EFFECT OF RHIZOBIAL STRAIN ON LENTIL N_2 FIXATION AND YIELD

E. Bremer[1], R.J. Rennie[2] and D.A. Rennie[1]
[1]Department of Soil Science, University of Saskatchewan
[2]Esso Chemical Canada, Lethbridge

The area seeded to lentil in Saskatchewan has increased from virtually zero in 1970 to 105 000 ha in 1986 (Saskatchewan Agriculture, 1986), and further increases are likely. Lentil crops generally do not require fertilizer N because they can obtain a large proportion of their N requirements from the atmosphere by forming a symbiotic relationship with bacteria of the species *Rhizobium leguminosarum*. However, there is a wide range in effectiveness between rhizobial strains, and the best strain also depends on legume cultivar (Nutman, 1984) and environmental conditions (Gibson, 1977). May and Bohlool (1983) found that only 5 of 31 strains of *R. leguminosarum* were highly effective with lentil. Rai et al. (1985) observed significant interaction of lentil genotype and rhizobial strain under salt stressed conditions. The objective of this study was to compare N_2 fixation and yield of lentil inoculated with different rhizobial strains.

Materials and Methods

Twenty strains were initially collected from various sources, and were maintained on TY agar slants. Suspensions of rhizobial cells were obtained by inoculating 50 ml of TY broth with cells from the slants and incubating the broth at 37°C for 48 hours. Lentil seeds were surface sterilized with a 10% bleach solution (5 min.), rinsed thoroughly with sterile water and then pre-germinated under sterile conditions for 2 days. Three to four lentil seeds were placed in a growth pouch (Northrup King Co.) and each seed was inoculated with 0.1 ml of rhizobial cell suspension. The cell count of the cell suspensions was always greater than 10^8 viable cells ml^{-1}. The growth pouches were then placed in a growth chamber with a 16 hr day and a day/night temperature of 22°C/18°C. Lentil seedlings were thinned to two per growth pouch after one week, and were alternately supplied with N-free nutrient solution (Manhart and Wong, 1979) and deionized water. Lentil plants were harvested at four to five weeks. Uninoculated lentil plants often had low levels of nodulation, but these nodules were generally ineffective. N_2 fixing activity was determined by the acetylene reduction method (Hardy et al., 1968). Lentil plants from each growth pouch were placed in a 0.9 l jar that was then sealed, and 100 ml of air was replaced with 90 ml of purified acetylene via a septum. After 20 minutes a one ml subsample was withdrawn and injected directly into a gas chromatograph equipped with a flame ionization detector, and ethylene concentration measured. Nodule number, nodule dry weight and total plant dry weight were also measured at this time. Ten strains with generally good nodulation and high levels of acetylene reduction were selected for further testing (Table 1).

Table 1: Strain #, source and most probable number (in prepared inoculant) of the *R. leguminosarum* strains tested in the field in 1986.

Strain #	Source	MPN
92A3*	Nitragin - Mississippi	$>3.55 \times 10^{11}$
128A12*	Nitragin - Brazil	4.36×10^{8}
175P1*	Nitragin	4.36×10^{8}
1-ICAR-SYR-Le-13	ICARDA - Syria	5.44×10^{10}
1-ICAR-MOR-Le-16	ICARDA - Morocco	$>3.55 \times 10^{11}$
1-ICAR-TUR-Le-18	ICARDA - Turkey	$>3.55 \times 10^{11}$
1-ICAR-SYR-Le-19	ICARDA - Syria	$>3.55 \times 10^{11}$
1-ICAR-SYR-Le-20	ICARDA - Syria	$>3.55 \times 10^{11}$
TAL 634	NifTal - Mississippi	$>3.55 \times 10^{11}$
99A1	Nitragin - Washington	$>3.55 \times 10^{11}$
Nitragin**		4.90×10^{8}

* Strains currently used in Nitragin inoculant type 'C' for pea and lentil
** Nitragin inoculant type 'C'

