THE EFFECT OF INOCULATION AND FERTILISER NITROGEN ON THE GRAIN YIELD AND NITROGEN CONCENTRATION OF DWARF BEAN (*PHASEOLUS VULGARIS* L.)

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ABSTRACT

Three methods of amending plant and soil nitrogen status of an experimental crop of *Phaseolus vulgaris* were; application of rhizobia to plots at sowing, addition of 100 kg N/ha at sowing and addition of a solution of 10 kg N/ha/wk during the six week period of pod development.

Many of the uninoculated plants became infected with rhizobia during growth of the plant so that comparisons between inoculated and uninoculated plants could not be made. However, application of nitrogen at sowing increased the seed yield from 249 to 324 g/m^2 and application of the nitrogen solutions during pod development increased grain yield from 258 to 316 g/m^2.

Grain nitrogen concentration was increased from 3.61 to 3.84 percent by application of nitrogen at sowing and from 3.54 to 3.90 percent by application of nitrogen during the pod filling period.

A positive correlation between seed yield and grain nitrogen concentration was obtained.

***Additional Key Words:** Rhizobium, yield components, nodulation*

INTRODUCTION

Leguminous crops complement cereal crops well for two reasons. Firstly they produce high protein seeds containing amino acids complementary to those in cereals so that, combined with cereals, they provide a balanced diet for man and animals. Secondly, when inoculated with the appropriate strain of rhizobia, they can fix atmospheric nitrogen. Thus legumes can be grown on soils of low nitrogen status and may also boost soil nitrogen reserves so that subsequent cereal crops may be less dependent on fertiliser nitrogen to achieve acceptable yields.

However, if an uninoculated legume is not infected with native soil rhizobia during growth, it will obtain its nitrogen requirement from nitrogen mineralised from soil organic matter, or from applied fertiliser nitrogen. Under conditions where it is not fixing nitrogen symbiotically, it is functioning in the manner of a cereal crop.

Cereals such as wheat and barley, which are dependent on root uptake of soil nitrogen generally show an inverse relationship between grain yield and seed nitrogen concentration but the two factors in nodulated nitrogen fixing legumes is not clear. Evans (1975) reported that most of the workers who had studied the relationship between seed yield and grain protein concentration in grain legumes (soyabeans; Johnson *et al.,* 1955; peas; Furedi, 1970; *Phaseolus vulgaris*, Tandon *et al.,* 1957; Rutger, 1970; Leleyi *et al.,* 1972) had shown a negative correlation between seed yield and grain nitrogen concentration.

However, as there was no mention of inoculation with the appropriate strain of rhizobia in any of these papers, it is possible that the plants were not adequately nodulated so they may have been dependent on soil nitrogen reserves. Under these conditions, negative correlations between seed yield and grain nitrogen concentration could be expected. If, however, plant nitrogen status could be amended by inoculating the plant with rhizobia or by addition of fertiliser nitrogen, positive correlations between these two yield parameters could be expected.

The purpose of this experiment was to compare grain yields of *Phaseolus vulgaris* grown under a range of nitrogen regimes and to examine the relationship between seed yield and grain nitrogen concentration.

Phaseolus vulgaris is not widely grown as a seed crop in the Manawatu but is more frequently grown as a crop for harvesting as a fresh vegetable or for freezing. It was chosen as the test legume because the crop had not been grown previously on the experimental area and there was little likelihood that native populations of *Rhizobium phaseoli* would be present. In addition, previous work had shown that the plant was difficult to nodulate (Owens y de Novoa, 1980; Quah Sin Hock, 1980) and it was therefore considered that accidental contamination between inoculated and non-inoculated plots would be unlikely to occur.

MATERIALS AND METHODS

Phaseolus vulgaris (cv. "Royal Streak") was sown in a 2^3 factorial with four replicates on a Tokomaru silt loam at Massey University.

