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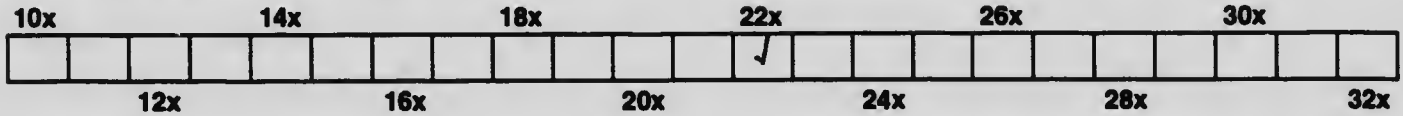

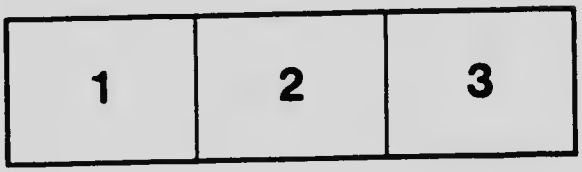

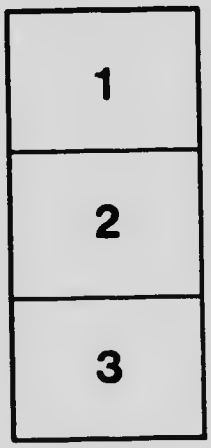

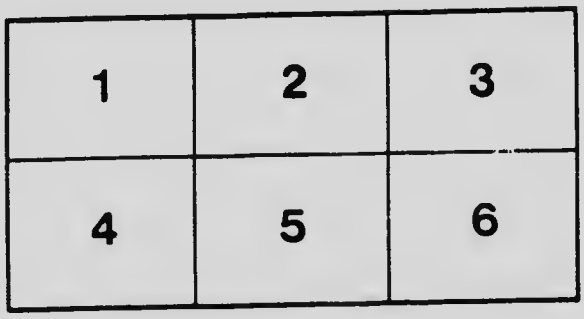

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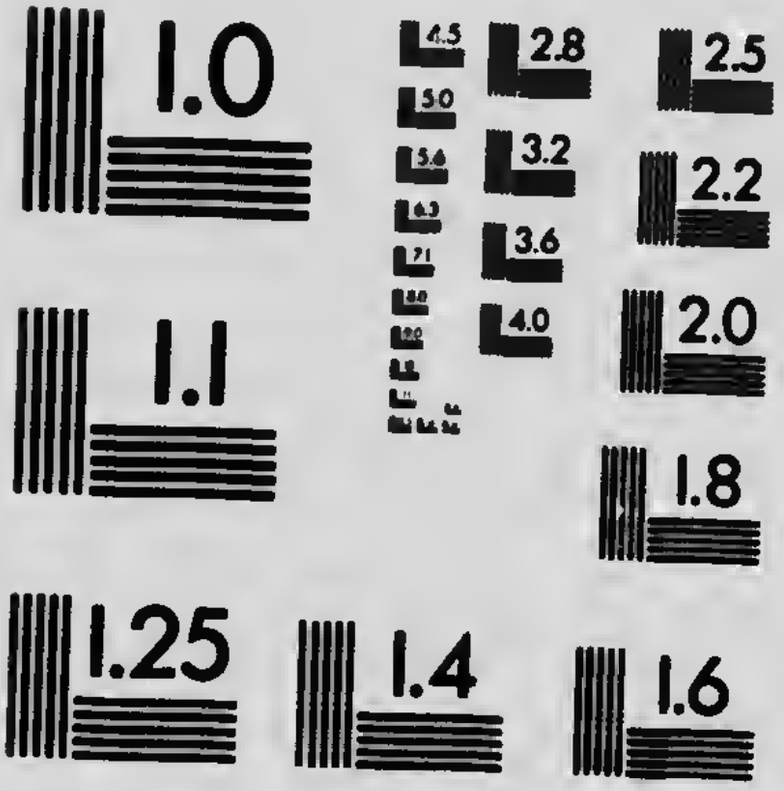

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ONTARIO DEPARTMENT OF AGRICULTURE

Ontario Agricultural College

BULLETIN 148

Co-operative Experiments
WITH
Nodule-forming Bacteria

BY

F. C. HARRISON, B.A., Professor of Bacteriology

AND

B. BARLOW, B.S., Demonstrator in Bacteriology

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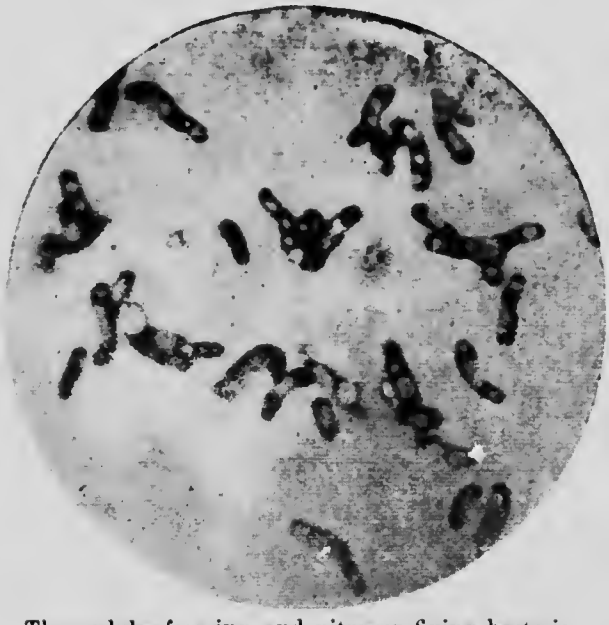
ONTARIO AGRICULTURAL COLLEGE AND EXPERIMENTAL FARM

CO-OPERATIVE EXPERIMENTS WITH NODULE-FORMING BACTERIA.

