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Title: EFFECTS OF INOCULATION WITH RHIZOBIUM AND NITROGEN FERTILIZER ON SNAP BEANS, SOYBEANS, AND LIMA BEANS

Abstract approved: Harry J. Mack

The responses of 'Oregon 1604' snap beans (Phaseolus vulgaris L.), 'Early Thorogreen' lima beans (Phaseolus lunatus L.), and 'Takii's Extra Early' soybeans (Glycine max L.) to two methods of inoculation with rhizobium (seedcoating and furrow treatments) and rates of nitrogen fertilizer were studied in field experiments. Nitrogen rates ranged from 0 to 84 kg/ha.

Nodulation was increased significantly by inoculation but decreased by nitrogen fertilization in three crops. Yield of snap beans was increased significantly from increasing nitrogen fertilizer but was not affected by inoculation. Neither nitrogen fertilizer nor inoculation influenced soybean yield.

For soybeans and lima beans, furrow inoculation resulted in significantly higher nodulation than the seedcoating treatment. Nodulation of snap beans and soybeans was significantly decreased

by nitrogen fertilizer in inoculated plots but not in check plots. Plant weight of lima beans was significantly increased by nitrogen fertilizer only when seedcoating inoculation was used.

Plant stands of snap beans were increased 118% by captan seed treatment but nodulation was significantly reduced.

Effects of Inoculation with Rhizobium and
Nitrogen Fertilizer on Snap Beans,
Soybeans, and Lima Beans

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

EFFECTS OF INOCULATION WITH RHIZOBIUM AND NITROGEN FERTILIZER ON SNAP BEANS, SOYBEANS, AND LIMA BEANS

INTRODUCTION

Legumes possess two systems to obtain their nitrogen: 1) They can fix nitrogen from the atmosphere through a symbiotic process involving Rhizobium bacteria, and/or, 2) assimilate inorganic nitrogen from the soil. What each system contributes to the nitrogen needs of the plant depends largely upon the effectiveness of the nitrogen fixation process, the growth habits of the legumes, and the amount of inorganic nitrogen available in the soil. If the soil is high in inorganic nitrogen, the legume absorbs soil nitrogen rather than obtaining it through fixation. A small amount of inorganic nitrogen is necessary for initial plant growth and may increase nodulation when seeds have been inoculated with rhizobium. With increasing costs of nitrogen fertilizer and a potential shortage, there is increased interest by growers in inoculation of legume seeds.

Inoculation of vegetable legumes is not widely practiced commercially. There is a large amount of research on soybeans, but not much consistent evidence has been found that snap beans and lima beans benefit from inoculation.

The purpose of this work was to study the effects of methods of inoculation with rhizobium and rates of nitrogen fertilizer, on snap beans, soybeans, and lima beans. An experiment was also conducted on snap beans to determine whether or not there was an interaction between a fungicide treatment and inoculation.

REVIEW OF LITERATURE

For many vegetable legume crops, nitrogen fertilization has become a common practice in enhancing yield. Rates of nitrogen commonly used on snap beans are 45 to 90 kg per hectare, and for lima beans are 85 to 115 kg per hectare (20). Many researchers have shown that good management and proper combination of efficient inoculation and nitrogen fertilization will give better yield and higher biologically fixed nitrogen on soybeans, but more information is needed on snap beans and lima beans.

Inoculation Effects

Inoculation is the addition of effective rhizobia to leguminous seeds prior to planting for the purpose of promoting symbiotic nitrogen fixation. This is necessary for most soils, because the effective rhizobia species are not always present in the soil, or if they are present, they do not exist in sufficiently high numbers for adequate nodulation. Some benefits which may be obtained from inoculation are: 1) early nitrogen deficiency is prevented, 2) the demand of the leguminous crop upon soil nitrogen is lessened, 3) crop yield, as measured in foliage or fruit, is increased, 4) the quality of the crop is improved (3). The source of inoculant affects the value of inoculation. Burton *et al.* (7) reported that ineffective rhizobia have often accounted for poor growth and low yield of certain leguminous plants, despite the presence of effective rhizobia in the rhizosphere. The efficiency of commercial inoculant depends on: 1) the use of properly selected, host-specific, rhizobial strains having high nitrogen-fixing efficiency, 2) the availability within the inoculant of large numbers of rhizobia per seed, 3) freshness in culture preparation, and 4) proper treatment of the seed with the inoculant at the time of planting (3).

Many experiments during the past 50 years, have shown that inoculated legumes produce more nodules, higher yield, and better quality of crop (18, 25, 29). However the amount of nitrogen that can be fixed by legume plants symbiotically with rhizobia is still being studied. Especially in field tests, the fixation depends upon many variables, such as soil moisture and oxygen content, soil type, planting date, light intensity, herbicide application, mineral nutrients in soil, depth of the root system, and soil pH (3, 8, 24, 25, 33, 34, 37). Under some conditions where the soil contains specific nodulation bacteria, inoculation is not beneficial. Response will be influenced by the soil situation and the previous crops or the cropping history. Elkins _et al_. (13) indicated that under the conditions of sufficient populations, _R. japonicum_ persists in southern Illinois soils for at least 10 or 11 years, resulting in good soybean nodulation and growth without inoculation. Ham _et al_. (16) found that soybean seed yields and seed protein percentage were not significantly increased by inoculating soybean seeds with a commercial rhizobia culture at planting time on five Minnesota locations in which the soils had produced nodulated soybeans previously. The uninoculated checks were adequately nodulated by rhizobia which were in the soil from previous soybean crops. Abel and Erdman (1) tested different strains of _R. japonicum_ on Lee soybeans in a soil where nodulated soybeans had grown previously. Treatments involving the strains were not measurably different from the uninoculated checks. Caldwell and Vest (10) evaluated 28 strains and two commercial preparations of _R. japonicum_ with five soybean cultivars and significant yield differences were found in three soils which were free of rhizobia. When inoculated seeds were planted in soil containing _R. japonicum_, there were no differences in seed yield and only five to ten percent of nodules were derived from the seed applied inoculant. Also, Abu-Shakra and Bassiri (2) reported soybeans grown on land planted the previous year with inoculated soybeans produced more nodules, seed yield and protein content concentration as compared to those grown on land that was planted with noninoculated soybeans.

