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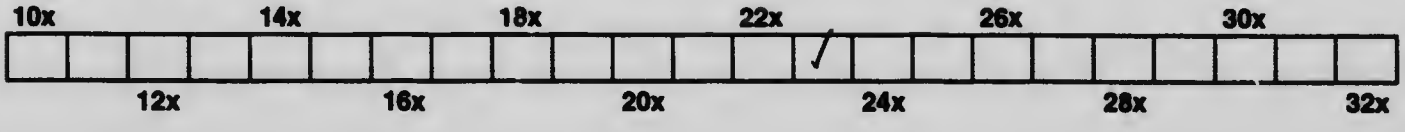

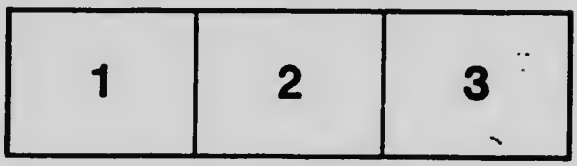

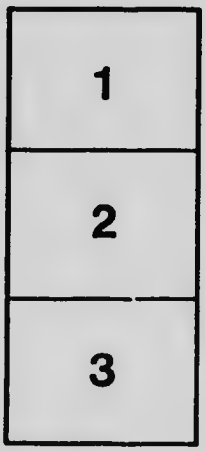

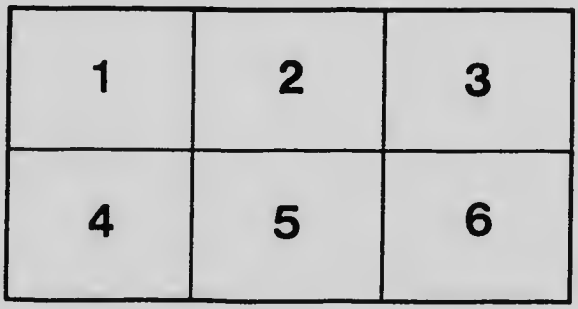

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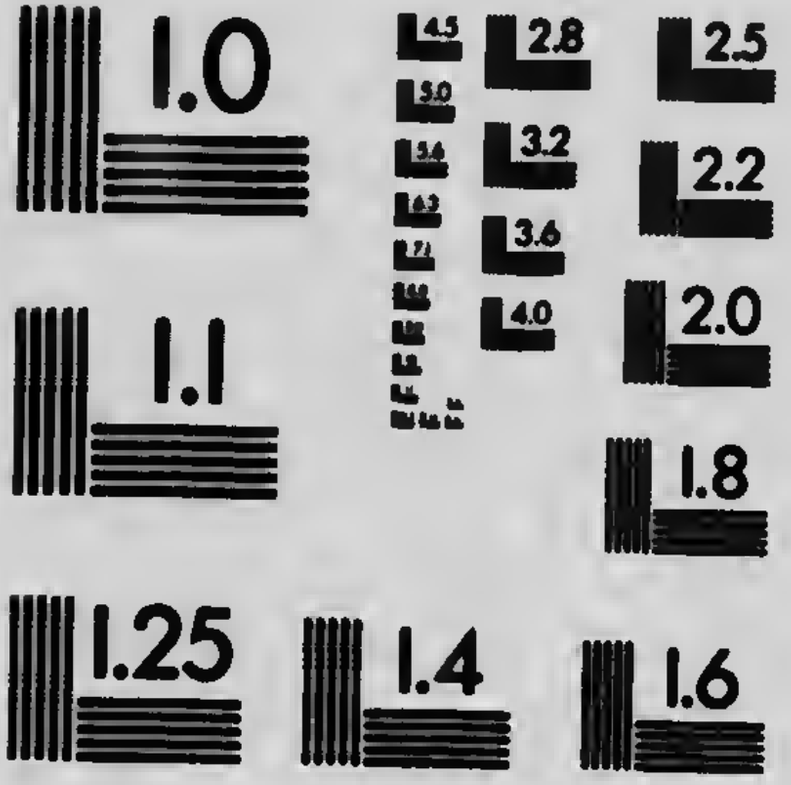

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BULLETIN 164.] [MARC 1908 ISSUE.

Ontario Department of Agriculture.

ONTARIO AGRICULTURAL COLLEGE.

LEGUME BACTERIA.

SEED INOCULATION BY CANADIAN FARMERS IN 1906 AND 1907.

By S. F. Edwards and B. Barlow.