By F. C. Harrison and B. Barlow.



The nodules-forming, and nitrogen-fixing bacteria.
(Magnified 1,500 times.)

The issue of a number of bulletins by the United States Department of Agriculture on "Beneficial Bacteria for Leguminous Crops," "Inoculation of Soil with Nitrogen-fixing Bacteria," and the publication of articles on this subject in some of the popular magazines, has called the attention of the Canadian farming community to the maintenance of soil fertility by the growth of legumes which have been treated with beneficial bacteria, and has resulted in the many requests for information to the Bacteriological Department of the College with demands for samples of the necessary bacteria for the purpose of treating various crops such as clovers, peas, beans, etc.

Hence, it seemed necessary to publish a report of what has been accomplished in this line at Guelph, together with a brief statement of the history of the discovery of the legume bacteria, their manner of growth, and how they may be utilized by the farmer.

The Importance of Nitrogen to the Farmer.

As is well known, nitrogen, the most important and costliest element that a farmer buys or uses for plant food, can be used by most plants combined only in the form of nitrates. The daily loss of this valuable fertilizer is enormous, and several scientists have predicted that in the course of a number of years our supply of nitrogen will be so appreciably used up that the growing of wheat and other crops will be a matter of difficulty. These men base their calculations on the loss of nitrogen which comes from the yearly cropping of land, the waste of the sewage from the great centres of populations, the loss by leaching from the soil, the action of the denitrifying bacteria, and other causes; and they also show that the great natural stores of this element are being quickly consumed for agricultural purposes. Thus the guano deposits are nearly all used up, and the South American saltpetre beds are being fast exhausted. Fortunately, however, there are vast stores of nitrogen in the air, the atmosphere containing about four out of every five parts of this element, and one of the great problems of modern agriculture has been to make this store of atmospheric nitrogen available to plants, and the peculiar ability of leguminous plants to assimilate the nitrogen of the air is the faculty which makes them so valuable to the farmer. The legumes are able to achieve this result by the aid of the bacteria which are associated with them in the nodules or tubercles on their roots.

Not only does the acquirement of the nitrogen from the air benefit the legume, but it also enriches the soil and renders available considerable combined nitrogen for the use of succeeding crops. Every practical farmer acknowledges these facts by introducing clover or some other legume in his rotation, knowing that the fertility of his soil is thus increased. As a result of the investigation of many Experiment Stations it has been shown that from 100 to 200 pounds per acre of nitrogen are added to the soil by the growth of a crop of legumes. Putting the matter into dollars and cents, the United States Department of Agriculture states that a crop of nodule-bearing legumes is equal to from 800 to 1,000 pounds of nitrate of soda per acre, which at the present rate for this fertilizer represents a value of from $20.00 to $25.00.

The Discovery of the Nodule Bacteria

The Romans were fully aware of the importance of growing legumes, and introduced such crops into their rotations, many writers in their time drew attention to the manure-like qualities of beans, vetches, etc. Thus, Pliny, a Roman writer, makes the definite statement that beans will fertilize the soil of a field or vineyard as well as the very best manure. The true reason of these facts was, of course, not known at that time, but was

attributed to the greater root development of the legumes and, in consequence, their ability to obtain more nourishment from the soil.

The tubercles or nodules on the roots of clovers, peas, etc., had been noticed for a considerable time (since 1687). Some thought they were of parasitic origin, and others saw in them simply excrescences or galls, and a few thought they were the normal growths of the plant; and it was not until 1886 that two German scientists, Hellriegel and Willfarth, showed that the development of the root nodules was intimately connected with the growth of the whole plant, and that the assimilation of the nitrogen of the atmosphere by legumes was associated with the development of the nodules or tubercles on the roots. Later investigators confirmed these results, and these discoveries were quickly followed by the detection of the bacteria in the nodules, their isolation and growth on artificially prepared food and the ability of these cultivated organisms to produce other nodules when brought into contact with suitable legumes growing in sterilized or germ free soil.

The first practical applications of these discoveries was the introduction of cultures or growths of these bacteria for application to the seeds of the various legumes. The trade name of *Nitragin* was given by Nobbe, the inventor of this method, to these cultures, and the product was extensively advertised and exploited by a German firm of manufacturing chemists. Numerous experiments were conducted with this material. Some succeeded and others failed, but after a few years' trial the manufacturers discontinued the sale of this article.

About 1902 the study of the root-nodule organism was undertaken by the Laboratory of Plant Physiology of the United States Department of Agriculture, and they perfected a method by which these organisms could be sent out to farmers and used for inoculating seed. Up to November 1904, over 12,000 packages of inoculating material were distributed, and the reports of 3,540 experimentors showed that 79 per cent. of these were successful.

