EVALUATION OF THE EFFECTIVENESS OF *RHIZOBIUM LEGUMINOSARUM* STRAINS FOR PEA UNDER FIELD CONDITIONS

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ABSTRACT

One hundred and eight isolates of *Rhizobium leguminosarum* were screened for effectiveness for pea under controlled environments. Eight superior strains plus the commercially available pea inoculants, Nitragin 'C', Grip-Inotec, and Rhizogen were tested at Waldheim and Brooksby for effectiveness with Tipu and Trapper pea as host plants. The experiment was a RCBD, laid out as a split plot with the two pea varieties as main plot treatments and rhizobial strain as the subplot treatments, replicated four times.

At Waldheim total dry matter ranged from 2429 kg/ha for uninoculated Trapper to 4024 kg/ha for Tipu inoculated with strain 128C79. Grain yield ranged from 1046 kg/ha for Trapper inoculated with strain 175G3 to 1665 kg/ha for Tipu inoculated with strain 175G3. Total grain-N ranged from 33.7 kg/ha for uninoculated Tipu to 55.5 kg/ha for Trapper inoculated with strain 128C56G. At this site, strain 175G3 appears to be a superior strain for Tipu but is the least effective strain tested for Trapper.

At Brooksby, no significant differences due to strain or cultivar were observed. Values for total dry matter, grain yield, and total N were around 75% of those values found at Waldheim.

A survey to assess nodulation was carried out and all uninoculated pea were nodulated. This would indicate the presence of indigenous *R. leguminosarum* nodulating pea or possible cross contamination from adjacent plots.

The below average yield and the absence of a yield response due to inoculation can largely be attributed to the extreme dry weather occurring at both sites. Nodulation of the uninoculated control has also reduced the effect of inoculation on yield.

INTRODUCTION

In the early seventies the pea production in Saskatchewan was of marginal importance. The average annual production around 5,000 tonnes was predominantly produced in the North-East of the province (Saskatchewan Agriculture, Agricultural Statistics, 1987). In the early and mid eighties the export market for pea increased dramatically due to a large increase in the demand for feed pea from the European Community. In a few years, the acreage of pea increased from 8,000 acres in 1971 to an estimated 500,000 acres in 1988. Future increases or decreases in pea production in Saskatchewan will largely depend on the demand and internal production of feed pea from the European Community.

As in other legumes symbiosis, the effectiveness of the pea-rhizobium symbiosis is dependent on the host plant as well as the bacterial component (Phillips, 1980). Significant differences in grain yield of inoculated legumes, i.e. two cultivars of lentil, with various different rhizobium strains were found in Saskatchewan (Bremer et al., 1987; Bremer et al., 1989). Furthermore, there are reports which suggest that each legume cultivar may require a specific rhizobial strain to ensure optimum yield (Bello et al., 1980; Rennie and Kemp, 1983).

Improved rhizobial strains have been obtained for lentil, and the existence of a more effective rhizobial strain for pea as compared with the commercial available inoculant is most likely, especially as it is known that strain selection for pea under Saskatchewan growing conditions has not yet been carried out.

MATERIALS AND METHODS

One hundred and eight isolates of *Rhizobium leguminosarum* were screened for effectiveness for Homesteader-pea under controlled environments. A final selection of eight superior strains was made for further testing under field conditions. Three commercially available inoculants for pea, i.e. Nitragin 'C', Grip (Inotec) and Rhizogen, were included in the field study. Nitragin 'C' inoculant for pea and lentil consists of two strains, 128C56G and 175G10B, of which the former is selected specifically for pea and the latter for lentil. Both strains and an uninoculated control were included in the study along with the others strains and the commercial inoculants.

