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Nitrogen Sources for Bean Seed Production[1]

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ABSTRACT

Beans (*Phaseolus vulgaris* L.) often respond to N fertilization; however, N fertilization is not practiced for maximum seed production in southern Idaho. This suggests that the symbiotic relationship and/or soil N sources can provide most of the N needed by this legume. Our objective was to evaluate the relative contribution of the symbiotic-nonsymbiotic N sources by studying the effects of N fertilization on the symbiotic N_2 fixation and seed yields under field conditions. Experiments were conducted on silt loam soils belonging to the Portneuf series (Xerollic Calciortnids). An acetylene reduction (AR) method was used to determine the effect of N fertilization treatments on the relative seasonal N_2 (AR) fixation. The symbiotic N_2 fixation was also estimated by the equation, $N_2 = N_{up} - (N_i + N_m - N_h) - \alpha N_f$, where N_{up} is the accumulated N uptake measured near physiological maturity, N_i and N_h are the amounts of soil NO_3-N in the root zone before planting and near physiological maturity, N_m is the N mineralized from soil organic N sources, and α is the recovery of the N fertilizer (N_f) applied. Estimates of the N fertilizer recoveries were obtained from two experiments using ^{15}N-depleted $(NH_4)_2SO_4$.

The symbiotic N_2 relationship contributed up to 90 kg N/ha, which was 40 to 50% of the total N found in bean plants near physiological maturity. The amount of symbiotic N_2 fixed decreased as the available soil N or fertilizer N increased, and increased as the N required by the individual cultivars increased. The response to N fertilization depended upon the cultivar, as well as on the N available from soil sources. Measured fertilizer N recoveries ranged from 7 to 33%. An average of 52% of the total N uptake near physiological maturity was taken up after the maximum symbiotic N_2(AR) rate occurred; while the seed contained an average of 60% of the total N uptake. A low N fertilization rate (< 50 kg N/ha) when the soil N_i was low (<50 kg N/ha) ensured an early vigorous plant growth but did not always increase seed yields. Higher N fertilization rates may be required on soils with lower amounts of mineralizable N.

Additional index words: Acetylene reduction, Fertilizer N utilization, N fixation, N uptake, *Phaseolus vulgaris*.

BEANS (*Phaseolus vulgaris* L.) utilize inorganic soil N or applied fertilizer N and N_2 fixed by a symbiotic relationship with *Rhizobium phaseoli*. Both the inorganic and symbiotic N sources seem necessary for maximum yields of seed legumes (5, 10); however, as the soil N or fertilizer N increases, that fixed by the symbiosis decreases (3, 8).

A relatively low amount of available N during the initial plant development generally enhances nodulation and plant growth (7). Recent studies indicate that foliar N applications during seed development may be beneficial to some legumes (6, 17), however, no consistent soybean [*Glycine max* (L.) Merr.] yield increases have occurred from several N application methods and N sources (23). In contrast, beans respond to N fertilization (2, 4, 11, 20, 26). This indicates that their symbiotic N_2 fixation process generally does not provide sufficient N for maximum yields. The symbiotic N_2 fixation capacity of beans is reported to be 40 kg N/ha as compared with 100 kg N/ha for soybeans (3, 9). Soybeans obtain 25 to 60% of their N requirements from the symbiotic fixation process.

Dry and garden beans are normally grown for seed production in southern Idaho without N fertilization and are nodulated from indigenous soil *Rhizobium* populations. Nitrogen fertilization is only recommended when large amounts of crop residue are being returned to the soil or if it is the first bean crop

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Table 1. Cultivars, soil N amounts, N fertilization rates and estimated efficiencies, N uptake, estimated seasonal N_2, and seed yields for different N treatments in each experiment.

