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# **Soybean Inoculation Studies**

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## *SUMMARY*

The results of the soybean inoculation studies reported in this bulletin were obtained under field conditions, and the experiments extended over a period of three years. These studies included the testing of different ways of applying inoculated soil to soybean seeds as well as to the soil, and also the testing of a number of commercial cultures, secured from different sources, on the inoculation of soybeans. Data have also been reported which show the relative efficiencies of soil from different sources, pure cultures of soybean bacteria, and different commercial cultures on the inoculation of different varieties of soybeans. The effects of inoculation, lime and lime and superphosphate, on the percentage of nitrogen in soybean plants have also been noted.

In some cases the experiments were repeated for two or three years, and the results obtained each year agreed remarkably well.

## *CONCLUSIONS*

When the results obtained in these studies are considered collectively for the three year period, the following conclusions seem justified:

(1) The efficiency of soil inoculation when applied to soybean seeds is proportional to the amount of soil which adheres to the seed coats.

(2) While all the different methods of applying inoculated soil produced inoculation, the method which gave the most consistent results was the soil paste method.

(3) Exposing either inoculated soybean seeds or inoculated soil to the direct rays of the sun for three, six or more hours did not result in any appreciable loss of inoculating efficiency.

(4) Moisture had little effect on the inoculation of soybeans. Planting moist inoculated soybeans in moist soil gave only slightly better results than planting dry inoculated soybeans in moist soil, or dry inoculated beans in dry soil.

(5) Increasing the amount of sugar in the solution used for making the inoculum gave a small increase in the degree of nodulation on soybeans.

(6) The sugar solution method proved to be inferior to the soil paste method for inoculating soybean seeds.

(7) Drying inoculated soybean seeds quickly proved little better than drying them slowly, or even very slowly.

(8) Compost soil (made by composting alternate layers of well-inoculated soybean roots with soil shaken from around them, prepared in the fall for use the following spring) produced more efficient inoculation than when inoculated soil from the same field was used in the spring.

(9) The degree of inoculation secured from the use of composted

soil and of ordinary inoculated soil by the soil paste method varied in proportion to the amount of soil used; the most efficient inoculation being secured with larger amounts of soil, up to 4 pints of soil per bushel of seed.

(10) Fresh soil from the field produced slightly better inoculation than similar soil which had been dried and pulverized.

(11) In 1925 and 1926 hydrated lime increased the degree of inoculation on Manchu soybeans as shown in Series 8 and 16. Lime and superphosphate gave a still further increase in inoculation.

(12) Different commercial cultures purchased from different manufacturers and dealers showed marked variation in ability to produce nodules on the roots of different varieties of soybeans.

(13) Some cultures seemed to be more efficient in inoculating one variety than others, but no single culture produced the best inoculation on all four varieties tested.

(14) While some cultures were consistently good in all of the tests, others proved to be very inefficient for the inoculation of soybeans.

(15) The 1925 results showed that the factory cultures gave, as a rule, much more efficient inoculation than the dealer cultures, but in 1926 the data showed no marked superiority in favor of the factory culture over the same brand of culture purchased from a dealer.

(16) Soaking of soybean seeds in a suspension of soybean bacteria overnight resulted in poorer inoculation than when the seeds were treated with the culture suspension and dried as quickly as possible.

(17) Manchu and Midwest soybeans were comparatively easy to inoculate with both the soil and culture methods. Dunfield soybeans were somewhat harder to inoculate than Manchu or Midwest soybeans, but Peking soybeans were extremely difficult to inoculate.

(18) Pure cultures of soybean bacteria isolated from Manchu, Dunfield and Midwest soybeans produced varying degrees of inoculation on Peking soybeans. The best inoculation on Peking soybeans, in general, was obtained from the use of soil taken from a field where this variety had been grown and where the plants had been well inoculated, altho, in 1926, two commercial cultures gave very good results.

(19) Indications that certain definite strains of soybean bacteria are required to produce the most efficient degree of nodulation on a given variety of soybean were obtained.

(20) The percentage of nitrogen in soybeans increased with increasing degree of nodulation, the highest percentage being found in the best inoculated plants.

(21) Hydrated lime alone and hydrated lime and superphosphate on this soil brought about some increase in the weights of plants, tops and roots, over the weights of the untreated plants and, while lime also increased the percentage of nitrogen in both tops and roots, superphosphate had no effect.

(22) Cultures of soybean bacteria, *provided they are efficient*, may give just as satisfactory results as soil inoculation. The soil paste method, using 2 to 4 pints of soil per bushel of seed was, however, more efficient than the *average* commercial culture tested. When compost soil was used, even better results were secured. If proper precautions are observed the compost soil paste method will rarely fail to bring about thoro inoculation of soybeans. Improvements in the preparation of commercial cultures for soybeans must obviously be made before successful inoculation from their use can be assured.

# Soybean Inoculation Studies

BY LEWIS W. ERDMAN AND F. SCOTT WILKINS\*

The difficulty often experienced in successfully inoculating soybeans is generally believed to be due to some unfavorable soil or seasonal condition, to the use of a poor inoculating material or to a combination of these factors. But even when the conditions are carefully controlled and all the usual precautions are taken frequently no inoculation occurs. It seems, therefore, that the inoculation of soybeans presents a different and more complex problem than is the case with other common legumes.

Recent investigations indicate the possible existence of more than one strain of the soybean organism, and some data also show that certain varietal characteristics of the plants may be of significance, since some varieties seem to be easier to inoculate than others. This indicates the necessity for observing unusual precautions in the preparation and use of inoculating material whether the pure culture or soil method is employed. Hence, fundamental studies are needed on the nature, habits and growth of the bacteria from the different varieties of soybeans in pure culture and under a wide range of soil and environmental conditions.

As a preliminary to investigations along this line and to secure more definite information on the inoculation of soybeans under field conditions in Iowa, the experiments reported in the following pages were carried out. These experiments included a study of the efficiency of a number of commercial cultures and of the value of inoculated soil when applied in different ways to the seed and to the soil. Four varieties of soybeans were used and cross inoculation tests were carried out both with inoculated soil and with pure cultures. The experiments were continued for three years. New locations in the field and, consequently, different soils were used each year.

## *HISTORICAL*

### THE USE OF SOIL AND COMMERCIAL CULTURES FOR SOYBEAN INOCULATION

Kirchner (18), as early as 1895, called attention to the fact that, of about 100 species of legumes growing in the botanical

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garden at Hohenheim, only the soybean failed to develop nodules. He believed that this was due to environmental conditions. Later he obtained some inoculated soil from Japan and in some pot and field experiments with two varieties of soybeans proved definitely that soybean bacteria were different from other legume bacteria and that soybeans could be inoculated by using soil from a field in which inoculated soybeans had been grown. Kirchner was probably the first investigator to use soil for the inoculation of soybeans.

In 1903 Hiltner and Störmer (15) made the first report on soybean inoculation experiments using pure cultures. In one of their experiments similar quantities of Dahlem soil and unlimed and limed moor soils were inoculated with a pure culture of soybean bacteria in January. In May definite amounts of these soils from 10 gms down to 0.00001 gm were used to inoculate sand cultures of soybeans. The best inoculation was secured with the limed moor soil, while with the unlimed moor soil the inoculation was very poor. In every case the best inoculation was secured when the largest quantity of soil was added.

Atwater and Woods (4) first suggested the use of soil infusion for the inoculation of soybeans. They carried out sand culture experiments, but the results were not very satisfactory. Of the inoculated plants, about one-third did not show any nodules, while the remainder had a very few small tubercles.

Munson (25) used tubercles from the previous year's crop for inoculation, introducing them with the soybean seed. Altho the crop was injured by frost and no weights were obtained, it was noted that the plants in the inoculated rows were larger and darker in color and bore many tubercles while the uninoculated plants showed no tubercles. The same author (26) later compared several Nitragin cultures and tubercles from soybean plants for inoculation, but practically no inoculation was secured, due, probably, to the tubercles being too dry.

Moore (23) stated that out of a total of 129 reports received from the soybean cultures sent out by the U. S. Department of Agriculture, 43 percent of the tests were failures; more failures being reported for soybeans than for any other crop.

Lipman (20), in experiments with Farmogerm and Nitragin, showed that, on soils well drained and properly supplied with moisture, lime, phosphates and potash, these cultures were capable of increasing the yields of such leguminous crops as had not been previously grown on the land.

Kisselbach (17) concluded that the soils at the Nebraska station possessed the soybean bacteria, as inoculation by both the culture and the soil methods failed to increase the yields materially.

Fellers (6) found that many of the commercial cultures tested failed to give satisfactory results with soybeans. He recom-

mended the soil transfer method for this crop, except when the commercial culture were known to be of good quality.

Later, Fellers (7) compared the relative efficiency of commercial cultures and a nodule infusion for the inoculation of soybeans and reported that the cultures gave just as good results as the nodule infusion. Kendall (16), using four commercial cultures for soybean inoculation, found that the inoculated plants showed a remarkable difference in color and also in the number of root nodules from the uninoculated plants.

In 1924, Fiske (9) reported the official tests on 33 samples of legume inoculants. Five were either fair or poor, while all the rest produced good inoculation. The following year (10), in similar tests made on 43 official samples, one gave negative results, one was poor, five produced only fair inoculation and the rest produced good inoculation.

#### METHODS OF APPLYING SOIL AND COMMERCIAL CULTURES

Hiltner and Störmer (15) described the two methods, proposed by Nobbe and Hiltner in 1896, for using Nitragin. In the one method, called soil inoculation, the culture was mixed with a quantity of soil which was then spread over and worked into the surface soil. In the other method, called seed inoculation, the seeds were moistened with the melted gelatin culture and then mixed with a small quantity of sand or soil to absorb the excess moisture and to prevent the seeds sticking together. In the majority of the field tests reported the soil inoculation method gave the better results. In an experiment in which inoculation was accomplished by mixing a pure culture and water with forest humus soil, with compost soil, with Dahlem soil and with quartz sand, plants taken from the quartz-sand-pure-culture inoculated plots averaged 11 nodules per plant, while all the others averaged less than 2 nodules per plant. It was concluded that these soil media did not serve as suitable carriers for the soybean bacteria.

In another field experiment, comparing the efficiency of a pure culture with that of a culture prepared by crushing fresh nodules in water, the same investigators found that soaking the seeds under water for five hours before applying the pure cultures increased the inoculation almost four times. When 10 times the normal inoculum was used in this way, it just about doubled the average number of nodules per plant, while the same amount applied in the usual way to unsoaked seed was twice as effective as the normal amount. When 10 times the normal inoculum of the crushed nodule infusion was used, the inoculation was doubled. Soaking the seeds under water before inoculation also led to better results when the nodule infusion was used. The pure culture gave a much better nodule formation than the infusion prepared from crushed nodules.

Otis (29), in inoculation tests with two varieties of soybeans in Kansas, used soil from Massachusetts, applying one-twentieth of a pint in each hill or pouring in one-sixth of a pint of a soil extract prepared by stirring thoroly 1 part of soil to 7 parts of water and allowing the soil to settle. Where the soil extract was used the tubercles on the Yellow Soy were numerous and well developed, while those on the Medium Green were scanty and rather inferior. Where the soil was used the two varieties were similarly inoculated. In a later pot culture experiment with four other varieties of soybeans no differences were noted between the soil inoculation and the soil extract methods. Increasing the amount of soil from one-twentieth of a pint to one and one-half pints per pot of soil did not produce any better inoculation. Successful inoculation was secured by using nodulated roots that had been left in the soil for about a month. When the nodulated roots were washed and air-dried for about a month before being used, much less satisfactory inoculation occurred.

Cottrell, Otis and Haney (5) prepared an inoculum by soaking  $\frac{1}{2}$  bu. of inoculated soil for 3 days in  $\frac{2}{3}$  of a barrel of water and mixed the soybean seed with the mud in the bottom of the barrel. They also drilled the seed with different amounts of soil and applied inoculated soil in different amounts broadcast before seeding and after seeding. The only satisfactory results were obtained by drilling the inoculated soil with the seed, and 375 pounds per acre produced the greatest number of nodules per plant. Where soil was broadcast at the rate of 1,000 pounds per acre, no inoculation was secured. It was found that dry inoculated soil kept for two years was as good as fresh soil.

Garman and Didlake (13) found that soybean seeds soaked overnight in a liquid suspension of the soybean bacteria were 100 percent inoculated, while shorter periods of soaking were less effective. In another experiment in which the seeds were soaked in the bacterial suspension for one hour, spread out, allowed to dry overnight and planted the following days, over three-fourths of the plants had nodules. When the inoculated seeds were not planted for four days, one week and two weeks, much less inoculation occurred and, when three weeks elapsed before the seeds were planted, no nodules were present.

Fellers, (8), on the other hand, inoculated soybean seeds with a nodule infusion and found that viable organisms remained on the seed coats for six to nine months. He showed that the bacterial cells were most rapidly destroyed the first few hours after inoculation.

Noyes and Cromer (28), in pot culture experiments, found that one pound or one-half pound of soil per acre was insufficient to inoculate soybeans. Four commercial cultures used in the proportion required per plant according to directions did



not give satisfactory inoculation. Applying a commercial culture at double the rate gave an average of 75 percent inoculation against 20 percent for no commercial inoculation.

Arny and McGinnis (3) found that, where water alone or 5, 20 or 30 percent glue and sugar solutions were used with soil as the inoculant, the plants generally were very slightly inoculated. Equal amounts of soil and seed gave satisfactory inoculation, while the commercial culture with few exceptions gave similar results. Storing the seed for a short time, or exposing the soil to the sun for one-half to five hours and exposing the commercial culture for one-half hour, did not affect the inoculation.

Nightingale (27), using 34 different strains of legume bacteria, studied the soil, water and milk methods of applying the bacteria to the seed and found that the soil method gave the best results, altho there was little difference between the soil and water methods.

Albrecht (1) found that the bacteria in the soil were effective for inoculation, regardless of whether or not the soil was dried in direct sunlight. The dried soil when stored for 30 months was as effective as the fresh moist soil gathered from the field.

Perkins (31) found the maximum nodulation of Virginia soybeans was secured when the number of infecting organisms was between 25 and 30 per seed. A rather definite number of organisms was required to produce maximum infection, and after a certain degree of infection was reached the plant was immune to additional infection.

Alicante (2) inoculated soybean seed with a bacterial infusion alone and with the bacterial infusion plus soil, or glue, or sugar and combinations of these materials. Some of the seeds were then planted immediately, others were planted at regular intervals and the last planting was made 2 months after inoculation. Nodule production was found to be fairly consistent, regardless of conditions and kind of treatment. The time of storage up to two months after treatment did not seem to influence nodule production. The use of sugar with the infusion was superior to the addition of glue or soil, the soil proving of some benefit but the glue having very little effect.

