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INOCULATION OF LEGUMES

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
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THE INOCULATION OF LEGUMES

By P. E. Brown

The soil-enriching effect of the growth of clover and other legumes has been recognized from the earliest days of agriculture. Even ancient Roman authors emphasized their value in regular rotations. Since then the use of leguminous crops to increase the yields from soils more or less worn out has gradually gained in favor. By the early part of the eighteenth century, clover had replaced the older bare fallow as a means of restoring fertility and it was a very common crop. At that time it was practically the only important fertilizer known and it received much attention and many theories were advanced about its growth and action.

EARLY EXPLANATIONS OF THE EFFECT OF LEGUMES

The explanations of the beneficial action of legumes were many and varied. The most common early idea was that the greater root systems of these plants brought up so much plant food from the lower soil layers that the succeeding crops were materially aided in their growth.

It was claimed later that all plants drew their nitrogen supply from the air and that the legumes, having broad leaves, took in more nitrogen and therefore left more in the soil for following crops. That this belief was wrong was soon shown when experiments proved that no other plants except legumes would grow in soils having no nitrogen unless that element were supplied to them in the form of nitrates. In the case of legumes it was not easy to draw conclusions for the results of various experiments were conflicting. Sometimes they would grow all right in soils containing no nitrogen and again they would not.

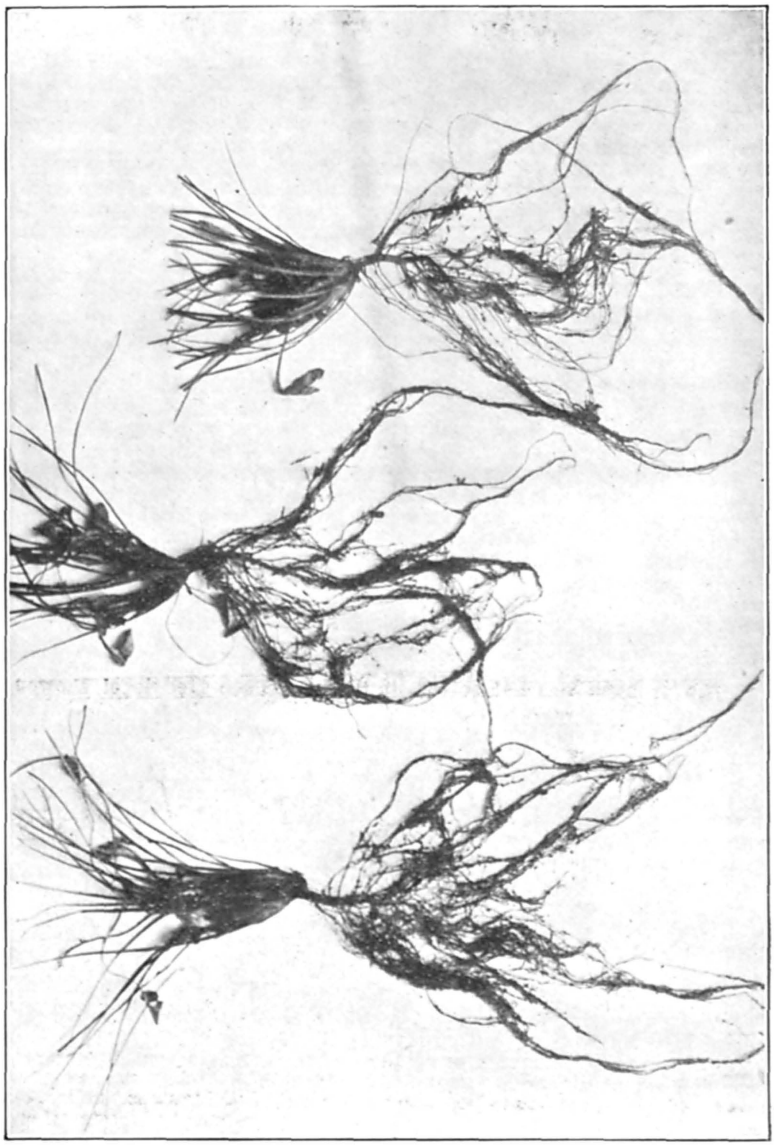
THE SOLUTION OF THE PROBLEM.

