Nitrogen Fixation of Several Grain Legumes in Saskatchewan

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Grain legumes have become increasingly important as a cash crop in many areas of Saskatchewan over the past decade. For example, the area seeded to lentils has increased from less than 8,000 ha in the early seventies to 60,000 ha last year. Similarly, the area planted to field peas has increased from less than 4,000 ha to 28,000 ha over the same time period (Saskatchewan Agriculture). Further increases are likely. There is also increasing interest in using grain legumes as green manures.

An important consideration in growing grain legumes is their ability to obtain atmospheric nitrogen through a close association with *Rhizobium spp.* The plant supplies nodules containing rhizobia with carbohydrates in exchange for simple amino acids containing nitrogen fixed from the atmosphere. The amount of nitrogen that is fixed this way may be quite large, depending on environmental conditions, crop variety and rhizobial strains. Few estimates of the amount of nitrogen fixed by grain legumes in Saskatchewan exist as accurate field estimates of nitrogen fixation are difficult to obtain.

Methods

A study was initiated under the Innovative Acres program to measure the growth and nitrogen fixation of several grain legumes under a range of conditions in Saskatchewan. The crops tested were Eston and Laird lentils, Trapper and Tara peas, and Aladin and Outlook fababeans. Johnston barley and Columbus wheat were grown as non-fixing controls. They were tested at four locations in 1984 and at six in 1985, with all the major soil zones being represented by at least one site in each year. Inoculated and uninoculated strips (2m x 50m) of each cultivar were planted at each site. Just after emergence urea (5% ^{15}N abundance) was injected in one meter sections of a row in each treatment at an equivalent rate of 7.5 kg ha^{-1}. At harvest two of these meters were combined for analysis, with five replications in each treatment. Additional forage samples of lentils and barley were taken from the inoculated treatments at earlier dates. All samples were weighed and analysed for %N and ^{15}N abundance. Soil samples were analysed for nitrate at all sites. Soil moisture depletion was measured at the 1985 locations.

As blocks were not randomized the validity of these estimates requires that environmental variations be randomly distributed across blocks. In particular, no gradients in available soil nitrogen or moisture should exist across blocks. This assumption appears reasonable for most of the sites tested.

Results

Nitrogen fixation could be estimated from this data by either the classical difference method or the ^{15}N isotope dilution method. Estimates of nitrogen fixation by the classical difference method were calculated as follows:

$$N\ fixed = N_{fs} - N_{nfs}$$

where: N = yield of N (kg ha^{-1})
fs = fixing crop
nfs = non-fixing crop

This method assumes that the fs and the nfs assimilate the same amount of soil nitrogen. This is rarely achieved as the control crop often explores a different soil volume and/or assimilates soil nitrogen with a different efficiency than the fixing crop (Rennie and Rennie,1983). The fs may simply substitute fixed nitrogen for soil nitrogen, with no change in total nitrogen.

The ^{15}N isotope dilution is based on the difference in the abundance of ^{15}N between the different nitrogen sources (Fig.1). The fixing crop obtains it's nitrogen requirements from air (nitogen fixation), soil and fertilizer nitrogen, while the nfs only obtains it's nitrogen requirements from soil and fertilizer nitrogen. As atmospheric nitrogen has a lower concentration of ^{15}N than soil or fertilizer nitrogen, the abundance of ^{15}N in plant material decreases with increasing dependence on fixed nitrogen. Nitogen fixation can therefore be calculated as follows:

$$\%\ Ndfa = \left(1 - \frac{\%^{15}N\ ex.\ in\ fs}{\%^{15}N\ ex.\ in\ nfs}\right) * 100$$

$$N\ fixed = N\ yield\ x\ \%Ndfa/100$$

where %Ndfa = % of crop nitrogen derived from atmospheric nitrogen
fs = fixing crop
nfs = non-fixing control