Thornton (30) reported that the number of nodules formed was increased when the dosage of inoculant was increased, but excessive bacteria had little effect. Storing the seeds between inoculation and sowing caused some loss in the nodule numbers. Weaver and

Frederick (31) in testing commercial inoculants with greenhouse soybeans, indicated that maximum total nitrogen fixed in plant tops was achieved at inoculation levels of 2×10^3 cells per seed for soil-grown plants and 1×10^5 for sand-grown plants. At these levels at least four taproot nodules were found. They suggested that taproot nodulation of soybeans is a qualitative characteristic that may be useful in determining the adequacy of nodulation and may indicate abundance of rhizobia for a soybean variety. For successful competition of nodulation in soils having different populations of soil rhizobia, they did another experiment and predicted that if the inoculant rhizobia are to form 50% or more of the nodules, an inoculant rate equivalent to at least 1,000 times the soil population must be used.

Plant density was also reported to influence nodulation. Weil and Ohlrogge (35) showed that thinned plants had significantly more nodule volume per plant, greater percentage of active red nodules, and a lower percentage of inactive green nodules during pod-filling.

Wilson (37) reported that inoculation depth strongly influenced the pattern of nodule development with most of the nodular tissue forming on roots at or near the depth of inoculation.

There have been no experiments done to compare methods of inoculation such as seedcoating or furrow treatment on the efficiency of nodulation.

Number and mass of nodules are the most common ways to show the effect of rhizobia or inoculation (1, 2, 13, 16). Abel and Erdman (1) showed the weight of nodules was not always proportional to yield. They related the difference of nodule weight to variations in plant density in the row, loss in sampling, or differential quality of numbers of bacteria in the inoculant. Their data suggested that weight of nodules alone did not necessarily indicate effectiveness of nodulation. Nodule size did not influence nitrogen fixation as Ayala and Giddens (5) noted on peanut plants; smaller nodules but a greater total weight of nodules was found on plants bearing the highest specific nitrogenase activity.

There have not been many experiments done on inoculation of vegetable legumes. Burton et al. (7) reported that bean (Phaseolus vulgaris L.) plant response to the inoculation treatment was not different at the 35-45-68 day harvests.

Rhizobia may be killed or inactivated by fungicides. There are many conflicting conclusions about the compatibility of rhizobia with seed protectants. Curley and Burton (12) explained that these contradictory results can be attributed to variation in methods, inattention to important variables, and insufficient concern for numbers of rhizobia applied, survival and numbers seeded, kind of seed, rhizobia species, and form of inoculant. With the same kind of fungicide and inoculation, the nodulation of peas was prevented under greenhouse culture but not in a field study (22). However, the viability of rhizobia was high under favorable planting conditions (28). Curley and Burton (12) indicated the *R. japonicum* was compatible with Thiram and Carboxin but not with Pentachloronitrobenzene and Captan under growth chamber conditions. Captan was less toxic, but tap root nodulation of plants was greatly reduced by this material. Nodulation was poor when these seeds were planted 24 hours after inoculation for all the treatments.

Nitrogen Fertilization Effects

Though legumes can fix nitrogen symbiotically, inorganic nitrogen is important for initial growth and vigor of plants, and it indirectly influences nodulation, even in the presence of adequate inoculation (4, 15, 18). High rates of applied nitrogen may not increase yield but normally reduces the symbiotic nitrogen fixation which results in fewer nodules and smaller nodule size or mass (17, 21, 23, 27, 34). For better results, rates of nitrogen application should be within the range where symbiotic fixation is not inhibited and should be referenced to the residual soil nitrogen. Allos and Bartholomew (4) tested several legumes with gravel culture in the greenhouse supplied at weekly intervals with varying amounts of tracer nitrogen. They found that all legumes responded in growth and in nitrogen uptake to the addition of inorganic nitrogen. They indicated that in some instances the growth increase resulting from fertilization caused increases in fixation of nitrogen. But when the applied nitrogen exceeded that necessary for the growth increase it tended to replace the fixation process. Weber (34) field tested nodulated and non-nodulated soybean isolines for seven years, and found that soybeans produced essentially equal nitrogen yields when adequate combined nitrogen was available. Nodule weight, number, and size were directly related to increased nitrogen fixation and inversely related to increased increments of applied nitrogen. Thakur and Hason (29) tested 0, 40, 80, and 120 kg nitrogen per hectare on soybeans with and without rhizobia inoculation. The grain yield was increased as nitrogen rates increased. Yields were increased for the inoculated treatment at each nitrogen rate except at 120 kg nitrogen per hectare. They suggested that with the application of 120 kg nitrogen per hectare the activity of the bacteria in fixing the atmospheric nitrogen might have decreased. Norman (26) found that inoculation increased soybean yield by about one-third over the uninoculated control at nitrogen levels of 53 and 176 kg nitrogen per hectare.

Time of nitrogen application also influences inhibition or promotion of nitrogen fixation. Hatfield et al. (18) tested inoculated soybeans with 10 meq per liter NO_3^- for 0, 2, 4, and 6 weeks from emergence and uninoculated plants for 6 weeks in a gravel culture system. Dry weight of inoculated plants with 0 and 2 weeks nitrogen application was significantly lower than that of inoculated plants receiving 4 and 6 weeks nitrogen. Dry weight of the stems and leaves was the same for the uninoculated plants having 6 weeks nitrogen as for the inoculated plants receiving 4 or 6 weeks nitrogen. The number of nodules was increased by the application of nitrogen for 2 weeks after seedling emergence and decreased most by the application of nitrogen 6 weeks for both inoculated and uninoculated treatments. Total nitrogen in the stem and leaves was the same for the inoculated plants receiving 4 and 6 weeks nitrogen as for the uninoculated plants with nitrogen. Hoover and Beard (19) tested four rates of ammonium sulfate (0 to 165 kg/ha) and three times of application (preplant, preplant and flowering, and flowering) on soybeans in California. They found that in early stages of growth, the plants in the plots with zero nitrogen were yellowish green early and symptoms disappeared later in the season. This chlorosis which was similar to the yellowing due to nitrogen deficiency contrasted sharply with the dark green color of the plants on the nitrogen fertilized plots. Application of 56 kg/ha of nitrogen or more before planting lowered the production of nodules. Up to 112 kg/ha of delayed nitrogen application did not change the number of nodules per plant but delayed application of 170 kg/ha nitrogen decreased nodulation. When nitrogen application was delayed nodules may have become established and remained effective even after nitrogen fertilization. Rates of nitrogen application affected nodulation in a linear manner with the lower rates associated with more nodules, while the highest nitrogen fertilizer rate (170 kg/ha) produced the lowest yield. Plants treated only at flowering produced a higher yield than those treated at preplant and flowering. Preplant treatment had lower yield than the unfertilized treatment. These results suggest that nitrogen application in the early stage of growth has the most influence on nodulation.

