastly the processes of nitrification annually convert large quantities of the ammonium salts and organic nitrogen compounds of the soil into nitric acid, whence a portion is used by plants and much is carried off in the drainage water as has been proved by Lawes and Gilbert and others. Only a small fraction of this great loss will again be returned in the rain water as ammonia and nitric acid which, as has been shown, falls far short of supplying the amount necessary for an ordinary crop. It was clear that the nitre beds of Chili and the Guano of the South Pacific could not long supply this great loss. Indeed it seemed probable that the nitrogen supply of the soil would have been exhausted centuries ago, unless there was some means by which plants could draw upon the unlimited supply of the nitrogen of the air.

For more than one hundred years this important question has been up for discussion. No scientific question has been so many times settled and so persistantly unsettled. In 1771 Dr. Joseph Priestly raised the question and finally came to the conclusion that "plants could assimilate a small amount of the free nitrogen of the air. Later, Ingenhousz confirmed the results of Priestley's experiments. Woodhouse and Senebier came to exactly the opposite conclusion, while de Saussure from his carefully conducted experiments decided that "plants not only did not take up free nitrogen" from the air "but on the other hand gave off nitrogen during active growth." In 1837 Boussingault began a series of extensive experiments which resulted in the conclusion that plants could not use the free nitrogen of the air in growth.

"In 1849 Ville of Paris objected to Boussingaults method of experimentation, that plants could not make a normal growth in such a confined body of air as that contained in a bottle or globe. He repeated Boussingault's experiments but used a room glazed with glass instead of a glass globe, and announced the result, that while cereals produced a crop containing only two or three times as much nitrogen as was contained in the seed from which they grew, colza, cress and sunflower produced in the crop 25 to 40 times as much nitrogen as was contained in the seed."# He concluded "that while certain kinds of plants have little or no power of taking up free nitrogen, other kinds have the power of combining with free nitrogen and using it" in growth. "Such contradictory results reached by two such distinguished scientists provoked a lively discussion and in the interests of harmony and to establish scientific truth, a commission was appointed by the French Academy, composed of such eminent men as Dumas, Regnault, Payen, Decaisne, Peligot, and Chevreul. The commission thought they "found evidence of some gain of nitrogen during the growth of plants and finally reported, # "that the experiment made at the Museum of Natural History by M. Ville is consistant with the conclusion which he has drawn from his previous labors."#

In 1857, 20 years after Boussingault began his experiments and while he was still working upon them, Lawes and Gilbert

R. C. Kedzie on "The Source of Nitrogen of Plants, Mich. Agr'l. Report, 1881-2, P. 379.

the greatest experimenters the world has ever known, began a series of experiments which resulted in the conclusion that # "plants grown in the absence of combined nitrogen except that contained in the seed have no power of taking up free nitrogen and combining it with other elements to form plant tissue."# It is not necessary to give a detailed account of the experiments, except to say that the work was so carefully and thoroughly done that the results were generally accepted and the question seemed settled beyond a doubt that the free nitrogen of the air was not in any sense a source of plant food. The plant which was able to breathe in carbon dioxide of the air, decompose it, and use the carbon as plant food must starve for the want of nitrogen which surrounds the plant on every side.

In 1876 M. Berthelot questioned the results obtained by Boussingault, Lawes and Gilbert, since their experiments excluded all micro-organisms and electrical action. The soil in its natural condition is subject to both these influences. Berthelot showed that free nitrogen was fixed by various organic compounds during the "silent electrical discharge" at ordinary temperatures during storms. At this time organic matters would absorb both oxygen and nitrogen. He concluded that through the influence of electricity micro-organisms as well as higher vegetation could fix free oxygen in the soil. I am not prepared to discuss the results obtained by Berthelot, but if we suppose them to be correct we must admit that the nitrogen thus stored in the soil would be available alike to all classes of plants.