To maintain and increase the fertility of his land, the farmer must put back into the soil as much plant food as he removes in his crops. It is true that most soils contain rich stores of plant food, and that through tillage and the biological agencies in the soil this food is placed at the disposal of plants. This supply is by no means inexhaustible, and as it is removed from the soil by successive cropping, methods must be adopted to compensate for the loss. Phosphorus, potash, and nitrogen may be applied to the soil in the form of commercial fertilizers or as barnyard manure. Nitrogen may also be secured from the vast supply in the atmosphere through the associative action of legumes (plants belonging to the bean family), and certain bacteria of the soil. These bacteria have the power to penetrate the roots of seedlings of leguminous plants, to multiply there, and in association with the plant, in some manner not yet fully understood, to take nitrogen from the air and store it up in the plant. Such plants when plowed under naturally increase the nitrogen content of the soil. Evidence of the activity of these bacteria may be seen in the small nodules or tubercles which form on the roots of these plants. (See Fig 1). Not all plants belonging to the order Leguminosæ are thus affected, but only those belonging to the sub-order *Papillionaceæ*. Of these, the commonly cultivated ones which man uses are: The clovers, alfalfa or lucerne, sweet white clover, lupines, vetches, beans, soy beans, peas, lentils, locust, sweet pea, and winter flat pea. Many others of the same order grow wild in meadow and forest.

This enrichment of the soil by the aid of the legumes is by no means a new thing. The phenomenon has been known for centuries, some of the earliest writers having dilated upon the manurial value of legumes. Only in recent years, however, have we attained knowledge of the bacteria associated with the legumes, and of their importance in the process of assimilation of atmospheric nitrogen. Although the bacteria can, under certain conditions, accumulate the nitrogen of the air apart from the legume, the legume *cannot* take the nitrogen from the air without the presence of the bacteria in its roots.

Fig. 1.—Red Clover. Inoculated and not inoculated. Fleming, Sask.

Methods have been devised whereby the bacteria infecting the common legumes may be distributed to farmers to inoculate the seed. In the United States, the Department of Agriculture began the distribution of

cultures in 1902, and have continued it each season. Commercial firms have engaged in the manufacture and sale of such cultures.

Fig. 2.—Alsike Clover. Inoculated and not inoculated. Fleming, Sask.

Distribution of Cultures in Canada.

The Bacteriological Department of the Ontario Agricultural College began the distribution of cultures to Canadian farmers in the spring of 1905, and during that season two hundred and forty-six cultures were sent out. A circular letter was sent to all who had received cultures, asking for a report of the success or failure of the experiment. These results were received and tabulated by Harrison and Barlow, and published with a short historical account and discussion as Bulletin No. 148 of the Ontario Agricultural College.

During the season of 1906, cultures were again sent out from this laboratory, three hundred and seventy-five being distributed as follows:

Ontario	121	Quebec	5
Nova Scotia	108	New Brunswick	4
United States	60	British Columbia	4
Alberta	24	South Africa	4
Manitoba	19	Porto Rico	1
Saskatchewan	14	England	1
P. E. Island	10		

As in the previous year, blanks were sent to recipients of the cultures, asking for a report as to their success or failure with the experiment.

The cultures sent to applicants in Nova Scotia were sent in the name of the Nova Scotia Agricultural College, and the reports were sent to Principal Cumming of that school.

All experimenters found the culture easy to apply, and a large number expressed a wish to continue the experiment.

A summary of the reports received follows:

Province.	Crop Grown.		Result.	
			Benefit.	No Benefit.
Ontario	Alfalfa	12	9	3
	Red Clover	14	10	4
	Peas	14	9	5
	Beans	11	6	5
	Soy Beans	3	2	1
	Alsike	1	1	
	Vetch	1	1	
	Sweet pea	1	1	
Alberta	Alfalfa	13	6	7
	Red Clover	3	1	2
	Peas	1	1	
Saskatchewan	Alfalfa	1		1
	Red Clover	2		2
	Peas	1	1	
	Beans	1		1
	Vetch	1		1
Manitoba	Alfalfa	2	2	
	Red Clover	8	6	2
Quebec	Alfalfa	3	3	
	Red Clover	1	1	
	Alsike	1	1	
United States	Alfalfa	5	3	2
	Red Clover	4	1	3
	Peas	3	2	1
	Beans	2	1	1
	Soy Beans	6	1	5
	White Clover	2	2	
	Vetch	1		1
New Brunswick	Red Clover	2	1	1

Thus of a total of 120 reports received, 72 showed a benefit to the crop by the application of the bacteria to the seed, as against 48 in which no benefit was apparent.

