# UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE AGRICULTURAL EXPERIMENT STATION CIRCULAR 86

COLUMBIA, MO.

MARCH, 1919

# SOIL INOCULATION FOR LEGUMES

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The present high prices for farm products have caused a wide-spread interest in those practices which promise to increase the productiveness of farm lands. Soil inoculation for legumes may be classed as such a practice, but its adoption has not been general because the beneficial effects of the practice are not yet fully appreciated. This is indicated by the many inquiries received by the Experiment Station. Farmers intending to practice inoculation for the first time wish to know when and why it is necessary, how it is carried out, and what beneficial effects it may have. It is the purpose of this circular to answer these questions.

## WHAT IS SOIL INOCULATION?

Inoculation of any kind deals with the introduction of bacteria, and the term "soil inoculation" refers to the introduction of certain desirable bacteria into the soil. Many years ago it was learned that certain bacteria have benefited effects on the soil, and that to realize these benefits in new fields it was necessary only to scatter on them small amounts of soil brought from a field containing the bacteria. The good effects of such treatment were marked, especially when leguminous crops such as lupines, cowpeas and beans were being grown. Small amounts of soil in which a legume had been grown, transferred to very poor soil newly seeded to the same legume, made the crop do well. It has long been known that beans will grow on soil too poor for other crops, and if the soil is inoculated, that the next crop to follow the beans will do better than if beans had not been grown. The reason for this has not been known so long, and was learned only when it was discovered how legumes differ from other crops.

#### LEGUMES DIFFER FROM NON-LEGUMES

Leguminous crops are very rich in protein. This is true of both their seed and hay. Alfalfa hay, for example, is as rich in protein as rye bran, while the soybean is richer in this respect than any cut

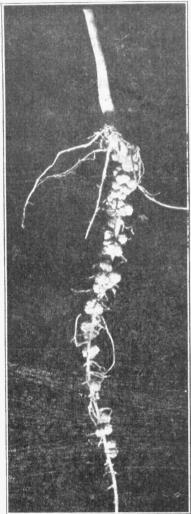


Fig. 1.—Roots of young cowpea plant. Nodules along main root.

of beef. For this reason the legumes have a very high feeding value, which makes them especially desirable as part of the ration for young animals. To produce this protein the plant must have large amounts of nitrogen, which is one of the plant-food elements commonly lacking in most soils, and the most costly of the fertilizer constituents. It is the ability of the leguminous plants to take these amounts of nitrogen from the air that makes them so valuable.

Besides being richer in protein, there is another significant difference between legumes and other plants. If a cowpea, bean, or any other leguminous plant is carefully dug up and its roots washed free of dirt, many wart-like growths will be seen. These are called nodules. They vary with the different legumes in color, size, shape and location on the roots. Red clover, for instance, bears small, club-shaped, flesh-colored nodules about the size of a pin-head which are distributed at random on all the roots; on the soybean and cowpea the nodules may be round and as large as a common pea, and are usually, altho not always, located on the upper part of the main root. Those on alfalfa often occur as

<sup>&</sup>quot;Inoculation may be worth more than manure to the alfalfa field."

clusters of club-shaped growths appearing on any part of the root system.

If the nodules are opened and a part of the inside examined under a microscope, numerous small bodies of rod-like form will be seen. Others may look like the letters "Y" and "T". All these are nitrogengathering or nitrogen-fixing bacteria, and are not found on common crop plants other than the legumes. The nodules must not be confused with the nodular or warty growths called crown-gall, which appear as a disease on a great variety of vegetable plants and fruit trees. Crown-gall is a disease injuring the plant and stunting its growth, while the nodules of legumes are normally found on the roots, increasing rather than reducing the size of the plant.

The fact that the bacteria inhabiting the nodules gather nitrogen and aid plant growth, is the greatest difference between legumes and non-legumes. Thru some unknown means the bacteria living within the nodules are able to take nitrogen from the soil air and give it to the plant. In return for this, the bacteria feed on the plant juices. The plant and bacteria work cooperatively, helping each other in a close relationship, called symbiosis. This close relation is not absolutely necessary for the existence of both, since either the plant or bacteria can live alone, but the growth of each one is greatly benefited by the presence of the other.

Legumes growing without the bacteria feed on the nitrogen of the soil in the same manner as corn, oats, wheat and grasses. By means of the bacteria living so helpfully in the nodules, the leguminous plants get a good share of their nitrogen from the bountiful supply of free or uncombined nitrogen in the soil air. It is this property which makes it possible for legumes to grow well on soils poor in nitrogen and yet contain a large percentage of nitrogen in their seeds, leaves and stems.