Experiment and cultivar	N_i†	N_h†	N_m†	N_f†	α†	N_{up}†	Estimated seasonal N_2(AR)	Estimated seasonal N_2†	Seed yield‡
	kg N/ha				%	kg N/ha			kg/ha
1. Slimgreen	40	32	78	0	–	178	17	92	3,020
	–	–	–	45	(51)§	185	25	76	3,220
2. Ouray	51	32	79	0	–	167	11	59	3,010
	–	–	–	45	(44)	174	11	56	3,220
3. UI-36	83	31	76	0	–	184	10	52	2,770
	–	–	–	56	(35)	180	10	29	2,630
4. UI-59	50	30	78	0	–	174	13	76	2,760a
	51	30	78	0	–	157	9	59	2,890ab
	58	31	78	0	–	178	11	72	3,020b
	–	–	–	45	(45)	189	8	63	3,300c
	–	–	–	134	(47)	194	6	25	3,250c
5. Manitou	47	40	78	0	–	142	8	58	2,020
	–	–	–	56	(38)	149	6	36	2,270
6. UI-76	48	27	78	0	–	180	19	81	3,000
	–	–	–	56	(51)	196	9	47	2,930
7. Viva	55	23	110	0	–	173	4	31	3,170
	–	–	–	56	32.7	181	3	20	3,290
	–	–	–	168	27.7	164	2	10	3,110
8. UI-1140	78	75	106	0	–	141	3	32	3,110
	–	–	–	56	7.5	139	4	26	2,960
	–	–	–	168	7.9	151	3	28	3,120
9a. Canyon	33	18	93	0	–	128	6	20	1,900
	–	–	–	112	(28)	128	3	10	2,080
9b. Bonus	33	30	93	0	–	178	18	82	2,900
	–	–	–	112	(46)	177	10	30	2,910
9c. Viva	33	20	93	0	–	177	20	70	3,120a
	–	–	–	112	(53)	208	2	42	3,400b
9d. Sanilac	33	18	93	0	–	144	9	35	2,830
	–	–	–	112	(40)	156	3	3	3,020
9e. UI-50	33	35	93	0	–	112	6	21	1,800a
	–	–	–	112	(28)	129	1	6	2,300b
9f. UI-114	33	33	93	0	–	152	10	59	3,470
	–	–	–	112	(47)	180	3	35	3,580

† N_i = initial NO_3-N; N_h = perharvest NO_3-N; N_m = mineralizable N; N_f = fertilizer N; α = fertilizer use efficiency; N_{up} = total N uptake; Est. N_2 = $N_{up} - (N_i + N_m - N_h) - \alpha N_f$.
‡ Seed yields within an experiment and cultivar followed by a different letter are significantly different at 95% probability level.
§ α estimated from data obtained in Exp. 7 and 8.

grown on a virgin desert soil (14). The objective of this study was to evaluate the symbiotic-nonsymbiotic N relationship of beans grown for seed on these soils. We are reporting here a summary of the effects of N fertilizer on the symbiotic relationship and on seed yields for this legume.

MATERIALS AND METHODS

Nine field experiments were conducted from 1974 to 1977 in southern Idaho on silt loam soils belonging to the Portneuf series (Xerollic Calciorthids). These soils have a calic layer at the 40 to 46-cm soil depth that restricts root penetration but not water movement. The fertilizer treatments were replicated four or five times and arranged in a complete block design. Individual treatment plots were 2.2 or 2.4 m × 15.2 m. Only one bean cultivar was planted in each experiment, except in Exp. 9 where six cultivars were compared (Table 1).

Ammonium nitrate was broadcast on the soil's surface and disced 10 to 15 cm into the soil before planting for all N treatments, except in Exp. 7 and 8, where ^{15}N-depleted $(NH_4)_2SO_4$ (0.0031 atom percent ^{15}N) was applied as the N fertilizer source. Phosphorus and zinc were uniformly applied at each location before discing when needed (14).

