EFFECTS OF ADDED NITROGEN, PHOSPHOROUS, AND POTASSIUM ON NODULATION AND YIELD OF CLARK 63 SOYBEAN

by

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This thesis is affectionately dedicated to

the memory of my departed parents

Nathaniel Adeyemo and Emily Omodebuola Rotimi

INTRODUCTION

In comparison with other crops the soybean is less sensitive to soil physical conditions, and is adapted to a wide textural range from sandy to clayey soils. Even organic soils are quite suitable to the production of the crop. The crop is grown very successfully on soils commonly used for corn (maize). Growth is limited, however, on humus-deficient clays, but good crops are possible even on claypan soils.

The soybean plant tolerates moderately free to slightly impeded drainage quite well but poorly drained soils are less suitable. Excess moisture does not kill the plant, but promotes profuse vegetative growth at the expense of seed production. Dry, overdrained soils with steep slopes are unsuitable, although the soybean can withstand drought quite well. Abundant available soil moisture throughout the growing season is most favorable.

Soybeans can tolerate considerable soil acidity and will grow on soils that are somewhat too acid for alfalfa and red clover. However, excessive acidity limits production considerably. The crop is grown over a soil pH range from 4.5 to 7.5, but from about pH 6.0 to 7.0 seems to be optimum. Highly acid soils must certainly be limed for best results, but it may be preferable to add the lime for the previous crop. The soybean plant is very sensitive to high salt content in the soil.

The nutrient requirements of soybeans are high by comparison with many other crops. In general, phosphorus and potassium are top priorities, but calcium too is highly important. Although it is a legume, the soybean, nevertheless, often responds to nitrogen application, more especially in the earlier stages of growth. It is highly important that the appropriate strain of nitrogen-fixing bacteria (*Rhizobium*) is present in the soil to supply the bulk of the nitrogen needed by the crop, and the inoculation of the seed with a suitable culture may be necessary on land not previously planted to soybeans. So far there is little evidence of micronutrient element problems associated with soybeans, but some cases of iron deficiency have been reported. On poor sandy soils, symptoms of magnesium deficiency have also been reported.

The soybean is a native of the Orient and has proved a valuable addition to American crop plants. Since its introduction into the United States it has spread over much of the territory east of the Mississippi river, becoming well established in the crop schemes of this area. The plant itself is high in nutritive value, the seeds especially so, making it particularly valuable as a livestock feed. The crop also has high commercial value, the seed furnishing oil and other products useful in industry. The soybean has now become the "Wonder Crop" in this country.

Although the soybean is still not the number one crop in Kansas, the acreage has increased more than 20-fold since 1940. The increased interest in the crop is due largely to the constant need for protein feed on the farm and the high cost of protein concentrates. Further increase in the acreage of soybeans is justified in the eastern one-fourth of Kansas.

Soil fertility, among many other environmental factors, stands out as an important factor affecting the production and establishment of soybeans in eastern Kansas. The influence of applied fertilizers (N, P, K), as expressed in seed yields, was the nucleus of the research project which was conducted at Kansas State University Agronomy Research Farm in Manhattan.

REVIEW OF LITERATURE

Nodulation and Nitrogen Fixation

The speed with which nodules are formed and become active in nitrogen fixation on young plants is impressive. Bergersen (4) observed the first nodules on Lincoln soybeans 9 days after planting and nitrogen fixation began about 2 weeks later. McAlister et al. (38) reported that even inoculated soybean plants may lack sufficient nitrogen after seed reserves are largely mobilized--about 2 weeks after planting. In the study of Bergersen (4) the seedlings became increasingly yellow until about 15 days after the start of nodulation, when they "became green overnight." There is lack of agreement as to how long active nitrogen fixation continues. Using winter-grown plants which flowered in 2 to 3 weeks after germination, Bergersen (4) found the total plant nitrogen increased at essentially a constant rate from about 2 weeks after the first nodules appeared until about 4 weeks later, when nodule decay started. By 58 days after planting, or 7 weeks after the first nodule was formed, nitrogen fixation has ceased. Summer-grown plants produced successive "crops" of nodules at intervals of about 14 days.

Magee et al. (37) observed nitrogen fixation by excised nodules "increased roughly with increasing size of the nodules from young, actively growing plants" but declined as the growth rate slowed with blooming. Bond (5), on the other hand, reported the highest daily rate of nitrogen fixation per plant during the period of pod set and development. Total weight of nodules increased throughout the vegetative life of the plant, and just before maturity totaled 2.5 grams per eight plants in pot culture. Ebertova (11) reported new pink active nodules originated continuously throughout the vegetative life of the plant, and in agreement with Bond (5), she found nitrogen fixation continuing until the seeds were nearly ripe. She may not have sampled frequently enough to detect the cyclic production of "crops" of nodules observed by Bergersen (4).

There is clearly a requirement for substantial amounts of nitrogen during the period of pod and seed development as reported by Hammond et al. (16), and Togari et al. (50), but Lathwell et al. (30) stated that soybeans grown in nutrient solution fail to accumulate enough nitrogen up to mid-bloom to suffice for subsequent growth.

Weiss (52) attributed the differences in ability of soybean lines to nodulate to differences in genetic constitution. Williams and Lynch (54) have found a nonnodulation response that is determined by a single gene pair. Clark (9) reported failure to nodulate is not due to absence of bacteria from nonnodulating plants. He was unable to transfer the ability to nodulate by approach-grafts below the first trifoliate leaf when plants were 3 to 4 weeks old. Tanner and Anderson (49) grafted root tips from genetic nonnodulators to roots of normal plants when the roots were 1 cm. long. The resulting root systems were partly nodulating and partly nonnodulating. In every case the nodulation pattern was determined by the genotype of the root segments. The importance of soil factors in nodulation was also indicated by Clark's work. He found bacterial strains which could produce nodules on the "nonnodulating" line in sand culture, although not in any of three soils used. Whether these nodules fixed nitrogen was not reported.

Sironval et al. (47) indicated that nodule development is influenced by processes in the leaf. More and heavier, as well as more effective, nodules were produced under long days than short. Sironval (47) stated that nodule formation appears to be parallel and related to chlorophyll formation. Bach et al. (3) showed the dependence of nitrogen fixation on photosynthesis, or in the case of sliced nodules, an external supply of sugar, and Kamata (29) showed that nodulation is promoted by foliar application of sugars.

Johnson et al. (28); Erdman et al. (12), reported that some strains of Rhizobium induces chlorosis, the severity depending on the soybean variety, the Rhizobium strain-variety combination, and environmental conditions. Johnson and Means (26) asserted that vigorous nitrogen fixation is required for the development of chlorosis. Although the association of chlorosis-inducing power and effectiveness in nitrogen fixation has not been established, Johnson and Means have used the chlorosis-inducing strains to obtain valuable information about other properties of the nodule system. Means et al. (39) showed that with rare exceptions, a single nodule contained only one bacterial strain. Johnson and Means (27) also reported that Rhizobium strains exhibit various degrees of competitiveness and maintain their relative rank under various competitive conditions.

Hawkes (18) obtained maximum yields with Lincoln variety of soybeans only when the plants were well nodulated, even though high nitrogen levels were supplied to uninoculated plots. Maximum yields could not be obtained from nodulation alone, however, added nitrogen being required. Nodulation seemed to facilitate phosphorus accumulation, in contrast to the decrease in phosphorus that resulted from an increase external nitrogen supply. Chesnin and Haghiri (8), on the other hand, found no yield differences between nodulating and nonnodulating lines in nutrient solutions. These workers asserted that nodulation reduced the potassium percentage in the roots but did not affect calcium, magnesium, and phosphorus levels. Lynch and

Sears (35) found no effect of inoculation on yields on eight soil experimental fields, although other treatments such as manure and lime gave yield increases.

Influence of Fertilizers on Nodulation

The proof of the influence of some nutrient elements on the effective nodulation of legumes and particularly soybeans have been reported by a number of workers. Wilson (55) found chlorides and phosphates stimulate nodule production. Sulfates had a harmful effect, as did the use of ammonia. Perkins (44) found phosphates were not essential to nodule formation. Scanton found that crop residues, manure, and potash did not materially affect nodulation.

Several investigators also reported some very interesting data concerning the use of lime. Nearly all reported increases in both yield of soybeans and increases in nodule production. Several of these investigators found that small applications of lime gave just as good results as large applications. Feller (13) using soil with a three ton limestone requirement found the application of lime in some cases almost doubled yield. Small quantities of lime were found to be practically as efficient as larger amounts. A tremendous excess of lime decreased yield. Perkins (44) found that lime materially increased nodule formation. Calcium application decreases soil acidity, which hinders the development of Rhizobium.

Wilson (55) reported phosphorous application in pot cultures with soil increased nodule production. The number of nodules on 30-day-old soybean plants was doubled by application of 962 pp2m monosodium- or monopotassium-phosphate. Heltz and Whiting (19) found a beneficial effect of phosphorous on nodule production in field experiments. Superphosphate at a rate of 150 pounds per acre and placed one inch above and to the side of the seed increased the number of nodules per plant from 37.8 to 49.6 on Miami sandy loam.