### IS INOCULATION ALWAYS NECESSARY?

"Must I inoculate my field to get a good crop?" is a question often asked and one not easily answered. To inoculate a leguminous crop at every seeding is both laborious and expensive, and if not needed it is a waste of inoculating material. On the other hand, the failure to inoculate, when inoculation is needed, is gross neglect and a significant loss in money. It is important to know when a leguminous crop should be inoculated, or when the bacteria must be introduced into the soil.

"When in doubt, inoculate."

Unfortunately, no chemical test or other rapid means can be used to determine when bacteria are needed on a field. The only certain method of answering this question is to grow the leguminous crop. If root nodules do not develop at all, or develop on only a few scattered plants, then that crop will be improved by inoculation. If, however, some few nodules develop on each plant, inoculation is not necessary. Under the latter conditions the growth of one crop of legumes will enable the few bacteria in the soil to multiply and produce numerous nodules during the next season of the crop, providing this legume is again seeded on the land within a few years. To test a soil in this manner requires time, and many farmers prefer to inoculate a leguminous crop rather than wait for the results of such an experiment. Altho no other test will decide the question with certainty, yet there are some general facts that may aid in the decision.

Such crops as red clover and cowpeas which have been grown in this state for many years probably need no inoculation, except in rare cases. In some isolated sections where neither of these two crops has ever been produced it may be possible that even red clover and cowpeas will do better if inoculated. No direct evidence for such statements is available, but the ease with which these two crops are started in the general farming districts of the state where they have been widely grown indicates that the inoculation for these crops is not the most important requisite in establishing them. Such, however, is not the case with sweet clover, alfalfa, soybeans, or with any other leguminous crop that is new to the district and has never been raised on the farm in question. These, as a rule, need to be inoculated when planted for the first time. None of the three crops last mentioned is native to the state, nor have any of them been extensively grown. On this account the bacteria adapted to each of them have not been widely scattered over the farming sections, and when a crop of alfalfa, sweet clover or soybeans is planted on a field for the first time it will need inoculation. The labor and expense required for this simple treatment are too small to trust to inoculation by chance, with the risk of a poor crop. Occasionally some seeds (arry a few bacteria into the field, which inoculate a few scattered plants and slowly spread to other parts of the field thru distribution by animals, flood waters, winds or other chance agencies, but this is too slow when artificial inoculation is so easy and costs so little.

The need of inoculation is not always shown by poor plant growth. Inoculation is necessary when the legumes make a fair

<sup>&</sup>quot;Grow legumes for food and improve the soil at the same time."

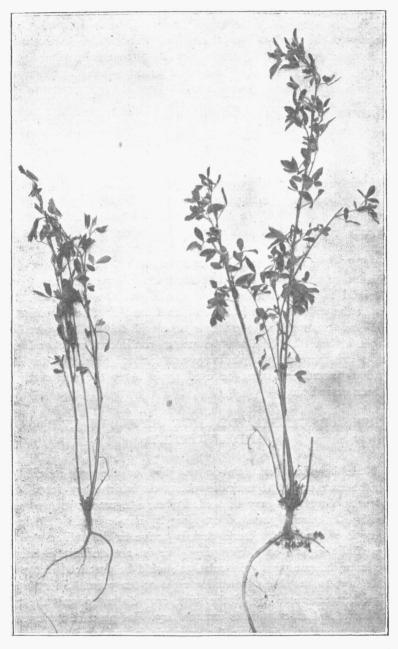


Fig. 2.—Alfalfa plants. Plant at left was not inoculated. Inoculated plant at right. Note the nodules on roots of the inoculated plant.

(Courtesy Illinois Experiment Station)

growth but fail to form nodules. Under such conditions the plants are using the nitrogen of the soil instead of drawing on the unlimited supply in the air. On very rich soils, uninoculated legumes may make a good growth by feeding on the soil nitrogen the same as other crops, and inoculation is the one treatment necessary to enable them to secure a part of their nitrogen from the air, and thus save the supply in the soil.

#### CONDITIONS MAY BE UNFAVORABLE TO THORO INOCULATION

Sometimes the first crop growth of a new legume is poor with only a few nodules on each plant, even tho it was given thoro inoculation. This may possibly be due to one of two causes: (1) The bacteria may be present in small numbers because they have not had time to multiply; or (2) some unfavorable soil condition keeps the legume plant and bacteria from doing better. In the former case. cultivation of the soil to distribute the bacteria, or a second crop of the legume to allow them to increase, will give the soil good infection. If the soil condition is at fault, this must first be remedied by special soil treatment before the legume will do well and the bacteria be of benefit. The most common conditions unfavorable to legumes are those of poor drainage and lack of lime. Under such conditions inoculation must be used again after the soil condition has been remedied.