Experiment 7 was sprinkler-irrigated whereas the other experiments were furrow-irrigated. Water was applied in the furrows in an alternating pattern in successive irrigations when about 55% of the available soil moisture remained at the 20 to 25-cm soil depth (indicated by tensiometers placed in the row).

The initial soil NO_3-N content was estimated from soil samples taken to the 46-cm depth from each replication before any fertilizer applications. In addition, four 1.3 cm × 46 cm soil cores were also taken from each treatment plot and composited by treatment across replications before analysis when the preharvest plant samples were taken. The mineralization of soil organic N was estimated by a buried polyethylene bag technique (22, 24). With this technique, unfertilized moist soil (~0.5 bar tension) was placed in 5 cm × 46 cm, 38-μm-thick polyethylene bags. The sealed bags were then buried in the plant rows in a vertical position with about a 2-cm soil covering. At selected time intervals, three or more individual bags were withdrawn for analysis. The NO_3-N changes in the bags approximates the N mineralized in these soils during crop growth. All soil samples were air-dried at an air temperature of about 35 C and then crushed to pass a 2-mm mesh screen before they were analyzed for NO_3-N (16).

The respective cultivars (Table 1) were planted at recommended seeding rates in late May in either 56 or 61-cm spaced rows (14). Stand counts were made in each treatment plot on a randomly selected 152-cm row section at about the early bloom growth stage, R2-R3 (13). Plant samples of 10 representative plants were taken from each treatment plot at an early vegetative growth stage (V2-V3) and at early bloom (R2-R3). The preharvest plant samples (R8-R9) consisted of the plants pulled from a 152-cm row section. All plant samples were dried at 60 C, ground to pass a 40-mesh sieve, and analyzed for total N by a semi-micro Kjeldahl procedure modified to include NO_3-N (1).

The plant N from Exp. 7 and 8 was also analyzed for atom percent ^{15}N with an AEI MS-20 isotope-ratio mass spectrometer, using conversion techniques (19). The percentage of N fertilizer recovered (α) was calculated from the equation, $[100(N_{up})(a-b)]/N_f(a-c)$, where N_{up} is the N taken up by the fertilized plants, N_f is the amount of ^{15}N-depleted fertilizer added, a and b are the atom percent ^{15}N of the plants grown on the nonfertilized soil and fertilized soil, and c is the atom percent ^{15}N of the ^{15}N-depleted fertilizer, respectively. Comparison of the atom percent ^{15}N of duplicate plant samples showed that the coefficient of variation was less than 1%.

The amount of symbiotic N_2 was estimated using the equation, Est. $N_2 = N_{up} - (N_i + N_m - N_h) - \alpha N_f$, where N_{up} is the N uptake by the plants on a treatment at the preharvest sampling, N_i and N_h are the soil NO_3-N contents of the nonfertilized treatment for the 0 to 46-cm soil depth before planting and at the preharvest sampling, N_m is the N mineralized from soil organic N, and α is the recovery of the fertilizer N (N_f) applied, respectively. As an approximation, the N fertilizer recoveries (α) in the other experiments were estimated from the ^{15}N data obtained in Exp. 7 and 8 with the equation, $\alpha = 93 - 66.3X$, where X is the ratio of $(N_i + N_m)/N_{up}$ ($r^2 = 0.96^{**}$). In using this relationship, we assumed (a) that the relative availabilities of the N fertilizers were similar in all experiments, (b) that the relative effects of the fertilizer N on the symbiotic relationship were similar for different cultivars, and (c) that the different cultivars take up N fertilizer in proportion to both their total N requirements and the N available from soil N sources. The low fertilizer N recoveries in Exp. 8 were probably due to its irrigation management which left some NO_3-N near or on the soil surface and sufficient N being available from N_i and N_m for crop growth without fertilization. A higher N fertilizer recovery in this experiment would have lowered the recoveries estimated for the other experiments.