Field evaluation was carried out at two sites, Waldheim and Brooksby, both located in the Black Soil zone. Soil characteristics are given in Table 1. Two pea varieties, Trapper and the semi-leafless Tipu, were chosen. Trapper-pea is one of the most popular pea varieties grown in Saskatchewan and Tipu is a new variety with promising characteristics. Seeding, on stubble, at the recommended seeding rate of 125 kg/ha occurred on May 24 at Waldheim and May 25 at Brooksby. Both sites were cultivated and seeding was carried out with a self-propelled 4 row double disc seeder with a row spacing of 30 cm. Phosphorus was applied at a rate of 50 kg P_2O_5/ha as triple superphosphate and was seed placed. Individual plots measured 6.0 by 2.4 m. Two outside rows on one site of the plot were substituted with flax. Flax was used as the non-N_2-fixing reference crop for measuring N_2-fixation using the ^{15}N-natural abundance method. The experiment was a randomized complete block design (RCBD), laid out as a split plot, with the two pea varieties as the main plot treatment and rhizobial strain as the sub-plot treatments, replicated 4 times.

Table 1. Soil characteristics at Waldheim and Brooksby.

Site	Texture	pH	NO_3†	P‡	K‡	SO_4-S‡	Precipitation (cm)
			---------- kg/ha ----------				
Waldheim	Clay loam	7.5	35	7	130	3	23.5
Brooksby	Clay loam	7.4	71	70	840	14	13.6

†Total in 60 cm
‡Total in 15 cm

R. leguminosarum strains were grown for three days in mannitol yeast extract medium (Nelson and Child, 1981), centrifuged and resuspended in half volume of fresh mannitol yeast extract medium. Sterile bags containing 96 g Class II peat (Nitragin Co., Milwaukee) and 8 g $CaCO_3$ were inoculated with 25 ml of bacterial culture, sealed and kneaded to distribute the bacteria evenly. The bags were stored for 2 weeks at 20°C prior to use. Samples for determination of number of colony forming units were taken at time 0 and after two week incubation; 0.1 ml of appropriate dilutions from 1 mg peat were spread on mannitol yeast plates and incubated at 30°C for 4 days and number of colonies counted.

Gum arabic was used as a sticker and after coating the seeds with the inoculant, seeds were further coated with lime to reduce sticking of the seeds. Uninoculated treatments were seeded first. After seeding of both pea varieties with one particular strain, the seeding belts and all other components of the seeder which might have been in contact with the seeds were cleaned with 95% ethanol in order to reduce cross contamination between the various strains. At time of seeding, subsamples of the two inoculated pea varieties were taken and tested for the number of colony forming units per seed which provides an indication of the quality of the inoculant. Colony forming units of the various rhizobial strains are reported in Table 2.

Table 2. Colony forming units on inoculated pea (Tipu and Trapper) at Waldheim and Brooksby.

Strain	Site			
	Waldheim		Brooksby	
	Tipu	Trapper	Tipu	Trapper
	colony forming units/seed			
128C56G	1.4*10.7	-	-	7.1*10.6
175G10B	2.3*10.5	-	-	1.2*10.5
Rhizogen	1.5*10.6	-	-	6.0*10.7
Nitragin C	7.6*10.7	-	-	6.8*10.7
Inotec	6.1*10.7	-	-	7.7*10.7
128C52	4.9*10.6	-	-	5.2*10.5
128C54	-	7.7*10.5	5.8*10.6	-
128C23	-	2.4*10.4	1.2*10.5	-
128C30	-	-	0.9*10.7	-
128C79	-	4.7*10.5	6.5*10.6	-
175G3	-	2.1*10.5	3.8*10.5	-
175G16	-	9.7*10.6	1.2*10.7	-
NA502	-	1.2*10.6	1.2*10.7	-

Weeds were controlled with Hoegrass (flamprop methyl) and Poast (Sethoxydem). Further weed control was carried out by hand.

Pea and flax were harvested at physiological maturity. The maturity of the pea was dependent on the inoculant used. Pea and flax of each sub plot were always harvested at the same day. The various harvest dates of Trapper and Tipu and the corresponding flax at the two sites are reported in Table 3. Harvest area for pea was 4.0 by 1.2 m, for flax 4.0 by 0.6 m. Plants were air dried, threshed and a subsample was further oven dried to convert air dry weight into oven dry weight. Pea (grain) was ground to pass a sieve of 0.2 mm and analyzed for total N by Kjeldahl (Bremer and Mulvaney, 1982). Flax was analyzed for total N without grinding. To determine the ^{15}N value of pea solely dependent on atmospheric N_2 as a N source, Trapper and Tipu were grown hydroponically in an N-free medium under controlled environments. N_2-fixation was measured according to Rennie and Rennie (1982).