R. C. Kedzie on "The source of Nitrogen of Plants, Mich. Agr'l. Report, 1881-2, p. 379.

The experiments of Deitzel's and Deherains are without definite results. Professor Frank concludes from his early experiments that there are two processes going on in the soil, one liberating nitrogen, the other bringing it into combination by the aid of vegetation. M. Joulie found very large gains of nitrogen in some cases. He was of the opinion that the nitrogen was first fixed in the soil by organisms. The nitrogen thus fixed could subsequently be used by plants. We are at once doubtful of the results since the large gains which he obtained was in the case of a polygonum (buckwheat) and not with plants of the leguminous family.

This brings us down to 1883 when Hellriegel bagan his famous experiments which were destined to reverse the decissions of a quarter of a century before by Boussingault, Lawes and Gilbert.

#The plan of Professor. Hellriegel's experiments is briefly as follows:- The plants experimented upon were grown in pots of sea sand; ~~the supply of~~ the sand first being washed to remove the nitrogen and then sterilized by subjecting to a temperature of 150° c. The seeds were sterilized by dipping in a solution of bichloride of mercury, then washed in boiled water and planted in the pots with sterilized tools. The plants were watered with distilled water in such away as to preclude the possible entrance of living organisms into the pots. The pots were supplied with all the elements of plant food necessary for growth except

These experiments are described at length by Prof. W. O. Atwater, Exp. Sta. Rec., Vol. V, No's 8 & 9.

nitrogen. If no nitrogen was added the plants would grow for a time then turn yellow and finally die. An analysis of the plant showed that in no case was there more nitrogen in the plant than was present in the seed at the beginning. The plant had grown until it used up the nitrogen of the seed. It was found that when varying amounts of nitrogen were added the growth of the plants was in proportion to the nitrogen added to the soil. This is illustrated by the following table giving the results of an experiment with serradella conducted in 1887.

	Nitrogen supplied	Yield of Vine and Seed.
pots	Grams	Grams.
1	0.000	0.078
2	0.056	2.883
3	0.112	6.540

In no case did he find that the nitrogen in the plant exceeded the nitrogen in the seed planted and that added to the soil. It was evident that under these conditions there was no assimilation of free nitrogen of the air. The conditions of these experiments were essentially the same as those of Boussingault and Lawes and Gilbert, twenty five years before and so far the results are exactly the same.

Hellriegel went a step farther and inoculated the sterilized soils with micro-organisms from rich soils.#

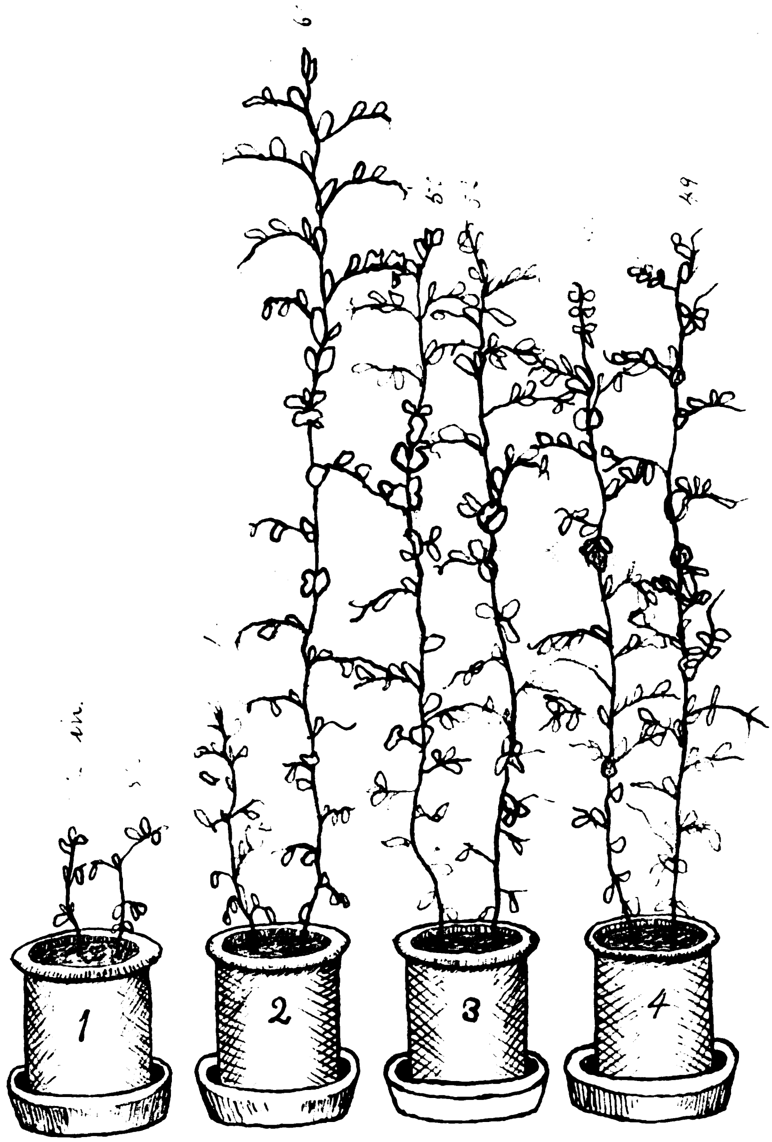
These micro-organisms were introduced by means of a "soil infusion" prepared by mixing a small amount of a cultivated soil with water and allowing it to settle. The almost clear water was poured off and used at the rate of 25 c. c. to each pot containing 9 lbs. of sand. Analysis showed that the 25 c. c. of solution contained a small amount of nitrogen varying from 3/10 to 7/10 of a m. g.

