Is it worth inoculating common bean (*Phaseolus vulgaris* L.)?

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Abstract

Legume inoculation is an established agricultural practice which has contributed to increased N_2 fixation and yield. Despite this evidence there is still an ongoing debate as to whether inoculation is necessary in grain legume production. Over the period 2003-05 a series of field experiments were conducted at Lincoln University, Canterbury, New Zealand to evaluate the effect of inoculation on common bean (*Phaseolus vulgaris* L.) production. Two white seeded bean cultivars, Scylla and T-49, were inoculated with six strains of *Rhizobium phaseoli* Dangeard, CC 511, RCR 3644, UK 2, H 20, PRF 81 and PhP 17 to determine their nodulation capabilities.

Nodulation was variable and appeared to be affected by cultivar and *Rhizobium* strain. The two planting areas used, which had similar cropping histories, gave contrasting results. In the first season (Paddock 1), when a peat based inoculum was used, no nodulation was observed. In the second season (Paddock 2) liquid inoculum was used and nodulation ranged form 0 to 7.3 nodules plant^{-1} at 21 days after sowing (DAS) to 0 to 75 nodules plant^{-1} at 70 DAS. Cultivar also significantly affected the number of nodules plant^{-1}; ranging between 2-5 nodules plant^{-1} at 21 DAS to 18-19 nodules plant^{-1} at 70 DAS.

Shoot dry matter (DM) over the two growing seasons ranged from 370-894 g m^{-2}, while green pod yield (taken at an average green seed length of 11 mm) ranged from 138 to 471 g m^{-2}. Total DM at final harvest ranged from 530 to 1,180 g m^{-2} and seed yield ranged from 266 to 635 g m^{-2}. Strains H 20 and PRF 81 consistently out performed the other strains in most measured parameters. The results confirm the difficulty of predicting nodulation, and question the benefits of inoculating *Phaseolus* beans in Canterbury.

Additional keywords: common bean, *Rhizobium,* liquid and peat based inoculum, nodulation, nodule number, green pod yield seed yield.

Introduction

Inoculation is a means of transferring selected or elite strains of root-nodule from the research test tube to the legume in the field (Date, 2001). Transfer can be achieved by applying the inoculant directly to the soil or by coating the seed at the time of sowing (Deaker *et al.*, 2004). The inoculation of legumes seeds is 'the success story' of applied soil microbiology as millions of hectares were inoculated since the beginning of this practice, making it the largest and oldest experience of voluntary microbial release and dissemination in the environment (Catroux *et al.*, 2001). In a recent review, Brockwell and Bottomley (1995), stated that world-wide production of legume inoculant is static or in decline and emphasised the importance of several limitations in the use of these inoculants. Of these limitations, failure of the inoculum to effect nodulation has been most frequently reported (Dapaah *et al.*, 2000; Date, 2000; Stephens and Rask, 2000).

When the specific rhizobia are absent, inoculation is known to enhance nodulation, plant growth, N_2 fixation and yield (Vlassak and Vanderleyden, 1997). On the other hand, when native bacteria exist in the field they often out-compete the inoculant strain, which only occupy a small proportion of nodules as observed in some areas of Latin America

(Vlassak and Vanderleyden, 1997; Aguilar *et al.*, 2001). Contrastingly, bean inoculation with *Rhizobium tropici* has been successful in Brazil (Hungria *et al.*, 2000; Mostasso *et al.*, 2002) and yield enhancement has been observed in the yield of *Rhizobium* inoculated beans and maize grown together (Pineda *et al.*, 1994). However, legume inoculation in New Zealand has shown extremely variable responses to yield and nodulation ranging from a 29 % increase in yield following chickpea inoculation (Hernandez and Hill, 1985) and profuse nodulation (Dapaah *et al.*, 2000), with pinto beans.