The problem was not solved until 1886. Then Hellriegel showed that the successful growth of legumes in soils containing no nitrogen depended on the nodules, or swellings, on their roots. Plants having such nodules grew well, even though no nitrogen was added to the soil; plants having no nodules died as soon as the little nitrogen in the seed was exhausted. It was clear, therefore, that the legumes which had nodules on their roots had some peculiar advantage, not only over all other plants, but also over other legumes on which no nodules were formed.

NODULES.

These nodules had been noticed many years before. They had been variously described as diseased roots, food store houses, or undeveloped buds, etc. More careful examination showed that they served as a home for bacteria, or minute microscopic plants. It was shown also that they consisted of pure cultures of bacteria, that is, they contained only a single kind.

Further experiments showed that when sterilized seed is planted in sterilized soil, that is, when seed and soil that have no bacteria are used, no nodules are formed on the roots of legumes. The peculiar power of legumes to grow on nitrogen-free soil was then shown to be due to the activities of bacteria which enter the roots, form nodules, take nitrogen from the air, and supply it to the plants in the necessary amounts.



Young Plants of Red Clover Well Inoculated.

RELATION BETWEEN LEGUMES AND BACTERIA.

The true relation between legumes and these bacteria was later shown to be one of mutual helpfulness, or symbiosis. The plants provide a home for the bacteria and also some food material and the bacteria in return place the great store of nitrogen in the atmosphere at the disposal of the plants.

Many experiments have since been carried on proving beyond doubt the correctness of these statements. The benefits to legumes from the nodules on their roots have been clearly shown. Indeed, these plants may grow more vigorously when they have abundant nodules than when nitrogen fertilizers are applied.

THE EFFECT OF LEGUMES ON SUCCEEDING CROPS.

The beneficial effects on succeeding crops due to the growth of legumes having many nodules has been demonstrated by comparing the yields of grain crops following such legumes with those of the same crops following other grains.

Increased yields were common on the soil which had previously borne the legume. This fact is of such general knowledge now that it is widely believed that proper rotations should contain a legume.

LEGUMES ACT LIKE A NITROGENOUS FERTILIZER.

Experiments carried on by many experiment stations in this country have shown that the growth of a leguminous crop acts like an application of a nitrogenous fertilizer. It has been estimated from the results obtained in sixteen states that the amount of nitrogen added to the soil by the growth of a well-inoculated legume amounts to 122 lbs. per acre. The Germans place their estimates somewhat higher, at 200 lbs. per acre. At this rate, it is evident that the growth of a crop of a nodule-bearing legume is equal to an application of 800-1000 lbs. of nitrate of soda which represents considerable money value at the present price of the nitrate.

METHODS OF SOIL INOCULATION

The importance of growing a legume in every rotation and the necessity of the legume having nodules on its roots, or being **inoculated,** if it is to take nitrogen from the air, are established facts. It is also clear that in many cases the bacteria necessary to produce these nodules are absent from soils and that in such cases the legumes draw all their nitrogen from the soil and therefore have no advantages over the more profitable cereal crops. These facts have led to the practice of **soil inoculation,** or putting into soils the necessary bacteria when they are lacking.

SOIL TRANSFER.

The first method used to supply bacteria to soils that need them is, quite naturally, to transfer soil known to contain the proper bacteria to the field to be inoculated. If a legume has been successfully grown on a soil and has shown abundant nodule formation, that soil is satisfactory for this method of inoculation.

While the nodule bacteria belong to the same species and are all called **Bacillus radicicola,** there are well defined strains or varieties especially adapted for certain legumes and it is more or less difficult for these special strains to adapt themselves to other legumes. In other words, certain varieties of the bacteria grow with a particular legume and will enter the roots of that legume only and no other.

One exception to this statement must be made, for it is now known that the bacteria for the nodules on sweet clover roots will inoculate alfalfa plants and vice versa.

CROSS INOCULATION NOT SUCCESSFUL.

Thus, to secure inoculation, for a particular legume, soil from a field where that legume has previously been successfully cultivated **must** be employed; no cross inoculation is possible between various legumes, such as clover and beans for example. The exception to this statement, as is noted above, consists in the fact that **soil from a sweet clover field will inoculate for alfalfa.**

THE VALUE OF THE SOIL TRANSFER METHOD.

The use of soil for inoculation has always proved of considerable value. Early experiments in Germany showed that crops are increased by inoculation and they have been very largely borne out in this country. While in most cases such crops as clovers, peas, and beans grow successfully without inoculation, others like alfalfa and soybeans make very little growth when the necessary bacteria are not supplied. When soil from a field where inoculated crops of these legumes have grown is spread uniformly over the area to be inoculated no difficulty is experienced in securing good crops. Failure to secure inoculation by the use of soil is very rare, provided care is taken to obtain well inoculated soil.