Nitrogen Treatments were as follows:

1. Seed was inoculated at sowing with *Rhizobium phaseoli* (Strain N.Z.P. 5479).

2. 100 kg N/ha was applied as calcium ammonium nitrate at sowing.
3. 20 kg N/ha was applied as a urea solution each week during the six week duration of pod development.

The area had been cropped previously to summer and winter cereals for three years with the intention of depleting soil nitrogen reserves. Reverted superphosphate (0:7:0:8) at 300 kg/ha and hydrated lime at 250 kg/ha were broadcast onto the plots a week before sowing. Maize and millet were sown around the perimeter of the experimental area on 10 November but, because temperatures were low, (Fig. 1) germination was slow and uneven and these shelter rows were resown with maize on 9 December. Drill widths of *Phaseolus vulgaris* were sown as guard plots between the experimental plots and the shelter on 16 December. Seeds were hand sown at 80 plants/m² in 15 cm rows in plots 1.5 m wide and 3 m long on 15 December 1980. Paths of 0.6 m were left around all plots to facilitate access (especially during the pod filling period when nitrogen solutions were applied) and to reduce possible movement of nitrogen and rhizobia during the growing period.

Alachlor (at 1*l*/ha) was applied to all plots and Thimet granules were broadcast after sowing. Although a few small nodules were found on plants within the guard rows of inoculated plots soon after the emergence, plots were watered and a second application of rhizobia as a slurry solution was made on 5 January to ensure that all plants within inoculated plots formed nodules.

As part of an additional study within this experiment, plants were taken from a 0.1 m² quadrat at each growth stage and nodule development on the sampled plants was assessed on a 1-5 scale (1 = poor, 5 = good).

Since flowering and pod filling occur concurrently in *Phaseolus vulgaris,* weekly applications of 10 kg N/ha/week began on 28 January, five days after flowering commenced and continued until plants began to senesce. To ensure retention of the applied nitrogen, all plots were irrigated and the nitrogen applied in 4 mm of water/ha to avoid foliar damage. Irrigation was applied with a large central sprinkler while a small sprinkler was used to irrigate plots on the perimeter of the area.

On 31 March, plants from a 2 m length of the central two rows were harvested and grain yield and components of yield determined. Seeds were ground to pass through 1 mm mesh and, following Kjeldahl digestion, nitrogen concentration of the seed was determined using an autoanalyser.

RESULTS

Although 80 seeds/m² were sown, counts made three weeks later indicated that there were 59.2 plants/m² established and at harvest, 57.4 plants/m². Some herbicide damage resulting from the application of Alachlor was apparent soon after establishment but this appeared to be confined to the first unifoliate leaf. Alachlor failed to give good control of all germinating weeds and some hand weeding of fathen (*Chenopodium album*); docks (*Rumex* spp.); Redroot (*Amaranthus* spp.) and Barnyard grass (*Echinochloa crus-gallia*) was required.

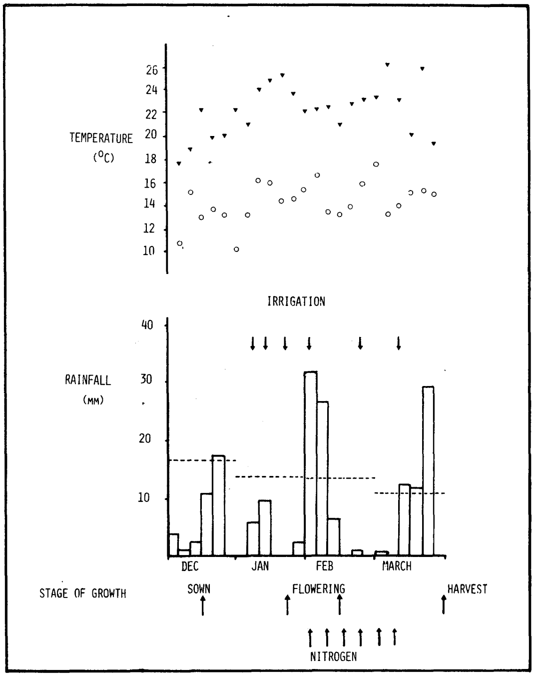

Figure 1: Maximum (▼) and minimum (o) temperature, actual and long term average rainfall (---) calculated for five day periods during the growth of *Phaseolus vulgaris.*

Thimet was found to control aphids for six weeks after application but after this time caterpillers (Silver Y moth, *Chrysodeixis eriosoma*) were noticed on the plants and derris dust was applied on 4 March.