The decision to inoculate is usually based on a demonstrated need from experimental plots or as an insurance against nodulation failure (Deaker *et al.*, 2004). Most soil sown to beans is reported to contain indigenous rhizobia (Graham, 1981; Vargas *et al.*, 2000) which may interfere with the establishment of the inoculated strain. As a consequence, the benefits of bean inoculation are usually questioned by most farmers due to doubt over the capacity of the practice to be translated in significant yields. These doubts have resulted in the use of N fertiliser becoming the common practice in legume production (Vargas *et al.*, 2000). This raises the question: Is inoculating beans worthwhile? The objectives of the present study were:

1. Evaluating the effectiveness of inoculation in field grown beans.
2. Evaluating the effect of nodulation on bean yield.
3. Selecting an effective strain suitable to current management practices in Canterbury.

Materials and Methods

Site and design

The two field experiments were located in the Horticultural Research Area, Lincoln University Canterbury, New Zealand on a Wakanui silt loam soil (Cox, 1978) in 2003-04 and 2004-05. Soil was of low to medium fertility in both seasons with the New Zealand MAF Quick Soil Test values for pH, Olsen P, Ca, Mg, K, Na and total N as shown in (Table 1). In the first season, a split plot completely randomised block design with three replicates was used with irrigation as the main plot. Treatments consisted of two common bean (*Phaseolus vulgaris*) cultivars, Scylla - a very early fresh green dwarf bean and T-49 - a navy bean grown for dry seeds, two *Rhizobium* strains CC 511 and RCR 3644, and a control of no rhizobia, two irrigation levels full and nil and two fertiliser nitrogen (N) levels, 0 and 150 kg N ha^{-1}. In the second season, a completely randomised block design with four replicates was used. Treatments included the same two common bean cultivars and five *Rhizobium* strains RCR 3644, UK 2, H 20, PRF 81, PhP 17 and a control.

The fields were previously in apples, followed by barley and oats. The area was harrowed, rotovated and rolled before sowing. In 2003-04, plots were 10 m long and 2.25 m wide, each plot contained 4 rows with 15 cm between rows and 5 cm between plants. Plots were mechanically planted with an expected plant population of 60 plants m^{-2}. In 2004-05, plots were 2 m by 0.75 m, with 0.3 m between plots and 0.5 m separating each replicates Experimental plots contained five rows, with 15 cm between rows and seed 10 cm apart

Table 1. MAF soil quick test values for paddocks in the Horticultural Research area (0 – 20 cm depth), Lincoln University, Canterbury. 2003-04 and 2004-05.

Season	pH	P	Ca	Mg	K	Na	Total N
2003/04	6.4	15	10	20	9	9	---
2004/05	6.3	28	9	21	14	8	0.20

Ca, P, K, Mg and Na are expressed in µg/g soil and total N as a percentage

within rows giving a population of 100 plants plot^{-1} (67 plants m^{-2}). Planting holes were prepared with a dibble board, 5 cm deep and seeds were sown by hand. After sowing, 2 ml of inoculant (3.4 x 10^{9} colony forming units (cfu's) ml^{-1}) was applied to the seed in each seed hole, lightly covered with soil and gently compacted. Irrigation was applied to eliminate any occurrence of water stress during plant growth.

Inoculum

In 2003-04, seed were inoculated with peat-based inoculum containing *Rhizobium tropici* CC 511 (1.70 x 10 9 cfu g^{-1} of moist peat) and *Rhizobium tropici* RCR 3644 (2.41 x 10 9 cfu g^{-1} of moist peat). Inoculant (240 g peat/100 kg seeds) was mixed into a slurry 20 g of peat inoculant to 40 ml of water in a plastic bag and gently applied to the seeds. Seeds were then air-dried for 3 hours before planting. In 2004-05 rhizobial isolates were grown on tryptone-yeast extract (TY) agar plates and incubated at 28 ^{o}C for 18 hours. The rhizobia were suspended in 100 µl of sterile water and transferred by pipette into a 250 ml sterile conical flasks containing tryptone-yeast broth. These were incubated at 150 rpm on a shaker at 28 ^{o}C for 18 hours. At sowing, 2 ml of the incubated concentrated solution was used as the field inoculum. The strains used in the field experiment were selected based on signs of early nodulation at thinning (14 days after sowing) in a preliminary experiment.

