NITROGEN, PHOSPHORUS AND RHIZOBIAL STRAIN RESPONSES OF LENTIL

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Abstract

Three field experiments were set out in 1987 to test the effect of nitrogen fertilizer, phosphorus fertilizer, and rhizobial strain on lentil yields and N_2 fixation. The following treatments were laid out in a split-split-plot design: main plot treatments of uninoculated, Nitragin 'C' inoculated, and strain 99A1 inoculated lentil; sub-plot treatments of 0 and 30 kg P/ha, and sub-sub plot treatments of 0, 10, 20, 40, or 80 kg N/ha. ^{15}N-enriched fertilizer was applied to a 1.05 m^2 microplot in each plot. Barley was used as the non-N_2 fixing reference crop. At all sites lentil inoculated with strain 99A1 had the greatest total dry matter yield, grain yield and N_2 fixed. Uninoculated lentil had a strong N response at all sites, 'C' inoculated lentil had a starter N response at Kindersley and 99A1 inoculated lentil had no N response at all. P responses were only observed at Foam Lake. Lentil receiving low amounts of N fertilizer obtained between 60 and 75 % of their N from the atmosphere at all sites. A good agreement was observed in estimating percent N derived from N_2 using the ^{15}N isotope dilution, A-value, or classical N-difference methods. The amount of fixed N in the seed ranged from 4 kg ha^{-1} under drought stressed conditions at Kindersley to 60 kg ha^{-1} under much better growing conditions at Foam Lake.

INTRODUCTION

Legumes may experience a period of N deficiency during early growth due to the lag before N_2-fixation is active (Gibson, 1977). The degree of N deficiency depends on crop species, growth conditions and soil N availability. This N-stress can be overcome through the application of small amounts of fertilizer N (10 to 15 kg N ha^{-1}), called 'starter nitrogen'. Under conditions of high N availability legumes will preferentially use soil N above fixed N_2 due to the apparent lower energy requirement of the former. Legumes grown under abundant soil N levels may outyield legumes dependent on atmospheric N_2 (Johnson and Hume, 1972; Harper, 1974), but additional costs of N fertilizer inputs will not be covered by this potential increase in yield.

P requirements for N_2-fixing legumes appear to be higher than for non-N_2-fixing legumes (Israel, 1987) and increased N accumulation due to added P has been reported for several legumes (Andrew and Robins, 1969; Singleton et al., 1985). In one study applications of fertilizer N increased the P response on total yield of subterranean clover (Robson et al., 1981).

Several methods are available for measuring N_2-fixation. The classical difference method attributes the difference in total N between N_2-fixing and non-N_2-fixing crops to N_2 fixation (Weber, 1966). The ^{15}N isotope dilution method assumes that N_2-fixing and non-N_2-fixing plants do not discriminate between ^{14}N and ^{15}N (McAuliffe et al., 1958), and therefore the dilution of ^{15}N in the N_2-fixing crop relative to a non-N_2-fixing crop is attributed to N_2 fixation. A similar approach is the A-value method, which applies different amounts of labelled N-fertilizer to N_2-fixing and non-N_2-fixing plant (Fried and Broeshart, 1975). Comparisons between the different methods for measuring N_2-fixation have been made and agreement (Broadbent et al., 1982; Vasilas and Ham, 1984,) as well as less satisfactory agreement (Talbott et al., 1982; Rennie, 1984) has been reported.

Adequate N_2-fixation for maximum lentil yields requires nodulation by effective strains of *R. leguminosarum*.. Numerous *R. leguminosarum* strains nodulate lentils but large differences in effectiveness are found (Bremer et al., 1987).

MATERIALS AND METHODS

The field study was carried out at three sites: Kindersley, Semans, and Foam Lake. All sites were located on cereal stubble land which had not been previously cropped to grain legumes. Sites were relatively free of indigenous *R. leguminosarum*; further soil characteristics are given in Table 1.

