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SOIL INOCULATION

By P. E. Brown

Soil inoculation is the introduction of certain desirable bacteria into the soil. As a practice it is very old, having been followed many years before its beneficial influence was understood. In reclaiming infertile land, the addition of fertile soil was often found helpful, especially for such crops as clover. The practice did not become general, however, until some thirty years ago when the reason for the soil-enriching properties of legumes was discovered.

At that time it was demonstrated that when clovers, vetches, alfalfa, cowpeas and all other legumes are associated with certain bacteria, these crops have the power of taking nitrogen from the air for their growth. It was demonstrated further that if the bacteria were introduced into soil deficient in nitrogen, legumes would grow satisfactorily on that soil and actually increase the amount of nitrogen in it. They not only take enough nitrogen from the air for their own growth, but store a surplus in the soil. Without the presence of the bacteria, however, the legumes do not thrive and they are not able to secure their supply of nitrogen from the air.

It was also demonstrated that not only must the necessary bacteria be present in the soil for this important work of the legumes, but they must enter the roots of the plants and form swellings or nodules there. Thru this means a state of mutual helpfulness is set up, called symbiosis. The plant supplies the bacteria with certain food materials and in return the bacteria draw nitrogen from the air and furnish it directly to the plant.

Later investigations led to the discovery that the nodules on *any* legume contain only one kind of bacteria; they are therefore called "pure cultures". The organisms which grow thus with all legumes have since been found to belong to the same species, and have been named *Bacillus radicicola*. They include, however, well defined strains or varieties, each especially adapted to grow with certain legumes, and it is more or less difficult if not impossible for them to adapt themselves to certain other legumes. Such adaptation apparently does occur *thru long periods of time*, but farm practice demands immediate results and hence the proper species of bacteria must be present in the soil if inoculation is to occur.

WHEN SOIL INOCULATION IS NECESSARY

There is no easy test for the presence of a particular kind of bacteria in a soil. The only way to be positive about the inoculating power of soil is to grow a legume and note whether there is an abundance of nodules on its roots. However, certain facts indicate rather definitely when inoculation should be practiced. In the first place, if the soil is poor and it has never borne a legume crop previously, the necessary bacteria are probably not present and they should be

introduced. If one legume has been grown on a soil and has been inoculated, that does not give assurance that the proper bacteria are present for any other legume and inoculation should be made. The bacteria which grow on the roots of certain legumes will not grow on the roots of certain others except in one case. Alfalfa and sweet clover do cross inoculate and the same bacteria grow in the roots of both these legumes. Successful inoculated crops of either of these plants will therefore insure inoculation of the other on the same soil.

If a legume has been grown successfully but its roots have no nodules, the soil should be inoculated in order that the plant may secure its proper supply of nitrogen from the air. On fertile soils legumes many times make entirely satisfactory growth without inoculation, drawing their nitrogen from the soil just like other crops.

If a legume made unsatisfactory growth even tho some nodules were found on its roots, inoculation should be practiced. The poor crop production indicates the absence of sufficient vigorous bacteria of the proper kind. Under such conditions growing the same crop for several years would probably be necessary before thoro inoculation would be accomplished.

Inoculation is also desirable when a legume is to be grown again on a certain field after several years of cropping to other plants. How long bacteria retain their efficiency in the soil is not definitely known, but experience indicates that reinoculation is desirable if the legume in question has not been grown for from five to seven years. In the absence of definite proof of the presence of the bacteria in a vigorous condition in the soil it is a wise precaution to introduce them and insure the proper growth of the legume.

WHEN INOCULATION IS UNNECESSARY

If satisfactory yields of a legume have been obtained recently on a soil and the plants carry a large number of root nodules, this indicates that inoculation has been thoro and the introduction of bacteria will be unnecessary. Within comparatively short periods of time bacteria apparently retain their efficiency and inoculation is not profitable, because it will not increase the yield or value of the crop.