#### THE EFFECT OF LIME AND SUPERPHOSPHATE\* ON INOCULATION, YIELD AND NITROGEN CONTENT OF SOYBEANS

Wilson (33) found that calcium compounds and phosphates were effective in stimulating nodule formation on soybean plants. Lipman and Blair (21) noted that lime increased the yield of soybeans and also the percentage of nitrogen.

Fellers (7) found that small amounts of lime were nearly as efficient in increasing the protein content of soybeans as larger

\*The term superphosphate is employed to refer to the ordinary commercial 16 percent acid phosphate.

amounts. Nodule production on soybeans was also stimulated on limed soils by the addition of superphosphate, but the effect of this fertilizer was not so marked on acid soils.

Mac Taggart (22) showed that phosphorus slightly increased the percentage of nitrogen in soybeans and alfalfa.

Perkins (30) noted that when lime was absent, or present in only small amounts, nodulation of soybeans was greatly limited. The addition of varying amounts of superphosphate showed no effect on the nodulation of young soybean plants.

Work at the Illinois Agricultural Experiment Station (24) showed that the inoculation of soybeans increased the yield on acid as well as on limed soils.

Fred, Whiting and Hastings (12) found that inoculation increased the nitrogen content of soybeans grown on various soils, sometimes doubling the percentage. Lime additions increased the nitrogen content of inoculated plants in some cases but not on all the soils studied.

Heinze (14) showed that inoculation increased the yield of soybean seeds and also that this plant responded to phosphorus fertilization.

#### THE QUESTION OF DIFFERENT STRAINS OF SOYBEAN BACTERIA

Voorhees (32) inoculated several varieties of soybeans with cultures of Nitrogerm and Farmogerm and found considerable difference in the inoculation secured on the different varieties. He concluded that different varieties of the same legume bear different and definite powers of resistance to association with the symbiotic bacteria.

Leonard (19) inoculated 19 varieties of soybeans with a pure culture of bacteria isolated from the Medium Yellow soybean and secured inoculation in all cases.

Fred and Bryan (11) found that pure cultures of organisms from Mammoth Yellow, Medium Green, Manchu and Haberlandt soybeans could be used interchangeably on these varieties, and the same was true for soil inoculation. Other field tests with additional varieties gave similar results. No evidence was secured to justify the conclusion that the nodule bacteria are specific.

Wright (34) studied eight strains of soybean bacteria and found them to be identical morphologically but quite different culturally and physiologically. He described two distinct types of organisms, designating them type A and type B. The former produced nodules which were usually centrally located on the root system and tended to become large and develop in clusters. The tendency of type B was to produce smaller scattered nodules on the lateral roots of the plants. Type B was able to withstand greater acidity than type A, but both were influenced by varia-

tions in the hydrogen-ion concentration of the cell sap of the host plant, which in turn is influenced by the reaction and composition of the soil.

Wright (35) later studied six strains of soybean organisms in greenhouse tests for three years with Ito San soybeans and found that the type A strains showed an average fixation of more than one and one-half times that of the type B strains. Field experiments with three varieties of soybeans confirmed the conclusion that there are two types of these organisms.

Investigations at the Illinois Agricultural Experiment Station (24) have indicated that soybean nodule bacteria from various sources differ in their abilities to fix atmospheric nitrogen.

## *EXPERIMENTAL*

### PLAN OF THE EXPERIMENTS IN 1924

The 1924 soybean inoculation experiments were located on an area of Carrington loam which showed a lime requirement of  $2\frac{1}{2}$  tons of ground limestone per acre of surface soil and on which soybeans had never been grown.

Single row plots were used thruout the entire experiment, each row being 56 feet long. The rows were 21 inches apart, and two guard rows planted with uninoculated seed separated adjacent plots. Duplicate plots were used for each treatment. Each plot was divided into three sections of 16 feet each, and each section was separated by a 4 foot alley or border. The first section received no soil treatment, the middle section received ground limestone at the rate of  $2\frac{1}{2}$  tons per acre and the third section received 250 pounds of superphosphate per acre in addition to the limestone. The limestone was applied about a week before planting, and the superphosphate was applied by hand the same day on which the planting was done.

The check plots and guard rows were planted with a Columbia planter, but all of the inoculated seeds were planted by hand, the beans being spaced 1 inch apart in the row. Contamination of the seed was prevented by cleansing the hands with a solution of mercuric chloride before planting each plot.

Data were secured showing the nodulation of the plants in all plots, but yields were not taken as the plots were too small for accurate results. Forty representative plants were carefully taken from each of the three sections of each plot and examined for nodules. They were then separated into four classes based upon the number and size of the nodules as follows:

- Class 0 No nodules present.
- Class 1 One to four very small nodules, or one to three small nodules, or one to two fair sized or large nodules.
- Class 2 Four to ten small nodules or two to six fair sized or large nodules.
- Class 3 More than 10 small nodules, or more than six fair sized or large nodules.

## TESTS WITH SOIL INOCULATION

### SERIES I—THE RELATIVE EFFICIENCY OF DIFFERENT METHODS OF APPLYING INOCULATED SOIL TO THE SEED AND TO THE SOIL.

Manchu soybeans were inoculated with soil in a number of ways as follows:

(1) The seeds were sprinkled with a glue solution (1 to 2 ounces furniture glue per gallon of water) and inoculated soil dust added and thoroly mixed until the seeds were perfectly dry.

(2) The seeds were sprinkled with a sugar solution (2 tablespoonfuls sugar to  $\frac{1}{2}$  pint water) and inoculated soil dust mixed with the seeds until they were thoroly dry.

(3) A soil-paste was prepared by mixing screened inoculated soil with water until it had the consistency of cream (about 3 or 4 pints of soil per bushel of seed). This was poured over the seeds which were mixed thoroly and spread out to dry.

(4) Inoculated soil and water (about 1 part soil to 5 parts water) were shaken for 5 minutes, the soil allowed to settle, and the seeds thoroly moistened with this extract.

(5) The seeds were sprinkled with milk to which sugar had been added (2 tablespoonfuls to  $\frac{1}{2}$  pint milk). Inoculated soil dust was then added and the seeds thoroly mixed until dry.

(6) A soil-paste was made by mixing screened inoculated soil with sufficient milk to give the consistency of cream. This was poured over the seeds which were thoroly mixed and spread out to dry.

(7) A muddy solution was made with milk by shaking about 1 part inoculated soil with 5 parts milk for 5 minutes. After the soil had settled, the solution was poured over the seeds which were mixed and allowed to dry.

All of the inoculations in this series were made on the morning of June 19, 1924, and plantings were made the same afternoon. The results are given in table I. These data as well as those presented in the next five tables represent averages of the duplicate plots receiving the same inoculation.

It is apparent from the data in table I that, where water was used, the soil-paste method produced the best inoculation of all the methods. The sugar solution method was second, the glue method third and the muddy water method last. Where milk was used similar results were obtained as with water, the paste method giving the best results. The use of sugar in the milk gave satisfactory results, altho there was no advantage over the sugar solution method. The results obtained with the muddy milk method were the poorest of all.

Apparently the efficiency of soil inoculation when applied to soybean seeds is proportional to the amount of soil which remains on the seed. More soil is held on the seeds when the soil paste method is used than when any of the other methods is followed, and the muddy water method allows retention of the smallest amount of the soil on the seeds.

Neither the lime nor the lime and superphosphate seemed to affect the inoculation of soybeans in this experiment.

TABLE I. THE EFFICIENCY OF DIFFERENT METHODS OF APPLYING INOCULATED SOIL TO MANCHU SOYBEANS

Plot No.	Inoculation treatment	Treatment of the soil													
		Check						Num-ber plants inocu-lated				Lime			
		Class number						Class number				Class number			
		0	1	2	3	%	%	0	1	2	3	0	1	2	3
1-2	Not inoculated	97.5	2.5	0.0	0.0	0.0	0.0	99.0	1.0	0.0	0.0	98.25	1.75	0.0	0.0
3-4	Soil-Glue method	27.5	40.0	27.5	5.0	72.5	0.0	25.0	36.25	38.75	0.0	23.75	40.00	32.5	3.75
5-6	Soil-Sugar solution method	12.5	38.75	40.0	8.75	87.5	0.0	16.25	37.5	37.5	8.75	11.25	23.75	55.0	10.0
7-8	Soil-Soil paste method	5.0	35.0	52.5	7.5	95.0	0.0	1.25	27.5	65.0	6.25	15.0	30.0	43.75	11.25
9-10	Soil-Muddy water method	58.75	38.75	2.5	0.0	41.25	0.0	62.5	35.0	2.5	0.0	43.75	43.75	12.5	0.0
11-12	Soil-Milk+sugar	23.75	31.25	40.0	5.0	76.25	0.0	11.25	30.0	52.5	6.25	15.0	32.5	47.5	5.0
13-14	Soil-Paste with milk	5.0	31.25	61.25	2.5	95.0	0.0	7.5	21.25	63.75	7.5	11.25	26.25	57.5	5.0
15-16	Soil-Muddy milk method	82.5	16.25	1.25	0.0	17.5	0.0	92.5	6.25	1.25	0.0	83.75	15.0	1.25	0.0

TABLE II. THE EFFECT OF DIRECT SUNLIGHT ON THE EFFICIENCY OF SOIL INOCULATION. (SUGAR SOLUTION METHOD)

Plot No.	Inoculation	Treatment of the soil													
		Check						Num-ber plants inocu-lated				Lime + superphosphate			
		Class number						Class number				Class number			
		0	1	2	3	%	%	0	1	2	3	0	1	2	3
17-18	Not inoculated	96.25	3.75	0.0	0.0	0.0	0.0	98.75	1.25	0.0	0.0	95.0	5.0	0.0	0.0
19-20	Soil-In shade for one hour	13.75	40.00	46.25	0.0	86.25	0.0	11.25	33.75	55.0	0.0	8.75	36.25	51.25	3.75
21-22	Soil-In sun for one hour	16.25	47.5	35.0	1.25	83.75	1.25	6.25	33.75	56.25	3.75	7.5	28.75	57.5	6.25

## **SERIES II—EFFECT OF DIRECT SUNLIGHT ON SOIL INOCULATION**

This series was planned to determine whether or not it is necessary to protect inoculated soybean seeds from direct sunlight. Two lots of Manchu soybeans were inoculated by the sugar solution method. One lot was exposed to the direct rays of the sun for one hour, the beans being worked over by hand at frequent intervals. The other lot was allowed to dry in the shade of a tree for one hour. Both lots were planted exactly one hour after treatment. The results of this experiment are shown in table II.

These data indicate that direct sunlight is not a harmful factor in lowering the efficiency of soil inoculation when the soil is applied to the seeds. The results were very similar under different soil treatments and the lime and superphosphate showed little effect on inoculation.

## **SERIES III—EFFECT OF KEEPING INOCULATED SOYBEANS FOR FOUR OR EIGHT DAYS BEFORE SEEDING**

The object of this series was to determine the longevity of the soybean organisms on inoculated seed when the sugar solution method was used. Three lots of Manchu soybean seed were used; one lot was inoculated on June 19, 1924; the second on June 23; and the third on June 26. All three lots were planted the same day, June 27. This made the intervals between inoculation and planting, eight days, four days and one day, respectively. The nodulation data for this experiment are found in table III.

The inoculation secured when the seeds were planted one day or four days after inoculation was better than that obtained after eight days, there being little difference in the results for the one and four day periods. Apparently inoculated soybeans may be held over for two, three or four days without any harmful effects.

Again the lime and superphosphate had no effect on the nodulation of soybeans.

## **SERIES IV—EFFICIENCY OF SOIL INOCULATION WHEN DRY AND MOIST SOYBEANS ARE PLANTED IN AIR DRY AND MOIST SOIL**

This series was planned to study the effect of moisture on the efficiency of soil inoculation (sugar solution method). The experiment was so arranged that both inoculated and uninoculated moist and dry Manchu soybeans were planted in dry and also in moist soil. The dry inoculated soybeans were treated the day before planting and allowed to dry overnight. The moist inoculated soybeans were planted immediately after inoculation. The moist uninoculated soybeans were obtained by thoroly moistening the seeds with sterile water just before planting. At the time

TABLE III. THE EFFICIENCY OF SOIL INOCULATION WHEN SOYBEANS ARE HELD OVER FOR 4 AND 8 DAYS AFTER INOCULATION

Plot No.	Treatment and time of planting	Treatment of the soil															
		Check				Num-ber plants inocu-lated				Lime				Lime + superphosphate			
		Class number				0 1 2 3				0 1 2 3				0 1 2 3			
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
23-24 25-26	Not inoculated Soil—Planted 1 day after inocu- lation	100.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	97.5	2.5	0.0	0.0
		16.25	41.25	41.25	1.25	88.75	23.75	88.75	32.5	43.75	0.0	0.0	26.25	32.5	40.0	1.25	67.5
27-28	Soil—Planted 4 days after inoc- ulation	21.25	41.25	35.0	2.5	78.75	12.5	78.75	53.75	31.25	2.5	2.5	15.0	38.75	40.0	6.25	61.25
		31.25	45.0	23.75	0.0	68.75	27.5	68.75	53.75	16.25	2.5	2.5	20.0	36.25	41.25	2.5	63.75

of planting the soil was practically air-dry. To obtain a moist soil condition a furrow was opened and the soil was thoroly moistened with clean well water. The seeds were then planted and covered at once.

The nodulation results obtained from this experiment are given in table IV.

Examination of the data for the plots in the no treatment section shows that slightly better nodulation was secured when dry inoculated soybeans were planted in moist soil than in dry soil. Where lime or lime and superphosphate was applied, however, the results were the opposite. Both on the no treatment and treated sections the best inoculation was secured by using moist inoculated beans planted in moist soil. While the differences were not extremely great, the results indicate that the best inoculation of soybeans will generally be secured when the moist inoculated seed are planted in moist soil.

There was some evidence of benefit from the lime and the lime and superphosphate treatments, but the results were not definite enough to permit of broad conclusions.

