LEGUME INOCULATION

BY

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DIVISION OF BACTERIOLOGY
DOMINION EXPERIMENTAL FARMS

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By A. Grant Lochhead, Ph.D.

Dominion Agricultural Bacteriologist, Dominion Experimental Farms

INTRODUCTION

Following upon the fundamental discoveries, made principally in the last two decades of the nineteenth century, which revealed the essential part which soil bacteria play in the maintenance of soil fertility, considerable attention was devoted to the question of adding cultures of bacteria to the soil or applying them to the seed with the object of exerting a stimulating effect on plant growth. Leguminous crops were the chief subject of study on account of the peculiar relationship which these plants bear to special groups of soil bacteria.

Many of the earlier attempts at soil or seed inoculation were unsuccessful, due to a lack of intimate knowledge of the bacteria concerned and of suitable methods for applying them. About the beginning of the present century, however, it was first demonstrated, in the field as well as in the laboratory, that practical use could be made of adding cultures of certain bacteria to soil or seed to make up for a natural deficiency of these bacteria in the soil. In later years further investigations have helped to explain why many of the earlier attempts, conducted both in Europe and America, were unsuccessful, and have developed improved methods of preparing and distributing cultures with the result that to-day the inoculation of legumes is a recognized aid to farming, founded upon scientific facts and proven by actual practice. Even yet much remains to be known concerning the relationship existing between growing plants and the microbial life of the soil, but with increasing knowledge will come still greater application to the problem of crop production of the many-sided activities of soil bacteria.

In Canada the use of legume cultures—"nitro-cultures" as they are frequently termed—has been increasing steadily in recent years. To encourage the more general practice of inoculation the Dominion Experimental Farms and certain of the agricultural colleges have been engaged in distributing cultures, while in addition, various commercial concerns have placed them on the market. The total number of cultures used in Canada, however, is still relatively small, and sufficient to inoculate but a small percentage (probably less than 5 per cent) of our total legume acreage. There is no doubt that much larger areas than are at present sown to treated seed could be advantageously inoculated. The purpose of this bulletin, therefore, is to give Canadian farmers a better understanding of the purpose of inoculation, the principles underlying the use of bacteria as aids to soil fertility, and furthermore, the limitations of inoculation.

HOW LEGUMES DIFFER FROM NON-LEGUMES

For many centuries it has been recognized that plants belonging to the leguminous family differ in a striking fashion from other cultivated plants in the effect which the crop growth produces upon the fertility of the soil. The custom of including a legume crop in the rotation was based upon the observations, made even in early times, that the productivity of the soil is noticeably better following a good growth of legumes—beans, peas, clovers, vetches, alfalfa, etc.—than after any non-leguminous crop. Even in the time of the Romans it was noticed that when certain legumes were grown in a field a subsequent crop of non-legumes showed improved growth. The legume had evidently left the soil richer. The explanation of the difference between legumes and non-legumes, however, was not found until towards the end of last century, when it was shown that these two groups of plants differ with respect to the manner in which they obtain their supply of nitrogen. It was discovered that whereas all non-legumes are dependent upon the supply of nitrogen in the soil, legumes, under certain conditions are able to make use of the nitrogen of the atmosphere. Four-fifths of the air is composed of nitrogen, and legumes, by drawing on this supply which is quite unavailable to other plants, are able to conserve in a large measure the supply of that element in the soil. Not only this; the nitrogen in the soil may be noticeably increased by the growth of a legume crop if the nitrogen so gained from the air is returned to the soil, either by ploughing under or by returning the manure after the crop is fed to animals.

LEGUMES REQUIRE BACTERIA

It was furthermore found that in order to make use of the atmospheric nitrogen legumes require the co-operation of bacteria, and that without the proper bacteria they are forced, like non-legumes, to depend upon the nitrogen of the soil.

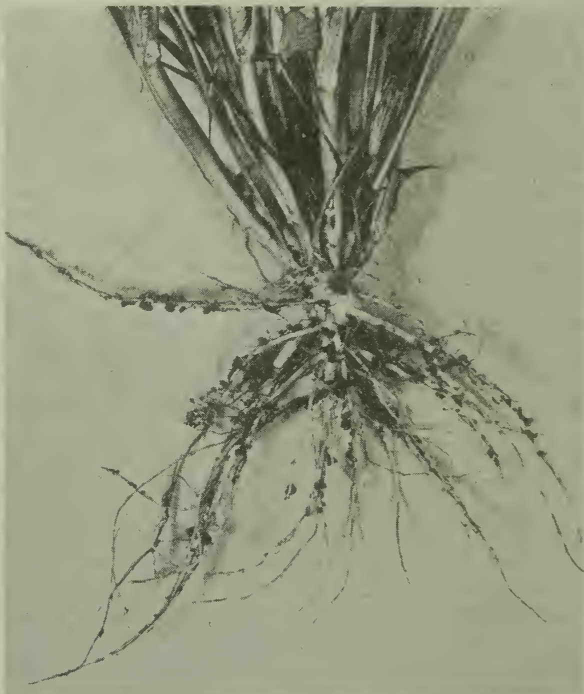

Fig. 1.—Red clover plant, showing nodules on roots.
(Photo by Dr. F. T. Shutt)

The nitrogen-gathering bacteria which enable legumes to utilize nitrogen from the air are situated inside the little swellings or outgrowths found in greater or lesser numbers on the roots of healthy plants. These are usually termed " nodules " and may vary in size, shape and location depending upon the plant, being generally seen, however, as roundish or club-shaped swellings, occasionally branching, usually observed best on young, vigorously growing plants. They are comparatively lightly attached to the root, and easily stripped off, but may be readily recognized when a legume plant is carefully dug up and the roots washed free from dirt. (See figs. 1, 2 and 3.)