The relative symbiotic N_2 fixation for the growing season was estimated by the acetylene reduction (AR) technique as described by Westermann and Kolar (25). This technique involves sampling part of the soil-root system (8 cm × 15-cm core surrounding the tap root) at selected times during the growing season, incubating the samples in 0.1 bar C_2H_2 (remainder atmospheric gases), and measuring the C_2H_4 produced. Seasonal nitrogenase activity (AR) profiles were developed for each treatment and the relative amount of N_2 fixed during the season was estimated by assuming a C_2H_4:N_2 molar ratio of 3:1 (9, 15).

RESULTS

The shapes of the seasonal AR profiles resembled those previously reported for this legume (25). The AR rates increased rapidly from the early development growth stage (V3), peaked at the start of seed development (R3-R4), and then decreased to near zero at physiological maturity (>R7) (Fig. 1). Nitrogen fertilization reduced the maximum AR rates, decreased the relative seasonal N_2 (AR) fixation (Table 1), and delayed the timing of the maximum AR rate.

The AR rate during early growth at the lower N fertilization rate was generally greater than the control when the initial soil NO_3-N (N_i) was less than 50 kg N/ha (data not shown). This caused the relative seasonal N_2 (AR) levels at the lower N fertilization rates to be nearly equal to those of the nonfertilized treatments. There was also some AR activity even at the highest N fertilization rates and when the N from soil sources (N_i and N_m) was relatively high.

Nitrogen fertilization generally reduced the number of nodules per plant at all AR samplings and the average nodule weight during early vegetative growth (Table 2). However, the nodule weights per plant of the lower N fertilization treatments were greater than those for the nonfertilized treatments when there was an early stimulation of AR activity. The N treatment did not affect specific nodule AR activity (μmoles C_2H_4/hour-g nodule), however, the activity tended to be lower in the highest N treatment. The specific activity decreased in all treatments with advanced plant ontogeny, particularly after the maximum AR rate was reached. This probably occurred because the percentage of inactive and decaying nodules in the assay sample also increased.

Seed yields were significantly increased by N fertilization only in Exp. 4 and for the Viva and UI-50 cultivars in Exp. 9 (Table 1). Seed yields tended to increase slightly with N fertilization when the initial soil NO_3-N content was less than 50 kg N/ha.

The N uptake increased linearly from early pod development until physiological maturity (Fig. 1). The N uptake for the nonfertilized treatments at the preharvest sampling was linearly related to $(N_i + N_m)$ and N_2 (AR) by the equation, $N_{up} = 2.0 + 0.82(N_i + N_m) + 4.3N_2(AR)$, $R^2 = 0.93^{**}$. This equation indicates that about 82% of the soil $(N_i + N_m)$ was taken up by the plants. The preharvest soil samples (N_h) showed that an average of 23% of the $(N_i + N_m)$ remained in the soil. This equation also indicates that the N_2(AR) underestimated the symbiotic N_2 fixation. A cross-sectional AR analysis of the soil-root profile over the plant row (8 cm × 15 cm × 61 cm), indicated that about 40 to 50% of the AR activity was contained in the soil-taproot sample (8 cm × 15 cm) in Exp. 4, 5, and 6. Roots and nodules were also found to a 46-cm soil depth which would further decrease the proportion measured. Thus, the N_2(AR) should only

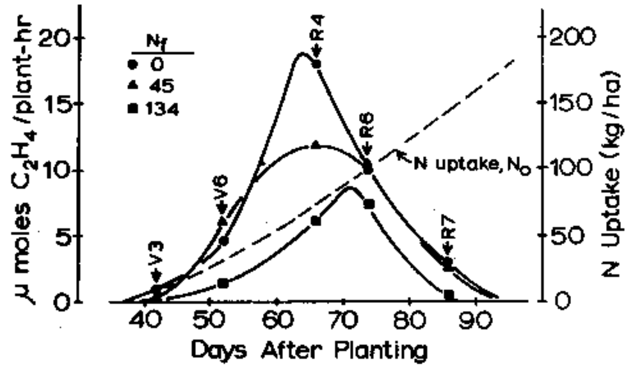

Fig. 1. Effect of N fertilization rate on seasonal profiles of nitrogenase activity (AR) and N uptake for the nonfertilized treatment (Exp. 4).