Sterile sphagnum peat moss was obtained by autoclaving Nitragin inoculant type 'B' (which contains rhizobial strains capable of nodulating clover but not lentil) three times at three day intervals in order to kill the existing rhizobia. The peat was then dried at 50°C for 3 hours, and added to sterile 'Whirlpak' bags at a rate of 100 g bag^{-1}. Fifty ml of rhizobial cell suspension was injected and mixed into each bag, and these were cured at 25°C for two weeks and stored at 4°C thereafter. Most probable number (Weaver and Frederick, 1982) of *R. leguminosarum* in the prepared inoculant was obtained for each strain (Table 1). Peat inoculated with sterile broth was free of *R. leguminosarum.*

The selected strains were tested at two locations during the summer of 1986. One site was located near Waldheim, approximately 60 km north of Saskatoon, and the other was located approximately 10 km south of Watrous (Table 2). Plots were set up in a split plot (RCB) design with four replications. Main plot treatment was lentil cultivar (Eston and Laird), and subplot treatment was rhizobial strain (commercial inoculant [Nitragin inoculant type 'C'], no inoculant and the 10 strain inoculants).

Table 2: Description of field plot sites

	Watrous	Waldheim
Great group	Orthic Dark Brown Chernozem	Orthic Black Chernozem
Association	Elstow-Weyburn loam	Oxbow loam
Planting date	May 22	May 21
Harvest date		
Eston	August 18	August 26
Laird	August 27	September 10
Spring soil NO_3^--N (kg ha^{-1})	18.5	55.6
Precipitation (cm)	21.2	15.0
Total water use (cm)		
Eston	23.2	22.2
Laird	25.2	23.1

The plot area had been rototilled in the fall, and no further cultivation was done in the spring. Phosphorus was banded in the plot area before seeding at a rate of 50 kg P_2O_5 ha^{-1}. Each plot was 1.5 x 3 m and consisted of four rows with a spacing of 30 cm. Eston and Laird lentil were seeded at rates of 38 kg ha^{-1} and 80 kg ha^{-1}, respectively. Gum arabic solution (30% wt/vol) was used as a sticker for the inoculum, and inoculum was applied at 0.2 g per 100 seeds (i.e. $>10^6$ rhizobia per seed) (Elgeba and Rennie, 1984). Treflan was applied and rototilled into the plot area in the fall of 1985, and any additional weed control was done by hand in 1986. In order to measure N_2 fixation by the ^{15}N isotope dilution method, urea (4.6337 atom% ^{15}N excess) was injected at a rate of 5 kg N ha^{-1} into the soil adjacent to two 1.2 m plant rows in each plot at approximately 12 days after planting (Rennie, 1986).

Nodulation was checked once in late June and again in late July. At maturity, two 1 m rows were harvested for grain yield, and two 1 m ^{15}N enriched rows were harvested for N and ^{15}N concentration. Ten plants were also harvested for natural levels of ^{15}N abundance for the following treatments: SYR-Le-13, 99A1 and no inoculant. All plant samples were dried at 60 °C until constant weight and then weighed and ground to pass a 2 mm sieve. N concentration (not including NO_3^--N) was measured by Kjedahl digestion and distillation (Bremner,1965). The ^{15}N content of the plant material was determined by LiOBr conversion of NH_4^+ to N_2 (Ross and Martin, 1970; Porter and O'Deen, 1977) followed by analysis of ^{15}N:^{14}N ratios on a VG Micromass 602D isotope ratio mass spectrometer.

Total yield was estimated from the total dry weight of the ^{15}N enriched and grain samples. Total N was calculated by multiplying total yield by N concentration (^{15}N enriched sample). Grain yield was calculated by multiplying total yield by the ratio of grain to total yield (grain sample). Straw N was calculated as the difference between total N and grain N. N_2 fixation was estimated by both the classical difference (CD) and the ^{15}N isotope dilution (ID) methods. N_2 fixation was also estimated for strains SYR-13 and

99A1 by the ID method using the natural ^{15}N enrichment of the soil (NA). N_2 fixation was calculated as follows (Rennie et al., 1978):

$$N_2 \text{ fixed (ID,NA)} = (1 - \text{at.\% } ^{15}N \text{ ex. [fs] / at.\% } ^{15}N \text{ ex. [nfs]}) * N \text{ [fs]}$$

$$N_2 \text{ fixed (CD)} = N \text{ [fs]} - N \text{ [nfs]}$$

where fs = fixing system
nfs = non-fixing system (uninoculated treatment)
N = N yield (kg ha^{-1})

Atom% ^{15}N excess was calculated with reference to the natural abundance of the atmosphere (0.3663 atom% ^{15}N).

