MANAGEMENT EFFECTS ON MATURITY, SEED YIELD, AND N_2-FIXATION IN DRY BEAN

K. Maxwell and B. Stevens, University of Wyoming

ABSTRACT

The association between dry beans (*Phaseolus vulgaris* L) and its associated Rhizobium symbiont (*R. phaseoli*) has been shown to be inefficient and as such, unable to provide adequate N for maximum bean production. Fertilization with inorganic N is sometimes recommended, but disadvantages of adding fertilizer N include delayed maturity and possible inhibition of N_2 fixation. It is unknown whether N fertilizer applications are most beneficial during early growth stages prior to nodulation, or during reproductive growth stages when N demand is the highest. Field studies were established at two sites with differing soil properties to compare fertilized, unfertilized and inoculated plantings for seed yield and pod maturation, nodulation, and N_2 fixation. Preplant broadcast, sidedress, and split applications of N fertilizer were evaluated. Yield responses were inconsistent among different N timing treatments, but delaying part or all of N application until the 4 to 6 trifoliate stage allowed beans to mature more quickly than when all N fertilizer was applied before planting. Inoculation of pinto bean seed, whether by seed treatment or in-furrow placement, failed to produce a yield response on a clay soil with a history of bean production.

INTRODUCTION

Among the goals of modern agriculture, one of the most important is increasing crop yields to meet the needs of a growing population. Dry beans (*Phaseolus vulgaris* L) are an important food crop throughout the world. Being leguminous, dry beans establish an intimate relationship with soil-borne rhizobium bacteria. Infection of the bean plant by a compatible strain of rhizobium causes nodules to form on roots wherein inert atmospheric dinitrogen is changed into a form that can be used by the plant. Nitrogen supplied to dry beans by the rhizobium symbiont is known to be insufficient to produce maximal yields (Piha and Munns, 1987).

Inoculating dry bean seed with more efficient bacterial strains has been suggested as a way to improve dinitrogen fixation, but results have been inconsistent because success is dependant on many variables (Vlassak and Vanderleyden, 1997). Applying supplemental nitrogen to dry beans has become a common practice, but yield response to nitrogen fertilizer is inconsistent (Moraghan et. al., 1991; Piha and Munns, 1987; Westermann et. al., 1981). Both dry bean variety and nitrogen application timing may affect whether a yield response occurs. Late maturing varieties of dry beans have been shown to achieve similar final harvest nitrogen accumulations and seed yields regardless of whether or not nitrogen was applied (Piha and Munns, 1997).

Potential disadvantages of nitrogen fertilization are that (1) nodule formation and dinitrogen fixation may be reduced, (2) fertilizer nitrogen may be leached from the soil profile, especially when furrow irrigation is practice, (3) bean maturity may be delayed, and (4) disease incidence may increase as a result of enhanced vegetative growth. These effects must be carefully weighed against the probability of an economic yield response when making nitrogen fertilizer management decisions. This study was designed to evaluate different nitrogen management practices on dry bean yield in the Big Horn Basin of Wyoming by examining the mechanisms associated with Rhizobia efficiency. Field studies were set up near Powell, Wyoming to examine fertilized, unfertilized and Rhizobia competition plantings for seed yield and pod maturation, nodulation, and N_2 fixation.

MATERIALS AND METHODS

Nitrogen Fertilization Field Experiments

The Northwest District of Wyoming, which encompasses the Big Horn Basin, harvests 10,000 acres of dry beans annually, (NASS, 2001). Park County is located in the Big Horn Basin and is the second largest dry bean production site in Wyoming (WASS, 2001). The study site was chosen in Park County near the town of Powell, because the environmental conditions are representative of bean production areas in the inter-mountain west. The arid climate and surface (furrow) irrigation methods commonly used in northwest Wyoming and surrounding areas, are favorable for dry bean production because humidity in the leaf canopy usually remains low and soil moisture can be controlled to some extent with careful irrigation management.

Procedure and Site Description: Heart Mountain

Three sites were selected in the Heart Mountain area, one in 2002 and two (1H and 2S) in 2003). Combined Ap and B-horizons are approximately one meter deep. Soil chemical properties were: pH, 8.2; organic matter, 0.8-1.3%; nitrate-N (0 to 12 inches), 7-10 ppm; bicarbonate-phosphorus, 13 ppm; exchangeable potassium, 113 ppm; and DTPA-zinc, 1.3 ppm. Previous crop was either sugar beet (2002 and 2003 1H) or barley (2003 2S). Preplant fertilizer treatments were applied concurrently with ethalfluralin (Sonalan) herbicide (0.94 pounds of active ingredient per acre) in mid-May. Inter-row cultivation and hand labor were used to eliminate any weeds not controlled by the preplant herbicide.