Sampling

In the first season, intermediate 0.2 m^{-2} quadrat samples were taken every 10-14 days from 23 DAS for biomass accumulation. At green pod yield and final harvest samples were taken from 1.0 m^{-2} quadrat samples for sorting into the measured parameters. In the second season, samples were taken from the three centre rows of each plot, starting from a randomly selected end. The first two rows of plants were omitted to eliminate border effects and the sample size was approximately six plants (two along and three across rows). A buffer of three plants was used between successive samplings. Samples were taken at 21 days after sowing (DAS), when 50 % of the plants had at least one open flower, when over 50 % of the plants had at least one green pod 5 mm long, and at green bean harvest, based on Heinz - Wattie's Australasia specifications (average seed length of approximately 11 mm from the centre-most seed in a sample of 25 randomly selected pods).

Measurement of growth parameters

At each sample date plants were collected for determination of total dry matter (TDM), shoot and root dry weight and nodule number. Plant tops were dried immediately after harvest in a forced air dryer at 70 ^{o}C to constant weight. Roots were washed in tap water, nodules counted and they were then dried. At green pod yield and final harvest samples were separated and the yield recorded on a DM basis.

The following characteristics were analysed statistically: total DM, nodules plant $^{-1}$ root dry weight, shoot dry weight green pod yield and seed yield. Weather data were recorded at the Broadfields Meteorological Station, 1.0 km from the experimental site. All statistical analyses were done using GenStat (GenStat Release 6.1 Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, UK).

Results

Climate

Rainfall over the 2003-04 growing seasons was 163 mm which was 31 % less than the long term average of 238 mm (Table 2). Minimum and maximum temperatures of approximately 20 ^{o}C and 10 ^{o}C respectively were recorded, which were similar to the long term average. Total rainfall in the second season was 202 mm, 16 % less than the long term average. Maximum temperature was similar to the long term average of 19 ^{o}C, while the minimum temperature was 1 ^{o}C less than the long term average 10 ^{o}C.

Table 2. Weather data for the 2003-04 and 2004-05 growing season and long term averages at Lincoln University, Canterbury, New Zealand.

	Dec	Jan	Feb	Mar	Apr	May	Total
Rainfall (mm)							
2003-04	1.2	21.0	43.6	36.8	60.4		163.0
2004-05		33.6	18.6	36.6	52.4	61.0	202.2
Long term mean	50.0	51.3	40.6	50.4	46.0	50.2	
Maximum daily temp. (°C)							
2003-04	21.8	23.6	20.2	20.0	15.9		
2004-05		21.7	23.4	19.9	16.5	14.3	N/A
Long term mean	20.9	21.9	21.8	20.1	17.3	14.3	
Minimum daily temp. (°C)							
2003-04	10	12.8	11	8.9	5.7		
2004-05		11.5	12.7	10.5	6.1	5.1	N/A
Long term mean	10.2	11.4	9.7	6.9	4.1	1.8	

Figure 1. Nodulation pattern of field grown common bean (*Phaseolus vulgaris* L.) cvs Scylla (left) and T-49 (right) inoculated with *Rhizobium* isolate H 20.

Inoculation response

Inoculation response was extremely variable over the two growing seasons. In experiment 1 no nodulation was observed during sampling, while in experiment 2 nodulation was observed from 21 days after sowing (DAS) until final harvest at 112 DAS (Figure 1). Nodules observed were light pink to red in colour denoting the presence of leghaemoglobin and were concentrated at the junction of the crown and secondary roots. Some control plants of both cultivars (no isolate applied) exhibited nodules, but were not as well nodulated as the inoculated plants. When present on control plants the nodules were small, round and white to pale pink in colour. Generally, the number of nodules plant^{-1} increased as the plants developed (Table 3), and inoculation significantly ($p < 0.001$) increased the number of nodules plant^{-1} at all sampling dates (Table 3).

Total dry matter (green pod)

The non-inoculated crop produced twice as much TDM at green pod in 2003-04 as in 2004-05 (Table 4). There was no difference in TDM production between the two cultivars (mean 868 g m^{-2}). In 2004-05, inoculation produced TDM values in the range 482 to 723 g m^{-2}. However inoculation had no effect, mean TDM was 581 g m-2 across treatments (Table 4). There was no significant interaction for TDM at green pod harvest in both seasons.

