Response of lentil to microbial inoculation and low rates of fertilization in the semiarid Canadian prairies

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Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, Swift Current, Saskatchewan, Canada S9H 3X2 (e-mail: gan@agr.gc.ca). *Received 29 June 2004, accepted 30 June 2005.*

Gan, Y., Hanson, K. G., Zentner, R. P., Selles, F. and McDonald, C. L. 2005. **Response of lentil to microbial inoculation and low rates of fertilization in the semiarid Canadian prairies**. Can. J. Plant Sci. **85**: 847–855. The use of microbial inoculation may increase nodulation and seed yield of annual legumes. A study was conducted to determine the effect of formulations (seed-applied powder vs. soil-applied granular inoculants), placement of granular inoculants in soils (applied in the seed-row vs. side-banded), and low rates of fertilizers in comparison to P-solubilizing microbes *Penicillium bilaii* on plant establishment, maturity, and seed yield of lentil (*Lens culinaris* Medik.) in the semiarid Canadian prairies. Green lentil was grown on a silt loam and a heavy clay soil in southwestern Saskatchewan from 1999 to 2002. Inoculated lentil with *Rhizobium* increased seed yield by 45% averaged across all 6 site-years. Granular soil inoculants increased lentil seed yield by 19% over seed-applied inoculants. Placement of soil inoculants in the seed row or side-bands produced similar results. On the silt loam soil, the use of rhizobial inoculants increased lentil seed yield by 15%, while the yield increase was 70% on the heavy clay. Starter N applied at a rate of 15 kg ha^{-1} increased seed yield by 13% for lentil grown on the heavy clay, but there was no effect on the silt loam. Phosphorus fertilizer applied at a rate 15 kg P ha^{-1} did not influence lentil establishment, growth or seed yield. Similarly, the P-solubilizing microbes *P. bilaii* did not influence plant growth or development, nor did it affect the seed yield of lentil. Soil granular rhizobial inoculants are preferred over seed-applied inoculants, fertilizers, or P-solubilizing microbes in lentil because of their strong and consistently positive impact on plant growth and seed yield in the semiarid Canadian prairies.

Key words: *Lens culinaris*, nodulation, seed yield, *Penicillium bilaii*, P-solubilizing microbes

Gan, Y., Hanson, K. G., Zentner, R. P., Selles, F. et McDonald, C. L. 2005. **Réaction de la lentille à l'inoculation et à une légère fertilisation dans la région semi-aride des Prairies canadiennes**. Can. J. Plant Sci. **85**: 847–855. L'inoculation pourrait accroître la nodulation et le rendement grainier des légumineuses annuelles. Les auteurs ont entrepris une étude afin de vérifier l'incidence de la formule (poudre appliquée à la graine ou inoculants granulaires appliqués au sol), de l'emplacement des inoculants granulaires appliqués au sol (planche de semis ou bande latérale) et d'une légère fertilisation comparativement à l'emploi de bactéries solubilisant le P (*Penicillium bilaii*) sur l'implantation, sur la précocité et sur le rendement grainier de la lentille (*Lens culinaris* Medik.) dans la région semi-aride des Prairies canadiennes. La lentille verte a été cultivée sur loam argileux et sur argile lourde dans le sud-ouest de la Saskatchewan de 1999 à 2002. L'inoculation avec *Rhizobium* a augmenté le rendement grainier de la lentille d'en moyenne 45 % pour les six années-sites. L'inoculant granulaire accroît le rendement grainier de la lentille de 19 % quand on l'applique à la semence. Le placement de l'inoculant dans la planche de semis ou en bande latérale donne des résultats analogues. Sur le loam limoneux, l'emploi d'un inoculant de *Rhizobium* accroît le rendement grainier de la lentille de 15 % contre 70 % sur l'argile lourde. L'application de N à raison de 15 kg par hectare au démarrage accroît le rendement grainier de 13 % sur l'argile lourde, mais le traitement n'a aucun effet quand la lentille est cultivée sur loam limoneux. L'application d'un engrais phosphaté au taux de 15 kg de P par hectare n'a aucune incidence sur l'établissement, la croissance et le rendement grainier de la culture. De même, la bactérie *P. bilaii* qui solubilise le P n'a aucun effet sur la croissance ou le développement de la plante, ni sur son rendement grainier. L'application d'inoculants granulaires de *Rhizobium* au sol donne de meilleurs résultats que l'application d'inoculants à la semence, la fertilisation ou l'application de bactéries solubilisant le P en raison de sa forte incidence, toujours positive, sur la croissance et le rendement grainier de la plante dans la région semi-aride des Prairies.

Mots clés: *Lens culinaris*, nodulation, rendement grainier, *Penicillium bilaii*, bactérie solubilisant le P

Increasing emphasis on environmental sustainability and economic growth has stimulated producers in Western Canada to include pulse and oilseed crops in their crop rotations (Johnston et al. 2002; Miller et al. 2002; Zentner et al. 2002). The area seeded to lentil (*Lens culinaris* Medik.) in this region has increased from less than 600 ha in 1970 to more than 720 000 ha in 2001 (Statistics Canada 2002). This annual legume is being used to diversify cereal-based crop rotations (Miller et al. 2003), replace conventional summerfallow and conserve soil quality (Zentner et al. 2001), and enhance productivity of subsequent cereal and oilseed crops (Gan et al. 2003). Lentil plants require stress in the later part of their life cycle to encourage seed set and hasten maturity, reflecting the indeterminate growth habit of this crop. In Western Canada, lentil production is concentrated in the semiarid Brown and Dark Brown soil zones where moisture stress in the later part of the growing season helps terminate its indeterminate growth.