Recently, however, some complaint has been made concerning the quality of the cultures sent out by the U. S. Department of Agriculture, the New York Experiment Station reporting the results of a bacteriological examination of 18 packages of treated cotton (the nodule-forming bacteria dried upon absorbent cotton) in which no living nodule bacteria were found.

The failure of these cultures was due to their method of preparation, but the N. Y. Experiment Station state that "they should not be understood as being opposed to the idea of treating the seed of legumes with living bacteria."

Distribution of Cultures in Canada.

During the spring of 1905, the Bacteriological Department of the Ontario Agricultural College sent out a number of samples of the nodule-forming bacteria for experimental purposes. These samples were sent out in small bottles, in such condition that all the farmer had to do was to mix the contents of the bottle with a measured quantity of water, and then apply to his seed ; this method doing away with the " building up" process

advocated by the U.S. Department of Agriculture, in which a package of treated cotton, containing dried bacteria, has to be put into a solution of chemicals, and the bacteria allowed to develop for a day or two before being applied to the seeds.

These samples were sent all over Canada, from Prince Edward Island to British Columbia, the number of cultures being as follows :

Ontario	76	Assiniboia	13
Nova Scotia	66	Alberta	12
New Brunswick	11	British Columbia	15
P. E. Island	17	United States	2
Cape Breton	1		
Quebec	10	Total	246
Manitoba	23		

They were also tested by Mr. Frank T. Shutt, Chemist, Experimental Farm, Ottawa ; by Principal M. Cumming, Agricultural College, Truro, N.S., by Mr. C. Jarvis, Assistant to Prof. L. H. Bailey, of Cornell Experiment Station, and by ourselves. At the end of the season a circular

Plot of alfalfa or lucerne, grown from seed untreated with nitro-culture. The mark on upright stick shows height to which the alfalfa grew in the adjoining treated plot. (Experiment conducted by Principal Cumming, Agricultural College, Truro, N.S.)

letter was sent to all who had received cultures, asking what success each experimenter had had. These reports may be summarized thus :—

Crop.	Total No. of reports received.	Inoculation successful, with increased growth of crop.	Organisms already present in the soil.	No advantage from inoculation.
Lucerne or alfalfa	59	43	1	15
Red Clover	47	1	1	15
Peas	12	7	1	4
Beans	9	5	.	4
Alsike	2	1	.	1
White Clover	1	1	..	..
Vetch	3	2	..	1
Soy Bean	1	1	..	..
	134	91	3	40

Alfalfa or Lucerne. Plot grown from seed treated with alfalfa nitro-culture. The stick in the centre of the plot is covered up to ten inches from the ground with alfalfa plants. (Experiment conducted by Principal Cumming, Agricultural College, Truro, N.S.)

In order to give the farmers an idea of these reports, a number of extracts are given from them which show the benefit some experimenters have obtained from the use of the nitro-cultures :

Taylor Village, N.B. I put three acres with the treated seed and three acres without the nitro-culture on a piece of heavy land, and in examining the plants from time to time I found that nodules were quite plentiful on the plants from the treated seed, and none on the plants from the untreated seed, and I also found the growth and thriftiness quite marked in favor of the plants from the nitro-treated seed. (Red Clover.)

River John, N.S. The plants from the treated seed were thicker and thriftier than those without the nitro-cultures, which were thin and spindly. The roots on the treated spot being loaded with nodules and very fibrous, while on the untreated there is far less root growth. (Red Clover).

Marshville, Ont. Enclosed please find samples of what I pulled this morning (July 4th) in different parts of the field, which I think is very satisfactory indeed. Nodules appeared when plants were just above ground. What I sowed in spring of 1904 has no nodules. (Red Clover).

NOTE.—The one sample showed numerous nodules, the other none.—Authors.

Colwood, B.C. The part treated with nitro-culture looks green and more vigorous than the untreated. Nodules are numerous on the treated portion. (Red Clover.)

Fingal, Ont. The rootlets of the plants treated with nitro-culture were simply clustered with nodules. Without nitro-culture, nodules were not so numerous, but were present. (Red Clover).

Colina, B.C. The clover plants were a lot better on the treated land. (Red Clover.)