Turnips, hemp, sunflower and oats derivedno benefit from the inoc ulation and died as soon as the nitrogen of the seed had been consumed, but with the leguminous plants such as peas, clover and vetbhes a very great change was soon apparent. The plants began to show a dark green color and grew rapidly. From this time on it was evident that the leguminous plants had plenty of nitrogen at their command, and developed rapidly and normally. An analysis of the plants showed that the nitrogen was many times greater than that contained in the seed planted.

The following sketch is from a photograph by Lawes and Gilbert showing the effect of soil infection upon peas. The soil in pots 1, 2 and 3 was sterilized nitrogen free quartz sand, to which all the elements of plant food were added except nitrogen. pot 1, was not infected. Pots 2 and 3 were infected with a soil ifusion as described in foot note on page 6. Pot 4 was garden soil. The peas were planted July 10th and before the end of July the plants in pots 2 and 3 showed a more rapid growth than in pot 1. The plants were photographed October 22, 104 days after planting

The plants in pot	1 (not infected)	were	8-1/4 and 8-1/2 ins. high
" " "	2 (infected)	"	14 and 60 " "
" " "	3 (infected)	"	50 " 52 " "
" " "	4 (garden soil)	"	44 " 49 " "

While the plants in the garden soil made a less extended growth than in pots 2 and 3, yet they were more figorous and flowered and produced seed which those in pots 2 and 3 did not do. In a



\# Popular Science, monthly, Vol. XXXVIII,
P. 49½--Manly Miles.

similar experiment with yellow lupines, like results were obtained except that the plants in pots 2 and 3 (infected) were more vigorous than in the lupine soil.

The lupine plants in pot 1 (not inocculated) were 1-1/2 and 2 ins.

"	"	"	2 (inocculated)	"	18	, 24	"
"	"	"	3 (" ")	"	20	, 26	"
"	"	"	4 (lupine soil)	"	16	, 18	"

Hellriegel planted nine kinds of seed, four non-leguminous, five leguminous in each of four different pots. Two of the pots A. and B. were infected with a soil infusion from a field where beets had been grown, the other two pots, C. and D. were infected with a soil infusion from a lupine field. The weight in grams of dry substance is shown in the following table:-

		Infected with Beet soil infusion		Infected with Lupine soil infusion	
Name of plant		Pot A.	Pot B.	Pot C.	Pot D.
Non-Leguminous	Turnip	0.010	0.017	0.006	0.015
	Hemp	0.025	0.055	0.047	0.046
	Sunflower	0.305	0.493	0.330	0.644
	Oats	0.257	0.153	0.140	0.238
Leguminous	Serradella	0.015	0.010	2.002	2.560
	Lupines	0.093	0.155	17.133	30.597
	Clover	2.213	3.241	0.363	1.589
	Vetch	15.971	6.132	6.678	5.181
	Peas	12.282	32.640	16.152	6.021

All four pots were sown April 18; A. and B. were harvested August 2, C. and D. August 20.