An analysis of variance for a split plot design was done for all measured crop parameters and means were compared by Duncan's multiple range test (Little and Hills, 1978).

Results and Discussion

All strains provided moderate to heavy nodulation, while the uninoculated treatment was virtually free of nodules (Table 3). This indicates that there were few indigenous rhizobia in these soils capable of nodulating lentils, and therefore also little competition with the introduced strains. Although there were significant differences in nodulation between strains, these were not closely related to the amount of N_2 fixed or dry matter accumulated (Table 4). N_2 fixation of Laird actually increased with decreasing nodulation, but this was primarily due to very heavy nodulation on an ineffective strain (SYR-13).

Table 3: Average nodule number and nodule ratings at the June and July sampling dates, respectively.

Strain #	June Eston	June Laird	July Eston	July Laird
	#nodules plant^{-1}		nodule rating*	
		Watrous		
Uninoc.	0.3_{d}**	0.0_{c}	0.0_{b}	0.0_{b}
92A3	17.8_{abc}	14.8_{b}	2.5_{a}	2.0_{a}
128A12	14.3_{bc}	17.0_{b}	2.3_{a}	2.0_{a}
175P1	11.3_{c}	27.3_{a}	2.0_{a}	2.5_{a}
SYR-13	19.8_{ab}	20.5_{ab}	2.5_{a}	2.8_{a}
MOR-16	13.8_{bc}	11.5_{b}	2.3_{a}	1.8_{a}
TUR-18	23.8_{a}	17.3_{b}	2.5_{a}	2.5_{a}
SYR-19	16.5_{bc}	17.3_{b}	2.3_{a}	2.8_{a}
SYR-20	11.3_{c}	18.3_{ab}	2.0_{a}	2.5_{a}
TAL 634	18.8_{ab}	16.8_{b}	2.0_{a}	2.5_{a}
99A1	20.0_{ab}	19.5_{ab}	2.3_{a}	2.3_{a}
Nitragin	16.0_{bc}	16.3_{b}	2.3_{a}	2.0_{a}
		Waldheim		
Uninoc.	0.0_{c}	0.3_{b}	0.0_{c}	0.0_{c}
92A3	7.5_{b}	24.8_{a}	2.1_{b}	2.3_{ab}
128A12	18.0_{a}	16.8_{a}	2.6_{a}	2.2_{ab}
175P1	8.8_{b}	20.0_{a}	2.1_{b}	2.5_{ab}
SYR-13	17.5_{a}	17.0_{a}	2.6_{a}	2.7_{a}
MOR-16	18.5_{a}	19.5_{a}	2.5_{ab}	2.5_{ab}
TUR-18	14.0_{ab}	16.5_{a}	2.5_{ab}	2.6_{a}
SYR-19	12.0_{ab}	17.8_{a}	2.4_{ab}	2.6_{a}
SYR-20	13.3_{ab}	20.8_{a}	2.3_{ab}	2.3_{ab}
TAL 634	13.0_{ab}	19.3_{a}	2.4_{ab}	2.0_{b}
99A1	19.8_{a}	18.0_{a}	2.4_{ab}	2.4_{ab}
Nitragin	12.8_{ab}	16.5_{a}	2.0_{b}	2.3_{ab}

* Rating system: 0 = no nodules; 1 = few nodules; 2 = moderately nodulated; 3 = heavily nodulated

** Values followed by a common letter within columns are not significantly ($P < 0.05$) different from each other.

Table 4: Correlation coefficients between nodulation and final harvest estimates of N_2 fixation and total dry matter.

