Effects of Certain Mineral Nutrients on Growth and Nitrogen Fixation of Inoculated Bean Plants, *Phaseolus vulgaris* L.

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Growth, pod yields, and nitrogen fixation of effectively inoculated bean plants were greatly enhanced in greenhouse sand cultures by high levels of phosphorus, potassium, calcium, and magnesium. The beneficial effects from high levels of mineral nutrients were most pronounced when the plants were grown in the presence of moderate amounts of inorganic nitrogen. Symbiotic nitrogen fixation was also increased by the presence of 200 p.p.m. of combined nitrogen in the sand cultures. Increased levels of mineral nutrients did not improve nitrogen fixation in bean plants nodulated by ineffective rhizobia, regardless of the presence or absence of inorganic nitrogen. High levels of mineral nutrients were favorable to uptake of added nitrogen by both inoculated and noninoculated plants.

Leguminous plants grown in the presence of adequate nutrients usually accumulate greater concentrations of basic mineral elements in their tissues than do nonleguminous plants (*4*, *7*). High concentrations of calcium, magnesium, phosphorus, and potassium in plant tissue are usually associated with a high nitrogen content (*12*, *15*). In the case of inoculated legumes, the high nitrogen content could result from stimulation of symbiotic nitrogen fixation by the high levels of mineral nutrients furnished the host plant.

Experiments were conducted to study effects of various levels of phosphorus, potassium, calcium, and magnesium on nitrogen fixation by the common bean (*Phaseolus vulgaris* L.) when inoculated with effective and with ineffective strains of rhizobia. The latter were included to determine the relationship, if any, between rhizobia efficiency and mineral nutrition of the host plant.

Phosphorus and Potassium Levels

Materials and Methods. In greenhouse experiments, stringless, green-pod beans were grown in 2-gallon glazed earthenware jars filled with 10 kg. of fine quartz sand. Each jar contained a glass watering tube which extended to the basal substrate. The lower end of this tube was wrapped with a small piece of borosilicate glass wool to prevent clogging. The jars containing the sand were autoclaved for 12 hours at 250° F. Nutrient solutions as shown in Table I were used.

A volume of 1800 ml. of nutrient solution was added to each jar. This was sufficient to provide three fourths of the total water-holding capacity of the sand. Moisture content was approximately maintained throughout the growth period by addition of distilled water as needed.

The mode of surface-sterilizing the seed has been described (*6*). Fifteen surface-sterilized seeds were planted, but only 10 plants were allowed to grow in each jar. There were four replicates in each treatment. One series of plants was inoculated with effective rhizobia, another with ineffective rhizobia, and a third noninoculated series served as controls. Bean yields, dry weights, and nitrogen contents of the plants were determined after a 7-week growth period.

Results. At the end of 4 weeks, the effectively nodulated plants were dark green in color, whereas the noninoculated plants and those with ineffective rhizobia showed nitrogen starvation.

At harvest, after 7 weeks of growth, the dry weights, pod yields, and nitrogen contents of the ineffectively inoculated plants were not appreciably different from those of similarly fertilized, noninoculated plants (Table II). This was to be expected, inasmuch as nitrogen was a growth-limiting element in all cases.

The plants supplied with ammonium nitrate showed symptoms of acute phosphorus deficiency at the 10-p.p.m. phosphorus level. Growth was stunted; the lower leaves were yellow, while the upper leaves were bluish green. These symptoms did not occur in plants that received 100 p.p.m. of phosphorus. The high levels of phosphorus and potassium were beneficial to nitrogen uptake by both inoculated and noninoculated plants.

At harvest time, the noninoculated and ineffectively inoculated plants which received 100 p.p.m. of phosphorus and 150 p.p.m. of potassium contained 62 and 72% more total nitrogen, respectively, than did those which received lesser amounts of these elements. The total nitrogen content of the plants which received ineffective strains of rhizobia did not differ from that of the noninoculated plants.