#### SERIES V—EFFICIENCY OF SOIL INOCULATION ON THREE VARIETIES OF SOYBEANS

Manchu, Ito San and







TABLE V. THE EFFICIENCY OF SOIL INOCULATION ON THREE VARIETIES OF SOYBEANS. (SUGAR SOLUTION METHOD)

Plot No.	Variety and treatment	Treatment of the soil																					
		Check						Lime						Lime + superphosphate				Number plants inoculated					
		Class number						Class number						Class number				Number plants inoculated					
		1		2		3		0		1		2		3		0		1		2		3	
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
47-48	Manchu—No inoculation	91.25	8.75	0.0	0.0	8.75	93.75	6.25	0.0	0.0	6.25	85.0	15.0	0.0	0.0	15.0						15.0	
49-50	Manchu—Soil inoculation	6.25	25.0	46.25	22.5	93.75	5.0	33.75	50.0	11.25	95.0	6.25	16.25	52.5	25.0	93.75						93.75	
51-52	Ito San—No inoculation	97.5	2.5	0.0	0.0	2.5	96.25	3.75	0.0	0.0	3.75	95.0	5.0	0.0	0.0	5.0						5.0	
53-54	Ito San—Soil inoculation	6.25	25.0	47.5	21.25	93.75	1.25	22.5	55.0	21.25	98.75	6.25	20.0	45.0	28.75	93.75						93.75	
55-56	Black Eyebrow—No inoculation	97.5	2.5	0.0	0.0	2.5	96.25	3.75	0.0	0.0	3.75	93.75	6.25	0.0	0.0	6.25						6.25	
57-58	Black Eyebrow—Soil inoculation	8.75	46.25	42.5	2.5	91.25	8.75	52.5	37.5	1.25	91.25	6.25	38.75	48.75	6.25	93.75						93.75	

Black Eyebrow soybeans were used in this series to study the efficiency of soil inoculation when the sugar solution method was employed on more than one variety of soybean. The seeds for each variety were inoculated on the morning of June 19, and planting was done the same afternoon. The nodulation data for this study are shown in table V.

The Manchu and Ito San soybeans consistently showed little difference in degree of nodulation, regardless of whether the plants were taken from the no soil treatment, lime, or lime and superphosphate sections. Very good inoculation was secured in classes 2 and 3 with both Manchu and Ito San varieties. The degree of nodulation was much greater with the Manchu and Ito San beans than with the Black Eyebrow variety. A much greater percentage of the Black Eyebrow soybeans fell into class 1, and only very few plants had sufficient nodules to place them in class 3. Thus it seems that some unknown varietal characteristic may be a factor in controlling the inoculation of soybeans.

**TESTS WITH SOIL AND COMMERCIAL CULTURES**  
**SERIES VI—RELATIVE EFFICIENCY OF SOIL AND COMMERCIAL**  
**CULTURES FOR THE INOCULATION OF BLACK**  
**EYEBROW SOYBEANS**

This series was planned to compare the relative efficiency of soil inoculation applied to the seed by the solution method, of soil broadcast down the row and of four commercial cultures for the inoculation of Black Eyebrow soybeans. For the soil treatment the inoculated soil was applied at the rate of 500 pounds per acre. The soybeans were planted immediately after the soil was applied and covered at once. All plantings in this series were made on the afternoon of June 19, and all seed inoculations, including the soil and four commercial cultures, were made the same morning. Directions as given on each package for the commercial cultures were carefully followed in making the inoculations. The results of these tests are given in table VI.

The plants from the check plots showed only slight inoculation on a few plants from each section. The best inoculation was obtained with the soybeans planted in immediate contact with the inoculated soil that was broadcast down the rows, altho some of the commercial cultures were practically as good. More plants were placed in class 3, when the soil broadcast method was followed, than in any other case. Good inoculation was secured from the sugar solution method, but the results were not as satisfactory as where the broadcast method was used. All four of the commercial cultures produced inoculation, but two cultures were much less effective than the other two. Lime alone did not seem to exert any noticeable effect on the inoculation of soybeans in this experiment and the lime and superphosphate treatment had only a slight beneficial effect.

**Plan of the Experiments in 1925**

The field used for the 1925 inoculation tests was slightly rolling in topography and, altho located on Carrington loam, was somewhat variable, especially in the amount of organic matter and nitrogen present. The acidity also varied, the lime requirement ranging from 2 to 3 tons of limestone per acre according to the Truog qualitative test.

The field was divided into two ranges running north and south across its entire length and separated from each other by a four foot alley. Each plot consisted of 4 rows, 20 feet long and 18 inches apart. In all cases, inoculations were made in duplicate, one plot for each treatment being located on the east and one on the west range. Every fifth plot was a check and was planted with uninoculated Manchu soybeans to note the presence, if any, of natural inoculation of soybeans in this field.

All check plots were planted with a hand planter, the seed being spaced about 1 inch apart in the row. All inoculated plots

TABLE VI. THE RELATIVE EFFICIENCY OF COMMERCIAL CULTURES AND SOIL ON THE INOCULATION OF BLACK EYEBROW SOYBEANS

Plot No.	Culture and treatment	Treatment of the soil																Num-ber plants inocu- lated	%
		Check				Lime				Lime + superphosphate				Lime + superphosphate					
		Class number				Class number				Class number				Class number					
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3		
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
59-60	No inoculation	97.5	2.5	0.0	0.0	96.25	3.75	0.0	0.0	3.75	93.75	6.25	0.0	0.0	6.25				6.25
61-62	Soil—Sugar solution method	8.75	46.25	42.5	2.5	8.75	52.5	37.5	1.25	91.25	6.25	38.75	48.75	6.25	93.75				93.75
63-64	Soil—Down the row, 500 lbs. per acre	0.0	21.25	56.25	22.5	1.25	22.5	62.5	13.75	98.75	0.0	16.25	58.75	25.0	100.00				100.00
65-66	Commercial culture No. 1	56.25	38.75	5.0	0.0	66.25	27.5	6.25	0.0	33.75	61.25	26.25	11.25	1.25	38.75				38.75
67-68	Commercial culture No. 2	0.0	37.5	56.25	6.25	100.00	8.75	32.5	50.0	8.75	91.25	1.25	20.0	65.0	13.75				98.75
69-70	Commercial culture No. 3	1.25	23.75	66.25	8.75	98.75	3.75	25.0	65.0	96.25	1.25	21.25	58.75	18.75	98.75				98.75
71-72	Commercial culture No. 4	50.00	40.00	10.00	0.0	47.5	41.25	11.25	0.0	52.5	47.5	27.5	22.5	2.5	52.5				52.5

were planted by hand as in the previous year and the same precautions used to prevent contamination.

The work was similar to that carried out in 1924 except that yields were secured and cross-inoculation studies were made using four soils taken from around the roots of plants of four varieties of soybeans. Tests were also made of commercial cultures secured from eight manufacturers. These cultures were used on three varieties of soybeans.

The experimental work has been divided into a number of series as in the 1924 work. Each series will be discussed separately.

For the nodulation data, 100 plants were carefully dug from the inner rows of each plot. These plants were classified into different classes based upon the number and size of the nodules on their roots as follows:

Class 0—All plants without nodules.

Class 1—Plants with one or two small or medium sized nodules.

Class 2—Plants with one or two large nodules, or in addition one or

two small or medium sized nodules, or three or four small or medium sized nodules.

Class 3—Plants with three or four large nodules, or five to nine small or medium sized nodules.

Class 4—Plants with three or more large sized nodules and in addition three or more small or medium sized nodules or ten or more nodules of any size.

A record was kept of the weight of the 100 plants which were used for the nodulation studies, and the remaining plants from the two inner rows were later dug up and weighed. The plot yields were calculated on an air-dry basis from the weights of all the plants taken from the two inner rows of each plot.

**SERIES VII—RELATIVE EFFICIENCY OF SOIL FROM AREAS  
WHERE DIFFERENT VARIETIES OF SOYBEANS HAD BEEN  
GROWN ON THE INOCULATION OF THE  
VARIOUS VARIETIES**

In this series four soils, taken from around the roots of four varieties of soybeans grown under widely different soil conditions, were used for inoculation. The soils were secured from fields that had grown the same variety of soybean for more than one year, and the soybeans were definitely known to be well inoculated. Manchu, Dunfield, Peking and Midwest soybeans were used and the cross inoculation efficiency of the soils was tested on each of the four varieties. Thus, inoculated soil from Manchu soybeans was used to inoculate Manchu, Dunfield, Peking and Midwest soybeans; the other varieties being treated similarly.

For the inoculation a sufficient amount of each soil was mixed with water to form a soil-paste having the consistency of cream. In the case of the Manchu soil, 10 grams were added to 7 cc. of water; with the Dunfield, Peking and Midwest soils, more moisture was present and 10 grams of soil and 4 cc. of water were used for the paste. This amount was enough to inoculate the one-half pound of soybeans required for each plot. After pouring the soil-paste over the seeds, they were mixed thoroly and spread out to dry. All the soybeans were planted the day after they were inoculated. The results obtained in these studies are shown in table VII.

Of the 10 uninoculated Manchu check plots scattered thru the series, only 7 percent of the plants were inoculated, and the majority of these showed only a few small nodules. This certainly shows that the soybean organism was not naturally present in this soil.

Noticeable differences were obtained when the various soils were used to inoculate the different varieties of soybeans. The best inoculation on the Manchu soybeans was obtained with soil taken from around the roots of the Dunfield soybeans, 97 percent of the plants being inoculated.

TABLE VII. THE EFFICIENCY OF SOIL INOCULATION ON DIFFERENT VARIETIES OF SOYBEANS

Plot No.	Source of soil used for inoculation	Manchu soybeans										Dunfield soybeans									
		Degree of inoculation										Degree of inoculation									
		Number of plants in each class										Number of plants in each class									
		0	1	2	3	4	Yield per acre (tons)	Num-ber plants inocu-lated	%			0	1	2	3	4	Yield per acre (tons)	Num-ber plants inocu-lated	%		
1 & a, b, c, d-2 & a, b, c, d	Check	93.0*	4.1	2.9	0.0	0.0	1.362	7.0				14.5	17.5	32.0	32.0	4.0	1.746	85.5			
3-4	Manchu soil	18.5	20.5	31.5	25.0	4.5	1.978	81.5				3.0	15.5	17.0	59.0	5.5	2.159	97.0			
5-6	Dunfield soil	3.0	14.0	26.0	46.5	10.5	2.272	97.0				13.0	27.5	35.5	16.5	7.5	2.107	87.0			
7-8	Peking soil	21.0	23.0	37.5	18.5	0.0	1.902	79.0				23.5	25.5	29.0	19.5	2.5	1.763	76.5			
9-10	Midwest soil	14.5	14.0	34.5	32.5	4.5	1.646	85.5													

Plot No.	Source of soil used for inoculation	Peking soybeans										Midwest soybeans									
		Degree of inoculation										Degree of inoculation									
		Number of plants in each class										Number of plants in each class									
		0	1	2	3	4	Yield per acre (tons)	Num-ber plants inocu-lated	%			0	1	2	3	4	Yield per acre (tons)	Num-ber plants inocu-lated	%		
1 & a, b, c, d-2 & a, b, c, d	Check	43.5	31.5	22.0	3.0	0.0	1.690	56.5				8.0	16.5	21.5	39.0	15.0	1.504	92.0			
3-4	Manchu soil	19.5	28.5	36.0	15.0	0.0	1.952	80.5				0.5	11.0	13.5	49.5	25.5	1.680	99.5			
5-6	Dunfield soil	14.5	17.5	33.0	27.5	5.5	1.832	83.5				3.5	18.0	21.5	36.5	20.5	1.897	96.5			
7-8	Peking soil	73.5	22.0	4.5	0.0	0.0	1.594	26.5				1.5	8.5	17.5	44.0	28.5					
9-10	Midwest soil																				

\*Average of 10 plots.

The Midwest soil produced slightly better nodulation on Manchu soybeans than did the Manchu soil, altho the yield per acre was less where the Midwest soil was used. The Peking soil did not produce as good inoculation as the other three soils on Manchu soybeans, but the yield obtained from the Peking soil plots was about the same as that for the Manchu soil plots.

The yield per acre when the Dunfield soil was used for the inoculation was higher than that obtained when the other soils were employed. In all cases, however, there was an increase over the yield on the uninoculated plots.

With the Dunfield soybeans the Dunfield soil produced by far the best inoculation. The Midwest soil gave the poorest inoculation. With Peking soil 86.0 percent of the plants were inoculated, but the inoculated plants were placed in the two poorest classes, 1 and 2. The Manchu soils produced very satisfactory inoculation being next in efficiency to the Dunfield soil.

Differences in yields were also noted in the case of the Dunfield soybeans. When the Dunfield and Peking soils were used, the yields were practically the same and much higher than those obtained where the Manchu and Midwest soils were used.

The best inoculation on the Peking soybeans was obtained from the use of Peking soil, altho the Dunfield soil again gave very satisfactory inoculation. The Manchu soil was much less effective and the Midwest soil produced only 26.5 percent inoculation. The plants from the Manchu and Midwest soil inoculated plots were very poorly inoculated, all but three being placed in classes 1 and 2. The yields of the Peking soybeans correlate closely with the inoculation data. The results confirm previous observations which have shown that Peking soybeans are the most difficult of all varieties to inoculate successfully.

Apparently the Midwest soybeans were easier to inoculate with soil than any of the other varieties tested. All the soils gave a high percent of inoculated plants, ranging from 92 to 99.5. A large percentage of the inoculated plants in all cases fell into the best inoculated classes, 3 and 4. The Manchu soil had the least effect and showed the smallest number of plants in classes 3 and 4. Very slight differences in the yields were secured under the various treatments.

It seems evident from this data that, while it is not necessary to obtain soil from the same variety of soybean to produce satisfactory inoculation, usually the best results may be secured when the particular variety of soybeans is inoculated with soil taken from around the roots of the same variety. Different varieties of soybeans certainly show decided irregularities in their susceptibilities to inoculation.

**SERIES VIII—EFFECT OF LIME AND SUPERPHOSPHATE ON  
THE YIELD AND DEGREE OF NODULATION ON  
MANCHU SOYBEANS**

In this series Manchu soybeans were grown on a soil of medium fertility and having a lime requirement of two tons of ground limestone per acre. The plots receiving the same treatment were replicated four times. Thus, 4 plots were used for checks, the remaining twelve were planted to Manchu soybeans inoculated with soil by the paste method as employed for Manchu soil in the previous series. Four of these 12 plots received an application of hydrated lime at the rate of 500 pounds per acre, and 4 received also an application of superphosphate at the rate of 200 pounds per acre; the remaining 4 were unfertilized. The results obtained from this study are presented in table VIII, the data representing the average of the four plots receiving the same treatment.