Table 3. The number of nodules plant^{-1} at the different harvest times

	Number of nodules plant^{-1}			
Treatment	21 DAS	40 DAS	54 DAS	70 DAS
Control	0.1	1.0	0.7	1.1
RCR 3644	0.0	0.9	0.5	0.5
UK 2	3.1	9.2	7.9	8.9
H 20	7.3	54.1	64.4	75.0
PRF 81	4.9	23.9	14.0	13.6
PhP 17	3.3	6.0	12.3	9.0
SEM	0.7	4.6	2.8	3.0
Significance	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
CV (%)	17.2	25.3	10.4	13.0

At 112 DAS the nodules were in an advance stage of decomposition and no counts were taken.

Table 4. The effect of inoculation, cultivar, fertiliser and irrigation on total dry matter at green pod harvest (g m^{-2}) of common bean in Canterbury, 2003-04 and 2004-05.

2003-04		2004-05	
Inoculation (IN)		Inoculation (IN)	
Nil	893	Nil	514
CC 511	819	RCR 3644	482
RCR 3644	890	UK 2	486
SEM	28.6	H 20	619
Significance	ns	PRF 81	723
		PhP 17	662
Cultivar (CU)		SEM	82.8
Scylla	875	Significance	ns
T-49	863		
SEM	23.4	Cultivar (CU)	
Significance	ns	Scylla	567
		T-49	595
Irrigation (IR)		SEM	47.8
Nil	894	Significance	ns
Full	841		
SEM	38.5		
Significance	ns		
Fertiliser (FE)			
0 (kg N ha^{-1})	855		
150 (kg N ha^{-1})	880		
SEM	23.4		
Significance			
CV (%)	6.1		26.6
Significant Interactions	nil		nil

Table 5. The effect of inoculation, cultivar, fertiliser and irrigation on green pod yield (g.m^{-2}) of common beans in Canterbury, 2003-04 and 2004-05.

2003-04		2004-05	
Inoculation (IN)		Inoculation (IN)	
Nil	463	Nil	165
CC 511	414	RCR 3644	164
RCR 3644	446	UK 2	175
SEM	16.4	H 20	234
Significance	ns	PRF 81	214
		PhP 17	214
Cultivar (CU)		SEM	22.2
Scylla	448	Significance	$p < 0.05$
T-49	433		
SEM	13.4	Cultivar (CU)	
Significance	ns	Scylla	184
		T-49	214
Irrigation (IR)		SEM	12.8
Nil	411	Significance	
Full	471		ns
SEM	21		
Significance	ns		
Fertiliser (FE)			
0 (kg N ha^{-1})	431		
150 (kg N ha^{-1})	451		
SEM	13.4		
Significance	ns		
CV (%)	6.5		17.7
Significant Interactions	nil		nil

Green pod yield

In 2003-04 and 2004-05 rhizobia inoculation had no effect on green pod yield, the grand mean being 441 g m^{-2} and 195 g m^{-2} respectively. In 2003-04, cultivar, irrigation and nitrogen had no effect on green pod yield (Table 5).

Total dry matter (final harvest)

In 2003-04, TDM production at final

harvest was not affected by inoculation or irrigation (Table 6). However both cultivar and N did have an effect. Nitrogen at 150 kg N ha^{-1} gave 12 % more TDM ($p < 0.001$) than control plots. In 2004-05, TDM was not affected by either factor. Over both seasons, Scylla produced 16 % more TDM than T-49. In 2004-05, the crop produced 59 % more TDM than the 2003-04 crop.

Seed yield

In the first season, seed yield was not affected by inoculation or irrigation. However there were significant ($p < 0.001$) response to cultivar and fertiliser (Table 7). Scylla (467 g m^{-2}) produced 76 % more seed yield than T-49 (266 g m^{-2}), while 150 kg N ha^{-1} produced 11 % more seed yield than the control. There was a significant ($p < 0.05$) cultivar by fertiliser interaction (Table 8). Scylla responded more to applied N than T-49.

Table 6. The effect of inoculation, cultivar, fertiliser and irrigation on total dry matter at final harvest (g m^{-2}) of common beans in Canterbury, 2003-04 and 2004-05.