Based on Welch's experiment (36) conducted at ten locations throughout Illinois, neither plow-down nor disk-in nitrogen fertilizer produced higher soybean yields. He also found that yields were not affected by nitrogen applied either at early flowering or at pod-filling.

When the soil is very low in nitrogen content, applied nitrogen will usually increase yield, because fixation processes do not supply sufficient nitrogen for maximum growth under this condition. The increased yield from applied nitrogen is found especially when the environmental conditions are unfavorable for the fixation process. Studies of Bhangoo and Albritton (6) on nodulated and non-nodulated soybean on a prelimed Calloway silt loam field indicated that applied or soil nitrogen, along with fixed nitrogen, was essential for maximum soybean yield on poorly drained soils low in organic matter with a strongly acid subsoil. Symbiotic nitrogen fixation dropped to zero when nitrogen rates exceeded 224 kg/ha. Burton _et al._ (8) noted that high levels of mineral nutrients were favorable to uptake of added nitrogen by both inoculated and noninoculated plants. On leached sandy-loam, alluvial and slightly acidic soil, field experiments showed that high rates of nitrogen reduced nodulation and increased the yield of soybean (11). Lyons and Earley (23) reported from their field study that rainfall and temperature conditions during the growing season apparently have a direct influence on the sufficiency of symbiotically fixed nitrogen for maximum yields, thus indirectly influencing the response to added nitrogen.

The quality of the soybeans is influenced by symbiotically fixed or applied nitrogen. Webber (33) showed that for nonnodulated soybeans applied nitrogen increased seed and dry matter yields, seed size, protein in seeds, plant weight, and lodging susceptibility, delayed maturity date and decreased oil content. But there were very small responses in nodulated lines with increasing nitrogen under the same conditions. He also suggested that symbiotic nitrogen was more biologically suitable for seed production than applied nitrogen.

The placement of applied nitrogen influences the inhibition of symbiotic nitrogen fixation. Harper and Cooper (17) noted that nodulation response was not strictly dependent upon internal nitrate content of the roots, indicating that application site may provide a means of partially alleviating the detrimental effect of applied nitrogen on nodulation. In other words, applied nitrogen may be less inhibitory to nodulation if not in direct contact with the nodulation zone. Under normal cultural practices the major development of nodules in soybeans occurs on roots near the soil surface (37).

Ghorashy *et al.* (14) reported no interaction differences between the response to nitrogen and inoculation treatments.

MATERIALS AND METHODS

Plot Design

Field experiments were in three locations during 1975 at the Oregon State University Vegetable Research Farm, Corvallis, on a Chehalis silt loam soil. The previous cropping history for location no. 1 was fallow or small grains for four or five years. Snap beans had been grown intermittently before then. In location no. 2 and 3, snap beans were grown as recently as two years before. The soil was plowed, tilled, and supplied with 60 kg phosphorus and 112 kg potassium per hectare banded at planting. The treatments were in a randomized block design with five replications. Each plot was a single row eight meters long, with one meter row spacing. Location no. 1 was planted on May 27 with a 2 (fungicide treatments) x 3 (inoculation methods) x 3 (nitrogen rates) factorial design. Location no. 2 was planted on June 10 with a 3 (nitrogen rates) x 3 (inoculation methods) design. Location no. 3 was planted on July 15 with a 2 (nitrogen rates) x 3 (inoculation methods) design. 'Oregon 1604' snap bean was used at all three locations, 'Takii's Extra Early' soybean was used at location no. 2, and 'Early Thorogreen' lima bean was used at location no. 3. For snap beans and lima beans 200 seeds were planted per plot, while 100 seeds of soybeans were planted per plot.

Seed Treatment

All seeds in location no. 3 were well coated with captan after slight moistening, and ready for inoculating or planting. At location no. 1 only half of the plots received the captan treatments. No seeds were treated with captan in location no. 2.

Inoculation

Materials for rhizobium inoculation were obtained from Nitragin Co., Milwaukee, Wisconsin. Rhizobium japonicum strain was used for soybeans, R. phaseoli for snap beans and lima beans. Two methods of inoculation, seedcoating and furrow treatments, were used for each crop. For the seedcoating method, 100 or 200 seeds were spread on a paper towel and slightly moistened with a light water spray. Then the moistened seeds and the powderlike seedcoating inoculant were added in a coin envelop and contents were shaken to obtain a uniform covering of inoculant on the seeds. In this way, the seeds were coated with inoculant and there was no loss of captan for those retreated. Seeds were planted by a belt planter after inoculating. In furrow treatments, the granular inoculant was put evenly with seeds on the planter belt and was deposited below the seeds in the seed furrow. Quantity of furrow inoculant per plot was 2.4 grams in location no. 1 and location no. 2, while 8.0 grams per plot were used for location no. 3. Rates of inoculant were slightly higher than those recommended by the supplier.

Nitrogen Applications

Ammonium nitrate was applied to supply 0, 17, and 84 kg nitrogen per hectare in location no. 1 and location no. 2, while 0 and 84 kg nitrogen per hectare were used in location no. 3. Materials were broadcast on the soil surface after planting. Sprinkler irrigation was supplied one or two days after planting. Soil samples were randomly taken 15 cm deep from check plots after planting and were analyzed by Oregon State University Soil Testing Laboratory. Stand counting was done after seedling emergence. Weeding was by hand as needed.

Color Rating of Foliage

Evaluation of leaf color was done about two weeks after planting. Scores ranged from one for light green or yellowish green to five for very dark green. Second ratings were about one month later. Only one rating was done in the soybean experiment because there were no noticeable color differences at the early stage.