It will be shown later, however, that other soil conditions must be favorable and climatic conditions must be satisfactory or any kind of inoculation will fail to accomplish results.

If soil and climatic conditions are favorable and well inoculated soil is employed, there can be no doubt but that it will be successful.

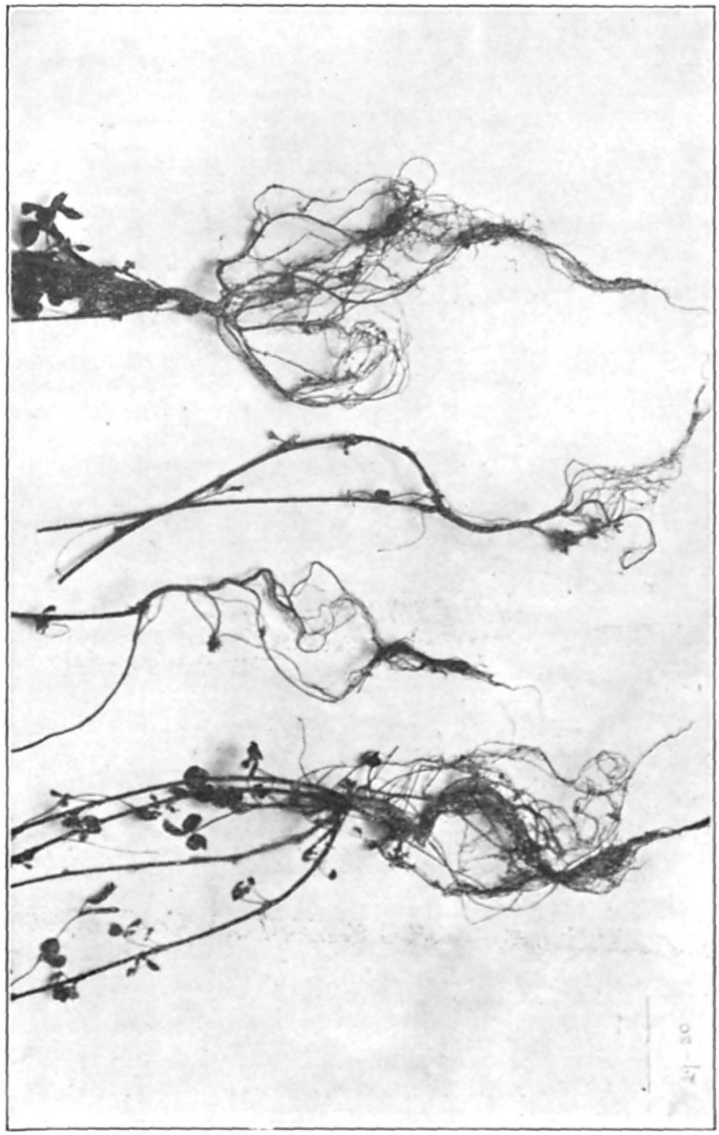
OBJECTIONS TO THE SOIL TRANSFER METHOD

Certain objections to the use of soil for inoculation have been offered from time to time. The first of these is that it is expensive if the large quantity of soil needed must be shipped from a great distance. Then there is the danger of transferring certain plant diseases, such as "leaf blight," or the "wilt" of cowpeas, which has been known to occur in the south. Objectionable weed seeds may also be transferred as, for example, seeds of Johnson grass, which has caused much trouble to southern farmers, particularly in Mississippi.

While undoubtedly there have been instances of such transfer of diseases and weed seeds, the dangers from the use of soil for inoculation have been largely overrated to make it easier to sell commercial cultures. If the soil used comes from a field where a **successful, healthy** crop of the particular legume has been grown, with **no objectionable weeds present** and **no plant diseases** evident, these objections are eliminated. There still remains the objection to soil because of its bulkiness and the cost of transportation. This in many cases cannot be overcome when the soil can not be secured in the surrounding territory.

PURE CULTURES.

The idea of using pure cultures for inoculation is not new. It originated soon after the discovery of the relations between bacteria and legumes. Pure cultures of nodule bacteria were obtained with little difficulty from the nodules of legumes. These grew readily on certain liquid and solid substances which contained the necessary food and it was suggested that the seed of legumes be inoculated with liquid media containing a vigorous growth of bacteria.