It is inside the nodules that the nitrogen-gathering bacteria perform their useful work, and by assimilating nitrogen from the air, manufacture compounds containing that necessary element which are passed on to and utilized by the

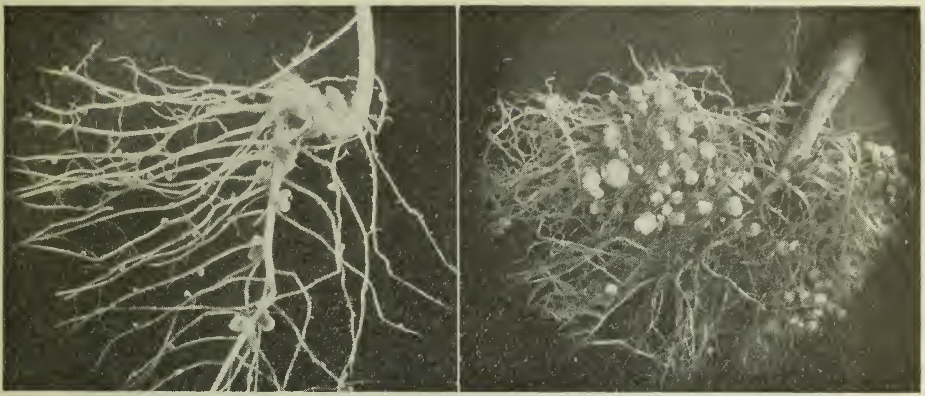

FIG. 2.—Nodules on root of young pea plant (left) and young bean plant (right).

FIG. 3.—Roots of soybean plants. Plants from uninoculated area show no nodules (left) while those from inoculated plot show well developed root nodules (right). (Photo by Mr. E. M. Straight.)

plant for growth. It is thus by reason of the presence of these highly beneficial bacteria that legumes are distinct from non-legumes. The bacteria actually gather the nitrogen but the legume reaps the benefit. (See fig. 4.)

THE LEGUME BACTERIA

The legume bacteria, like all others, are microscopic in size, being quite invisible to the naked eye. Seen under the microscope they are found to vary somewhat in shape and size, not only with the variety of legume but also according to whether they are living in the nodule, in the soil or in an artificial culture grown in the laboratory. Varying with the condition they may appear as small round bodies or as straight rod-like forms, or very frequently, especially inside the nodule of a vigorously growing plant, as irregular Y- or T-shaped forms. (See fig. 5.)

FIG. 4.—Alfalfa plants growing in sand containing essential plant nutrients except nitrogen. To the pot on the left nodule bacteria were added and the alfalfa was able to make normal growth through the utilization of atmospheric nitrogen. The pot on the right was not inoculated and the plants failed through lack of nitrogen.

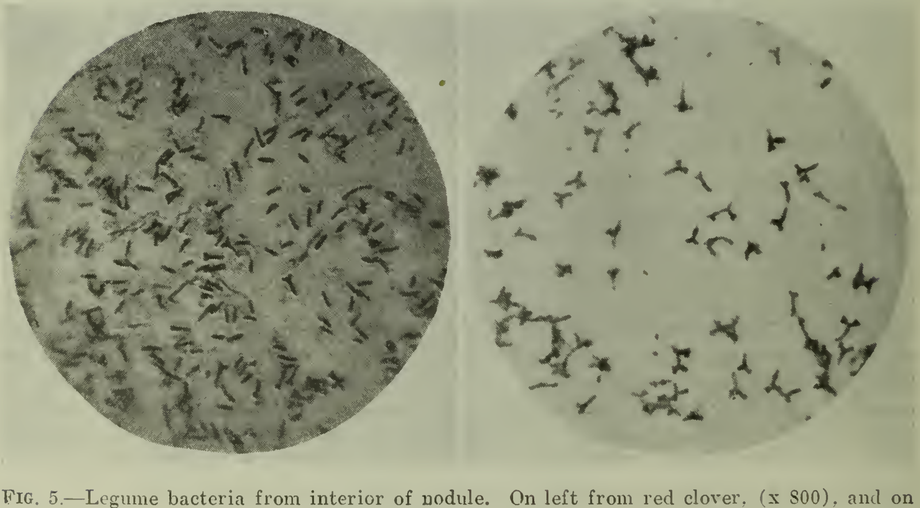

FIG. 5.—Legume bacteria from interior of nodule. On left from red clover, (x 800), and on right from pea, (x 1000).

