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Nitrogen levels and *Rhizobium* inoculation and yields of native white bean (*Phaseolus vulgaris* L.)[1]

Gerardo Mangual Crespo, Robert Kluson and Eduardo C. Schröder[2]

ABSTRACT

The combined effect of N fertilization and *Rhizobium* inoculation on bean (*Phaseolus vulgaris* L., cv. Bonita) nodulation and yield was studied in the semi-arid southern region of Puerto Rico. Six N treatments: 0, 22, 45, 90, 180 and 360 kg/ha were tested in inoculated and noninoculated plots arranged in a split-plot design with 4 replications. The application of 22 kg/ha of N in the inoculated plots increased plant nodulation 4 and 8 weeks after planting. Higher rates of N fertilization reduced the number and size of bean nodules at both sampling dates. On the other hand, dry beans consistently increased with applications of 0 to 180 kg/ha of N in the presence of *Rhizobium*.

INTRODUCTION

Beans, *Phaseolus vulgaris* L., are an important grain legume worldwide. In the Caribbean Basin and Central America, beans are the main source of vegetable protein and together with rice form the basis of many traditional dishes.

The majority of bean producers are small farmers with limited economic resources using a subsistence farming system. Under these conditions yields are usually low ranging from 400 to 600 kg/ha in Latin America and averaging about 33% of U.S. yields. On the other hand, Latin America is the leading bean producing region, with approximately 4 x 10^6 metric tons per year or 35% of the total world production (1).

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[2] Associate Horticulturist (Retired), Department of Horticulture; Research Assistant; and Associate Professor, respectively, Department of Agronomy and Soils, College of Agricultural Sciences, University of Puerto Rico, Mayagüez, P. R.

As legumes, beans are involved with the biological fixation of atmospheric nitorgen (N) through a symbiosis with *Rhizobium phaseoli* bacteria. The maximization of the N fixing system would contribute to overcome the problem of low N content in tropical soils which affect bean yields. Because of the limited economic resources available on small farming systems, bean inoculation with *R. phaseoli* strains is one management practice that could be instituted.

However, it is well recognized that the bean-*Rhizobium* symbiosis is generally low in the proportion of fixed N because of poor nodule production and a short cycle of N fixing activity (5, 9). On the other hand, greenhouse studies on N fixation in beans have demonstrated the importance of cultivar-strain interactions on nodulation and yield responses. Field inoculation trials of *R. phaseoli* have not always been successful and have produced extremely variable seed production responses (9).

Nitrogen fertilization of beans is a frequently recommended practice because of the urgent need to increase yield. Lugo-López et al. (12) reported white bean yields in Puerto Rico of 1460 kg/ha with N applications of 160 kg/ha of a complete fertilizer formula. However, cultivar responses to applied N have been shown to differ greatly (6, 8).

An important aspect of supplemental N fertilization of beans is the effect on the bean-*Rhizobium* symbiosis. High N rates generally inhibit bean nodulation and N fixing activity, whereas amounts of 5 to 15 kg/ha can promote nodule development (9). The bean symbiotic responses to N fertilization depend on such factors as soil, cultivar, *Rhizobium* strain and source of N (15).

Franco (6) has reviewed the conflicting results of N fertilizer on beans and has recommended that a more basic approach, besides yield response, is necessary for an explanation. The objective of this study was to evaluate the effect of various levels of N with and without the *Rhizobium* bacteria on the native white bean cultivar grown in the semiarid southern region of Puerto Rico.

MATERIALS AND METHODS

The experiment was conducted at the Fortuna Research and Development Center, in Juana Díaz, Puerto Rico. The experiment was planted in November 1981 and harvested in February 1982. The soil was a San Antón clay loam (Cumulic Haplustoll) with a pH of 7.2, containing 15 p/m N (NO_3), 18 p/m P and 368 p/m K. The plots consisted of four rows, 5.5 m long 60 cm apart. The two outside rows were used as border.

Six levels of N, 0, 22, 45, 90, 180, and 360 kg/ha, supplemented with P, K and Mg at the rate of 229, 112 ,and 34 kg/ha, respectively, were tested in *R. phaseoli* inoculated and noninoculated plots. The source of N was ammonium sulfate. The fertilizer was incorporated into the soil at the time of planting. A control treatment with neither N nor any other basal fertilizer was also included. Inoculation was done with granular peat (Soil Implant "D", Nitragin Co., Wisconsin)[3] which was applied at the rate of 1.14 g/m under the seed. The treatments were replicated four times in a randomized split-plot design. The main plots were the N fertilizer levels and the subplots the inoculated and noninoculated treatments.

The Bonita cultivar was hand sown at a planting rate of 13 seeds/m. Weed growth was suppressed by hand hoeing. To avoid moisture stress we sprinkler irrigated plants. Five plants from the guard rows in each plot were sampled for nodulation at 4 and 8 weeks after planting. Because of a lack of homogeneity of variances, the recorded values for number of nodules and dry weight were adjusted with a square root transformation. Yield estimates were calculated from the harvest of the two middle rows.

RESULTS AND DISCUSSION

Nitrogen fertilization and *Rhizobium* inoculation produced a combined effect on bean nodulation and yield. Table 1 shows the transformed means of nodule number and nodule dry weight. The application of 22 kg/ha of N increased nodulation on *Rhizobium*-inoculated beans 4 and 8 weeks after planting. However, a reduction in number and weight of nodules was detected with greater application rates of N. The inhibition of nodule number was significant at 90 and 360 kg/ha at 4 weeks. At 8 weeks after sampling, 90 and 360 kg/ha of N showed lower nodule dry weights than those of the control or 0 N/ha. However, the intermediate N level of 180 kg/ha did not significantly reduce nodulation. Saito et al. (15) previously reported inhibition of nodulation in beans with 50 kg N/ha, whereas Dean and Clark (4) demonstrated depressed nodulation with N additions as low as 20 kg/ha. These conflicting results on the inhibition level of N fertilization on *P. vulgaris* nodulation showed the importance of evaluating a comprehensive range of N levels and other factors.

