

## **Final Report for Wyoming Bean Commission**

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### **Effect of Various Granular Rhizobia Inoculants on Yield of Dry Bean Cultivars under Sprinkler Irrigation**

Our research during the past several years has indicated minimal yield effects from adding N fertilizer to dry bean. The results are similar to other published studies reported by teams in North Dakota. It is well known that dry bean roots host root nodules that contain rhizobia bacteria. With energy supplied by the plant, these rhizobia convert atmospheric N<sub>2</sub> into ammonia (a process called N<sub>2</sub>-fixation) and subsequently ureides and amino acids for transport to shoots. Thus, given this N<sub>2</sub>-fixation capacity and given that some plant available N (e.g., NO<sub>3</sub>-N) is present in the soil throughout the growing season, it is not surprising that N fertilizer effects are inconsistent. Our previous research at Powell using furrow irrigation (2019 to 2022) compared N rates of 0, 40, and 80 lbs of N per acre showed no significant yield gains or loss with N application. Those tests have included multiple pinto varieties because we wanted to see whether or not the response to N was variety dependent (so far, we have not found variety/cultivar or maturity to affect the response).

Most dry bean fields in Wyoming have a history of having planted dry bean within the three previous years and it is generally assumed that the soil contains enough “native” rhizobia to preclude the need for inoculant. Nevertheless, among the factors that have not been thoroughly tested, related to dry bean N fertility, are commercial rhizobia preparations. The objectives of this trial were to compare four products alongside two check treatments (an unfertilized check and an N-fertilized control) and to include multiple varieties.

#### **Materials and Methods**

The experiment was located at the University of Wyoming Research and Extension Center in Powell, Wyoming. The soil, a Garland clay loam (fine, mixed, mesic: Typic Haplarid), was prepared by roller harrow and leveled in the spring. The entire plot area received 70 units of P, 60 units of K, 43 units of sulfate-S, and 5 units of Zn on 27 April 2023. Chemical weed control consisted of a preplant incorporated chemical treatment of 2 pints of Sonalan, 3 pints Eptam, and 1 pint of Orro applied on 31 May 2023. Cultivation occurred during the growing season when appropriate.

The study was divided into three segments, each one-half acre in size. One segment received full irrigation throughout the season, a second segment received slight deficit irrigation, and the third segment received severe deficit irrigation. A sprinkler irrigation system was used and delivered 1.0-inch per week for the full irrigation but only 0.8-inch and 0.6-inch for the two reduced irrigation segments. Deficit irrigation rates were not started until July in order to allow a good stand establishment.

Seed was planted on 8 June in two side-by-side three-row plots that were 5.5 feet wide and 20 feet long using a six-row Almaco planter equipped with hoppers to deliver in-furrow products. Row spacing was 22-inch and seeding rate was approximately 90K seed per acre. The experimental design was a split plot with two to three replicates for the six-row-wide main plots (inoculant, described later). Seven cultivars and three experimental lines were included in each main plot. Each irrigation section contained a total of 160 plots and overall, there were 480 plots.

Inoculant Treatments. Four inoculants and the two check treatments were tested in 11-foot wide strips (Table 1). For planting, and in order to minimize cross contamination, the two check treatments were sown first, followed by sowing the plots of four inoculant treatments. The inoculant was delivered in-furrow along with the seed. The hopper boxes were vacuumed out between each inoculant planting to minimize contamination. From all indications on the product specification sheets, N-Charge and N-Dure were similar products; thus, each of those were only tested in two strips whereas the other treatments were evaluated in three test strips.

Table 1. Treatments used for the inoculant test and associated details. Exceed, N-Charge, and N-Dure contained *Rhizobium leguminosarum* biovar *phaseoli* whereas Lallemand contained two strains of *Rhizobium tropici*. During equipment calibration, the original inoculant formulations did not flow smoothly from the hopper box, through the tubing, and into the furrow. Thus, inoculants were mixed with sand within the boxes. Thus, inoculants were mixed with sand within the boxes. After a further series of test calibrations, settings were chosen so that the sand served as a carrier and approximately 35 lbs of sand was delivered per acre. From all indications on the product specification sheets, N-Charge and N-Dure were similar products; thus, each of those were only tested in two strips whereas the other treatments received three test strips. A photo of the hopper boxes and one with sand is shown in Figures 1 and 2.