Fig. 3.—The cultures are sent in glass bottles accompanied by directions for their use.

EXTRACTS FROM REPORTS, 1906.

Big Fork, Ont. Area of plots, each one-eighth acre. Yield on treated plot, 120 pounds; on untreated plot, 101. Soy Beans.

M[illegible]villa, Ont. At the time of harvesting the nurse crop (barley), where the culture was used, the plants were very thick, and the binder cut several inches off the top of the plants. Where the culture was not used, the plants were thin, weak, and of a sickly yellow color. At a distance of half a mile you could easily tell the difference up to the middle of October. Where it was not used, the stubble was not covered at that date, while on the other part of the field there was a thick mat of stems and luxuriant leaves. Alfalfa.

Houghton, Ont. We have had the driest summer we have ever had, and I have more clover where I sowed the treated seed than there is altogether in this neighborhood. I believe the culture helped it grow stronger so it stood the drought. Red Clover.

Thorndale, Ont. The plants on the treated plots are very strong with very numerous nodules, while those on the untreated plot are weakly and dying, and have no nodules. We never have been able to secure a stand of alfalfa before. Alfalfa.

Chesley, Ont. The plants grown from treated seed were much more vigorous than those from untreated seed. The yield was larger, and the beans of better quality. Field Beans.

Dorion, Ont. The treated plants are a beautiful dark green, large and very strong, while the untreated are very small, weakly, and pale in color. Red Clover.

Grimsby, Ont. The crop on the untreated plot was only about half as large as that on the treated plot. Field Beans.

Highland Grove, Ont. In the first part of the season the plants nearly all turned yellow, and I could find no nodules. Then that on which I used the culture turned green, and I have found nodules on them. The plants where the culture was not used seem to have dried off entirely, and that the culture was used on has made a good growth, and has thickened out a lot since I cut the grain with which it was sown. Alfalfa.

Meaford, Ont. The plants on the treated plot made a decidedly better growth. Red Clover.

Rylstone, Ont. The crop on the treated plot looks twenty-five to fifty per cent. better than on the untreated plot. The farm has been rented for fifteen years, and the tenant that had the place before me said he could not raise clover on that field and that I was only throwing away the seed. Now there is no better piece of clover in the neighborhood. Red Clover.

Verner, Ont. The treated plot yielded over six bushels more per acre than the untreated. The treated plants were six inches longer, and stronger than on the untreated plot, with much more numerous nodules. Peas.

Hensall, Ont. Area of plots, one-fortieth acre. Yield on treated plot, four and one-half bushels. Yield on untreated plot, four bushels. Garden peas.

Maplewood, Ont. The amount of crop on the treated plot was easily double the amount of the untreated plot. Beans.

Aurora, Ont. The crop on the treated plot is very heavy, and on the untreated not so rank. The crop on the treated plot is the best catch I have ever had. Red Clover.

Markdale, Ont. Growth on the untreated plot not one-third of that from the treated seed. Vetch.

Morpeth, Ont. The crop on the part treated with culture was five bushels to the acre better than where it was not used. Crop on sandy soil. Beans.

Garry Owen, Ont. Plants on treated plot are more vigorous than those on untreated plot. Clover (Mammoth).

Big Fork, Ont. The treated plot yielded at the rate of four hundred and forty pounds more per acre than the untreated plot. Soy Beans.

Calgary, Alta. Nodules were numerous on treated plot, but absent on the untreated. The crop on the treated plot was so heavy I had the greatest difficulty in binding it. It was a bumper. Peas.

Bon-Accord, Alta. The plants on the untreated plot are not as long by six inches as on the treated plot. The piece on which the culture was used is a long way ahead of the untreated piece. Alfalfa.

Fig 4.—Root of Red Clover, showing nodules, natural size.

Fig. 5.—Root of Hairy Vetch, showing nodules, natural size.

Claresholm, Alta. Plants on the treated plot were very dark green in color; those on the untreated plot are pale and not healthy. Alfalfa.

England. The plot sown with untreated seed seemed a good deal affected by the dry weather, while the treated plot seemed to be quite unaffected by the drought. The plants looked a good color and grew stouter than on the untreated plot. Alfalfa.