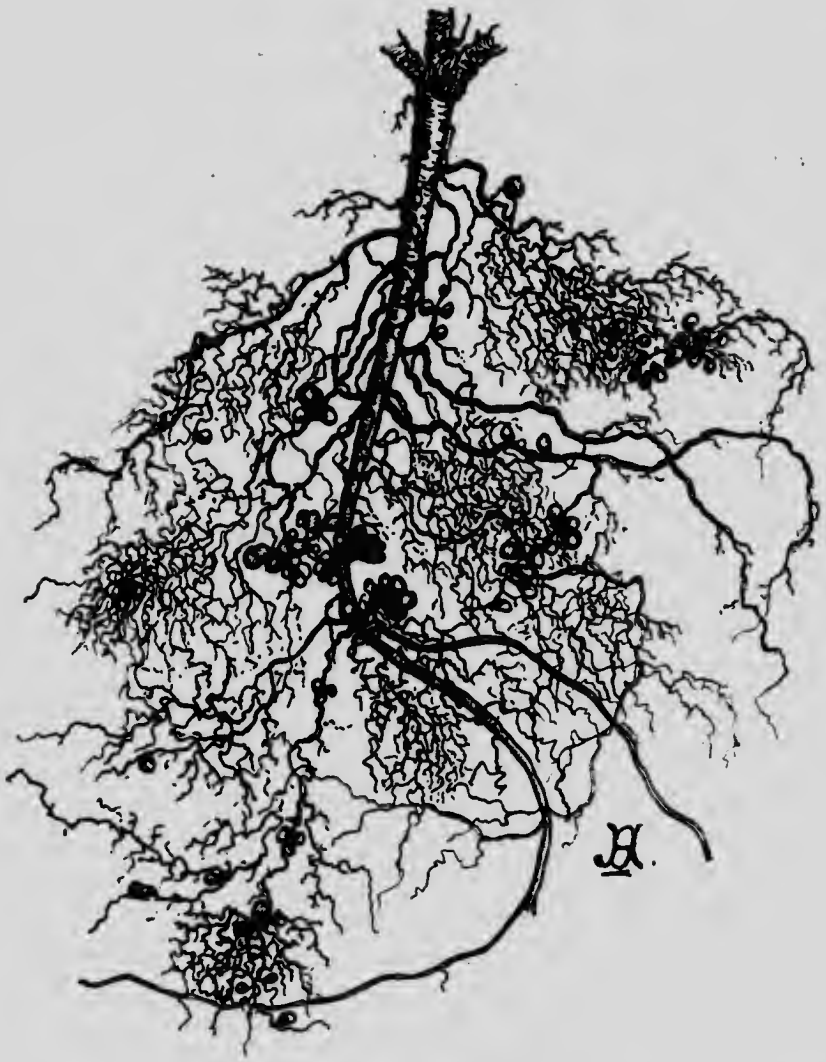
Emerson, Man. On some plants treated the nodules were quite numerous. Those having nodules were very vigorous, bnt the others have made but small growth. (Red Clover.)

Neepawa, Man. The vigor of plants from seed treated with nitro-culture was very good. The untreated seed produced pale and short plants. (Red Clover.)

Edmonton, Alta. Numerous nodules on plants from treated seed and very healthy stand. With the untreated seed the plants were sickly with a few healthy stalks. (Red Clover.)

Deep Brook, N.S. Soon after seeding I commenced examining plants on the treated patch and found nodules present which increased in size and number each week as the season advanced. There was a marked difference between the plants from treated and untreated seed in favor of the former. (Red Clover.)

Berwick, N.S. The plants from treated seed were quite vigorous, those from untreated only half as high. (Red Clover.)



Root of Sanfoin, showing nodules.

Cypress River, Man. Plants from treated seed very strong with numerous nodules, without nitro-culture much smaller and weaker. (Red Clover.)

Stamford, Ont. Vigor of plants from treated seed good, without nitro-culture no catch. (Red Clover.)

Meadowville, N.S. Very vigorous plants with numerous nodules from the treated seed, only fair stand from untreated. (Red Clover.)

Port Williams, N.S. In the early part of the season the division line between the inoculated plants and the uninoculated was very marked.

Nodules were very numerous on the treated plants but only a few present on some of the untreated. (Red Clover.)

Nauteig, N.B. Very vigorous and numerous nodules from the treated seed; not nearly so vigorous without the nitro-culture. (Beans.)

Cedar Springs, Ont. I am convinced the application was a success. (Beans.)

Mount Salem, Ont. The nodules on the beans not treated were small, about the size of pin-heads, and pods on plants were short. On the plants treated the nodules were as large as small peas, growing tight against one another. The pods on the plants were longer and the plants withstood dry weather better than those not treated, being green and flourishing when the others were dried up. The beans when ripe were bright and even in the pods. I exhibited half a bushel of these at the East Elgin Fair and secured first prize on them. (Beans.)

Cedar Springs, Ont. The nodules were larger and more numerous on the plants from the treated seed. Those of the untreated had nodules, but they were not so large and easily one-third less in number. (Beans.)

Cross Roads, N.S. Nodules present on the plants from treated seed, fairly vigorous growth. Without nitro-culture the plants were poor and no nodules were present. (Vetch.)

Waterville, N.S. The plants from treated seed were very strong, many roots were a solid mass of nodules. From untreated seed plants were not so good and had very few nodules. (Vetch)

Rapid City, Man. Plants from treated seed strong, numerons nodules; from untreated seed plants only about half as big. (Alsike clover.)

Barrie, Ont. I found the culture very satisfactory, the yield of peas being fully ten per cent. more from the seed treated with culture. (Peas.)