Table 1. Soil characteristics of Foam Lake, Semans, and Kindersley

Site	texture	pH	NO_3	P	K	S	Precipitation
			---------- kg/ha			----------	cm
Foam Lake	loam	7.7	21.5	29.5	398	9.9	23.8
Semans	loam	7.6	11.9	44.7	658	8.4	20.5
Kindersley	clay	7.4	8.1	47.5	638	12.1	15.5

Treatments were laid out in a split-split plot design, with main plots arranged in a randomized complete block design. Laird lentil (*Lens esculenta* Moench. var. Laird) was used at all sites. There were four main plot treatments: uninoculated, inoculated with Nitragin 'C' inoculant, inoculated with *R. leguminosarum* strain 99A1, and a non-N_2-fixing reference crop barley (*Hordeum vulgare* L). Sub-plot treatments were P at 0 kg P_2O_5 ha^{-1} and 30 kg P_2O_5 ha^{-1} banded beside the seed row and in the form of triple superphosphate. Sub-sub-plot treatments were 5 levels of N: 0, 10, 20, 40, and 90 kg N ha^{-1} in the form of urea at Foam lake and 0, 10, 25, 50, and 90 kg N ha^{-1} in the form of NH_4NO_3 at Semans and Kindersley. Treatments were replicated 4 times; experimental units were 1.8 by 3.6 m in area. Laird lentil and barley were seeded in early May at a rate of 80 and 70 kg ha^{-1}, respectively. Gum arabic was used as a sticker for the inoculant; inoculant was applied at a rate which supplied >10^6 rhizobia per seed. Weeds were controlled by a Treflan applied the previous fall and by hand. In all sub-subplots receiving N, ^{15}N-enriched fertilizer was applied to an area 1.0 by 1.05 m. Atom % ^{15}N excess of the urea applications were 4.6337, 2.3168, 1.1584, and 0.5148 for 10, 20, 40, and 90 kg N ha^{-1}, respectively. For the ammonium nitrate applications the values were 4.6337, 2.3168, 0.9267, and 0.5148 atom % ^{15}N excess for 10, 20, 50, and 90 kg N ha^{-1}, respectively. Barley was used as the non-N_2-fixing reference plant at Foam Lake and Semans; uninoculated lentil was used as the reference crop at Kindersley. At the 0 level of N application, the natural ^{15}N enrichment of soil was used to measure N_2-fixation by the isotope dilution method. In late August all treatments at Kindersley and Foam Lake and the inoculated treatments at Semans were treated with Reglone (6,7-dihydrodipyrido [1,2-a:2',1'-c]pyrazinediium ion) to promote ripening. Plants were harvested at physiological maturity, dried at 60 °C until constant weight, weighed and threshed. Total N, including nitrate and nitrite, was determined by micro-Kjeldahl analysis (Bremner and Mulvaney, 1982). ^{15}N analysis was carried out by conversion of NH_4 to N_2 by LiBrOH (Ross and Martin, 1970, Porter and O'Deen, 1977) and the $^{15}N/^{14}N$ ratio determined by a VG Micromass 602E isotope ratio mass spectrometer. Atom % ^{15}N excess was calculated using the value of 0.3663 atom % ^{15}N of atmospheric N_2 as background (Mariotti, 1983). Calculations from ^{15}N data were as follows:

%Ndff = [atom % ^{15}N excess (plant) / atom % ^{15}N excess (fertilizer)] * 100
where %Ndff = percent N derived from fertilizer

%FUE = (%Ndff * seed N) / 100
where %FUE = % fertilizer use efficiency in the seed

A = [(1-%Ndff) / %Ndff] * rate of fertilizer N applied
where A = A value

%Ndfa (isotope dilution method) = [1- (atom % ^{15}N excess (f) / atom % excess (nf)] *100
where %Ndfa = percent N derived from the atmosphere
f = N_2-fixing crop
nf = non-N_2-fixing crop

%Ndfa (Classical difference method) = [total N (f) - total N (nf)] / total N (f) * 100

%Ndfa (A value method) = {[A (f) - A (nf)] * %FUE (f) / 100} / total N

N_2 fixed = (%Ndfa * seed or total N (f)] / 100

RESULTS AND DISCUSSION

Grain yields in this study ranged from very poor at Kindersley to above average at Foam Lake (Table 2). Germination was poor at Kindersley and Semans due to dry conditions and poor seeding equipment. Premature desiccation with Reglone also contributed to the low grain yield at Kindersley. Due to an accidental herbicide application at Kindersley, the barley treatment was lost.