The results reported from Germany form a contrast to the modest and sometimes inconsistent responses to phosphorus obtained in the United States. Being aware of American results with calcium, this element was applied in most German trials while the phosphorus application was varied. Ludecke (33) applied phosphosphorus as $CaHPO_4$ at the rates of 11, 33.4, 66.6 and 111 pp2m (parts per two million) and K_2SO_4 at three rates of application in pot cultures with quartz sand. The 10-fold range of phosphorus application resulted in a 10-fold increase in number of nodules and a 15-fold increase in their weight after 120 days. Maximum nodulation was not reached. It was further found that the nitrogen content of the nodules increased from 4.06 percent to 4.8 percent due to phosphorus application. The amount of nitrogen assimilated per gram of nodules was increased from 51 to 155 milligrams. This represents a 3-fold increase in activity of nitrogen fixation due to phosphorus application and a 45-fold increase in total amount of nitrogen fixed per plant. Ludecke et al. (33) also reported similar trends in other experiments. Poscherieder et al. (45) also found a distinct increase in the percent nitrogen due to phosphorus application.

Yields as Affected by Mineral Nutrient Application

Excellent correlation between crop yields and the application of proper nutrients to the soil have been shown by Smith et al. (48). Sears (46) estimated that 88, 26, and 16 pounds of nitrogen were added per acre when the crop was used as green manure, hay with manure returned to the soil, or seed with straw left in the soil. When seed or straw was removed, or the crop without manure being returned to the soil, losses of 3 and 30 pounds respectively of nitrogen per acre occurred. Sears' estimations were based on a crop of 20 bushels of seed, or 4,500 pounds of hay per acre. Approximately two-thirds of the nitrogen requirements of the plant were assumed to be met from the atmosphere.

Norman and Dranpitz (43) found the probable range of nitrogen fixation on average prairie soils to be in the order of 20 to 35 percent of the total used by the plant.

Soybeans were found to have heavy demands on both macro- and micronutrient elements. Sears showed that a crop of soybeans removed somewhat more phosphorus and magnesium and about three times more potassium and seven times more calcium than crops of corn, oats or wheat with comparable yields. Norman (43) also reported that the nitrogen required by soybeans was somewhat in excess of what was needed by comparable yields of corn. But, soybeans had the faculty of entering into a symbiotic relationship with *Rhizobium japonicum*, to supplement its supply of nitrogen.

Morgan, as quoted by Jackson (25) reported a minimum pH of 5.0 was necessary for growth of soybeans and the desirable range was from pH 5.2 to 7.0. According to Albrecht (2), soybeans require more calcium than any other element for nodulation, and as such its supply was the principal factor for obtaining the appropriate pH. At low levels of calcium saturation, Albrecht found the phosphorus in the seed was lost to the substrate and there was no nitrogen fixation during a six week period. When 0.2 m. e. calcium was supplied for each plant, all phosphorus was retained and there was a good growth of the plants with satisfactory nodulation. Lipman and Blair (31), Albrecht and Davis (2), Moser (41), Horner (20), and others have also emphasized the importance of calcium for the nodulation and nitrogen balance of the soybean plants.

According to some workers, a high calcium content has usually been accompanied by a low potassium content and vice versa in soybeans. Loehwing (32) reported the additions of calcium may cause starvation of potassium on soils low in the element.

Fred et al. (15) reported a nitrogen hunger period in young soybean plants after cotyledonary nitrogen was exhausted and before the nodules supplied an adequate quantity. Either by shading the plants or by addition of combined nitrogen, fixation of nitrogen by Rhizobium was initiated. It appears that under conditions of low available soil nitrogen, nitrogen fixation from nodulation can furnish nitrogen for soybean yields of at least 60 bushels per acre with fixed nitrogen amounting to as much as approximately 160 pounds per acre. On soils high in available nitrogen, the amount of nitrogen supplied by the fixation process appears to be as low as 10 pounds per acre.

Application of nitrogen tends to retard nodulation of seed-inoculated soybeans in Rhizobia-free soil. Abel (1) reported that applications of 100 pounds of nitrogen at planting time in the Imperial Valley of California, where Rhizobia were not present in the soil, resulted in poorly nodulated soybeans which produced yields of 6 to 16 bushels per acre with 27 to 34 percent protein on a dry matter basis. Omitting nitrogen application at planting time permitted good nodulation and resulted in yields of 35 to 40 bushels per acre with 40-45 percent protein content of the seed.

Hardy (17) reported an extensive trial in Arkansas in which nitrogen was applied in a factorial experiment with phosphorous and potassium showed no significant response from nitrogen applied at different stages of plant development. These results are typical of the many trials showing no appreciable benefit from nitrogen fertilizer on well-nodulated soybeans.

Weber (51) measured soybean nodulation and what it means in terms of seed production and nitrogen. To do this, two soybean lines that are identical, except one forms nodules on its roots and the other does not, were used. Several pairs of isolines with differing maturities for evaluating nodulation under varying conditions were developed. In the development of the nodulated (nod) and nonnodulated (nonnod) soybean isolines, Weber first showed that the nod and nonnod performed alike when the nonnod was given sufficient nitrogen.

At Ames, Weber (51) compared nod and nonnod soybeans with varying rates of applied nitrogen on each and with the following: (a) with and without drought conditions; (b) ample moisture and with nitrogen partially immobilized with 20 tons of corncobs per acre. When cellulose such as corncobs is applied, normally available soil nitrogen is partially immobilized ("tied up").

Soybeans with drought yielded about half those without drought. Adequate moisture not only increased seed yield but more than double the percentage of nitrogen fixed by symbiosis. The nod line produced about the same yield with or without applied fertilizer nitrogen. Added fertilizer nitrogen greatly decreased the amount of nitrogen fixed because increasing nitrogen in the soil decreased nodulation. The nonnod line increased in yield with added fertilizer nitrogen. This line got all its nitrogen from the soil. These results suggest that, under good growth conditions encountered, fixed nitrogen in nodulated soybeans may approach 40 percent of the total nitrogen produced.

Weber (51), from the results obtained at Ames, summarized that well nodulated soybeans do not need nitrogen fertilization. Nodulation and nitrogen-fixation are decreased by high soil nitrogen.

Howell (22) reported finding soybean varieties which differ in their responses to high levels of phosphorous. Some varieties tolerate high phosphorous, whereas others do not (21). The difference was first observed with nutrient solutions in the greenhouse (23), where the development of toxicity symptoms by sensitive varieties can be easily and reversibly controlled by manipulation of the nutrient solution. Fletcher et al. (14) also observed the differences in the soil in the greenhouse and in the field, although the effects of high phosphorous in the soil are less severe and less persistent than in nutrient solutions. The reaction of sensitive varieties to high phosphorous is called "phosphorous toxicity," a term which tends to obscure the participation of other nutrient elements in the physiological system leading to differences in varietal response. Nitrogen proved to be the most effective element in alleviating the symptoms of phosphorous toxicity.

MacTaggart (36) reported lime and phosphorous together increased the weight and total nitrogen content of soybeans more than lime alone. Heltz and Whiting (19) claimed rock phosphate stimulated nodule production. Wilson (55) found a correlation between liming of soils and the phosphorous content of the soybeans raised on them. Hutching (24) suggested a relatively close relationship between calcium, phosphorous and nitrogen in the soybean production. With adequate calcium or the appropriate pH level, applications of phosphorous resulted in greater concentration of phosphorous in the plant tissues. In the early stages of growth, however, phosphorous was not found to be a significant factor.

Approximately 16 pounds of phosphorous are present in 40 bushels of soybean seed. Welch (53) asserted North Carolina results indicated that a yield response was obtained from applied phosphorous when soil tests showed less than 40 pounds available phosphorous in the soil. Nelson (42), on a coastal plain soil in North Carolina, obtained 6.4 bushels per acre of soybeans on a soil low in available phosphorous, but 33.8 bushels with added phosphorous. Bray (7) drew response curves which show that with 10 pounds of available phosphorous per acre soybean yields will be 75 percent of the maximum expected, whereas with 30 pounds of available phosphorous, soybean yields will be at 98 percent of maximum. This response to phosphorous assumes other elements to be in adequate supply.

The ratio of phosphorous to potassium may be as important in some cases as the phosphorous level. Miller et al. (40), on a Dickinson fine sandy loam in Iowa with a pH of 6.6 and testing low in available phosphorous and very low in available potassium, showed that over 80 percent of the variation in soybean yield which they obtained was accounted for by the variation in phosphorous and potassium content of some plant parts. The greatest yield increase was obtained from heavy applications of both phosphorous and potassium, but the greatest yield depression resulted from heavy additions of phosphorous and no addition of potassium. The variety Harosoy used in the study is sensitive to high levels of phosphorous in relation to other elements in the nutrient solution.

In so far as the response to the potassium fertilization is concerned, Bower and Pierre (6) working on high lime soils claim soybeans are intermediate between corn and sorghum (very responsive to potash) on the one hand and sweetclover and buckwheat (not responsive to potash) on the other. Although soybeans drew heavily on calcium, their potassium requirement was also found to be high, resulting in an intermediate calcium-magnesium: potassium ratio.

Forty bushels of soybean seed contain approximately 50 pounds of potassium. North Carolina data suggest that response to applications of potassium is likely when the available soil potassium is less than 75 pounds per acre. Response curves drawn by Bray (7) suggest that when the soil tests show 50 pounds of potassium per acre, soybean yield will be approximately 50 percent of the maximum whereas with a soil test of 200 pounds, the soybean yield will be about 97 percent of maximum.

Rouse reported that on a Kalmia sandy loam soil in Alabama high in phosphorous but low in potassium, the 5-year average seed yield increase from 50 pounds of added potassium was 50 percent. Studies in North Carolina on a soil very low in potassium showed a four-fold yield increase from potassium. The addition of potassium caused greater retention of pods, increased the degree of pod filling, and improved seed quality. Nelson et al. (42) claimed the application of 120 pounds per acre of potassium increased oil content about 2 percent and reduced protein content 5 percent.