Treatment	Number of Strips	Notes
Unfertilized Check	3	na
N Fertilized Check	3	60 units of N as urea, broadcast/incorporated
Exceed	3	Applied 135 g per 1000 linear foot (granular)
Lallemand	3	Applied 5.5 g per 1000 linear foot (peat-based)
N-Charge	2	Applied 12.4 g per 1000 linear foot (peat-based)
N-Dure	2	Applied 11.3 g per 1000 linear foot (peat-based)
Total	16	



Figure 1. (left) and Figure 2 (right). Hopper boxes that held the inoculant (Fig. 1) and close-up of sand-inoculant mixture (Fig. 2). Note the domed cone planter to the left of Fig. 1 that distributed seed for one of the rows of the three-row plots.

Visual estimates for days to bloom (50 percent of plants showing bloom) and days to maturity (50 percent of the plants with one buckskin pod) were made. Canopy health was recorded by measuring NDVI (normalized difference vegetation index) with a RapidSCAN CS-45 unit on 6 July and 21 July.

To compare the effects of the treatments on biomass and nodule formation, three genotypes were selected from the moderate irrigation section. Thus, shoots were severed from one-linear-foot of row (typically four plants) and the roots from those plants were excavated to the fullest extent possible. Because the main goal of the study was to compare accurately the inoculant effects, and in order to process the plant and nodule sampling across the sixteen inoculant strips a quick time frame before the nodules decayed, we sampled only one genotype per day. Thus, LPID3 was sampled on 23 Aug, Rattler was sampled on 30 August, and Monterrey was sampled on 6 September. After nodules were removed by hand and counted and weighed (fresh weight only), roots and shoots were dried at 60°C or 140°F for two days and weighed (dry biomass).

At maturity, 10-feet of plants from two rows from each plot (but not the edge rows) were uprooted by hand, allowed to dry in the field for several hours, and those plants were threshed with a Zurn research plot combine later that day. The harvested seeds were cleaned with a Clipper unit and then hand-picked to remove dirt clods and bean chaff. Samples were then weighed for clean seed yield per plot and seeds per pound, and average seed size in milligrams (the reciprocal of seeds per pound) was determined.

Harvested seed from each plot were tested for protein, fat, starch, and fiber using a Perten DA7250 system. Values presented in this report used an existing calibration available from Perten. We expect to use an updated calibration for future research.

## Results and Discussion

Stand establishment was good, with excellent soil and weather conditions. The growing season can be described as wet and cool. June, July, Aug high/low temps (°F) were: 74/42, 84/45, and 84/42, respectively (5 days in July and 7 days in Aug were +90°F).

Canopy health, as measured by NDVI on 6 July and 21 July, is shown in Tables 2 and 3. No effects from the six treatments were observed (higher values are considered healthier canopies).

Nodule number, nodule weight, root weight, and aboveground biomass for LPID3 as affected by inoculant treatment is provided in Table 4. Likewise, those same traits for Rattler and Monterrey are provided in Tables 5 and 6. No statistically significant effects on the four traits were found due to inoculant treatment.

Yield was not significantly affected by treatment within any of the three irrigation sections (Table 7). However, there was a trend for N-Charge to have a numerically higher yield than the other five treatments within each irrigation section. Yields of Max and Othello were greater than Cowboy and SV6139GR and likewise, those four cultivars yielded greater than the other six entries (Table 8).

Protein concentration of the harvested seed was unaffected by the inoculant treatments (Table 9). However, protein concentration of seed from our experimental lines (LPID3, LPID7, and LPID9) was greater than the other seven entries in the trial (Table 10). This higher concentration of protein in seed of LPID-3, LPID-7, and LPID-9 may be related to the fact that one of the parents for those lines is Long's Peak. There was a trend for deficit irrigation to increase protein concentration.

The number of seed per pound was not appreciably affected by the inoculant treatments (Table 11). As far as the number of seed per pound, Max showed its typical lower value (indicating larger seed) and PT9-5-6 and LPID-7 had a greater number of seed per pound (Table 12).

Maturity was unaffected by the inoculant treatments but as expected, Othello and Max matured earlier than Monterrey and Rattler (Tables 11 and 12). The earlier maturing lines yielded higher than later maturing lines (Fig. 3).

Table 2. NDVI of the six treatments on 6 July 2023 at Powell.

<b>Treatment</b>	<b>100% Irrigation</b>	<b>80% Irrigation</b>	<b>60% Irrigation</b>	<b>Average</b>
Untreated	0.22	0.23	0.23	0.23
Plus N	0.22	0.23	0.24	0.23
Exceed	0.20	0.21	0.21	0.21
Lallamand	0.21	0.22	0.23	0.22
N-Charge	0.23	0.23	0.23	0.23
N-Dure	0.23	0.25	0.24	0.24
LSD (0.05)	ns	ns	ns	ns
CV (%)	8	8	8	-
Mean	0.23	0.23	0.23	0.23

Table 3. NDVI of the six treatments on 21 July 2023 at Powell.