"Thus under absolutely the same experimental conditions, the infusion of beet soil was ineffective with the non-laguminous plants, also with the serradella and lupines, but exerted a good effect upon the peas, vetches and clover as will be seen by the table. The lupine soil infusion was also ineffective on the non-leguminous plants but exerted a beneficial influence upon the

growth and assimilation of nitrogen by all the legumes employed. Its effect being doubtful only in the case of clover."# This painted to the important fact that the leguminous plants stood alone in this peculair power to secure nitrogen from the air. The non-leguminous plants in the same pot with the leguminous plants grew until the nitrogen in the seed was consumed, then died.

To prove beyond a doubt that this power whihh some plants have to obtain nitrogen from the air was due to the action of the organisms introduced in the soil infusion, Hillriegel conducted two experiments. In the first he sterilized the infusion before applying, by heating to 70° c. and found that there was no increase in nitrogen as is shown by the following tabulated results of an experiment with lupine in 1888.

Nos.	Treatment	Dry matter produced	Nitrogen acquired from the air.
#1	Soil infusion sterilized	0.926 gra	-0.007 grams
2	Soil infusion sterilized	1.008 "	-0.007 "
3	Soil infusion not sterilized	42.681 "	1.147 "
4	Soil infusion not sterilized	40.574 "	1.054 "

In the second experiment Hellriegel grew peas in such a way that the roots of each plant grew intwo pots, about half in one and half in the other. The soil in both pots was sterilized by boiling and received the same kind of treatment except that one of the pots was inoculated with soil infusion which was not sterilized, while the second pot was treated with the same amount of the infusion but which had been previously sterilized by heating.

W. O. Atwater, Exp. Sta. Rec., Vol. V. No. 9.
E. S. R., Vol. V. No. 9, P. 844 by W. O. Atwater.

This experiment was repeated several times and in every instance but one the half of the roots grown in the soil affected with living organisms were well supplied with tubercles while the other half of the roots were destitute of tubercles in every case. The plant which failed to develope tubercles died.

It was now apparent to Hellriegel that there was a direct relation between the assimilation of atmospheric nitrogen and the formation of tubercles. Where no organisms were present no tubercles were formed, and where no tubercles were formed no atmospheric nitrogen was fixed by the plant. It still remained for Hillriegel to determine whether the source of the nitrogen thus acquired was the combined or the free nitrogen of the air. He grew plants in a closed glass vessel so arranged that only free nitrogen was admitted to the plant.# The nitrogen free sand was inoculated and on June 6, one pea was planted. The rapidity of growth and gain of nitrogen will be seen from the following figures which show the weight of the plant dried at 100° c.

First cutting, August 31	Seed	0.376	grams
	Vines	6.173	"
Second cutting, October 4	Vines	2.320	grams
	Roots	1.290	"
Total		10.159	"

In this experiment, the air in the vessel at the beginning was not analyzed or purified and therefore contained a small amount of combined nitrogen. The amount of combined nitrogen in 100 liters of air (the amount which the vessel held) has never exceeded 1 mg. at the station. This error was avoided in the subsequent experiments and like results were obtained.

Nitrogen Balance.#

Combined nitrogen in the air of the vessel at beginning less than	0.0001	grams
In the sand	0.0000	"
In nutritive solutions and twice distilled water	0.0000	"
In the soil infusion	0.0002	"
In the seed	0.0081	"
Total nitrogen supplied	0.0084	"

Nitrogen found at the end of the experiment.