Timing of the main physiological growth stages are outlined in Fig. 1. The first flowers opened on 23 January and counts made on this date indicated that neither inoculation nor application of 100 kg N/ha at sowing affected the number of plants flowering. Small pods 3-4 cm long and 2-3 mm in width were apparent by 28 January and flowering continued until 20 February.

TABLE 1: Nodulation score (1 = poor, 5 = good) for *Phaseolus vulgaris* at three stages of growth.

	Stage of growth		
	Vegetative	Flowering	Senescing
I-	1.1	1.3	1.4
I+	2.3**	2.1*	1.6
N_s-	2.3	1.9	1.8
N_s+	1.1**	1.5	1.1*
N_p-	1.5	1.5	1.6
N_p+	1.9	1.9	1.3
$SE_{\bar{x}}$	0.25	0.28	0.18

TABLE 2: Grain yield, yield components and grain nitrogen concentration in *Phaseolus vulgaris*.

	Grain yield g/m^2	Pods/plant	Beans/pod	Bean size (g)	Grain nitrogen concentration %
I-	279.4	3.26	3.75	0.400	3.70
I+	293.9	3.18	3.87	0.414	3.74
N_S-	248.9	2.93	3.47	0.391	3.61
N_S+	324.4**	3.51**	3.95**	0.422**	3.84**
N_P-	257.7	3.08	3.65	0.397	3.54
N_P+	315.5**	3.36**	3.97**	0.416	3.90**
$SE_{\bar{x}}$	14.90	0.0950	0.068	0.0095	0.037
Interactions	—	—	—	I x N_S I x N_P	—

Soil cores taken at sowing indicated a pH of 6.1. Scores made during vegetative growth (Table 1) showed that, as expected, plants in plots to which rhizobia were applied were better nodulated than plants in uninoculated plots and that nodulation was suppressed in plants which received 100 kg N/ha at sowing. Although the differences between treatments were maintained throughout the experiment, they became less significant as the growing season progressed.

As the plants began to senesce, nodulation scores fell and the reductions in the nodulation of inoculated plants may have been hastened by the weekly applications of 10 kg N/ha made during the podding period (Table 1).

Grain yields of 294 g/m^2 were obtained from plants inoculated with rhizobia (Table 2), while applications of nitrogen at sowing increased grain yields from 249 to 324 g/m^2. Seed yields were increased from 258 to 315 g/m^2 as a result of weekly applications of nitrogen during pod development. Both applications of fertiliser nitrogen increased grain yields and the components of yield (Table 2). While application of nitrogen at sowing suppressed nodulation of inoculated plants (Table 1), these plants produced larger seeds than uninoculated plants (Table 3). It is possible that application of 10 kg N/ha/week during the pod filling period failed to supply as much nitrogen to the plant as the symbiotic system it suppressed so that seed size was reduced (Table 4).

TABLE 3: Effect on bean size (g) of Rhizobial inoculation and nitrogen fertiliser applied at sowing.

	N applied at sowing	
	—	+
Inoculation		
—	0.3960	0.404
+	0.3860	0.414
$SE_{\bar{x}}$	0.0136	
Significance	*	

Nitrogen concentration of seed from inoculated plants was 3.74%, while that of plants which received nitrogen at sowing, and during grain filling, was 3.84 and 3.90% respectively (Table 2).

Seed yield and grain nitrogen concentrations were positively correlated ($r = 0.65$ $P \leq 0.01$). The regression equation accounted for 39.8% of the variation and is as follows:

Grain nitrogen =
3.039 + grain yield (g/m^2) concentration (%) (I)

Identification of the values for the main treatments within the regression plotted in Fig. 2 shows that all three methods of amending plant nitrogen tended to increase yield and grain nitrogen concentration beyond that of the controls.