All of the effectively inoculated plants showed symptoms of phosphorus deficiency at the 10-p.p.m. phosphorus level, regardless of the presence or absence of ammonium nitrate; however, the symptoms were most pronounced in the former case. At harvest, there were no marked differences in dry weight or nitrogen content attributable to phosphorus level, except where ammonium nitrate was present. Here the plants in treatment 6 which received 100 p.p.m. of phosphorus had approximately 50% greater dry weight and

Table I. Nutrient Combinations and Concentrations

Treatment[a] No.	Element, P.P.M.		
	P[b]	K[c]	N[d]
1	10	15	0
2	10	150	0
3	100	15	0
4	100	150	0
5	10	15	500
6	100	150	500

[a] Secondary elements were added uniformly in all treatments. Elements and concentrations, p.p.m.: Calcium, 150; magnesium, 50; iron, 15; boron, 0.5; copper, 0.1; manganese, 1.0; zinc, 0.4; molybdenum, 0.02. All nutrient solutions were adjusted to approximately pH 6.8 with NaOH solution and autoclaved at 250° F. for 0.5 hour before adding to the sand.
[b] Source, H_3PO_4.
[c] Source, K_2SO_4.
[d] Source, NH_4NO_3.

Table III. Effect of Levels of Phosphorus and Potassium on Growth and Nitrogen Content of Inoculated Bean Plants

(Data are averages of four replicates, 10 plants each)

Treatment No.[a]	Pods: Green wt., g.	Pods: Total N, mg.	Total Plants[b]: Dry wt., g.	Total Plants[b]: Total N, mg.	Nitrogen fixed,[c] mg.
		Not Inoculated			
1	5.5	15	6.4	86	
2	2.3	6	6.2	68	
3	3.0	6	8.2	91	
4	4.5	8	8.2	85	
5	9.9	35	9.7	246	
6	29.9	103	14.1	400	
		Inoculated with Ineffective Strains			
1	4.4	12	7.0	105	
2	3.5	8	5.9	72	
3	3.8	8	8.7	99	
4	3.4	7	8.3	96	
5	16.7	59	9.8	227	
6	21.7	71	14.3	390	
		Inoculated with Effective Strains			
1	13.3	38	10.1	235	149
2	11.1	33	10.2	195	127
3	18.8	61	11.9	276	185
4	21.7	67	11.4	208	123
5	16.5	64	11.5	345	99
6	45.2	170	18.7	589	189

[a] See Table I for composition of treatment solutions.
[b] Includes roots.
[c] Data on nitrogen fixed were obtained by subtracting corresponding figures for total nitrogen in noninoculated series from those obtained with effective strains. Apparently the ineffective rhizobia caused no nitrogen fixation.

Table II. Nutrient Concentrations and Combinations

Treatment[a] No.	Element, P.P.M.: Mg[b]	Ca[c]	N[d]
1	25	100	0
2	100	300	0
3	200	400	0
4	25	100	200
5	100	300	200
6	200	400	200
7	25	100	400
8	100	300	400
9	200	400	400

[a] Phosphorus and potassium levels, 100 p.p.m. and 150 p.p.m., respectively, were uniform in all treatments. Concentrations of trace elements were the same as given in [a] Table I.
[b] Source. $MgSO_4 \cdot 7H_2O$.
[c] Source. $CaCl_2 \cdot 2H_2O$; $Ca(NO_3)_2 \cdot 4H_2O$.
[d] Source. KNO_3, $NaNO_3$, $Ca(NO_3)_2 \cdot 4H_2O$.

nitrogen content than those supplied with 10 p.p.m. of phosphorus. Green pod yields were increased 41% in treatment 3 and 174% in treatment 6 as a result of increasing the phosphorus level from 10 to 100 p.p.m.

In treatments 1 to 4, where nitrogen was the limiting element of growth, 15 p.p.m. of potassium were apparently adequate for growth. There was no beneficial effect from increasing the potassium level from 15 to 150 p.p.m.

On the other hand, with plentiful nitrogen, the effectively inoculated plants that received the upper levels of potassium and phosphorus in treatment 6 contained 70% more total nitrogen and produced 65% greater pod yields than did those provided with the lower dosages. These increases were considerably above those of the noninoculated and ineffectively nodulated plants in similarly treated series. It appears that the effectively nodulated plants fixed more atmospheric nitrogen when grown in the presence of inorganic nitrogen.