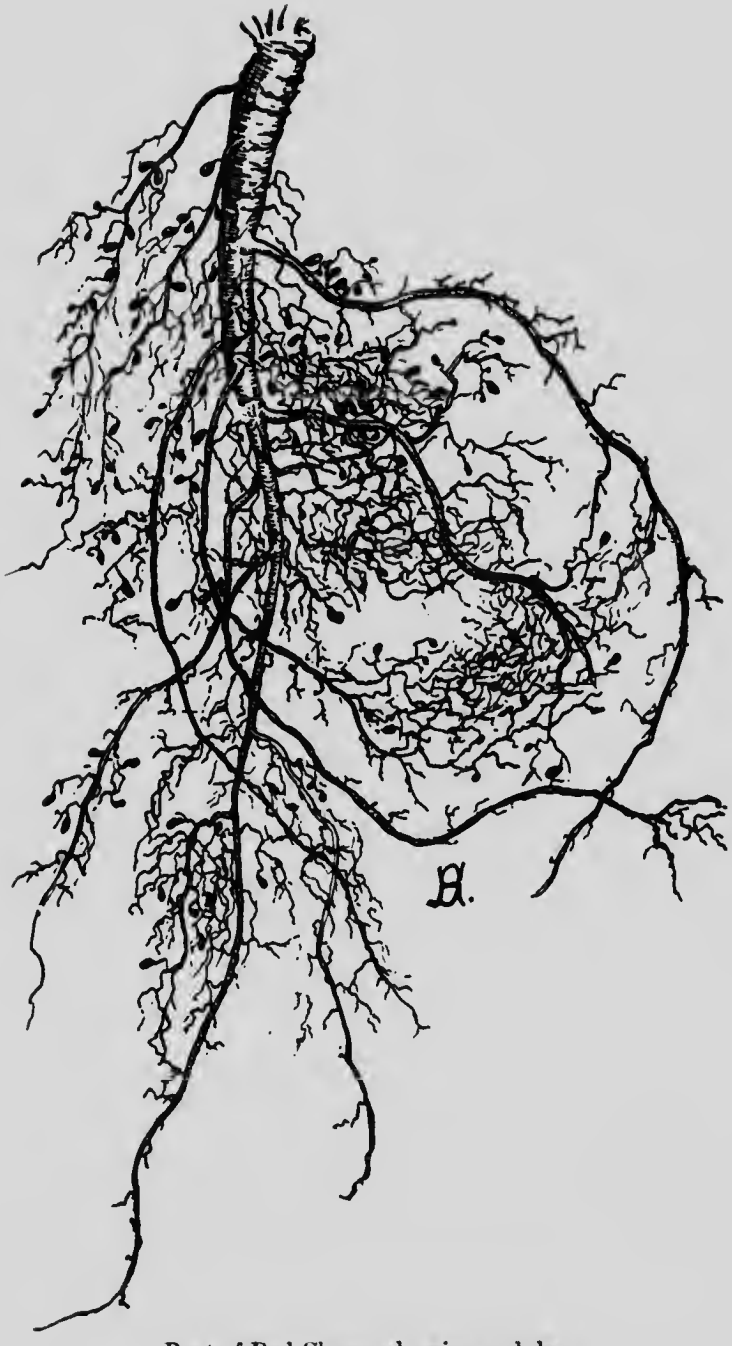
Ruthven, Ont. Very vigorous plants from the treated seed with numerous nodules. Without treatment the plants were about the usual size and few nodules. (Peas.)

Colquitz, B. C. I find that in the portion of ground where this culture was used, plants seem to be thriving and have the nodules on the roots, while, at the same time, on ground seeded to alfalfa close by on which the culture was not used, the plants are sickly looking and have no nodules upon the roots. (Alfalfa.)

Lion's Head, Ont. The alfalfa seed sown by me this spring, and treated with nitro-culture obtained from you, has grown without a check. That without the culture came up in patches, but did no more. As the alfalfa has failed here before I attribute my success to the culture. (Alfalfa.)

Round Hill, N. S. From treated seed the growth of plants was 25 inches, from seed untreated about one inch. (Alfalfa.)

Windsor Mills, Que. Plants from treated seed strong and healthy, those without nitro-culture only fair.



Root of Red Clover, showing nodules.

Harding, Man. There was quite a marked difference between what was treated and what was not treated, and I consider the result was very good. (Alfalfa.)

Mount Stewart, P. E. I. Plants from treated seed were dark green and quite vigorous. From untreated seed the plants were yellow and unthrifty. (Alfalfa.)

Colquitz, B.C. I am not a believer in nitro-culture, or, at least, was very skeptical as to the treatment being of any value whatever. I have been unable, however, to shut my eyes to the fact as shown by my own land. The land had been well sub-soiled in preparation to a depth of from 16 to 18 inches, and the portion upon which the nitro-culture was not used was treated in every way as well as the other. The plants from the treated seed were very strong and sown with nurse crop are now about 8 inches high. From untreated seed the plants are weak in appearance and only about 3 inches high. (Alfalfa.)

Centretown, Ont. The plants from treated seed sown on very light sandy soil were healthy and grew rapidly. Those from untreated seed were delicate and of stunted growth. (Alfalfa.)

Cloverdale, B.C. Plants from treated seed are healthy in color and 18 inches high, from untreated seed 4 to 6 inches high and yellow. I may say in conclusion that the quarter acre of untreated seed was planted on better land which had been under cultivation in root crops for two years and had been slightly manured with barnyard manure and lime previously. The treated seed was planted on absolutely new forest land which is almost void of humus and available nitrogen. This speaks well for the cultures. (Alfalfa.)

Charlottetown, P.E.I. The boundary between the two plots is distinctly marked, that growing from the inoculated seed is a richer green and of stronger growth. (Alfalfa.)

Brule, N.S. The difference between treated and untreated plants was more marked after the first cutting. The treated are now about one foot high and of a dark green healthy color. The untreated are only a few inches high and except in a few scattered bunches are yellow and sickly. (Alfalfa.)

Marshville, Ont. I sowed two acres last spring without nitroculture and the roots are smaller than what I sowed this spring with treated seed. (Alfalfa.)