The inoculation which occurred on the plants from the check plots was probably due to the close proximity of some plots that had been inoculated by broadcasting soil, and the inoculation might have been brought about by wind blown soil. In the plots where the seed was inoculated but no fertilizer added, 78.5 percent of the plants were inoculated. The influence of lime on the inoculation was very noticeable, and a total of 93.25 percent of the plants on these plots were inoculated. Also, on these plots a larger percentage of the inoculated plants were in classes 3 and 4; evidently the lime led to the production of larger nodules and a greater number of nodules.

A still further benefit was noted when superphosphate was used with the lime. In this case 95.5 percent of the plants were inoculated and exactly twice as many plants were placed in class 4 as where lime alone was applied.

Inoculation increased the yield 0.349 ton over the check plots; the lime increased the yield 0.437 ton over the inoculation alone;

TABLE VIII. THE EFFECT OF LIME AND SUPERPHOSPHATE ON THE YIELD AND DEGREE OF INOCULATION OF MANCHU SOYBEANS

Plot No.	Inoculation and fertilizer treatment	Degree of inoculation						Yield per acre (tons)
		Number of plants in each class					Number-plants inoculated	
		0	1	2	3	4		
		%	%	%	%	%		
11-12 11a-12a	Check—No inoculation—No fertilizer	71.25	9.25	17.50	2.00	0.0	28.75	1.880
13a-14a	Soil inoculation—No fertilizer	21.50	14.00	33.75	25.00	5.75	78.50	2.229
15-16 15a-16a	Soil inoculation + lime	6.75	14.25	31.75	38.50	8.75	93.25	2.666
17-18 17a-18a	Soil inoculation + lime + superphosphate	4.50	10.75	30.00	37.25	17.50	95.50	2.806



TABLE IX. THE EFFECT OF DIRECT SUNLIGHT ON THE EFFICIENCY OF SOYBEAN BACTERIA IN INOCULATED SOIL

Plot No.	Soil treatment and time of sun exposure	Degree of inoculation						Yield per acre (tons)
		Number of plants in each class					Number-plants inoculated	
		0	1	2	3	4		
		%	%	%	%	%	%	
19-20	Check—No inoculation	36.50	18.25	29.00	16.25	0.00	63.50	1.842
19a-20a								
21-22	Soil 500 lbs. per acre; harrowed immediately	8.50	12.50	23.25	43.00	12.75	91.50	2.279
21a-22a								
23-24	Soil 500 lbs. per acre; harrowed after 3 hours	21.25	21.25	21.50	30.00	6.00	78.75	2.195
23a-24a								
25-26	Soil 500 lbs. per acre; harrowed after 6 hours	17.75	19.75	21.75	34.75	6.00	82.25	2.217
25a-26a								
27-28	Soil 500 lbs. per acre; not harrowed at all	14.75	14.50	20.00	45.00	5.75	85.25	1.842
27a-28a								

and superphosphate and lime increased the yield by 0.140 ton of soybeans per acre over the lime alone.

These data indicate that lime and superphosphate may increase the inoculation of Manchu soybeans, and inoculation plus these treatments may distinctly increase crop yields.

#### SERIES IX—EFFECT OF DIRECT SUNLIGHT ON THE EFFICIENCY OF SOYBEAN BACTERIA IN INOCULATED SOIL

This series was similar to series II of the 1924 experiments except that, instead of exposing inoculated soybean seed to the direct rays of the sun, inoculated soil at the rate of 500 pounds per acre was broadcast and allowed to remain exposed to the sun for three or six hours before being harrowed, or not harrowed at all. Four plots were used as checks and received no inoculation, and four plots were used for each of the treatments.

All the plots were planted on May 29. The soil was very dry to a depth of 3 inches. On May 31, there was a light rain and on June 3 and June 7 heavy rains which undoubtedly washed the soybean bacteria down to the young soybean plant roots. The results of this experiment are given in table IX, the figures in all cases representing the average yield of the four plots receiving the same treatment.

On the check plots it was found that 63.5 percent of the plants were inoculated, the inoculation undoubtedly coming from near-by plots.

The best inoculation was secured when the soil was harrowed in immediately. In this case 91.5 percent of the plants were inoculated, and over 50 percent of these plants were in the two best nodulation classes. The number of inoculated plants from the plots on which the soil was exposed to the sun for 3 and 6 hours was 78.75 and 82.25 percent respectively. This would suggest that the sun had exerted a harmful effect and possibly killed



some of the organisms, yet, when the data obtained from the plot where the inoculated soil was not harrowed in at all is examined, it may be noted that 85.25 percent of the plants were inoculated and 45 percent were sufficiently inoculated to be placed in class 3. These data indicate, as other investigators have already shown, that the harmful effect of sunlight on the legume organisms has been overestimated in the past.

Inoculation in this series gave small increases in the yield of soybean hay, only where the soil was left exposed to the sun and not harrowed was no increase secured. The largest increase was obtained on the plot where the soil was harrowed in immediately, but the differences were not great.

From these results it may be concluded that there is slight danger of over exposing inoculated soil to the direct rays of the sun after it has been broadcast. While it is probably best to harrow the soil in immediately, little loss of efficiency of the bacteria will be noticeable if the inoculated soil is not harrowed for three or six hours or even longer.

**SERIES X—EFFECT OF DIFFERENT CONCENTRATIONS OF..  
SUGAR ON THE EFFICIENCY OF SOIL INOCULATION  
BY THE SOIL PASTE METHOD**

In this series three concentrations of sugar were used in the soil paste, namely; one, two and four tablespoonfuls for each pint of water. The inoculated soil was used at the rate of two pints per bushel of soybeans, and a sufficient quantity of the respective sugar solutions was used to give the desired consistency. The treatments were made in duplicate, and the average results are given in table X.

Only 15 percent of the plants were inoculated in the check plots where the different amounts of sugar were used in preparing the soil paste for inoculation. Somewhat more efficient inoculation was secured with the larger amounts of sugar than with the smaller amounts, but the differences were not very great. The inoculations increased the yield of soybeans, but there was little correlation with the amount of sugar used.

TABLE X. THE EFFECT OF DIFFERENT CONCENTRATIONS OF SUGAR ON THE EFFICIENCY OF SOIL INOCULATION

Plot No.	Amount of sugar added to soil inoculation	Degree of inoculation					Number-plants inoculated	Yield per acre (tons)
		Number of plants in each class						
		0	1	2	3	4		
		%	%	%	%	%		
29-30	Check—No inoculation	85.0	8.0	7.0	0.0	0.0	15.0	1.122
31-32	One tablespoonful sugar	30.5	19.5	38.0	12.0	0.0	69.5	1.633
33-34	Two tablespoonfuls sugar	29.0	17.5	35.5	18.0	0.0	71.0	1.496
35-36	Four tablespoonfuls sugar	20.5	17.5	40.5	21.5	0.0	79.5	1.671

As a whole, these results furnish some evidence that increasing the amount of sugar in making the inoculum has some effect on increasing the degree of nodulation of soybeans.

**SERIES XI—EFFECT OF DRYING INOCULATED SOYBEANS QUICKLY AND SLOWLY ON THE EFFICIENCY OF SOIL INOCULATION BY THE SOIL PASTE METHOD**

In this test the soybeans were inoculated by the soil paste method, and the plots were duplicated. Enough seeds for two plots were inoculated and immediately spread out to dry. The soybeans for two plots were inoculated and placed in a milk bottle which was left uncovered. In this way the seeds dried slowly. Where the soybeans were dried very slowly the inoculated seeds were placed in a milk bottle in which was inserted a rubber stopper containing two small holes about  $\frac{1}{4}$  of an inch in diameter.

The average results are presented in table XI. There were slight differences in the results with different methods of drying and when the seeds were dried very slowly the poorest inoculation occurred. The difference between drying quickly and slowly was not definite, however. When the total number of plants is considered, very little difference was noted between the different rates of drying the seeds, and the same thing may be said for the yield of soybean hay per acre.

**SERIES XII—EFFICIENCY OF DIFFERENT METHODS OF USING SOIL FOR THE INOCULATION OF MANCHU SOYBEANS**

In the fall of 1924 a quantity of well-inoculated soybean roots were dug from a field of Manchu beans, care being taken not to lose many of the nodules. A box, approximately 2 feet square and 10 inches deep, with the bottom removed was placed in the ground with the top of the box nearly level with the surface of the ground. In the bottom of the box was placed a 2-inch layer of the soybean roots, with the nodules and above them a layer of 2 inches of soil which had shaken from the soybean roots in harvesting was next added. This procedure was repeated, alternat-

TABLE XI. THE EFFECT OF DRYING INOCULATED SOYBEANS QUICKLY AND SLOWLY ON THE EFFICIENCY OF SOIL INOCULATION

Plot No.	Treatment of Seed	Degree of inoculation						Yield per acre (tons)
		Number of plants in each class					Number of plants inoculated	
		%	%	%	%	%		
37-38	Check—No inoculation	85.0	8.0	7.0	0.0	0.0	15.0	1.122
39-40	Seed dried quickly	19.5	12.5	30.5	36.5	1.0	80.5	1.502
41-42	Seed dried slowly	19.0	16.0	33.0	32.0	0.0	81.0	1.489
43-44	Seed dried very slowly	23.5	14.0	43.5	19.0	0.0	76.5	1.529

TABLE XII. THE EFFICIENCY OF DIFFERENT METHODS OF USING SOIL FOR THE INOCULATION OF MANCHU SOYBEANS

Plot No.	Treatment of soil for inoculation	Degree of inoculation						Yield per acre (tons)
		Number of plants in each class					Number of plants inoculated	
		0	1	2	3	4		
		%	%	%	%	%	%	
45-46 45a-46a	Check—No inoculation	78.50	11.00	8.75	1.25	0.50	21.50	1.247
47-48 47a-48a	Compost soil—(Soil paste method)	17.50	26.75	25.50	29.00	1.25	82.50	1.506
49-50 49a-50a	Soil—(Soil paste method)	23.25	27.75	32.25	16.75	0.00	76.75	1.505
51-52 51a-52a	Soil—(Sugar solution method)	29.25	23.00	31.25	15.75	0.75	70.75	1.412
53-54 53a-54a	Soil—Broadcast 500 lbs. per acre	3.00	6.75	17.00	58.50	14.75	97.00	1.535

ing a layer of roots and a layer of soil, until the box was nearly full. This mixture of soil and soybean roots with the nodules was allowed to remain outdoors during the winter and spring months by which time the nodules and roots had almost completely decayed. This compost was used the following spring to inoculate a number of plots in the 1925 experiments. This mixture will be referred to as compost soil.

Plots receiving the same inoculation treatment were replicated four times. The soybeans were inoculated in the following different ways: (a) the compost soil was used at the rate of 1 pint per bushel of seed to make a soil paste with water; (b) inoculated soil, taken just before planting time from the same area in the field from which the soil came that was used in making the compost soil, was used at the rate of 1 pint of soil per bushel of seed to make a soil paste; (c) an identical amount of the same soil as used in (b) was mixed with a sugar solution, containing one tablespoonful of sugar per pint of water to form a soil paste; (d) the same soil as was used in (b) was broadcast at the rate of 500 pounds per acre and harrowed in. Four plots were left untreated to serve as checks.

The results of this experiment are given in table XII. The figures in all cases represent the average of the four plots receiving the same treatment.

The check plots in this series were all found to be slightly inoculated; contamination probably coming mainly from those plots which had been inoculated with the broadcast soil.

The compost soil used as a soil paste produced a more efficient inoculation than when inoculated soil was used either by the soil paste or the sugar solution method; 82.5 percent of the plants being inoculated where the compost soil was used, while the other methods gave only 76.75 and 70.75 percent inoculation, respectively.

Broadcasting the soil for inoculation gave a 97.0 percent inoculation, and almost three-fourths of the plants were placed in the two highest classes. The superiority of broadcasting the soil over the other methods tested is probably due to the fact that a much larger number of organisms was introduced with the soil in an application of 500 pounds per acre than where only 1 pint of the soil was used to inoculate a bushel of soybean seed.

The experiment also shows definitely the greater inoculating efficiency of the compost soil used as soil paste over ordinary inoculated soil applied in the same way.

**SERIES XIII—EFFICIENCY OF DIFFERENT AMOUNTS OF SOIL  
AND OF COMPOST SOIL ON THE DEGREE OF INOCULA-  
TION OF MANCHU SOYBEANS**

In this test Manchu soybeans were inoculated with compost soil and ordinary inoculated soil used according to the soil paste method. The amounts of soil used were  $\frac{1}{2}$  pint, 1 pint,  $1\frac{1}{2}$  pints, 2 pints and 4 pints per bushel of seed. Each of the treatments was replicated four times and there were 10 check plots. The results given in table XIII are the averages from all the plots in each treatment.

A few plants from the check plots were inoculated, but the area where this series was planted was certainly free from natural inoculation. The compost soil produced much superior inoculation to that brought about by the ordinary inoculated soil. Even where  $\frac{1}{2}$  pint of this compost soil was used, 93.5 percent of the plants were inoculated and with the larger amounts the inoculation was more complete. Altho the total number of inoculated plants did not vary much with the different amounts of soil, the degree of nodulation varied considerably. With increasing amounts of soil, more and more plants were placed in the two best classes; the largest number being in these classes when 4 pints of soil were used.

With the ordinary soil inoculation there was the same tendency for the degree of inoculation on the plants to become better as the amount of soil used increased. The differences, however, were not nearly so large nor as regular as with the compost soil.

When the yields per acre are studied, it may be noted that in every case inoculation brought about an increase. Increasing the amount of compost soil used for inoculation brought small but consistent increases in yield of soybean hay per acre, the largest amount of soil giving the largest yield. Rather consistent increases were also noted for the different amounts of inoculated soil with one exception in the case of the  $1\frac{1}{2}$  pint amount. A more efficient inoculation was obtained with the compost soil than with the ordinary soil and greater yields were also secured.

These results, as a whole, indicate the value of compost soil for the inoculation of soybeans, and they show that yields may correlate with degree of nodulation.