Yield	June: # of nodules		July: nodule rating	
	Eston	Laird	Eston	Laird
		Watrous		
Total DM	0.06	-0.19	-0.17	-0.50
N_2 Fixed	0.22	-0.27	-0.07	-0.57*[1]
		Waldheim		
Total DM	0.13	0.10	-0.12	-0.52
N_2 Fixed	0.18	0.08	-0.10	-0.65**

[1] Significant at *$P < 0.1$; **$P < 0.05$

ID and CD estimates of %Ndfa and N_2 fixed were very similar ($R > 0.96$; slope not significantly different from one; y-intercept not significantly different from zero). NA estimates of %Ndfa for strains SYR-13 and 99A1 were less precise than the ID or CD estimates (Table 5), but may have been more accurate because the ^{15}N enrichment of the soil was more uniform (Witty, 1983). Ranking of the strains for N_2 fixation is probably the same for all methods, and ID estimates of N_2 fixation were used for comparing strains because they were available for all treatments and were the least variable.

The amount of N_2 fixed as measured by the ID method ranged from 17 to 105 kg ha^{-1} at Watrous and from 0 to 61 kg ha^{-1} at Waldheim (Table 6). Most rhizobial strains fixed about the same amount of N_2, but two strains (175P1, SYR-13) fixed significantly less and one strain (99A1, Watrous only) fixed significantly more for Eston lentil. One strain (SYR-13) also fixed significantly less for Laird lentil. Ranking of strains was the same at both sites, even though N_2 fixation was lower at Waldheim due to higher soil NO_3^- levels.

Table 5: Comparison of three methods of estimating %Ndfa for strains SYR-13 and 99A1. The mean of the uninoculated treatment was used in all calculations.

		NA[1]	ID[2]	CD[3]
			Watrous	
Eston	SYR-13	48.5 ±22.5	35.7 ±4.5	33.5 ±4.0
	99A1	64.1 ± 3.9	75.1 ±2.5	76.7 ±1.7
Laird	SYR-13	65.9 ±16.1	41.9 ±4.6	39.7 ±6.7
	99A1	61.3 ±15.3	75.9 ±2.9	60.5 ±5.2
			Waldheim	
Eston	SYR-13	35.4 ±16.5	-17.6 ± 8.0	-25.3 ±29.3
	99A1	62.0 ±26.0	35.7 ± 6.2	33.1 ± 8.5
Laird	SYR-13	42.9 ± 4.9	-13.7 ± 8.4	-0.6 ±11.7
	99A1	60.5 ±17.0	34.7 ±14.8	33.4 ±17.0

1 ^{15}N isotope dilution using the natural ^{15}N enrichment of the soil
2 ^{15}N isotope dilution using soil enriched with ^{15}N fertilizer
3 Classical difference method

Nitragin inoculant type 'C' compared favorably with the introduced strains, and only strain 99A1 fixed significantly more N_2 than the commercial inoculant (Eston lentil, Watrous only) (Table 6). Strains 92A3, 128A12 and 175P1 are supplied in Nitragin inoculant type 'C', and these were also tested seperately in this experiment. 175P1 was much poorer for Eston lentil at both sites (Fig. 1), but was not significantly different from the other strains for Laird lentil. 128A12 consistently fixed more N_2 than 175P1 or 92A3, and generally fixed as much or slightly more than the commercial inoculant. May and Bohlool (1983) also reported that 175P1 was less effective for the lentil cultivars they tested. However, the presence of the less effective strain did not significantly reduce lentil N_2 fixation for the multistrain inoculant.

Table 6: %Ndfa (% of total N from atmospheric N_2), N_2 fixed (^{15}N isotope dilution), total dry matter and grain yield of Eston and Laird lentil inoculated with various rhizobial strains.