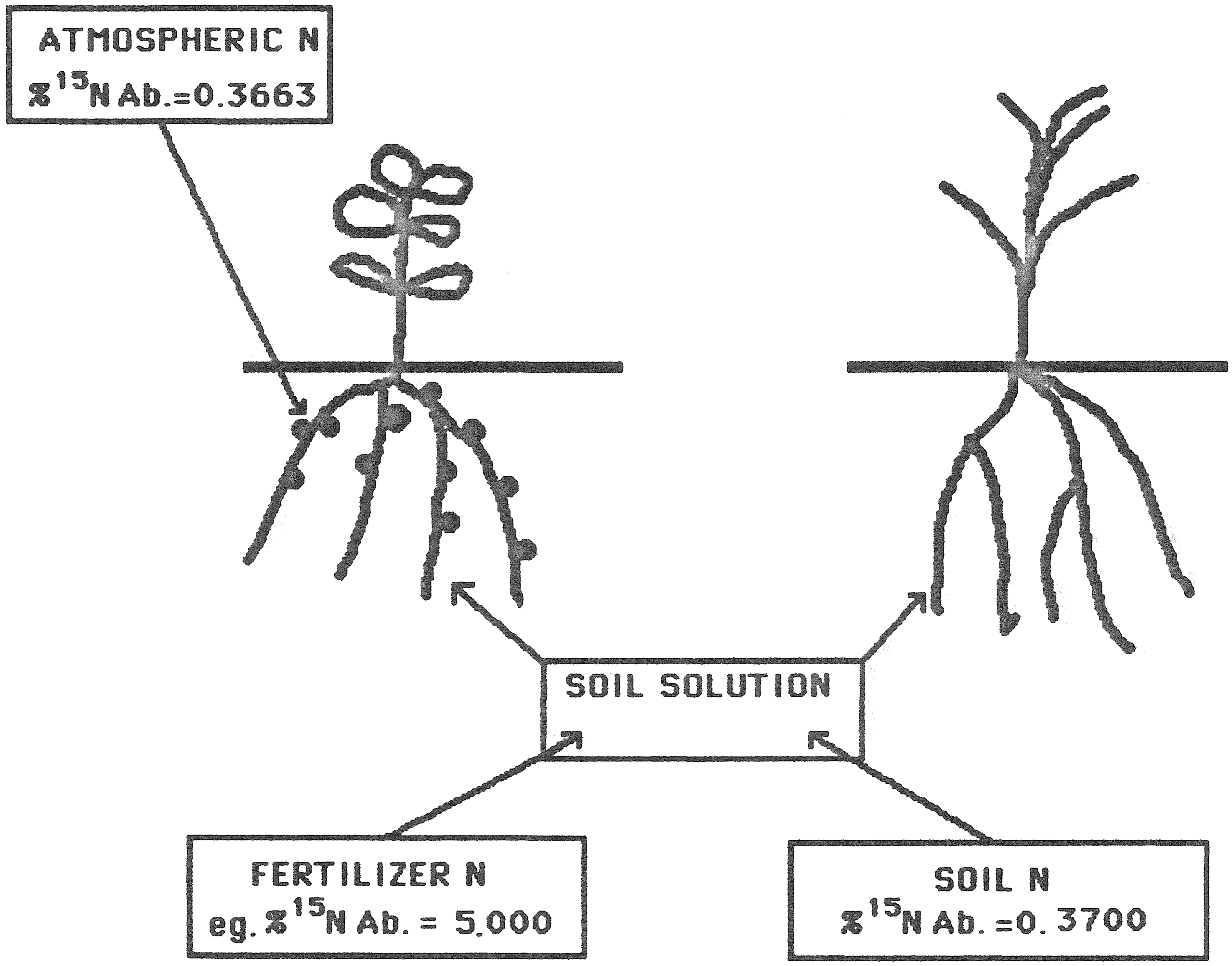

Fig.1 % ^{15}N Ab. of nitrogen sources for a fixing and a non-fixing crop.

This calculation assumes that the the control crop obtains the same ratio of $^{15}N:^{14}N$ from soil solution as the fixing crop. This assumption is met if the ^{15}N abundance of available soil nitrogen is constant for both crops, or if both crops utilize soil nitrogen in a similar pattern (Rennie and Rennie, 1983).

As ^{15}N enriched urea was injected at a localized point the enrichment of ^{15}N in the soil solution varied both spatially and temporally, although in a similar pattern for both the fs and the nfs. Samples taken in June, approximately six weeks after planting and three to four weeks after the addition of ^{15}N enriched urea, showed that barley had a much lower ^{15}N abundance than lentils (Table 1). This is due to the barley obtaining less of it's nitrogen requirements from fertilizer than lentils as barley explores a larger soil volume to meet it's larger nitrogen requirements, and perhaps as barley accumulates more nitrogen prior to the addition of ^{15}N enriched urea. Thus, estimates of nitrogen fixation using barley as a control were invalid at these earlier sampling dates.