TABLE XIII. THE EFFECT OF USING DIFFERENT AMOUNTS OF SOIL AND OF COMPOST SOIL ON THE INOCULATION OF MANCHU SOYBEANS

Plot No.	Amount and kind of soil per bushel of soybeans	Degree of inoculation					Number-plants inoculated	Yield per acre (tons)
		Number of plants in each class						
		0	1	2	3	4		
		%	%	%	%	%		
55 & a, b, c, d 56 & a, b, c, d 57-58 57a-58a 59-60 59a-60a 61-62 61a-62a 63-64 63a-64a 65-66 65a-66a 67-68 67a-68a 69-70 69a-70a 71-72 71a-72a 73-74 73a-74a 75-76 75a-76a	Check—No inoculation*	74.92	14.25	7.33	2.75	0.75	25.08	1.477
	Soil—½ pint	36.00	17.50	30.60	14.80	1.10	64.00	1.652
	Compost soil—½ pint	6.50	20.00	34.70	36.70	2.10	93.50	1.908
	Soil—1 pint	21.50	22.20	36.30	17.50	2.50	78.50	1.719
	Compost soil—1 pint	6.70	13.30	25.50	51.30	3.20	93.30	1.942
	Soil—1½ pints	21.70	23.80	36.80	16.70	1.00	78.30	1.687
	Compost soil—1½ pints	3.80	11.20	20.00	56.30	8.70	96.20	2.083
	Soil—2 pints	19.80	20.70	33.70	24.70	1.10	80.20	1.733
	Compost soil—2 pints	2.00	11.20	21.80	55.00	10.00	98.00	2.151
	Soil—4 pints	13.30	20.00	36.20	28.50	2.00	86.70	1.802
	Compost soil—4 pints	0.50	7.20	10.30	67.70	14.30	99.50	2.256

\*Average of all checks.

#### SERIES XIV—RELATIVE EFFICIENCY OF DRY PULVERIZED AND FRESH SOIL FOR THE INOCULATION OF MANCHU SOYBEANS

In this series one lot of seed was inoculated with fresh field soil by the soil paste method, using the soil at the rate of 1 pint per bushel of seed. Another lot was inoculated with the same amount of soil and in the same manner, except that the soil was air dried and ground to a fine powder in an iron mortar. The treatments were replicated four times, while the checks were duplicated. The average results are given in table XIV.

This test shows that the fresh soil produced slightly better inoculation than the dry pulverized soil. It seems advisable to use fresh soil direct from the field whenever possible in order to secure the best results but, if the soil does become dry before it can be used, no very serious loss in efficiency would necessarily follow.

#### SERIES XV—RELATIVE EFFICIENCY OF EIGHT DIFFERENT COMMERCIAL CULTURES FOR THE INOCULATION OF THREE VARIETIES OF SOYBEANS

This series was planned to determine whether or not certain of the commercial cultures are better adapted to one variety of

TABLE XIV. THE RELATIVE EFFICIENCY OF DRY PULVERIZED AND FRESH SOIL FOR THE INOCULATION OF MANCHU SOYBEANS

Plot No.	Treatment	Degree of inoculation						Yield per acre (tons)
		Number of plants in each class					Number-plants inoculated	
		0	1	2	3	4		
		%	%	%	%	%	%	
77-78	Check—No inoculation	73.5	13.50	7.5	4.00	1.50	26.5	1.349
79-80	Soil—Dry pulverized	29.5	20.75	39.5	9.75	0.50	70.5	1.615
79a-80a								
81-82	Soil—Fresh from field	23.5	24.00	38.5	13.25	0.75	76.5	1.618
81a-82a								

soybeans than to another, to compare the most important varieties of soybeans in Iowa from the standpoint of relative ease of securing inoculation, and to determine the relative efficiency of the more important cultures which are sold in Iowa.

The cultures used were purchased direct from the manufacturer, and also wherever possible a duplicate culture was bought on the open market from a dealer. This was done to determine whether any loss of efficiency occurs during the period of storage by the dealers. Thirteen cultures were tested of which seven were obtained from factories and six from dealers.

Directions, which accompanied each culture, were carefully followed in making the inoculations, and care was taken to secure the proper amount of inoculum for the seed for each plot. Duplicate plots for each variety of soybean were used for each culture. Every fifth plot was planted with uninoculated Manchu soybeans and served as a check. All of the seeds for the treated plots were inoculated on the morning of May 29.

The results are given in table XV. As no uninoculated Dunfield and Peking soybeans were planted for checks, all plots planted with these varieties having 94 or more uninoculated plants were averaged together to give the check yields for these varieties in table XV. The average of 19 check plots of the Manchu soybeans showed that this field was practically free of the nodule organisms of soybean.

Wide variations were noted in the efficiency of the different cultures to produce nodules on Manchu soybeans. The best inoculation was obtained from the use of factory culture No. 2, while the dealer culture No. 7 was next. Several of the cultures tested proved very inefficient.

In all but one case, where a comparison could be made of the same brand of culture, the factory culture was more efficient than the dealer culture. This would indicate the desirability of using fresh cultures but does not throw any light on the question of how long a culture may be kept and still be satisfactory for use.

TABLE XV. THE EFFICIENCY OF DIFFERENT COMMERCIAL CULTURES ON MANCHU, DUNFIELD AND PEKING SOYBEANS

Plot No.	Culture number and source	Manchu soybeans						Yield per acre (tons)
		Degree of inoculation						
		Number of plants in each class					Number plants inoculated	
		0	1	2	3	4		
		%	%	%	%	%	%	
83a, b, c, d, e, f, g, h, 84a, b, c, d, e, f, g, h, i	Check No Inoculation	94.1 <sup>1</sup>	3.0	2.9	0.0	0.0	5.9	1.801
85-86	Culture 1 Factory	27.5	13.5	22.0	20.5	16.5	72.5	1.710
87-88	Culture 1 Dealer	95.5	0.5	4.0	0.0	0.0	4.5	1.900
89-90	Culture 2 Factory	7.0	9.0	34.0	38.0	12.0	93.0	1.699
91-92	Culture 2 Dealer	55.5	9.5	29.0	5.0	1.0	44.5	2.206
93-94	Culture 3 Factory	97.5	2.5	0.0	0.0	0.0	2.5	1.502
95-96	Culture 3 Dealer	98.5	1.0	0.5	0.0	0.0	1.5	1.971
97-98	Culture 4 Factory	84.0	10.0	6.0	0.0	0.0	16.0	1.217
99-100	Culture 4 Dealer	33.0	14.5	32.5	14.0	6.0	67.0	2.643
101-102	Culture 5 Factory	48.0	14.0	29.0	9.0	0.0	52.0	1.602
103-104	Culture 5 Dealer	90.5	6.5	3.0	0.0	0.0	9.5	2.656
105-106	Culture 6 Factory	86.5	6.5	6.0	0.0	1.0	13.5	1.277
107-108	Culture 7 Dealer	13.0	11.0	27.0	33.0	16.0	87.0	1.838
109-110	Culture 8 Factory	49.0	8.5	35.5	5.0	2.0	51.0	1.649
Dunfield Soybeans								
—	Check No Inoculation	96.6 <sup>2</sup>	1.5	1.8	0.0	0.1	3.4	2.919
85-86	Culture 1 Factory	59.5	18.5	18.5	2.0	1.5	40.5	2.684
87-88	Culture 1 Dealer	90.0	2.5	7.5	0.0	0.0	10.0	3.092
89-90	Culture 2 Factory	72.5	18.5	7.0	1.5	0.5	27.5	2.464
91-92	Culture 2 Dealer	93.0	4.5	2.5	0.0	0.0	7.0	3.318
93-94	Culture 3 Factory	93.5	4.0	2.5	0.0	0.0	6.5	2.817
95-96	Culture 3 Dealer	96.5	2.0	1.5	0.0	0.0	3.5	2.897
97-98	Culture 4 Factory	92.0	3.5	4.5	0.0	0.0	8.0	3.372
99-100	Culture 4 Dealer	97.0	2.5	0.5	0.0	0.0	3.0	2.658
101-102	Culture 5 Factory	34.5	14.5	28.0	17.5	5.5	65.5	3.620
103-104	Culture 5 Dealer	98.0	0.5	1.5	0.0	0.0	2.0	2.759
105-106	Culture 6 Factory	89.0	5.0	5.5	0.5	0.0	11.0	3.524
107-108	Culture 7 Dealer	95.0	1.0	3.5	0.0	0.5	5.0	3.365
109-110	Culture 8 Factory	73.0	11.5	12.5	3.0	0.0	27.0	3.327
Peking Soybeans								
—	Check No Inoculation	98.1 <sup>2</sup>	1.4	0.5	0.0	0.0	1.9	1.629
85-86	Culture 1 Factory	74.0	14.5	10.5	0.5	0.5	26.0	2.319
87-88	Culture 1 Dealer	100.0	0.0	0.0	0.0	0.0	0.0	1.372
89-90	Culture 2 Factory	78.0	16.5	5.5	0.0	0.0	22.0	2.196
91-92	Culture 2 Dealer	87.0	10.0	3.0	0.0	0.0	13.0	1.484
93-94	Culture 3 Factory	97.0	1.0	2.0	0.0	0.0	3.0	2.175
95-96	Culture 3 Dealer	99.5	0.5	0.0	0.0	0.0	0.5	1.197
97-98	Culture 4 Factory	100.0	0.0	0.0	0.0	0.0	0.0	2.093
99-100	Culture 4 Dealer	97.5	2.5	0.0	0.0	0.0	2.5	1.702
101-102	Culture 5 Factory	87.0	9.5	3.5	0.0	0.0	13.0	1.685
103-104	Culture 5 Dealer	97.5	2.0	0.5	0.0	0.0	2.5	1.312
105-106	Culture 6 Factory	93.5	3.0	3.5	0.0	0.0	6.5	1.561
107-108	Culture 7 Dealer	95.0	4.0	1.0	0.0	0.0	5.0	1.554
109-110	Culture 8 Factory	77.5	17.5	4.0	1.0	0.0	22.5	1.360

<sup>1</sup>Average of 19 checks.<sup>2</sup>Average of plots No. 95-96, 99-100, 103-104, and 107-108.<sup>3</sup>Average of plots No. 87-88, 93-94, 95-96, 97-98, 99-100, 103-104, and 107-108.



The yields of Manchu soybeans were increased in a few instances by the inoculation but, in general, there was no influence from the inoculation and no relation between nodulation and yield.

None of the 13 cultures tested proved very efficient for the inoculation of Dunfield soybeans. In fact only four cultures produced satisfactory results, one factory culture, No. 5, inoculating 65.5 percent of the plants. All four cultures which produced satisfactory inoculation on Dunfield soybeans were factory cultures; none of the dealer cultures producing nodules on more than 10 percent of the plants.

There was a wide variation in yields of Dunfield soybeans and no relation to nodulation was found. Many cultures had no effect on the crop yield.

Only three cultures produced fair inoculation on the Peking soybeans, and these were all factory cultures. It seems that the Peking soybeans are more difficult to inoculate, at least with commercial cultures, than other varieties, and it is possible that the proper strains of soybean organisms are not present in the cultures tested. In four cases the yields were increased, but there was no correlation between nodulation and yield.

These results indicate that some commercial cultures are better adapted to one variety of soybeans than to others. They show, too, that cultures vary widely in inoculating efficiency and that cultures obtained direct from the factory were apt to be more efficient in producing nodules than the same brand of culture purchased from a dealer.

#### Plan of the Experiments in 1926

The 1926 soybean inoculation experiments were made on an area of Carrington loam having a lime requirement of  $2\frac{1}{2}$  tons per acre, as shown by the Truog test. It was believed that soybeans had never grown on this field. The field had received an application of approximately 10 tons of barnyard manure in the fall of 1925.

The field was laid out in eight ranges of plots, running north and south, with  $4\frac{1}{2}$  foot border strips separating each range. Each plot consisted of 5 rows, 30 feet long and 18 inches apart. One-half of each plot, except those in the lime and superphosphate series, was limed at the rate of 5,200 pounds of ground limestone per acre shortly before planting. Every fifth plot was planted with uninoculated soybeans of the same variety used in the adjacent treated plots.

All plantings were made with Columbia hand planters in a manner approximating closely actual field conditions. The planters were sterilized with commercial denatured alcohol after seeding a group of plots inoculated with the same culture. Cul-



tivation was done with hand cultivators, which were sterilized with a mercuric chloride solution to prevent contamination between plots.

The studies in 1926 were grouped into nine series; duplicating as nearly as possible the work in 1925. However, the tests with commercial cultures and the cross inoculation studies were more extensive than the 1925 studies.

The results obtained in 1926 included data on nodulation studies, yields per acre of soybean hay, and a number of determinations for nitrogen on plants from different varieties showing varying degrees of inoculation. The inoculated plants were separated into seven classes, following the scheme of classification proposed in 1926 by the senior author. This classification is as follows:

Class No.	Number and size of nodules per plant	Class No.	Number and size of nodules per plant
0	No nodules present	5	31 to 40 small
1	1 to 5 small	1	medium + 26 to 35 small
2	1 medium	2	medium + 21 to 30 small
	6 to 10 small	3	medium + 16 to 25 small
	1 medium + 1 to 5 small	4	medium + 11 to 20 small
	2 medium	5	medium + 6 to 15 small
	1 large	6	medium + 1 to 10 small
3	11 to 20 small	7	medium + 1 to 5 small
	1 medium + 6 to 15 small	8	medium
	2 medium + 1 to 10 small	1	large + 21 to 30 small
	3 medium + 1 to 5 small	1	large + 1 medium + 16 to 25 small
	4 medium	1	large + 2 medium + 11 to 20 small
	1 large + 1 to 10 small	1	large + 3 medium + 6 to 15 small
	1 large + 1 medium + 1 to 5 small	1	large + 4 medium + 1 to 10 small
	1 large + 2 medium	1	large + 5 medium + 1 to 5 small
	2 large	1	large + 6 medium
4	21 to 30 small	2	large + 11 to 20 small
	1 medium + 16 to 25 small	2	large + 1 medium + 6 to 15 small
	2 medium + 11 to 20 small	2	large + 2 medium + 1 to 10 small
	3 medium + 6 to 15 small	2	large + 3 medium + 1 to 5 small
	4 medium + 1 to 10 small	2	large + 4 medium
	5 medium + 1 to 5 small	3	large + 1 to 10 small
	6 medium	3	large + 1 medium + 1 to 5 small
	1 large + 11 to 20 small	3	large + 2 medium
	1 large + 1 medium + 1 to 15 small	4	large
	1 large + 2 medium + 1 to 10 small	6	All plants showing a greater number of different sized nodules than listed in class 5
	1 large + 3 medium		
	1 large + 3 medium + 1 to 5 small		
	1 large + 4 medium		
	2 large + 1 to 10 small		
	2 large + 1 medium + 1 to 5 small		
	2 large + 2 medium		
	3 large		

Fifty plants were dug from the four center rows on both the limed and unlimed sections of each plot. Only the four center rows were harvested to determine the yield per acre of soybean hay. As the lime had no effect on the nodulation data nor on the yields the results are not given separately.