On the average they measure approximately one ten-thousandth of an inch in length. Like other bacteria, the legume organisms have an enormous capacity for multiplication, provided conditions are favourable to their growth, and in a single nodule millions may be found. Practical use is made of this capacity for increasing in the manufacture of nitro-cultures, where by sowing a comparatively small number in suitable nutrient material, countless millions may be developed within a short time.

The legume bacteria, if present in adequate numbers in the soil when the young legume seedling develops, and sufficiently close, are able to penetrate the rootlet of the plant, and becoming established at favourable spots, commence to increase in numbers. The nodule tissue develops, the characteristic swellings appear, and if a nodule is examined during the course of the season of active growth of the plant, it will be found to be filled with a mass of bacteria engaged in assimilating nitrogen for the benefit of the plant. (See fig. 6.)

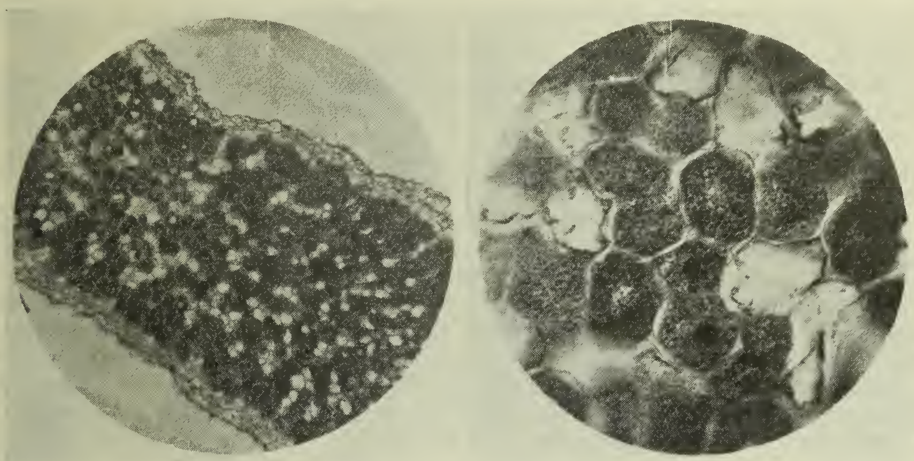

FIG. 6.—Cross-section of nodule from root of red clover plant. On left (magnification 60 diameters) are seen dark areas representing cells of nodule tissue filled with legume bacteria. On right, at a higher magnification, (600 diameters) the bacteria are more easily recognizable.

Towards the end of the growing season, the nodules, whose attachment to the root is comparatively light, begin to decay, the bacteria inside returning to the soil, where they remain ready to reinfect the crop the next season or to enter the rootlets of a new crop and begin anew another season of nitrogen-gathering. Without a suitable host plant the nodule bacteria remaining in the soil tend to decrease in numbers, particularly in acid soil, and after the lapse of a few years will usually be depleted in numbers to such an extent that unsuccessful reinfection of a new crop may not be possible without reinforcements in the form of inoculation.

VARIETIES OF LEGUME BACTERIA

Although the bacteria associated with the various types of leguminous plants are all very closely related and cannot be definitely disinguished by ordinary laboratory procedure, yet through the course of time different varieties or strains have become so adapted to certain legumes or groups of legumes that they are unable to cause nodules on plants outside their particular groups. The alfalfa nodule bacteria, for instance, are capable of producing nodules on sweet clover as well as alfalfa, but totally incapable of doing so on the common clovers, or on peas, vetches or beans. The pea bacteria are useless as far as alfalfa, clovers, or beans are concerned, although they are adaptable to vetches. Soybean bacteria produce nodules on soybeans alone. It is therefore important to know, when using a culture, just which legumes may be successfully inoculated with the particular strain of bacteria, and considerable attention has been given to determining how far bacteria from a given legume are capable of inoculating other legumes, or in other words, to what extent legumes cross-inoculate. This has been worked out for a large number of legumes, many of them foreign to Canada. As far as those which are cultivated to a greater or lesser extent in Canada are concerned, legumes may be divided into the following groups which represent the limits of cross-inoculation:—

1. Red clover group, including red, white, crimson, mammoth red, altaswede and alsike clovers.
2. Alfalfa group, including alfalfa, white sweet clover, yellow sweet clover, yellow trefoil.
3. Pea group, including field and garden peas, sweet peas, vetches, lentils and broad beans.
4. Bean group, including garden and field beans, navy and scarlet runner beans.
5. Soybean.
6. Cowpea group, including cowpeas, lima beans, jack beans, partridge pea, beggar weed.
7. Lupines and serradella.

VARIATIONS IN EFFICIENCY OF DIFFERENT STRAINS

Not all strains of legume bacteria of the same cross-inoculation group are alike in the benefit they bring to the host plant. Just as different breeds of animals or plants vary in some important characteristic, so do different strains of legume bacteria vary with respect to their nitrogen assimilating capacity and so in their ability to aid a given legume crop. While some strains may be very efficient in benefiting the host plant, others may be of comparatively little value as nitrogen gatherers while certain strains may even act as parasites on the host plant, giving no benefit whatever. It is thus of importance that the strain of bacteria used for inoculation be an efficient one, capable of active nitrogen assimilation. It is of interest to note that in general the most efficient strains produce larger nodules, grouped more towards the top of the root system, while the less efficient strains tend to produce smaller nodules distributed over the entire root system.