Inoculation strongly and consistently affected lentil yields and N_2 fixation at all sites (Table 3). Inoculation increased lentil grain yield at all sites, with the greatest response at Foam Lake, which had the best growing conditions, and the lowest at Kindersley, which had the poorest growing conditions (Table 3). The economic return was much higher for the inoculated treatments at Foam Lake than for the uninoculated treatment (Fig. 1). Similar results were obtained at the other two sites. This study again demonstrates the benefit of inoculation.

Table 2: Inoculation and P responses by lentils on total dry matter, total grain yield, total N grain, total kg N-fixed, harvest index, % N grain, FUE and % of N derived from N_2 in grain at Foam Lake, Semans, and Kindersley.

Treatment	Dry matter	Grain yield	N grain	Ndfa	HI	N grain	FUE	Ndfa
	kg ha^{-1}				%			
Foam Lake								
Uninoculated	3796 a[†]	1338 a	34.2 a	6.4 a	35.7 a	2.87 a	10.2 a	21.1 a*
Commercial	5136 b	2048 b	62.8 b	31.7 b	40.1 b	3.45 b	11.0 a	49.8 b
99A1	6166 b	2268 b	76.4 c	48.0 c	37.1 ab	3.82 c	9.6 a	62.4 c
P0	4773 a	1785 a	53.9 a	27.1 a	37.7 a	3.35 a	8.6 a	45.2 a
P1	5293 b	1984 b	61.7 b	31.4 a	37.5 a	3.43 a	9.9 b	45.8 a
Semans								
Uninoculated	2914 a	1046 a	29.4 a	9.7 a	36.2 a	3.05 a	10.1 a	33.9 a*
Commercial	3506 ab	1203 ab	37.2 b	19.6 b	35.2 a	3.44 b	9.1 a	52.7 b
99A1	4066 b	1418 b	46.9 c	28.8 c	35.5 a	3.71 c	9.2 a	61.4 c
P0	3573 a	1239 a	37.3 a	18.1 a	35.3 a	3.38 a	9.9 a	47.2 a
P1	3418 a	1206 a	38.4 a	20.6 a	35.9 a	3.42 a	9.1 a	51.4 a
Kindersley								
Uninoculated	2059 a	394 a	10.7 a	NAΨ	17.1 a	3.02 a	7.1 a	NA
Commercial	2236 ab	441 a	14.0 a	2.7 a	17.7 a	3.73 a	6.4 a	33.4 a**
99A1	2627 b	501 a	16.7 a	7.2 b	18.5 a	3.85 b	4.9 a	45.4 b
P0	2336 a	463 a	14.2 a	5.3 a	18.2 a	3.52 a	6.2 a	40.4 a
P1	2278 a	428 a	13.4 a	4.6 a	17.3 a	3.55 a	6.0 a	38.3 a

[†]Values with same letter within site are not significantly different ($\alpha = 0.05$)

ΨNA - not available

*% Ndfa at Foam Lake and Semans using barley as the non-N_2-fixing control

**% Ndfa at Kindersley using the uninoculated lentil as the non-N_2-fixing control

Table 3. Significance of analysis of variance for the effect of rhizobial strain, phosphorus, and nitrogen fertilization on lentil.

Source	Dry matter	Grain yield	HI	N-grain	% N-grain	% FUE	% NdfA	kg NdfA
	-------	-------	-------	------- significance of F test -------	-------	-------	-------	-------
Foam Lake								
Strain (S)	***	***	***	***	***	*	***	***
Phosphorus(P)	***	***	NS	***	NS	**	NS	NS
Nitrogen(N)	***	***	***	NS	NS	**	***	***
SxP	NS	NS	NS	*	*	NS	NS	NS
SxN	***	***	NS	**	NS	NS	NS	NS
PxN	NS	NS	NS	NS	NS	NS	NS	NS
SxPxN	NS	NS	NS	NS	NS	NS	NS	NS
Semans								
Strain (S)	***	***	NS	***	***	***	***	***
Phosphorus (P)	NS	NS	NS	NS	NS	NS	NS	NS
Nitrogen (N)	NS	NS	NS	NS	*	***	***	***
SxP	NS	NS	NS	NS	NS	NS	NS	NS
SxN	NS	NS	NS	NS	NS	NS	NS	NS
PxN	NS	NS	NS	NS	NS	NS	NS	NS
SxPxN	NS	NS	NS	NS	NS	NS	NS	NS
Kindersley								
Strain (S)	*	NS	NS	NS	***	NS	*	***
Phosphorus (P)	NS	NS	NS	NS	NS	NS	NS	NS
Nitrogen (N)	*	**	*	***	***	***	***	NS
SxP	NS	NS	NS	NS	NS	NS	NS	NS
SxN	NS	NS	NS	NS	***	NS	NS	NS
PxN	NS	NS	NS	NS	NS	NS	NS	NS
SxPxN	NS	NS	NS	NS	NS	NS	*	NS