In the pea plant	0.2335	grams
In the soil	0.0207	"
Total found	0.2542	"
Total supplied	0.0084	"
Gain	0.2458	"

The gain was 0.2458 grams of combined nitrogen, for which the free nitrogen of the air was the only source. Laurent and Schloessing recent experiments fully confirm these results. In their experiments a known amount of free nitrogen was allowed for the plant. At the end of the experiment it was found that the plant's gain in nitrogen was the air's loss. Thus after years of "patient scientific thoroughness" Professor Hellriegel, Director of Bernberg Experiment Station announced the results of "certainly the most important discovery for agricultural science."

The one fundamental truth that some plants under certain conditions could utilize atmospheric nitrogen has never been disputed by the many experimenters who have entered the field since Hellriegel announced his results. Many subsidiary questions, though questions of much importance have arisen and been widely discussed. The discussions though active have not been bitter

P. 847, E. S. R., Vol. V. No. 9.

and all agree that much remains to be worked out.

Where does the fixation of nitrogen take place? Is it in the soil or in the plant? It has been claimed by Berthelot and Andre and others that the fixation of nitrogen must first take place in the soil by means of organisms and electrical action . Without denying the statement of Berthelot and Andie that nitrogen may be fixed in the soil, we have abundant evidence that nitrogen thus fixed is not the only source of nitrogen for plants, if indeed it is the source of any considerable amount of it. The results of analyses of the soils by Lawes and Gilbert and Schloessing and others where plants had accumulated large amounts of nitrogen showed no gain of nitrogen in most cases.# If the nitrogen was first accumulated in the soil it must have been taken up by the plant as fast as formed which is not a reasonable supposition. Again in Hillriegel's experiments where he grew 9 sorts of plants, 4 non-leguminous and 5 leguminous, the nitrogen if formed in the soil would have been available alike to both classes of plants, but the non-leguminous plants in the same pot with the leguminous plants failed to secure any nitrogen. It can hardly be supposed that if the nitrogen was formed in the soil that the leguminous plants were better able to secure it than the non-leguminous. This is contrary to all experience.
It is well known that the application of nitrogenous fertilizers produce a much greater gain in the case of non-leguminous, than with leguminous crops.

In several instances there is unmistakable evidence of a small gain in the soil which will be spoken of later.

The question arises if the fixation of free nitrogen takes place in connection with the plant as we must believe that it does, where is the important function performed? No less an authority than Professor Frank of Berlin who has written more upon this subject than any other scientist claims that the fixation takes place in the cell protoplasm through"a powerful act of the machinery of the leguminous plant , urged to the necessary expenditure of energy by the stimulating action of the organisms in the roots." This view is also held by Prazmowski, Hillriegel and others. Frank claims that other plants than legumes are able to assimilate nitrogen, but that the leguminous plants have the power in a greater degree than non-leguminous plants, which is due to the stimulating action of the organisms in the tubercles of the roots. To show that the assimilating action is not due to the tubercles, Professor Frank gives the results of experiments which in his opinion show that other plants such as oats, potatoes, mustard, spurry, turnips, buck beans and norway maple are capable of fixing free nitrogen

We cannot avoid a feeling of doubt as to the reliability of Professor Frank's results,since his experiments were mostly conducted in the open air. The plants were simply sheltered from rain, and were accessable to the combined nitrogen of the air. Professor Frank gives us the general conclusions but fails to support them by giving an account of methods,and results obtained by analysis. Not only are the results of Boussingault and Lawes and Gilbert's experiments of thirty five years ago,entirely contrary to Frank's results,but the more recent and"exceedingly" carefully conducted experiments of Laurent and Schloessing,show no fixation of nitrogen in oats, tobacco, cress, mustard, cabbage, spurry and potatoes. Many of these are the very same plants Frank experimented with.

Schloessing and Laurent went further, By an ingenious contrivance they managed to grow leguminous plants so that no nitrogen was accessible to the roots of the plants. The leaves were left exposed to the free nitrogen of the air. The soil was inoculated with organisms. The cell protoplasm of the plants had every opportunity to fix the free nitrogen of the air, but in every case the plants died for the want of nitrogen. On the other hand, where the roots had access to atmospheric nitrogen, tubercles were formed and the plants fixed nitrogen. Again when the conditions were reversed and the atmosphere about the leaves was deprived of its nitrogen, hydrogen being substituted in its place, the plants developed normally showing that the nitrogen was assimilated in connection with the roots. The results of Schloessing Sons and Laurent are confirmed by Kosch and Kossowitsch later.