Abbreviations: **GDD**, growing degree-day; **GSP**, growing season precipitation; **TSP**, triple superphosphate

Like most annual legumes, lentil can provide a part of its own N requirement through symbiotic N_2 fixation when the plants are inoculated. The use of granular soil inoculants increases seed yield compared with peat-based seed inoculants in dry pea (*Pisum sativum* L.) (McKenzie et al. 2001a; Clayton et al. 2004a, b) and in chickpea (*Cicer arietinum* L.) (Kyei-Boahen et al. 2002; Gan et al. 2005). Little is known about the effect of formulation and placement of inoculants in the soil on lentil production under semiarid growing conditions.

Studies conducted in the 1970s showed that annual legumes may require a high level of N fertility to achieve maximum yield (Sosulski and Buchan 1978). In these earlier studies, nodulation and nitrogenase activity were generally poor even when the legume was inoculated with a rhizobial strain. Indigenous populations of *Rhizobium* for legumes may be present in the prairie soils, but these indigenous populations may be ineffective for inducing N_2 fixation under semiarid environments (Kucey and Hynes 1989). Small doses of N fertilizers applied to an annual pulse are beneficial if nodule initiation is delayed (Mahon and Child 1979). In dry pea, application of fertilizer N at 20 to 60 kg N ha^{-1} increased seed yield by an average of 9% in one-quarter of 58 trials conducted in Alberta (Mckenzie et al. 2001a). When spring soil NO_3-N (0–30 cm depth) was less than 20 kg N ha^{-1}, the use of fertilizer N increased pea yield by an average of 11% in one-third of the trials. Similarly, application of fertilizer N increased dry bean (*Phaseolus vulgaris* L.) seed yield proportionally in southern Manitoba (McAndrew and Mills 2000). Most producers in Western Canada inoculate the seed or the soil with a rhizobial strain and provide little or no fertilizer N to their lentil crops. Due to the lag period between rhizobial root colonization infection and the onset of nodule functioning, the young lentil plants may require a small dose of additional N (i.e., starter-N) from external sources to achieve vigorous vegetative growth and establish N_2-fixing symbiosis.

Penicillium bilaii Chala., isolated from a Canadian prairie soil (Asea et al. 1988), has been shown to increase inorganic P uptake, dry matter accumulation, and seed yield in wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.), and field bean (Kucey and Leggett 1989; Downey and van Kessel 1990; Vessey and Heisinger 2001). The possible mechanism by which *P. bilaii* increases the availability of inorganic P to plants appears to arise from an increase in soil acidification and exchange reactions (Asea et al. 1988; Cunningham and Kuiack 1992), or increase in root hairs (Vessey and Heisinger 2001). These studies encouraged investigation of whether a lentil crop would respond to *P. bilaii* similarly as do other crops in the semiarid Canadian prairies. This is of practical importance because P is an essential element required for efficient N_2-fixation in legume, while some seeding systems lack the capacity to apply P fertilizer due to application of granular inoculants. Some producers often face the dilemma of whether to apply fertilizer P or granular inoculants with their two-tank seeding systems. If *P. bilaii* shows positive effect on lentil, then these producers could inoculate their lentil seed with *P. bilaii* to meet the crop need of P while still applying granular rhizobial inoculants with their second tank of the drill. The objectives of this study were to determine (i) the effect of rhizobial inoculant formulations and the methods of placement in soil on lentil establishment, maturity, and seed yield under the semiarid environments of Western Canada, and (ii) the response of lentil to low doses of N and P fertilizers and *Penicillium bilaii* applied with the seed.

MATERIALS AND METHODS

Field experiments were conducted in southwestern Saskatchewan, at Swift Current (50°17′N, 107º48′W) from 1999 to 2001, and at Stewart Valley (50º36′N, 107º48′W) from 2000 to 2002. The soil at Swift Current was a Swinton silt loam, an Orthic Brown Chernozem (Ayres et al. 1985) with a surface pH of 6.5 (saturated paste 0–15 cm depth), and at Stewart Valley the soil was a Sceptre heavy clay, a Rego Brown Chernozem (Ayres et al. 1985) with a pH of 6.8.

Experiment Design

The experiments consisted of four factors: (i) the formulation of rhizobial inoculants (peat-based powder applied to the seed vs. granular inoculants applied to the soil), (ii) placement of granular inoculants in the soil (applied in the seed-row vs. side-banded), (iii) starter N and P applied at rates of 15 kg ha^{-1}, and (iv) application of *Penicillium bilaii* where the wettable powder was mixed into water and applied to the seed as a liquid at a rate of 20 kg seed g^{-1} of culture of the fungus (wt/wt = 3–22%). These four factors, alone or in combination, were represented in 15 treatments (Table 1), which were layed out in a randomized, complete block design with four replicates. For the purpose of simplification, the word "inoculant" in the following text refers to "rhizobial inoculant" unless otherwise specified. Laird (1999) and CDC Glamis (2000–2002), the most popular lentil cultivars in Western Canada (Anonymous 2002), were used in the experiment. For seed inoculation treatments, Nitragin Nitrastick C® (Nitragin Inc., Brookfield, WI) peat-based powder was applied to the seed at the rate of 4 g kg^{-1} of seed. For soil inoculation treatments, Nitragin Soil Implant C® (Nitragin Inc., Brookfield, WI) granules were applied to the soil at the rate of 5.6 kg ha^{-1}. The two formulations of inoculants contain identical *Rhizobium* strains, with a minimum of 100 million viable cells of *Rhizobium leguminosum biovar vicea* per gram. In the side-banding treatments, the inoculant was placed 4 cm to the side of the seed row. To avoid potential cross-contamination of inoculants between treatments, the seed compartments on the drill were cleaned between treatments using a high-pressure air flow followed by running sand through the hoses and the openers.