Rhizobial strain	Eston				Laird			
	%Ndfa	N_2 fixed	DM	Grain	%Ndfa	N_2 fixed	DM	Grain
		------- (kg ha^{-1}) -------				------- (kg ha^{-1}) -------		
Watrous								
Uninoc.	-	-	2450_{f}*	921_{d}	-	-	2926_{d}	946_{a}
92A3	67_{ab}	63_{c}	4496_{cd}	2053_{b}	65_{d}	57_{a}	4807_{b}	1222_{a}
128A12	68_{a}	68_{bc}	4896_{bc}	2081_{b}	74_{ab}	75_{a}	5597_{ab}	1170_{a}
175P1	45_{c}	31_{d}	4129_{de}	1681_{c}	67_{cd}	65_{a}	5332_{ab}	1396_{a}
SYR-13	36_{d}	17_{e}	3598_{e}	1461_{c}	42_{e}	25_{b}	3956_{c}	1343_{a}
MOR-16	70_{ab}	84_{b}	5392_{ab}	2343_{ab}	66_{cd}	68_{a}	5479_{ab}	1378_{a}
TUR-18	71_{ab}	80_{bc}	4837_{bc}	2095_{b}	69_{bcd}	67_{a}	4982_{ab}	1180_{a}
SYR-19	65_{b}	73_{bc}	5077_{bc}	2311_{ab}	72_{abc}	66_{a}	5224_{ab}	1290_{a}
SYR-20	66_{b}	70_{bc}	4973_{bc}	2170_{ab}	68_{bcd}	63_{a}	5270_{ab}	1594_{a}
TAL 634	65_{b}	72_{bc}	5054_{bc}	2410_{a}	69_{bcd}	62_{a}	5458_{ab}	1490_{a}
99A1	75_{a}	105_{a}	5771_{a}	2451_{a}	76_{a}	68_{a}	5277_{ab}	1000_{a}
Nitragin	70_{ab}	79_{bc}	5200_{abc}	2273_{ab}	72_{abc}	68_{a}	5547_{a}	1555_{a}
Waldheim								
Uninoc.	-	-	5560_{cd}	2326_{cd}	-	-	5427_{b}	2198_{a}
92A3	20_{ab}	27_{ab}	6195_{bc}	2577_{abc}	27_{a}	35_{a}	7277_{a}	2188_{a}
128A12	32_{a}	45_{a}	6646_{ab}	2716_{abc}	37_{a}	52_{a}	7621_{a}	1676_{a}
175P1	3_{b}	5_{bc}	5403_{d}	2388_{bcd}	30_{a}	39_{a}	7227_{a}	1756_{a}
SYR-13	-18_{c}	-13_{c}	4863_{d}	1964_{d}	-14_{b}	-14_{b}	5806_{b}	2109_{a}
MOR-16	36_{a}	53_{a}	6836_{ab}	2820_{ab}	24_{a}	33_{a}	7315_{a}	2064_{a}
TUR-18	33_{a}	45_{a}	6621_{ab}	2945_{a}	34_{a}	44_{a}	7512_{a}	2002_{a}
SYR-19	25_{a}	36_{a}	6845_{ab}	2657_{abc}	29_{a}	38_{a}	7244_{a}	2110_{a}
SYR-20	30_{a}	43_{a}	7392_{a}	2946_{a}	39_{a}	58_{a}	7935_{a}	2448_{a}
TAL 634	28_{a}	38_{a}	6239_{bc}	2681_{abc}	36_{a}	55_{a}	7540_{a}	1927_{a}
99A1	36_{a}	52_{a}	6707_{ab}	2615_{abc}	35_{a}	61_{a}	8346_{a}	1723_{a}
Nitragin	29_{a}	44_{a}	6981_{ab}	2868_{a}	31_{a}	46_{a}	7456_{a}	2196_{a}

* Values followed by a common letter within a column are not significantly ($P < 0.05$) different from each other.