EXPERIMENTAL PROCEDURES

Greenhouse Rhizobium Strain Evaluation

Eleven different Rhizobium strains were tested for their effectiveness to inoculate and cause nodulation on Clark 63 soybean variety. Five strains: W.9, 5565, 5566, Nodogen humus, and Nodogen liquid were obtained from Nodogen Laboratories, Princeton, Illinois. Two strains: 61A65 and 61A69, were procured from the Nitragin Company, Incorporated of Milwaukee, Wisconsin. Four strains identified as 5A40, 5A41, 5A42, and 5A43 were obtained from Agricultural Laboratories, Incorporated of Columbus, Ohio. All Rhizobium strains are isolates from different varieties and from different areas and soil types. Each culture was either in humus, agar or liquid solution.

The bacteria growing on agar in test tubes were prepared in the following way to facilitate thorough inoculation. To make a good solution the organisms were transferred from the culture tubes into a quart jar and 5 ml of tap water added. The solution was thoroughly mixed and applied to sixty healthy seeds with intact seed-coats.

About 3 grams of humus inoculants was placed in a quart jar and mixed into a slurry with 5 ml of water. The seeds were mixed with the slurry.

Five ml of the liquid inoculant was mixed with 60 preselected seeds in a quart bottle.

Forty-eight 9-inch pots were filled with the soil collected from the site selected for field planting on the Agronomy farm. Ten seeds inoculated with each of the 11 inoculants were planted in each pot. Each treatment was replicated 4 times and completely randomized. The pots were placed on a bench in the greenhouse. Adequate moisture was supplied throughout the study so that the plants were at no time under moisture stress.

After 10 days of growth, the plants were thinned to 5 well spaced plants in each pot. The plants were allowed to grow for 24 more days. On the 34th day of growth, the roots of the five plants in each pot were removed, washed, and placed in labeled quart bottles containing 5 percent formaldehyde solution until nodule counts were made.

Field Experimental Design

The greenhouse work was conducted to enhance the selection of an inoculum to be used in treating the seeds for the field plantings. After putting the results of the greenhouse studies into statistical analysis, strain 5566 from Nodogen Company was selected and used in treating all seeds planted in the field experiment.

Inoculum solution was prepared by mixing the bacteria growing on agar with one pint of water in a quart jar. The seeds were then soaked in the culture solution a few minutes before planting in the field. Gum arabic was used as a sticking agent. All plots were planted on May 31, 1966 with a Columbia hand planter, and the seeds were spaced about one inch apart.

There were 18 treatments randomized in 4 replicates. The plots were in a 2 x 3 x 3 factorial design i.e. 2 levels of nitrogen, 3 of potassium, and 3 of phosphorous in all possible combinations. The nitrogen and potassium fertilizers were broadcast by hand while the phosphorous was banded a few inches from the seeds with a Columbia hand planter.

Plantings were made in 3 row plots, the rows were 36 inches apart and 21 feet long and harvested plot was 16 feet. The nitrogen was supplied as ammonium nitrate (33.5-0-0) and the potassium as muriate of potash (0-0-60).

These were broadcast 2 days ahead of planting. The phosphorous was supplied as triple superphosphate (0-46-0) which was banded 3 days after planting. The design of experiment and layout of the different plots are shown in Appendix I.

Weeds were removed from the plots by the use of a cultivator and also by hand hoeing.

Sampling Methods

When vegetative growth had ceased, 5 equally spaced plants were carefully dug, the roots severed and preserved in 5 percent formaldehyde solution for subsequent nodule counts.

Data on plant height, number of nodes, and pods were collected from each plot. Five well spaced plants were selected from each plot for the observations made on the above listed characteristics. These observations and samplings were made after the plants had ceased to grow vegetatively.

Lodging notes were taken on each plot and were scored from 1 to 5; 1 assigned to plots with all plants erect, and 5 to plots where most of the plants were down.

Harvest and Yield Determination

The rows to be harvested were selected and tagged with the proper label designation. The middle row was harvested in each plot. One 16 feet row in each plot was harvested separately with a hand mower, and threshed with a nursery thresher. The seed weight in grams per plot was determined and this was later converted into bushels per acre using the conversion factor of 0.0334 (Grams x 0.0334 = Bu/A).

Seeds from all 4 replications for each treatment were composited, and samples of at least 75 grams from each were taken and sent to U.S. Regional Soybean Laboratory in Urbana, Illinois for oil and protein analysis.

Size of seed was determined and expressed in grams per 100 seed. The seeds used for the determination were taken from the composited seeds from each treatment. Seed quality was rated from 1 to 5. The factors considered in estimating seed quality were: objectionable colors, damage, seed development, disease, wrinkled seed, and general appearance of the seeds.

RESULTS AND DISCUSSION

Greenhouse Nodulation Study

The eleven strains of *Rhizobium japonicum* were evaluated in the greenhouse study for their relative ability to inoculate and cause nodule formation on the roots of Clark 63 soybean (*Glycine max*). The results are shown in Table 1.

The preliminary study was prompted by the well-established concept of strain variation and host specificity which exist in Rhizobium: Legume symbiotic relationship. Analysis of results in Table 1 shows four strains produced significantly more nodules at 5 percent level than strain 5A41 (agar) which had the lowest nodulation of all the strains tested. Strains 5566 (agar), 61A69 (humus), 61A65 (humus), and 5A43 (agar) produced 5 or more nodules per plant over strain 5A41. Strains 5566 from Nodogen, and 61A65 from Nitragin produced significantly higher number of nodules than the control. The peat-base inoculants as a group produced 1.3 more nodules per plant than inoculants on agar, and 0.9 more nodules per plant than the liquid inoculant. This slight increase in nodulation might be due to better

Table 1. Comparative ability of 11 strains of Rhizobium japonicum to inoculate and cause nodulation on soybean variety Clark 63.

Rhizobial strain		No. of plants	Nodule counts		
			Total/4 Reps.	Ave./Rep.	Mean
W9	(Agar)	20	603	150.8	30.2
5565	(Agar)	20	582	145.5	29.1
5566	(Agar)	19	643	160.1	33.8**
Nodogen	(Humus)	17	482	120.5	28.4
Nodogen	(Liquid)	20	599	149.8	30.0
61A69	(Humus)	19	551	137.8	31.5*
61A65	(Humus)	15	494	123.5	32.9**
5A40	(Agar)	16	431	107.8	26.9
5A41	(Agar)	20	509	127.3	25.5
5A42	(Agar)	20	601	150.0	30.1
5A43	(Agar)	20	619	154.8	31.0*
Not inoculated		20	468	117.0	23.40

L.S.D. (.05) Between strains = 5.32 Nodules/plant.

L.S.D. (.05) Control vs. strains = 8.55 Nodules/plant.

*Significant differences between strains.

**Significant increase over control at 5% level.

and longer retention of bacteria on the seeds after planting by the humus carrier. A photograph of a nodulating and a non-nodulating soybean is shown in Plate I.

Strain 5566 was selected for the inoculation of seeds planted in the field fertility study. This strain was selected because it produced the highest number of nodules (33.8 nodules per plant), and it produced large effective nodules on the soybean roots (Plate III). Effectiveness of Rhizobial strain is evidenced by the bright pigment present in the nodules (Plate II).

The greenhouse strain evaluation study shows that strains of R. japonicum differ in their effectiveness to inoculate and nodulate the roots of the same soybean variety. The control plants had nodules formed mostly on the lateral roots while roots of inoculated plants had clusters around the tap root. The soil used in the study probably had residual bacteria present and this could account for the relatively high number of nodules on the non-inoculated plants. The non-inoculated plants produced 23.4 nodules per plant and all inoculants produced more nodules than the control, but not all show a significant increase when analysis of variance was computed as shown in Table 1. All the inoculant treatments are compared with the control graphically in Fig. 1.

Field Fertility Experiment

The field experiment, conducted at the Agronomy Research Farm at Manhattan, Kansas, was designed to study the influence of no nitrogen and 50 pounds of nitrogen per acre with 3 levels of P_2O_5 (0, 40, 120 pounds per acre), and 3 levels of K_2O (0, 50, 100 pounds per acre) on nodulation, yield

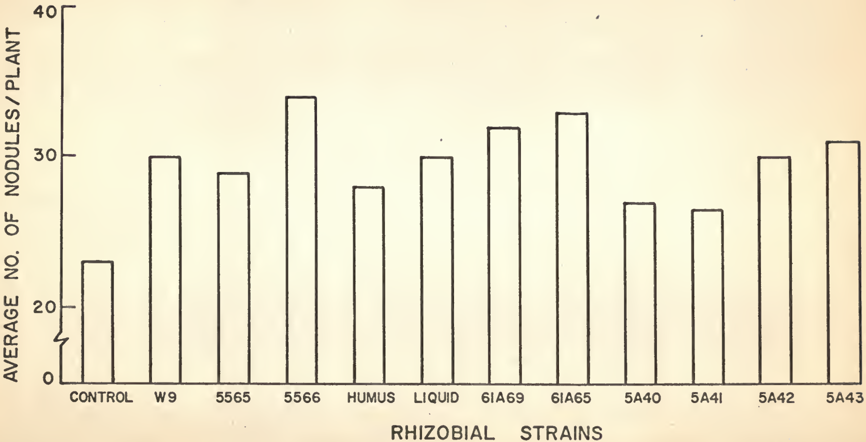


Fig. 1. Relative effectiveness of eleven Rhizobial strains in nodulating the roots of Clark 63 soybean.