At harvest lentils contained a lower ^{15}N abundance than barley. This is due to both increased nitrogen fixation and greater assimilation of soil nitrogen by lentils during the period between the June and August samplings. Estimates based on ^{15}N dilution showed significant quantities of nitrogen were fixed. These estimates assume that barley obtained the same ratio of $^{15}N:^{14}N$ from soil solution over the whole growing season as lentils, but this assumption could not be checked from the information collected. As the uninoculated treatments were also effectively nodulated, they could not be compared with barley as non-fixing controls.

Table 1 Nitrogen yield and % ^{15}N abundance at Tisdale for two sampling dates of barley and lentils.

Sampling date	Cultivar	N Yield (kg ha^{-1})	% ^{15}N Ab.	% NdfFert	Calculated % NdfA
June 27	Barley	73	0.53	3.5	
	Eston	26	0.86	10.6	-201
	Laird	25	0.86	10.6	-201
August 22	Barley	112	0.49	2.6	
	Eston	153	0.42	1.1	55
	Laird	167	0.43	1.3	40

The ^{15}N isotope dilution method gave better estimates of nitrogen fixation than the classical difference method. Fewer anomalous values were obtained by the dilution method than by the difference method as only about 6% of the dilution estimates were significantly below zero while about 19% of the difference estimates were significantly below zero. Wheat and barley had significant differences in nitrogen yield at 6 sites, but only at 2 sites for %^{15}N abundance. At sites where the uninoculated treatments were ineffectively nodulated the uninoculated treatments were more similar in % ^{15}N abundance than in nitrogen yield to the cereal controls. These observations indicate that the differences between fixing and non-fixing crops were greater for nitrogen uptake than for the ratio of ^{15}N:^{14}N assimilated from soil solution, suggesting that the assumptions of the dilution method were more closely met than those of the difference method. On average, estimates of %Ndfa derived from the ^{15}N isotope dilution method were about 20% higher than the estimates based on the classical difference method, but this did vary quite widely.

Factors affecting nitrogen fixation

Nitrogen fixation requires the presence of adequate numbers of effective rhizobia. At three of the ten sites inoculation did not increase the amount of nitrogen fixed significantly as native rhizobia were able to nodulate the uninoculated treatments, but at the remainder of the sites inoculation was effective (Fig. 2).

High soil nitrate levels inhibit nitrogen fixation as the plant preferentially assimilates soil nitrogen. A negative correlation was observed between spring soil NO_3 levels and %NdfA for most crops tested (Table 2).