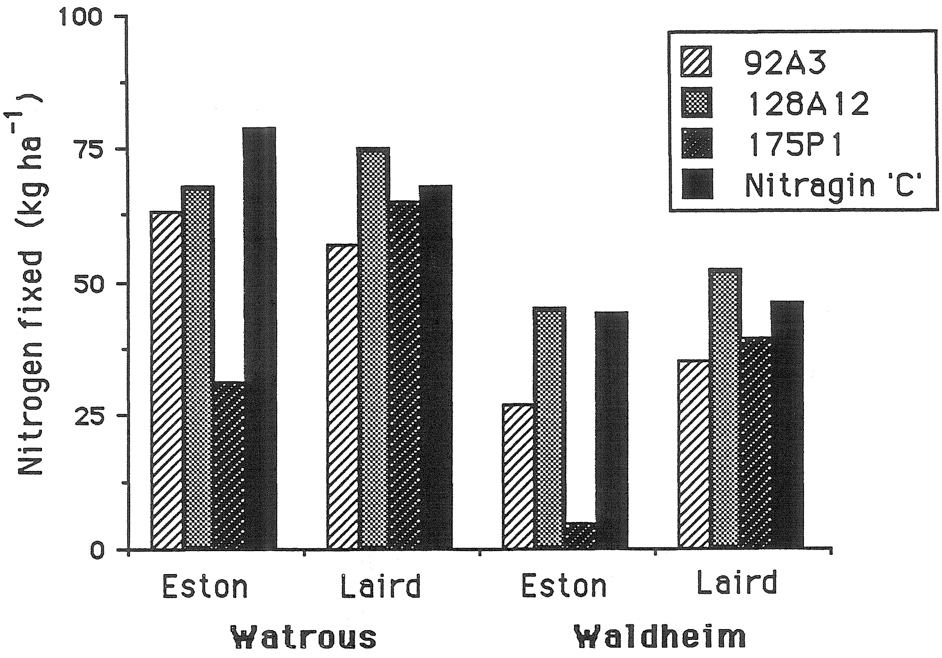

Figure 1: N_2 fixed (estimated by ^{15}N isotope dilution) by lentil inoculated with Nitragin inoculant type 'C' and it's component strains

The strains which supported the highest levels of N_2 fixation also supported the greatest accumulation of dry matter (Fig. 2). N_2 fixation was not as closely related to grain yield because the most effective strains supplied sufficient N to encourage vegetative growth at the expense of reproductive or seed growth. This type of response occurred because lentil require a period of stress to encourage seed set, and the relatively moist conditions in 1986 combined with greater N supply by the more efficient strains minimized plant stress. Although more effective strains did not always increase grain yield, they did increase the amount of N in crop residues dramatically (Fig. 3). A proportion of this would be available for subsequent crop growth.

Growth-room and field measurements of plant growth and N_2 fixation were more highly correlated for Eston lentil than Laird lentil. This may be partially due to greater strain differences in Eston lentil. Growth-room nodulation was inversely related to dry matter accumulation and N_2 fixation in the field for both Eston and Laird lentil. Acetylene reduction and plant growth in growth pouches were positively correlated to field measurements for Eston lentil but were not related to field measurements for Laird lentil. Future selection of rhizobial strains in the growth-room should probably be based on acetylene reducing activity at a slightly later time period (e.g. 6 weeks).