<b>Treatment</b>	<b>100% Irrigation</b>	<b>80% Irrigation</b>	<b>60% Irrigation</b>	<b>Average</b>
Untreated	0.62	0.67	0.65	0.65
Plus N	0.68	0.67	0.69	0.68
Exceed	0.67	0.66	0.69	0.67
Lallamand	0.66	0.60	0.62	0.63
N-Charge	0.70	0.70	0.68	0.69
N-Dure	0.69	0.71	0.70	0.70
LSD (0.05)	0.05	0.05	0.05	-
CV (%)	7	9	8	-
Mean	0.67	0.67	0.67	0.67

Table 4. Nodule and morphological traits of LPID3 as affected by inoculant treatments per foot of row on 23 August 2023 at Powell. No statistically significant differences were found.

<b>Treatment</b>	<b>Nodule Number</b>	<b>Nodule Weight (g)</b>	<b>Root Biomass (g)</b>	<b>Aboveground Biomass (g)</b>
Check	87	0.42	5.4	102
Plus N	216	1.39	5.6	113
Exceed	170	0.81	5.4	107
Lallemand	129	0.71	5.1	126
N-Charge	60	0.38	5.0	118
N-Dure	117	0.96	4.9	105

Table 5. Nodule and morphological traits of Rattler as affected by inoculant treatments per foot of row on 30 August 2023 at Powell. No statistically significant differences were found.

<b>Treatment</b>	<b>Nodule Number</b>	<b>Nodule Weight (g)</b>	<b>Root Biomass (g)</b>	<b>Aboveground Biomass (g)</b>
Check	85	0.30	6.9	124
Plus N	155	0.28	7.3	128
Exceed	147	0.64	10.3	169
Lallemand	105	0.53	8.0	137
N-Charge	153	0.41	7.5	132
N-Dure	62	0.25	6.1	110

Table 6. Nodule and morphological traits of Monterrey as affected by inoculant treatments per foot of row on 6 September 2023 at Powell. No statistically significant differences were found.

<b>Treatment</b>	<b>Nodule Number</b>	<b>Nodule Weight (g)</b>	<b>Root Biomass (g)</b>	<b>Aboveground Biomass (g)</b>
Check	31	0.11	5.9	101
Plus N	53	0.20	7.2	125
Exceed	41	0.14	7.7	127
Lallemand	30	0.09	5.7	95
N-Charge	46	0.12	5.9	102
N-Dure	86	0.20	7.3	127

Table 7. Yield of the six treatments at Powell as affected by inoculant and irrigation regime.

<b>Treatment</b>	<b>100% Irrigation</b>	<b>80% Irrigation</b>	<b>60% Irrigation</b>	<b>Average</b>
	----- lbs/a -----			
Untreated	2819	2726	2113	2553
Plus N	2973	2680	2222	2625
Exceed	2973	2519	2141	2544
Lallamand	2878	2637	2165	2560
N-Charge	3118	2836	2303	2752
N-Dure	2901	2778	1947	2542
LSD (0.05)	ns	ns	ns	na
CV (%)	6	7	14	na
Mean	2936	2680	2145	2596

Table 8. Yield of six cultivars and four experimental lines in the inoculant test in 2023 at Powell. Values are the average of 16 separate plots.

Entry	Irrigation Rate			Average
	60%	80%	100%	
	----- lbs / acre -----			
Cowboy	2272	2869	3202	2781
LPID3	2078	2513	2942	2511
LPID7	1927	2409	2596	2311
LPID9	1662	2208	2592	2154
Max	2473	3057	3023	2851
Monterrey	2088	2651	2925	2555
Othello	2797	3023	3122	2981
PT9-5-6	2000	2566	2868	2478
Rattler	1977	2481	2987	2482
SV6139GR	2260	2988	3106	2785
Average	2145	2680	2936	2587
LSD(0.05)	227	255	220	na

Table 9. Protein concentration as affected by the inoculant treatments.

Treatment	Irrigation			Average
	60%	80%	100%	
Check	19.8	18.0	17.0	18.3
Plus N	20.5	18.3	17.5	18.8
Exceed	20.4	18.0	17.5	18.6
Lallemand	19.6	17.9	17.0	18.2
N-Charge	20.4	18.3	17.5	18.7
N-Dure	19.9	18.1	17.3	18.4
LSD (0.05)	ns	ns	ns	na
Average	20.1	18.1	17.3	18.5

Table 10. Protein concentration (from the Perten DA7250 unit) of the six cultivars and four experimental types.