Professor Frank answers by saying that plants must be very vigorous and near the "maturing point before they have power to energetically seize and fix the atmospheric nitrogen." But as we have already seen, this will not apply to leguminous plants which have the power to fix free nitrogen at an early stage in their development. In the case of peas, Lawes and Gilbert found that within twenty days after planting, nitrogen was being assimilated. It is further shown by the analysis of Lawes and Gilbert that during certain stages of their development, the tubercles contain a much higher percent of nitrogen than the other parts of the plant, and in some cases higher than the highly nitrogenous seeds.

Professor Frank admits this, but claims that the increased amount in the tubercles is not sufficient at any time to account for the large gain in the plant, which is taken from the air. He further contends that if the nitrogen fixation takes place in the tubercles alone, they must yield a gradual supply to the plant, a supposition which he claims has no advocates.

We will grant for the present that no nitrogen is formed in the tubercles until they have reached a certain stage in their development. But Professor Frank's argument looses much of its force when we remember that the tubercles on the growing plant, are in various stages of development. I found the tubercles very much more uniform in development during the month of May and early June than later, but even then in most cases there were plenty of young tubercles just forming by the side of those which were three and four weeks old. At the present time (July 25) it would be difficult to find a plant from April seeding, that does not contain both the newly formed tubercles and those which are being absorbed.

While there is diversity of opinions among those who have given the matter much attention, yet the evidence at hand strongly indicates that the fixation process takes place in the tubercles. But whether the <u>protoplasm</u> or the <u>organism</u> is the chief factor in the process is by no means settled. H. Marshall Ward favors the protoplasm theory of Frank, but does not agree with him that the protoplasm in the cells outside the tubercles and in non-leguminous flowering plants have this power. Ward's modification of Frank's theory briefly stated is to the effect that the protoplasm in some way fixes the free nitrogen through the stimulating effect of the organisms. In proof of this theory M. Ward cites the wonderful powers which protoplasm is admitted to have of disorganizing and reorganizing the materials of plant food. He thinks it not unreasonable to go a step farther and suppose that the protoplasm can in some way force this "notoriously inert" element (nitrogen) into combination with other substances, especially when urged to such great activity as is shown by the alkaline reaction of the tubercle contents.

M. Gonnerman in a recent article on the probable number of organisms capable of forming tubercles says, "it seems probable that the plant itself without symbiosis can take up and assimilate free nitrogen; the bacteria may, however, assist the plant in contributing to its higher nitrogen content."#

On the other hand there is some evidence that the organisms when not in contact with the protoplasm can fix free nitrogen. Berthelot claims to have established beyond a doubt that several species of soil bacteria, as well as the organisms of leguminous tubercles cultivated separately, have this power. In one case there was an increase of 50 percent. Beyerinck while regarding

E. S. R. Vol. VI. No. 9, P. 784.

it as probable that the nodule organisms fix atmospheric nitrogen admits that he does not prove it. Laurent and Immendorf both failed to satisfy themselves that the organisms can flourish without organic compounds of nitrogen. Lawes and Gilbert think that the fixation is probably due directly to the organisms, and "if this should eventually be established, we have to recognize a new power of living organisms--that of assimilating an elementary substance."# "Neither experience in practical agriculture, nor the nitrogen statistics of soils and crops, points to the conclusion that there is a gain of nitrogen to any material extent under the agency of microbes within the soil independently of leguminous growth."#

It is known that the organisms do not fix nitrogen in the nitrogen-free sand cultures, but this is not proof that they might not fix nitrogen in rich soils where other substances could perhaps take the place of the protoplasm in the tubercles. On the one side, the fact that the plant in the absence of the organisms fixes no free nitrogen, and on the other side, the evidence that little if any is fixed by the organisms in the soil, strongly indicates that the organisms and the protoplasms are both essential factors in the process, but the part played by each is unknown.