EXPLANATION OF PLATE I

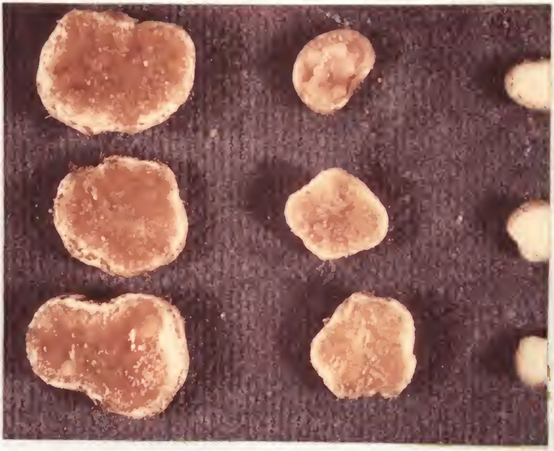
Nonnodulated plants are shown on the left and well nodulated plants to the right.

PLATE I

EXPLANATION OF PLATE II

A view of the cross-section of nodules from left to right showing effective, moderately effective, and ineffective nodulation and nitrogen fixation.

PLATE II



EXPLANATION OF PLATE III

A close view of well nodulated soybean roots brought about by the use of R. japonicum strain 5566 grown in the greenhouse study.

PLATE III

EXPLANATION OF PLATE IV

A photograph depicting a well-nodulated field-grown soybean.

PLATE IV

and yield components, chemical qualities, and other agronomic characteristics of Clark 63 soybean. Data and analysis of variance are shown in Tables 6-12, Appendix II. The results obtained on each factor are discussed in the succeeding paragraphs.

Nodulation

Statistical analysis shows that addition of 50 lbs per acre of nitrogen alone and in combination with the various levels of phosphorous and potassium, significantly decreased the number of nodules per plant. Plots which received no nitrogen treatment as a group produced 9.4 nodules per plant more than plots where 50 lbs of nitrogen per acre was added. The different levels of phosphorous stimulated nodulation with the highest number of nodules per plant (42 nodules per plant) produced from plots which received 120 lbs phosphorous per acre (Fig. 2). Increased levels of potassium application did not seem to affect nodulation. Plots which received 50 lbs and 100 lbs potassium per acre each averaged 9 nodules per plant. Figure 3 shows the influence of potassium levels (lbs per acre) with and without nitrogen application, on nodulation. The graph shows that plots with no potassium and no nitrogen applications had the highest number of nodules per plant when compared with plots which received 50 lbs and 100 lbs potassium per acre with the addition of 50 lbs of nitrogen per acre. The plots which received no fertilizer application averaged about 10 nodules per plant more than those receiving 50 lbs and 100 lbs of potassium per acre. Statistical analysis shows that potassium application significantly decreased nodulation at the 5 percent level as shown in Table 7, Appendix II.

The applications of phosphorous in combination with 50 lbs of nitrogen

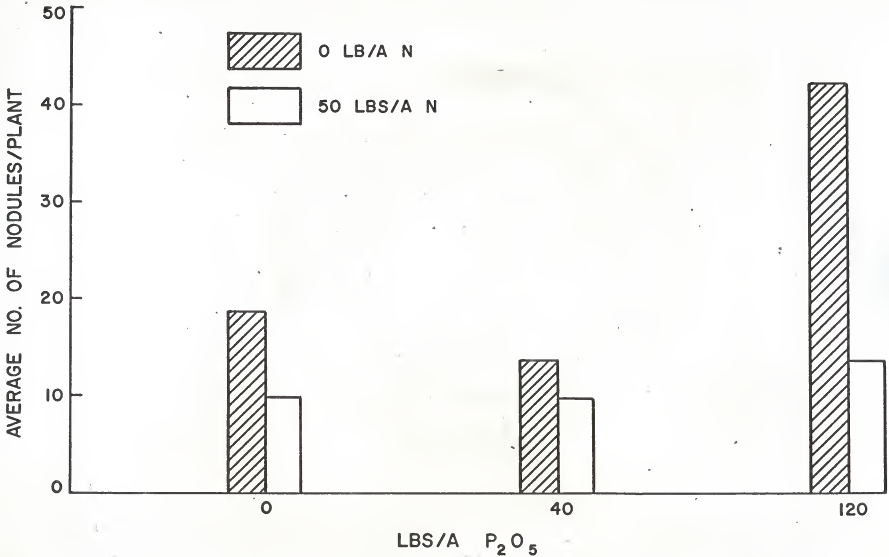


Fig. 2. Effect of applied P_2O_5 with and without nitrogen on nodulation of Clark 63 soybean.

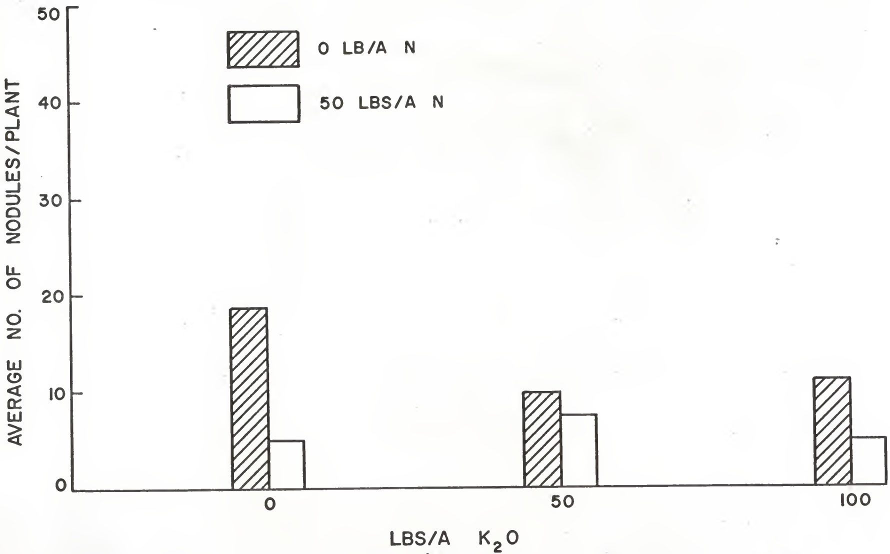


Fig. 3. Effect of applied K_2O with and without nitrogen on nodulation of Clark 63 soybean.

per acre significantly depressed nodule formation. Phosphorous levels with levels of potassium significantly depressed nodulation, however, the depressing effect is not as pronounced as the effect produced where potassium alone or in combination with 50 lbs of nitrogen per acre were applied. Application of phosphorous alone seems to be conducive to good nodulation.

Seed Yield

Table 2 shows data on seed yield and the yield components, plant height, number of nodes per plant, number of pods per plant, and seed weight (grams per 100 seeds).

Analysis of variance shows no significant differences in seed yield between the fertilizer applications. Plots which received 50 lbs of nitrogen per acre averaged only 0.2 more bushels per acre than those receiving no nitrogen. The effect of different levels of phosphorous with levels of potassium is shown in Fig. 4. Plots which received 40 lbs of phosphorous per acre average 2.0 bushels more than plots receiving no phosphorous application, while equaling the yield for plots treated with 120 lbs of phosphorous per acre. Where 50 lbs of potassium per acre was added, plots receiving 40 lbs of phosphorous per acre produced 3.0 bushels more than where no phosphorous was applied, however, with 120 lbs of phosphorous per acre, yield was reduced from 35 bushels to 31 bushels per acre. There was no increase in seed yield where 100 lbs of potassium per acre was added with 40 lbs of phosphorous per acre, however, with 120 lbs of phosphorous per acre the seed yield was increased by 2.0 bushels per acre.

Figure 5 shows the effects of levels of phosphorous, and potassium with 50 lbs of nitrogen per acre. Seed yield was reduced by 3.0 bushels per

Table 2. Effect of different levels of applied P_2O_5, K_2O with and without 50 lbs N per acre on yield and yield components of Clark 63 soybeans.

Treatment	Average				Seed weight (G/100 seeds)
	Seed yield (Bu/A)	Plant height (Inches)	Nodes per plant	Pods per plant	
0-0-0	31	35	19	33	14.6
0-40-0	33	35	22	42	14.5
0-120-0	33	35	21	39	14.2
0-0-50	32	35	21	39	14.0
0-40-50	35	36	23	47	14.1
0-120-50	31	34	20	38	14.4
0-0-100	32	36	19	36	13.9
0-40-100	32	34	18	35	14.3
0-120-100	34	37	22	43	14.6
50-0-0	35	39	25	51	15.3
50-40-0	32	36	19	37	14.8
50-120-0	33	37	21	40	14.7
50-0-50	30	35	18	33	14.8
50-40-50	33	37	22	43	15.0
50-120-50	35	37	22	47	14.3
50-0-100	34	38	22	44	13.5
50-40-100	34	38	22	44	14.9
50-120-100	29	35	18	30	14.4
$LSD_{.05}$	NS	NS	4.5	8.5	NS

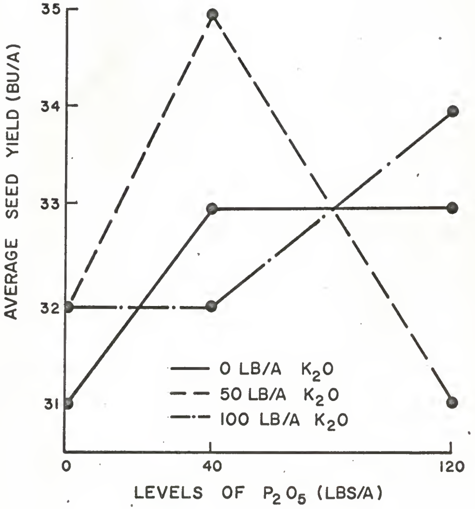


Fig. 4. Effect of levels of P_2O_5 and K_2O on seed yield of Clark 63 soybean.

