Nitrogen fixation in peas (*Pisum sativum* L.), lupins (*Lupinus angustifolius* L.) and lentils (*Lens culinaris* Medik.)

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Abstract

Growers of legume crops in New Zealand generally rely on indigenous soil rhizobia for inoculation. This contrasts with most other parts of the world where legume seeds are normally inoculated with effective commercial rhizobial strains. A field experiment investigated the amount of nitrogen (N) fixed by indigenous and commercial strains of rhizobia. Peas, lupins and lentils were grown either with a commercial inoculum or without inoculation. Nitrogen fixation was measured using the ^{15}N dilution method with barley as the reference crop. The results showed that the total amount of N derived from N_2 fixation was 34, 122, 119 and 25 kg N ha^{-1} for green peas, dry peas, lupins and lentils, respectively. Inoculation had no effect on N_2 fixation, plant growth, plant N content or seed yield. Isolation of rhizobial DNA from inoculated and uninoculated plant roots showed that the rhizobia in all treatments originated from the soil rather than from the applied inoculant. The quantity of N fixed minus the quantity of N removed in harvested grain and stover was negative for all crops. The N balance for barley was the most negative with twice as much N removed compared with dry peas and lupins. While the legumes were not restorative in terms of soil N fertility, they were less depletive than barley, particularly if the stover was incorporated into the soil.

Additional key words: *inoculation, legume, N balance, soil N*

Introduction

On New Zealand arable farms, legumes are commonly grown in rotation with other crops as a source of N. Traditionally, the legume phase has been a clover/grass sward grown for 2-5 years but there is now increased pressure to reduce the length of this restorative phase and to use other options for maintaining fertility under continuous cropping. Grain legumes such as peas, lupins and lentils are one option used by farmers. However, agronomic trials indicate an overall removal of N in the harvested grain and stover with little contribution of N to subsequent crops (Haynes *et al.*, 1993). These trials have also shown that grain legumes grown in Canterbury fix lower rates of N compared with overseas, with <40% of the N they accumulate coming from N_2 fixation.

Nitrogen fixation depends on the establishment of a successful association between the host and the N_2 fixing rhizobium. Since New Zealand farmers generally rely on indigenous soil rhizobia rather than inoculating seed with effective strains, it is possible that the indigenous rhizobia are insufficient for, or ineffective in, nodulating grain legumes. In this study we investigated the influence of inoculation on plant growth, seed yield and N_2 fixation in four legume crops (green peas, dry peas, lupins and lentils). A further objective of the study was to construct N balances for the various crops to identify the potential for improving the N fertility of the soil.

Material and Methods

The field experiment was carried out at Lincoln University on a Templeton silt loam. The site was selected for its low N fertility. Chicory had been grown at the site for two years prior to the establishment of the trial; no N was applied to the chicory. Peas (harvested green and dry) cv. Aztec (240 kg/ha), lupins cv. Feste (190 kg/ha), lentils cv. Titore (60 kg/ha) and barley cv. Regatta (140 kg/ha) were sown in a randomised block design on 3rd November 1995. Plots were 5.4 m x 4.5 m with four replicates. Each legume had a commercial inoculation or no inoculation treatment. Peas and lentils were inoculated with *Rhizobium leguminosarum* biovar *viciae* strain su-391 and lupins with *Bradyrhizobium* sp *lupinus* strain wu-425. Seeds were slurry inoculated according to the suppliers recommendations (10^7 cells per seed) and sown within 24 hours. The trial was irrigated throughout December and January to ensure adequate moisture for plant growth.

The amount of N_2 fixed was measured using the ^{15}N isotope dilution technique which compares ^{15}N enrichment in legume crops with a reference crop (Peoples and Herridge, 1990). The ^{15}N was applied as urea (20% enrichment) in an aqueous solution at 3 kg N /ha to microplots 1 m^2 within each plot.

The treatments were harvested when plants were judged to be physiologically mature i.e., 90% of pods had turned brown. The green peas were harvested when the seeds reached a tenderometer reading of 105. All above-ground portions of the plants were hand harvested from the 1 m^2 microplot. These samples were separated into grain, pods and stover then oven dried and weighed. Root material was carefully extracted from a 0.2 m^2 area of each subplot to a depth of 40 cm. The roots were washed free of soil, oven dried and weighed. Plant samples were ground finely (<0.5 mm) and their total N content and $^{15}N/^{14}N$ ratio determined using a Tracermass stable isotope analyser in conjunction with a Roboprep-CN biological sample converter. Nitrogen fixation was calculated from the $^{15}N/^{14}N$ ratio of the legume plants compared with the barley reference plants (Fried and Middelboe, 1977).

DNA material from the rhizobia isolated from root nodules of inoculated and uninoculated plants was compared with that from rhizobia of the original inoculum. Suspensions of crushed nodules or original inoculum were streaked onto G/RDMplates. DNA extracted from the rhizobial cells was digested with the restriction enzymeEcoRI and then separated by agarose gel electrophoresis (Kelstrup, 1996). The gels were stained with ethidium bromide, visualised under ultra violet light and photographed.