*,**,*** Significant at the 0.05, 0.01 and 0.005 probability levels, respectively, NS = not significant.

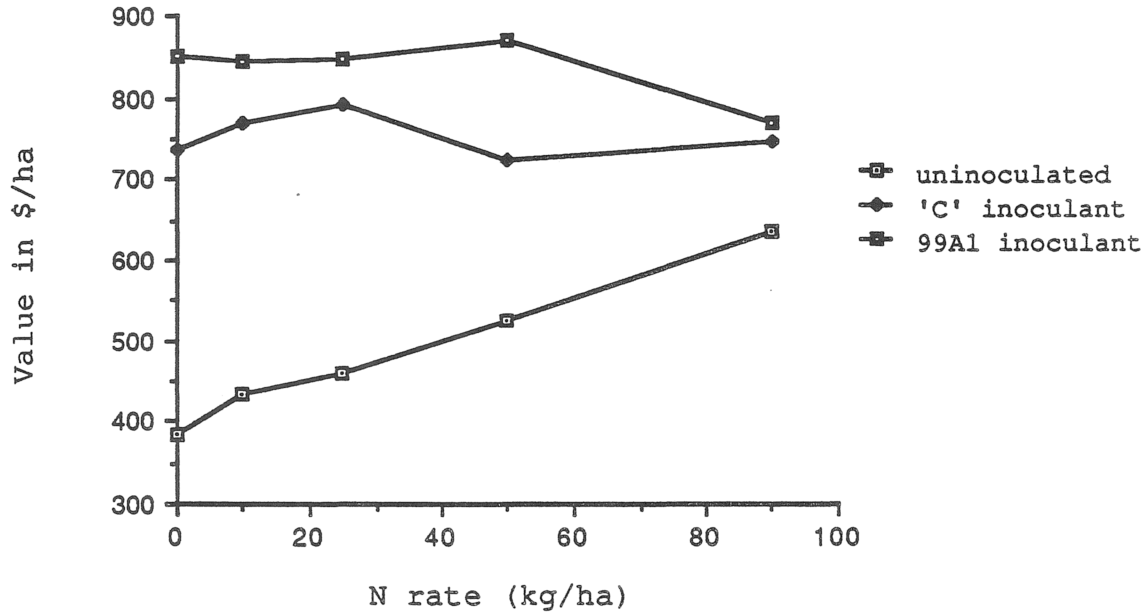

Fig. 1 Economic benefit of inoculation and N application at Foam Lake. Value was calculated from a price \$375 t^{-1} for lentil and a cost of \$0.44 kg^{-1} for N.

Strain 99A1 inoculant increased grain yields relative to the 'C' inoculant at all sites, although only significantly at Semans (Table 2). Thus, there may be a significant benefit in substituting this strain for the strains currently provided in commercially available inoculant. However, further testing to determine the response under a wider range of conditions will be required. The competiveness of strain 99A1 for nodulation compared to indigenous rhizobia must also be determined.

Significant responses to P fertilizer were only observed at Foam Lake (Table 3). At this site P significantly increased total dry matter, grain yield and grain N, but did not significantly affect N_2 fixation or interact with N fertilizer. The response at Foam Lake can be attributed to lower levels of available P (Table 1) and to increased P demand due to more favorable growth conditions. According to soil test guidelines, 20 kg P_2O_5 ha^{-1} would have been recommended at Semans and Kindersley, but dry weather conditions may have minimized P demand at those two sites.