The relation between the organism and the plant seems to be one of true symbissis. However during the early stages of nodule formation the action is largely parasitic, the organisms developing at the expense of the plant, as is shown by the pale yellow appearance and arrested growth of the plants in the sand cultures.

Jr. Royal Agricultural Soc. P. 695-692.

From recent experiments it would seem that leguminous plants are not the only ones which can assimilate free nitrogen. H. Marshall Ward in an article on Fixation of nitrogen "# says, "the experiments of Nobbe, Schmid, Hiltner, and Hotter show that Eleagnus plants, the roots of which develope nodules due to the invasion of a funguss totally differnet from the one causing the leguminous nodules, also fix and assimilate the free nitrogen of the air, as shown by their growing and flourishing much better and more rapidly than Eleagnus plants side by side with them, but not infected with the root organisms." "It will be interesting to see if further research shows similar results with any of the physiologically similar root-growths, due to very different fungi, met with in Taxodium, Podocarpus, Alnus, Juncus and many other plants." In reviewing the more recent works of Nobbe and Hiltner, Walter H. Evans who is at the head of the Department of Botany and Diseases of Plants, says # "the ability to assimilate the free nitrogen of the air as possessed by tubercle -bearing plants such as legumes, Alders, Eleagnus, Podocarpus etc. is recognized." Nobbe and Hiltner further claim that only those plants which show an increased nitrogen content in the leaves and stems above ground are able to assimilate free nitrogen.

There is now little doubt that some of the algæ have the power to fix free nitrogen when affected with the proper bacteria. It has been observed by Hillriegel and others that when the nitrogen free sand cultures became affected by an algae

Nature Vol. 49, P. 513.
E. S. R. Vol. Vl, P. 381 1895.

growth there was a small gain of nitrogen in the soil. Professor Frank has held this view for some years and the recent experiments of Laurent and Schloessing, shows that not only were the green algae able to fix gasseous nitrogen but that some of the mosses possessed this power in a marked degree. The still more recent experiments of Kosch and Kossowitsch who repeated this work with green and blue-green algae, using purely inorganic solutions confirm the results of Laurent and Schloessing. Later than this Kossowitsch arrives at a somewhat differnet conclusion. This time he was able to separate the algae from all bacteria and secure pure cultures. There was no gain of nitrogen in any of the entire series of experiments, but when they were mixed with soil bacteria and fungi there was in some cases a considerable increase of nitrogen.

Are there few or many tubercles forming organisms? Bearing upon this question, are the interesting observations of Professor Bolley # relative to the distribution of tubercles on native and introduced leguminous plants of the Dakotas. He examined a great number of plants and everywhere found the native plants well supplied with tubercles, while many of the introduced plants bore no tubercles. This was particularly the case with common red clover. He claims, however, that red clover thrives and forms tubercles when it is preceded by white clover, which does well in Dakota and never fails to be supplied with many tubercles.

In the case of two tropical legumes grown in France by

Agricultural Science 7, 58, 1893.

C. Naudin, no tubercles were formed while several species of Australian plants bore tubercles in profusion. My own observations in 1892 and again during the present year confirm the above results in a general way. For example sainfoin which thrives well in England does very poorly here and seldom forms any tubercles. Also tubercles are rarely found on lupine plants here while they are very abundant in England. On the other hand several introduced plants as lathyrus silvestris and horse bean produce tubercles in great numbers.

Recently several series of experiments were conducted by Nobbe, Schmid and Hiltner in pure quartz sand, with inoculations of pure cultures from various legume tubercles. In one series, peas and common locust were inoculated with cultures from peas, common locust, alfalfa, Vicia sepium, and Caragana arborescens. Tubercles were formed on the peas from all the inoculations, while on the locust only those receiving the cultures from the tubercles of locust and Caragana produced any. In another series lathyrus latifolius was inoculated with pure culture from peas, vetch and locust. Only the first and second produced tubercles. The tabulated results of a third series of experiments by the same authors is shown below. In this experiment, locust, Acacia lophantha, Villous vetch, and peas were inoculated by cultures of two-year-old tubercles of locust, two-year-old tubercles of Caragana, tubercles of vetch and peas.# The peas met with an accident in the last part of the experiment and had to be omitted from tables 2 and 3.