TABLE 4: Effect on bean size (g) of Rhizobial inoculation and fertiliser nitrogen applied during pod fill.

	N applied during pod fill	
	—	+
Inoculation		
—	0.377	0.423
+	0.418	0.409
$SE_{\bar{x}}$	0.0136	
Significance	*	

DISCUSSION

Phaseolus vulgaris L. was chosen as the test legume because previous work at Lincoln College had suggested that even when supplied with suitable strains of rhizobia, nodules did not form readily (Owens y de Novoa, 1980; Quah Sin Hock, 1980). It was hoped therefore that there would be little or no contamination of uninoculated plants. Despite great care to avoid contamination during sowing of the inoculated seed and the slurry inoculation made after emergence, some plants within uninoculated plots formed nodules. Contamination increased as the season progressed (Table 1) and probably resulted from splash from rainfall and irrigation (Fig. 1) or from surface runoff. Nevertheless nodulation scores from inoculated plants were higher throughout the growing season than from those inoculated later as a result of contamination.

As plants began to senesce, nodulation fell. A method of assessing nodulation which is more quantitative than a simple 1-5 score is needed to investigate the I x N_S x N_p interaction which occurred during this sampling (Table 1).

As a result of nodulation of uninoculated plots, true comparisons between inoculated and uninoculated plants can not be drawn. However, the effect of time of application of fertiliser nitrogen can still be studied.

Grain yields of up to 324 g/m² were obtained from *Phaseolus vulgaris* (Table 1). These are similar to grain yields obtained from a number of legumes grown in the Manawatu by Withers (1979) (*Lupinus spp.* 43-462 g/m²; *Pisum sativum:* 423 g/m²; *Vicia faba* 281 g/m²) and in Canterbury (Hill *et al.,* 1977; Owens de y Novoa, 1980). Grain yields of up to 622 g/m² have been obtained by Goulden (1976).

As inoculated legumes are able to use nitrogen mineralised from soil organic matter, fertiliser nitrogen, and nitrogen fixed symbiotically, they are therefore not dependent on any one source of nitrogen. As nitrogen from once source becomes more available, the plant requirement for nitrogen from the other sources is reduced. Thus application of nitrogen at sowing or during pod development tended to reduce nodule number (Table 1).

The response of a legume to nitrogen amendment is influenced by its current nitrogen supply so that very variable responses to additional nitrogen during growth have been reported in a number of legumes. Garcia and Hanway (1976) reported increases in grain yields of soyabeans when N was applied during the podding period while Welch *et al.* (1973) reported no consistent increase in grain yield of the crop. In lupins (Herbert and Dougherty, 1978) and in field beans (Witty *et al.,* 1980), applications of fertiliser nitrogen during the grain filling period caused reductions in yield.

The current nitrogen supply to the plant may also influence the relationship between seed yield and grain nitrogen concentration. Should soil nitrogen reserves be depleted before plant growth is complete, negative correlations between these two parameters may occur and it is suggested that this may explain the negative correlations reported by Evans (1975). Positive correlations can occur if the nitrogen supply to the plant is maintained during life of the plant (Fig. 2).

The situation in a nodulated grain legume appears to be more complex as the relationship between seed yield and grain nitrogen concentration is dependent on the distribution of carbohydrate within the plant. Pate (1979) indicated that during grain filling, 54% of the photosynthate produced in *Lupinus albus* was diverted to