Alfalfa Inoculated With Sweet Clover Bacteria.

Sweet Clover Inoculated With Alfalfa Bacteria.

In 1895 the commercial preparation of pure cultures for seventeen legumes was begun in Germany and they were put on the market. These cultures consisted of bottles of nourishing jelly on which the particular bacteria were growing. The directions called for dissolving the contents of the bottle in lukewarm water, moistening the seed with that and drying the seed in a cool place before planting. This Nitragin was quite widely tested, both abroad and in this country, and was expected to revolutionize the method of soil inoculation, but the results were very disappointing and the commercial preparation was soon discontinued.

The United States Department of Agriculture then undertook the problem and prepared so-called "cotton cultures" which consisted in a growth of bacteria dried on cotton. The cultures were sent out for use with a package of sugar and salt. The latter were dissolved in water, and when the cotton was placed in the solution, the bacteria multiplied and a material suitable for inoculating the seed was obtained. These cultures were a failure also, due to the fact that the drying of the bacteria on the cotton killed many and weakened others so that they were of no value.

DIFFICULTIES IN THE PREPARATION OF PURE CULTURES.

The difficulty with most pure cultures is that when bacteria are grown in the laboratory on artificial media, they lose what is known as their **physiological efficiency**, that is, they become weakened and in that condition are unable to force an entrance into the roots of the plants.

In this connection it may be said that legumes resist to a certain extent the entrance of bacteria into their roots. If the bacteria are weak, they are kept out unless the plants are also very weak. The stronger and more vigorous the plants, the stronger must the bacteria be to force their way in. Thus the effects of inoculation are always more clearly shown on legumes growing in a nitrogen-poor soil, not only because without the inoculation the crop would be small, but also because the crop is weaker and hence the roots are more easily entered by bacteria. The nodule bacteria become weak when grown in a medium containing an abundance of nitrogen, for in such a case they are not obliged to draw upon the nitrogen of the atmosphere for support. Thus in soils very rich in nitrogen, the bacteria gradually lose their power to fix the nitrogen from the air. For this reason pure cultures for inoculation are grown on nitrogen free media.

NEW METHODS OF PREPARING CULTURES.

Recently bacteria have been bred to a high state of efficiency by growing them on such nitrogen-free media and then on the roots of legumes in the laboratory. This has led to the production of pure cultures which are much more promising than any previously prepared.

Cultures of highly vigorous organisms prepared by a commercial concern have been tested somewhat extensively and in many cases have given quite satisfactory results. Not in all cases, however, was inoculation accomplished and frequently soil proved better. Some failures occurred when all precautions were taken to make soil conditions satisfactory, and when the weather conditions could not be held responsible; hence the difficulty must be charged to the cultures.

Another preparation has recently been placed on the market which gives indications of considerable value. It has not been tested enough yet to permit of positive statements, but the cultures undoubtedly contain vigorous organisms, and the preparation is well

Young Crimson Clover Plants With Large Nodules.

calculated to preserve the bacteria in an efficient form. It is reasonable to expect that the successes which have been reported in certain sections from the use of their cultures should be repeated.

TESTS NOT CONCLUSIVE.

Experiments carried on during the last year at the Iowa Agricultural Experiment station to test pure cultures on cowpeas and soybeans do not permit of definite conclusions, owing to an early frost which stopped the growth of the plants.

Before the frost, the inoculated plots were much greener and more healthy and had an abundance of nodules. At the time of the frost, they were still in a vigorous growing condition while the plants on the uninoculated plots were mature.

While the weights of the crops obtained on these plots did not show any benefits from the inoculation, the nitrogen content of the inoculated plants was much greater than that of the uninoculated. A longer season of growth would probably have given larger yields also for the inoculated plots. Further tests of this material and comparisons of its value with that of soil should be made. If such tests show superior or equal value, then the pure cultures should be advocated to avoid the labor and expense involved in obtaining soil for inoculation.

SOIL TRANSFER STILL RECOMMENDED.

Until that time, however, in view of the uncertainty attendant upon the use of pure cultures, soil transfer should still be recommended.

Precautions to be observed. The precautions already noted with regard to obtaining soil should be kept in mind and fields where plant diseases or objectionable weeds are prevalent should be carefully avoided. Care should be taken also that the soil be obtained from fields where the particular legume has been grown successfully with abundant inoculation, except that in the case of alfalfa, soil from a sweet clover field may be used.