2003-04		2004-05	
Inoculation (IN)		Inoculation (IN)	
Nil	625	Nil	948
CC 511	640	RCR 3644	1227
RCR 3644	6245	UK 2	1000
SEM	12.6	H 20	900
Significance	ns	PRF 81	1117
		PhP 17	1072
Cultivar (CU)		SEM	116
Scylla	730	Significance	ns
T-49	530		
SEM	10.3	Cultivar (CU)	
Significance	$p < 0.001$		
		Scylla	1066
Irrigation (IR)		T-49	1022
Nil	656	SEM	67.0
Full	604	Significance	ns
SEM	25.9		
Significance	ns		
Fertiliser (FE)			
0 (kg N ha^{-1})	595.3		
150 (kg N ha^{-1})	664.8		
SEM	10.3		
Significance	$p < 0.001$		
CV (%)	5.7		6.5
Significant Interactions	nil		nil

Table 7. The effect of inoculation, cultivar, fertiliser and irrigation on seed yield at final harvest (g m^{-2}) of common beans in Canterbury, 2003-04 and 2004-05.

2003-04		2004-05	
Inoculation (IN)		Inoculation (IN)	
Nil	363	Nil	442
CC 511	375	RCR 3644	635
RCR 3644	363	UK 2	522
SEM	8.9	H 20	505
Significance	ns	PRF 81	557
		PhP 17	545
Cultivar (CU)		SEM	66.4
Scylla	467	Significance	ns
T-49	266		
SEM	7.2	Cultivar (CU)	
Significance	ns	Scylla	572
		T-49	497
Irrigation ((IR)		SEM	38.3
Nil	354	Significance	ns
Full	380		
SEM	14.9		
Significance	ns		
Fertiliser (FE)			
0 (kg N ha^{-1})	344		
150 (kg N ha^{-1})	381		
SEM	7.2		
Significance	$p < 0.001$		
CV (%)	6.9		6.1
Significant Interactions	CU x FE		nil
	$p < 0.05$		

Table 8. The cultivar by fertiliser interaction for seed yield at final harvest (g m^{-2}) of common beans in Canterbury, 2003-04.

	Fertiliser	
Cultivar	0 (kg N ha^{-1})	150 (kg N ha^{-1})
Scylla	433	501
T-49	254	278
SEM	10.2	
CV (%)	6.9	

Discussion

Justification for the use of inoculants is based on the premise that the proper rhizobia are not always present in the soil, or if present, they do not exist in sufficiently high numbers to bring about the best result (Graham, 1981; Catroux *et al.*, 2001). The maintenance of an effective population of rhizobia in the soil is dependent on the successive cropping of the appropriate leguminous plant (Hungria *et al.*, 2003); considering that the fields planted were coming out of apples (*Malus* spp.) would suggest that the population of rhizobia in the soil would be very low or non existent. This could have resulted in a lack of nodulation had inoculation with the commercially recommended and available strains not been applied. Inoculation with strains RCR 3644 and CC511 in a peat-based formulation did not produce nodules. Reasons for nodulation failure with the application of suitable inoculum are numerous and include acids soils and acidity factors, soil (root) temperatures, inoculant quality, calcium and phosphorus deficiencies and appropriate skills and technology as recently been reviewed (Date, 2000).

Subsequent inoculations in a follow-up field experiment, with the same strains, and some additional selections resulted in nodulation. However, the field was different and the inoculum was applied in a liquid form instead of as a peat-based formulation. Although peat is recognised as the most reliable carrier (Date, 2001), the liquid (broth) cultures produced the best nodulation results implying that some factor other than carrier affected the nodulation results. Adverse conditions such as sowing inoculated seeds in high temperatures and hot dry conditions (Hungria and Vargas, 2000; Date, 2001), as experienced in these experiments, are known to reduce the survival of *Rhizobium* on the seed and thus affect nodulation. When nodules were present they varied in colour from light pink to red. This nodule colour indicates the presence of leghaemoglobin, which forms at active sites for nitrogen fixation by the bacteria (Vance, 1983), suggesting that isolates used in the experiment formed effective nodules.