In late May, bean seed was planted 1.75 inches deep into pre-formed beds spaced 22 inches apart. GTS900 (Gen-Tec Seeds, Ltd.) and 'Maverick' pinto varieties were planted at the 2002 and 2003 2H locations, respectively, and 'Beryl' great northern beans were planted at the 2003 1S location. Plots were irrigated according to typical furrow irrigation methods using a gated pipe delivery system. The frost-free growing period for the area is 107 days.

Procedure and site description: Powell Research and Extension Center

Soil samples at the Powell Research and Extension Center (REC) were analyzed using the same procedures as were used for the Heart Mountain sites. Chemically properties for the two Powell REC sites were: pH, 7.8; organic matter content (Ap horizon), 1.2-1.3%; nitrate-N (0 to 12 inches), 9-11 ppm; bicarbonate-phosphorus, 13 ppm; and exchangeable-potassium, 176 ppm. Soil textural class is clay (hydrometer method) and slope is less than one percent. The frost-free growing period for the area is 107 days. Previous crop in at both sites was barley. Ethalfluralin (Sonalan) and Eptam (S-ethyl dipropylthiocarbamate) herbicides were applied at rates of 0.75 and 1.75 pounds of active ingredient per acre. Inter-row cultivation and hand labor were used to eliminate any weeds not controlled by the preplant herbicide.

Pinto beans were planted in late May to a depth of 2.5 inches in pre-formed, pre-irrigated beds spaced 22 inches apart. Maverick pinto bean was planted in 2002 and both Maverick and

Focus pinto bean varieties were planted in 2003. Plots were irrigated according to typical furrow irrigation methods using a gated pipe delivery system.

Experimental Design.

All field experiments were established using a randomized complete block experimental design with five replications. Treatments allowing evaluation of nitrogen application rate, timing and placement variables were included (Table 1). In 2003, the effects of nitrogen fertilizer treatments on two different pinto varieties (‘Maverick’ and ‘Focus’) were compared, but only at the Powell REC location. Granulated inoculant (Nitragin Soil Inoculant +) applied at 9 lb/acre in the seed row was added in 2003 as a treatment at the Powell REC site.

Preplant nitrogen fertilizer was applied as dry ammonium nitrate (34-0-0) and post-emergence (side dress) nitrogen fertilizer was applied either as dry ammonium nitrate or as liquid urea-ammonium nitrate (32%N). Dry fertilizers were applied to each plot by hand while liquids were applied using a knife-type injection applicator. All dry fertilizers were immediately incorporated by tillage to minimize volatilization losses. At the Powell REC 2003 site, granulated rhizobium inoculant was applied in the seed row using chemical metering attachments on the planter implement.

Table 1. 2003 Experimental Design at the Powell REC. Design of the 2002 experiment was the same except only one variety was used and no rhizobium inoculant was applied.

‘Maverick’ Pinto Bean	‘Focus’ Pinto Bean
Unfertilized Check Plot	Unfertilized Check Plot
Seed-row-applied Inoculant	Seed-row-applied Inoculant
N Application Timings Preplant Sidedress Split application	N Application Timings Preplant Sidedress Split application
N Application Rates 60 lb/acre 100 or 120 lb/acre	N Application Rates 60 lb/acre 100 or 120 lb/acre

Data collection

All plants were harvested during the first two weeks of September for yield determination. A laboratory assay (ureide analysis) will be conducted to estimate the proportion of total plant nitrogen fixed by Rhizobia. Plants selected for ureide analysis were collected between the early flowering (R1) and early pod set (R3) growth stages. Stems and petioles collected from the field were dried, ground, and prepared for ureide testing (data not shown).

Field Experiment to Compare Rhizobium Strains

An indigenous population of Rhizobia typically exists in soils with a history of dry bean production. Newer Rhizobium strains or materials that stimulate existing bacterial populations may enhance nitrogen fixation. An experiment was conducted to compare the effectiveness of these management practices. Six treatments were arranged in a randomized complete block design with five replications. The inoculants included two single strains and a mixed strain (Table 2), which were applied either in the seed row as a granular material or directly to the seed as a peat-based suspension (liquid). A non-nutritive carbohydrate solution was also included as a treatment.

Table 2. Bacterial strains applied in field study for comparing inoculants.