Table 3. Harvest dates of pea at Waldheim and Brooksby.

Strain	Site			
	Waldheim		Brooksby	
	Tipu	Trapper	Tipu	Trapper
128C56G	Sept. 15	Sept. 15	Sept. 8	Aug. 21/Sept. 8
175G10B	Sept. 15	Sept. 15	Aug. 29	Aug. 21/Sept. 8
Rhizogen	Sept. 1	Sept. 1	Sept. 8	Aug. 21/Sept. 8
Nitragin C	Sept. 1	Sept. 15	Aug. 29	Aug. 21/Sept. 8
Inotec	Sept. 15	Sept. 15	Sept. 8	Sept. 8
128C52	Sept. 1	Sept. 15	Aug. 29	Aug. 21/Sept. 8
128C54	Sept. 1	Sept. 1	Aug. 29	Aug. 21/Sept. 8
128C23	Sept. 1	Sept. 1	Aug. 29	Aug. 21/Sept. 8
128C30	Sept. 15	Sept. 15	Aug. 29	Sept. 8
128C79	Sept. 1	Sept. 15	Sept. 8	Aug. 21/Sept. 8
175G3	Sept. 15	Sept. 15	Aug. 29	Sept. 8
175G16	Sept. 1	Sept. 15	Aug. 29	Aug. 21/Sept. 8
NA502	Sept. 1	Sept. 15	Aug. 29	Aug. 21/Sept. 8
Uninoculated	Aug. 22	Aug. 22	Sept. 8	Sept. 8

RESULTS AND DISCUSSION

The year of 1988 was characterized by severe drought which reduced germination and yield below the long term average of 1675 kg/ha (Saskatchewan Agriculture, 1988). Due to lack of moisture the growth of pea was stressed and the demand for N became limited. The effect of inoculation on yield was much reduced.

Physiological maturity and subsequently date of harvest were dependent on the strain used. For example at Waldheim, pea inoculated with strain 128C54, Rhizogen, and 128C23 was harvested 2 weeks earlier than pea inoculated with the remaining strains (Table 3). It is possible that rhizobial strains which prolong the growth period for pea for two weeks may increase grain yield. However, in this study it appears that time of physiological maturity had no significant effect on total grain yield. Pea inoculated with early maturing strains did not consistently yield less than pea inoculated with strains which delayed maturing (Table 4). A potential disadvantage can be that strains which do prolong the growth period of the pea will delay harvest and may cause problems when frost comes early.

Total dry weight at Waldheim ranged from 2429 kg/ha for uninoculated Trapper to 4024 kg/ha for Tipu inoculated with strain 175G3 (Table 4). Grain yield ranged from 1046 kg/ha for Trapper inoculated with 128G3 to 1665 kg/ha for Tipu inoculated with 175G3. For Waldheim, it appears that strain 175G3 is more effective on Tipu than the commercially available strain and the grain yield increase as compared with the commercial C inoculant is 35 %. However, this particular strain is the least effective strain for Trapper and compared with Trapper inoculated with commercial C inoculant, grain yield decreased by 27%. Similar observations have been found for other legumes where a specific strain was more effective for one cultivar but less for another (Rennie and Kemp, 1983).

Total N ranged from 33.7 kg N/ha for uninoculated Tipu to 55.5 kg N/ha for Trapper inoculated with strain 128C56G. As with total grain yield, total N was lower than in most other studies reported from the Prairies (Bremer et al., 1988) and this is most likely directly related to the drought.

Around 40 % of the N was derived from N_2-fixation and was independent of pea cultivar and strain (Table 4). Apparently, the amount of available soil N was not sufficient to meet the plant N requirements, even under the poor growing conditions as encountered in 1988. The amount of N_2-fixed averaged around 20 kg N/ha and was also independent of pea cultivar and strain (Table 4).