St. Catharines, Ont. The culture was used on alfalfa sown in April on fall wheat about 15 pounds per acre. The stand at present is considered good, a difference being evident in favor of the nitro-culture treated seed. (Alfalfa.)

Treesbank, Man. Nodules are plentiful on the part sowed with the treated seed, but I have not been able to find any on the other strip seeded

with untreated seed. Furthermore, the second growth on the untreated strip is now sickly looking and much shorter than the rest. (Alfalfa.)

In addition to these reports, special mention may be made of the results obtained by Principal Cumming, of the Nova Scotia Agricultural College, Mr. F. T. Shutt, Chemist, Central Experimental Farm, Ottawa, and Mr. C. Jarvis, Assistant in Horticulture, Cornell University Experi-

Peas grown in swamp soil. The roots on plant marked "0" had no nodules. Peas planted in the other two pots marked "Culture" and "Lime and Culture" were treated with nitro-culture. The pot with lime was treated at the rate of 1,000 pounds of lime to the acre. The roots of these two plants had numerous nodules.

ment Station, as these gentlemen are accustomed to carrying out experimental work.

Principal Cumming writes as follows :—

"The only positive results we have to report are with your alfalfa culture. Our red clover was sown just in time to catch the drought, and neither that treated with culture or left untreated amounted to much.

"The peas and vetches all grew very rank, and it was impossible to notice any difference between the treated and untreated plots. With the alfalfa we were more successful, having sown it just at the right time. We conducted the experiment in duplicate, and had side by side plots that were untreated with culture, plots treated with United States Department of Agriculture culture, and plots treated with your culture. For some reason or other, we got no decided results with United States culture; possibly it was not rightly handled. Of this I am not sure, for I was away from home when the seed was treated. The most marked results were found on a piece of land, which was seeded with a nurse crop of barley. The plot that was treated with your culture has, at the present time, a most vigorous growth of alfalfa, the plants being on an average of eight inches high, which, considering the dry season, is very good. On the untreated plot adjoining the alfalfa will not average more than one inch in height, and the plants are rather sickly looking.

"I sent you by last night's mail a representative plant from each plot, and you will readily observe the decided difference in the nodular growth. I have sent a picture of the two plots to the "Farmer's Advocate" asking them to make plates.

"Our most striking results are on a piece of land which was in roots last year, and consequently was free from weeds of all kinds when the alfalfa was sown. We tried a duplicate experiment on a piece of land, which was ploughed out of sod after having been down fifteen or twenty years. We grew six plots on this piece of land; two untreated, which amounted to almost nothing; two treated with United States culture, which are little, if any better, and two treated with your culture, which are so strikingly superior that you can pick out the plot almost as far away as you can see.

"In the light of these results we are very anxious to continue our work and would like especially to treat red clover seed as successfully as we seem to have treated alfalfa."

The results obtained at the Dominion Experimental Farm were as follows :—

Pot Experiments. Clover, sown May 6th., 3 cuttings.

Total weight of green crops from untreated seed...374.7 grams.
" " inoculated seed..450.7 "
" " inoculated soil...440.4 "

A difference of about 17 per cent. and 15 per cent. respectively in favor of inoculated

Pot Experiments. Alfalfa, sown May 6th., 3 cuttings.

Total weight of green crop from untreated seed....204.5 grams.
" " inoculated seed....217.3 "
" " inoculated soil.....249.1 "

Soy Bean Plant. The one on the left is from untreated seed, that on the right from seed treated with nitro-culture.

A difference of about 6 per cent. and 18 per cent. respectively in favor of inoculation.

Plot Experiments. Clover sown May 9th. Each plot 22 by 33 feet.

	lbs.	ozs.
Hay crop from untreated seed (2 cuttings).....	46	11
" " " inoculated seed (2 cuttings).....	59	13

A difference of a little more than 22 per cent. in favor of inoculated seed.

Plot Experiments. Alfalfa, Sown May 9th. Each plot 22 by 33 feet.

	lbs.	ozs.
Hay crop from untreated seed (3 cuttings).....	88	4
" " " treated seed (3 cuttings)	62	13

A difference of nearly 30 per cent. in favor of the untreated seed.

Mr. Shutt makes no explanation of this difference in favor of the untreated seed, but writes :

"The nodules on the roots of the untreated alfalfa appeared to be just as numerous as on the roots of the inoculated.

"The soil is a light, sandy loam, fairly rich in humus. It has in all probability carried legumes, but not for some years."

Mr. C. Jarvis, of the Department of Horticulture, Cornell University, tried the culture on the Mohawk variety of bean. Each row was 20 feet long. His results are as follows :—

"1. Mohawk Beans untreated—30 plants, no nodules.

"2. Beans treated with fresh culture and sown at once—27 plants, 12 with nodules.

"3. Beans seeded two weeks later but treated same time as in Experiment 2.—35 plants, 2 with nodules.