### LEGUME BACTERIA DO NOT LIVE IN THE SOIL INDEFINITELY

These beneficial bacteria do not live in the soil indefinitely in the absence of the leguminous plants on which they feed. For this reason inoculation may be needed when a legume is grown again on land after several years of cropping by non-legumes. However, the length of time the bacteria remain active in the soil in the absence of the legume on which they live is not definitely known. Experience indicates that reinoculation should be practiced if the desired legume has not been grown for eight or ten years. Short lapses of time between reseeding of the same crop do not permit the bacteria to die, and inoculation is unnecessary. Its practice under such conditions would not increase the crop yields and would be unprofitable.

#### CROSS INOCULATION

Inoculation for one legume crop will not necessarily serve for all If one legume crop has been grown on a soil and was thoroly inoculated with many nodules on its roots, this gives no assurance that any other kind of legume will grow without the special treatment of inoculation. Some different kinds of legumes inoculate each other, but legumes do not cross-inoculate universally. have a single kind of bacteria which will grow on no other plant, and they must be inoculated with that specific kind of bacteria for their best growth, while others have one kind of bacteria common to two or more types of plants. It is a well-established fact that alfalfa can be inoculated with the bacteria from sweet clover, and that the cowpea may be cross-inoculated with the wild partridge pea. Likewise, red clover, white clover and all other true clovers will inoculate each other. Sovbeans, however, have a particular kind of bacteria not vet found on any other legume. According as the common legumes will cross-inoculate, or as they have the same kind of bacteria in common, they may be separated into the following groups:

- 1. The true clovers, including red, white, alsike, crimson and mammoth red.
- 2. Alfalfa, yellow and white sweet clovers, bur clover, and black medic or yellow trefoil.
  - 3. Cowpea, partridge pea, peanut, velvet bean and Japan clover.
  - 4. Garden, field and sweet peas, and vetches.
  - 5. Garden beans.
  - 6. Soybeans.

Because sweet clover and alfalfa inoculate each other many persons are apt to think that simply seeding sweet clover will inoculate the soil for alfalfa. Such is not the case. If proper bacteria have never been introduced artifically or by chance they must be applied when the first of these two crops on the soil is sweet clover as well as when it is alfalfa. Sweet clover often gets into alfalfa and smothers it, because conditions favorable for alfalfa are also favorable for sweet clover. The bacteria that nourish the alfalfa also nourish the sweet clover, and the sweet clover makes a much more rank growth, so that the alfalfa is smothered out. The soil is, however, no better supplied naturally with the bacteria for sweet clover than it is with those for alfalfa the first time one of these

"The principle of inoculation has never been condemned, but the methods of distributing bacteria have."

crops is grown; and if they must be added to the soil for the alfalfa, the need of inoculation cannot be met by substituting sweet clover. If the soil is to be put into alfalfa eventually, there may be some advantages in first seeding it to sweet clover for a season or two, since the latter crop seems to be less delicate than alfalfa. It estab-

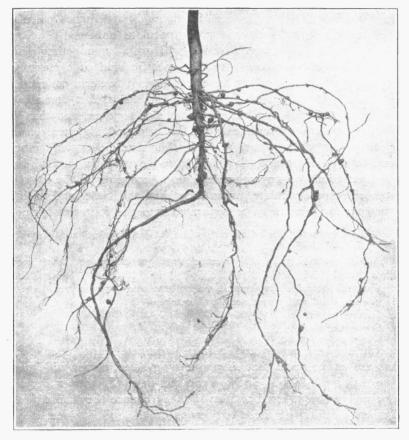


Fig. 3.—Inoculated roots of cowpea plant. Nodules distributed over the entire root system.

lishes itself over weeds more readily and helps distribute the bacteria for the alfalfa. It cannot, however, generate the bacteria needed for the alfalfa if they are not put there either by chance or by artificial inoculation, and the venture of establishing inoculation for sweet clover is as great as establishing it for alfalfa.

#### HOW TO INOCULATE

Soil may be inoculated by two methods: (1) By transferring soil from a field where the same legume has been growing with plenty of nodules, and (2) by the use of pure cultures, or artificial cultures, grown especially for this purpose.