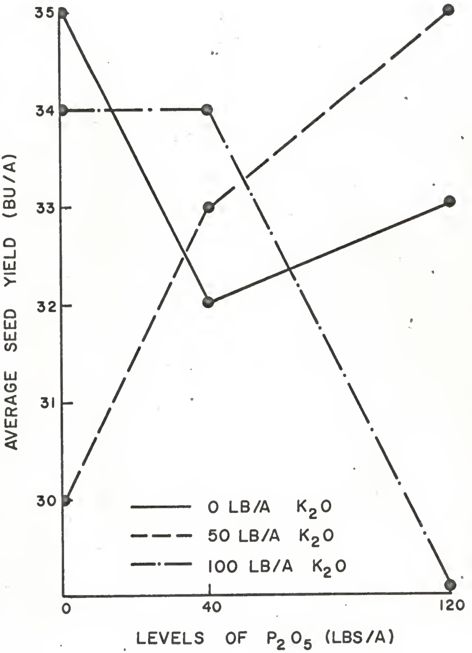


Fig. 5. Effect of levels of P_2O_5 and K_2O with the addition of 50 lbs N per acre on seed yield of Clark 63 soybean.

acre where 40 lbs of phosphorous per acre was added, and by 2.0 bushels per acre with the application of 120 lbs of phosphorous per acre. The addition of 50 lbs of potassium per acre with 50 lbs of nitrogen increased yield with increasing levels of phosphorous. However, the addition of 100 lbs of potassium and 50 lbs of nitrogen per acre decreased seed yield with increasing levels of phosphorous. The detailed results are shown in Appendix II, Table 8. The average seed yield ranged from 29 to 35 bushels per acre but this increase in yield is not statistically significant at the 5 percent level.

Plant Height

The application of different levels of nitrogen, phosphorous, and potassium apparently did not have a significant effect on the plant height as evidenced by the analysis of variance shown in Appendix II, Table 11. The influence of applied levels of phosphorous and potassium on plant height of Clark 63 soybean is illustrated in Fig. 6. Application of different levels of potassium with phosphorous did not affect plant height and a straight line graph obtained. The average plant height for all fertilizer treatments was 35 inches. Where 50 lbs potassium was added per acre, the plant height increased by 1 inch with the application of 40 lbs of phosphorous per acre, but with 120 lbs of phosphorous average plant height decreased by 1 inch. Average plant height of plots which received 100 lbs of potassium was shorter by 2 inches with the addition of 40 lbs of phosphorous and was taller by 1 inch with the application of 120 lbs of phosphorous per acre.

Nitrogen in combination with phosphorous and potassium increased plant height by approximately 2 inches over the no nitrogen treated plots; however the increase was not statistically significant. Figure 7 shows the

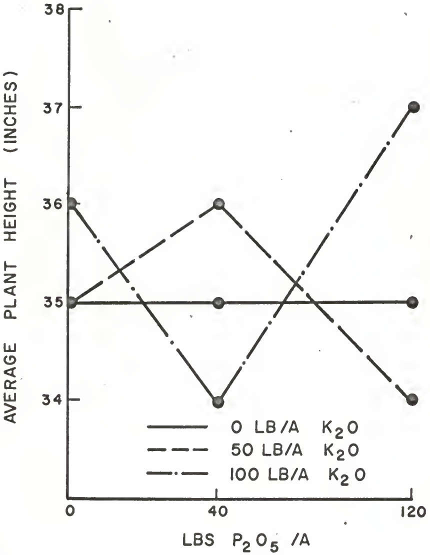


Fig. 6. Effect of applied levels of P_2O_5 and K_2O on plant height of Clark 63 soybean.

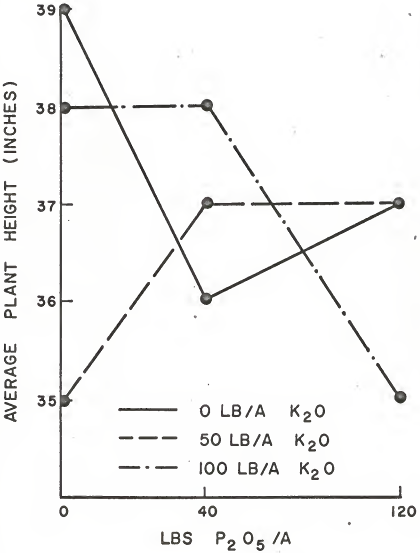


Fig. 7. Effect of applied levels of P_2O_5 and K_2O with 50 lbs N per acre on plant height.

effect of applied levels of phosphorous and potassium with 50 lbs of nitrogen per acre on plant height. The graph in Fig. 7 shows an increase of 2 inches in plant height when 50 lbs of potassium was applied with 40 lbs of phosphorous but there was no increase in height with 120 lbs of phosphorous. Increasing levels of phosphorous with 100 lbs of potassium shortened plant height by approximately 3 inches from an average of 38 inches for 100 lbs of potassium with no phosphorous to 35 inches for 100 lbs of potassium with 120 lbs of phosphorous per acre.

Nodes Per Plant

Data on average number of nodes per plant is shown in Table 2 and in Appendix II, Table 9. Application of nitrogen did not significantly increase the number of nodes per plant as can be seen from Table 9, Appendix II. However, plant in plots with nitrogen treatment averaged approximately 0.44 more nodes per plant than plots receiving no nitrogen treatment. This result can be justified by the increase in the number of pods per plant in nitrogen treated plots which is discussed in the next section on pods per plant. The different levels of potassium did not significantly affect the average number of nodes per plant. However, when potassium was applied with phosphorous, it increased the average number of nodes per plant significantly as shown in the analysis of variance in Appendix II, Table 9. Figure 8 also shows graphically the effect of phosphorous alone and in combination with various levels of potassium.

Addition of 50 lbs of nitrogen with levels of potassium significantly reduced the number of nodes per plant. But the addition of nitrogen with various levels of phosphorous did not appreciably affect the number of

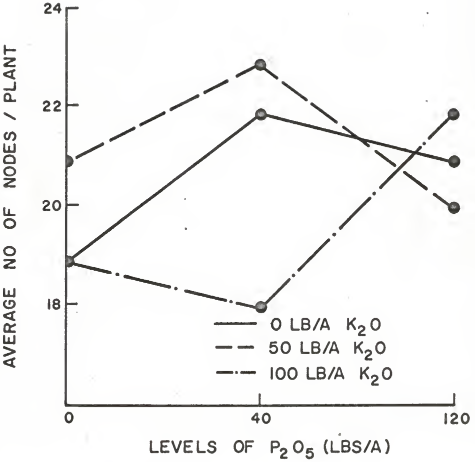


Fig. 8. Effect of levels of applied P_2O_5 and K_2O on average number of nodes per plant.

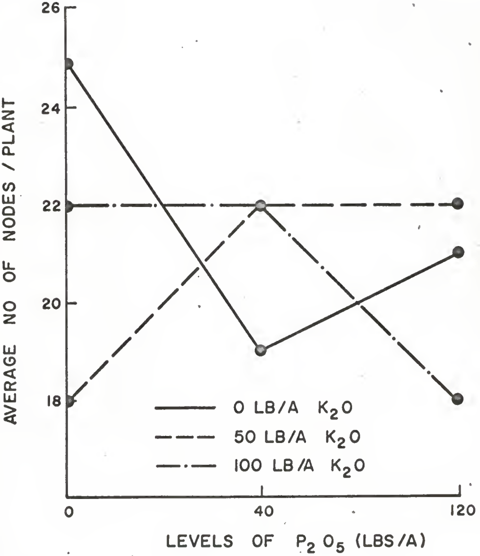


Fig. 9. Effect of levels of applied P_2O_5 and K_2O with 50 lbs N/acre on average number of nodes per plant.

nodes per plant as is evidenced by the analysis of variance shown in Appendix II, Table 9 and in Fig. 9.

Pods Per Plant

Application of 50 lbs of nitrogen significantly increased the number of pods per plant when compared with the check plot. Averaging the number of pods per plant for plots which received 50 lbs of nitrogen per acre, there is an increase of approximately 2 pods per plant more than plots without nitrogen fertilization. The effect of different levels of applied phosphorous and potassium on the average number of pods is shown graphically in Fig. 10 and the detailed result is shown in Table 2. Statistical analysis of the results show levels of phosphorous significantly increased pod formation, while average number of pods decreased with increasing levels of potassium. This decrease is significant as can be seen from the analysis of variance in Appendix II, Table 10. Levels of potassium with levels of phosphorous did not significantly affect pod formation. As can be seen in Fig. 10, the rise in trend lines is counteracted by the descending lines.