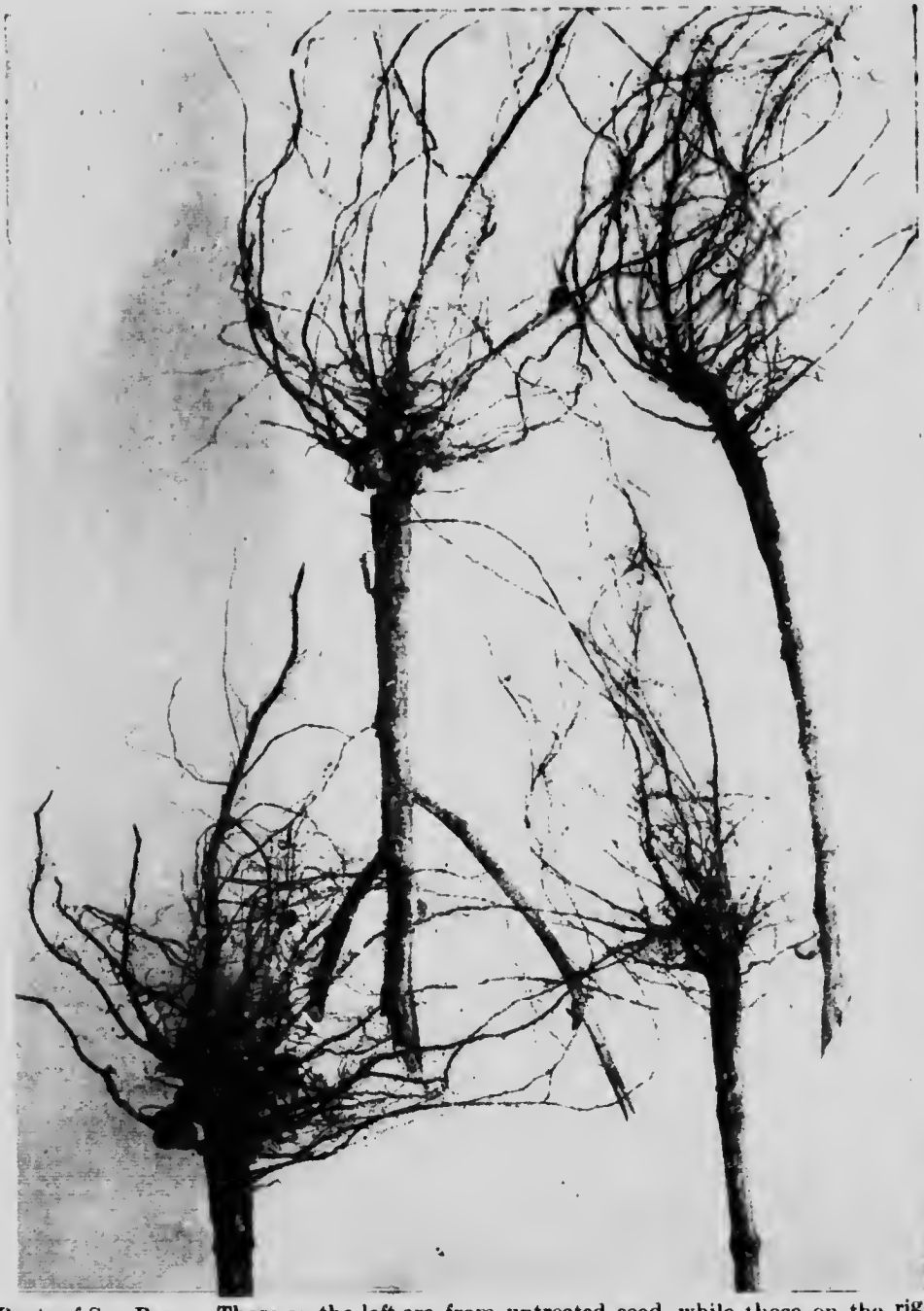
"4. Beans untreated sown at same time as in Row 3—37 plants, 7 with nodules.

"5. Beans treated with culture 1 month old and sown in a moist condition—31 plants, 6 with nodules.

"6 Beans untreated sown at same time as No. 5—35 plants, 3 with nodules."

As will be seen from above the best results were obtained from fresh culture used according to directions; the remaining experiments were conducted on Mr. Jarvis' own initiative. Mr. Jarvis remarks: "Had some other variety of bean been used in place of Mohawk, which is a very hardy, strong growing variety, a difference in *growth* may have been apparent."

As the Bacteriological Department intends to continue experimental work along this line, and will send out nitro-cultures in the spring of 1906, some information is needed regarding the use of the cultures and their application; hence a few notes are given for the guidance of those who desire to experiment with these beneficial bacteria.



THIS ILLUSTRATION IS UPSIDE DOWN

Roots of Soy Bean. Those on the left are from untreated seed, while those on the right are from seed treated with nitro-culture. Note the nodules on the roots.

It should be thoroughly understood that these nitro-cultures are of use for legumes only, such as the clovers, beans, peas, and vetches, or, as they are sometimes termed, the pod-bearing plants. We have had a number of applications for nitro-culture for inoculating wheat, turnips, etc., but these bacteria are of no use whatever for such crops.

Inoculation—and by inoculation we mean the treatment of the soil or seed with the nodule forming bacteria—is necessary when the land has never before been seeded down to a leguminous crop, or if legumes have grown in it without forming the characteristic nodules or tubercles, which is proof that the nodule bacteria for that particular crop are not present in the soil. In such cases as these the farmer may well resort to inoculation.