Seeding and Plot Management

Prior to seeding each year, available soil nitrate-N in the experimental areas was measured to a depth of 60 cm, and bicarbonate extractable soil P to a depth of 30 cm (Table 2). To minimize the potential effect of indigenous *Rhizobia* from the soil, the fields selected had no legume crops grown in the previous 5 yr. Plots were seeded directly into wheat

Table 1. Treatment structure and the components for lentil tests conducted in southwestern Saskatchewan, 1999–2002

Treatment[z]		No. of tanks in seeders	Description and components of treatments				
Name	Code		Formulation of inoculants	Placement of inoculants	Fertilizer N (kg ha^{-1})	Fertilizer P (kg ha^{-1})	*Penicillium bilaii*
1T0I0N0P	1	1	None	None	0	0	No
1Tptsr0N0P	2	1	Peat	With seed	0	0	No
1TPb0N0P	3	1	None	None	0	0	Yes
1TPbsr0N0P	4	1	Peat	With seed	0	0	Yes
1Ttag0N0P	5	1	Peat	With seed	0	0	Yes
2Tgrsr0N0P	6	2	Granules	Seed row	0	0	No
2TPbgrsr0N0P	7	2	Granules	Seed row	0	0	Yes
2Tptsr0N15P	8	2	Peat	With seed	0	15	No
2Tptsr15N15P	9	2	Peat	With seed	15	15	No
3TPbgrsr0N15P	10	3	Granules	Seed row	0	15	Yes
3TPbgrsr15N15P	11	3	Granules	Seed row	15	15	Yes
3Tgrsr0N15P	12	3	Granules	Seed row	0	15	No
3Tgrsr15N15P	13	3	Granules	Seed row	15	15	No
3Tgrside0N15P	14	3	Granules	Side-banded	0	15	No
3Tgrside15N15P	15	3	Granules	Side-banded	15	15	No

[z]For example, the treatment #7 "**2TPbgrsr0N0P**" means the drill has two tanks, with *Penicillium bilaii* (Pb) applied to seed, granular inoculant (gr) applied in the seed row (sr), and zero rates of fertilizer N and P (0N0P).

Table 2. Agronomic information for lentil [z] crops tested at Swift Current (SC) and Stewart Valley (SV), Saskatchewan, 1999–2002

	Seeding date		Soil (15-cm depth) temperature at seeding (°C)		Residual soil N, P [y]	Kernel weight	Seeding rate	Harvest date	
Year	SC	SV	SC	SV	(kg ha^{-1})	(mg seed^{-1})	(kg ha^{-1})	SC	SV
1999	May 25	–	17	–	16, 20 (SC)	69	99	Sep. 10	–
2000	May 03	May 04	16	12	9, 28 (SC)				
					18, 9 (SV)	60	84	Aug. 11	Aug. 17
2001	Apr. 26	Apr. 30	10	9	15, 18 (SC)				
					17, 10 (SV)	63	88	Aug. 10	Aug. 10
2002	–	May 17	–	11	20, 18 (SV)	61	85	–	Sep. 10

[z]Laird in 1999 and CDC Glamis in the other years.
[y]Soil nitrate-N to a depth of 60 cm and bicarbonate extractable soil P to a depth of 15 cm.

stubble at a depth of 4 to 6 cm using a 2-m-wide hoe press drill equipped with three separate tanks for seed, fertilizers, and granular inoculants. At seeding, the noon soil temperature at the 10-cm depth was between 9 and 13°C. Target plant population was 120 plants m^{-2}, with the seeding rate determined based on seed size, pre-seeding germination tests, and an estimated field emergence rate of 75%. Each plot was 7.5 m long and consisted of eight rows with 25-cm row spacing.

The plot areas received a broadcast application of ethalfluralin at a rate of 850 g a.i. ha^{-1} and a spray application of glyphosate at a rate of 200 g a.e. ha^{-1} for pre-seeding weed control. In-crop weeds were controlled using sethoxydim at a rate of 460 g a.i. ha^{-1}. Deltamethrin was applied in mid-July to control grasshoppers in 2001 and 2002.

Data Collection and Statistical Analysis

Plant counts were conducted 2 wk after initial seedling emergence in two randomly selected 0.5-m^2 quadrats per plot. Canopy height was measured prior to maturity. Plant maturity was recorded when >90% of the pods in a plot turned a brownish tan color and seed moisture content reached 300 to 350 g kg^{-1}. The center six rows of each plot (9.0 m^2) were harvested with a plot combine when the crop had dried sufficiently for satisfactory threshing. The seed samples were air-dried, cleaned, weighed, and seed yield was presented on a dry basis. Mean seed mass was based on two 1000-seed assessments.

Root nodules were evaluated at flowering on 10 plants that were randomly collected from each plot. The plant root-soil matrix was excavated to a soil depth of approximately 40 cm, and was placed in water and soaked to aid in soil removal while retaining the nodules on the roots. After initial soil removal, the plants were placed in bags with some water and transferred to a cold room (4ºC) until the time of evaluation within 48 h. The number of root nodules was counted on each individual plant and total nodule dry mass was determined.