Table 2. Effect of N fertilization treatment on the number of nodules per plant, fresh nodule weight per plant, and specific nodule AR – activity (Exp. 4).

Days after planting (Growth stage)	N applied (kg/ha)†		
	0	45	134
	no. nodules/plant		
42(V3)	33 a	15 b	10 b
52(V6)	108 a	64 b	33 c
66(R4)	273 a	149 b	128 b
	mg nodules/plant		
42	32 a	5 a	3 a
52	181 b	254 a	69 c
66	855 a	572 b	370 c
	μmole C_2H_4/hour-g nodule		
42	25.6 a	25.0 a	28.0 a
52	25.0 a	24.6 a	20.6 a
66	21.1 a	20.5 a	16.4 a

† Numbers within a growth stage followed by a different letter are significantly different at 95% probability level.

be considered relative since it did not contain the complete nodule-root system nor was an attempt made to quantify the C_2H_4:N_2 ratio.

The N_2 fixation estimated from the plant N characterization equation increased curvilinearly as the N_2(AR) increased (Fig. 2). This indicates that the N_2(AR) method used underestimates the actual symbiotic N_2 fixation. The actual N_2 fixation also appears to be underestimated by the plant N characterization equation at the higher N_2 fixation levels. The regression equation for only the unfertilized treatments resembled the combined equation for N_2 (AR) up to 18 kg N/ha in Fig. 2.

DISCUSSION AND CONCLUSION

The maximum N contributed by the symbiotic relationship was estimated to be between 80 and 90 kg N/ha under the conditions of our study (Table 1, Fig. 2). The symbiotic N_2 fixed depended upon the amount of soil N available ($N_i + N_m$) and the N requirements of the individual cultivars. The effect of available soil N can be seen by comparing the Viva cultivar in Exp. 7 and 9, where the available soil N decreased from 165 to 126 kg N/ha, while the estimated N_2 fixed increased from 31 to 70, respectively. The effect of the cultivar's N requirement can also be seen by comparing Canyon and Viva in Exp. 9 where the N_{up} increased from 128 to 177 kg N/ha, while the estimated N_2 fixed increased from 20 to 70, respectively. The relationship between the estimated N_2 fixed and the ratio $(N_i + N_m)/N_{up}$) was Est. $N_2 = 179-149\ [(N_i + N_m)/N_{up}]$, $r^2 = 0.72^{**}$, for the nonfertilized treatments. A similar relationship was found between the ratio and N_2(AR). Separating the effects of N_i and N_m where the ratio was less than 0.9 showed that the N_m was about 30% more effective than N_i in reducing the estimated N_2 fixed. This might be expected since N_m would be released from soil organic N sources during the entire growing season, whereas N_i would have its greatest effect during early crop development and may be subjected to greater losses.

The N requirements of edible legumes and soybeans during seed growth requires translocation of N from vegetative tissues. This translocation is thought to eventually limit seed yields (12, 18, 21). In our study, an average of 52% of the N contained in the nonfertilized plants at the preharvest sampling was taken up by the plants after the maximum symbiotic N_2(AR) rate occurred. The seeds also contained an average of 60% of the preharvest N. In the example given in Fig. 1, an average of 3.5 kg N/ha-day was taken up by the N_o plants between the maximum N_2(AR) and physiological maturity. That rate of N uptake was equivalent to the N required by the seed filling process if it took place over the same time interval. In practice, the seed probably develops over a shorter time interval so part of its N requirements would come from the mobilization and translocation of N from the vegetative plant parts.