Calcium and Magnesium Levels

The growth and nitrogen contents of the effectively inoculated plants in treatments 1 to 4 (Table II) were surprisingly low as compared to those obtained in earlier studies where vermiculite was used as the substrate (*6*). In these studies, effectively nodulated bean plants grown without inorganic nitrogen contained 600 mg. of nitrogen per 10 plants, whereas in the foregoing experiment using quartz sand, the plants contained less than one half of this amount. Since vermiculite contains about 22% of magnesium oxide (*2*), it was suspected that the 50 p.p.m. of magnesium supplied in the sand cultures was inadequate.

Experimental Procedure. To explore the possibility that magnesium was a determining factor, three levels—25, 100, and 200 p.p.m.—of magnesium were established in a second pot culture experiment similar to the preceding one, except as indicated. Because of the importance attached to the necessity for a satisfactory calcium-magnesium ratio (*9*, *10*) and also because of the possible synergistic (*1*, *16*) and antagonistic (*5*, *8*) absorption effects of these elements on one another, the calcium portion was varied to provide calcium–magnesium ratios of 4 to 1, 3 to 1, and 2 to 1. The nutrient combinations are shown in Table III.

The basic salts used in preparing nutrient solutions, KH_2PO_4, KNO_3, and $Ca(NO_3)_2$, were used at the maximum level possible without exceeding the desired concentration of any particular element. Calcium in treatments 1 to 3 which received no combined nitrogen was provided by $CaCl_2$. In the high nitrogen treatments, $NaNO_3$ provided the needed extra nitrogen. All magnesium was supplied as $MgSO_4.7H_2O$. Concentration of sodium was kept to a minimum.

All nutrient solutions were adjusted to approximately pH 6.8 with 0.1N NaOH or H_2SO_4 as needed. The methods of surface-sterilizing seed and planting have been described. One series of plants was inoculated with effective strains of rhizobia and the other with ineffective strains.

Results. All plants were uniformly green and thrifty up to the fifth week, when yellowing began in the ineffectively inoculated plants grown without added nitrogen. When harvested at 7 weeks, all of the ineffectively inoculated plants showed symptoms of nitrogen starvation.

The data (Table IV) give no indication that high levels of calcium and magnesium increase nitrogen fixation in ineffectively inoculated plants. Growth and nitrogen content of plants which received 100 p.p.m. of magnesium and 300 p.p.m. of calcium did not differ from those of plants which received greater amounts of these elements.

Uptake of fertilizer nitrogen by ineffectively inoculated plants was increased by raising the levels of magnesium from 25 to 100 p.p.m. and of calcium from 100 to 300 p.p.m. The starting levels of 25 p.p.m. of magnesium and 100 p.p.m. of calcium are apparently too low for maximum uptake of nitrogen from the substrate. Nitrogen content of plants furnished moderate and high levels of magnesium and calcium were directly related to amounts of nitrogen added to the jars.

The effectively inoculated plants remained green throughout the growth period, although there was considerable variation in height and vigor due to the different treatments. In general, plants which received inorganic nitrogen grew faster and more luxuriantly than the others.

The effects of levels of calcium and magnesium on growth and nitrogen content of the effectively nodulated plants varied in respect to the level of nitrogen. In the series which received no inorganic nitrogen, total growth and total nitrogen content of plants were uniform regardless of the levels of calcium and magnesium (Table IV). On the other hand, where 200 p.p.m. of nitrogen were added, high levels of calcium and magnesium had a decided beneficial effect on growth and total nitrogen in plants. Plants which received 100 p.p.m. of magnesium and 300 p.p.m. of calcium contained more than twice as much nitrogen as did those receiving only 25 p.p.m. of magnesium and 100 p.p.m. of calcium. In treatment 6, where 200 p.p.m. of magnesium and 400 p.p.m. of calcium were provided, the plants contained almost three times as much nitrogen as did those in treatment 4 (see Figure 1 for growth).