Analysis of variance was performed on the data set using the GLM procedure of SAS (SAS Institute, Inc. 1996), with blocks as random effects and treatments and site-year as fixed effects. The results were presented separately by site-year when the treatment × site-yr interaction was significant. Single degree-of-freedom contrasts were used to determine significance in maturity (Table 6), seed yield (Table 7), and seed mass (Table 8) for the following pre-planned comparisons: (a) inoculants applied either to the seed or soil vs. uninoculated check (Code #1 vs. #2 or 6); (b) seed- vs. soil-

Table 3. Weather data, growth stage and growing degree-days for lentil [z] grown at Swift Current (SC) and Stewart Valley (SV), Saskatchewan, 1999–2002

		Growing season (May 01 – Aug. 31)		Growing degree days (5°C basis) from seeding to:			Julian days from seeding to:		
Year	Site	Total precipitation (mm)	Mean temperature (°C)	1st-flower	Mid-flower	Maturity	1st-flower	Mid-flower	Maturity
1999	SC	257	14.8	458	624	–	49	63	95.6
2000	SC	259	15.6	377	562	980	55	70	93.3
2001	SC	121	17.0	443	714	1078	56	75	91.9
2000	SV	190	15.8	422	601	1029	57	70	96.8
2001	SV	80	17.0	427	688	1042	53	72	91.2
2002	SV	260	15.2	355	589	1092	40	56	95.6
Mean		208[y]	15.6[y]	414	630	1044	51.6	67.7	94.1

[z]Laird in 1999 and CDC Glamis in the other years.
[y]Means from 1963 to 2002 at Swift Current, not available at Stewart Valley since the trials were on a producer's field.

Table 4. ANOVA mean squares for the agronomic traits of lentil at Swift Current (SC) and Stewart Valley (SV), Saskatchewan

Source	df	Plant density	Plant height	Days to maturity	Seed yield ($\times 10^4$)	Seed mass
Year	3	18 966***	4523***	93***	3200***	2170***
Site	1	187	50**	17***	92***	269***
Year × site	1	15 488***	47**	1000***	17*	150***
Treatment	14	192	7	2*	19***	12**
Year × treatment	41	138	8	2*	4	16**
Site × treatment	14	66	5	2*	11***	5

*, **, ***Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

applied inoculants (Codes #2, 8, 9 vs. #6, 12, 13); (c) granular inoculants applied in the seed-row vs. side-banded (Codes #12, 13 vs. #14, 15), (d) starter N vs. non-N treatments (Codes #8, 10, 12 vs. #9, 11, 13) and P vs. non-P treatments (Codes #2, 6, 7 vs. #8, 10, 12); and (e) treatments with *Penicillium bilaii* vs. those without *P. bilaii* (Codes #1, 6 vs. #3, 7). Contrasts also were used to determine differences in response between the heavy clay at Stewart Valley and the silt loam soil at Swift Current when treatment × site interaction was significant.

RESULTS AND DISCUSSION

Weather Conditions and Overall Plant Growth and Development

Growing season precipitation (GSP) in 1999 and 2000 at Swift Current was 24% higher than the long-term (1960–2000) average, whereas in 2001 it was only 58% of normal (Table 3). At Stewart Valley, 2000 had near normal GSP and temperature, while in 2002 GSP was 25% higher than the long-term average with slightly below-normal temperatures. In contract, in 2001 GSP was only 38% of normal with above (5%) average temperatures. These contrasting environments provided excellent conditions to evaluate the lentil responses to inoculation.

Growing degree-day (GDD) requirements for lentil plant development did not differ significantly among site-years except in 2002 at Stewart Valley where delayed seeding (May 17) lowered the GDD requirements for lentil to flower (Table 3). On average, the crops required 52 days or 414 GDD to reach 1st flower, 68 d or 630 GDD to mid-flower, and 94 d or 1044 GDD to full maturity. As expected, site-year had significant effects on most of the yield-related variables (Table 4), and in most cases where the treatment effect was significant the site-year × treatment interaction was also significant.

Lentil established well during the 4 yr of the experiments, and plant density ranged from 67 to 115 plants m^{-2} (Table 5), with the highest density being in 2001 at Stewart Valley and the lowest in 1999 at Swift Current. Within a given site-year, there was no significant difference in plant density among treatments, suggesting that inoculation and low dose of fertilization used in this study have no direct impacts on plant establishment of lentil in this semiarid region.

On average, lentil reached full maturity 94 d after seeding (Table 5) with the earliest maturity observed in 2001 (91 d), whereas severe drought in that year advanced maturity by 3 d and shortened plant height by as much as 20% from the average of the other site-years. Consequently, seed yield in 2001 was only 25% of the yield obtained in other years at Swift Current and was only 38% as high at Stewart Valley. Seed harvested from the 2001 crops was heavier than that obtained in other years (Table 5) due to fewer pods produced on each plant (data not shown). However, the heavier seed did not compensate for the yield loss caused by severe drought in 2001.

Effect of Formulations and Placement

The use of inoculants and low doses of fertilizers did not influence lentil maturity at Swift Current, but at Stewart Valley, the lentil crop matured 1.5 to 2.7 d earlier when the crop received no inoculants compared with those inoculated with granular inoculants (Table 6). The early maturity of non-inoculated lentil at Stewart Valley was probably due to

Table 5. Overall means of agronomic traits for lentil [z] grown at Swift Current (SC) and Stewart Valley (SV), Saskatchewan, 1999–2002

Year	Site	Plant density (plants m^{-2})	Plant height (cm)	Days to maturity (d)	Seed yield (kg ha^{-1})	Seed mass (mg $seed^{-1}$)
1999	SC	67	–	95.6	1110	58.1
2000	SC	103	42.3	93.3	1680	54.9
2001	SC	98	31.0	91.9	353	69.6
2000	SV	89	42.3	96.8	1756	59.6
2001	SV	115	29.2	91.2	532	70.2
2002	SV	87	37.0	95.6	1035	64.6
LSD (0.05)		<0.01	<0.01	<0.01	0.03	<0.01
[y] *P* value in contrast						
SC vs. SV		0.36	0.01	0.18	<0.01	<0.01

[z] Laird in 1999 and CDC Glamis in the other years.
[y] Single degree-of-freedom contrast; 2000 and 2001 data only.