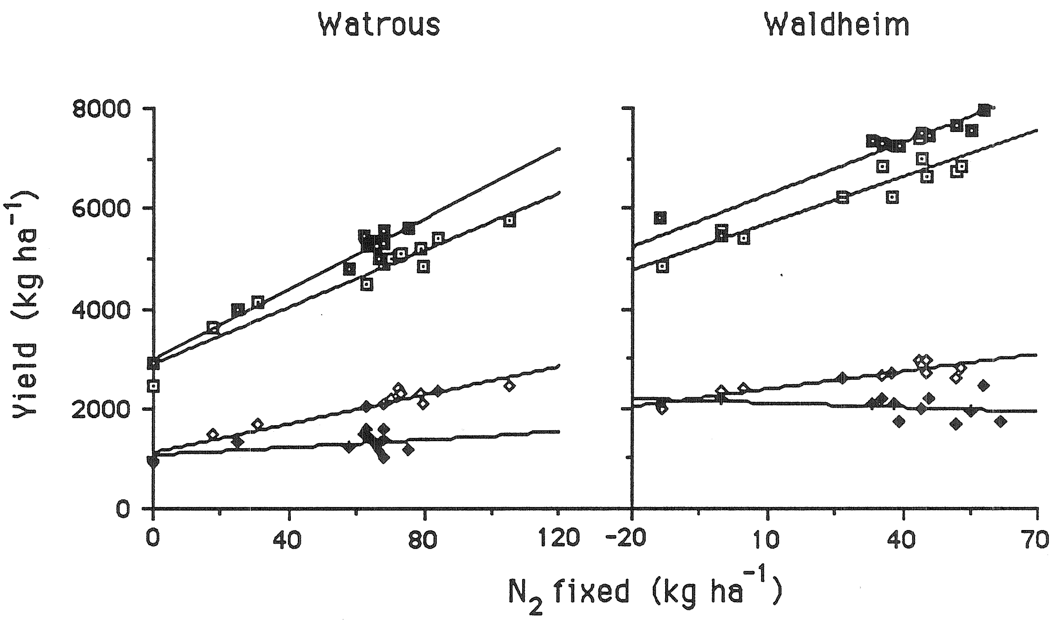

Fig. 2: Dry matter and grain yield of Eston and Laird lentil as a function of N_2 fixed (▫ Eston-DM; ▪ Laird - DM; ◊ Eston - grain; ♦ Laird-grain)

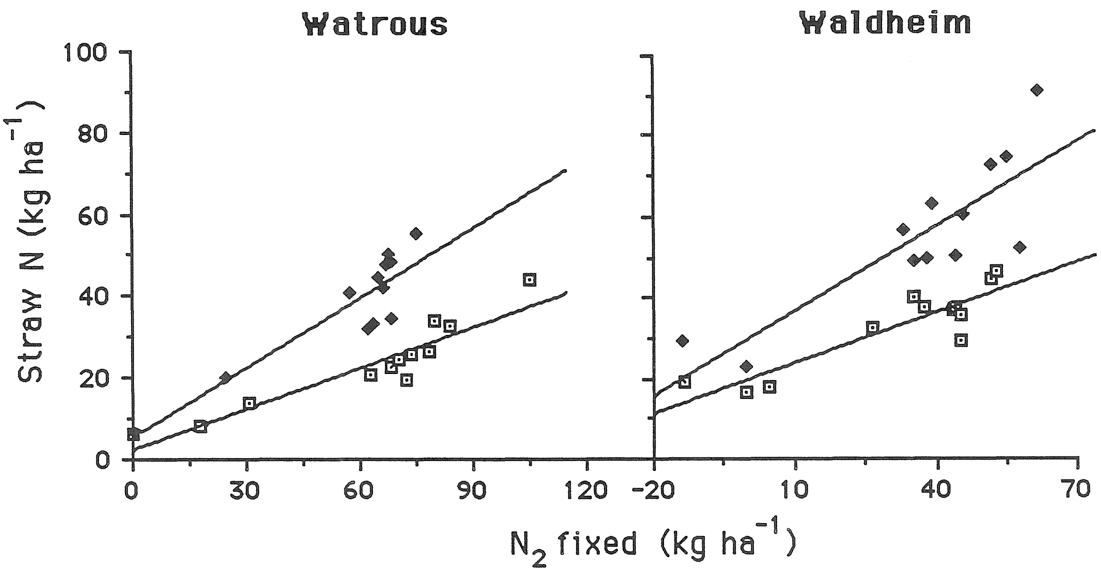

Fig.3: Increase in straw N with increasing N_2 fixation for Eston (□) and Laird (♦) lentil at Watrous and Waldheim

Table 7: Correlation between growth-room and field measurements of lentil inoculated with ten different strains of rhizobia.

Growth-room measurement	Coefficients of correlation: Eston Total DM	Eston N_2 fixed	Laird Total DM	Laird N_2 fixed
		Watrous		
#nodule plant^{-1}	-0.48	-0.43	-0.12	-0.03
C_2H_4[1] plant^{-1}	0.80***[2]	0.73**	0.06	0.08
DM plant^{-1}	0.64**	0.59*	0.00	0.14
nodule DM plant^{-1}	-0.70**	-0.63*	-0.58*	-0.60*
		Waldheim		
#nodule plant^{-1}	-0.54	-0.46	-0.04	-0.15
C_2H_4 plant^{-1}	0.74**	0.77***	0.22	0.04
DM plant^{-1}	0.69**	0.62*	0.30	0.12
nodule DM plant^{-1}	-0.78***	-0.68**	-0.40	-0.57*