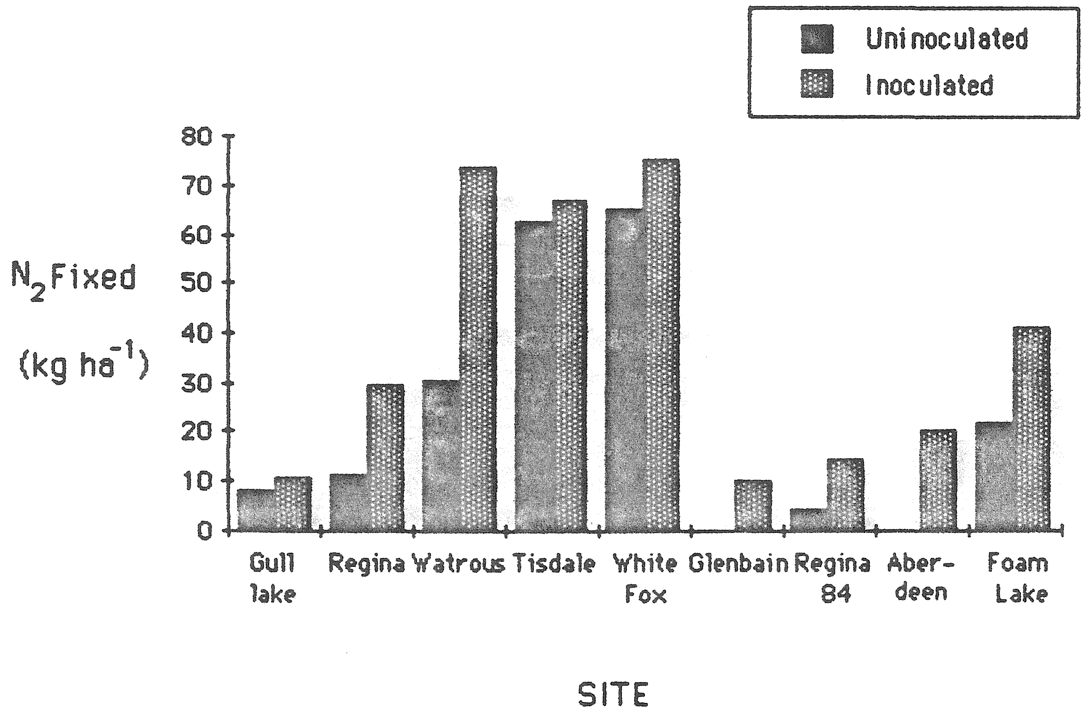

Fig. 2: Effect of inoculation on the amount of nitrogen fixed by Laird lentils at 9 locations in 1984 and 1985.

Table 2: Coefficients of correlation of water use and spring soil NO_3 with pea and lentil crop parameters in 1985 (5 sites).

Cultivar	Independent factor	Coefficients of Correlation of Crop Parameters: Total Yield	N Yield	%NdfA	N Fixed
Eston	WU [1]	0.74	0.66	0.81	0.69
	NO_3 [2]	0.64	0.69	0.39	0.64
Laird	WU	0.70	0.68	0.19	0.80
	NO_3	0.66	0.70	-0.71	0.20
Trapper	WU	0.76	0.83	0.58	0.89
	NO_3	0.63	0.52	-0.66	0.01
Tara	WU	0.70	0.77	0.25	0.85
	NO_3	0.62	0.57	-0.88	0.12

If $r > 0.88$ then $P < .05$
If $r > 0.73$ then $P < .1$

[1] WU = Total water use
[2] NO_3 = Spring soil nitrate (ppm) at 0-15 cm
Correlation coefficient of WU to NO_3 is 0.146

Water use was positively correlated with not only yield but also with the dependence of most crops on fixed nitrogen (Table 2). This may be due to an increase in both the amounts of nitrogen required and the availability of energy (photosynthate) under less water stressed conditions. Although the correlations of water use and soil nitrate with %NdfA were generally not significant, together they accounted for about 80% of the observed variation in %NdfA of peas and lentils in 1985.

There were significant differences in growth and nitrogen fixation between cultivars. At higher levels of available moisture fababeans had the highest yields of dry matter and fixed nitrogen, followed by peas and then lentils (Table 3). Under more drought stressed conditions peas had the highest yields of dry matter and fixed nitrogen, followed by lentils, and then fababeans, which failed to survive at most of these sites. Nitrogen fixation ranged from 5 to 25 kg ha^{-1} for peas and lentils in the Brown soil zone to 140 to 160 kg ha^{-1} for fababeans and 70 to 100 kg ha^{-1} for peas and lentils in the Gray and Gray-Black transition soil zones.

Conclusions

The ^{15}N isotope dilution method was a better estimater of nitrogen fixation than the classical difference method. Dilution estimates averaged about 20% higher than difference estimates. However, further work is still required to establish appropriate controls.

Significant quantities of nitrogen were fixed by grain legumes in Saskatchewan, ranging from 10 to 40 kg ha^{-1} in the Brown soil zone to 80 to 160 kg ha^{-1} in the Gray and the Gray-Black Transition soil zones. The

Table 3: Yields of above ground dry matter and fixed nitrogen of lentils, peas and fababeans in Saskatchewan.

Area of province	Crop	Yield (T ha^{-1})	%NdfA	N fixed (kg ha^{-1})
South (Brown soil zone)	Lentils	0.5-1.5	30-50	5-30
	Peas	0-3	40-60	0-40
	Fababeans	0-0.8		
North (Gray and Transition soil zones)	Lentils	5-8	50-70	60-80
	Peas	5-9	60-80	80-120
	Fababeans	7-10	75-85	120-160

amount of nitrogen fixed increased with inoculation, reduced water stress, and lower soil nitrate levels. Temperature may also have been a factor, but was not documented in this study. Fababeans fixed the most nitrogen under wetter conditions, but peas and lentils fixed more under water stressed conditions.

References

Rennie, R.J. and D.A. Rennie. 1983. Can. J. Microbiol. 29:1022-35.

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