Amount of Soil to Use. Three hundred to five hundred pounds of soil per acre should be secured and thoroughly distributed on the field to be seeded, and disked in. As has already been stated, under satisfactory soil and climatic conditions, if well inoculated soil is obtained with the above precautions the success of the inoculation is practically assured.

WHEN INOCULATION IS NECESSARY

Since the best results with legumes can be obtained only when the proper bacteria are associated with the plants, and since the necessary nodule bacteria are not present in all soils, it is evident that inoculation is essential when a legume is to be grown on a poor soil which has never borne one previously.

Inoculation is necessary also if a legume possessing no nodules on its roots has been grown in the soil. In some cases on apparently quite fertile soils, legumes will make but a poor growth in the absence of nodules.

If a legume grown previously has made poor growth, even though nodules have been shown on its roots, the soil should be inoculated. Experience has shown that it may take several crops of the same legume, showing increasing numbers of nodules on the roots, before inoculation will be extensive enough.

Inoculation should also be performed in cases where another legume has been grown on the field except, as has been noted, in the case of alfalfa and sweet clover.

Occasionally soils upon which a legume has previously been successfully grown will not produce an inoculated crop of the legume. This may be due to a loss of vigor in the bacteria left in the soil and the introduction of vigorous forms will insure inoculation.

It is practically impossible to determine this condition in a soil and experimental inoculation is the only way to find out the need of a crop in this direction. Hence, if a legume was grown successfully inoculated, several years before, the soil should be inoculated again to insure success.

WHEN INOCULATION IS UNNECESSARY.

Inoculation is unnecessary where average yields of the particular leguminous crops are being obtained and the roots are well inoculated. Under those conditions, it is practically certain that the proper bacteria are present in sufficient numbers, and in a high state of vigor.

CONDITIONS NECESSARY FOR SUCCESSFUL INOCULATION

Contrary to a somewhat prevalent belief, the inoculation of a legume is not all that is necessary to insure the success of a crop. If other important soil conditions are not carefully observed, inoculation will be entirely unsuccessful.

Selecting the Seed. In the first place, the seed should be very carefully selected, as poor seed will cause a poor crop in spite of the best of soil conditions, the presence of vigorous bacteria and the best climatic conditions.

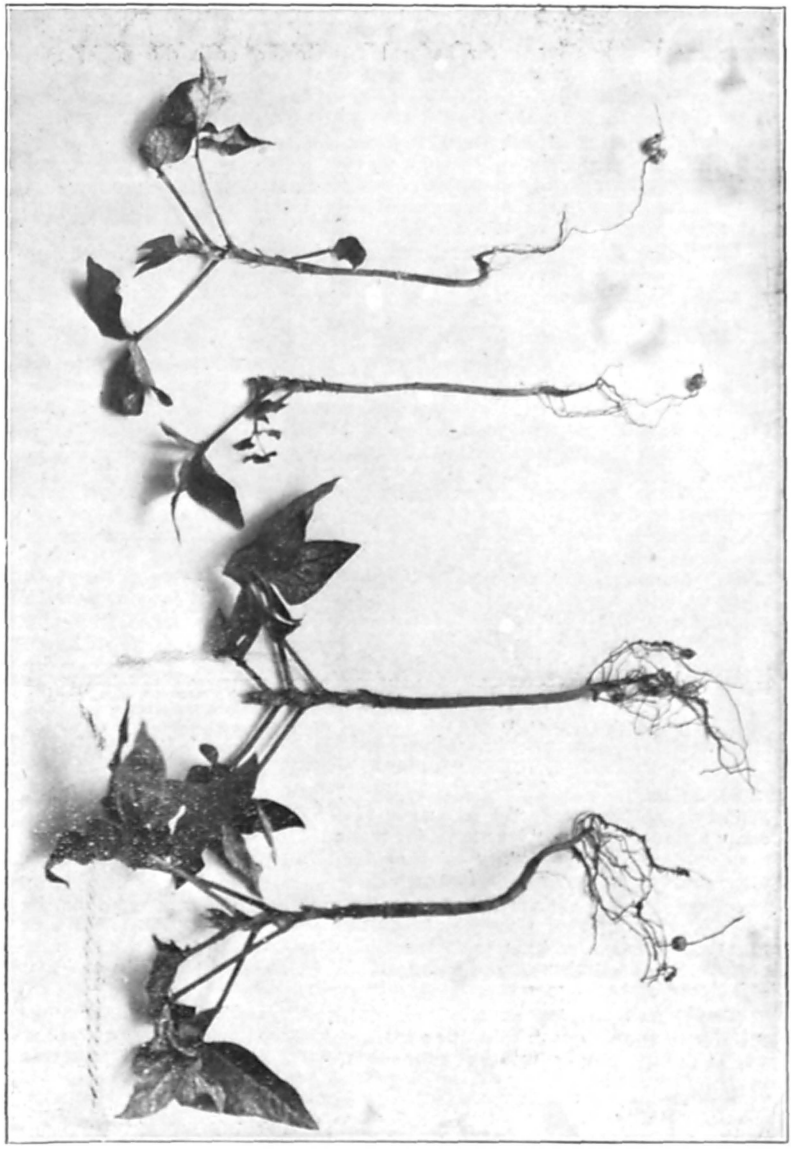
Preparing the Seed Bed. Then the seed bed should be prepared very carefully to insure the best mechanical conditions of the soil.