Rhizobium Strain	Application Method	Host Plant	Manufacturer
127K44	Liquid	*P. vulgaris* var. 'Maverick'	Nitragin
127K89	Liquid	*P. vulgaris* var. 'Maverick'	Nitragin
127K105 127K44 127K89 Combination	Liquid or Granular	*P. vulgaris* var. 'Maverick'	Nitragin

Rhizobia Competition Treatments
1. Strain 127K44 (liquid)
2. Strain 127K89 (liquid)
3. Mixed liquid inoculant
4. NEB supplement (microbial stimulant)
5. Mixed granular inoculant: 127K105, 127K44, and 127K89 combined
6. No treatments: indigenous Rhizobium strains only

Data Collection.

All plants were harvested during the first two weeks of September for yield determination. Plants selected for ureide analysis were collected during the early pod fill (R4) growth stage. Plants selected for ureide analysis were collected between the early flowering (R1) and early pod fill (R4) growth stages. Stems and petioles collected from the field were dried, ground, and prepared for ureide testing (data not shown). Nodules were also collected from roots of randomly selected plants from each plot in preparation for and immunoassay to determine the strain(s) of Rhizobium bacteria contained in the nodules. Plants, including roots, were removed with soil intact and transported to the laboratory facility in buckets containing sufficient water to cover the root ball. Once at the laboratory, soil was removed from the roots with a gently stream of water and 10 nodules were removed and desiccated for future processing.

RESULTS

Seed yield

Application of N fertilizer significantly ($P<0.1$) increased seed yield in three of six site-years (Tables 3 and 4), with the average advantage ranging from 246 to 414 lb/acre. Fertilizer application timing did not affect yield at all and fertilizer application rate had an inconsistent effect (Table 4). The yield response to added N was inconsistent and relatively small. This sporadic response cannot be explained by high residual nitrate-N levels, which ranged from 7 to 11 ppm. University of Wyoming guidelines call for N applications of 40 to 60 lb N/acre when soil residual N levels are in this range. Variation in N_2-fixation efficiency or organic N mineralization may have influenced yield response, but laboratory analyses to test these hypotheses are still being conducted. It should be noted, that seed yield with no added N was very good, ranging from 2941 to 3947 lb/acre for the pinto varieties and 1940 lb/acre for the great northern variety. These high check yields indicate that N supply, whether from soil N or fixed N, was near optimum levels.

Bean yield was not enhanced by use of a commercial multi-strain granular inoculant applied in the seed furrow (Tables 3, 4 and 5), nor by individual strains applied as a liquid seed treatment (Table 5). This suggests that the indigenous population of Rhizobia was adequate to maximize nodulation – a result that was expected because dry beans had been grown previously on all sites. A microbial stimulant (NEB-SO) applied to the seed caused an apparent yield increase, but this difference was not statistically significant (Table 5).

Maturity

The maturity response of dry bean to N fertilizer application was more consistent than was the yield response. Nitrogen fertilization delayed maturity in three of four site-years for which data was collected. In some cases the negative effect was less when N was added as a sidedress application following development of the fourth trifoliate leaf (Tables 3 and 4). Maturity was significantly delayed at a high N application rate (120 lb N/acre) compared to the 60 lb N/acre rate in only one of four site-years. While the effect of fertilizer N on maturity was somewhat inconsistent, it was more pronounced at the Heart Mountain sites, where the soil was much coarser in texture than at the Powell REC site.

N_2 Fixation.

Results of laboratory assays estimating nodulation efficiency and N_2 fixation are still being completed, and will be reported on the poster if possible.

CONCLUSIONS

These results indicate that applying nitrogen to dry beans under conditions similar to those in northwest Wyoming will increase seed yield in some cases, but differences in response were difficult to explain based on measured soil characteristics. Application of a *Rhizobium* inoculant or a biological stimulant (NEB-SO) failed to increase seed yield relative to a check treatment, indicating that the inoculant strains used in this study did not increase yields significantly in a soil with an established Rhizobia population. Nitrogen application generally lengthened the maturation period. Delayed (sidedress) application of a part or all of the recommended amount of N fertilizer tended to lead to higher seed yield and earlier maturity, but the benefit was small and inconsistent. Increasing N application rate delayed maturity in most cases, but delayed applications had less effect on maturity than did preplant applications. Data showing the effects of N management practices on nodulation and N_2 fixation may be viewed on our poster.

REFERENCES

Moraghan J.T., J.A. Lamb, and W. Albus. 1991. Nitrogen fertilizer requirements of navy beans in the northern great plains. J. Prod. Agric. 4:204-208.

Piha, M.I. and D.N. Munns.1987. Nitrogen fixation capacity of field-grown bean compared to other grain legumes. Agron. J. 79:690-696.