Moosejaw, Sask. The treated seed bore thick sturdy vines with very dark green leaves; the untreated seed much lighter in color and vine. I did not find a single nodule at any time on the roots of the untreated plot. The treated seed always bore nodules in increasing number as the season advanced, the roots were three or four times as large as the untreated. Peas.

Fleming, Sask. I enclose four samples of clover, two treated with culture and two not treated; one sample of each grown on high ground, and one of each on low ground. (See Figs. 1 and 2.) Red Clover.

Sheffington, P.Q. First cutting yielded two tons per acre on treated plot, one and one-quarter tons per acre on untreated plot. Alfalfa.

Treesbank, Man. Numerous nodules were present on the treated plot, while the untreated plot showed only a few. Alfalfa.

Baldwin, Man. The clover seed treated with the culture showed more vigorous roots and consequently better growth than the seed not so treated. Red Clover.

Idaho Experiment Station, Moscow, Idaho, U.S.A. In pot experiments with white clover, garden pea, garden bean, and red clover, in every case the treated seed developed plants having more numerous nodules than the untreated seed.

Iowa College of Agriculture, Ames, Iowa, U.S.A. In pot experiments with white clover, soy bean, and alfalfa, nodules were present on the plants growing from the treated seed, while those growing from untreated seed showed no nodules.

Langhorne, Pa., U.S.A. The plants on the treated plot are vigorous with numerous nodules, while those on the untreated plot are weak and no nodules are present. Alfalfa.

N. Y. State College of Agriculture, Ithaca, N.Y., U.S.A. Most of our trials with nitro-culture have been failures. The O.A.C. cultures were a success in the one trial we gave them this year. Alfalfa.

Dundee, Mich., U.S.A. The plants on the inoculated plot averaged one-third larger and stronger than those on the uninoculated plot. Peas.

Cultures were sent to nearly every experiment station in the United States. In many cases reports from these stated that the soil seemed to be well inoculated with the nodule-forming bacteria and the culture was of no benefit.

Four cultures were also sent to the Government Farm of Orange River Colony.

Fig. 6.—Nodule bacteria of Sweet Pea from culture on agar, showing the flagella or whips by which the cells move. Magnified 2,000 diameters.

Fig. 7.—Legume bacteria from nodule on Alfalfa. Magnified 1,500 diameters.

Distribution of Cultures in 1907.

During the spring of 1907 the distribution of cultures was continued and reports were received, a summary of which is given in the following table:

Province.	Alfalfa.	Red Clover.	Beans.	Soy Beans.	Alsike.	Peas.	Vetches.
Ontario	170	54	5	1	2	29	1
Quebec	11	3	2	.....	.....	3	.....
Nova Scotia	3	4	4	.....	2	2	.....
New Brunswick	5	3	.....	.....	.....	.....	.....
P. E. Island	3	.....	.....	.....	.....	.....	.....
Newfoundland	1	.....	.....	.....	.....	.....	.....
Manitoba	1	5	.....	.....	.....	2	.....
Saskatchewan	4	2	.....	.....	.....	.....	.....
Alberta	18	.....	.....	.....	.....	.....	1
British Columbia	6	4	.....	.....	.....	2	.....
United States	8	1	.....	1	2	.....	.....
England	.....	5	.....	.....	.....	.....	.....
Scotland	.....	1	.....	.....	1	.....	.....
Total	230	82	11	2	7	38	2

Cultures Sent Out for the Agricultural College, Truro, N.S.

Province.	Alfalfa.	Red Clover.	Beans.	Alsike.	Peas.	Vetches.
Nova Scotia	41	38	1	7	12	2
New Brunswick	1	5	.....	1	.....	.....
P. E. Island	4	4	.....	2	.....	.....
Total	46	47	1	10	12	2

As during 1906, cultures were prepared in this laboratory for the Truro Agricultural College, reports of such cultures being received by that College.

The total number of cultures sent with the exception of those sent for the Truro Agricultural College, which will not be further considered, was 372. In answer to a request for a report upon the use of the cultures, two hundred and fifty-seven replies were received. In many cases no untreated seed was planted for comparison. Owing to a late, wet spring some farmers did not sow the crop intended; in some cases the entire crop failed by reason of the unfavorable season, etc. Eliminating, then, all reports which did not give actual results as to the success or failure of the cultures to aid the crop, the number available for judging results is 124, the results being shown in the table which follows:

Results of Seed Inoculation.

