# The Relation of Varieties of the Soybean to Various Strains of the Rhizobia.

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## INTRODUCTION.

Variation in nodule formation among different varieties of soybeans (Glycine max (L) Merr) has been reported from different parts of the United States. In many cases a certain variety has failed to show inoculation when treated with a culture of known origin, while among different varieties growing side by side one has been inoculated and the other has remained free or practically free of nodules.

Leonard (16) concluded that a culture of bacteria isolated from a single variety of soybeans will form nodules on other varieties. In his experiments the organisms were isolated from the medium yellow variety of soybean.

The ability to form nodules varies among the different kinds of soybeans. Apparently certain varieties are more easily inoculated than others. It is also true that the time of ripening of one variety may influence the infection and subsequent formation of nodules. For instance, an early and late variety may show differences in inoculation as a result of variation in plant food at different times of the year. Repeated investigations have proved that the formation of nodules is influenced to a considerable degree by the reaction and salt concentration of the soil water as well as by other factors.

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Kirchner (15) called attention to the fact that, of about 100 species of legumes growing in the botanical garden at Hohenheim, only the soybean failed to develop nodules. He believed that this was due to environmental conditions. Later he obtained some soil from Japan which contained the organism for soybean, and in some pots and field experiments with two varieties of soybeans proved definitely that soybean bacteria were different from other legume bacteria and that the soybean would produce nodules by using soil from a field in which soybeans bearing nodules had been grown. Kirchner was probably the first investigator to use soil for the inoculation of soybeans.

In 1903 Hiltner and Strömer (13) 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. 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) 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.

Moore (18) stated that out of a total of 129 reports received from the soybean cultures sent out by the U. S. Department of Agriculture, 43 per cent of the tests were failure; more failures being reported for soybeans than for any other crop.

Lipman (17), 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.

Fellers (8) found that many of the commercial cultures tested failed to give satisfactory results with soybeans. He recommended the soil transfer method for this crop, except when the commercial cultures were known to be of good quality.

Later, Feller (9) compared the relative efficiency of commercial cultures and nodule infusion for the inoculation of soybeans and reported that the cultures gave just as good results as the nodule infusion. Kendal (14), 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 when compared with uninoculated plants.

In 1924, Fiske (11) reported the official test on 33 samples of legume inoculants. Five were either fair or poor, while all the rest produced good inoculation.

Hiltner and Störmer (13) described the two methods, proposed by Nobbe and Hiltner in 1896, for using nitragin. In one method, called soil inoculation, the culture was mixed with a quantity of soil which was then spread over and worked into the 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 average 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 nodulation almost four times. 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 (21), 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 scantly and rather inferior. Where the soil was used the two varieties were similarly nodulated. In a later pot culture experiment with four other varieties of soybeans no differences were noted between the soil inoculation method and the soil extract of inoculation method. 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 nodulation. 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 nodulation occurred.

Garman and Didlake (12) found that soybean seeds soaked overnight in a liquid suspension of the soybean bacteria were 100 per cent inoculated, while shorter periods of soaking were less effective. In another experiment in which the seeds were soaked and dried over-night and planted the following day, 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 (10) 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 (20) 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 nodulation. Applying a commercial culture at double the rate gave an average of 75 per cent of nodulated plants against 20 per cent for no commercial inoculation.

Arny and McGinnis (3) found that, where water alone or 5, 20, or 30 per cent 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 hour, did not affect the inoculation.

Nightingale (19) studied the soil, water and milk methods of applying the bacteria to the seed and found that the soil method gave the best results, although there was little difference between the soil and water methods.

Perkins found that 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 (1) 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, regardlesss 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.

Voorhees (24) inoculated several varieties of soybean with cultures of Nitrogerm and Farmogerm and found considerable difference in the nodulation obtained on the different varieties. He concluded that different and definite powers of resistance to association with the symbiotic bacteria were measurable.