METHODS OF SOIL INOCULATION

Soil may be inoculated by two methods: By the transfer of soil from a field where the *same* legume has previously been successfully grown and inoculated or by the use of commercial preparations which are on the market.

The soil transfer method was the first to be employed and its value has been demonstrated in many experiments and in extensive practice. The method followed is to scatter 300 to 500 pounds of the inoculated soil over each acre of the field to be seeded and disk it in thoroly before seeding. Inoculated soil should not be allowed to remain exposed to heat and light but should be mixed with the surface soil immediately for the desirable bacteria are rather readily killed by exposure to the sun.

Certain other precautions should be observed. The *same* legume must have been grown on the soil which is to be used, for cross inoculation between legumes occurs only in the case of alfalfa and sweet clover. Further, the legumes grown in the soil used must have been well inoculated as shown by the presence of many root nodules. Also, the soil should be free from plant diseases and this may be determined by a careful observation of the crop grown on it. Finally, no

objectionable weed seeds should be present in the soil. If these precautions are followed and the soil and climatic conditions are favorable, successful inoculation can be practically guaranteed.

OBJECTIONS TO THE SOIL METHOD

There are, however, certain objections to the soil transfer method of inoculation. If the soil must be shipped any great distance the cost of transportation and spreading will be rather large. If soil that is safe cannot be secured in the immediate vicinity, other methods of inoculation may be more desirable.

The "glue" method is a modification of the soil transfer method and has been advocated as a cheap but effective method of inoculating the seed. Soil, meeting the requirements mentioned above, is dried in the dark, pounded to a fine powder and sifted. The seed is sprinkled with a 10 per cent solution of glue (one pound of furniture glue to one gallon of water) or with a solution made sticky by dissolving sugar in water and is stirred until each seed is moist. Dry soil is then sifted over the seed in sufficient amount to absorb all the moisture. The seed is then mixed thoroly, screened and planted within a day. Small seeds inoculated in this way must be sifted carefully to prevent clusters from remaining together.

Several questionable features of this method may render it undesirable from the field standpoint. In the first place the glue which is employed may contain substances which will injure the bacteria and reduce their efficiency. Drying the soil before using may reduce the vigor of the organisms and result in only partial inoculation. Exposure to light or high temperatures may kill the organisms or lessen their efficiency. While this method has been used to some extent in certain states no definite comparative results have been secured under field conditions and this station is not ready to recommend it as always reliable. Consequently, if the use of soil is considered too laborious and expensive it would be better to procure a satisfactory, tested pure culture and inoculate the seed. With the present price of certain commercial cultures, less expense and labor may also be involved with more certain returns secured.

PURE CULTURES

Soon after the discovery that the nitrogen gathering power of legumes depends upon the presence of bacteria in the nodules on the roots, it was suggested that pure cultures be used for inoculation, thus avoiding the difficulties attendant upon the use of soil. Pure cultures of the various organisms were secured from nodules and various liquid and solid substances prepared in a sterile condition were found to support the growth of these organisms. Commercial preparations were made and put on the market, but the results secured in practice showed that there were many difficulties to be overcome before inoculation with pure cultures could be made as safe and generally satisfactory as the use of soil. The difficulty has been with the material upon which the bacteria were grown to make commercial "pure cultures". The bacteria seem to lose their efficiency or vigor when grown on materials containing nitrogen, and when introduced into the soil in such a weakened condition are unable to enter the legume roots. When the plants are very weak inoculation will occur with weak bacteria but if, as is usually the case, the legumes are rather vigorous it is necessary that the organisms be vigorous and active. Then, too, in diluting the cultures before applying them to the seed,

in drying and in other steps connected with the actual inoculation, the bacteria are often weakened or killed and other bacteria frequently enter and drive out the beneficial organisms.