INOCULATING WITH THE PROPER LEGUME BACTERIA

If legumes, therefore, are to benefit from the supply of nitrogen in the air through the agency of the nitrogen-gathering bacteria, it is necessary for the soil to contain a sufficient supply of bacteria of an efficient strain peculiar to the group to which the legume being cultivated belongs. There may be a scarcity in the soil of bacteria adaptable to the crop being sown, and if there is any reason to suspect this it is advisable to add the bacteria, or in other words to inoculate.

WHEN IS INOCULATION NECESSARY?

There is no quick method of testing for the presence of the proper strain of legume bacteria in a soil, in fact the only way of making certain is to grow the legume and note whether there is an abundant supply of nodules on the roots. There are, however, certain circumstances which indicate more or less definitely whether inoculation is required in a given case.

There is reason to suspect a scarcity of the proper bacteria in the soil when neither the crop in question nor others of the same cross-inoculation group have been previously cultivated. In this case inoculation is strongly recommended. Furthermore, if previous crops have been unsuccessful, with the roots showing none or but a few nodules, inoculation is well advised, especially if soil and climatic conditions are otherwise favourable to the special legume grown.

Again, on soils of more than usual fertility it may happen that a legume is found to develop successfully without root-nodules. Under such conditions the crop is drawing its nitrogen wholly from the soil like other plants and gradually depleting that supply. Inoculation, under such circumstances, may allow the crop to secure part of its nitrogen from the air and thus lessen the drain on that valuable plant food in the soil, leaving it richer for subsequent non-legumes.

Inoculation is furthermore advisable when a legume is to be grown after a lapse of several years. The nodule bacteria multiply in the soil only by developing in the roots, and if a legume is absent from the soil, the bacteria peculiar to that legume group tend to die off. Although experiments have shown that some bacteria may remain alive after a long period of years, yet their numbers, under average conditions, become seriously depleted after two or three years, more particularly if the soil has any tendency towards acidity. Acidity is unfavourable to the bacteria as well as to the legume itself, and in case a sour soil is being limed preparatory to sowing alfalfa or other legume, inoculation is to be advised.

It is therefore not only in cases where the legume bacteria are completely absent from a soil that inoculation is to be recommended. Even in cases where some nodules may be produced inoculation will frequently be of definite value in producing a much more abundant supply of nodules, and permitting of much more active nitrogen fixation than otherwise possible. When there is any doubt the best policy is to inoculate, since inoculation costs little in comparison with the price of seed, and in many cases may be the deciding factor in success.

METHODS OF INOCULATION

A number of methods have been devised for adding to the soil the proper bacteria for the legume being planted. These fall into two general procedures which consists of (1) the transfer of soil from an inoculated field to the field being sown either directly or by mixing first with the seed, and (2) the application of cultures of the bacteria to the seed just before planting, and are known respectively as the " soil method " and the " pure culture method." Of these, the latter is the one ordinarily favoured and coming into more general use.

SOIL METHODS

The inoculation of a field with soil from an area already inoculated may be made by (*a*) the direct soil transfer method, or (*b*) the soil-seed method.

Direct Soil Transfer Method.—This method was the first to be employed, and had been carried on for years with success before the principles underlying its application had been understood. It consists simply in the transfer of soil from a field where the same legume or legumes of the same cross-inoculating group have been grown successfully, and the incorporation of this with the soil of the new field at the rate of 200 to 500 pounds per acre.

In effecting the transfer the soil may be sifted and drilled in, or scattered on the surface and harrowed in before the legume is seeded. In taking soil for inoculation it is advisable to remove soil only to a depth of five or six inches. discarding, however, the topmost inch or two, as bacteria are adversely affected by direct sunlight and drying and consequently the layers immediately below the surface should contain larger numbers of active bacteria. For the same reason it is well to mix well with the new soil after applying without unnecessary delay.

With this method, the chief precautions to be observed are firstly, that the soil used is well inoculated, as evidenced by the presence of abundant root-nodules on the crop, and secondly that the proper legume has been selected. At best, however, this method of transporting soil is cumbersome and often expensive in time and labour, especially if the soil must be shipped any great distance. In addition, there is the danger that along with the old soil, objectionable weed seeds, insects and plant diseases may be transferred to the new area. Although the soil transfer method has certain drawbacks and is used much less extensively than the pure culture method, yet it may be employed with advantage in many cases, especially when the distance of haulage is short, and under circumstances where, for one reason or another, pure cultures are not available.