At Brooksby, no significant differences due to inoculant strain or cultivar were found (Table 5). Total dry weight ranged from 1920 kg/ha for uninoculated Trapper to 2561 kg/ha for Tipu inoculated with strain 128C56G or 128C23. Grain yield ranged from 803 kg/ha for Tipu inoculated with strain 128C54 to 1158 kg/ha for Trapper inoculated with strain 175G10B. For total N those figures were 18.1 kg/ha for Tipu inoculated with 128C54 and 38.1 for Tipu inoculated with Rhizogen. The percentage of N_2-fixed was rather low and ranged from 0.4 to 31.1 %, showing a large variability. With one exception, the amount of N_2-fixed for every pea-rhizobium symbiosis tested was smaller than 10 kg/ha. All these values were lower than those at Waldheim (Table 4).

A survey to assess nodulation was carried out in August. At both sites, the uninoculated control was nodulated and judging by the reddish color of the inside of the nodule most probably did fix N_2. Due to the presence of nodules on the uninoculated control, the possible increase in yield of inoculated pea as compared with the uninoculated control might have or been reduced substantially. At this time, it has yet to be established if

Table 4. Yield and percent grain N of pea at Waldheim.

Pea	Strain	Dry weight	Grain				
		kg/ha	kg/ha	% N	kg N/ha	% Ndfa	kg Ndfa
Tipu	128C56G	3235	1256	3.30	41.9	46.2	19.4
	175G10B	3295	1284	3.44	43.6	47.2	20.6
	Rhizogen	2841	1100	3.15	34.0	43.2	16.1
	Nitragin C	3106	1238	2.58	32.4	30.7	11.2
	Inotec	3185	1346	2.70	36.7	35.6	12.5
	128C52	3473	1456	2.92	43.8	42.3	21.5
	128C54	3012	1250	3.15	39.6	45.3	18.4
	128C23	2925	1254	2.74	34.6	47.3	16.1
	128C30	3334	1314	3.00	40.4	31.2	13.6
	128C79	2895	1248	2.79	34.8	47.9	16.3
	175G3	4024	1665	2.99	49.5	39.7	19.7
	175G16	3160	1354	2.98	38.5	35.2	14.9
	NA502	3175	1349	2.84	39.8	48.2	19.5
	Uninoc	3400	1418	2.47	33.7	37.1	12.3
Trapper	128C56G	3524	1522	3.67	55.5	52.1	29.2
	175G10B	3339	1390	2.99	41.8	47.3	19.3
	Rhizogen	2556	1094	2.76	30.2	43.6	13.0
	Nitragin C	3321	1429	2.91	42.3	41.8	17.4
	Inotec	3041	1327	3.20	42.4	48.2	22.5
	128C52	3372	1390	3.02	41.8	47.5	19.3
	128C54	2657	1214	3.38	41.6	43.6	19.1
	128C23	2588	1147	3.22	36.4	35.7	13.3
	128C30	2906	1312	3.08	38.7	41.2	15.6
	128C79	2559	1063	3.65	38.8	41.2	16.9
	175G3	2613	1046	3.63	38.0	49.6	19.2
	175G16	2722	1186	3.14	37.0	29.3	10.6
	NA502	2830	1225	3.08	38.6	21.1	7.9
	Uninoc	2429	1087	3.25	35.1	39.1	13.4
	LSD (<0.05)	800	369	0.81	14.3	15.6	7.6

Table 5. Yield and percent grain N of pea at Brooksby.