An estimate of the relative contribution of the different N sources to the total N uptake at the preharvest sampling for different N-need-indexes is shown in Fig. 3A and B. The estimated N_2 fixed was used as a measure of the symbiotic N_2 fixation. Deviations outside the ranges of 130 to 200 kg N/ha for N_{up} and 118 to 184 kg N/ha for ($N_i + N_m$) may significantly change the proportionality factors.

The proportion of N from symbiotic N_2 fixation decreased curvilinearly as the available soil N increased (Fig. 3A and B). Nitrogen fertilizer (Fig. 3B) decreased the proportion of N contributed by the symbiotic N_2 fixation, while it had only minor effects on the portion obtained from the available soil N sources. Similar results were recently reported in a study with soybeans (3).

Some of the variability in our study was due to a N fertilization by cultivar interaction. This interaction is illustrated in Exp. 9, where we compared six cultivars. Paired comparisons of cultivars with similar N_2(AR) and N_{up} (Bonus vs. Viva; and Canyon vs. UI-50), showed that N fertilization significantly increased the seed yields and N_{up} of Viva and UI-50 but did not for Canyon or Bonus. In addition, N fertilization reduced the N_2(AR) of Canyon and Bonus by about 50% and those of Viva and UI-50 by about 90%. Both Bonus and Viva are considered to have a relatively higher N_2(AR) as compared with Canyon and UI-50 (23). These data indicate that some host-symbiont

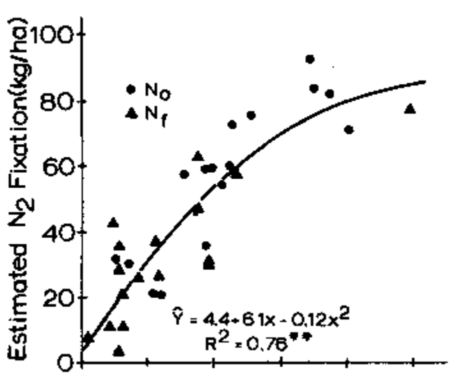

Fig. 2. Relationship between the estimated N_2 fixed and the N_2(AR).

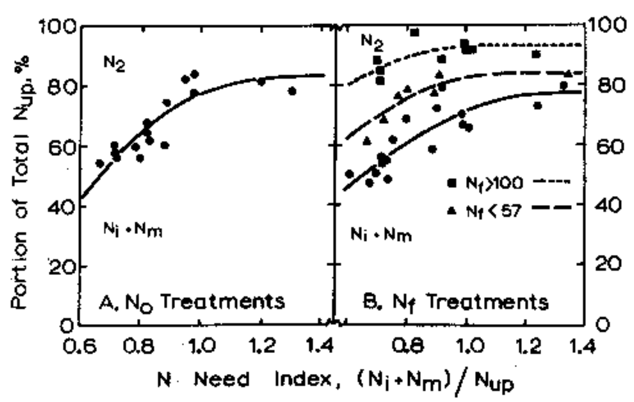

Fig. 3. The percentage portion of total preharvest N uptake as affected by the N Need Index for the nonfertilized treatments (A) and the fertilized treatments (B). Dashed lines are handdrawn through the data points. The portion between each dashed and solid line is that N from the N fertilizer.

relationships are more sensitive to N fertilization than others. In addition, some host-symbiont relationships supplied sufficient N, when combined with the N available from soil sources, for the cultivars to reach their genetic field potential without N fertilization.

As a consequence of the N fertilization by cultivar interaction, it appears difficult to predict the need for N fertilization for bean seed production. There is some justification to recommend a low N fertilization rate to ensure an early vigorous plant growth, particularly when the initial amounts of soil NO_3-N are less than 50 kg N/ha. In any case, the N fertilization rates would not need to be greater than 40 to 50 kg/ha. A significant factor contributing to the lack of a response to N fertilization in these soils is the magnitude of the soil N mineralized during crop growth. Nitrogen fertilization might be required on soils with lower mineralizable N or initial soil NO_3-N levels.

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