Soil—Seed Method.—A modification of the above method consists in applying inoculated soil to the seed itself instead of directly to the field to be sown. The soil used for inoculation should be first sifted and pulverized. It may be then mixed directly with the seed until every seed is dirty. A variation of this procedure consists in moistening the seeds before the soil is applied with water or preferably with a solution of sugar or sweetened skim-milk, to make the seeds sticky. For a bushel of seed a pint of liquid, water or skim-milk, in which two tablespoonfuls of sugar have been dissolved will usually suffice. After the seed is thoroughly mixed with the liquid, sifted inoculated soil is sprinkled over the seed and mixed until each seed has a coating of soil. The seed is then spread out in a thin layer to dry before being bagged.

The use of glue for preparing a solution for moistening the seed is sometimes advocated. It appears to have no advantage over the use of a sugar or skim-milk solution and is less convenient to prepare. All of the modifications of the soil method of inoculation have been found to work satisfactorily, provided the soil used is well inoculated, this being a more important consideration than the mode of application to the seed.

PURE CULTURE METHOD

THE PREPARATION OF PURE CULTURES

Soon after it was discovered that the nitrogen-gathering ability of legumes depended upon the presence of bacteria in the root-nodules, and that the proper bacteria were lacking in some soils, attempts were made to prepare cultures of bacteria in the laboratory for the purpose of treating soil or seed. Many of the earlier attempts to apply pure cultures were attended with little or no success, due to a lack of understanding of the proper means of propagating the bacteria to

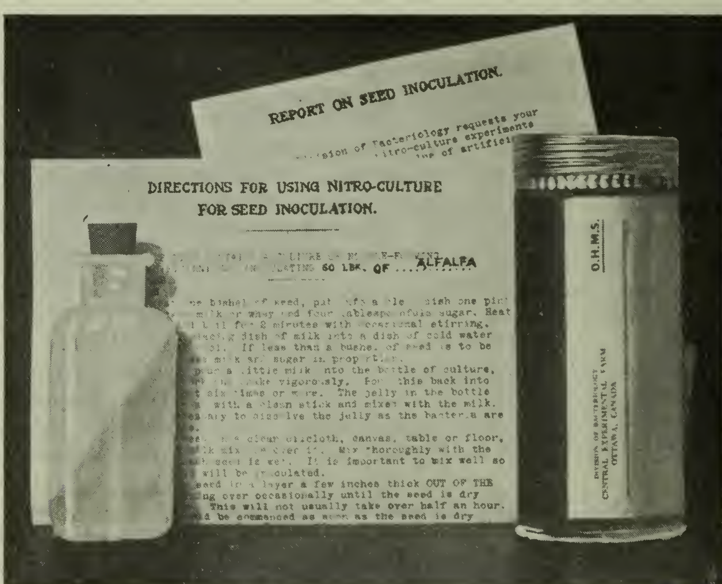

Fig. 7.—Nitro-culture as prepared by the Division of Bacteriology for distribution to farmers for inoculation of legume seed.

insure a supply of organisms which would retain their vigour under artificial conditions and be capable of producing nodules upon reaching the soil. Subsequent study of the requirements and habits of the legume bacteria have resulted in so improving the methods of preparing pure cultures that inoculation by this means is now recognized as a distinct practical aid to agriculture. The pure culture method has the advantage of simplicity and greater convenience, and is now regarded as the most generally satisfactory means of legume inoculation.

Pure cultures are so called because they contain only one kind of bacterium. Bacteria are taken from the nodules, and with care taken to prevent all outside contamination, are allowed to grow on materials containing suitable food ingredients. The progeny of a single active bacterium is selected for further propagation for the preparation of nitro-cultures. These are prepared in several forms, the bacteria being allowed to multiply either in a suitable liquid, on the surface of a nutrient jelly, or occasionally mixed with sterilized soil or humus. Cultures made by the Division of Bacteriology at the Central Experimental Farm are prepared by cultivating the bacteria on the surface of a jelly solidified in bottles. (See. fig. 7.) Under suitable temperature conditions the bacteria multiply enormously and in the course of a few days a whitish slime is apparent on the surface, consisting of countless millions of bacteria, all of one kind. Corresponding with the various cross-inoculation groups of legumes described on page 8, different varieties of legume bacteria must be propagated and kept distinct, so that each individual culture consists of bacteria capable of inoculating legumes of but a single group. In appearance, however, the various cultures are much the same.

The distribution of nitro-cultures by the Division of Bacteriology is essentially for educational and experimental purposes, the object being to encourage the more widespread use of cultures by furnishing sufficient to farmers to make a trial of inoculation on a practical scale. Only one culture is sent to each applicant, and that with the understanding that he reports the result of his inoculation test. Nitro-cultures may also be obtained from some of our provincial agricultural colleges, while in addition various commercial concerns have placed on the market satisfactory cultures.

INOCULATING WITH A PURE CULTURE

When inoculating with pure cultures of bacteria, the seed rather than the field soil at large is treated. Treating the seed is not only easier but much more effective than treating the soil, since by applying the bacteria to the seed, they find themselves upon planting just where they are needed, namely, in close proximity to the young plants. Liquid cultures are simply poured over the seed and well mixed, while cultures on jelly, those most widely used in Canada, are first mixed in some liquid before being poured on the seed.