Leonard (16) 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 found that pure cultures of organisms from Mammoth Yellow, Medium Green, Manch 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 obtained to justify the conclusion that the nodule bacteria are specific.

The accumulated data indicate that the ability to form nodules varies among the different kinds of soybeans. Apparently certain varieties are more easily inoculated than others. It is also true that the time of ripening of one variety may influence the infection and subsequent formation of nodules. For instance, an early and late variety may show differences in inoculation as a result of variation in plant food at different times of the year. Repeated investigations have proved that the formation of nodules is influenced to a considerable degree by the reaction and salt concentraction of the soil water as well as by other factors.

## Experimental.

### *Isolation of Cultures.*

The seeds of the soybean employed were sterilized in a solution of calcium hypochlorite (Wilson 26). The length of time which the seeds were treated varied with the different species of seed and with the treatment which was given to the seed to permit them to absorb water. In general, a solution of about ten per cent of calcium hypochlorite was allowed to stand on the seed from fifteen to thirty minutes. The seeds were then washed with sterile water and placed in Erlenmyer flasks which contained sterile sand where they were allowed to dry before the inoculation was added. The inoculation was added in the solution which was used to wet the sand. The solution contained 0.05% $KH_2PO_4$ and 0.05% sucrose. Twenty cc. of solution were added for each hundred grams of sand.

Within three to five weeks after planting, a well defined contrast was noticeable in size and color of the inoculated plants. The uninoculated plants were smaller and at the end of four to five weeks decidedly yellow. At this stage the plants were removed from the flasks and examined for nodules. For reisolation of the organism the nodules were immersed in a solution of calcium hypochlorite, in which the odor of chlorine was barely detectable, for three to five minutes, depending on the size of the nodule. The nodules were then washed in sterile water, and placed in a petri dish containing a few drops of sterile water. The nodules were crushed with a sterile spatula. Loop dilutions were made from the macerated nodule to three other petri dishes which also contained a few drops of sterile distilled water. Approximately ten cc. of Ashby's (2) medium was then added to each petri dish.

The composition of Ashby's medium is :—

| | | |
|---|---|---|
| Manitol ($C_6H_8(OH)_6$) ... ... ... | 10.0 | gr. |
| Monopotassium phosphate ($KH_2PO_4$) ... | 0.2 | " |
| Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) ... | 0.2 | " |
| Sodium chloride ($NaCl$) ... ... ... | 0.2 | " |
| Calcium sulphate ($CaSO_4 \cdot 2H_2O$) ... ... | 0.1 | " |
| Calcium carbonate ($CaCO_3$) ... ... | 5.0 | " |
| Distilled water ... ... ... | 1,000 | cc. |

After incubating for one week at 25°C., transfers were made to slopes of the same medium. As soon as sufficient growth occurred, the culture were stained with saturated alcoholic gentian violet. The rhizobium remains unstained in most instances when water is not present. The back-ground is stained a deep gentian violet. All cultures which did not show this typical staining characteristic were discarded. Those which appeared to be pure cultures of Rhizobium when examined microscopically were checked for nodulation on the original host plant, as well as the plant from which they were last isolated. Those failing to symbiose with the plant were discarded.

In each series of plantings adequated controls were grown. In no case were nodules found on those plants which were not inoculated.

## Presentation of Data.

*Symbiosis Between Various Strains of the Rhizobia and Soybean Plants.*

In making this test of symbiosis between various strains of the rhizobia and soybeans, the varieties were obtained from the Department of Genetics, Cornell University. They were described very largely by serial numbers. These numbers together with the names of certain varieties are listed in Table 1.

The (+) signs in the squares in the table indicate that the plants and the bacteria were symbiosing as evidenced by the appearance of nodules. The (–) signs indicate no nodules were found on the roots of the plants.