Inoculation is also desirable if a farmer wishes to grow a new kind of legume on land even though it has previously grown excellent crops of a different species of legume. Thus, although red clover may have been successfully grown, with a large number of nodules on the roots, the bacteria from these nodules may not infect alfalfa or lucerne if it were planted in the clover soil, and hence, inoculation with bacteria taken from the afalfa nodules, would be desirable. So also, when introducing the culture of vetches, of field beans, and soy beans, it would be necessary to treat the seed with bacteria taken from these plants. In some cases, bacteria derived from closely related species are mutually available, thus bacteria from sweet clover (*Melilotus*) are capable of infecting afalfa. It seems especially desirable in Ontario to inoculate the field bean and soy bean, as experiments have shown that Ontario soils are not so generally infected with the bacteria appropriate for these plants as for the other legumes, and there is less possibility of their becoming inoculated from the bacteria of closely related wild legumes, on account of the relative scarcity of the latter plants.

The employment of nitro-cultures is recommended also on soils which produce a weak growth of legumes even when nodules are present, the more vigorous bacteria of the culture aiding the plant to fix more nitrogen and in those cases where the leguminous crop is not producing the highest yield.

A word of warning is necessary with regard to failures in the use of these nitro-cultures, and also with regard to their use as a remedy for lack of care in preparation and cultivation of the soil. No benefit may be expected from the use of nitro-cultures if they are improperly prepared, the directions for their use are clearly set forth, and should be strictly followed. The farmer simply invites failure, if, for example, as some have done, he disregards the directions and uses the culture on turnip seed, instead of for the legume for which the culture was prepared.

Failure of plants to form nodules, even when the seed is inoculated, may result if:

1. The soil is too acid or too alkaline. Lime should be first applied if the soil is too acid.

2. If some fertilizer with a caustic action is brought into contact with the treated seed.

Little benefit may result from innoculation :

1. If the soil does not contain sufficient of the other necessary plant foods, especially potash and phosphoric acid.

2. If the soil is too rich in nitrogen; in this case it is better to plant crops that are nitrogen feeders rather than nitrogen accumulators

3. If the soil is not properly cultivated and freed from weeds.

4. If the soil is already thoroughly inoculated with the nitrogen-fixing bacteria.

Methods of Inoculating.

There are three methods of inoculating the crop :—

1. By transfer of soil. 2. By treating the soil. 3. By treating the seed.

In the first case, soil which is known to contain the nodule-producing organisms is scattered over the land where it is desired to grow a crop of legumes. In other words, the land is top-dressed with soil from an infected field. This method has given good results, but is expensive when the soil has to be brought any distance, and in certain sections of the country such a practice is fraught with danger, as weed seeds and certain diseases are apt to be transferred from one field to another by this means.

In the second method, a quantity of soil is moistened with large quantities of culture, and this is mixed with more dry soil, and then used as a top dressing on the land that is to be planted. We have not tried this method of soil inoculation, and it is not convenient for our method of culture distribution. For small gardens, liquid cultures may be used and applied by means of a watering can to the young plants, but it is far better to have the bacteria on the seed so that the plant may become inoculated when they commence to grow.

The third method, seed treatment, consists in moistening the seed with a culture or growth of the desirable bacteria, allowing the seed to dry for a *short* time and immediately sowing it. We recommend that cultures supplied by us be used for seed inoculation.

Directions for the use of Nitro-cultures Supplied by the Bacteriological Department of the Ontario Agricultural College.

The culture is sent you with the understanding that it is to be used for experimental purposes and that you will use it as directed and report to us your success or failure.

1. For every 60 pounds of seed to be treated take one and one-half pints of clean cool water in a small pail.

2. Pour some of the water into the bottle, shake the bottle and pour back the water into the pail. Repeat this until the culture is all rinsed from the bottle into the pail and the water in the pail is clouded. The jelly-like substance in the bottle is agar, it will not dissolve, but may be broken up and mixed in the water.

3. Pour the water from the pail over the seed and mix thoroughly.

4. Spread out the seed to dry in a clean place out of the sunshine.

5. The seed will dry in an hour and may be planted in the usual manner as soon as it is dry.

6. Do not add water to the culture in the bottle until you are ready to plant your seed.

7. Some untreated seed should be planted for comparison and it is well to plant this first.

8. After the seedlings are one month old, look for nodules on the roots. During the season note number and size of nodules and vigor of plant growth from treated and untreated seed.

ANNOUNCEMENT.

For the spring of 1906 the Bacteriological Department is preparing to send out a *limited* number of cultures for the inoculation of the following legumes: Red Clover, Alsike Clover, Alfalfa or Lucerne, Field Peas, Vetches, Field Beans and Soy Beans, Those desiring cultures will please make application according to the following blank form.

Application for Nitro-culture.

I should like to conduct an experiment with Nitro-culture for Red Clover, Alfalfa or Lucerne, Vetches, Soy Beans, Alsike Clover, Field Peas, Field Beans.

(Strike out those *not* wanted).

State probable date of seeding.

If the material is sent to me I shall endeavor—

1. Carry on the test according to the instructions received.
2. Exercise care and accuracy in the work.
3. Report the results of the experiment as soon as possible after harvest, whether successful or not.

Name .....

Post Office..... Express Office.....

County ..... Province .....

This sheet when filled out should be addressed to the Bacteriological Department, Agricultural College, Guelph, Ontario, and will require *2 cents* postage whether the envelope is sealed or not.

LIS[T]

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