Table 6. Days to maturity for lentil [z] grown at Swift Current (SC) and Stewart Valley (SV), Saskatchewan, 1999–2002

Treatment [y] Name	Code	1999 SC	2000 SC	2001 SC	2000 SV	2001 SV	2002 SV	SC vs. SV [x]
		(d)						
1T0I0N0P	1	95.0	93.8	95.8	97.5	89.3	94.0	0.51
1Tptsr0N0P	2	96.2	93.5	94.5	97.5	90.5	96.3	1.00
1TPb0N0P	3	96.4	93.5	95.8	97.3	89.5	94.3	0.55
1TPbsr0N0P	4	96.0	93.5	96.0	96.8	90.8	96.0	0.58
1Ttag0N0P	5	–	92.5	95.3	97.3	92.3	96.5	0.58
2Tgrsr0N0P	6	95.8	92.8	95.0	96.5	92.8	95.5	0.55
2TPbgrsr0N0P	7	95.2	93.5	96.5	96.3	91.8	95.8	0.51
2Tptsr0N15P	8	95.0	93.8	97.0	96.5	91.0	95.8	0.36
2Tptsr15N15P	9	94.6	92.5	96.0	96.0	90.3	95.3	0.55
3TPbgrsr0N15P	10	95.6	93.3	96.0	96.8	92.0	95.8	0.88
3TPbgrsr15N15P	11	96.0	93.3	96.3	96.8	91.5	95.8	0.72
3Tgrsr0N15P	12	95.8	93.5	96.5	97.3	92.0	96.0	0.83
3Tgrsr15N15P	13	96.0	93.3	96.3	96.3	91.8	95.5	0.62
3Tgrside0N15P	14	95.0	93.5	96.8	97.0	92.3	96.5	0.77
3Tgrside15N15P	15	95.8	92.8	94.3	96.5	91.0	95.8	0.86
LSD (0.05)		1.54	1.36	2.36	0.84	0.92	0.83	N/A
Contrast (P value) [x]								
Uninoc vs. peat	1 vs. 2	0.12	0.71	0.29	1.00	0.01	<0.01	N/A
Uninoc vs. granules	1 vs. 6	0.30	0.15	0.53	0.02	<0.01	<0.01	N/A
Seed vs. soil	2, 8, 9 vs. 6, 12, 13	0.18	0.83	0.90	1.00	<0.01	0.73	N/A
G seedrow vs. sideband	12, 13 vs. 14, 15	0.36	0.60	0.30	1.00	0.44	0.20	N/A
N = 0 vs. N = 15	8, 10, 12 vs. 9, 11, 13	0.52	0.10	0.14	0.02	<0.01	0.04	N/A
P = 0 vs. P = 15	2, 6, 7 vs. 8, 12, 10	0.55	0.53	0.09	0.73	1.00	1.00	N/A
Non-*P. bilaii* vs. *P. bilaii*	1, 6 vs. 3, 7	0.65	0.67	0.15	0.09	0.53	0.73	N/A

[z] Laird in 1999 and CDC Glamis in the other years.
[y] Treatment consists of five factors: (a) # of tanks in a drill, (b) inoculant formulation, i.e., peat-based powder (pt) vs. granular soil inoculant (gr), (c) inoculant placement, i.e., in the seed-row (sr) vs. side-banded (side), (d) amount of fertilizer N and P, and (e) *Penicillium bilaii (Pb)*. For example, the treatment "2TPbgrsr0N0P" means the drill has two tanks, with *Penicillium bilaii* applied to seed, granular inoculant applied in the seed row, coupled with zero rates of fertilizer N and P.
[x] Single df contrast; 2000 and 2001 data only.

earlier depletion of available soil nutrients in the heavy clay compared with the crops at Swift Current. Often the main limitation for crop development on the dry Brown, silt loam soil at Swift Current is water rather than nutrients (Campbell et al. 2004).

The use of inoculants increased lentil seed yields by 45% averaged across the 6 site-years, although the data from some of the Swift Current site-years were not statistically significant (Table 7). A portion of the increased seed yield was due to increased seed mass (Table 8). In 4 of 6 site-years, the use of inoculants increased seed mass significantly. Between the two formulations of inoculants, the granular soil inoculants increased the seed yields by 3 to 38% in 5 of 6 site-years compared with seed-applied inoculants.

The positive influence of inoculation on seed yield and seed mass in lentil was partly due to the inoculation promoting nodule formation. Measurements at flowering showed that the inoculated lentil produced 9.6 nodules $plant^{-1}$ with nodule dry mass of 9.2 mg $plant^{-1}$, which were, respectively, 92 and 76% greater ($P < 0.01$) than those measured on lentil that was not inoculated. Between two formulations of inoculants, the lentil plants with granular soil inoculants produced 12.6 nodules $plant^{-1}$ with nodule dry

Table 7. Seed yield for lentil[z] grown at Swift Current (SC) and Stewart Valley (SV), Saskatchewan, 1999–2002.