Nitrogen fertilization significantly increased grain yield of uninoculated lentil at all sites (Fig. 2). Except for a starter N (10 kg N ha^{-1}) effect on lentil inoculated with 'C' inoculant at Kindersley, no significant N responses in total grain yield were observed for inoculated lentil. This would indicate that *R. leguminosarum* was capable of providing the host plant with sufficient N to maximize plant growth. Furthermore, it is apparent from this study that applying higher levels of fertilizer N to inoculated legumes has no economic benefit (Fig. 1). Thus, inoculated lentils should not receive any fertilizer N beyond the level of starter N.

Harvest index (HI), the percentage of grain to total dry matter, was between 35 and 40 at Foam Lake and Semans but only around 18 at Kindersley (Fig. 3). The low HI at Kindersley was at least partly due to the time of desiccation by Reglone. The experimental plot was located in a 75 ha lentil field seeded about 2 weeks earlier. By the time the larger field was mature enough to be desiccated by airplane, the small plot experiment was still in the pod fill stage, especially the

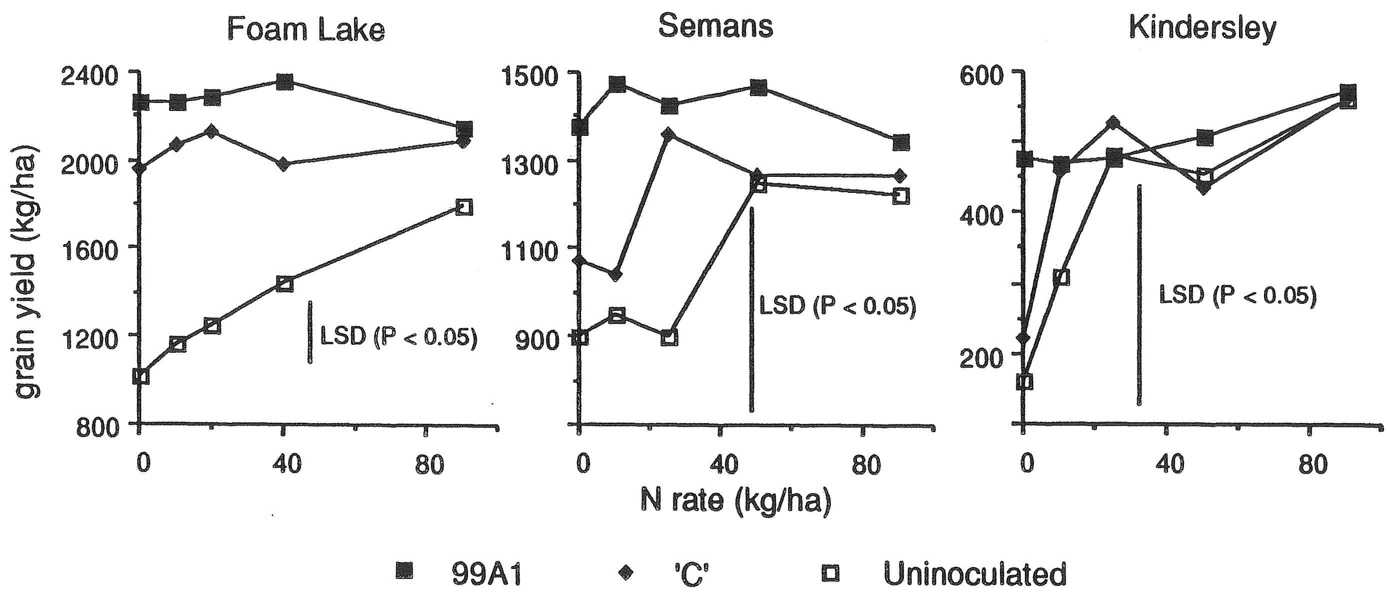

Fig. 2 Effect of N on lentil grain yield at all sites.

treatments inoculated with strain 99A1. Thus, pod fill was aborted abruptly, and grain yield was low. All treatments at Foam Lake and the inoculated lentil at Semans were also desiccated but at a later growth stage, and no apparent grain losses were observed.