To overcome these difficulties, nitrogen-free materials were used and the bacteria grown on these in making "pure cultures". These cultures have recently come into the market and have proven quite uniformly satisfactory. Many of the older preparations have been discontinued or remodeled because of their uncertainty. Cultures are now being prepared by growing the organisms alternately on nitrogen-free materials and on the roots of the particular legume, thus breeding the bacteria up to a high state of efficiency.

The improved methods of preparation of pure cultures and the difficulties attendant upon the securing of well inoculated soil in so many cases has made it seem advisable to test some of the cultures now on the market and to compare their value with that of soil.

IOWA EXPERIMENTS

Two experiments were carried out with alfalfa and one each with cowpeas and soybeans. The cultures used in the tests were "Nitragin," prepared by the Nitragin Co. of Waterloo, Iowa; "Farmogerm", prepared by the National Soil Improvement Co., Charlottesville, Va.; a culture prepared by the Bureau of Plant Industry of the United States Department of Agriculture; "Nitrogerm," prepared by the H. K. Mulford Co., of Glenolden, Penn.. and soil obtained from a field where the same legume had previously been grown and well inoculated.

A cross inoculation of alfalfa and sweet clover was tested and also an inoculation with soil plus a commercial culture.

These tests were all carried out on one-twentieth acre plots, the first test with alfalfa in 1914, the second on different plots in 1915 and the tests with cowpeas and soybeans also in the latter year. The soils were well supplied with lime in each case and all the precautions necessary to secure a satisfactory crop growth were observed.

THE ALFALFA TEST

In the first alfalfa test the crop was seeded in the spring of 1913 with barley as a nurse crop and the yields obtained the following season. In the second test, the alfalfa was seeded in August, 1914. Three cuttings were made in the case of both alfalfa experiments, but these will not be considered separately here. The total yields per acre in pounds are given for all the crops and also the total nitrogen in the crops in pounds per acre.

The table on the next page gives the results of the tests, both for yields and for nitrogen present in the crops.

This table indicates that inoculation for any of these legumes brought about some increase in the crop growth. There were some variations in the effects of the different cultures on the various crops and in the case of the alfalfa, on the same crop when grown on different soils.

While no definite comparison of the values of the various cultures can be made it may be concluded that all the cultures tested gave uniformly satisfactory results. Inoculation with soil was slightly superior in most instances but the differences were too small to be distinctive. In fact, the relative value of the pure cultures and of the soil is so nearly equal that the choice between them should undoubtedly be based on the cost of the cultures and the ease with which soil may be secured rather than on the crop yields alone.

YIELD PER ACRE IN POUNDS OF ALFALFA, COWPEAS AND SOYBEANS WITH DIFFERENT INOCULATION MATERIALS.

	Uninoculated Soil	Inoc. Soil	Inoc. Nitragin	Inoc. Farmogerm	Inoc. U. S. Culture	Inoc. Soil + Nitragin	Inoc. Nitrogerm	Inoc. Nitragin Culture of Sweet Clover
Alfalfa 1914	6413.00	7402.00	6912.00	6988.00	6800.00	.....	7268	7272
Alfalfa 1915	6933.40	7305.40	7283.00	7250.80	6774.80	7583.00		
Cowpeas	5158.00	6938.40	5216.40	4799.80	5179.40	5238.00		
Soybeans	5336.00	6158.00	*4221.60	5198.80	6858.40	*3859.60		
TOTAL N. IN CROP PER ACRE POUNDS								
Alfalfa 1914	160.06	187.04	170.76	176.14	171.88	.....	186.08	170.76
Alfalfa 1915	201.28	221.72	215.90	218.20	204.62	217.72		
Cowpeas	88.26	133.42	116.24	94.12	117.00	121.62		
Soybeans	108.22	156.40	*106.60	141.32	151.84	*114.52		

The cross inoculation of alfalfa with sweet clover proved quite as satisfactory as when alfalfa cultures were used. A pure culture used in addition to soil for inoculation showed some slight effect but not sufficient to warrant the use of a pure culture on a soil already well inoculated.