# TESTS WITH SOIL FOR INOCULATION

## SERIES XVI—EFFECT OF LIME AND SUPERPHOSPHATE ON THE YIELD AND DEGREE OF NODULATION ON MANCHU SOYBEANS

This series was similar to Series VIII in the 1925 experiments, except that compost soil was used at the rate of 3 pints per bushel of seed instead of the ordinary inoculated soil. Hydrated lime was added at the rate of 500 pounds per acre and superphosphate at the rate of 200 pounds. The treatments were replicated three times, and the results in table XVI are the averages of all plots in the same treatment.

Altho 57.5 percent of the plants on the check plots were inoculated, 47.0 percent were placed in the two poorest nodulation classes. With the compost soil inoculation, 96.7 percent of the plants were inoculated and 44.7 percent were placed in the best nodulation class, the remaining plants being rather evenly distributed in classes 2, 3, 4 and 5. The lime and superphosphate showed slight beneficial effects on the degree of inoculation, but the differences were not large enough to be significant.

The yields of soybean hay were not appreciably increased over the checks by the inoculation or the treatments altho slight increases were noted.

## SERIES XVII—EFFECT OF DIRECT SUNLIGHT ON THE EFFICIENCY OF SOYBEAN BACTERIA IN INOCULATED SOIL

The plan of the experiment in this series was the same as that of Series IX in the 1925 tests, except that two additional plots were added to compare the relative efficiency of 250 pounds of soil and 1,000 pounds of soil, broadcast and harrowed in immediately, in bringing about inoculation. Soil broadcast at the rate of 500 pounds per acre was exposed to the sun for three or six hours; either harrowed in immediately or not harrowed at all.

TABLE XVI. THE EFFECT OF LIME AND SUPERPHOSPHATE ON THE YIELD AND DEGREE OF INOCULATION OF MANCHU SOYBEANS

Plot No.	Inoculation and fertilizer treatment	Degree of inoculation							Number plants inoculated	Yield per acre (tons)
		Number of plants in each class								
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%		
1-2	Check—No inoculation—	42.5	28.0	19.0	6.5	3.0	1.0	0.0	57.5	1.748
1a	No fertilizer									
3-4	Compost soil inoculation—	3.3	5.0	11.0	13.7	12.0	10.3	44.7	96.7	1.816
3a	No fertilizer									
5-6	Compost soil inoculation	1.8	4.3	7.3	15.0	10.3	13.0	48.3	98.2	1.729
5a	+ lime									
7-8	Compost soil inoculation	1.0	3.0	6.0	8.7	17.3	12.0	52.0	99.0	1.847
7a	+ lime + superphosphate									

TABLE XVII. THE EFFECT OF DIRECT SUNLIGHT ON THE EFFICIENCY OF SOYBEAN BACTERIA IN INOCULATED SOIL

Plot No.	Soil treatment and time of sun exposure	Degree of inoculation							Number plants inoculated	Yield per acre (tons)
		Number of plants in each class								
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%		
9-10 9a	Check—No inoculation	14.0	17.3	23.9	18.8	12.1	5.6	8.3	86.0	2.221
11-12 11a	Soil 250 lbs. per A. Harrowed immediately	1.0	6.3	10.3	14.3	14.7	11.7	41.7	99.0	2.178
13-14 13a	Soil 500 lbs. per A. Harrowed immediately	4.7	13.0	10.3	10.0	15.0	16.0	31.0	95.3	2.377
15-16 15a	Soil 500 lbs. per A. Harrowed after 3 hours	7.4	6.0	8.0	16.0	13.7	10.3	38.6	92.6	2.315
17-18 17a	Soil 500 lbs. per A. Harrowed after 6 hours	2.2	9.6	10.3	14.3	8.6	17.0	38.0	97.8	2.282
19-20 19a	Soil 500 lbs. per A. Not harrowed at all	7.0	6.0	11.7	16.3	16.3	14.0	28.7	93.0	2.132
21-22 21a	Soil 1,000 lbs. per A. Harrowed immediately	0.6	2.0	5.7	9.0	14.7	11.7	56.3	99.4	2.059

The average results for the three plots receiving the same treatment are given in table XVII.

Altho the check plots were inoculated, possibly due to previous applications of manure carrying the proper bacteria or to the introduction of the organisms by soil carried over by the wind from inoculated plots, all of the soil inoculations produced a more efficient nodulation. The 250 pounds of soil produced slightly better inoculation than the 500 pound application and about the same as the 1,000 pounds, altho the latter amount gave more plants in the best inoculation class. Where the soil was exposed to the sun for three or six hours, the results were about the same as where the soil was harrowed in immediately. It seems, therefore, that there is little danger of seriously reducing the efficiency of inoculated soil by exposure to the direct rays of the sun. Even where the soil was not harrowed in at all, excellent results were obtained.

#### SERIES XVIII—EFFECT OF DIFFERENT CONCENTRATIONS OF SUGAR ON THE EFFICIENCY OF SOIL INOCULATION BY THE SOIL PASTE METHOD

This series was an exact duplicate of Series X in the 1925 tests. One, two and four tablespoonfuls of sugar was used in preparing the inoculum for the inoculated plots. The average results for the three plots receiving the same treatments are given in table XVIII.

The results obtained show somewhat greater inoculation when four tablespoonfuls of sugar were used in the inoculum, but the other amounts were only slightly less effective. No effects of the different methods were noted on the yields. It seems, as

TABLE XVIII. THE EFFECT OF DIFFERENT CONCENTRATIONS OF SUGAR ON THE EFFICIENCY OF SOIL INOCULATION

Plot No.	Amount of sugar added to soil inoculation	Degree of inoculation							Number plants inoculated	Yield per acre (tons)
		Number of plants in each class								
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%		
23-24 23a	Check—No inoculation	9.6	14.7	17.3	22.7	12.7	10.0	13.0	90.4	2.221
25-26 25a	One tablespoonful sugar	3.1	4.7	12.0	16.0	13.7	11.0	39.5	96.9	2.276
27-28 27a	Two tablespoonfuls sugar	5.0	3.7	8.7	13.3	17.3	16.0	36.0	95.0	2.180
29-30 29a	Four tablespoonfuls sugar	0.0	2.4	8.0	16.3	16.3	12.7	44.3	100.0	2.268

was concluded from the 1925 test, that increasing the amount of sugar in the solution used for making the inoculum may have some effect in increasing the degree of nodulation on soybeans.

#### SERIES XIX—RELATIVE EFFICIENCY OF DRY, PULVERIZED AND FRESH SOIL FOR THE INOCULATION OF MANCHU SOYBEANS

This series was an exact duplication of Series XIV in the 1925 tests. The dry pulverized soil was prepared in the same manner as in 1925, and soil taken from the same source fresh from the field was used for comparison. The results are found in table XIX.

The data show that the fresh soil produced slightly better inoculation than the same soil which had been dried and pulverized. No influence on crop yields was observed, however, and, while it seems desirable to use fresh soil whenever possible, no serious loss in efficiency of inoculation necessarily follows if the soil has been allowed to become dry before it is used.

#### SERIES XX—EFFICIENCY OF DIFFERENT AMOUNTS OF SOIL AND COMPOST SOIL ON THE DEGREE OF INOCULATION OF MANCHU SOYBEANS

This series was similar to Series XIII in the 1925 tests, except that two additional plots were added to test the efficiency of 8 pints of ordinary soil and 8 pints of compost soil on the inoculation of Manchu soybeans. The results of this experiment are given in table XX.

Altho the plants on the check plots in this series were well inoculated, the efficiency of the different soil and compost soil inoculations is plainly evident. A more definite idea of the relative efficiency of the various inoculations in this experiment is shown by the results given for the number of plants in Class 6 rather than by the figures for the percent of inoculated plants.

TABLE XIX. THE RELATIVE EFFICIENCY OF DRY PULVERIZED AND FRESH SOIL FOR THE INOCULATION OF MANCHU SOYBEANS

Plot No.	Treatment	Degree of inoculation								Yield per acre (tons)
		Number of plants in each class							Number plants inoculated	
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%	%	
31-32 31a	Check—no inoculation	23.1	18.3	22.3	19.3	9.0	6.3	1.7	76.9	2.221
33-34 33a	Soil—Dry pulverized	3.0	6.0	9.7	14.0	25.3	15.0	27.0	97.0	2.001
35-36 35a	Soil—Fresh from field	2.5	4.3	9.3	15.3	19.3	16.0	33.3	97.5	2.153

The compost soil was somewhat superior to the ordinary soil in every case, except one, but the differences were not very large, except where one pint of soil was used.

As in the earlier tests, there was a tendency for the degree of inoculation to be increased as the amount of soil used in the inoculum was increased. There were two exceptions where the differences were slight.

The yields were increased by the inoculations, but the differ-

TABLE XX. THE EFFECT OF USING DIFFERENT AMOUNTS OF SOIL AND OF COMPOST SOIL ON THE INOCULATION OF MANCHU SOYBEANS

Plot No.	Amount and kind of soil per bushel of soybeans	Degree of inoculation								Yield per acre (tons)
		Number of plants in each class							Number plants inoculated	
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%	%	
37-38 37a	Check—No inoculation	19.3	14.0	21.8	20.3	10.2	6.4	8.0	80.7	1.968
39-40 39a	Soil—½ pint	11.3	14.7	23.0	18.0	12.3	9.7	11.0	88.7	2.100
41-42 41a	Compost Soil—½ pint	5.8	11.3	15.3	21.0	17.3	10.3	19.0	94.2	2.058
43-44 43a	Soil—1 pint	14.4	11.0	10.7	17.3	22.3	11.3	13.0	85.6	2.165
45-46 45a	Compost soil—1 pint	1.6	9.0	15.0	15.7	15.7	12.3	30.7	98.4	1.222
47-48 47a	Soil—1½ pints	3.7	4.7	11.0	17.0	16.0	17.6	30.0	96.3	2.105
49-50 49a	Compost soil—1½ pints	9.3	6.7	17.3	19.0	18.3	8.7	20.7	90.7	2.500
51-52 51a	Soil—2 pints	0.2	8.0	16.0	16.7	20.7	12.7	25.7	99.8	2.102
53-54 53a	Compost soil—2 pints	2.0	4.3	10.7	17.7	16.3	11.3	37.7	98.0	2.127
55-56 55a	Soil—4 pints	2.7	4.7	7.7	8.3	16.0	15.3	45.3	97.3	2.021
57-58 57a	Compost soil—4 pints	0.7	5.0	8.0	17.0	11.0	12.3	46.0	99.3	2.385
59-60 59a	Soil—8 pints	0.3	4.3	6.3	16.0	15.7	9.7	47.7	99.7	2.005
61-62 61a	Compost soil—8 pints	0.3	1.0	4.7	12.3	13.0	13.7	55.0	99.7	2.330

TABLE XXI. THE EFFICIENCY OF DIFFERENT METHODS OF USING SOIL FOR THE INOCULATION OF MANCHU SOYBEANS

Plot No.	Treatment of soil for inoculation	Degree of inoculation								Yield per acre (tons)
		Number of plants in each class							Number plants inoculated	
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%	%	
63-64 63a	Check—No inoculation	14.9	14.5	18.5	20.5	14.2	7.7	8.7	84.1	2.135
65-66 65a	Compost soil—(Soil paste method)	0.7	1.7	7.3	7.0	10.0	11.3	62.0	99.3	2.204
67-68 67a	Compost soil—(Sugar solution method)	1.6	5.0	5.7	8.7	11.0	12.3	55.7	98.4	2.135
69-70 69a	Soil—(Soil paste method)	0.9	5.0	9.7	13.0	14.7	12.0	44.7	99.1	2.208
71-72 71a	Soil—(Sugar solution method)	3.0	3.0	8.0	18.0	14.3	12.7	41.0	97.0	2.216
73-74 73a	Soil—Broadcast 500 lbs. per A.	0.6	2.7	6.7	13.0	13.7	10.3	53.0	99.4	2.199

ences with the various methods of inoculation are too small to be considered definite.

#### SERIES XXI—EFFICIENCY OF DIFFERENT METHODS OF USING SOIL FOR THE INOCULATION OF MANCHU SOYBEANS

The plan of this series was the same as that of Series XII in the 1925 tests, except that the two soils were used at the rate of 3 pints per bushel of seed, and the efficiency of compost soil, when applied by the sugar solution method, was tested. The results are given in table XXI.

From the data it seems that the best results were secured by the soil paste method using the compost soil. The sugar solution method proved to be slightly inferior to the soil paste method in the case of both soils; the differences being most noticeable in the number of plants in the best nodulation class. The broadcast soil inoculation gave very similar results to those secured by the soil paste method. There were no definite effects on yields from the different methods of inoculation.

#### SERIES XXII—EFFECT OF DRYING INOCULATED SOYBEANS QUICKLY AND SLOWLY ON THE EFFICIENCY OF SOIL INOCULATION BY THE SOIL PASTE METHOD

This series was similar to Series XI in the 1925 tests, except that the soil was used at the rate of 3 pints instead of 1 pint per bushel of seed. The results are found in table XXII.

These results confirm those obtained in 1925 in showing that drying the inoculated seeds quickly gave slightly better results than drying them slowly or even very slowly, as is shown by the number of plants in class 6. The differences obtained, however, were not great and any one of the three methods of drying

the seeds should give satisfactory results. All the inoculations increased the yield of soybeans, but the different methods gave very similar results.

### CROSS INOCULATION STUDY OF SOIL AND PURE CULTURES

#### SERIES XXIII—EFFICIENCY OF INOCULATED SOIL FROM DIFFERENT SOURCES AND PURE CULTURES OF SOYBEAN BACTERIA ON THE INOCULATION OF FOUR VARIETIES OF SOYBEANS

The plan of this series was very similar to that of Series VII in the 1925 tests. Well inoculated soil, taken from four fields where Manchus, Dunfield, Midwest and Peking soybeans had been grown, respectively, was used on these same varieties of soybeans. The soil paste method of applying the soil to the seeds was followed, using 3 pints of soil per bushel of seed.

Cross inoculation tests were also made on the four varieties of soybeans, using three pure cultures of soybean bacteria which had been isolated from nodules of Manchus, Dunfield, and Midwest soybeans. All of these cultures had been tested previously for purity and ability to produce nodules on soybean plants. For this work these cultures were transferred to yeast-mannitol agar slants in eight-ounce prescription bottles. When ready to use, the contents of each bottle were thoroughly shaken with 1 pint of tap water and the necessary aliquot of this inoculum was mixed with the proper amount of seed for each plot, so that the total suspension in the bottle would be used to inoculate 1 bushel of seed. All inoculated seeds were planted the same day the inoculations were made.

The average results obtained on three plots for each treatment and six check plots are presented in table XXIII.