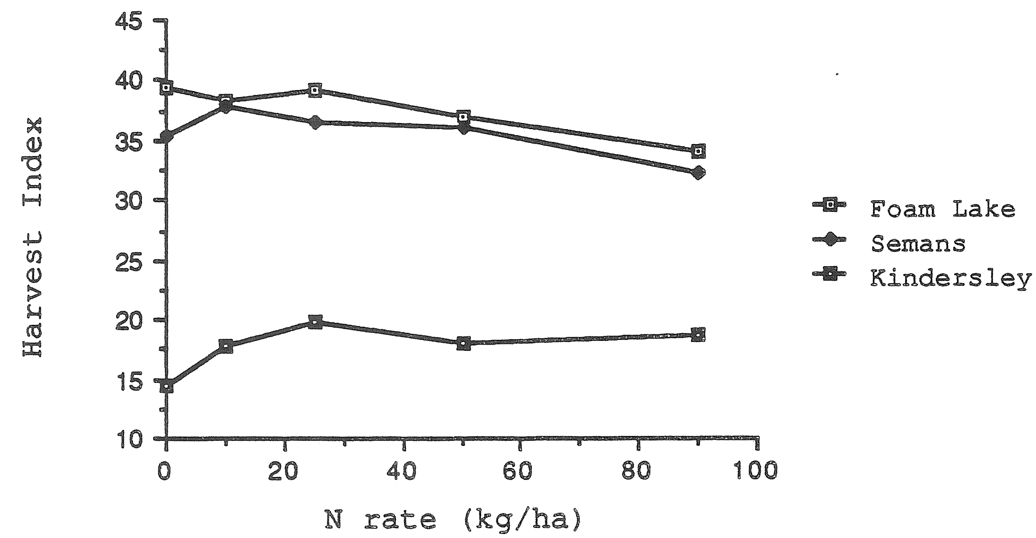

Fig. 3 Response of harvest index to N application

At Foam Lake and Semans higher N rates reduced the HI. Higher N applications apparently stimulated vegetative growth but not reproductive growth. A similar effect may be caused by introduction of more effective rhizobial strains, such as 99A1. In this study lentil inoculated with strain 99A1 matured later than the uninoculated lentil or lentil inoculated with 'C' strains. This may pose problems when frost comes early and may increase the need for desiccation.

Lentil inoculated with strain 99A1 were more active N_2-fixers at all sites compared to 'C' inoculated lentil (Fig. 4). At high rates of N application N_2-fixing activity was reduced significantly but was never inhibited completely. Similar responses have been observed with other legumes (Semu and Hume, 1979; Mahon and Child, 1979). Higher rates of fertilizer N did not increase total N in the grain but merely substituted atmospheric N with fertilizer N (Fig. 5). Similar results have also been found in soybean (Vasalis and Ham, 1984).

Total N_2 fixed in seed at the 0 N level ranged from 51 kg N ha^{-1} at Foam Lake for lentil inoculated with 99A1 to only 4 kg N ha^{-1} at Kindersley for lentils inoculated with 'C' inoculant. These are within the range of 10 to 73 kg N ha fixed by the whole plant reported for lentil grown under Saskatchewan conditions (Bremer et al., 1988). The low amount of N_2 fixed at Kindersley was due to drought stress, late seeding and premature desiccation.