The method at present recommended consists in mixing the contents of the nitro-culture bottle with an appropriate amount of sweetened skim-milk, the mixture being poured over the seed to be treated. The amount of milk required depends upon the quantity of seed being treated, enough being used to moisten all the seed without unduly wetting it. One pint is usually sufficient for a bushel of seed. The moistened seed is thoroughly mixed by hand, or by turning over with a shovel if large in amount and then spread in a thin layer to dry, out of the sunlight. When dry the seed is ready for planting. Each seed should be covered with a thin film of milk containing large numbers of bacteria. Water may be used in place of milk, but the latter is recommended for several reasons. The milk, especially if sweetened, forms a slightly sticky residue which helps keep the bacteria adherent to the seed, and in addition provides the bacteria on reaching the soil with readily available nourishment which tends to keep them vigorous.

It is advisable to sow without delay after inoculating the seed. The practice of inoculating legume seed sometimes attempted by dealers before shipment to customers is not as satisfactory as that of inoculating at the time of sowing. Experiments at the Central Experimental Farm[1] have shown that the numbers of living bacteria adhering to the seed decrease on storage and likewise the capacity to form nodules. Although even after six months' storage some bacteria will remain alive capable of producing nodules on planting, yet their numbers decrease (see table 1).

TABLE 1.—NODULE FORMATION, INOCULATED ALFALFA SEED PLANTED AFTER DIFFERENT PERIODS OF STORAGE

Length of storage	Average number of nodules per plant
Planted immediately after inoculation	37·9
Stored 1 week before planting	23·9
" 3 weeks before planting	20·7
" 6 " "	17·3
" 2 months "	15·7
" 4 " "	11·3
" 6 " "	10·7
Seed not inoculated	2·3

If it is necessary for any reason to keep inoculated seed more than a few days or a week, reinoculation is recommended for best results. Inoculated seed, however, even though it has been stored for much longer periods, is better than uninoculated seed.

The legume cultures now being manufactured by various commercial concerns are, in general, satisfactory. Since they appear in a number of forms, usually as cultures on jelly (agar) or cultures in soil or humus, the directions for their use will naturally vary somewhat. In almost all cases, however, the seed to be treated is moistened as the inoculant is applied, and if instructions are followed they will be found generally dependable provided they are reasonably fresh. With all inoculants the efficiency tends to decline with age and now many are labelled with the date beyond which they are not considered reliable.

DRY INOCULATION

A recent development in inoculation methods has been the appearance of the so-called "Dry inoculant" by means of which it is claimed that seeds may be satisfactorily inoculated without being moistened. Several forms of cultures for dry inoculation have appeared, the preparation being usually a dry, finely pulverized, dark grey or black material to be applied to the seed by mixing dry. While such preparations represent the handiest type of inoculant their efficiency is still a matter of doubt. It is a well known fact that the legume bacteria are adversely affected by drying. Furthermore, comparative tests on the capacity of dry inoculants to produce nodules as compared with cultures on jelly, soil, humus etc., which have so far been conducted at the Central Experimental Farm and other experiment stations have shown a distinct superiority on the part of the latter. As its efficiency still remains to be demonstrated, therefore, we are not prepared to recommend dry inoculation in preference to the older method.

BENEFICIAL EFFECTS OF INOCULATION

The amount of nitrogen assimilated from the atmosphere by a well inoculated stand of legumes will obviously vary with the crop, the nature of the soil and the climatic conditions. Naturally the greatest benefit will be reaped in the case of leguminous crops best adapted to the soil and climate. Furthermore, if the soil itself contains much readily available nitrogen, as nitrates,

[1] Report of the Dominion Agricultural Bacteriologist for 1926, p. 7-10.

the legume may use this to a larger extent, assimilating less from the air than in cases where the soil is less rich in available nitrogen. Under average conditions, however, it is estimated that approximately two-thirds of the nitrogen in a crop of legumes grown on soil of average productivity is obtained from the atmosphere. It can be said that with a well inoculated legume crop the nodule bacteria may be responsible for a gain of 40 to 100 pounds or more of nitrogen per acre. Both legume crop and soil may reap an advantage.

GAINS OF INOCULATED LEGUMES

The beneficial effect of inoculation upon a legume crop may be noted in an increased yield or in better quality or both. From what has already been said it will be evident that many factors are involved in determining the extent to which the benefits of inoculation will be manifest and so in practice wide variations may be found. The effect on crop yield has been made the subject of tests at different Experimental Stations. In tables 2 and 3 respectively are summarized the results of inoculation experiments conducted at Kapuskasing, Ontario[1] and Beaverlodge, Alberta[2]. The data in table 2 point to a moderate, though definite increased yield due to inoculation sufficient to justify treating the seed. In table 3 the effect of inoculation is more strikingly demonstrated, in which case it is to be assumed that the preponderating limiting factor affecting the growth of the legumes tested was the presence of the appropriate nodule bacteria.