[1] estimate of N_2 fixing activity

[2] significant at *$P < 0.1$, **$P < 0.05$, ***$P < 0.01$

Conclusions

Commercially available inoculant (Nitragin inoculant type 'C') was as effective as most of the strains tested in this study for supporting N_2 fixation and yield in the lentil cultivars currently grown in Saskatchewan. Some potential for improvement also exists. Strain 99A1 was more effective than commercial inoculant for Eston lentil at Watrous, and other more effective strains may have been missed due to the screening technique used. Moisture is usually more limiting during the growing season than it was in 1986, and this may cause greater differences between strains, at least in grain yield. Approximately 120 rhizobial strains are currently being compared for lentil in growth-room tests by Dr. Louise Nelson at the Plant Biotechnology Institute in Saskatoon. Several of these strains will be compared in the field in 1987.

Acknowledgements

This project was initially funded by the Saskatchewan Pulse Crop Development Board and NSERC, and is continuing through joint funding from the Saskatchewan Agriculture Development Board and Esso Chemical Canada Ltd. Special thanks also to Dan Klassen and George Swerhone for technical assistance.

References Cited

Bremner, J.M. 1965. Total nitrogen. *In* C.A. Black (ed.) Methods of Soil Analysis. Agronomy 9: 1149-78.

Elegba, M.S. and Rennie, R.J. 1984. Effect of different inoculant adhesive agents on rhizobial survival, nodulation, and nitrogenase (acetylene-reducing) activity of soybeans (*Glycine max* (L.) Merill). Can. J. Soil Sc. 64:631-6.

Gibson, A.H. 1977. The influence of the environment and managerial practices on the legume-rhizobium symbiosis. pp. 393-450. *In* Hardy, R.W.F. and A.H. Gibson. A Treatise on Dinitrogen Fixation. John Wiley and Sons, New York.

Hardy, R.W.F., R.D. Holsten, E.K. Jackson and R.C. Burns. 1968. The acetylene-ethylene assay for N_2-fixation: laboratory and field evaluation. Plant Physiol. 43: 1185-207.

Little, T.M. and F.J. Hills. 1978. Agricultural Experimentation. John Wiley and Sons, New York.

Manhart, J.R. and P.P. Wong. 1979. Nitrate reductase activities of rhizobia and the correlation between nitrate reduction and nitrogen fixation. Can. J. Microbiol. 25:1169-74.

May, S.N. and B.B. Bohlool. 1983. Competition among *Rhizobium leguminosarum* strainsfor nodulation of lentils (*Lens esculenta*). Appl. Env. Micro. 45:960-5.

Nutman, P.S. 1984. Improving nitrogen fixation in legumes by plant breeding; the relevance of host selection experiments in red clover (*Trifolium pratense* L.) and subterranean clover (*T. subterraneum* L.). Plant Soil 82: 285-301.

Porter, L.K. and W.A. O'Deen. 1977. Apparatus for preparing nitrogen from ammonium chloride for nitrogen-15 determinations. Anal. Chem. 45: 514-6.

Rai, R., S.K.T. Nasar, S.J. Singh and V. Prasad. 1985. Interactions between *Rhizobium* strains and lentil (*Lens culinaris* Linn.) genotypes under salt stress. J. Agric. Sci., Camb. 104:199-205.

Rennie, R.J., D.A. Rennie and M. Fried. 1978. Concepts of ^{15}N usage in dinitrogen fixation studies. p. 107-33 In Isotopes in biological dinitrogen fixation. International Atomic Energy Agency, Vienna.

Rennie, R.J. 1986. Comparison of methods of enriching a soil with nitrogen-15 to estimate dinitrogen fixation by isotope dilution. Agron. J. 78:158-63.

Ross, P.J. and A.E. Martin. 1970. A rapid procedure for preparing gas samples for N-15 determinations. Analyst 95: 817-22.

Saskatchewan Agriculture. 1986 Specialty Crop Report.

Weaver, R.W. and L.R. Frederick. 1982. *Rhizobium*. pp.1043-69 *In* Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties - Agronomy Monograph no.9 (2nd edition).

Witty, J.F. 1983. Estimating N_2-fixation in the field using ^{15}N labelled fertilizer: some problems and solutions. Soil Biol. Biochem. 15:631-9.