All of the check plots, except in the case of the Peking soy-

TABLE XXII. THE EFFECT OF DRYING INOCULATED SOYBEANS QUICKLY AND SLOWLY ON THE EFFICIENCY OF SOIL INOCULATION

Plot No.	Treatment of seed	Degree of inoculation								Yield per acre (tons)
		Number of plants in each class							Number plants inoculated	
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%	%	
75-76 75a	Check—No inoculation	10.0	10.0	20.0	23.7	14.0	8.7	13.3	90.0	2.089
77-78 77a	Seed dried quickly	5.0	2.7	2.3	7.0	14.0	7.0	62.0	95.0	2.281
79-80 79a	Seed dried slowly	0.0	3.7	3.7	7.7	11.7	13.0	59.6	99.4	2.301
81-82 81a	Seed dried very slowly	1.0	1.3	4.7	10.0	13.7	11.7	57.6	99.0	2.365



TABLE XXIII. THE EFFICIENCY OF INOCULATED SOIL FROM DIFFERENT SOURCES AND PURE CULTURES ON THE INOCULATION OF DIFFERENT VARIETIES OF SOYBEANS

Plot No.	Source of soil and culture used for inoculation	Manchu soybeans									Yield per acre (tons)
		Degree of inoculation									
		Number plants in each class								Number plants inoculated	
		0	1	2	3	4	5	6	7		
		%	%	%	%	%	%	%	%		
83-84 83a, b	Manchu soil	3.3	4.6	9.3	16.6	15.3	17.3	33.6	96.7	1.889	
85-86 85a, b, 86a, b	Manchu culture	5.0	9.9	9.4	16.9	14.6	10.6	33.6	95.0	1.996	
87-88 87a, b, 88a, b	Dunfield soil	3.1	5.6	12.1	19.6	18.6	12.0	29.0	96.9	2.033	
89-90 89a, b, 90a, b	Dunfield culture	3.8	5.0	9.0	18.6	16.3	15.0	32.3	96.2	2.065	
91-92 91a, b, 92a, b	Midwest soil	2.3	7.6	15.0	18.6	22.3	9.6	24.6	97.7	2.086	
93-94 93a, b, 94a, b	Midwest culture	7.5	8.0	12.3	22.3	18.6	7.0	24.3	92.5	2.035	
95-96 95a, b, 96a, b	Peking soil	3.6	6.6	16.0	16.0	12.6	10.6	34.6	96.4	2.121	
97-98 97a, b, 98a, b	Check	27.1	10.3	19.8	19.8	8.3	3.1	2.6	72.9	1.905*	

### Dunfield soybeans

[illegible]

TABLE XXIII. (Continued)

Plot No.	Source of soil and culture use for inoculation	Degree of inoculation							Number plants inoculated	Yield per acre (tons)
		Number of plants in each class								
		0	1	2	3	4	5	6		
		%	%	%	%	%	%	%		
Peking soybeans										
83-84 83a, b, 84a, b	Manchu soil	73.9	20.3	4.3	1.2	0.3	0.0	0.0	26.1	2.170
85-86 85a, b, 86a, b	Manchu culture	59.0	22.9	7.9	6.2	4.0	0.0	0.0	41.0	2.323
87-88 87a, b, 88a, b	Dunfield soil	71.8	11.3	9.0	5.6	2.0	0.0	0.3	28.2	2.469
89-90 89a, b, 90a, b	Dunfield culture	34.6	10.6	13.6	13.6	10.3	7.0	10.3	65.4	2.335
91-92 91a, b, 92a, b	Midwest soil	80.5	9.0	5.3	3.3	1.0	0.6	0.3	19.5	2.173
93-94 93a, b, 94a, b	Midwest culture	52.8	18.0	17.0	7.6	2.6	2.0	0.0	47.2	2.277
95-96 95a, b, 96a, b	Peking soil	16.2	18.0	14.6	19.6	18.0	7.0	6.6	83.8	2.363
97-98 97a, b, 98a, b	Check	96.7	2.8	0.5	0.0	0.0	0.0	0.0	3.3	1.928*
Midwest soybeans										
83-84 83a, b, 84a, b	Manchu soil	1.4	9.6	19.9	13.3	14.6	8.6	32.6	98.6	1.815
85-86 85a, b, 86a, b	Manchu culture	2.1	14.9	13.6	18.9	15.3	7.6	27.6	97.9	1.822
87-88 87a, b, 88a, b	Dunfield soil	2.1	14.0	16.0	17.6	13.3	12.0	25.0	97.9	1.752
89-90 89a, b, 90a, b	Dunfield culture	1.5	8.0	14.0	16.6	16.0	7.6	36.3	98.5	1.892
91-92 91a, b, 92a, b	Midwest soil	2.2	4.0	10.3	14.3	14.3	11.3	43.6	97.8	1.796
93-94 93a, b, 94a, b	Midwest culture	5.1	6.6	17.0	14.0	17.7	11.0	28.6	94.9	2.115
95-96 95a, b, 96a, b	Peking soil	2.5	6.0	12.0	12.3	13.0	7.6	46.6	97.5	1.730
97-98 97a, b, 98a, b	Check	24.5	19.0	22.6	14.8	10.1	4.5	4.5	75.5	1.903*

\*Average 6 plots.

beans, were inoculated. However, only relatively few plants were well inoculated and the majority were in the poorest nodulation classes. The results for the different inoculations, however, are quite definite.

The Manchu soil, Dunfield soil, Peking soil, and the Manchu and Dunfield cultures all produced about the same degree of inoculation on Manchu soybeans. Since one-third of the total number of plants on these plots was in the best nodulation class, all of these inoculations certainly produced most satisfactory results. The Midwest soil and the Midwest culture gave about the same results, but they were not quite as efficient as the other inoculation treatments.

The Peking soil produced the best inoculation on Dunfield soybeans, but the Manchu soil and the Manchu culture were only slightly less effective. The other inoculations were much less satisfactory, the Midwest soil being the poorest.

The most efficient inoculation of the Peking soybeans was brought about by the Peking soil. The other treatments were all much less effective, the Midwest soil again being the poorest.

With the Midwest soybeans all of the inoculation treatments produced excellent results; the Peking soil and the Midwest soil giving a somewhat more efficient inoculation than the other treatments.

Inoculation of the Peking soybeans resulted in increased yields of soybean hay in every case, but the inoculation of the other varieties did not always give increased yields. In some cases increases were secured while in others, no effects were noted.

As has been stated earlier, these results indicate that different varieties of soybeans vary considerably in their susceptibility to the most successful inoculation by soils from different sources. Pure cultures isolated from different varieties of soybeans also show marked differences in their relative efficiency to produce nodulation on different varieties of soybeans.

### *TESTS OF COMMERCIAL CULTURES AND SOIL*

#### **SERIES XXIV—A TEST OF THE RELATIVE EFFICIENCY OF DIFFERENT COMMERCIAL CULTURES AND SOIL INOCULATION FOR INOCULATING MANCHU, DUNFIELD, MIDWEST AND PEKING SOYBEANS**

This series was similar in plan to that of Series XV in 1925, altho the test in 1926 was much more extensive. Four varieties of soybeans were used and all inoculation treatments were made in duplicate; the plots receiving the same treatments being placed as far apart in the field as possible.

The experiment involved the testing of 10 different brands

of cultures manufactured by 9 commercial companies. One culture was secured direct from the factory and wherever possible one or two more cultures of the same brand were purchased on the open market. Three cultures from each of six companies, two cultures from two companies and two brands from one company were used in the test.

One culture prepared in the Soil Bacteriology Laboratory of the Iowa Agricultural Experiment Station was also included twice in the test, seeds being inoculated as usual and dried as quickly as possible or allowed to soak in the bacterial suspension overnight and dried the next morning. This culture consisted of three strains of soybean bacteria which were isolated from Manchu, Dunfield and Midwest nodules. The individual strains had been used separately in Series XXIII in the 1926 tests.

In addition to the 10 commercial cultures and the 1 station culture, the following soil inoculations were tested:

1. Composted soil (Soil paste method using 3 pints of soil per bushel of seed)
2. Inoculated soil (Soil paste method using 3 pints of soil per bushel of seed)
3. Inoculated soil (Broadcast at the rate of 300 lbs. per A.)
4. Inoculated soil (Broadcast at the rate of 500 lbs. per A.)
5. Inoculated soil (Broadcast at the rate of 1,000 lbs. per A.)

In making the inoculations the directions accompanying each culture were carefully followed, care being exercised that the proper amount of inoculum would be used on the quantity of seed needed for each plot. The Station culture was prepared in 8-ounce prescription bottles and after shaking the contents of each bottle with 1 pint of clean tap water an aliquot was used.

All of the seeds for the inoculated plots were treated with the cultures and soil and planted the same day. The average results are given in table XXIV.

The check plots of the Manchu, Dunfield and Midwest soybeans were so well inoculated in all cases that there is little evidence of value from the various inoculations.

Examination of the data obtained on the Manchu soybeans, shows that 12 of the 26 commercial cultures produced nodulation on more than 80.0 percent of the plants. Six plots which had been inoculated with commercial cultures were not examined for nodules. Eight commercial cultures produced very efficient nodulation, over 90.0 percent of the plants being inoculated. As a general rule, the cultures which produced the highest percentage of inoculated plants also had the largest number of plants in the best nodulation classes. These data show that the efficiency of the different cultures varied greatly. Some were very poor while others gave excellent inoculation









TABLE XXIV (Continued).

PEKING SOYBEANS

99-100	Com'l. culture 1 (Factory)	92.5	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5	1.782
101-102	Com'l. culture 1 (Dealer 1)	97.5	2.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.228
103-104	Com'l. culture 1 (Dealer 2)	97.0	2.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	1.991
105-106	Com'l. culture 2 (Factory)	19.5	20.5	12.0	13.0	11.0	8.5	15.5	80.5	15.5	80.5	30.5	2.105
107-108	Com'l. culture 2 (Dealer 1)	81.5	10.0	7.0	7.0	0.5	0.0	0.5	0.0	0.0	0.0	18.5	2.235
109-110	Com'l. culture 3 (Factory)	86.0	10.0	2.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	14.0	1.795
111-112	Com'l. culture 3 (Dealer 1)	90.0	8.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	2.014
113-114	Com'l. culture 3 (Dealer 2)	91.0	7.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.0	2.278
115-116	Com'l. culture 4 (Factory)	95.0	4.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	5.0	2.003
117-118	Com'l. culture 4 (Dealer 1)	91.0	8.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.0	1.943
119-120	Com'l. culture 4 (Dealer 2)	92.0	7.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	2.190
121-122	Com'l. culture 5 (Factory)	81.5	6.5	5.5	4.0	2.0	0.5	2.0	0.5	0.0	0.5	18.5	1.953
123-124	Com'l. culture 5 (Dealer 1)	99.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	2.598
125-126	Com'l. culture 6 (Factory)	73.0	9.0	9.5	5.0	2.5	1.0	0.0	1.0	0.0	0.0	27.0	2.126
127-128	Com'l. culture 6 (Dealer 1)	64.5	11.5	15.5	6.0	2.0	0.0	2.0	0.0	0.5	35.5	35.5	2.192
129-130	Com'l. culture 6 (Dealer 2)	73.0	10.5	9.0	7.5	0.0	0.0	0.0	0.0	0.0	0.0	27.0	2.273
131-132	Com'l. culture 7 (Factory)	94.0	3.5	1.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	6.0	2.030
133-134	Com'l. culture 7 (Dealer 1)	97.0	1.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	1.998
135-136	Com'l. culture 8 (Factory)	83.5	14.5	1.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0	16.5	2.169
137-138	Com'l. culture 8 (Dealer 1)	87.5	9.0	3.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	12.5	2.178
139-140	Com'l. culture 8 (Dealer 2)	94.0	5.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	6.0	2.269
141-142	Com'l. culture 9 (Factory)	91.5	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.5	1.890
143-144	Com'l. culture 9 (Dealer 1)	94.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	2.226
145-146	Com'l. culture 9 (Dealer 2)	95.0	3.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	2.090
147-148	Com'l. culture 10 (Factory)	10.5	14.0	16.5	18.5	19.0	8.5	13.0	89.5	5.0	13.0	89.5	2.418
149-150	Com'l. culture 10 (Dealer 1)	46.0	19.5	15.0	10.0	6.0	2.5	2.5	2.5	1.0	54.0	2.273	
151-152	Expt. Station culture 11	61.0	16.5	15.0	7.0	0.0	0.5	0.0	0.0	0.0	0.0	39.0	2.193
153-154	Expt. Station culture 11												
	Seeds soaked overnight												
155-156	Compost soil (4 pts. per bu. seed)	82.5	13.0	3.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	17.5	2.366
157-158	Soil (4 pts. per bu. seed)	51.5	17.0	14.0	13.0	3.0	0.0	0.0	1.5	48.5	1.5	48.5	1.755
159-160	Soil broadcast (300 lbs. per acre)	57.0	8.0	2.5	2.0	0.5	0.0	0.5	0.0	0.0	0.0	13.0	2.402
161-162	Soil broadcast (500 lbs. per acre)	55.5	20.0	12.0	9.5	2.5	0.5	0.5	0.0	0.0	0.0	44.5	1.970
163-164	Soil broadcast (1000 lbs. per acre)	62.0	16.0	10.0	9.0	1.5	1.5	0.0	0.0	0.0	0.0	38.0	2.103
165-166	Check (Seed not inoculated)	48.0	37.0	9.0	5.5	0.5	0.5	0.0	0.0	0.0	0.0	52.0	2.268
		90.8	5.9	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.2	1.991

and compared favorably with the results obtained from the Station culture and the various soil inoculations.

When the Station culture was applied to the seeds in the usual way, excellent results were obtained. But, when the soybeans were soaked in the bacterial suspension overnight, poorer nodulation occurred. All of the soil inoculations produced efficient inoculation and the compost soil gave the most efficient inoculation of all treatments.

The majority of commercial cultures were not very effective in producing nodules on Dunfield soybeans, only nine producing nodules on 75.0 percent or more of these plants. Some cultures, efficient for Manchu soybeans, were almost without effect on Dunfield beans.

The Station culture, too, was not nearly so effective on Dunfield soybeans as on Manchu. Soaking the Dunfield soybean seeds in the bacterial suspension overnight again proved to be harmful to the efficiency of this culture.

Of various soil inoculations the broadcast soil at the rate of 300 pounds per acre was the most effective, the larger soil application proving less efficient. The compost soil did not show up as well as the 300 pounds of soil broadcast.