Pea	Strain	Dry weight	Grain				
		kg/ha	kg/ha	% N	kg N/ha	% Ndfa	kg Ndfa
Tipu	128C56G	2561	996	2.94	29.4	4.5	1.4
	175G10B	2038	832	2.44	20.6	10.2	0.9
	Rhizogen	2524	966	2.93	28.5	9.0	2.5
	Nitragin C	2333	920	2.65	24.3	0.5	0.1
	Inotec	2298	884	2.44	21.9	4.9	0.8
	128C52	2350	953	2.57	24.3	0.4	0.1
	128C54	1899	803	2.29	18.1	14.3	2.2
	128C23	2561	1009	2.94	29.4	8.2	2.1
	128C30	2313	974	2.58	24.2	11.3	3.1
	128C79	2495	1017	3.19	32.6	3.7	1.0
	175G3	2437	1004	2.33	23.4	8.8	2.1
	175G16	2082	882	2.99	26.1	6.1	1.6
	NA502	2453	1051	2.52	26.1	23.0	5.1
	Uninoc	2252	924	3.02	27.4	16.7	4.5
Trapper	128C56G	2342	1085	2.77	29.3	24.1	7.3
	175G10B	2354	1158	3.03	35.4	14.6	5.6
	Rhizogen	2450	1142	3.35	38.1	10.2	3.3
	Nitragin C	2066	997	3.18	29.6	26.6	7.2
	Inotec	2099	998	2.95	28.8	21.7	5.7
	128C52	2155	1031	2.98	30.8	5.7	2.5
	128C54	2201	1017	2.69	26.8	12.2	3.8
	128C23	1996	1000	2.89	28.5	15.4	4.5
	128C30	2280	999	3.00	29.8	26.4	7.5
	128C79	2213	1134	2.89	32.3	31.1	10.1
	175G3	2093	919	2.90	26.1	15.7	4.1
	175G16	2171	1042	3.01	31.3	20.3	6.8
	NA502	2191	1056	2.75	28.4	29.7	8.8
	Uninoc	1920	861	3.20	27.6	21.4	5.6
	LSD (<0.05)	NS	NS	NS	NS	NS	NS

the nodules formed on the uninoculated control plants were indeed formed by the introduced strains or were due to cross contamination by indigenous *R. leguminosarum*. Furthermore, it is unknown if all or only some of the nodules formed on the inoculated pea were formed by the introduced strains. A possible explanation for the absence of an inoculation response could be given by the presence of competitive indigenous strains. Such indigenous strains might be less effective but more competitive than the selected strains thus outcompeting all the introduced strains during the initial process of nodule formation.

At both sites no significant differences among the three commercially available inoculants were observed. As with the eight selected strains, the superiority of the commercial inoculant will only become apparent when the growing conditions are more ideal than those encountered in 1988, the demand for N by the plant is maximal and the presence of available N is low. Those growing conditions did not prevail at the two sites selected in 1988.

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LITERATURE CITED

Agricultural Statistics. 1987. Saskatchewan Agriculture, Economics Statistics, Regina.

Bello, A.B., W. A. Ceron-Dias, C.D. Nickell, E.O. El Sherif, and L.C. Davis. 1980. Influence of cultivar, between-row spacing, and plant population of fixation of soybeans. Crop Sci. 20: 751-755.

Bremer, E., C. van Kessel, and R. Karamanos. 1989. Nitrogen, phosphorus and rhizobial strain responses by lentil. Can. J. Soil Sci. (in press).

Bremer, E., C. van Kessel, L. Nelson, R.J.Rennie, and D.A.Rennie. 1989. Selection of *Rhizobium leguminosarum* strains for lentil (*Lens culinaris*) under growth room and field conditions. Submitted for publication.

Bremer E., R.J. Rennie, and D.A. Rennie. 1988. Dinitrogen fixation of lentil, field pea and fababean under dryland conditions. Can. J. Soil Sci. 68: 553-562.

Bremner, J.M., and C.S. Mulvaney. 1982. Nitrogen-Total. *In* Methods of Soil Analysis. (Eds., A.L. Page, R.H. Miller and D.R. Keeney). pp. 595-624. American Society of Agronomy, Madison, WI.

Nelson, L.M., and J.J. Child. 1981. Nitrogen fixation and hydrogen metabolism by *Rhizobium leguminosarum* isolates in pea root nodules. Can. J. Microbiol. 27: 1028-1034.

Phillips, D.A. 1980. Efficiency of symbiotic nitrogen fixation in legumes. Ann. Rev. Plant Physiol. 31:29-49.

Rennie, R.J., and G.A. Kemp. 1983. N_2-fixation in field beans quantified by ^{15}N isotope dilution. II. Effect of cultivars of beans. Agron. J. 75: 645-649.

Rennie, R.J., and D.A. Rennie. 1983. Techniques for quantifying N_2-fixation in association with non-legumes under field and greenhouse conditions. Can. J. Microbiol. 29: 1022-1035