Province.	Alfalfa.		Red Clover.		Alsike Clover.		Peas.		Beans.		Sweet Pea.	
	Benefit.	No Benefit.	Benefit.	No Benefit.	Benefit.	No Benefit.	Benefit.	No Benefit.	Benefit.	No Benefit.	Benefit.	No Benefit.
Ontario	36	28	6	11	2	....	2	2	1	....	....	....
New Brunswick	2	....	1	....	....	....	....	....	....	....	....	....
Saskatchewan	1	2	....	....	....	....	....	....	....	....	....	....
Alberta	1	1	....	....	....	....	....	....	....	....	....	....
P. E. Island	2	....	....	....	....	....	....	....	....	....	....	....
British Columbia	1	2	....	2	....	....	....	....	....	....	1	1
Quebec	3	1	....	....	....	....	....	....	....	1	1	....
Manitoba	1	1	....	1	....	....	....	1	....	....	....	....
Nova Scotia	1	....	2	....	....	1	....	....	2	....	....	....
Indiana, U. S. A.	....	1	....	....	....	....	....	....	....	....	....	....
England	....	....	....	1	....	....	....	....	....	....	....	....
Ohio, U. S. A.	....	....	....	....	1	....	....	....	....	....	....	....
Total	48	36	9	15	3	1	2	3	3	1	2	1

As seen from the table computing the total number of reports available, the number in which benefit was derived from the application of the culture to the seed is to the number in which no benefit was apparent as 67 to 57.

Extracts from Reports, 1907.

Five Islands, N.S. Numerous nodules on treated seed and few on untreated seed. Beans.

Marsville, Ont. Would never sow clover again without using culture. The treated plot made very rapid growth, but there was no catch on the untreated plot. Nodules very numerous on the treated plants. Red Clover.

Rocklin, N.S. Treated plants had many nodules and vigorous growth, but few nodules on untreated plot. Red Clover.

Eden, Ont. The untreated seed grew fairly well, but the treated seed far surpassed it for thickness and growth. Red Clover.

Vankleek Hill, Ont. Treated plants made greater growth, longer and stouter roots, more numerous root fibres than the untreated. Red Clover.

McNamee, N.B. Treated plants more vigorous and with numerous nodules. Red Clover.

Springhill, N.S. Plants on treated area strong and with many nodules, while those on the untreated plot were tender and few nodules. Red Clover.

Bluevale, Ont. Treated plants were very vigorous and of a fine, healthy appearance; untreated plants were not so vigorous and there were some yellow patches. Alfalfa.

Whiteside, Ont. Most unfavorable season for growth of clover in this section for twenty-nine years. Treated seed vigorous, and untreated seed a failure. Alfalfa.

Thamesford, Ont. Marked difference between treated and untreated seed. Three nodules on the treated plants to one on the untreated. A splendid catch and much pleased with it. Alfalfa.

Fig. 8—Small unbranched form of the nodule-forming organism. From nodule of Lucerne. Magnified 1,000 diameters.

Fig. 9.—Branched form of the nodule-forming organism. From a nodule from Hairy Vetch. Magnified 1,500 diameters.

North Wiltshire, P. E. Island. No nodules on the untreated plants, but very numerous on the treated plants, which are also very strong. Alfalfa.

Caledonia Springs, Ont. No nodules on the untreated plants, but numerous on the treated ones. Treated plants very strong and many roots fourteen inches long. Alfalfa.

Don, Ont. A marked differrence between the treated and untreated seed in favor of the former. Treated plants healthy and vigorous. Alfalfa.

Hurondale, Ont. Far better catch with treated seed and stronger plants, although the untreated seed was sown on the best part of the field. Alfalfa.

Clachan, Ont. During the whole season the treated seed showed up the best. Alfalfa.

Jarvis, Ont. Nodules more numerous and plants stronger and greener on the treated seed. Untreated seed turned yellow. Alfalfa.

Lakefield, Ont. Treated plants very strong and of good color. Untreated plants sickly looking. Alfalfa.

Arthur, Ont. Can see division where seed was treated and untreated. Treated plants are more vigorous and of a better color than untreated plants. Alfalfa.

Moira, Ont. Where the culture was applied, the stand is thick and healthy at present, although very small on the untreated part—being thin and sickly looking, much the same as my last year's seeding. Alfalfa.