TABLE 2.—EFFECT OF INOCULATION ON YIELD OF ALFALFA
(Kapuskasing)

—	Yield per acre		
	1921 seeding		1923 seeding, dry weight, 1924
	Cured hay, 1922	Dry weight, 1923	
	lb.	lb.	lb.
Treated	3,940	7,465	5,740
Untreated	2,480	6,967	4,980

TABLE 3.—EFFECT OF INOCULATION ON YIELD OF VARIOUS LEGUMES
(Beaverlodge)

Nature of crop	Crop from areas clipped in year of seeding (yield of hay per acre)						Second year crop from areas not clipped in year of seeding	
	Inoculated			Not inoculated				
	1st year	2nd year	Total, two years	1st year	2nd year	Total, two years	Inoculated	Not inoculated
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
Alsike	3,840	428	4,268	560	80	640	1,142	400
Common red clover	2,880	1,282	4,162	320	0	320	2,631	0
Sweet clover	3,520	2,140	5,660	1,360	160	1,520	4,708	480
Alfalfa	....	....	....	....	....	....	3,364	1,120

An interesting experiment to note the effects of inoculation by the soil transfer and pure culture methods respectively is summarized in table 4.

[1] Ballantyne, S. Report of the Superintendent, Dominion Experimental Station, Kapuskasing, Ontario, for 1924.
[2] Albright, W. D. Report of the Superintendent, Dominion Experimental Sub-Station, Beaverlodge, Alberta, for 1921.

Although both methods resulted in good crop gains, inoculation with pure cultures gave somewhat higher figures than when soil was employed. On distinctly acid soils, however, the soil transfer method appears to have the advantage over the pure culture method, the experiments of Alway and Nesom[1] indicating an increasing superiority of the former method with increasing lime deficiency of the land being seeded.

TABLE 4.—EFFECT OF INOCULATION BY SOIL AND PURE CULTURE METHODS ON YIELD OF LEGUMES[2]

Treatment	Yield of cured hay per acre			
	Sweet clover			Alfalfa
	First cutting	Second cutting	Total, two cuttings	One cutting only
	lb.	lb.	lb.	lb.
Uninoculated	853	773	1,626	1,520
Soil inoculated	933	1,306	2,239	2,640
Nitro-culture inoculated	1,226	1,760	2,986	3,320

Apart from any influence which it may have upon the yield of a legume crop, inoculation has a beneficial effect upon the quality of the crop. The supply of the element nitrogen is an important factor in determining the amount of protein in a plant, and in the case of such crops as alfalfa, clovers, peas, soybeans, etc., it has been demonstrated that the protein content may be strikingly increased by inoculation, in proportion as the roots are provided with nodules carrying active nitrogen assimilating bacteria. Quite apart from any question of yield, then, inoculation tends to make a crop a more valuable feeding stuff.

FIG. 8.—Alfalfa plots, Lacombe, Alta. At left, uninoculated and at right, inoculated plot.

EFFECT ON SOIL FERTILITY

Long before the real function of the nodule bacteria was recognized, it was noticed that legumes act in some way as soil enrichers. It is obvious, however, from what has been said that their capacity for soil improvement will depend upon whether they are successfully inoculated. The actual amount of nitrogen which may be added to the soil will depend largely upon how much of the crop goes back to the soil. The nodule bacteria supply nitrogen to the plant, and not directly to the soil, so the method of handling the crop and the proportion of it which returns will decide to what extent the soil itself is enriched. The maximum enriching effect will be obtained by ploughing under the crop as green manure or by returning the manure after the crop is used for feeding. In experiments conducted by Shutt[3] at the Central Experimental Farm, it was found that field soil which had grown red clover for two years which was then turned under

[1] Alway, F. J. and Nesom, G. H. Univ. of Minn. Agr. Exp. Sta. Tech. Bull. 46. 1927.
[2] Albright, W. D. Report of the Superintendent, Dominion Experimental Sub-Station, Beaverlodge, Alberta, for 1922.
[3] Shutt, F. T. Report of the Dominion Chemist in Annual Report on Experimental Farms for the year 1911-1912, p. 145.

showed a gain of 175 pounds of nitrogen per acre, an annual gain of 87 pounds per acre. At the end of a ten-year period during which this crop was cultivated continuously, the plot being dug over and resown every second year, the nitrogen content of the soil, reckoned to a depth of 4 inches, had almost doubled. The average annual increase of nitrogen was slightly over 50 pounds per acre. On the other hand, the complete removal of the crop may leave the soil little or no richer in nitrogen. In any case, however, the cultivation of the inoculated legume will tax the soil's nitrogen to a less extent than a non-legume or uninoculated legume crop.

CONDITIONS AFFECTING SUCCESS OF INOCULATION

INOCULATION BUT ONE FACTOR IN LEGUME PRODUCTION

Although in some cases inoculation may mean the difference between success and failure, yet it should always be remembered that it is but one factor in successful legume production. Even the best of cultures will be unsuccessful if the other conditions necessary for a good legume stand are not met. An inoculated crop requires the same care in seed selection and in the preparation and tillage of the soil as an uninoculated crop. When good soil and climatic conditions prevail, and when the crop is cared for properly, inoculation is most likely to bring benefit. It should be emphasized that the purpose of inoculation is not to supply an easy way to grow legumes, but to provide for a better growth through the addition of bacteria which help the plants to make the most of a good environment. The legume bacteria cannot supply the crop with lime or with such plant food as potash or phosphates. Inoculation, therefore, is most effective on soils sufficiently well limed and containing adequate supplies of plant mineral food.

