FIELD NITROGEN FIXED BY NODULE BACTERIA AND SOME SYMBIOTIC RELATIONSHIPS EXHIBITED BY TWO SOYBEAN SELECTIONS

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ABSTRACT.—Two sister selections of soybeans under varying soil nitrogen levels fixed amounts of nitrogen comparable to values reported by other workers using other measurement techniques. Preliminary observations are reported on differences shown by the symbionts under laboratory conditions.

A number of methods have been used to determine the amount of nitrogen fixed by legumes in the symbiotic process under field conditions. Many early workers compared the nitrogen levels of nodulated and non-nodulated legumes as a criterion of fixation. (Virtanen and Linkola, 1947) compared the nitrogen content of plants nodulated with effective strains with those nodulated with ineffective strains. Using peas they found that under field conditions, more than 88 lbs. N/A was fixed annually. The generalization most widely used to describe the contribution of nodule bacteria to the leguminous crop has been that of (Hopkins, 1910). It is generally stated that ⅓ of the nitrogen in the crop comes from the soil and the remaining ⅔ from the fixation of atmospheric nitrogen.

Other workers have attacked this problem using the N_{15} isotope to measure the nitrogen made available to the plant through the fixation process when supplied with varying amounts of soil or fertilizer nitrogen. (Norman and Krampitz, 1946) using the N_{15} technique with soybeans reported that the fixation process at any level of supplied nitrogen was not completely halted nor was all of the inorganic nitrogen recovered in the crop. The presence of fertilizer nitrogen largely resulted in a dimunition of the amount of atmospheric nitrogen fixed in the symbiotic process. (Thornton, 1947) using tracer techniques with inoculated lespedeza and soybean plants found a reverse relationship between the fixation of atmospheric nitrogen and the amount of inorganic nitrogen made available to the plant. From these experiments an estimate was made that in prairie soils of average nitrogen content not more than 30% of the nitrogen in the plant is air derived.

A more extensive experiment utilizing 7 leguminous and 4 non-leguminous plants was conducted by (Allos and Bartholomew, 1955). Nitrogen was added as ammonium sulfate and was enriched with the N_{15} isotope to the extent of 1.65 atom percent. Nitrogen fixation decreased while fertilizer nitrogen uptake increased as the quantity of available nitrogen to the plants was increased. As in previous observations no total inhibition of fixation or total absorption of the fertilizer nitrogen was achieved. Nitrogen fixation was largest in the soybean and peanut plants which were also the plants with the greatest total growth. In the case of the soybean plant they state that "When nitrogen was applied in quantities equal to or in excess of that absorbed by the soybean crop, fixation from the atmosphere did not constitute more than 30 to 40% of the total absorbed. When nitrogen was applied equal to 10 to 15% of that absorbed by the crop, fixation contributed from 80 to 90% of the total absorbed."

Williams and Lynch (1954) reported in 1953 two strains of soybeans that varied only in their ability to form nodules. One of these is abundantly nodulated, while the other strain bears no nodules under field conditions. Genetic studies indicate that the inability to nodulate was due to a single recessive gene. Sears and Lynch (1951) have made some preliminary reports on these varieties when studied in the field. It was found that given adequate sources of nitrogen and other nutrients, the two sister strains gave comparable yields of soybeans in the field. Some

of these data have been reported elsewhere (Sears and Lynch, 1951) and are given here for comparison purposes with other techniques used for the measurement of nitrogen fixed by nodule bacteria under field conditions.

Methods and Materials

The two sister strains varying only in the ability to produce nodules were used to determine the contribution of nodule bacteria to the soybean crop and to determine what percentage of the nitrogen in nodulated soybeans is obtained from the air. The soybean strain that bears no nodules is designated as the low protein selection, while the nodulated strain is designated as the high protein selection.

The two sister strains were inoculated with the same strain of nodule bacteria and seeded in plots that differed only in their nitrogen content. Other nutrients were supplied in adequate amounts. The soil treatments used are given in Table I.