#### THE SOIL METHOD

The use of inoculated soil was the first means of introducing the proper legume bacteria into a field, and this was accompanied with such good results that it has become established as good procedure. By this method, from 300 to 500 pounds of inoculated soil are scattered over each acre of the field and disked or harrowed in before seeding. The soil so distributed is collected from the surface six inches of a field on which the same legume—or one which cross inoculates with it—has been grown recently with many nodules. Extensive exposure of this soil to the sun before scattering is thought to be detrimental to the bacteria, but definite evidence fails to prove that this is as harmful as might be thought. Useless exposure to the sun, however, should be avoided.

In case only small amounts of soil are available it may be applied to the seed rather than to the field. Soil collected as just mentioned is dried and sifted over the seed which has been moistened (not wet) with a ten per cent glue solution (one pound of liquid furniture glue to one gallon of water, or one pound of dry glue to three gallons of water). Dry, powdered soil is added while the moist seed is stirred until every grain has become coated and the seed has a dirty appearance. After drying rapidly to prevent molding and loss of vitality, the seed is ready to be sown. The smaller seeds, such as alfalfa and sweet clover will need to be screened before seeding in order to break up the clusters of seeds held together by the glue.

Some objections have been made to the soil inoculation method, but under proper precautions the objections are not serious. The method may be laborious, and rather expensive when no thoroly infected soil is available in the immediate vicinity. It can also introduce noxious weed seeds or dangerous plant diseases, but these can be avoided by using uninfested soil. When any of these undesirable

<sup>&</sup>quot;Bacteria on legumes not only work for nothing, but even pay for the privilege."

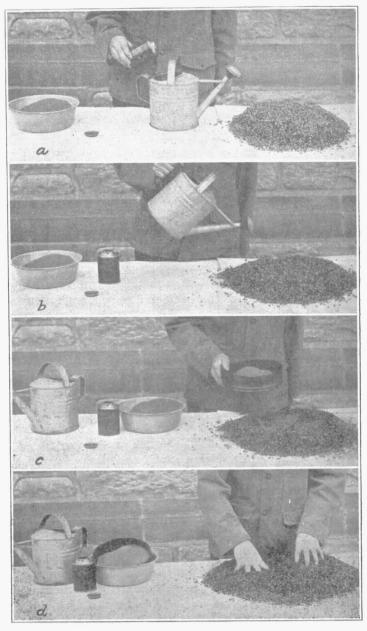


Fig. 4.—Inoculating legume seeds by soil method: a, Making the glue solution; b, sprinkling the seed with glue solution; c, sifting infected soil on the moistened seed; d, mixing the soil and seed before spreading it to dry.

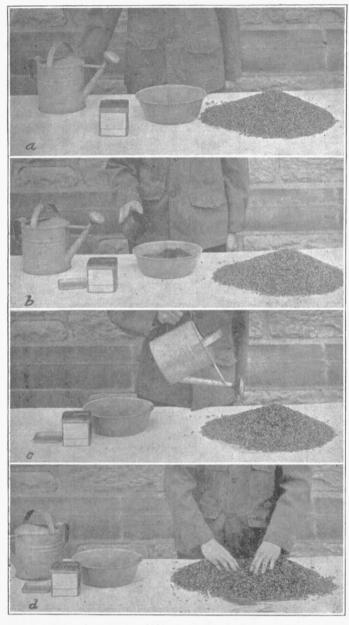


Fig. 5.—Inoculating legume seeds by artificial culture method: a, materials needed—water, culture, clean pan, and seed; b, adding water to the culture; c, sprinkling the culture on the seed; d, mixing the moist seed and spreading it to dry.

conditions prevail, the pure-culture method might well be used; but whenever good soil can be had for the same cost as pure cultures, the soil method is doubtless preferable. It has given a greater degree of success in past experiences.

#### PURE OR ARTIFICIAL CULTURES

The pure-culture method, like the soil method, is based on the principle of scattering the bacteria on the field. The bacteria are grown especially for such use. When bacteria were discovered to be the cause of the nitrogen-gathering power of legumes, attempts were soon made to grow pure cultures of them to avoid the difficulties of the soil method. For several years the pure cultures were in ill repute, but recently improved methods of propagation and distribution have overcome the former opposition and tests of commercial cultures by many experiment stations have found them generally reliable.

The desired bacteria are separated from the nodule and grown on neutral jelly in the laboratory, where they increase rapidly. These are sent to the farmer in various ways, sometimes in solution, sometimes on vegetable jelly, but more often on sterilized soil or sand. The method used is immaterial, provided the bacteria are alive. In using the culture it is diluted with water, sprinkled on the seed and allowed to dry.