Experimental treatments employed had no effect on TDM (Table 3) or pod yield (Table 4) at green pod harvest. Although inoculation with *Rhizobium* generally improves the N nutrition of legumes and increases yield (Jahan and Talukdar, 2005), positive growth response is not universal. Jahan and Talukdar (2005) reported depressed growth in French beans with effective inoculation. Compared with TDM at green pod yield over the two seasons, successful inoculation with effective *Rhizobium* isolates (season 2) resulted in depressed growth, measured at TDM. Depressed growth, as reported here, could be a function of the inability of the symbiosis to fix adequate amounts of N to have an effect on growth, highlighting the unpredictability of the symbiosis, or the effect of environmental and management conditions.

Bean plants, especially the early maturing, bush (erect) types used in these experiments have become very efficient at using mineral N for growth (Redden and Herridge, 1999). This could account for the lack of response at green pod harvest for the measured parameters. In studies with *P. vulgaris* and soybean (*Glycine max*) it was proposed that the low capacity for N_2 fixation and growth response to inoculation was associated with inefficient nodule function rather than low nodule numbers (Piha and Munns, 1987). The efficiency in using mineral N in this crop was most likely developed simultaneously with a decline in the efficiency of the nodule function through the breeding and selection of elite lines in high N fertility soils (George and Singleton, 1992).

Total dry matter and seed yield at final harvest were influenced by cultivar and fertiliser application in 2003-04. The average TDM of around 650 g m^{-2} observed in the first season was similar to previously reported values (Dapaah *et al.*, 1995) with pinto beans in Canterbury. A cultivar effect in relation to

inoculation with some *Rhizobium leguminosarum* strains was also reported (Rennie and Kemp, 1983a, 1983b). Scylla is a more vigorous cultivar than T-49 as shown by its higher TDM production throughout the experiment.

There was also a highly significant correlation between seed yield and TDM (data not shown). High TDM is a known prerequisite for high seed yield in grain legumes, e.g. pinto beans (Dapaah *et al.*, 2000) and field peas, lentils, chickpea and lupin (Ayaz *et al.*, 2004). The significant cultivar by fertiliser interaction observed for seed yield (Table 8) indicated genotypic differences in cultivar responses to nitrogen. The cultivars used were Scylla a very early maturing fresh bush bean grown for green immature pods and T-49 an erect bush type grown for its small white seeds.

Most of the commercial bean cultivars are developed through breeding and selection in high fertility soils (Redden and Herridge, 1999), which possibly resulted in a decline in the efficiency of nodule function. Reduced nodule function could result in the plant becoming more reliant of fertiliser N, hence the N fertiliser recommendation of 75-150 kg N ha^{-1} for culinary and navy bean production (Redden and Herridge, 1999). These results support the suggestions *P. vulgaris* has been bred as a vegetable crop reliant on fertiliser N and the capacity for N_2 fixation may not be necessary (Redden and Herridge, 1999).

Legume inoculation is a simple and practical means of ensuring effective nitrogen fixation (Date, 2000), and the most visible response of symbiotic nitrogen fixation is the formation of nodules (Jahan and Talukdar, 2005). The presence of nodules does not necessarily mean that the final outcome will be increased nitrogen fixation, growth and yield. The literature is punctuated with numerous cases of lack of yield response to inoculation (Dialoff and Saint-Smith, 1981; Huntington *et al.*, 1986), although a few authors have reported increased seed yields (Taylor *et al.*, 1983; Bengtsson, 1991; Kyei-Boahen *et al.*, 2005). Before inoculation is considered as an option the farmer must consider a number of scenarios (Date, 2000). In spite of the progress that has been made in the selection of effective *Rhizobium* strain (Laguerre *et al.*, 1993; Hungria *et al.*, 2000; Hungria *et al.*, 2003) there is still conflicting reports with regards to the use of *Rhizobium*-legume symbiosis as a tool to improve plant growth and yield. It is unlikely that these arguments will subside in the near future, and until the scientific community of agronomist, microbiologists, plant physiologists and rhizobacteriologists come together to investigate the mechanism of symbiosis in grain legumes the question of "Is it worth nodulating bean" will continue to trouble growers.

Conclusion

Production of green beans in Canterbury is quite variable and this variability is a function of season, cultivar, environmental conditions and management.

Inoculation with increased nodulation does not guarantee increased production in beans; although seed yields ranging between 14 and 43 % more than the control plants was observed.

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