Treatment I was used to demonstrate that the two soybean selections had equal yielding abilities. The straw in treatment V was used to reduce the soil nitrogen supply available to the soybean plants. Some of the beans and plant parts were harvested for chemical analyses while others were harvested for yield comparison purposes.

Laboratory studies of the nutritional requirements of these microorganisms were carried out on various media to which vitamins or casein were added. Cultural characteristics such as pigment production, rate of growth, slime production, and colonial characteristics were recorded.

Results and Discussion

The seed yields and nitrogen contents of the entire plants, including roots are given in Table 2. It can be seen that the greatest benefit from the nodule bacteria occurs on soils that have low nitrogen levels. In fact the use of nitrogen fertilizers actually decreases the nitrogen available from nodulation. It also suggests that the soybean plant can attain maximum yields by nodulation alone if other nutrients are not limiting. These results are in conformity with those of (Thornton, 1947) and (Allos and Bartholomew, 1955) who report an inverse relationship between the amount of nitrogen available to the plant and the amount fixed in the symbiotic process. If one uses the estimate that 1.5 pounds of soil nitrogen is sufficient to produce a bushel of corn and if the corn plant can obtain amounts of nitrogen from the soil equivalent to that obtained by the soybean plant then the following conclusions can be reached. On land having a nitrogen fertility level sufficient to produce a 40 bushel corn crop per acre nearly two-thirds of the soybean nitrogen requirement will be secured from the air. On land of 100 bushel per acre status, only about one-fifth of the nitrogen in the nodulated soybean crop will be from the atmosphere. These results also coincide with the results reported by (Allos and Bartholomew, 1955) in that the soybean plant gave little or no growth response to nitrogen fertilizer treatments.

It should be pointed out that under field conditions the low protein selection was never observed to be nodulated. However, in sand and vermiculite a few inefficient nodules were found on the low protein selection when grown in the

Table 1.—Soil Treatment Practices.

Treatment	Previous Treatment	Additional Treatment
I.....	Birdsfoot trefoil green manure crop.....	NH_4NO_3 300 lb. per acre
II.....	Same as Treatment I.....	none
III.....	Grass sod fertilized with $(NH_4)_2SO_4$.....	none
IV.....	Grass sod—no nitrogen fertilizer.....	none
V.....	Same as Treatment IV.....	5-ton oat straw mulch

TABLE 2.—Yields and Nitrogen Content of Soybeans on Soils of Different Nitrogen Fertility Levels.

Treatment	Soybean Selection		Total N		N from Air
	High Protein	Low Protein	High Protein	Low Protein	
	bu./A	bu./A	lbs./A	lbs./A	%
I	38.6	38.3	.....	.....	.....
II	36.2	33.0	.....	.....	.....
III	36.6	33.1	199	158	21
IV	36.0	29.2	192	102	47
V	35.5	20.9	177	58	63

greenhouse. A study of those few strains of soybean nodule bacteria that would effect some inefficient nodulation, as against the many strains that would not, yielded some general differences in cultural and biochemical characteristics of these *Rhizobia*. The few nodulating strains produced a thin slimy growth with a distinct pigment, they grew at a faster rate, elaborated indole-3-acetic acid from casein and had fewer vitamin requirements (especially B vitamins) than did the non-infective strains. These differences and others are now being studied to ascertain the physiological bases for the nodulation of leguminous plants.

This experiment was unique in that two sister strains of soybeans varying in nodulating ability were used to determine the contribution made by nodule bacteria to a crop under field conditions and varying levels of soil nitrogen. With very inadequate supplies of soil nitrogen as much as 63% of the nitrogen in the crop comes from the air by bacterial fixation. Under high nitrogen levels only about 21% is so obtained (Sears and Lynch, 1951).

In this era of increasing populations the use of efficient nodule bacteria in many areas of the world should be of considerable economic importance.

ACKNOWLEDGMENT

A part of this work was done in conjunction with O. H. Sears, Dep't. of Agronomy, University of Illinois.

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Manuscript received July 3, 1967.