Entry	Irrigation			Average
	60%	80%	100%	
Cowboy	19.6	17.2	16.7	17.8
LPID-3	20.7	19.3	18.4	19.5
LPID-7	22.1	20.8	19.9	20.9
LPID-9	22.8	20.1	19.0	20.6
Max	18.2	16.5	16.1	16.9
Monterrey	20.6	18.2	17.4	17.7
Othello	17.8	16.1	15.7	16.5
PT9-5-6	19.8	17.3	15.7	17.6
Rattler	20.8	18.2	17.3	18.8
SV6139GR	19.5	17.5	16.9	18.0
LSD (0.05)	0.9	0.7	0.6	na
Average	20.1	18.1	17.3	18.5

Table 11. Number of seed per pound as affected by the inoculant treatments.

Treatment	Irrigation			Average
	60%	80%	100%	
Check	1460	1353	1301	1371
Plus N	1466	1360	1344	1390
Exceed	1483	1368	1388	1413
Lallemand	1455	1337	1350	1379
N-Charge	1420	1363	1333	1372
N-Dure	1489	1359	1317	1388
LSD (0.05)	51	41	44	na
Average	1464	1357	1341	1387

Table 12. Number of seed per pound of the six cultivars and four experimental types.

Entry	Irrigation			Average
	60%	80%	100%	
Cowboy	1429	1338	1325	1364
LPID-3	1257	1189	1155	1200
LPID-7	1613	1471	1465	1516
LPID-9	1545	1466	1381	1464
Max	1371	1245	1244	1286
Monterrey	1474	1363	1354	1397
Othello	1501	1396	1382	1426
PT9-5-6	1596	1484	1434	1505
Rattler	1343	1281	1281	1302
SV6139GR	1530	1385	1381	1432
LSD (0.05)	65	51	56	na
Average	1464	1357	1341	1387

Table 13. Maturity (dap) as affected by the inoculant treatments.

Treatment	Irrigation			Average
	60%	80%	100%	
Check	90	92	93	92
Plus N	90	91	92	91
Exceed	91	92	93	92
Lallemand	91	91	92	91
N-Charge	92	92	93	92
N-Dure	90	93	94	92
LSD (0.05)	2	2	2	na
Average	91	92	93	92

Table 14. Maturity (dap) as affected by the six cultivars and four experimental types.

Entry	Irrigation			Average
	60%	80%	100%	
Cowboy	90	92	94	92
LPID-3	91	94	94	93
LPID-7	93	96	98	96
LPID-9	99	98	98	98
Max	78	79	78	78
Monterrey	95	97	97	96
Othello	77	78	77	77
PT9-5-6	95	96	97	96
Rattler	96	96	98	97
SV6139GR	90	92	94	92
LSD (0.05)	3	3	3	na
Average	91	92	93	92

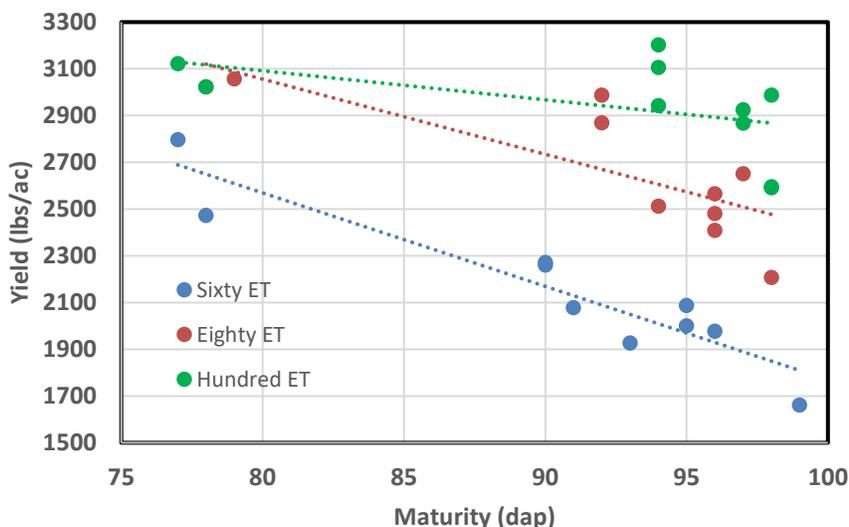


Figure 3. Relationship between yield and maturity in Powell 2023 as affected by the three irrigation rates. Each data point represents the yield of the 10 genotypes averaged across all of the inoculant treatments.

### Summary

None of inoculants or the plus N treatment had conspicuous effects on yield or other traits. The possible exception was N-Charge that had numerically higher yield across all three irrigations. The early entries outyielded the later-maturing entries. Seed protein concentrations were greater in the later-maturing lines, especially our progeny lines. Water stress reduced yield as expected but increased seed protein concentration, perhaps due to a dilution effect.

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