The advantages of this method are its simplicity and ease of performance. No great expense is involved in making the cultures. With pure cultures there is no liability of introducing dangerous diseases, destructive insects or noxious weed seeds. They must not be used carelessly, however, since the bacteria are living organisms and die when the cultures are stored too long or subjected to excessive heat or cold. Directions for using are always supplied and if followed with reasonable care, this method of inoculation should be successful.

# SMALL ACREAGES OF LEGUMES ADVISABLE FOR BEGINNERS

Whenever a new legume is seeded for the first time it is good policy to attempt it on a small acreage. Such procedure offers a "Well inoculated legumes can use the nitrogen of the soil air, other crops cannot."

chance to become acquainted with the habits of the crop and the best methods of handling it, without the chance of a heavy loss of money and labor in case of failure. If this small area is inoculated, soil may be taken from it to inoculate larger fields on years following. It is, however, well to leave a part of this small area uninoculated and if the treated part has dark green plants with many nodules on the roots while the untreated has poorer plants or fails to develop nodules, the soil needs inoculation for the crop. Such procedure with the small acreage of a new legume has the following advantages: First, it involves no great risk of a heavy loss in labor and money; second, it determines at a very small cost whether the legume crop will do well; third, it tests the soil to see if inoculation is necessary; and fourth, if such treatment is necessary, it furnishes the soil from the inoculated part of the field as good material for inoculating the rest of the farm. Venturing on a small scale is far better policy than trying a large one, and any one who is seeding a new legume for the first time will do well to be guided by this principle. trying a few acres to find out how successful the crop is in the locality, rather than investing large sums in seed and labor to be lost in possible crop failure.

#### BENEFITS FROM INOCULATION

As previously mentioned, inoculation enables legumes to make a good growth on poor soil by taking nitrogen from the air. It also enables the plants to take up larger amounts of potash and phosphorus, and to give larger yields. By this practice the total crop is increased and its percentage of protein becomes higher, with a resulting higher feeding value.

Besides larger yields, and a higher protein content in the crop, inoculation causes greater root growth. This with the nodules included gives the root system also a high total nitrogen content; and since the root system remains in the soil legumes do not exhaust the soil nitrogen as rapidly as other crops. The legume, by taking nitrogen from the air, is a soil enricher and when plowed under serves as a nitrogen fertilizer. How much nitrogen a single crop of legumes plowed under will add to an acre of soil varies widely, and figures varying from twenty to one hundred and fifty pounds have been given. It is generally believed that about three-fourths of the nitrogen in the roots and tops of leguminous plants, grown on aver-

age soil, may come from the air. Regardless of what this amount may be, the fact remains that properly inoculated legumes use the nitrogen of the atmosphere without depleting the nitrogen in the soil, while in the absence of the bacteria they may drain the soil of its nitrogen the same as any other crop.

#### POSSIBLE FAILURE OF INOCULATION

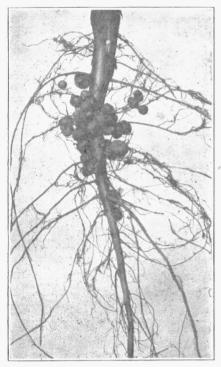


Fig. 6.—Roots of soybeans with good inoculation. Distribution along main roots.

Inoculation, while giving striking benefits, is by no means a cure-all for soil troubles. The bacteria concerned require satisfactory soil conditions if they are to flourish. In a very sour soil they do poorly and their growth can readily be improved by lime. If a farmer is uncertain regarding the acidity of his soil, it is well to have the soil tested before seeding a leguminous crop. This test is made gratis for Missouri farmers by the Experiment Station when soil samples are taken according to the Station's directions.

For a legume, as for any other crop, the seed bed should be well prepared, the soil well drained, and its acidity neutralized by the use of lime. When all these requirements are met, and the inoculation is given in addition, the legume crop should be successful. Well inoculated

legumes should find a place in every system of rotation in order to assist in keeping the soils permanently fertile.

#### DISTRIBUTION OF CULTURES

The laboratory of the Department of Soils, University of Missouri, is growing the bacteria for soybeans, sweet clover and alfalfa for distribution among the farmers of the state. These are sent out

at the cost of production, which is twenty-five cents an acre, delivered by parcel post or express. The bacteria are distributed on sterile sand, diluted with water, and applied to the seed or soil. Complete directions for use are sent with them. The cultures are recommended where no infected soil is readily available. Requests for cultures should state the number of acres, or pounds of seed, and the kind of legume to be planted. Reports of the results with the material will be appreciated.

"The greater proportion of legumes that can be turned under, either directly or in the form of manure, the easier it will be to maintain the fertility of the soil."