Nodule Counting

After full bloom and beginning of formation of small pods, ten plants from each plot were carefully removed from the soil, leaving as much as possible of the upper root system intact. Roots were then washed over a fine screen to avoid nodule loss. Nodules were counted and the plants were weighed. Nodules were divided into two sizes: large (2 mm diameter and larger) and small (less than 2 mm in diameter).

Harvesting

Plots were harvested by hand once to simulate machine harvest. A center part of the row 3 m in length was harvested for yield. Location no. 1 was harvested on August 4, location no. 2 snap beans on August 19 and soybeans on September 20, and location no. 3 snap beans on September 22. Lima beans were not harvested because the late planting did not permit adequate maturity. Beans with pods were weighed and stand of the 3 m row was counted. Soybeans were harvested with pods when pods were fresh green. Snap bean pods were graded into various sieve sizes with a mechanical bean grader.

RESULTS

The results of soil analysis from the experimental plots are shown in Table 1. Range in pH was 5.9 to 6.0 for all three locations. Nitrogen soil tests ranged from 0.09 to 0.22 percent in location no. 1, from 0.14 to 0.16 percent in location no. 2, and from 0.25 to 0.20 percent in location no. 3. Phosphorus levels were lowest in location no. 2 and ranged from 27 to 28 ppm. Phosphorus levels were similar for locations no. 2 and no. 3 and ranged from 48 to 61 ppm. Potassium content was lowest in location no. 1 ranging from 156 to 186 ppm, highest in location no. 3 ranging from 268 to 304 ppm, and was from 216 to 274 ppm in location no. 2. Calcium content was also lowest in location no. 1 and ranged from 11.6 to 13.5 meq/100 g, highest in location no. 3 ranging from 16.5 to 17.7 meq/100 g, and was from 14.7 to 16.5 meq/100 g in location no. 2. Magnesium content ranged from 5.5 to 6.6 meq/100 g for location no. 1, 6.5 to 7.0 meq/100 g for location no. 2, and 6.4 to 6.9 meq/100 g for location no. 3.

Snap Beans

Location No. 1

The effects of fungicide, inoculation and nitrogen fertilizer on leaf color, yield, plant weight, and nodulation are shown in Table 2 and Table 3. Fungicide did not influence leaf color, but average

Table 1. Soil Mineral Analysis of Samples from Experimental Sites.

Site*	pH (%)	N (%)	P (ppm)	K (ppm)	Ca (meq/100 g)	Mg (meq/100 g)
Location no. 1						
Replication 1	5.9	0.22	27	174	13.1	6.1
2	5.9	0.11	28	174	13.5	6.6
3	5.9	0.11	27	186	13.1	6.3
4	6.0	0.10	27	180	12.5	6.1
5	6.0	0.09	27	156	11.6	5.5
Location no. 2						
Replication 1	6.0	0.16	49	274	14.7	6.5
2	5.9	0.15	48	216	16.5	7.0
3	5.9	0.15	48	216	16.1	7.0
4	6.0	0.14	58	240	16.3	6.9
5	5.9	0.14	52	244	15.9	6.9
Location no. 3						
Replication 1	5.9	0.15	54	268	16.9	6.6
2	5.9	0.15	55	280	17.1	6.5
3	5.9	0.16	50	286	17.7	6.9
4	5.9	0.15	61	304	16.7	6.4
5	6.0	0.20	54	292	16.5	6.6

*Samples were randomly taken 15 cm deep.

Table 2. Effects of Fungicide, Inoculation, and Nitrogen Fertilizer on Leaf Color, Yield, Plant Weight, and Nodulation of Snap Beans, Location no. 1.[a]

Treatments and L. S. D.	Leaf color[b]		Yield of pods			Wt/plt (g)	No. of nodules/plt	
	2 wks	6 wks	T/ha	per plt (g)	sieve size (% 1-4's)	after blooming	large[c]	small
Fungicide treatment								
with	3.5	4.0	11.79	39.05	80.32	50.14	14.3	27.8
without	3.4	3.9	9.28	66.67	73.62	73.91	19.2	43.5
N rates (kg/ha)								
0	2.6	3.0	8.89	53.05	75.87	53.27	21.4	33.2
17	3.5	3.9	10.89	49.66	77.20	60.40	18.9	41.9
84	4.3	5.0	11.82	55.87	77.84	72.41	9.9	26.8
Inoculation								
Check	3.5	3.8	10.39	56.87	75.71	59.71	15.2	32.8
Seedcoating	3.4	4.0	10.81	50.79	77.35	59.47	19.6	39.9
Furrow	3.5	4.0	10.41	50.93	77.86	66.90	15.5	34.3
L. S. D. at 5%[d]								
at 1%								
N rate	--	--	0.81	NS	--	9.90	5.5	11.9
			(1.08)	(NS)		(13.16)	(7.4)	(NS)
Fungicide	--	--	0.66	6.13	--	8.09	4.5	9.7
			(0.87)	(8.15)		(10.75)	(NS)	(12.9)

[a] Values are means of five replications with 18 treatments.

[b] Leaf color rated from 1 for light green to 5 for dark green.

[c] Nodules larger than 2 mm diameter.

[d] No significant difference was shown in inoculation treatments. Interactions are shown in Appendix Table 1.

Table 3. Effects of Fungicide, Inoculation, and Nitrogen Fertilizer on Leaf Color, Yield, Plant Weight, and Nodulation of Snap Beans, Location no. 1.[a]