Treatment[y] Name	Code	1999 SC	2000 SC	2001 SC	2000 SV	2001 SV	2002 SV	SC vs. SV[x]
		(kg ha $^{-1}$)						
1T0I0N0P	1	1014	1745	248	1359	210	691	0.10
1Tptsr0N0P	2	1038	1891	295	1399	442	1051	0.36
1TPb0N0P	3	1146	1444	281	1525	205	786	0.99
1TPbsr0N0P	4	1035	1672	321	1797	475	998	0.52
1Ttag0N0P	5	–	1736	374	1645	625	903	0.36
2Tgrsr0N0P	6	1055	1685	397	1707	653	1081	0.11
2TPbgrsr0N0P	7	1195	1682	339	1824	592	1047	0.08
2Tptsr0N15P	8	1126	1820	353	1728	465	953	0.50
2Tptsr15N15P	9	1125	1628	411	1605	524	1211	0.63
3TPbgrsr0N15P	10	1253	1714	395	1860	667	1016	0.01
3TPbgrsr15N15P	11	1058	1669	386	1954	673	1278	<0.01
3Tgrsr0N15P	12	1161	1476	434	1851	653	1067	<0.01
3Tgrsr15N15P	13	1072	1605	375	2129	725	1162	0.02
3Tgrside0N15P	14	1090	1729	383	1980	468	964	0.03
3Tgrside15N15P	15	1168	1790	302	1971	611	1320	<0.01
LSD (0.05)		178.5	366.5	125.2	337.2	87.7	282.8	N/A
Contrast (P value) [x]								
Uninoc vs. peat	1 vs. 2	0.78	0.46	0.46	0.81	<0.01	0.01	N/A
Uninoc vs. granules	1 vs. 6	0.64	0.88	0.02	0.04	<0.01	0.01	N/A
Seed vs. soil	2, 8, 9 vs. 6, 12, 13	0.99	0.10	0.18	<0.01	<0.01	0.70	N/A
G seedrow vs. sideband	12, 13 vs. 14, 15	0.84	0.07	0.17	0.90	<0.01	0.78	N/A
N = 0 vs. N = 15	8, 10, 12 vs. 9, 11, 13	0.25	0.89	0.47	0.48	<0.01	<0.01	N/A
P = 0 vs. P = 15	2, 6, 7 vs. 8, 12, 10	0.11	0.50	0.17	0.09	0.20	0.56	N/A
Non-*P. bilaii* vs. *P. bilaii*	1, 6 vs. 3, 7	0.09	0.11	0.98	0.02	0.67	0.98	N/A

[z]Laird in 1999 and CDC Glamis in the other years.
[y]Treatment consists of five factors: (a) # of tanks in a drill, (b) inoculant formulation, i.e., peat-based powder (pt) vs. granular soil inoculant (gr), (c) inoculant placement, i.e., in the seed-row (sr) vs. side-banded (side), (d) amount of fertilizer N and P, and (e) *Penicillium bilaii (Pb)*. For example, the treatment "2TPbgrsr0N0P" means the drill has two tanks, with *Penicillium bilaii* applied to seed, granular inoculant applied in the seed row, coupled with zero rates of fertilizer N or P.
[x]Single df contrast; 2000 and 2001 data only.

mass of 11.9 mg plant^{-1}, which were 47 and 45%, respectively, greater ($P < 0.01$) than those measured on lentil with seed-applied inoculants. Other researchers have reported similar observations in pulse including dry pea (Clayton et al. 2004a) and chickpea (Kyei-Boahen et al. 2002; Gan et al. 2005), but our results show that the lentil response to granular inoculants is more pronounced than either dry pea or chickpea.

Granular inoculants applied to the soil allow *Rhizobium* to become more uniformly distributed in the rooting zone, encouraging more nodules to be formed on lateral roots. Kyei-Boahen et al. (2002) observed that chickpea with granular soil inoculants produced 220% more nodules on the lateral roots compared with seed-applied inoculation. These authors found a strong correlation between lateral root nodules and seed yield. Nodules formed on lateral roots contribute significantly to N_2-fixation during the later stage of plant development (Wolyn et al. 1989). The mobility of *Rhizobium* in the rhizosphere is often limited, particularly when soils are dry (Caetano-Anolles et al. 1992). Under dry field conditions, nodules are mostly formed on the radical root and a few on lateral roots when the inoculum is delivered through seed treatment, whereas plants receiving soil-applied inoculants produce nodules throughout their entire root systems. In the present study, lateral roots were not separated from the radical root because the whole rooting system of lentil plants was more or less a mass of lateral root matrix.

Responses of lentil to inoculation were stronger and more consistent on the heavy clay at Stewart Valley than those on the silt loam at Swift Current in this study (Table 7). The use of inoculants increased the seed yield of lentil by an average of 15% at Swift Current, while it was 70% at Stewart Valley. Similarly, on the clay soil, soil inoculation increased lentil seed yield by 26% over seed inoculation, while on the silt loam, soil inoculation performed similarly to seed-applied inoculation (only 2% difference in yield). The greater response to soil inoculation on the heavy clay was probably due to greater water-holding capacity of the clay that had permitted better root development and *Rhizobium* colonization. Hynes et al. (2001) observed that colonization of the rhizosphere of dry pea was increased significantly when soil moisture was high. Furthermore, Postma et al. (1989) observed that the survival of *Rhizobium* in fine-textured soil was greater than that in coarse-textured soils. In addition to soil water status, the difference in response to inoculants between the two sites observed in the present study may also be related to other factors such as soil organic matter, and the history of crop production, which may affect *Rhizobium* colonization and development.

In some site-years, nodules were found on non-inoculated lentil roots at the time of sampling (flowering stage), suggesting that indigenous populations of *Rhizobia* exist in these soils. However, the indigenous populations apparently

Table 8. Plant population and seed mass of lentil[z] grown at Swift Current (SC) and Stewart Valley (SV), Saskatchewan, 1999–2002