Charlton, Nipissing, Ont. There was a marked difference in the two plots. The treated seed plants showing a very dark green and hardly any yellow leaves, whereas the others always looked a pale green and are at present about ripening. Several neighbors are taking an interest in this experiment, and it is likely that next spring a few more and bigger applications for culture may be sent from here. Alfalfa.

Fig. 10.—Colony on agar of nodule-bacteria from Garden Bean. Magnified 200 diameters.

Fig. 11.—Colony on agar of nodule-bacteria from Garden Bean. Magnified 100 diameters.

Rawdon, P.Q. Many nodules on the treated seed, but very few on the untreated. Treated plants much more vigorous and longer than untreated. Alfalfa.

Creelman, Sask. Treated plants sturdy and deep rich green in color; untreated not nearly so sturdy nor nearly so deep a green color and later in flowering. Alfalfa.

Raymond, Ont. Numerous nodules and more vigorous plants from the treated than from the untreated seed. Alfalfa.

Loring, Ont. Plants from treated seed vigorous and nodules fairly numerous, none on the untreated seed. Alfalfa.

St. Thomas, Ont. More thrifty and stronger plants from the treated seed than from the untreated. Alfalfa.

Source of Cultures Distributed.

The cultures which we have sent out to Canadian farmers have been isolated and cultivated in this laboratory, and each culture was from its appropriate host plant. Thus, the alfalfa culture was isolated from a nodule on the root of an alfalfa plant, the vetch culture from the vetch nodule, and so for the red clover, white clover, pea, field bean, etc.

When a pure culture was once obtained it was transplanted at intervals and a record of each transplantation was kept, so that each mother culture, and therefore each culture which we sent out, can be traced back to its original nodule.

The cultures were isolated in the spring and summer of 1904, and had, therefore, been grown on artificial media from two years and a half to nearly three years, and in that time had been transplanted several times. The longest period between two successive transplantations was in some cases more than a year.

Fig. 12.—Colony on agar of nodule-bacteria from Alfalfa Root. Magnified 200 diameters.

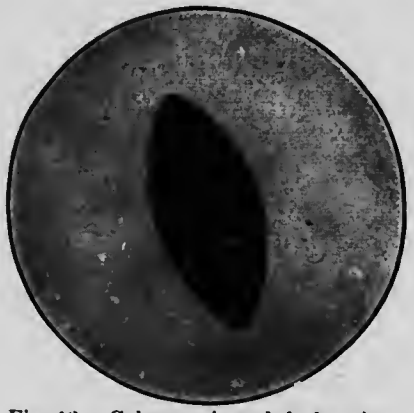

Fig. 13.—Colony of nodule-forming bacteria from Flat Pea. Magnified 100 diameters.

In preparing the culture media, we took pains to exclude combined nitrogen, and the media was all nitrogen poor. Five of the eight cultures were proved, that is, they formed nodules on the roots of their appropriate host plants in the absence of all other bacteria.

During the spring of 1908, cultures of the nodule-forming bacteria will again be distributed from this laboratory to Canadian farmers upon receipt of twenty-five cents for each culture, which is sufficient to treat sixty pounds of seed. It is expected further that the recipients of the cultures will report the results of his experiment, sowing some uninoculated seed for comparison.

Those who intend to use the culture should read what follows:

These bacteria under natural conditions combine the free nitrogen of the air only in association with plants of the bean family. Therefore it

is only a waste of time and material to apply the culture to potatoes, oats, wheat, etc. As plainly stated on each bottle, the cultures are carefully prepared for one species of plant, and if used for other species failure of the experiment may be expected. Plain directions for the use of the culture accompany each bottle, and these directions must be implicitly followed if good results are to be expected.

When Inoculation is of Benefit.

If a crop is thriving it indicates that either the soil is plentifully inocuated with the bacteria necessary to produce nodules on that particuar species, or else that the soil already contains an abundant supply of nitrogen upon which the plants can live. In either case the use of artificial cultures would be of little if any benefit. On the other hand, if the crop fails to thrive and upon examination no nodules are found on the roots, it is an indication that the culture should be used. Sometimes the use of the culture proves beneficial to the crop when a few nodules are present. Of course, failure to thrive may be due to other causes than lack of nitrogen. The soil may lack available potash or phosphoric acid, or may be deficient in lime. Inoculation does not and cannot remedy this.

When it is intended to sow seed of a legume which never has been grown upon the soil, inoculation of the seed should prove beneficial. This is true even if other legumes have been grown on the same soil, as the bacteria forming root nodules on one species do not necessarily form nodules on the roots of other species.