Treatments and L. S. D.	Leaf color[b] 2 wks	Leaf color[b] 6 wks	Yield of pods T/ha	Yield of pods per plt (g)	Yield of pods sieve size (% 1-4's)	Wt/plt (g) after blooming	No. of nodules/plt large[c]	No. of nodules/plt small
With fungicide[d]								
N rates (kg/ha)								
0	2.7	3.1	10.22	33.65	80.83	40.33	15.1	32.1
17	3.5	3.9	12.11	40.64	78.51	51.40	19.1	34.3
84	4.4	5.0	13.03	42.85	81.62	58.69	8.7	17.0
Inoculation								
Check	3.5	3.9	12.33	39.67	79.80	49.09	11.5	26.4
Seedcoating	3.5	4.1	11.91	38.71	80.60	50.09	18.9	34.6
Furrow	3.4	3.9	11.12	38.77	80.56	51.24	12.4	22.4
Without fungicide[d]								
N rates (kg/ha)								
0	2.5	2.8	7.56	72.45	70.91	66.21	27.7	44.4
17	3.4	3.9	9.66	58.67	75.89	69.39	18.8	49.5
84	4.2	4.9	10.60	68.89	74.06	86.13	11.2	36.6
Inoculation								
Check	3.4	3.7	8.45	74.06	71.61	70.33	18.9	39.1
Seedcoating	3.3	3.9	9.71	62.87	74.09	68.85	20.2	45.2
Furrow	3.5	4.0	9.69	63.08	75.16	82.55	18.6	46.2
L. S. D. at 5% at (1%)								
N rate			1.15 (1.53)	10.62 (14.11)	--	14.00 (18.61)	7.8 (10.4)	16.9 (NS)
Inoculation	--	--	1.15 (1.53)	10.62 (14.11)	--	14.00 (18.61)	7.8 (NS)	16.9 (22.12)

[a] Values are means of five replications with 18 treatments.
[b] Leaf color rated from 1 for light green to 5 for dark green.
[c] Nodules larger than 2 mm diameter.
[d] Average number of plts/plot was 184 for fungicide treatments, and 85 for no fungicide treatments.

number of plants per plot was increased 118% by the fungicide treatment. The fungicide treatment significantly increased total (T/ha) yield (25%), but decreased yield per plant (41%), weight per plant (32%), and numbers of large nodules (26%) and small nodules (36%). The sieve size distribution showed that maturity was delayed by higher stand levels as a result of fungicide application than at lower stand levels where no fungicide was applied. Sieve sizes were not affected by nitrogen or inoculation treatments. Leaf color scores were higher in both ratings when nitrogen fertilizer was increased. The scores of the first observation were almost the same for different inoculation treatments, but in the second observation, inoculated treatments had higher scores than the check.

At the higher nitrogen fertilizer rate (84 kg/ha), yield (T/ha) and weight per plant were increased and number of large and small nodules were decreased, significantly. Highest yield per plant was obtained in 84 kg/ha nitrogen treatment, but differences in nitrogen treatments were not significant. Inoculation methods did not show any significant difference in observations made, but highest yield (T/ha) and numbers of large and small nodules occurred in the seed-coating treatment. Check plots had highest yield of pods per plant and the furrow treatment had highest weight per plant.

Interactions of fungicide, inoculation and nitrogen fertilizer are shown in Appendix Table 1. Inoculation treatments produced significantly lower yields (T/ha) with fungicide but higher yields without fungicide than that of the check (noninoculation) treatment. With the fungicide treatment, yield per plant was significantly highest in 84 kg/ha nitrogen treatment, but when no fungicide was used, the zero nitrogen treatment produced the highest yield per plant. Inoculation and nitrogen fertilizer interaction was significant in affecting number of small nodules at the 17 kg/ha nitrogen rate but not at other nitrogen rates.

Location No. 2

The effects of inoculation and nitrogen fertilizer on leaf color, yield, plant weight and nodulation are shown in Table 4. No fungicide was used and the average number of plants per plot was about 57 which indicated that only about one-fourth of the seeds emerged. Leaf color as influenced by applied nitrogen or inoculation followed the same trend as in location no. 1, except that the scores of the first rating were higher in this location.

Certain trends were noted although nonsignificant: yield (T/ha) and weight per plant were increased with increased nitrogen fertilizer and were highest when inoculated with the seedcoating method. Highest yield per plant and lowest percentage of 1-4 sieve sizes were at the 17 kg/ha nitrogen rate. Check plots with no inoculation had highest weight of pods per plant and highest percentage of pods in

Table 4. Effects of Inoculation and Nitrogen Fertilizer on Leaf Color, Yield, Plant Weight, and Nodulation of Snap Beans, Location no. 2 (no fungicide treatment).[a]

Treatments and L. S. D.	Leaf color[b]		Yield of pods			Wt/plt (g) after blooming	No. of nodules/plt	
	3 wks	6 wks	T/ha	per plt (g)	sieve size (% 1 - 4's)		large[c]	small
N rate (kg/ha)								
0	3.4	4.5	8.52	93.65	26.40	98.32	5.5	41.6
17	3.9	4.3	8.77	99.61	25.87	100.57	4.0	50.4
84	4.8	4.8	9.80	92.66	26.94	103.89	1.5	31.5
L.S.D. at 5%	--	--	NS	NS	--	NS	2.4	NS
at (1%)			(NS)	(NS)		(NS)	(3.2)	(NS)
Inoculation[d]								
Check	4.1	4.5	8.88	105.39	27.22	99.49	4.6	43.4
Seedcoating	3.9	4.6	9.57	91.02	24.94	111.08	3.2	45.1
Furrow	4.1	4.5	8.63	89.51	27.05	92.21	3.2	35.0

[a]Values are means of five replications with 9 treatments.

[b]Leaf color rated from 1 for light green to 5 for dark green.

[c]Nodules larger than 2 mm diameter.

[d]No significance was shown in inoculation treatment.

size 1-4. Number of large nodules was significantly decreased at 84 kg/ha nitrogen fertilizer rate. Nitrogen treatment did not significantly affect other results. However, there was a tendency for increased nitrogen to decrease nodulation while yield and plant weight were increased. The inoculation treatments did not show any significant difference from the check but the seedcoating treatment produced the highest yield, plant weight, and most nodulation. There was no significant interaction of nitrogen fertilizer and inoculaton, as shown in Appendix Table 2.

Location No. 3

Effects of inoculation and nitrogen fertilizer on leaf color, yield, plant weight, and nodulation are shown in Table 5. All seeds were treated with fungicide, and emergence was about 95%. Leaf color scores were higher when treated with nitrogen in both ratings but were not influenced by inoculation. Pod sieve size percentage of 1-4 was decreased with nitrogen fertilizer and highest in the seedcoating inoculation treatment.