Growth of the effectively inoculated plants which received 400 p.p.m. of nitrogen was no better than that of plants furnished only 200 p.p.m. of this element. The growth under treatment 9, where 200 p.p.m. of magnesium and 400 p.p.m. of calcium were applied along with 400 p.p.m. of nitrogen, was inferior to that under treatment 6, where only 200 p.p.m. of nitrogen were added. This was possibly due to a depression in the uptake of potassium caused by the high concentration of calcium and magnesium and the consequent imbalance between nitrogen and potassium. In treatment 6, potassium uptake was probably also depressed, but the nitrogen-potassium balance was not upset because of the smaller quantity of nitrogen added.

Differences in pod yields resulting from the high calcium-magnesium treatments were more marked than was total growth, for both the effectively and ineffectively inoculated plants. As evidenced in treatments 2, 5, and 8 of the effectively inoculated series, green weights of the pods from plants given 300 p.p.m. of calcium and 100 p.p.m. of magnesium were 171, 203, and 1050% higher at the 0-, 200-, and 400-p.p.m. nitrogen levels, respectively, than were those of the same series of plants which received smaller amounts of these elements (treatments 1, 4, and 7).

The highest yield of green pods was obtained from effectively inoculated plants which received 400 p.p.m. of calcium, 200 p.p.m. of magnesium, and 200 p.p.m. of nitrogen. Lowest pod yields in this effectively inoculated series resulted from applications of 400 p.p.m. of combined nitrogen, 100 p.p.m. of calcium, and 25 p.p.m. of magnesium, treatment 7. Large amounts of nitrogen, coupled with inadequate supplies of calcium and magnesium, depressed pod formation.

Table IV. Effect of Levels of Magnesium and Calcium on Growth and Nitrogen Fixation by the Bean Plant

(Data are averages of four replicates, 10 plants each)

Treatment No.[a]	Pods: Green wt., g.	Pods: Total N, mg.	Total Plants[b]: Dry wt., g.	Total Plants[b]: Total N, mg.	Total Plants[b]: Nitrogen fixed,[c] mg.
Inoculated with Ineffective Strains					
1	2.7	4	7.8	95	
2	2.9	5	8.0	93	
3	3.2	5	9.7	127	
4	2.8	7	14.5	210	
5	11.5	25	14.8	186	
6	9.6	17	17.5	216	
7	3.4	8	10.9	193	
8	25.8	69	24.3	399	
9	23.7	57	22.9	327	
Inoculated with Effective Strains					
1	8.4	25	13.4	395	300
2	22.8	76	15.2	382	289
3	19.5	66	14.2	384	257
4	9.6	27	13.9	253	43
5	29.1	96	20.8	551	365
6	39.2	110	25.0	658	442
7	3.0	7	15.6	320	127
8	34.8	134	23.4	516	117
9	31.0	76	23.0	369	42

[a] Table III for composition of treatment solutions.
[b] Includes roots.
[c] Data on nitrogen fixed were obtained by subtracting corresponding figures in ineffective inoculated series from those obtained with effective strains.

Figure 1. Effect of varying levels of calcium and magnesium on growth of effectively inoculated bean plants.

Levels of phosphorus, 100 p.p.m., potassium, 150 p.p.m., and nitrogen, 200 p.p.m. were uniform for all treatments. Age of plants, 7 weeks.

Mg 25 Ca 100 | Mg 100 Ca 300 | Mg 200 Ca 400

Discussion

The results of this study emphasize several important considerations in the culture of garden beans. Conclusive evidence is given that properly nourished bean plants are highly benefited by nodulation with effective rhizobia. The desirability of using inoculants in bean culture has not always been fully appreciated by bean growers. No evidence was obtained that the nitrogen-fixing ability of ineffective rhizobia was improved by raising the levels of phosphorus, potassium, calcium, and magnesium furnished the host plant.