Treatment[y]		Plant	Seed mass						
Name	Code	population	1999 SC	2000 SC	2001 SC	2000 SV	2001 SV	2002 SV	SC vs. SV[x]
		(Plants m^{-2})	(mg $seed^{-1}$)						
1T0I0N0P	1	92.2	58.4	57.2	68.0	61.9	65.5	56.7	0.39
1Tptsr0N0P	2	93.0	57.2	55.4	70.3	61.0	68.3	64.4	0.33
1TPb0N0P	3	96.9	56.6	58.8	69.3	61.4	65.0	62.0	0.90
1TPbsr0N0P	4	94.7	57.0	54.7	69.8	58.8	69.8	64.3	0.21
1Ttag0N0P	5	96.8	–	53.7	69.5	59.6	72.0	64.3	0.01
2Tgrsr0N0P	6	90.8	57.0	53.7	71.5	59.3	73.3	67.6	0.06
2TPbgrsr0N0P	7	95.8	58.4	52.8	70.0	59.4	72.5	66.2	0.01
2Tptsr0N15P	8	96.8	59.2	56.1	67.0	60.2	69.3	62.9	0.15
2Tptsr15N15P	9	89.7	60.2	56.0	68.3	60.7	68.0	66.9	0.10
3TPbgrsr0N15P	10	92.0	59.4	56.3	68.3	58.4	72.8	64.9	0.04
3TPbgrsr15N15P	11	92.8	58.0	53.0	70.3	57.6	72.0	68.5	0.06
3Tgrsr0N15P	12	92.8	58.6	53.6	70.3	59.0	71.0	62.8	0.01
3Tgrsr15N15P	13	87.3	56.6	55.7	72.0	59.0	71.0	67.2	0.49
3Tgrside0N15P	14	94.1	58.0	55.2	69.0	58.8	71.5	61.8	<0.01
3Tgrside15N15P	15	95.8	59.0	52.6	70.3	58.6	71.8	68.9	0.01
LSD (0.05)		5.7	2.38	3.47	3.91	1.90	2.02	5.82	N/A
Contrast (P value) [x]									
Uninoc vs. peat	1 vs. 2	0.83	0.32	0.34	0.24	0.37	0.01	0.01	N/A
Uninoc vs. granules	1 vs. 6	0.31	0.24	0.04	0.08	0.01	<0.01	<0.01	N/A
Seed vs. soil	2, 8, 9 vs. 6, 12, 13	0.08	0.04	0.12	0.02	0.01	<0.01	0.49	N/A
G seedrow vs. sideband	12, 13 vs. 14, 15	0.01	0.29	0.50	0.26	0.68	0.36	0.86	N/A
N = 0 vs. N = 15	8, 10, 12 vs. 9, 11, 13	0.08	0.56	0.26	0.11	0.78	0.37	<0.01	N/A
P = 0 vs. P = 15	2, 6, 7 vs. 8, 12, 10								
Non-*P. bilaii* vs. *P. bilaii*	1, 6 vs. 3, 7	0.68	0.03	0.21	0.07	0.21	0.55	0.12	N/A
		0.04	0.77	0.99	0.82	0.12	0.88	0.43	N/A

[z]Laird in 1999 and CDC Glamis in the other years.
[y]Treatment consists of five factors: (a) # of tanks in a drill, (b) inoculant formulation, i.e., peat-based powder (pt) vs. granular soil inoculant (gr), (c) inoculant placement, i.e., in the seed-row (sr) vs. side-banded (side), (d) amount of fertilizer N and P, and (e) *Penicillium bilaii (Pb)*. For example, the treatment "2TPbgrsr0N0P" means the drill has two tanks, with *Penicillium bilaii* applied to seed, granular inoculant applied in the seed row, coupled with zero rates of fertilizer N and P.
[x]Single df contrast; 2000 and 2001 data only.

were not sufficient to induce N_2 fixation effectively under the semiarid conditions.

In this study, granular inoculants were placed in the seed-row and were compared with side-banding practices. The results showed that the placement of inoculants in the soil did not affect plant establishment, seed yield, or seed mass in any site-years (Table 7), suggesting that lentil producers have options to apply granular inoculants either in the seed-row or to the side of seed rows using whatever seeding systems that can accommodate fertilizers and inoculants.

These results strongly indicate that lentil grown under the semiarid environments of Western Canada prefers soil-applied granular over seed-applied inoculants. Use of granular soil inoculants also has more economic benefits. A simple calculation using (i) the current market prices for inoculants ($44 ha^{-1} for soil-applied and $7 ha^{-1} for seed-applied inoculants), (ii) average lentil seed yield (1500 kg ha^{-1}), and (iii) lentil grain prices (39¢ kg^{-1}) suggests that the extra net income obtained from increased seed yield with granular soil inoculants is far in excess of the added cost associated with the use of granules products.

Effect of Starter N and P

Starter N at the rate of 15 kg ha^{-1} did not affect plant establishment in lentil (Table 5). However, the low dose of fertilizer N advanced plant maturity by an average of 0.5 to 1.3 d at Stewart Valley. Fertilizer N promoted lentil plant growth in the earlier part of the life cycle. We observed that fertilized plots were greener than those without fertilization prior in the early growth stage. Lafond et al. (2002) had some similar observations on lentil crops grown in the Parkland region of Western Canada. Using a GreenSeeker instrument that measures Normalized Difference Vegetation Index (NDVI, the ratios of the infra-red and red bands), Lafond (personal communication) found that the chlorophyll content of the lentil canopy at flowering was significantly greater when the crop was fertilized with a small amount of N.

The use of starter fertilizer N increased lentil seed yield by an average of 13% at Stewart Valley (Table 7). The highest response of seed yield to starter N was in 2002 when there was a 24% yield advantage with N fertilization. The large portion of the increased yield was due to the increased seed dry mass (Table 8). Although the addition of starter N reduced the number of nodules by 67% and nodule dry mass by 150% in lentil (data not shown), the strong response of lentil to fertilizer N overshadowed the negative impact on nodulation. The reduction in number and dry mass of nodules was expected because the plants did not require as much N_2-fixation when soil N was available. In comparison, there was no effect of starter fertilizer N on the yield of inoculated lentil at Swift Current. Often a low availability of soil water on the dry Brown soil at Swift Current limits nutrient uptake by annual crops.