Results and Discussion

All the crops established at plant densities close to that expected to give optimum yields, although there were some disease problems in the lentils and lupins. Nodules were visible 13-14 days after sowing and were well formed on all roots. Grain yields for the legume and barley crops were within the range expected for Canterbury.

Inoculation had no effect on grain yield (Table 1), plant growth or N concentration (Table 2). The pattern of nodulation and number of effective nodules was similar on both inoculated and uninoculated plants (Kelstrup, 1996). Furthermore, similar patterns in DNA isolated from plant nodules were observed in inoculated and uninoculated plants. These DNA patterns were different from those isolated from the applied inoculum which suggests that the rhizobia present in the soil were more successful in nodulation than the applied strains. The introduced strains may not have survived long enough in the soil or may not have been sufficiently competitive enough with the indigenous population to occupy a significant proportion of the nodules effectively.

The mean amounts of N_2 fixed in this study were 34, 112, 119 and 25 kg N/ha for green peas, dry peas, lupins and lentils (Table 3). Because there were no differences between inoculated and uninoculated plants, the data for these treatments have been averaged. The percentage of accumulated N derived from N_2 fixation (%Ndfa) ranged from 62 to 68% (Table 4). These %Ndfa values are higher than the 22-38% measured by Haynes *et al.*, (1993) possibly due to the higher N fertility at their site which would have reduced N_2 fixation (Herridge *et al.*, 1984). The %Ndfa values reported in our study are similar to those measured in Australia (Evans *et al.*, 1989) and California (Larson *et al.*, 1989). While the %Ndfa was similar among all crops, the proportion of plant N removed in the grain (nitrogen harvest index,

Table 1. Mean grain yields for the legume and barley crops (kg/ha).

Crop	Inoculated	Uninoculated	SEM
Green peas	490	510	150
Dry peas	3420	4230	150
Lupins	2250	2420	150
Lentils	610	1050	150
Barley	Barley	5630	280

Table 2. Nitrogen concentration in legume and barley grain at harvest (%).

Crop	Inoculated	Uninoculated	SEM
Green peas	4.7	4.7	0.06
Dry peas	4.0	4.1	0.06
Lupins	4.8	4.5	0.06
Lentils	3.9	4.0	0.06
Barley		1.5	0.06

Table 3. Nitrogen accumulation and amount of fixed-N removed in grain and stover in legume and barley crops (kg N/ha).

N	Green peas	Dry peas	Lupins	Lentils	Barley
Total N accumulated	56	180	184	36	119
Fixed N_2 accumulated	34	123	119	25	-
Soil N accumulated	22	57	65	11	119
N_2 fixed - N removed (grain + stover)	-19	-57	-64	-11	119
N_2 fixed - N removed (grain only)	11	-34	11	-8	-84

Table 4. Proportion of plant N derived from biological N_2 fixation (%Ndfa) and proportion of total N removed in harvested grain (NHI) for legume and barley crops (%).

Crop	% Ndfa	NHI
Green peas	62	43
Dry peas	68	87
Lupins	65	59
Lentils	68	91
SEM	3.9	0.2
Barley		71
SEM		1.2

NHI) (Table 4) was affected by maturity at harvest. The lowest NHI was for green peas due to the shorter growing period and the fact that the green peas were harvested before translocation from plant residues to the seeds was complete. The higher NHI values for the other crops indicated that a larger proportion of the N was removed in the grain.

An N balance was calculated for each crop by subtracting the amount of N removed in the grain and stover from the amount of N fixed (Table 3). The N balance was negative for all crops due to the large amount of N removed at harvest. Barley had the greatest negative balance, while the grain legumes removed less soil N as they fixed 59-71% of their N requirement. Quite large amounts of N (up to 72 kg N/ha) were removed in the legume stover at the end of the experiment. When incorporated into the soil this offset the N losses resulting in a positive N balance for green peas and lupins (Table 3).

Incorporating grain legume residues into the soil has been shown to be beneficial in terms of improving the N economy of the soil (Askin *et al.*, 1985; Francis *et al.*, 1994). However, in this experiment, the amounts of N returned to the soil were small (<70 kg N/ha in the stover) and the net N balance where stover was incorporated was only 11 kg N/ha. Since the total amount of N in the topsoil is 3000-3500 kg N/ha at this site, incorporating stover will not have a large effect on the total soil N pool. Consequently the role of grain legumes appears to be for maintaining rather than increasing soil N.

Conclusions

Inoculating pea, lupin and lentil plants with specific rhizobia appears to have had little effect on the agronomic yield and N_2 fixation. Although the plants were well nodulated, this was probably due to indigenous rhizobia rather than introduced strains.

In general, grain legumes do not exhaust the soil of N to the same extent as a cereal crop. The grain legumes in this study fixed a relatively large amount of their total N and therefore removed less soil N than barley. Returning the stover to the soil was beneficial in offsetting this loss. However, grain legumes do not appear to be have a major role in improving the N fertility of the soil.

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