Vlassak, K.M. and J. Vanderleyden. 1997. Factor influencing nodule occupancy by inoculant rhizobia. Crit. Rev. Plant Sci. 16:163-229.

Westerman D.T., G.E. Kleinkopf, L.K. Porter, and G.E. Leggett. 1981. Nitrogen sources for bean seed production. Agon. J. 73:660-664

Table 3. The effect of N fertilizer application rate and timing on maturity and seed yield of 'Maverick' and 'Focus' pinto bean varieties. Powell Research and Extension Center, Powell, WY, 2002 and 2003.

		'Maverick' 2002		'Maverick' 2003***	'Focus' 2003***
Nitrogen Timing	Nitrogen Rate (lb/ac)	Maturity** (% mature pods)	Yield	Yield	Yield
Check	0	58.8	3090	3868	3947
Granular Inoculant	0	--	--	4030	3916
Preplant	60	59.1	3484	4039	4239
	100	38.6	3287	--	--
	120	--	--	4229	3935
Sidedress	60	50.7	3550	4099	3868
	100	50.7	3711	--	--
	120	--	--	4600	4241
Split (PP+SD)	60(30/30)	42.9	3506	4075	3757
	100(50/50)	45.7	3365	--	--
	120 (60/60)	--	--	4090	3943
Split (starter + SD)	60(20/40)	47.1	3560	--	--
	100(20/80)	51.1	3571	--	--
ANOVA PR>F					
Rep		0.0014	0.0324	0.5884	0.4000
Treatment		0.7729	0.5455	0.4897	0.3303
orthogonal comparisons					
check v N applied		0.2647	0.0558	0.0640	0.6150
preplant v sidedress		0.8309	0.2220	0.1210	0.0374
preplant v split		0.6095	0.8022	0.7565	0.7981
60 lb N/acre v 100 (120) lb N/acre		0.2792	0.8699	0.2334	0.8480

* Averaged across two N application rates within each N timing treatment.

**Maturity was measured by removing six plants from each plot and manually counting total and mature (buckskin colored) pods.

***Maturity data not available

Table 4. The effect of N fertilizer application rate and timing on maturity and yield of 'Maverick' and 'Focus' pinto bean varieties. Heart Mountain area, Powell, WY, 2002 and 2003.

		'GTS 900' pinto, 2002		'Maverick' pinto, 2003 (1H)		'Beryl' great northern, 2003 (2S)	
Nitrogen Timing	Nitrogen Rate (lb/ac)	Maturity** (% mature pods)	Yield	Maturity** (% mature pods)	Yield	Maturity** (% mature pods)	Yield
Check	0	56.2	2941	79.3	3104	66.2	1940
Preplant	30	--	--	59.7	2892	57.1	2370
	60	30.5	2931	50.3	2705	53.2	2140
	120	16.9	3369	43.5	2967	46.3	2217
Sidedress	30	--	--	65.3	3230	59.0	2108
	60	59.1	3017	65.4	2873	58.1	2102
	120	30.9	3021	65.5	2731	48.2	2193
Split (PP+SD)	60(30/30)	54.8	3459	66.4	3248	54.8	2146
	120 (60/60)	37.7	2938	47.1	2738	58.2	2214
Split (starter + SD)	60(20/40)	31.8	2815	--	--	--	--
	120(20/100)	31.7	3084	--	--	--	--
ANOVA PR>F							
Rep		0.6934	0.0155	0.0587	0.5945	0.0023	0.9439
Treatment		0.0001	0.0568	0.0591	0.4340	0.1558	0.3030
orthogonal comparisons							
check v N applied		0.0008	0.3667	0.0137	0.4163	0.0235	0.0313
preplant v sidedress		0.0002	0.3640	0.0314	0.5980	0.4713	0.1557
preplant v split		0.0001	0.7353	0.2256	0.4545	0.7763	0.4709
60 lb N/acre v 120 lb N/acre		0.0304	0.0051	0.6007	0.9478	0.4716	0.2375

* Averaged across two N application rates within each N timing treatment.

**Maturity was measured by removing six plants from each plot and manually counting total and mature (buckskin colored) pods.

Table 5. Effect of various inoculants and a seed treatment on the yield of 'Maverick' pinto beans. Powell Research and Extension Center, Powell, WY 2003.

Treatment	Yield
Check	4087
NEB-SO	4305
Mixed Liquid Inoculant	4120
Strain 127K44	4032
Strain 127K89	4021
Mixed Dry Inoculant	4001
ANOVA P>F	
Rep	0.4851
Treatment	0.7632