The addition of 50 lbs of nitrogen per acre with levels of potassium significantly increased pod formation when compared with the effect of the combination of all fertilizer elements acting together. The influence of added nitrogen was not significant with the application of levels of phosphorous. The increase in pod formation on plants in plots which received 50 lbs of nitrogen alone is highly significant. These plots produced the greatest number of pods averaging 51 pods per plant. It is significant to note that plots which received 50 lbs of nitrogen per acre averaged 18 pods per plant more than the check plot. As mentioned in the preceding paragraph

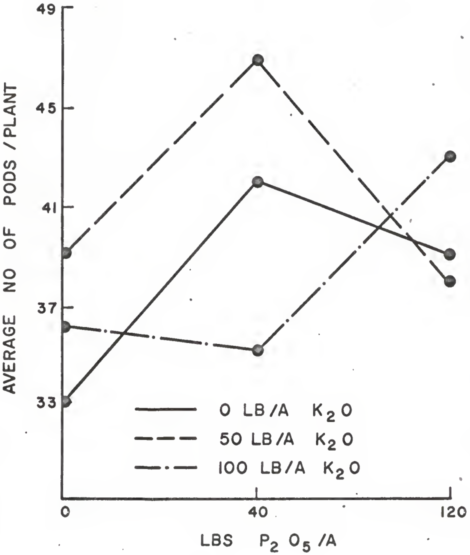


Fig. 10. Effect of levels of applied P_2O_5 and K_2O on average number of pods per plant.

the average for all plots which received various levels of phosphorous and potassium in addition to 50 lbs of nitrogen per acre averaged approximately 2 pods per plant more than plots without applied nitrogen. The combination of nitrogen with levels of phosphorous and potassium significantly increased the number of pods per plant. The results discussed in this paragraph are shown graphically in Fig. 11.

Seed Weight

The seed weight is the weight in grams per 100 seed and it is considered a yield component. By taking samples from combined seeds from the four replicates it was not feasible to run an analysis of variance on seed weight. The figures reported for seed weight in Table 2, and Appendix II, Table 6 are averages for the four replications for each treatment.

Plots which received phosphorous and potassium with 50 lbs of nitrogen per acre weighed 3.5 grams more than those without nitrogen treatment. This weight increase is an average of approximately 0.4 grams per treatment. Nitrogen alone had a slight effect on seed weight with an average seed weight of 15.3 grams which represents the highest weight for all treatments. All weights are 14 grams and above except weights of 13.9 and 13.5 grams recorded for treatments 0-0-100 and 50-0-100 respectively. These slight decreases in seed weight may lead to the conclusion that high levels of potassium depressed seed weight in this particular experiment, however, this is only an assumption.

Seed Quality

Seed quality was rated and numbers assigned considering seed

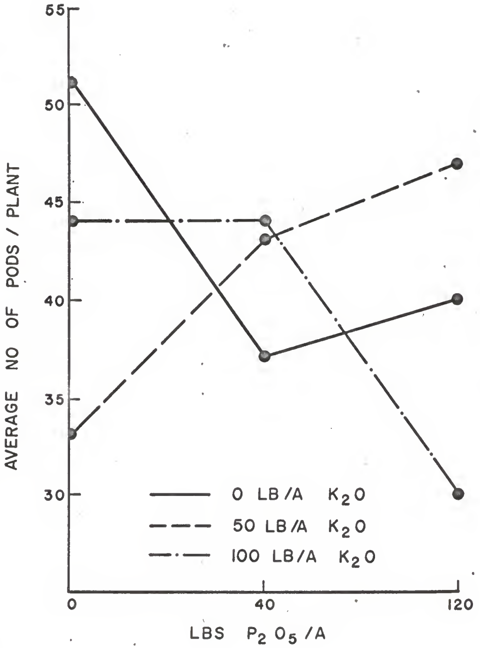


Fig. 11. Effect of levels of applied P_2O_5 and K_2O with 50 lbs N per acre on average number of pods per plant.

development, damage, disease, wrinkling, and objectionable colour for the variety Clark 63. The scale is arbitrarily set from 1 to 5 where a rating of 1 indicates very good seeds which have developed normally and without unusual traits to mar the usual characteristics for the seed. Analysis of variance was not made on seed quality because the variation resulting from the various fertilizer treatments was quite negligible. Seeds from all treatments rated from very good to good. Notes taken on seed quality are included in Table 3.

Lodging

There was essentially no lodging in any of the treatments. However, plots receiving 50 lbs of nitrogen alone had a lodging score of 1.5 which was the highest score for all the treatments. The differences in lodging between the treatments were not large enough to be statistically significant. Results are presented in Table 3 and the analysis of variance is shown in Appendix II, Table 12.

Maturity

Maturity was recorded as the date when approximately 95 percent of the pods were ripe and most of the leaves had dropped, and is expressed as days earlier (-) or later (+) than the average for the variety. All plants in plots which did not receive nitrogen treatment matured on the same date (October 4), 127 days after planting. Plants in plots which received 50 lbs of nitrogen alone matured approximately 3 days later than the plots without nitrogen treatment. This is expected because high levels of nitrogen tend to delay maturity of most crops. Phosphorous fertilization did not

Table 3. Effect of different levels of applied P_2O_5, K_2O with and without 50 lbs N per acre on some agronomic characteristics of Clark 63 soybeans.

Treatment	Seed quality*	Lodging**	Maturity***
0-0-0	2	1.3	0
0-40-0	1	1.2	0
0-120-0	1	1.3	0
0-0-50	2	1.3	0
0-40-50	2	1.4	0
0-120-50	1	1.2	0
0-0-100	2	1.3	0
0-40-100	1	1.2	0
0-120-100	1	1.3	0
50-0-0	1	1.5	+3
50-40-0	2	1.3	0
50-120-0	2	1.4	0
50-0-50	2	1.3	+2
50-40-50	1	1.3	0
50-120-50	2	1.4	0
50-0-100	1	1.3	+2
50-40-100	2	1.3	0
50-120-100	1	1.2	0
$LSD_{.05}$	-	NS	-

*Seed quality is rated according to the following scale considering seed development, damage, disease, wrinkling, and objectionable colour for the variety:
1 = very good; 2 = good; 3 = fair; 4 = poor; 5 = very poor.

**Lodging is rated from 1-5
where 1 rating = almost all plants are erect
5 rating = almost all plants are prostrate
2-4 ratings = intermediate.

***0 indicates the date check plot matured (October 4); +2 and +3 indicate days later than the check.

delay maturity while potassium in addition to 50 lbs of nitrogen delayed maturity by 2 days. Table 3 includes the data collected on maturity. The fertilizer treatments did not exert any great influence on maturity of the soybeans because there were no plots maturing before October 4 nor was there any treatment which delayed maturity in excess of 3 days later than October 4.

Protein and Oil Content

The data reported in Table 4 and Appendix II, Table 6 are averages of all four replications for each treatment. Analysis of variance was not made on the percent protein and oil since these were not reported on replication basis, however, there are some slight differences attributable to the fertilizer treatment.

Nitrogen fertilization did increase the percent protein. Plots which received 50 lbs of nitrogen per acre in addition to the two levels of phosphorous and potassium averaged about 0.5 percent more protein than plots without nitrogen fertilization. Increasing levels of phosphorous was found to have progressively increased the protein content. With the application of 50 lbs of potassium per acre, increasing levels of phosphorous increased the protein content of the seeds, while 100 lbs of potassium slightly decreased the protein content with increasing levels of phosphorous. The effects of the different levels of nitrogen, phosphorous, and potassium on protein content are shown graphically in Figs. 12 and 13.

The oil content was decreased by the application of nitrogen fertilizer. Plots which received 50 lbs of nitrogen in addition to the two levels of phosphorous and potassium lowered the oil content of the seeds by 0.6

Table 4. Effect of different levels of applied P_2O_5, K_2O with and without 50 lbs N per acre on protein and oil content of Clark 63 soybeans.

Treatment	% Protein	% Oil
0-0-0	39.3	22.2
0-40-0	38.5	22.6
0-120-0	38.8	22.5
0-0-50	38.6	24.7
0-40-50	38.8	22.0
0-120-50	39.0	22.9
0-0-100	38.7	22.2
0-40-100	38.6	22.0
0-120-100	38.4	22.7
50-0-0	39.5	22.6
50-40-0	39.5	21.5
50-120-0	39.4	22.3
50-0-50	39.5	22.0
50-40-50	38.8	22.0
50-120-50	39.2	22.0
50-0-100	39.2	22.5
50-40-100	38.6	21.8
50-120-100	39.7	21.9

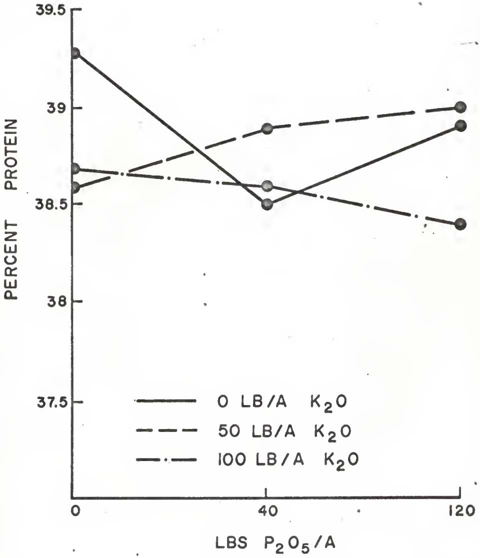


Fig. 12. Effect of levels of applied P_2O_5 and K_2O on protein content of Clark 63 soybean.