Nitrogen treated plots produced significantly higher yield of pods (T/ha) and weight per plant, but fewer nodules per plant. No significant difference appeared in inoculation treatments, but the seedcoating treatment had the highest yield of pods (T/ha), yield per plant, weight per plant, and number of small nodules. Number of

Table 5. Effects of Inoculation and Nitrogen Fertilizer on Leaf Color, Yield, Plant Weight, and Nodulation of Snap Beans, Location no. 3 (all treated with fungicide). [a]

Treatments and L. S. D.	Leaf color[b]		Yield of pods			Wt/plt (g) after blooming	No. of nodules/plt	
	2 wks	7 wks	T/ha	per plt (g)	sieve size (% 1-4's)		large[c]	small
N rates (kg/ha)								
0	3.5	3.9	9.93	30.63	58.48	35.29	19.4	46.2
84	4.7	4.4	11.05	34.93	56.55	48.91	13.9	38.3
L.S.D. at 5%	--	--	1.01	NS	--	9.85	5.6	NS
at (1%)			(NS)	(NS)		(13.44)	(NS)	(NS)
Inoculation[d]								
Check	4.1	4.2	10.45	32.07	57.70	38.10	16.3	37.5
Seedcoating	3.9	4.1	10.90	34.02	59.46	45.23	16.6	43.8
Furrow	4.3	4.2	10.13	32.25	55.39	42.96	17.1	43.4

[a]Values are means of five replications with 6 treatments.

[b]Leaf color rated from 1 for light green to 5 for dark green.

[c]Nodules larger than 2 mm diameter.

[d]No significant difference was shown in inoculation treatments.

large nodules was highest in the furrow treatment but differences were not significant. There was no significant interaction between nitrogen fertilizer and inoculation in all observations, as shown in Appendix Table 3.

Soybeans

Location No. 2

Effects of inoculation and nitrogen fertilizer on leaf color, yield, plant weight, and nodulation are shown in Table 6. Germination was very good even without fungicide. Early stage leaf color did not show noticeable differences. Later stage leaf color was increased as nitrogen rate was increased, and was a little higher in the inoculated plots.

As nitrogen fertilizer was increased, numbers of large and small nodules were significantly decreased. Weight of plants was significantly higher when treated with 84 kg/ha nitrogen than at the 0 and 17 kg/ha rates. Highest pod yield (T/ha) and yield per plant were obtained in the 84 kg/ha nitrogen treatment too, but these were not significant. Numbers of large and small nodules were significantly highest in the furrow treatment. Though non-significant, the highest pod yield (T/ha), and yield per plant were obtained in the furrow

Table 6. Effects of Inoculation and Nitrogen Fertilizer on Leaf Color, Yield, Plant Weight, and Nodulation of Soybeans, Location no. 2 (no fungicide treatment).[a]

Treatments and L.S.D.	Leaf color[b] 8 wks	Yield of pods		Wt/plt (g) after blooming	No. of nodules/plt	
		T/ha	per plt (g)		large[c]	small
N rates (kg/ha)						
0	3.3	3.61	25.61	53.53	12.5	4.5
17	3.7	3.74	26.37	50.96	8.8	3.4
84	4.8	3.99	29.73	65.37	5.1	2.1
L.S.D. at 5%[d]	--	NS	NS	10.87	5.3	1.3
at (1%)		(NS)	(NS)	(NS)	(NS)	(1.8)
Inoculation						
Check	3.8	3.74	26.16	59.56	1.4	0.3
Seedcoating	3.9	3.68	27.16	56.01	5.0	1.7
Furrow	4.1	3.95	28.39	54.29	19.9	8.0
L.S.D. at 5%	--	NS	NS	NS	5.3	1.3
at (1%)		(NS)	(NS)	(NS)	(7.2)	(1.8)

[a]Values are means of five replications with 9 treatments.

[b]Leaf color rated from 1 for light green to 5 for dark green.

[c]Nodules larger than 2 mm diameter.

[d]Interaction of nitrogen x inoculation on number of small nodules is shown in Appendix Table 2.

treatment, but plant weight was highest in check plots with no inoculation.

There was a significant interaction of nitrogen fertilizer and inoculation treatment on number of small nodules: increased nitrogen rates significantly decreased number of small nodules in the furrow treatment but not in the seedcoating and check treatments, as shown in Appendix Table 4.

Lima Beans

Location No. 3

Effects of inoculation and nitrogen fertilizer on leaf color, plant weight, and nodulation are shown in Table 7. Scores of leaf color differed little with inoculation treatment but were higher with nitrogen treatment in both ratings.

Nitrogen treatment did not significantly affect number of nodules but the trend was for number of large nodules to be decreased and number of small nodules to be increased by nitrogen fertilizer. Weight per plant was significantly higher in nitrogen treated plots. Weight per plant, and numbers of large and small nodules were significantly higher for the furrow inoculation treatment than for the check and seedcoating treatments. Nitrogen and inoculation interaction was significant in weight per plant as shown in Appendix Table 5.

Table 7. Effects of Inoculation and Nitrogen Fertilizer on Leaf Color, Plant Weight, and Nodulation of Lima Beans, Location no. 3 (all treated with fungicide).[a]

Treatments and L.S.D.	Leaf color[b]		Wt/plt (g) after blooming	No. of nodules/plt	
	2 wks	8 wks		large[c]	small
N rates (kg/ha)					
0	4.1	3.9	25.48	5.5	3.8
84	4.9	4.7	29.49	3.8	4.6
L.S.D. at 5%[d]	--	--	2.81	NS	NS
at (1%)			(3.83)	(NS)	(NS)
Inoculation					
Check	4.5	3.7	24.20	0.5	0.1
Seedcoating	4.5	4.3	28.01	3.1	1.3
Furrow	4.4	4.9	30.24	10.3	11.2
L.S.D. at 5%	--	--	3.44	2.7	6.1
at (1%)			(4.69)	(3.7)	(8.4)

[a]Values are means of five replications with 6 treatments.

[b]Leaf color rated from 1 for light green to 5 for dark green.

[c]Nodules larger than 2 mm diameter.

[d]Interaction of nitrogen x inoculation on weight per plant is shown in Appendix Table 3.

In making comparisons between experiments, snap beans had the smallest nodules but highest number of nodules per plant while soybean nodules were largest on the average. Lima bean nodules were about the same size as those of soybeans but lima beans usually had the lowest number of nodules.