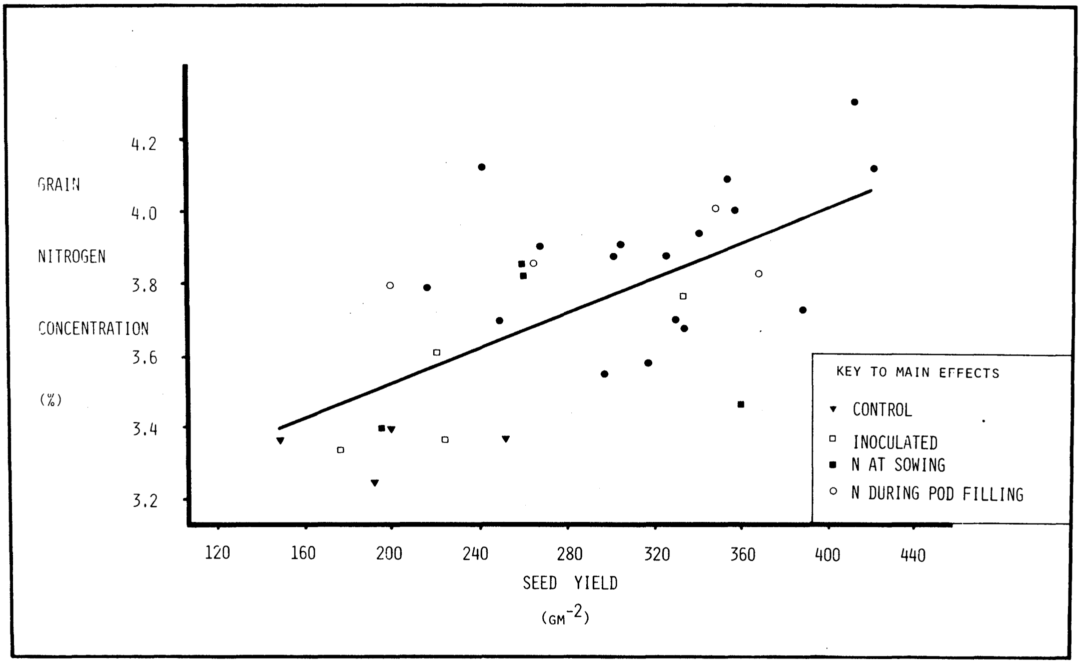

Figure 2: The relationship between seed yield and grain nitrogen concentration in *Phaseolus vulgaris.*

the root, while in the cowpea (*Vigna unguiculata*) only 14% of the photosynthate was sent there. The cowpea was able to divert a larger proportion of its carbohydrate to the seeds than the lupin but supply of fixed nitrogen from the nodules was lower than in the lupin. In addition, nitrogen tends to be mobilised from plant tissue to the seed early in its development so that later additions of carbohydrate from photosynthesis occuring during pod fill 'dilute' the grain nitrogen. Some workers with legumes support the hypothesis that mobilisation of nitrogen from the leaves during pod fill leads to their senescence, the abscission of nodules, and ultimate 'self destruction' of the plant (Sinclair and de Wit, 1975). Others, such as Hardy *et al.* (1971) have noted that indeterminate cultivars of soyabeans fixed only 10% of their total nitrogen before flowering and that nitrogen fixation during the flowering and pod filling period is thus an important factor. Positive correlations between seed yield and grain nitrogen concentration in inoculated fieldbeans (*Vicia faba* L.) have been calculated by Newton (1980) from data published by Magyarosi and Sjodin (1976). The slopes of the regression equations are similar to that presented in Equation 1.

Further work is needed to document for each grain legume the conditions under which nitrogen fixation is able to supply the plant requirement for nitrogen (especially during the pod filling period) and the extent to which nitrogen fixation may increase seed yields and grain nitrogen concentration.

CONCLUSIONS

When supplied with *Rhizobia phaseoli* (strain N.Z.P. 5479), dwarf beans (*Phaseolus vulgaris*) nodulated more readily than was previously expected. Grain yields of up to 324 g/m^2 were obtained which are similar to yields of other grain legumes grown in the Manawatu.

The relationship between seed yield and grain nitrogen concentration is dynamic and is dependent on such factors as availability of soil nitrogen, amount of nitrogen fixation and production of, and competition for, assimilate within the plant. The positive correlation between seed yield and grain nitrogen concentration obtained suggests that, in this experiment, grain yield was not limited by nitrogen.

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