## TABLE 1.

### Symbiosis Between Various Strains of the Rhizobia and Soybean Plants.

| Varieties of Soybean | Strains of the Rhizobia | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Albizzia julibrissin | Amphicarpa monoica | Apios tuberosa | Baptisia australis | Dalea alopecuroides | Glycine max | Lespedeza striata | Phaseolus vulgaris | Vigna sinensis |
| 53933 | — | + | — | — | — | + | — | — | — |
| 54608 | — | — | — | — | — | ++ | — | — | + |
| 65341 | — | — | — | — | — | ++ | — | — | + |
| 79602 | + | + | — | — | — | ++ | — | — | + |
| 79620 | — | — | — | — | — | + | — | — | + |
| 79746 | + | — | — | — | — | ++ | — | — | + |
| 79773 | — | + | — | — | — | ++ | — | — | — |
| 85340 | — | — | — | — | — | + | — | — | + |
| 86023 | — | — | — | — | — | ++ | — | — | + |
| 88803 | — | — | — | — | — | — | — | — | — |
| 89154 | — | — | — | — | — | ++ | — | — | + |
| 90566 | + | — | — | — | — | + | — | — | + |
| 90579 | — | — | — | — | — | + | — | — | — |
| 92468 | + | — | — | — | — | ++ | — | — | + |
| 92634 | — | + | — | — | — | ++ | — | — | + |
| 92637 | + | — | — | — | — | ++ | — | — | + |

## TABLE 1.

SYMBIOSIS BETWEEN VARIOUS STRAINS OF THE RHIZOBIA AND SOYBEAN PLANTS.

| Varieties of Soybean | Strains of the Rhizobia | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Albizzia julibrissin | Amphicarpa monoica | Apios tuberosa | Baptisia australis | Dalea alopecuroides | Glycine max | Lespedeza striata | Phaseolus vulgaris | Vigna sinensis |
| 65344 | + | — | — | — | — | ++ | — | — | + |
| 92640 | — | — | — | — | — | + | — | — | + |
| 92681 | + | — | — | — | — | + | — | — | — |
| 92686 | — | — | — | — | — | ++ | — | — | + |
| 92716 | — | — | — | — | — | + | — | — | + |
| 92719 | — | — | — | — | — | ++ | — | — | + |
| 92791 | + | — | — | — | — | ++ | — | — | + |
| 92868 | — | — | — | — | — | + | — | — | — |
| 96194 | — | — | — | — | — | ++ | — | — | + |
| 96200 | — | — | — | — | — | + | — | — | + |
| Bansei | + | + | — | — | — | ++ | — | — | + |
| Cayuga | + | + | — | — | — | ++ | — | — | + |
| Delicious | — | — | — | — | — | — | — | — | + |
| Dunfield | + | — | — | — | — | ++ | — | — | + |
| Early Michigan | + | + | — | — | — | ++ | — | — | + |
| Black Eyebrow | — | — | — | — | — | + | — | — | — |

## TABLE 1.

SYMBIOSIS BETWEEN VARIOUS STRAINS OF THE RHIZOBIA AND SOYBEAN PLANTS.

| Varieties of Soybean * | Strains of the Rhizobia | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Albizzia julibrissin | Amphicarpa monoica | Apios tuberosa | Baptisia australis | Dalea alopecuroides | Glycine max | Lespedeza striata | Phaseolus vulgaris | Vigna sinensis |
| Hakkaido | + | + | — | — | — | ++ | — | — | — |
| Hiro | — | — | — | — | — | + | — | — | + |
| Kura | — | — | — | — | — | ++ | — | — | + |
| Manchu | + | + | — | — | — | ++ | — | — | + |
| Mandell | — | — | — | — | — | ++ | — | — | + |
| Minsoy | — | — | — | — | — | + | — | — | + |
| Osoya | — | — | — | — | — | ++ | — | — | + |
| Soja graciles | — | — | — | — | — | — | — | — | — |
| Shiro | — | — | — | — | — | ++ | — | — | + |
| Suru | + | — | — | — | — | + | — | — | + |
| Waseda | — | + | — | — | — | ++ | — | — | + |
| Wilson | — | — | — | — | — | ++ | — | — | + |

*++ = 50% or more of plant nodulated.
\+ = Less than 50% of plant nodulated.
— = No nodules.