DISCUSSION

Soil pH, mineral content, and environmental factors appeared to be favorable for rhizobial growth in these experiments but snap bean nodulation was not significantly increased by inoculation methods (there was a slight increase in some cases). However, number of nodules per plant for soybeans and lima beans was significantly increased by seedcoating or furrow inoculation treatments of rhizobia.

A larger amount of furrow inoculant was used for snap beans in location no. 3 than in locations no. 1 and no. 2, but the nodulation was not increased by the higher amount of inoculant. Thronton (30) suggested that adding excessive bacteria gave no increase in nodulation. No significant difference was shown by methods of inoculation on snap bean nodulation, but a little higher nodulation was obtained by the seedcoating method. The efficiency of the inoculation methods then was dependent upon crops.

There was no consistent relationship between yield and number of nodules per plant when no nitrogen fertilizer was applied. The higher nitrogen rate of 84 kg/ha usually produced the highest yield of crops but consistently reduced the number of nodules produced compared to the 0 and 17 kg/ha nitrogen rates. In field studies with soybeans it has also been found that preplant application above about 50 kg/ha have depressed nodulation (19).

The 17 kg/ha nitrogen rate tended to increase nodulation but it was not statistically significant. A small amount of applied nitrogen was reported to be helpful to initial growth and vigor which resulted in higher nodulation (4, 8, 18). Up to 50 ppm of nitrate nitrogen (15) and 11 kg/ha nitrogen (20) was suggested to be beneficial to nodulation.

Results of snap bean and lima bean leaf color ratings appear to indicate that early plant growth depended on fertilizer while later plant growth was more influenced by fixed nitrogen which was affected by the early growth of plants. A similar tendency was also reported by Hoover and Beard (19).

Snap Beans

The leaf color, yield, and plant weight were significantly increased with increased nitrogen fertilizer even though nodulation was decreased. The snap bean nodules did not appear to contribute much in providing nitrogen for plant growth and yield.

The average nodule size was small, and the activity was only about 1/100 of that of soybean nodules, as shown in Appendix Table 6, and as CIAT (9) reported, the fixation period for most bean cultivars was short. At 18-20^{o}C few cultivars initiated fixation prior to 28 days after planting, and in most, activity began to decline at about 10 weeks. This period compares unfavorably with the 90-105 day fixation period reported for soybean. For these reasons the benefit of nodules to snap bean yield probably is not as great as to the soybean yield.

There was no assessment of rhizobial populations or strains in soils at the various locations or measure of the effectiveness of the commercial inoculant used. Snap beans had not been grown during the preceding five or six years at location no. 1, although some were grown prior to that. In locations no. 2 and no. 3, snap beans were produced about two years previously. There were no large differences in number of nodules per plant for uninoculated treatments at the three locations when plant spacing was comparable.

Only one cultivar of snap beans, 'Oregon 1604', was used. It was developed in the Oregon State University snap bean breeding program under local conditions of generally cool temperatures, with adequate irrigation and nitrogen fertilizer applications. Different species, cultivars and accessions show variable nitrogen fixation ability and some of the indeterminate or climbing types appear to be more efficient than the shorter season, determinate types (9). Emphasis is being placed in some improvement programs on location or development of more effective strains of rhizobia and more responsive cultivars to inoculation. If these efforts are successful and increased nodulation will increase yields, then inoculation will probably become more of a widespread practice than it is now. In most of the snap bean growing areas of the U.S. current practice is to apply

20 to 60 kg nitrogen per hectare and seeds are not inoculated.

Average yield of snap bean pods was 27 percent higher for the seed treatment with captan fungicide than with no fungicide treatment in location no. 1. The major reason appears to be that plant stands were markedly reduced when no fungicide was used. The trend was for inoculation to decrease yield in the fungicide treatment and increase yield slightly when no fungicide was applied.

Effects of fungicide on nodulation are not clearly demonstrated because of the differential in plant spacing or plant population caused by fungicide treatment. Earlier work by Curley and Burton (12) indicated that rhizobia was incompatible with captan and this may also be indicated by nodulation data from location no. 1. However, larger plants at the lower population density produced more nodules per plant than at the higher population when fungicide was used. Also, if incompatibility were a major problem, the furrow treatment, where there was less contact with rhizobia inoculant with the fungicide, should have produced a greater number of nodules per plant than the seedcoating treatment. This was not the case. In location no. 3, fungicide was used for all treatments and there was no advantage of the furrow treatment over seedcoating in affecting nodulation. Further work is needed to clarify the incompatibility question.

Soybeans

Nodulation increased with inoculation and significantly decreased with nitrogen fertilizer. The nodule activity was in the normal range reported for soybean nodules, as shown in Appendix Table 7, but the leaf color and yield were not related to nodulation. Only plant weight was significantly increased when treated with 84 kg/ha nitrogen. The early stage leaf color was not affected by the applied nitrogen. Neither higher nitrogen fertilizer nor more nodules resulted in better plant growth and seed yield. Response of 'Takii's Extra Early' soybean for best plant growth and seed development to local climatic conditions has not been determined but temperatures may have been too cool.

Weaver and Frederick (31) suggested that tap root nodulation of soybean is a qualitative characteristic that may be useful in determining the adequacy of nodulation. Counting of tap root nodules in addition to regular nodule counting may be of value in future studies.

Lima Beans

Either applied nitrogen or inoculation increased leaf color and plant weight, but no pod yields were taken. The furrow treatment produced highest plant weight and nodule number, and was higher than the higher nitrogen rate. It is also not clear why the furrow treatment was much better than the seedcoating treatment.