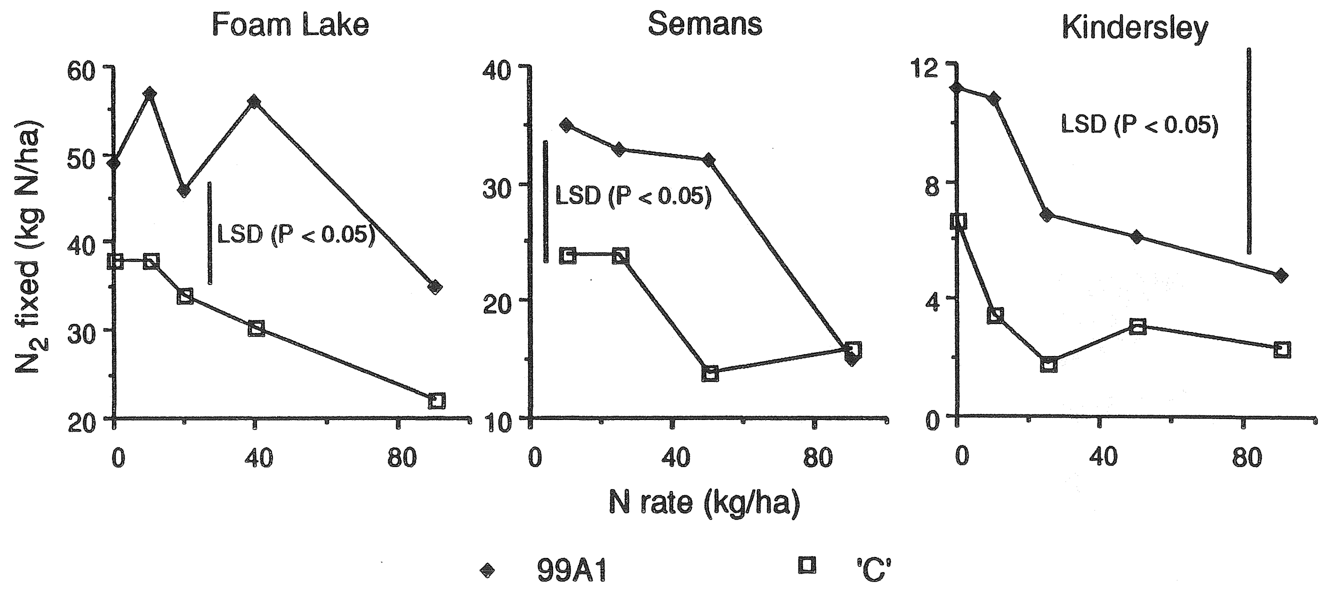

Fig. 4 Effect of N on N_2 fixed by inoculated lentil at all sites.

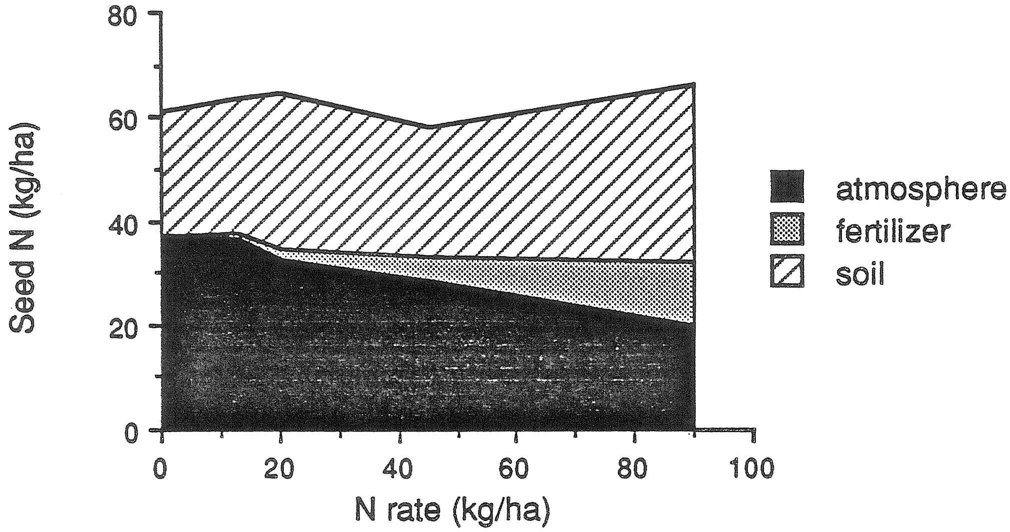

Fig. 5 Amount and source of seed N of 'C' inoculated lentil at Foam Lake.

In this study the classical N-difference method, the ^{15}N-isotope dilution method and the A-value method could be compared using either uninoculated lentil or barley as the reference crop. Comparisons between the methods were made at Foam Lake and Semans for inoculated lentil in the 10 kg N ha^{-1} treatment. The isotope dilution method using barley as the reference crop tended to be higher, while all the other estimates tended to be comparable (Table 4). The use of uninoculated lentil as the reference crop generally gave lower estimates of %NdfA compared with barley as the reference crop, and is at least partly due to sparse nodulation of uninoculated lentil at these sites.

Table 4. Comparison of the different methods for measuring N_2-fixation at Foam Lake and Semans.