## Discussion.

Most of the early investigators were of the opinion that any strain of Rhizobium would symbiose with all leguminous plants, but that a given strain of bacteria may be better adapted to a species of leguminous plants than some other strain. Breall ( 5 ) reported nodulation of pea, lupine, and alfalfa with the bacteria isolated from alfalfa in 1888. Ward in 1889 reported cross-inoculation between peas and broad bean. In 1890-91 Laurent reported that he had been able to produce nodules on peas with thirty-six strains of Rhizobium isolated from as many plant species.

Prazomowski (22) was the first to show that a definite species of legumes will not symbiose with all strains of the rhizobia. He failed to obtain symbioses with lupine and Rhizobium isolated from pea nodules. Nobbe, Schmid, Hiltner, and Hotter confirmed and extended the work of Prazmowski to include organisms isolated from Pisum, Robinia, Cytisus, and soil in which Gledissia had grown. None of these organisms symbiosed with either the pea or bean bacteria, but not with lupine or Robinia bacteria.

Later investigators came to the conclusion that the specificity between bacteria and plant was definite enough to consider the strains of bacteria which symbiose with a particular plant or group of plants as a species of the root nodule bacteria. Fred, Baldwin, and McCoy published a review of the literature in which the various groups and species are discussed. They consider the early work which does not agree with this grouping as being of little value, due to faulty technique. Wilson (25), Raju (23) and others, using improved technique, have shown that the bacteria which are adapted to one host plant may symbiose with other plants, provided conditions are suitable. Wilson's work indicated that nearly all plants will symbiose with several strains of bacteria, although the percentage of plants infected may be very low. If a plant is grown in a soil which is devoid of bacteria adapted to that plant, it may symbiose with bacteria which are not well adapted to the plant.

Allen and Baldwin (2) have shown that bacteria from red clover which are not affective become more effective with each passage through the plant. Therefore, we may expect a flora which is adapted to a plant to develop, although the 'strains present when the seeds are planted may not be well adapted to that plant.

Burrill and Hansen (6) suggested that the ability of a strain of Rhizobium to symbiose with a certain host plant may be due to the enzymes or other differences in the root sap which cannot be determined by chemical methods. If this is true, it might be expected that growing the bacteria in the extract of a plant would cause them to become adapted to that plant in much the same way as when the bacteria are grown in the nodules of that plant. Burke and Hohl (7) obtained negative results with the extract of alfalfa and the bacteria from vetch and red clover. Their extract was obtained, however, by adding distilled water to the tissue and grinding in a mortar. Dilution might cause the effect of enzymes of the plant to be less intense than that in the nodules.

In considering the adaptation of a strain of Rhizobium to a legume, the variations in individual plants must be overlooked. The characters of the individual plant which permits symbiosis may be slightly different from the other individuals of the same species. This difference may be great enough to permit a poorly adapted strain to symbiose with one plant which all of the others growing under the same conditions may produce no nodules. During this symbiotude the characters of the bacteria are changed to such an extent that the bacteria will symbiose with a larger per cent of the plants on the next exposure.

On consulting the table in connection with symbiosis between various strains of the Rhizobia and soybean plants, it may be seen that the strain of Rhizobium from Glycine max seem more adapted to Glycine, Vigna, Albizzia and Amphicarpa in the order named.

## CONCLUSIONS.

The conclusions drawn from the data presented in this paper may be summarized as follows:—

1. The plants with which a strain of Rhizobium symbioses vary according to the plants with which it has previously symbiosed.

2. Some cultures seemed to be more efficient in inoculating one variety than the others, but no single culture produced the best inoculation on all forty-four varities tested.

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

4. 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.

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