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APPENDIX

Appendix Table 1. Interactions of Fungicide (F), Inoculation (I), and Nitrogen Fertilizer (N) on Yield, Plant Weight, and Nodulation of Snap Beans, Location no. 1.[a]

Treatments and L. S. D.	Yield of pods T/ha	Yield of pods per plt (g)	Wt/plt (g) after blooming	No. of nodules/plt large	No. of nodules/plt small
$F_1I_0N_0$	10.74	34.11	40.66	14.4	40.2
$F_1I_0N_{17}$	12.40	39.46	40.20	14.1	25.1
$F_1I_0N_{84}$	13.83	45.45	66.40	6.0	14.1
$F_1I_cN_o$	9.95	32.11	41.30	17.9	34.6
$F_1I_cN_{17}$	12.58	40.19	52.82	26.6	49.7
$F_1I_cN_{84}$	13.16	43.82	56.16	12.3	19.4
$F_1I_fN_0$	9.95	34.75	39.04	12.8	21.6
$F_1I_fN_{17}$	11.37	42.28	61.18	16.4	28.1
$F_1I_fN_{84}$	12.06	39.28	53.50	7.9	17.6
$F_oI_oN_o$	7.22	70.94	66.96	22.0	42.7
$F_oI_oN_{17}$	9.51	67.32	57.06	15.1	23.6
$F_oI_oN_{84}$	8.63	83.92	86.98	19.4	51.0
$F_oI_cN_o$	7.56	72.21	62.24	35.4	54.4
$F_oI_cN_{17}$	9.84	52.25	59.10	17.9	51.0
$F_oI_cN_{84}$	11.73	64.16	85.20	7.3	30.2
$F_oI_fN_o$	7.91	74.21	69.42	25.6	36.1
$F_oI_fN_{17}$	9.66	56.43	92.02	23.5	73.9
$F_oI_fN_{84}$	11.46	58.61	86.22	6.8	28.6
L.S.D. at 5%					
IxF	1.14	NS	NS	NS	NS
IxN	NS	NS	NS	NS	20.7
FxN	NS	10.62	NS	NS	NS

[a]Values are means of five replications.

Appendix Table 2. Interactions of Nitrogen Fertilizer (N), and Incoulation (I) on Yield, Plant Weight, and Nodulation of Snap Beans, Location no. 2.[a]

Treatments	Yield of pods T/ha	Yield of pods per plt (g)	Wt/plt (g) after blooming	No. of nodules/plt large	No. of nodules/plt small
I_oN_o	7.62	90.81	93.02	7.2	45.4
I_cN_{17}	8.72	114.31	105.50	5.1	50.0
I_oN_{84}	10.29	111.04	99.94	1.5	34.8
I_cN_o	10.13	91.99	109.46	4.9	42.1
I_cN_{17}	8.52	91.36	110.82	2.3	55.9
I_cN_{84}	10.09	89.72	112.96	2.3	37.2
I_fN_o	7.85	98.16	92.48	4.4	37.3
I_fN_{17}	9.06	93.17	85.38	4.6	45.3
I_fN_{84}	9.01	77.20	98.76	0.7	22.4

[a]Values are means of five replications. No significant difference was shown on interactions.

Appendix Table 3. Interactions of Inoculation (I), and Nitrogen Fertilizer (N) on Yield, Plant Weight, and Nodulation of Snap Beans, Location no. 3.[a]

Treatments	Yield of pods T/ha	Yield of pods per plt (g)	Wt/plt (g) after blooming	No. of nodules/plt large	No. of nodules/plt small
I_oN_o	9.93	30.48	30.62	18.1	35.8
I_oN_{84}	10.96	33.66	45.58	14.5	39.2
I_cN_o	10.36	32.30	37.98	22.2	57.2
I_cN_{84}	11.46	35.75	52.48	10.9	36.5
I_fN_o	9.51	29.12	37.26	18.0	45.6
I_fN_{84}	10.76	35.38	48.66	16.2	39.1

[a]Values are means of five replications. No significant difference was shown on interactions.

Appendix Table 4. Interactions of Nitrogen Fertilizer (N), and Inoculation (I) on Yield, Plant Weight, and Nodulation of Soybeans, Location no. 2.[a]

Treatments	Yield of pods T/ha	Yield of pods per plt (g)	Wt/plt (g) after blooming	No. of nodules/plt large	No. of nodules/plt small
I_oN_o	4.26	23.50	57.0	1.5	0.4
I_oN_{17}	3.61	25.40	45.4	1.3	0.2
I_oN_{84}	4.28	29.57	76.4	1.5	0.3
I_cN_o	3.59	24.31	52.2	8.5	2.4
I_cN_{17}	3.59	26.94	53.9	3.0	1.2
I_cN_{84}	3.86	30.21	61.9	3.5	1.4
I_fN_o	3.95	29.03	51.4	27.5	10.7
I_fN_{17}	4.04	26.76	61.4	22.0	8.7
I_fN_{84}	3.86	29.39	57.8	10.3	4.7
IxN L.S.D. at 5%	NS	NS	NS	NS	2.3
at 1%	NS	NS	NS	NS	(3.1)

[a]Values are means of five replications.

Appendix Table 5. Interactions of Inoculation (I) and Nitrogen Fertilizer (N) on Plant Weight and Nodulation of Lima Beans, Location no. 3.[a]

Treatments and L.S.D.	Wt/plt (g) after blooming	No. of nodules/plt large	No. of nodules/plt small
I_oN_o	22.44	0.6	0.1
I_oN_{84}	25.96	0.4	0.1
I_cN_o	23.54	4.2	1.2
I_cN_{84}	32.48	2.0	1.3
I_fN_o	30.46	11.6	10.0
I_fN_{84}	30.02	9.0	12.4
IxN L.S.D. at 5%	4.86	NS	NS

[a]Values are means of five replications.

Appendix Table 6. Snap Bean Nodule Nitrogenase Activity at 26°C.[a]

Replication	n moles[b] C_2H_4 formed per GFN hr.	
	Control	Furrow inoculated
1	136.41	117.86
2	80.79	61.93
3	80.36	94.69
4	105.56	no material
Mean ±	100.78 ±26.51	91.49 ±28.10

[a] Samples were taken at full bloom in Location no. 2 without nitrogen applied plots, and determined by the acetylene reduction activity. Values are average of 3 plts. Nitrogenase activity determined by Dr. T. M. Ching, Crop Science Dept., OSU.

[b] 1 n mole = 1×10^{-9} moles.

Appendix Table 7. Soybean Nodule Nitrogenase Activity at 26°C.[a]

Treatment	n mole[b] C_2H_4 per GFN hr.
Control	1.84×10^4
Furrow inoculated w/o N	1.26×10^4
Seedcoating w/o N	9.53×10^3

[a] Samples were taken at full bloom determined by the acetylene reduction activity. Values are average of 3 plts. Nitrogenase activity determined by Dr. T. M. Ching, Crop Science Dept., OSU.

[b] 1 n mole = 1×10^{-9} moles.