Site	Percent N derived from N_2-fixation					
Strain	ID†		A-value[y]		N-difference[w]	
	B*	U*	B	U	B	U
Foam Lake						
99A1	61.2	34.7	44.9	45.2	54.9	49.9
'C'	76.2	59.9	66.2	66.4	62.2	58.0
Semans						
99A1	72.7	41.1	61.6	46.0	44.9	33.8
'C'	78.4	53.3	69.6	57.2	61.7	54.0

†Lentil and non-N2-fixing reference plant received 10 kg N/ha
[y]Lentil and non-N2-fixing reference plant received 10 and 90 kg N/ha, respectively
[w]Lentil and non-N2-fixing reference plant received 10 kg N/ha
*Barley (B) or uninoculated lentil (U) used as the non-N_2-fixing reference plant

% FUE was around 10 at all sites for lentil and 15 for barley at Foam Lake and Semans, respectively (Fig. 6). No apparent difference in %FUE between urea-N (Foam Lake) and ammonium-nitrate-N (Semans and Kindersley) was observed. Generally %FUE decreases with increasing rates, but this was not observed in this study (Fig. 7). The low %FUE was probably due to broadcasting of fertilizer on the surface and the lack of precipitation in the spring. Incorporation would have likely increased the effectiveness of N fertilizer applications.

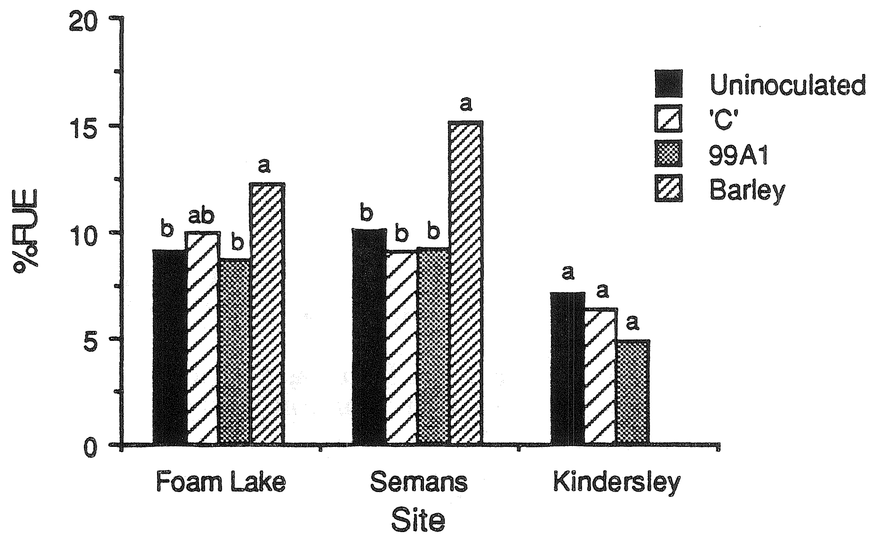

Fig. 6 Effect of crop on % fertilizer use efficiency for N at each site.

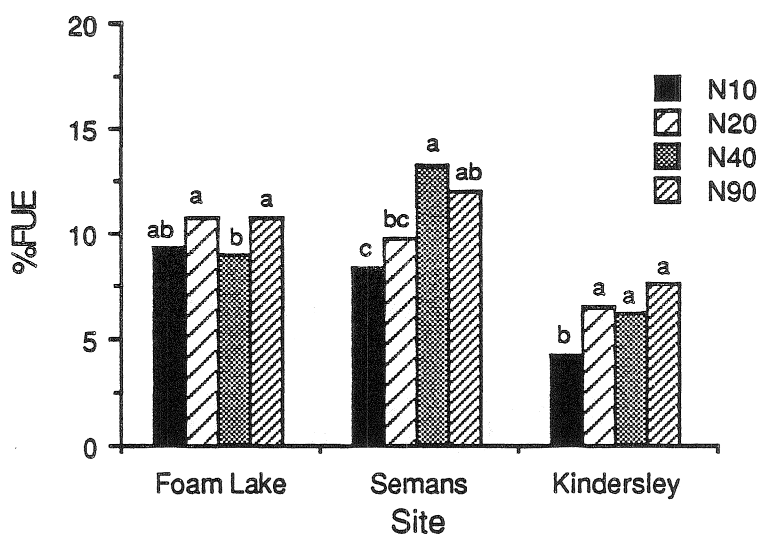

Fig.7 Effect of rate of N on % fertilizer use efficiency for N at each site.

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