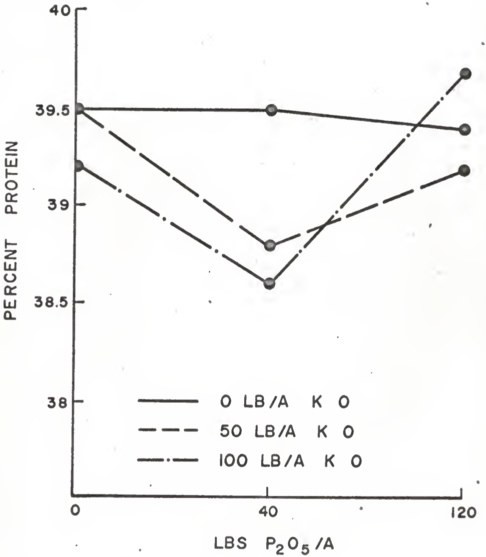


Fig. 13. Effect of levels of P_2O_5 and K_2O with the addition of 50 lbs N on the protein content of Clark 63 soybean.

percent when compared with plots receiving no nitrogen treatment. The influence of nitrogen addition on oil content is shown in Fig. 15. Plots which received only 50 lbs of potassium per acre (0-0-50) sharply increased the oil content of the seeds (24.7 percent). The reason for the erratic increase in oil content is not known, but it is interesting to note that these plots did not receive nitrogen fertilization. However, with increasing levels of phosphorous application the oil content declined back to the level for most of the other treatments which is from 22.0 to 22.6 percent. The effect of the different levels of phosphorous and potassium is clearly illustrated in Fig. 14.

SUMMARY

Two experiments were conducted. The first one was in the greenhouse designed to evaluate the relative effectiveness of eleven strains of Rhizobium japonicum to inoculate and cause nodule formation on roots of Clark 63 soybean (Glycine max). Statistical analysis of results showed 4 strains significantly produced more nodules per plant than the other strains at the 5% level. However, only 2 strains produced significantly more nodules than the non-inoculated plants. Strain 5566 from Nodogen Laboratory in Princeton, Illinois, and strain 61A65 from Nitragin Company of Milwaukee, Wisconsin produced approximately 10 and 9 nodules per plant more than the non-inoculated plants. Strain 5566 was selected to be used on seeds for a field planting study. Peat-base (humus) inoculants produced approximately 1.3 more nodules per plant than the agar and liquid inoculants.

The second experiment was conducted in the field to study the response of inoculated soybean plants to fertilizer treatments at Manhattan, Kansas.

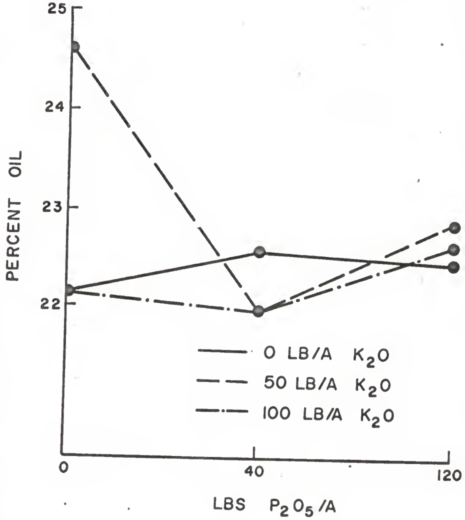


Fig. 14. Effect of levels of P_2O_5 and K_2O on oil content of seeds of Clark 63 soybean.

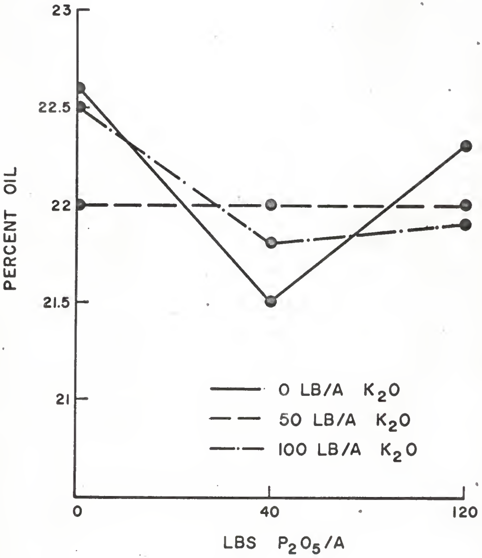


Fig. 15. Effect of levels of P_2O_5 and K_2O with the addition of 50 lbs N on oil content of seeds of Clark 63 soybean.

Statistical analysis of results obtained on nodulation showed the addition of 50 lbs of nitrogen per acre alone and in combination with levels of phosphorous and potassium significantly decreased nodulation. Where 50 lbs of nitrogen was applied alone, nodulation was decreased by 9 nodules per plant when compared with plants in the check plot. All levels of phosphorous stimulated nodule formation and the highest number of nodules per plant (42 nodules) was produced from plots receiving 120 lbs of phosphorous per acre. Increased levels of potassium application did not seem to affect nodulation.

Seed yield was not affected appreciably by added fertilizer treatments. The addition of 50 lbs of nitrogen per acre with levels of phosphorous and potassium averaged 0.2 of a bushel more per acre than plots which received no nitrogen. The differences between treatments were too small to be significant.

Plant height was not significantly affected by the fertilizer treatments, however, the plants in nitrogen treated plots were 2 inches taller than those in plots without nitrogen.

Application of nitrogen and phosphorous increased seed weight, while increasing levels of potassium seemed to have a decreasing effect. The differences in seed weight between treatments were too small to be significant. Seed quality for all treatments was rated either as very good or good and did not appear to be affected by fertilization.

Plots which received 50 lbs of nitrogen alone averaged 6 more nodes per plant than plants from the check plot. Application of nitrogen with levels of phosphorous and potassium averaged approximately 1 more node per plant than plots without nitrogen. Neither phosphorous nor potassium, when applied alone, had a significant influence on the number of nodes per plant.

The average number of pods per plant was significantly increased by the application of nitrogen alone. Plots receiving 50 lbs of nitrogen per acre with levels of phosphorous and potassium produced approximately 2 more pods per plant than plots without nitrogen. Levels of phosphorous significantly increased podding, and so did levels of potassium but to a lesser extent. However, when phosphorous and potassium were applied together, there was no significant influence on the average number of pods per plant. The increase in number of pods per plant effected by the application of nitrogen alone was significant statistically at the 5% level. Plants in plots receiving nitrogen alone averaged approximately 51 pods per plant as compared to an average of 33 for plants in the check plot.

Fertilizer applications did not appreciably affect lodging and none of the plots had plants that were down. Nitrogen treated plots had the highest lodging score of 1.5. The differences in lodging ratings were not statistically significant. All plots which received no nitrogen matured at the same time (October 4) while plots receiving 50 lbs of nitrogen matured 3 days later than plants in the check plot. Addition of 50 lbs and 100 lbs of potassium with 50 lbs of nitrogen delayed maturity by 2 days.

Analysis of variance was not made on protein and oil content of seeds because samples for analysis for percent protein and oil were taken from composited seeds of all four replications. Nitrogen fertilization with levels of phosphorous and potassium increased protein content by 0.6 percent over plots which received no nitrogen. Phosphorous alone increased the protein content while potassium treated plots contained slightly less protein.

Oil content was decreased by 0.5 percent where nitrogen with levels of phosphorous and potassium was applied. Potassium appeared to increase oil content while it was decreased slightly by levels of phosphorous.

Adverse weather conditions which persisted throughout the growing season may have limited the responses obtained from the fertility study. There was approximately 7.0 inches of precipitation from planting to maturity, and the limited supply of moisture was accompanied by temperatures over 100^{o}F for 10 days between the last day in June till the middle of July. The average maximum temperature was 95.9 degrees for month of July while August had a maximum high average of 84.7 degrees. The highest temperature recorded was 108^{o}F on July 19.

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APPENDIX I

The effects of applied fertility on soybean nodulation and yield was conducted at Kansas State University's Agronomy Research Farm.

The set up and outline of the various treatments is given below. The design of experiment is also outlined in the appendix.

SOYBEAN FERTILITY--MANHATTAN, 1966

Treatments and plots receiving them.

	Treatment--N, K_2O-Broadcast P_2O_5-Band			Plots			
	N	P_2O_5	K_2O	Block I	Block II	Block III	Block IV
1.	0	0	0	8	17	1	5
2.	0	40	0	18	3	8	2
3.	0	120	0	11	1	5	14
4.	0	0	50	16	11	18	3
5.	0	40	50	13	7	14	1
6.	0	120	50	14	18	4	13
7.	0	0	100	9	4	13	4
8.	0	40	100	4	5	16	9
9.	0	120	100	6	8	3	7
10.	50	0	0	10	10	11	12
11.	50	40	0	17	12	9	16
12.	50	120	0	12	6	15	10
13.	50	0	50	7	2	17	18
14.	50	40	50	2	14	12	8
15.	50	120	50	3	9	7	11
16.	50	0	100	15	13	2	6
17.	50	40	100	5	15	6	17
18.	50	120	100	1	16	10	15

1. N as NH_4NO_3 (33.5-0-0);
2. P_2O_5 as 0-46-0
3. K_2O as 0-0-60

DESIGN OF EXPERIMENT

	2 x 3 x 3 Factorial Design					
	N_0			N_1		
	K_0	K_1	K_2	K_0	K_1	K_2
P_0	$N_0P_0K_0$	$N_0P_0K_1$	$N_0P_0K_2$	$N_1P_0K_0$	$N_1P_0K_1$	$N_1P_0K_2$
P_1	$N_0P_1K_0$	$N_0P_1K_1$	$N_0P_1K_2$	$N_1P_1K_0$	$N_1P_1K_1$	$N_1P_1K_2$
P_2	$N_0P_2K_0$	$N_0P_2K_1$	$N_0P_2K_2$	$N_1P_2K_0$	$N_1P_2K_1$	$N_1P_2K_2$

Soil pH = 6.8

There are 18 treatments altogether

We have 4 blocks, the analysis of variance using Randomized Block Design for 18 treatments:

Sources of Variation	
Between Blocks	3 D.F.
Treatments Break down treatments into linear, quadratic etc. components each with 1 d.f.	17 D.F.
Error	51
Total	71

APPENDIX II

Table 6. Effect of different levels of P_2O_5 and K_2O with and without nitrogen on nodulation, yield, agronomic and chemical properties of Clark 63 soybean.