SOME CAUSES OF NON-SUCCESS

Failure of inoculation to benefit a crop may be assigned to a number of different causes. Information on this point is being accumulated by the Division of Bacteriology through reports of field tests returned by farmers using nitro-cultures. A summary of the reports on the inoculation of a number of different leguminous crops is presented in table 5. It will be observed that the reports, representing all sections of Canada, are distinctly favourable to inoculation. This is seen, not only in the case of alfalfa, the most outstanding case of success, but also for other legume crops.

TABLE 5.—SUMMARY OF REPORTS OF LEGUME INOCULATION

—	Alfalfa	Sweet clover	Red and alsike clover	Field peas
Total reports received	885	245	257	58
Benefit through inoculation	687	181	202	41
Percentage showing benefit	80·3	73·9	78·6	70·7
Legume grown for first time	700	191	132	40
Benefit through inoculation	591	144	108	27
Percentage showing benefit	84·4	75·4	81·8	67·5
Cases reporting no benefit	168	64	55	17
Treated and untreated crops grew well	84	20	18	7
"No catch" reported	35	16	14	3
Failure due to drought	20	12	14	4
Crop winter killed	1	2	....	....
No reason given	28	14	9	3

In general inoculation was more successful where the legume was grown for the first time. Our reports indicate, however, that beneficial effects of inoculation were observed in many instances where the crop or a legume of the same cross-inoculation group had been previously cultivated, thus supporting the view that inoculation may be of value even when the soil may already contain a certain supply of the bacteria in question.

In many cases where no benefit is noted, the apparent non-success may be due to the soil being already sufficiently inoculated. In such cases the treated and untreated plants grow equally well.

In other instances, however, where crop growth is poor with treated as well as untreated seed, adverse climatic or soil conditions may be responsible for lack of success. Drought, poor drainage, soil sourness, etc., are outstanding causes for legume failures, even with treated seed, the adverse conditions affecting the bacteria as well as the plants themselves. Good drainage and liming are frequently necessary before a good legume stand can be established, the amelioration of the soil from these improvements favouring the crop, not only directly, but also indirectly by fostering the nitrogen-gathering bacteria, which then have a much better chance of surviving when introduced into the soil. When a soil is being limed for legume cultivation it is best to add the lime well in advance of seeding to give it sufficient time to react with the soil before inoculated seed is planted. Adding lime together with treated seed may result in the destruction of large numbers of legume bacteria.

In still other cases, non-success may be simply the result of using inferior seed, the crop being thus handicapped from the start. The remedy in such cases is obvious. In considering inoculation it should always be remembered that legume bacteria are living things and may be quite powerless to overcome detrimental factors; also that the better the seed, soil and cultural conditions, the better will be their chance to perform their useful work.

THE INOCULATION OF NON-LEGUMINOUS CROPS

When the relationship of the root-nodule bacteria to legume crop cultivation had become known, and soil and seed inoculation for legumes had been proven practicable, frequent attempts were made to devise cultures of bacteria which would prove of equal value in the case of non-leguminous crops. Although numerous trials have been conducted with the object of adapting root-nodule bacteria to the roots of non-legumes, yet up to the present they have not been attended with success. Our cultivated non-legumes—cereals, root-crops, etc.—are all forced to obtain their nitrogen directly from the soil.

The legume bacteria, it should be pointed out, are, however, by no means the only useful soil bacteria. In all soils bacteria are necessary for the best production of any crop. They play an indispensable part in the preparation of food for all plants, helping to change it from an unavailable form into a soluble form capable of being used by the growing plant. Numerous types of bacteria are engaged in this work, some breaking down the remains of vegetable matter left in the soil, helping to render manures and fertilizers available, and some even helping to increase the soil's nitrogen by gathering supplies of that element from the atmosphere without being in association with any plants.

From time to time cultures are brought on the market, usually under various trade names, stated to contain beneficial soil bacteria for the treatment of crops of all types, non-legumes as well as legumes. The claims put forth on behalf of such preparation are usually very extravagant, and are, as far as present-day science knows, quite unwarranted.

With the single exception of the legume root-nodule bacteria there is no indication of a scarcity of useful bacteria in an otherwise good soil. If they are lacking in a soil, the soil does not favour their growth due to some natural defect such as sourness, lack of drainage, want of humus, etc. Only when such a defect is corrected will the useful bacteria flourish; hence adding bacteria (other than legume bacteria) will be of little or no avail in abnormal soils and superfluous in normal soils.

Lack of success following the use of cultures for miscellaneous crops has sometimes tended to bring into disrepute the practice of inoculation. Hence it is very important for the farmer to realize that so far cultures prepared for non-leguminous crops have not been proven successful, and that whatever future research may bring forth, at the present time inoculation is to be recommended only in the case of legumes.

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