Cultural studies of the bacteria have shown them to be very closely dependent on the air supply, especially with reference to nitrogen. Deprived of the proper amount of air, the organisms soon die. Thorough cultivation puts an abundance of air into the soil for the growth of the bacteria and therefore should be practiced.

Regulating Water Content of Soil. Cultivation also conserves the moisture content of the soil by preventing undue loss through evaporation. Experience has shown that water in the proper amount is essential for nodule formation. Increasing the moisture content of a soil up to that amount increases the number of nodules formed. More than enough water, however, will check the development of nodules by shutting out the air. Thus soils which are very wet should be thoroughly drained before attempting to grow legumes on them.

Eliminating Objectionable Weeds. Thorough cultivation before seeding is also an important factor in killing out weeds which may later prevent a good stand of the legume.

Correcting Acidity. The relative acidity ("sourness") or alkalinity, or reaction, of the soil, should be carefully determined before seeding to a legume. All legumes, and particularly alfalfa and clover, are sensitive to acid conditions in the soil. If the litmus paper test shows the soil to be acid, even though only slightly so, applications of ground limestone should be made to the extent of 2000-6000 lbs. per acre, depending on the degree of acidity.



Young Cowpea Plants With Characteristic Nodules.

To make the litmus paper test, secure a small sample of soil free from roots and grass and put it in a clear water glass. Insert two strips of blue litmus paper in the soil so that they are half covered. Then add pure water very carefully until the soil is thoroughly saturated. After the test has stood for several minutes, remove the paper and rinse thoroughly. If that portion of it which was in contact with the soil has become red then the soil is acid and would be benefited by liming. The litmus paper may be bought at almost any drug store.

Supplementing Plant Food Deficiencies. Again, if the soils are deficient in phosphorus or potassium, the best crop yields of legumes cannot be obtained any more than is the case with other crops. In such cases, phosphatic and potassic fertilizers must be resorted to in order to remedy the deficiency.

Choosing the Legume. There is one other point to be considered in the growth of legumes and that is the selection of a legume for a particular locality.

Experience has shown that certain legumes are well adapted for certain climates while others would prove a complete failure under the same conditions. Consequently, the legumes which are known to be adapted to certain climatic conditions should be chosen, for the greatest care in every detail will not accomplish the successful production of any legume not adapted. to the locality.

CONCLUSION.

If, then, good seed is secured, the seed bed is thoroughly prepared with the elimination of weeds which might prove a nuisance, proper regulation of the water content is provided, the absence of acid conditions is assured and the necessary phosphorus and potassium compounds are present in the soil, inoculation should prove of value in most cases. It will be unnecessary, however, to inoculate in cases where the legume has previously been successfully grown.

In short, in preparing for a leguminous crop the farmer should use all the precautions which the majority are now employing for the growth of all crops. Then if thorough inoculation is performed by the use of soil from a carefully examined source, climatic conditions being favorable, optimum yields should be obtained.

EXPERIMENTS SUGGESTED.

It is highly desirable that the pure cultures prepared for soil inoculation should be tested at various localities and any farmer who contemplates growing legumes for which inoculation will be necessary, will perform a service to the state if he will arrange to test the efficiency of some pure cultures.

This may be accomplished by dividing the field into sections, as uniformly located as possible, inoculating one section with soil and planting the other with seed inoculated as directed with the pure culture. The weights of the crops should be obtained and the relative efficiency of the two methods of inoculation ascertained.

Such co-operation on the part of the farmers may assist materially in the adoption of a pure culture method for inoculation which will simplify the difficulties connected with the growth of legumes very appreciably.