If soil once becomes thoroughly inoculated as indicated by a successful crop and the presence of numerous nodules, the use of artificial inoculation with later seedings is considered unnecessary if a three-year to five-year rotation is followed.

It should be definitely understood that the use of artificial inoculation with bacterial cultures will in no way compensate for carelessness in selection of seed, preparation of the soil, or subsequent care of the crop.

Advantages of Seed Inoculation.

There are certain advantages over other methods to be gained in the inoculation of seed. Soil may be transplanted from a field which has successfully grown legumes, and used as a top dressing on the field to be sown, but the method, especially if the soil is brought from a distance, is cumbersome and expensive. Furthermore, noxious weed seeds may be carried in such soil, and gain a foothold in sections not previously infested.

When inoculation of the seed is practised, the bacteria remain in intimate association with each seed, and the chances of early root infection and consequent nitrogen assimilation are enhanced. The cultures distributed from this laboratory are for seed inoculation.

The cultures are sent in glass bottles securely packed in a mailing case, each one accompanied by a sheet of directions for its use. (Fig. 3.)

A copy of these directions follows:

Directions for the Use of Cultures Supplied by the Bacteriological Department of the Ontario Agricultural College.

This bottle contains bacteria for inoculating.....seed.

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. *Follow directions carefully.*

1. For every sixty 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 thoroughly and pour back the water into the pail. Repeat this six or eight times. The jelly-like substance in the bottle will not dissolve, but may be broken up with a clean stick and stirred 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 the 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, search for nodules, "little bunches," on the roots. Examine for nodules again after three months. During the season note number and size of nodules and vigor of plant growth from treated and untreated seed.

REPORT OF NITRO-CULTURE (See Figs. 1 and 2).

Kind used.—Red Clover, Alsike Clover, White Clover.

Character of land.—Loam.

Amount of treated seed.—One hundred and fifty pounds.

Amount of untreated seed.—Four pounds.

	With Nitro-culture.	Without Nitro-Culture.
Area of land planted	25½ acres	½ acres.
Nodules present or absent	Lots of nodules	None.
Few or numerous nodules	Numerous	None.
Vigor of plants	Good	Poor.
Amount of crop		

Did you find the culture easy to apply? Yes.

Would you like to continue the experiment? Yes.

Do you consider the use of Nitro-cultures practical? Yes.

Remarks on the weather during the growth of plants: June, very wet; July, moderate; August, September, October, very dr

Name.—Harry Campbell. *Post Office* [illegible] *Province.*—Sask.

The treated clover is doing splendid[ly] the untreated looks spindly and poor. The treated has stood the dry weather far better than the untreated, which has nearly disappeared. I will report the amount next year.

REPORT OF EXPERIMENT WITH NODULE-FORMING BACTERIA FOR LEGUMES.

Crop seeded.—

Character of land.—

Amount of treated seed.—

Amount of untreated seed.—

——	With culture.	Without culture.
Area of land planted.....		
Nodules present or absent, at one month.....		
Few or numerous nodules after three months		
Vigor of plants after three months.....		

Do you think your crop has been benefitted by the culture?

Remarks on the weather during the growth of plants.

Your Name. Post Office. Province.

NOTE.—If desired, write additional notes on the back of this sheet. Please fill out this report and return promptly to *Laboratory of Bacteriology, Ontario Agricultural College, Guelph, Canada.*

APPLICATION FOR NODULE-FORMING BACTERIA.

I desire to conduct an experiment with nodule-forming bacteria for :— Alfalfa or Lucerne, Red Clover, Alsike Clover, White Clover, Vetches, Peas, Field Beans (Strike out those not wanted.)

Pounds or bushels of seed to be inoculated.

(Each bottle is sufficient for 60 pounds of seed).

Probable date of seeding.

If the culture is sent to me, I will—

1. Carry on the experiment according to the instructions received.

2. Exercise care and accuracy in the work.

3. Report the results of the experiment soon after harvest, whether successful or not.

Name..... Post Office.....

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

Enclosed find..... cents to pay for culture.

This sheet when filled out should be addressed in a sealed envelope to the *Bacteriological Laboratory, Agricultural College, Guelph, Canada.*

In order to avoid annoying mistakes and delays, write plainly in filling out the application.

NOTE—This application form can be detached along the perforated line.