Many factors are involved in bean responses of nodulation to supplemental N. Awonaike et al. (2) observed that the inhibition effect on nodulation is dependent on host genetic factors. Guss and Döbereiner (10) found that nodulation inhibition due to applied N varied greatly among *R. phaseoli* strains. The form of N added is also an important factor. MacLeod and Ormrod (14) showed that ammonium-treated white beans developed leaf chlorosis and necrosis, whereas nitrate-supplied plants had greater growth.

[3]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

TABLE 1.—*Effect of nitrogen fertilization and* Rhizobium *inoculation on* Phaseolus vulgaris *nodulation*

N level	4 weeks after planting		8 weeks after planting	
	Nodule number	Nodule dry weight	Nodule number	Nodule dry weight
kg/ha	$\sqrt{\#/plant}$	$\sqrt{g/plant}$	$\sqrt{\#/plant}$	$\sqrt{g/plant}$
0	3.0	.056	2.1	.074
22	3.6	.062	2.5	.087
45	2.8	.050	1.4	.044
90	2.0	.022	1.3	.019
180	2.9	.048	1.6	.039
360	1.8	.018	1.4	.025
Control	3.0	.068	1.7	.066
L.S.D. (.05)	1.0	NS	NS	.048

The ANOVA results indicated that *Rhizobium* inoculation positively influenced the degree of nodulation at both sampling dates. Table 2 shows the transformed means of nodule number and nodule dry weight. The stimulation effect was significant for all nodulation measurements, except nodule weight at 8 weeks.

Vegetative yields were not increased at 4 weeks over those of the control (4.9 g/plant) by the basal fertilization with 0 kg N/ha (5.6 g/plant), and no consistent response was observed with increasing levels of N. At 8 weeks vegetative yields were not increased over the control (24.6 g/plant) by N additions up to 360 kg N/ha (29.9 g/plant). The inoculation effect averaged over N treatments did not produce greater vegetative yields at either 4 weeks (6.0 vs. 5.6 g/plant) or 8 weeks (27.9 vs. 25.4 g/plant).

Table 3 shows that seed yields did not increase at greater N levels or with inoculation. The yields of 180 and 360 kg/N treatments suggest an effect of applied N, but the lack of significance does not support conclusions which promote the use of high N fertilization. The observed yields are lower than those obtained in the same area in previous experiments (13), but are higher than average yields in the Caribbean Basin and Central America (1).

The results of the present study demonstrated that the supply of N from both the symbiosis and soil was not a limiting factor for yield. High N applications did not produce significantly increased yields and, therefore, would not be economically beneficial. Lindemann and Hibner (11) failed to increase bean yields with applied N and attributed this lack of response to high levels of available soil N. Laboratory analysis of the soil of this study also indicated adequate levels of soil N. Cackett (3) observed increased bean yields resulting from N fertilization that were attributed to site-specific soil conditions which inhibited nodulation until after the onset of flowering. Nodulation was not similarly delayed in the present

TABLE 2.—*Inoculation effects on dry bean response of nodulation at two sampling dates*

Inoculation	4 weeks after planting		8 weeks after planting	
	Nodule number	Nodule dry weight	Nodule number	Nodule dry weight
	$\sqrt{\#/plant}$	$\sqrt{g/plant}$	$\sqrt{\#/plant}$	$\sqrt{g/plant}$
No	2.0	.037	1.5	.049
Yes	3.5	.057	2.0	.058
LSD	.01	.05	.05	NS

TABLE 3.—*Nitrogen fertilization and inoculation effects on dry bean yield*[1] *response*

N level	Inoculated	Nonincoulated
kg/ha	*kg/ha*	
0	1767	1761
22	1831	1730
45	1827	1446
90	1921	1805
180	1991	1931
360	1968	1960
Mean	1884	1772

[1] Main effects of nitrogen fertilization and inoculation are not significant at the 5% level in all cases.

study. Improved bean response to N fertilization has been suggested by Fernández et al. (5), who reported increased seed yields with supplemental N in the nitrate form and applied after flowering when N fixation activity normally decreases rapidly. Low levels of N applied at planting (e.g., 22 kg N/ha), however, was an appropriate management practice because nodulation was consistenly enhanced. In addition, the significant increases in nodulation due to *Rhizobium* inoculation justified continued recommendation in the production of dry beans.

RESUMEN

Niveles de nitrógeno, inoculación con *Rhizobium* y rendimientos de la habichuela blanca (*Phaseolus vulgaris*)

Se estudió el efecto de cantidades de abono nitrogenado sobre la respuesta simbiótica y el rendimiento de la habichuela seca (*Phaseolus vul aris* L., cultivar Bonita). El experimento se sembró en un suelo Mollisol en la región semiárida del sur de Puerto Rico. Se evaluaron seis tratamientos de N: 0, 22, 45, 90, 180 y 360 kg./ha. en parcelas divididas en un diseño *de parcelas divididas con 4 repeticiones. La aplicación de 22 kg./ha.* de N aumentó el número de nódulos 4 semanas después de la siembra y el peso de los nódulos a la octava semana. Las cantidades más elevadas de abono nitrogenado redujeron el número y el tamaño de los nódulos en ambos muestreos. Por otro lado, los rendimientos de habichuela seca aumentaron consistentemente con aplicaciones de 0 a 180 kg./ha. de N en presencia de *Rhizobium*.

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