Results also signify that the best growth response to effective inoculation and increased mineral nourishment was obtained with a treatment of 200 p.p.m. of inorganic nitrogen. With this nitrogen plants grew more rapidly and had larger leaves than did those grown without it. Presumably, the denser foliage and, hence, increased photosynthetic capacity of the plants were beneficial to symbiotic nitrogen fixation. No improvement in plant growth or total nitrogen content of the plants resulted from increasing the level of inorganic nitrogen from 200 to 400 p.p.m. It appears that high levels of inorganic nitrogen suppress symbiotic nitrogen fixation.

Apparently, the bean plant requires rather large amounts of divalent cations for maximum growth and best response to inoculation with rhizobia. Further study to determine a proper balance between potassium and the divalent cations, calcium and magnesium, appears warranted.

A point of practical importance to bean growers is the beneficial effect of high levels of calcium, magnesium, and phosphorus on pod yields. Increasing the level of phosphorus from 10 to 100 p.p.m. caused a 41% increase in pod yield from effectively inoculated plants, although the total nitrogen content of the plants was not materially affected (Table II). The responses in pod yields resulting from high levels of calcium and magnesium were even more spectacular. Plants furnished 300 p.p.m. of calcium and 100 p.p.m. of magnesium produced almost three times the yield of green pods harvested from those which received only 100 p.p.m. of calcium and 25 p.p.m. of magnesium.

In general, the results reported here agree with those obtained with other leguminous plants. Roberts and Olsen (*14*) showed that inoculated red clover plants fixed little nitrogen in the absence of ample potassium. Peanut plants grown on fertile soils fixed more nitrogen than did those grown on poor soils (*13*). Lynch and Sears (*11*) noted improved efficiency of rhizobia of *Lotus corniculatus* as a result of high fertility treatments given the host plant. In experiments of a similar nature, Ash (*3*) showed that alfalfa grown in the presence of 100 and 200 p.p.m. of phosphorus and potassium, respectively, fixed the largest amounts of nitrogen. On the other hand, the nitrogen-fixing ability of an ineffective strain was improved little by high fertility treatments.

All of the afore-mentioned results seemingly point to the conclusion that both effective rhizobia and adequate plant food are essential for best nitrogen fixation. Whereas nitrogen-fixing abilities of mediocre strains may be improved by high fertility treatments, fertilization is not a good substitute for effective rhizobia, or vice versa.

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INSECTICIDE ANALYSIS

The Colorimetric Determination and Paper Chromatography of Some Aromatic Carbamates

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The formation of naphthol blue from 1-naphthol and *p*-nitrobenzenediazonium fluoborate has been used as the basis of a colorimetric method of analysis for Sevin (1-naphthyl *N*-methylcarbamate) (*3*). Since there are many other compounds which will react with a suitable diazonium salt to produce a colored product, this study was undertaken to establish a method of analysis for similar carbamate or other pesticidal compounds having a potentially reactive moiety. Four cyclic carbamates (Geigy Chemicals Co.) which have such a potentially reactive moiety were obtained. Upon hydrolysis, Isolan [dimethyl 5-(1-isopropyl-3-methyl pyrazolyl) carbamate], Dimetilan [dimethyl 5-(3-methyl pyrazolyl) carbamate], and Pyrolan [dimethyl 5-(1-phenyl-3-methyl pyrazolyl) carbamate] yield pyrazols. The fourth insecticidal carbamate, Dimetan (dimethyl 5,5-dimethyl-3-oxo-1-cyclohexen-1-yl carbamate) hydrolyzes to the 5,5-dimethyldihydroresorcinol. Another insecticidal carbamate, H-5727 (3-isopropyl phenyl *N*-methylcarbamate) (Hercules Chemical Co.), yields a reactive phenol. The moieties released by hydrolysis are coupled with *p*-nitrobenzenediazonium fluoborate to give colored products suitable for colorimetric estimation.

Although the diazo coupling reaction is not a general one (*4*), several examples of phenols, which will react under the conditions outlined to give a suitable colored compound, are given in Table I. Carbamates or other pesticides containing these phenols may thus be determined by this method. In general, it is first necessary to unmask the phenol by