Experiment Station Record, Vol. VI, No. 6, P. 505.

Transpiration from time of inoculation to harvest.

	Inoculated with pure cultures from--			
	Robinia	Acacia	Vicia	Pisum
	Cc.	Cc.	Cc.	Cc.
Robinia pseudacacia........	3,570	1,136	1,425	1,396
Acacia lophantha...........	1,538	3,805	1,205	1,511
Vicia villosa..............	934	1,097	4,978	1,277
Pisum sativum..............	1,380	1,034	1,265	1,849

Average height of plants at harvest.

	Inoculated with pure cultures from--			
	Robinia	Acacia	Vicia	Pisum
	Mm.	Mm.	Mm.	Mm.
Robinia....................	131	50	50	50
Acacia.....................	80	295	62	75
Vicia......................	350	400	1,126	450

Chemical analysis of plants.

	Inoculated with pure cultures from--			
	Robinia	Acacia	Vicia	Pisum
Robinia dry substance, grams	7.402	1.158	0.858	1.479
Robinia nitrogen..... Mg.	232.100	16.600	13.500	21.100
Acacia dry substance, grams	1.953	6.943	1.248	1.817
Acacia nitrogen..... Mg.	17.000	109.800	16.200	19.700
Vicia dry substance.. grams	.883	.866	9.133	1.033
Vicia nitrogen...... Mg.	12.900	14.700	264.000	22.600

"From the above table it will be seen that in all but one case each plant was most favorably affected when it was inoculated with bacteria from the tubercles of its own species."

In a fourth series 21 different leguminous plants were inoculated with pure culture bacteria from tubercles of peas and locust. Of the 21 species inoculated with bacteria from peas, only three, the vetch, lentils and beans developed many normal tubercles.

The crimson clover and locust had a few scattering tubercles. The remaining 16 species bore no tubercles. Of those inoculated with locust bacteria only the locust gave good results. The beans had many small tubercles and the red clover a very few scattering ones. The other 18 species were unaffected by the inoculation.

These investigations, however, conclude that the "differences between the forms is not sufficient to entitle them to be ranked as separate species of bacteria and agree with Beyerinch that there is but one species, Bacillus radiciola, which becomes more or less modified by the different host plants on which the tubercles are grown.

On the other hand Albert Schneider, Frank and others have gone so far as to name several different species.# Their classification is based upon a microscopical study of the organisms, both in the tubercles and in pure cultures.

Other evidence such as variations in shape, size, color and markings of tubercles on different species of legumes, as recorded by myself and others could be presented upon this question; but the multiplicity of forms which have been found among the bacteroids, together with the facts that they are constantly undergoing modifications and are under abnormal conditions, makes it very apparent that the question of whether there are few or many species, must remain unsettled until their life history has been worked out.

The morphological data is most extensive. Over one

Torrey Bot'l. Club Vol. 19, P. 213.

hundred investigators in more than two hundred papers have touched upon this phase of the question. Among these are to be found our greatest scientists, such as Frank,# H. Marshall Ward, Schloessing, Laurent, Hiltner, Atkinson, Beyerinck, Schneider and others. These papers together with the extensive drawings, represent many years of work.

In the words of George F. Atkinson## of Cornell University, "The record presents a discouraging volume of conflicting testimony. It would indeed be a misfortune should all these pains-taking and laborious investigations be so much at variance as appears from the examinations of the contributions." When more is known regarding the life history of the organisms, many of the theories and apparent facts which at present seem to be so much at variance with each other, will no doubt be harmonized.

Professor Frank alone has presented over twenty papers on the question of the fixation free nitrogen.
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The above references are to papers which I have consulted in preparing this thesis. For a complete list of references to January 1, 1894, see Bulletin No. 29 of Illinois Experiment Station, P. 311, by Albert Schneider.