The results indicate that the Dunfield soybeans are not so easy to inoculate as the Manchu. There is also evidence to support the belief that different varieties of soybeans require different strains of bacteria for the best inoculation.

With the Midwest soybeans, 21 out of the 26 commercial cultures produced nodulation on more than 75.0 percent of the plants. In the majority of cases, those cultures which were very efficient in producing inoculation on Manchu soybeans were also very efficient for Midwest soybeans. But more of the cultures were effective on Midwest beans than on the Manchu beans. Apparently, therefore, the Midwest soybeans are easier to inoculate.

The Station culture produced about the same degree of nodulation on Midwest soybeans as it did on Dunfield soybeans but was not so efficient on the Manchu beans. With the Midwest soybeans, soaking them overnight again lowered the efficiency of the inoculation by the Station culture.

All the soil inoculations produced very efficient nodulation on Midwest soybeans. The 300 pound broadcast application was just as efficient as the 1,000 pound treatment, and the compost soil was no more efficient than the ordinary inoculated soil.

Only six of the commercial cultures produced nodulation on more than 20.0 percent of the Peking soybean plants, but two of these were very efficient. Evidently Peking soybeans are by far the most difficult to inoculate of the four varieties tested.

The Station culture did not do so well on the Peking soybeans

TABLE XXV. EFFECT OF COMMERCIAL CULTURES ON NITROGEN CONTENT OF MANCHU SOYBEAN PLANTS SHOWING VARYING DEGREES OF NODULATION

Plot No.	Inoculation treatment	Percent of nitrogen				
		Class number				
		0	1	2	3	4
		%	%	%	%	%
37- 38	Check	1.05	.....	.....	.....	.....
85- 86	Com'l. culture No. 1 (Factory)	0.94	1.12	1.39	1.95*	.....
89- 90	Com'l. culture No. 2 (Factory)	1.10*	1.27	1.65	1.95*	2.18
97- 98	Com'l. culture No. 4 (Factory)	1.00	.....	1.23	.....	.....
X	Check	1.08	.....	.....	.....	.....
101-102	Com'l. culture No. 5 (Factory)	1.20*	1.06	1.51	1.70	.....
107-108	Com'l. culture No. 7 (Factory)	1.04*	1.11	1.83	1.63	1.95
109-110	Com'l. culture No. 8 (Factory)	1.08	.....	1.23	.....	.....
X	Check	1.12*	.....	.....	.....	.....
91- 92	Com'l. culture No. 2 (Dealer)	1.12	1.28	1.40	1.66**	.....
99-100	Com'l. culture No. 4 (Dealer)	1.30	1.29	1.56	.....	1.88*
X	Check	1.28	1.24	.....	.....	.....
103-104	Com'l. culture No. 5 (Dealer)	1.34*	1.46	.....	.....	.....
	Average all plots	1.12	1.20	1.47	1.78	2.00

\*Five plants used for analysis. \*\*Four plants used for analysis.

either, altho only three commercial cultures surpassed it in inoculating efficiency. Soaking of the Peking soybeans proved harmful to the soybean bacteria in the Station culture.

The 1,000 pound broadcast soil application gave more efficient nodulation on the Peking soybeans than any of the other soil inoculations, altho the compost soil and the 300 pound broadcast soil application were nearly as good.

The results of the tests of the different cultures show no marked superiority of the factory culture over the same brand of culture purchased from the dealer. There was, however, a great variation in the efficiency of the several cultures manufactured by the same manufacturer. Good cultures give just as good inoculation as the best soil methods and sometimes better.

The variations in yields among the different treatments were not great. In many cases the inoculations gave small increases, but this was not always the case. The results, as a whole, cannot be considered definite.

#### NITROGEN CONTENT OF SOYBEANS HAVING DIFFERENT DEGREES OF NODULATION

In order to study the effect of the different inoculation treatments on the nitrogen content of soybeans and also to note any correlation between the nitrogen content of the plants and the degree of nodulation, nitrogen determinations were made on a number of samples of soybean plants grown in the 1925 tests. The samples were ground and analyzed by the modified Gunning method.

In table XXV data are presented showing the analyses of samples of Manchu soybean plants taken from plots which had been inoculated with different commercial cultures. The whole

TABLE XXVI. SHOWING WEIGHT AND NITROGEN CONTENT OF MANCHU SOYBEANS, TOPS AND ROOTS, IN FIVE CLASSES OF NODULATION

Class No.	Manchu soybeans					
	Tops		Roots		Tops and roots	
	Weight (gms.)	Nitrogen (percent)	Weight (gms.)	Nitrogen (percent)	Weight (gms.)	Nitrogen (percent)
0	34.50	1.243	5.00	0.656	39.50	1.168
1	28.37	1.370	4.50	0.790	32.87	1.288
2	31.75	1.533	4.87	1.288	36.62	1.500
3	30.37	1.798	5.37	1.587	35.74	1.765
4	36.00	1.916	6.83	1.943	42.83	1.920

plant was analyzed, and the data represent the average of duplicate determinations on 10 plants, except where otherwise noted.

The soybeans in class 0 from the check plots showed slight variations in percent of nitrogen, which indicate differences in amounts of available nitrogen in this soil. The analyses of the uninoculated plants from the plots treated with commercial cultures agreed closely with the analyses of the check plants. While considerable variation was noted for the analyses of the plants in classes 2, 3 and 4 from the different plots, the average results from all plots showed a gradual but steady increase in percent of nitrogen from class 0 to class 4. The data indicate that as the intensity of inoculation increased the percent of nitrogen in the plants also increased. This increase amounted to about 0.3 percent per class for the three best nodulation classes. The plants in class 4 were found to contain 2.0 percent nitrogen, which was not quite double the percent of nitrogen found in the uninoculated plants.

The data given in table XXVI show the weight and nitrogen content of Manchu soybeans, tops and roots, in five classes of nodulation. These data represent the average results of analyses of plants from four plots which had been inoculated with different commercial cultures. Ten plants from each class are represented in the weight data.

The tops of the uninoculated plants weighed more than the tops of the plants in classes 1, 2 and 3. The same is true for the roots, except in class 3. The tops and roots of the 10 plants in class 4 weighed a little more than the uninoculated plants.

TABLE XXVII. SHOWING WEIGHT AND NITROGEN CONTENT OF DUNFIELD SOYBEANS, TOPS AND ROOTS, IN FIVE CLASSES OF NODULATION

Class No.	Dunfield soybeans					
	Tops		Roots		Tops and roots	
	Weight (gms.)	Nitrogen (percent)	Weight (gms.)	Nitrogen (percent)	Weight (gms.)	Nitrogen (percent)
0	71.25	1.430	8.25	0.677	79.50	1.344
1	67.50	1.445	7.50	0.665	75.00	1.366
2	77.00	1.442	8.25	0.837	85.25	1.398
3	88.50	1.610	11.50	1.035	100.00	1.543
4	77.00	1.660	10.00	1.045	87.00	1.590

The data given in table XXVI show that the percent of nitrogen in the tops of these plants increased with increasing intensity of inoculation; the difference between the uninoculated plants and those of class 4 amounting to 0.67 percent. The percentage of nitrogen in the roots also increased as the degree of nodulation was increased. This was to be expected because of the presence of the nodules on the roots. With the roots, the differences in the percent of nitrogen among the different classes were much greater than with the tops. The soil on which these plants were grown showed a total nitrogen content ranging from 2,500 to 3,000 pounds per acre.

The data showing the weight and nitrogen content of Dunfield soybeans, both tops and roots, in five classes of nodulation are found in table XXVII. These plants were taken from two plots, inoculated with different commercial cultures. They were grown on soil showing an average nitrogen content of 3,800 pounds of nitrogen per acre.

From the weights recorded for 10 plants in each class it may be noted that inoculation increased the weight of tops over the checks in all classes, except one. Rather decided gains in weights of roots were also noted for the plants in classes 3 and 4. Had these results been calculated on an acre basis they would have shown very distinct increases in yields of soybean hay and plant roots in favor of the two best nodulation classes.

The percent of nitrogen in the tops and roots was gradually increased with increasing degree of nodulation. However, the

TABLE XXVIII. EFFECT OF SOIL INOCULATION, LIME AND SUPERPHOSPHATE ON WEIGHT AND NITROGEN CONTENT OF MANCHU SOYBEANS

Class No.	Seed inoculated with soil					
	Tops		Roots		Tops and roots	
	Weight (gms.)	Nitrogen (percent)	Weight (gms.)	Nitrogen (percent)	Weight (gms.)	Nitrogen (percent)
0	34.00	1.350	5.50	0.622	39.50	1.248
1	30.25	1.392	4.50	0.777	34.75	1.322
2	33.25	1.542	6.25	1.227	39.50	1.496
3	40.25	1.740	7.25	1.475	47.50	1.695
4	52.00	1.697	9.75	1.627	61.75	1.686
Seed inoculated with soil + lime (Hydrated) 500 lbs. per A.						
0	38.50	1.705	5.00	0.792	43.50	1.598
1	22.50	1.892	3.50	0.942	26.00	1.594
2	33.25	1.810	6.25	1.357	39.50	1.749
3	41.75	2.080	7.00	1.720	48.75	2.025
4	57.00	2.002	10.00	1.375	67.00	1.951
Seed inoculated with soil + lime + superphosphate 200 lbs. per A.						
0	38.00	1.615	5.00	0.610	43.00	1.512
1	39.25	1.712	5.25	0.797	44.50	1.604
2	43.25	1.867	5.75	1.232	49.00	1.815
3	48.75	2.025	7.75	1.630	56.50	1.972
4	71.00	2.157	13.50	1.790	84.50	2.098

difference in the percent of nitrogen from class to class was not nearly so large as it was with the Manchu soybeans. It is believed that the difference in total nitrogen in the soils is sufficiently great to account for this variation.

In table XXVIII are given the results of analyses made on samples of Manchu soybeans taken from three of the plots in Series VIII in the 1925 tests. One plot was untreated, one received 500 pounds of hydrated lime per acre and the other was treated with lime and 200 pounds of superphosphate per acre. All were seeded with inoculated soybeans.

In all cases inoculation increased the weights of the tops and roots of the soybean plants in classes 3 and 4. Lime had a tendency to increase the weight of tops and roots and the lime and superphosphate showed a still further increase. Usually, the weight of tops and roots increased with increasing degree of nodulation. With the soybean roots on the limed plots the weight of the best inoculated plant roots was exactly double that of the uninoculated roots. On the lime and superphosphate treated plots the weight of the best inoculated plant tops was nearly double that of the uninoculated plant tops, and the weight of the roots from the same plants was more than double, showing a very decided advantage due to the inoculation.

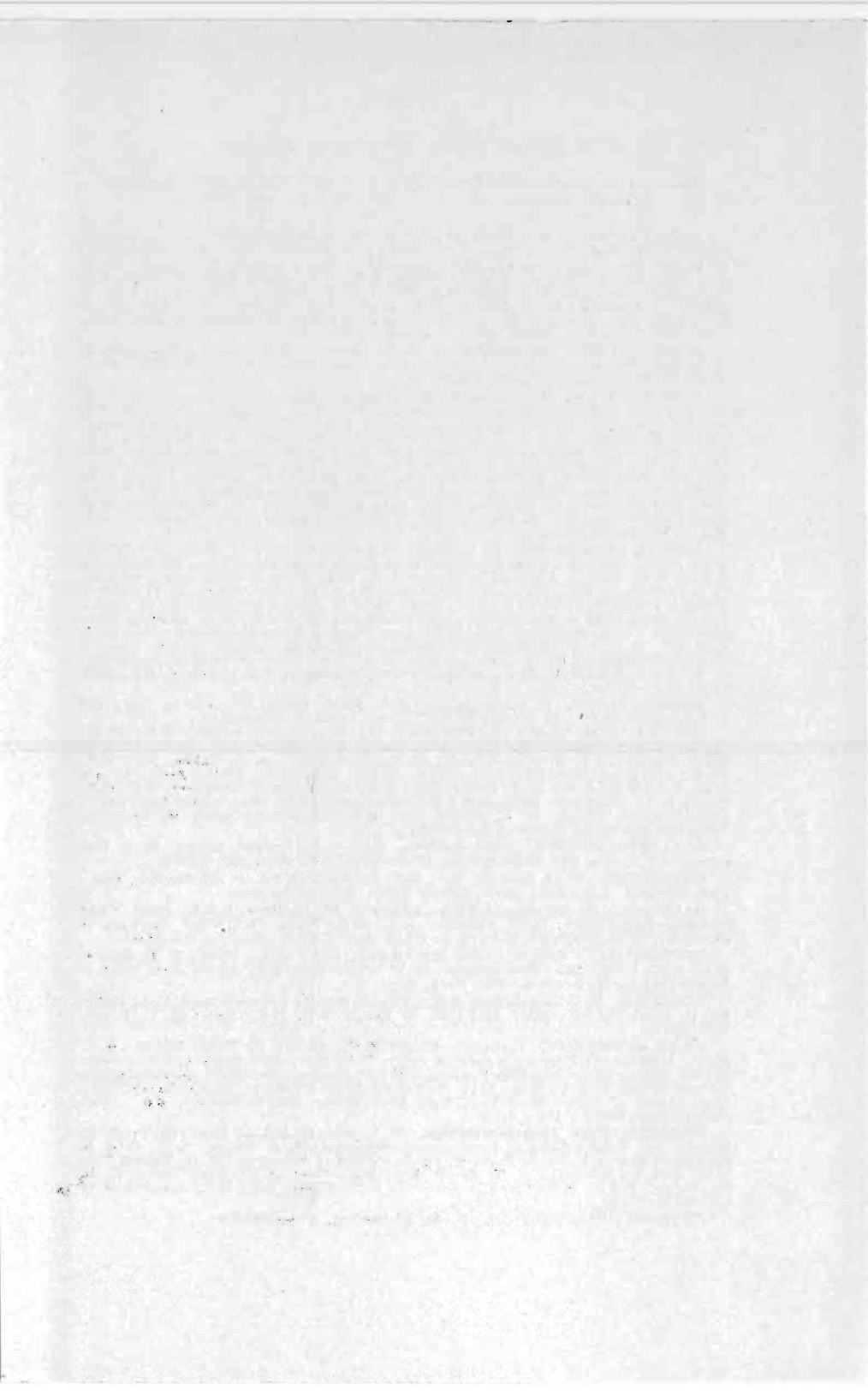
These data indicate that the percent of nitrogen in both the tops and roots of soybean plants increased as the degree of inoculation increased. Lime increased the percent of nitrogen in both the tops and roots of these soybean plants, but superphosphate had no effect.

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