Inoculation of the legumes increased their nitrogen or protein content as well as their yields. Again, however, the effects of the different cultures are not sufficiently distinctive to warrant a conclusion regarding the relative merits of the various pure cultures and of soil for inoculation. There is apparently a tendency toward equalization between the total crop yield and the nitrogen content. When the crop is larger, the nitrogen content is somewhat lower and vice versa.

These experiments are of value in that they show rather definitely that the particular pure cultures tested gave satisfactory inoculation for the legumes used and practically as good results were secured with them as by the use of well-inoculated soil.

HOW TO INOCULATE

The choice between the two methods of inoculation must, therefore, rest upon the expense and labor involved in their use. If soil is available in the immediate vicinity and can be secured for the labor and expense of hauling and spreading it over the field to be seeded, it is the cheaper and safer method of inoculation. Such soil must of course be secured from a field where the same legume has been grown and well inoculated and where no objectionable weeds or plant diseases have been present. In addition, soil used for inoculation should be disked in at once in order to avoid weakening and killing of the bacteria.

If it is not possible to secure well-inoculated soil in the neighborhood and it must be shipped or hauled from a distance the use of commercial cultures would probably prove cheaper. Satisfactory cultures have recently been placed on the market at a cost of about 50 cents for an amount sufficient for an acre instead of the former price of $2.00, and at such a price they prove less expensive than soil. In large amounts the cost of such cultures is reduced to about 33 cents per acre.

*Seeded June 8. All other plots seeded May 27. Delay in securing cultures. Crops harvested on same date.

With cultures of about equal value with soil available at such a figure, the objections to their use are largely removed. The amount of labor involved in their use is unquestionably smaller. The cultures are merely mixed with a sugar solution according to directions and poured over the seed, which is then dried over night in a cool dark room and seeded the next day in the usual way.

Many commercial preparations are now on the market. Unfortunately not all have been fully tested but those which have been tested for efficiency have proven satisfactory. Before employing any new material, however, farmers are urged to inquire of the Soils Section of the Iowa Agricultural Experiment Station as to its value. Besides the cultures tested in the experiments reported in this bulletin, "Nitragin", "Farmogerm", "Nitrogerm" and the United States Department of Agriculture culture, the culture of "Legume Bacteria" supplied by the Edwards laboratories at Lansing, Michigan, has been tested and proven quite satisfactory. Other cultures are being tested and reports on their efficiency will be available later. Farmers are advised to employ those cultures which may be secured at the least cost, provided they are recommended here or elsewhere by the Soils Section. The relative efficiency of the various cultures thus far tested is practically the same for all and a choice can, therefore, only be made on the basis of the expense involved.

CONDITIONS NECESSARY FOR SUCCESSFUL INOCULATION

The inoculation of a legume will not insure a satisfactory crop altho when other conditions are satisfactory for crop growth, it will insure the utilization by the legume of the nitrogen of the atmosphere and consequently conserve the supply in the soil. It will also increase the crop yield and the protein content of the crop. General soil conditions must be satisfactory or inoculation will be unsuccessful and the crop will fail for inoculation will not make up for lack of care in the preparation of the soil, in the choice of seed or in the treatment of the crop.

The conditions necessary for successful inoculation are the same as those necessary for the successful growth of the legume. The soil must be carefully prepared to insure proper moisture and aeration conditions, to correct acidity by the use of lime, to remedy plant food deficiencies by the proper additions and to insure good mechanical condition by the use of manure. Then if the seed has been carefully selected and a legume adapted to the particular soil and climatic conditions is chosen, successful inoculation and good crop growth will result.

When all these conditions are met, then and then *only* will inoculation prove successful. With sucessful inoculation, satisfactory growth of legumes may be insured and the fertility of the soil increased. Well inoculated legumes should occupy a prominent place in all rotations if soils are to be kept permanently fertile for by their use the nitrogen and organic matter content of the soil can be maintained at a minimum expense.