Treatment	Average					
	Nodules/ plant	Seed yield (Bu/A)	Plant height (Inches)	Nodes/ plant	Pods/ plant	Seed weight (G/100 seeds)
0-0-0	19	31	35	19	33	14.6
0-40-0	16	33	35	22	42	14.5
0-120-0	42	33	35	21	39	14.2
0-0-50	9	32	35	21	39	14.0
0-40-50	16	35	36	23	47	14.1
0-120-50	18	31	34	20	38	14.4
0-0-100	9	32	36	19	36	13.9
0-40-100	16	32	34	18	35	14.3
0-120-100	23	34	37	22	43	14.6
50-0-0	5	35	39	25	51	15.3
50-40-0	11	32	36	19	37	14.8
50-120-0	16	33	37	21	40	14.7
50-0-50	7	30	35	18	33	14.8
50-40-50	10	33	37	22	43	15.0
50-120-50	12	35	37	22	47	14.3
50-0-100	5	34	38	22	44	13.5
50-40-100	9	34	38	22	44	14.9
50-120-100	8	29	35	18	30	14.4
$LSD_{.05}$	7.86	NS	NS	4.49	8.49	----

Table 6 (Cont.).

Treatment	Seed quality	Lodging	Maturity	% Protein	% Oil
0-0-0	2	1.3	0	39.3	22.2
0-40-0	1	1.2	0	38.5	22.6
0-120-0	1	1.3	0	38.8	22.5
0-0-50	2	1.3	0	38.6	24.7
0-40-50	2	1.4	0	38.8	22.0
0-120-50	1	1.2	0	39.0	22.9
0-0-100	2	1.3	0	38.7	22.2
0-40-100	1	1.2	0	38.6	22.0
0-120-100	1	1.3	0	38.4	22.7
50-0-0	1	1.5	+3	39.5	22.6
50-40-0	2	1.3	0	39.5	21.5
50-120-0	2	1.4	0	39.4	22.3
50-0-50	2	1.3	+2	39.5	22.0
50-40-50	1	1.3	0	38.8	22.0
50-120-50	2	1.4	0	39.2	22.0
50-0-100	1	1.3	+2	39.2	22.5
50-40-100	2	1.3	0	38.6	21.8
50-120-100	1	1.2	0	39.7	21.9
$LSD_{.05}$	-	NS	-	----	----

Table 7. Analysis of variance on nodulation.

Sources of variance	D.F.	Mean square	Decision
	Average number of nodules/plant		
Replicates	3	20.533	N.S.
N	1	138.880	H.S.
P_2O_5	2	90.495	S.
K_2O	2	368.040	H.S.
N x P_2O_5	2	331.725	H.S.
N x K_2O	2	300.430	H.S.
P_2O_5 x K_2O	4	250.040	H.S.
N x P_2O_5 x K_2O	4	425.020	H.S.
Error	51	13.56	

Table 8. Analysis of variance on seed yield.

Sources of variance	D.F.	Mean square	Decision
Replicates	3	11031.00	S.
N	1	857.00	N.S.
P_2O_5	2	2364.50	N.S.
K_2O	2	628.50	N.S.
N x P_2O_5	2	849.50	N.S.
N x K_2O	2	2566.00	N.S.
P_2O_5 x K_2O	4	652.25	N.S.
N x P_2O_5 x K_2O	4	1157.00	N.S.
Error	51	13.72	

N.S. = Not significant at = .05

S. = Significant at = .05

H.S. = Highly significant at = .05

Table 9. Analysis of variance on average number of nodes per plant.

Sources of variance	D.F.	Mean square	Decision
Replicates	3	4.440	N.S.
N	1	15.580	N.S.
P_2O_5	2	21.240	S.
K_2O	2	12.000	N.S.
N x P_2O_5	2	2.775	N.S.
N x K_2O	2	21.835	S.
P_2O_5 x K_2O	4	19.463	S.
N x P_2O_5 x K_2O	4	8.128	N.S.
Error	51	5.020	

Table 10. Analysis of variance on average number of pods per plant.

Sources of variance	D.F.	Mean square	Decision
Replicates	3	13.90	N.S.
N	1	234.40	H.S.
P_2O_5	2	219.00	H.S.
K_2O	2	69.90	S.
N x P_2O_5	2	11.50	N.S.
N x K_2O	2	380.90	H.S.
P_2O_5 x K_2O	4	1.88	N.S.
N x P_2O_5 x K_2O	4	127.00	H.S.
Error	51	16.48	

Table 11. Analysis of variance on average plant height.

Sources of variance	D.F.	Mean square	Decision
Replicates	3	34.603	S.
N	1	.010	N.S.
P_2O_5	2	9.385	N.S.
K_2O	2	6.760	N.S.
N x P_2O_5	2	2.720	N.S.
N x K_2O	2	20.515	N.S.
P_2O_5 x K_2O	4	5.973	N.S.
N x P_2O_5 x K_2O	4	11.228	N.S.
Error	51	6.47	

Table 12. Analysis of variance on lodging.

Sources of variance	D.F.	Mean square	Decision
Replicates	3	.0418	N.S.
N	1	.0138	N.S.
P_2O_5	2	.0154	N.S.
K_2O	2	.0116	N.S.
N x P_2O_5	2	.0126	N.S.
N x K_2O	2	.0087	N.S.
P_2O_5 x K_2O	4	.0058	N.S.
N x P_2O_5 x K_2O	4	.0172	N.S.
Error	51	.0199	

EFFECTS OF ADDED NITROGEN, PHOSPHOROUS, AND POTASSIUM ON NODULATION AND YIELD OF CLARK 63 SOYBEANS

by

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The concept of strain variation and host specificity which has been reported to exist in the symbiotic relationship between bacteria and leguminous plants prompted the evaluation of eleven strains of Rhizobium japonicum. The study was conducted in the greenhouse in Spring, 1966. Seeds were inoculated and immediately planted in pots with soil taken from the site of a field experiment. Roots were removed after 34 days of growth and nodule counts made.

Four strains produced significantly more nodules per plant than the others; however only 2 strains produced significantly more nodules at the 5% level. The soil used had been planted to soybeans in prior years and apparently nodule formation on the non-inoculated plants may be attributed to the residual bacteria in the soil. Strains 5566 and 61A65 produced approximately 10 and 9 more nodules per plant respectively than the non-inoculated plants. Strain 5566 was used to inoculate the seeds for field planting. Peat-base inoculants produced 1.3 more nodules per plant than the liquid inoculants.

The thesis also describes the field fertility experiment conducted at Manhattan during 1966 growing season. Inoculated seeds of Clark 63 soybeans were planted on soils where nitrogen (50 lbs/A), phosphorous (40; 120 lbs/A), and potassium (50; 100 lbs/A) were applied. Nitrogen was supplied as ammonium nitrate, potassium as muriate of potash, and both were broadcast by hand. Phosphorous was supplied as triple superphosphate and was banded near the rows.

Data on nodulation showed nitrogen application alone depressed nodulation by approximately 9 nodules per plant, while increased levels of phosphorous produced more nodules per plant. Plots receiving 120 lbs of phosphorous produced an average of 42 nodules per plant.

Seed yield was not affected appreciably by added fertilizer treatments. However, 50 lbs of nitrogen with levels of phosphorous and potassium averaged 0.2 of a bushel more per acre than plots receiving no nitrogen. Plants in nitrogen treated plots were 2 inches taller though not significant than those where nitrogen was not applied. Nitrogen and phosphorous increased seed weight, while increasing levels of potassium appeared to have no effect. Seed quality did not appear to be affected by fertilization.

Plants in plots receiving 50 lbs of nitrogen alone averaged 6 more nodes per plant than those from the check plot. Neither phosphorous nor potassium had a significant influence on the number of nodes per plant. Plots receiving 50 lbs of nitrogen per acre with levels of phosphorous and potassium averaged 2 more pods per plant than plots without nitrogen. The increase in number of pods effected by 50 lbs of nitrogen alone was significant at the 5% level. Levels of phosphorous and potassium separately increased podding, however when applied together, the effect was not significant.

Fertilizer applications did not appreciably affect lodging nor maturity, but where nitrogen was applied the greatest amount of lodging occurred. Nitrogen treated plots matured approximately 3 days later than the check plot.

Nitrogen with levels of phosphorous and potassium increased protein content by 0.6% over the plots not treated with nitrogen. Phosphorous alone increased the protein content over the check plot while it was decreased by potassium.

Oil content was decreased by 0.5% where nitrogen was used in combination with phosphorous and potassium. Potassium increased oil content by 1.3% over the check plot.

Adverse weather conditions which persisted throughout the growing season may have limited the responses obtained from the fertility study. There was approximately 7.0 inches of precipitation from planting to maturity, and the average maximum temperature was 95.9 degrees for month of July while August had a maximum high average of 84.7 degrees.