Further increases in the rate of fertilizer N may not be necessary for annual pulses. Lafond et al. (2002) found no effect of N on lentil yield when conditions were dry or when fields had a long history of continuous cropping with adequate fertilizers applied to previous crops, whereas fertilizer N at a rate of 30 kg ha^{-1} promoted early growth and increased lentil seed yield only when residual soil NO_3-N was below 10 kg N ha^{-1}. Clayton et al. (2004a) reported that dry pea inoculated with granular soil inoculants produced 22% higher biomass yield than an uninoculated check when starter N was <20 kg ha^{-1}, while only a 3% increase in biomass yield was realized when fertilizer N was increased from 20 to 40 kg ha^{-1}. McConnell et al. (2002) found that application of 100 kg N ha^{-1} of fertilizer to uninoculated dry pea maintained shoot biomass similar to the granular inoculated plots. It is apparent that N requirements of annual pulses are mostly met by N_2 fixation when the crop is well inoculated with an appropriate *Rhizobium* culture. However, if the plants do not produce effective nodules for any reasons (e.g., inoculum failure, low number of *Rhizobium* per soil mass), application of fertilizer N is needed to satisfy the N requirements of lentil crops.

Phosphorus applied at a rate of 15 kg P ha^{-1} did not affect any plant growth parameter, nor did it affect the yield-related variables in this study (Tables 6, 7, and 8). In 4 of 6 site-years, the small dose of P increased lentil seed yield by an average of 4% compared with non-P check, but this magnitude of increase was not statistically significant ($P = 0.21$). Pre-seeding bicarbonate extractable soil P was between 9 and 28 kg ha^{-1} (Table 2). These amounts of residual soil P may be sufficient for lentil crops under the semiarid conditions. McKenzie et al. (2001b) determined the response of dry pea to triple superphosphate (TSP) fertilizer in 52 trials across Alberta, and concluded that TSP at 13 kg P ha^{-1} was sufficient to maximize pea productivity when soil test P levels (Modified Kelowna extractable-P, equivalent to bicarbonate extractable soil P in our study) were less than 30 kg P ha^{-1}. Karamanos et al. (2003) also studied various rates of P on dry pea at multiple sites in Alberta, and found no significant yield increase when the soil P was greater than 20 kg ha^{-1} in the soil. In chickpea, application of low doses of P had no (Gan et al. 2005) or little (Walley et al. 2005) effect on seed yields, although it usually increased biomass production.

Penicillium bilaii

In the present study, *P. bilaii* was applied to the seed at the recommended rate and was compared with treatments that received no *P. bilaii*. The results indicated that the use of *P. bilaii* in lentil did not affect plant establishment, crop growth, or seed yield (Tables 6, 7, and 8). The only exception was in 2000 at Stewart Valley where the use of *P. bilaii* increased lentil seed yield by 6% compared with plots that did not receive *P. bilaii* (Table 7). The reason for the positive response at this specific site-year was not known, but it is noteworthy that bicarbonate extractable soil P at this site was 9 kg ha^{-1} (Table 2), the lowest among the 6 site-years of the study. Research is needed to elucidate possible interaction between soil P status and the functionality of *P. bilaii* in semiarid conditions.

Although it is believed that *P. bilaii* increases P availability to plants by lowering soil pH and dissolving soil calcium P (Asea et al. 1988; Cunningham and Kuiack 1992), research by Selles (1993) and Soon (1991) showed that at the rates of fertilizer P used in prairie crop production systems, the availability of inorganic forms of P is governed mainly by sorption processes in soils rather than the solubility of calcium forms of P. This may partly explain why lentil grown in these soils did not respond to *P. bilaii* in the present study. Some previous studies have shown that *P. bilaii* inoculation of crops such as wheat, canola, and field bean can increase P uptake, dry matter accumulation and seed yield (Kucey and Leggett 1989; Downey and van Kessel 1990). However, the host–growth promotion caused by *P. bilaii* cannot always be attributed to an increase in plant P status (Heisinger 1998). Gulden and Vessey (2000) reported that dry pea inoculated with *P. bilaii* produced 30% more root hairs, but 14% lower shoot P concentration, compared with pea plants that were not inoculated with *P. bilaii*. It is apparent that P uptake in dry pea is unrelated to root hair production of the plants inoculated with *P. bilaii.* There exists a need to study the interaction between *P. bilaii* and host plants pertaining to host root morphology in lentil. Such a type of study may help us to understand other mechanisms by which lentil roots may react to the inoculation of *P. bilaii* under the semiarid conditions.

CONCLUSIONS

Indigenous populations of *Rhizobia* exist in the soils of the semiarid prairies of western Canada, but these indigenous populations apparently are not sufficient to induce effective N_2 fixation in lentil. The use of rhizobial inoculants in lentil significantly increased seed yields (15–45%) compared with non-inoculated crops. Granular soil inoculants outperformed seed-applied inoculants in seed yield (2–26%), with granules placed in the seed-row performing similarly to side-banded granules. Lentil grown on a heavy clay soil responded to inoculants more strongly and consistently than lentil on a silt loam soil. The greater water-holding capacity of the heavy clay promoted greater nodule formation, increasing N_2-fixation and crop yield in response to enhanced N nutrition. For air seeders with a two-tank delivery system, the use of granular inoculants will preclude application of fertilizers, while low doses of fertilizer N are somewhat beneficial to lentil crop particularly on the heavy clay. In this case, use of granular soil inoculants is the priority over N fertilization. Using a P-solublizing inoculant such as *Penicillium bilaii* may not be as effective as a P fertilizer alternative for lentil in this semiarid region. In a one-tank seeding system, lentil seed can be pre-inoculated with peat-based powder inoculants without using *P. bilaii.* In a three-tank seeding system more options are available to accommodate multiple requirements, thus granular inoculants can be coupled with low doses of fertilizers N and P. The use of granular soil inoculants may result in a more uniform distribution of *Rhizobium* in the root zone, a more effective nodulation, and increased seed yield.

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