The importance of inoculant type or application rate for nitrogen fixation by grain legumes in cropping systems of south-eastern Australia

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Abstract

The increasing use of liquid and granular inoculants in Australia provides greater ease of use for inoculation of legumes and separates rhizobia from potentially toxic seed-applied fungicides. The aim of these studies was to test the efficacy of different inoculant types or different rates of inoculant application on nodulation, nitrogen fixation and grain yield. In multiple field experiments, faba bean and lupin were inoculated with peat slurry applied to seed, peat/attapulgite granular inoculants, and peat inoculant applied as a liquid to furrows or were sown un-inoculated. In a further study the rates of seed inoculant application varied from no inoculant to 100 times the typical rate of inoculation. Nodulation patterns differed according to inoculation method, with greater crown nodulation from seed-applied peat slurry inoculants and greater nodule mass on lateral roots in granular or liquid inoculant treatments. Nodule numbers increased in response to inoculant rate, although differences were not observed in the presence of a large population of soil rhizobia. Legumes inoculated with liquid inoculants fixed a similar proportion of nitrogen to those with standard peat slurry inoculants; faba bean inoculated with granules fixed less nitrogen. Nitrogen fixation increased with increasing seed inoculation rates. Grain yields increased due to application of some inoculant types and increased in response to inoculation rate. These studies highlight the importance of establishing effective symbioses to maximise the nitrogen benefits of using pulses in cropping systems.

Key Words

Rhizobia, nodulation, ^{15}N natural abundance, granules, lupin, faba bean

Introduction

In Australia, peat inoculant applied to the seed as a slurry (‘peat slurry’) is the most commonly used method to inoculate grain legumes with rhizobia. Australian peat slurry inoculants are generally of high rhizobial quality and quantity and, under suitable sowing conditions and when applied appropriately, provide excellent nodulation of legumes (Hartley et al. 2005).

In addition to peat inoculants, the types of carriers and formulations for delivery of rhizobia have recently increased. Liquid inoculants are routinely used for soybean and the winter pulses. The use of granular inoculants has also recently increased in Australia. Liquid and granular inoculants are soil-applied, usually in the seeding furrow. Soil placement confers a range of advantages, as the inoculant is physically separated from the seed, which means that inoculant can be supplied without the concerns for seed-applied chemicals that are toxic to rhizobia (e.g. fungicides and insecticides) (Denton et al. 2009). Granular inoculants typically increase nodulation on lateral roots and have been shown to enhance seed yield relative to seed-applied inoculants in Canada (Kyei-Boahen et al. 2002; Gan et al. 2005). The efficacy of granular inoculants is known to vary according to the presence of soil rhizobia (Denton et al. 2009), but the capacity of granular inoculants to improve N_2 fixation in legume crops in Australian conditions is not known.

Our aim was to compare N_2 fixation of legume crops inoculated with liquid and granular inoculants with conventional peat-based slurry inoculants. We hypothesised that grain legumes would fix N_2 equally effectively with liquid, granular and peat slurry inoculants, and that background populations of soil rhizobia would influence the response to inoculation. Experiments were established in soils known to contain a population of rhizobia and in soils that contained no rhizobia and the potential for a response to inoculation was investigated.

Methods

Site characteristics

The sites were established at Mininera, Vic, (37? 36'S, 142? 57'E) and Rutherglen, Vic, (36? 3'S, 146? 27'E). Mininera soil was a Vertic Stagnic Solonetz with a pH of 5.5 (H_20) and Apr-Oct rainfall was 294 mm in 2008 and 453 mm in 2009. The Rutherglen soil was a Cutanic Lixisol of pH 5.0 and the growing season rainfall was 205 mm in 2008 and 322 mm in 2009. There was 28 kg N/ha in the top 20 cm soil at Rutherglen and 43 kg N/ha at the Mininera site. The Rutherglen site had soil populations of rhizobia able to nodulate lupin and faba bean, but rhizobia able to nodulate lupin and faba bean were not detected at Mininera using the most probable number technique (Brockwell 1963).

Trial Design and Management

In the experiments testing inoculant type, peat-slurry inoculants were applied to seed as a standard slurry inoculant or injected into furrows as a liquid at the same inoculation rate, using 100 L water /ha. Commercially available peat/attapulgite clay-based granular inoculants were used after storage according to manufacturer's recommendations. Un-inoculated treatments were also used. Faba bean (*Vicia faba* L.) cv. Farah was sown at the site at 210 kg/ha and narrow-leafed lupin (*Lupinus angustifolius* L.) cv. Mandelup at 120 kg/ha. In the following year, wheat was sown over the plots. In the second set of experiments, faba bean seed was inoculated with different concentrations of commercially-available freeze dried inoculant, to establish treatments with a normal inoculation rate, and rates of inoculation that were ten-fold lower or higher: 0, 0.001, 0.01, 0.1, 1, 10 and 100 times normal inoculation rate. N_2 fixation was measured using the ^{15}N natural abundance method (Unkovich et al. 2008), with wheat (*Triticum aestivum* L. cv. EGA Gregory sown at 80 kg/ha) and canola (*Brassica napus* L. cv. Thunder sown at 7 kg/ha) as non-N_2-fixing reference species.

Plots (10–15 m in length) were sown into moist soil using an eight-row cone seeder with 180 mm row spacing. Six replicates were sown using a randomised complete block design. Care was taken to minimise contamination between treatment plots during sowing and sampling by decontaminating the cone seeder and sowing tubes with ethanol between treatments. Weeds, insects and fungal pathogens were controlled by chemical spray applications, as required, at rates according to manufacturers' recommendations.

Sampling

Ten plants were sampled from each plot 10 and 20 weeks after sowing by excavating the entire root system if possible. Roots were assessed for nodule number and nodule mass. Peak shoot biomass was determined prior to seed set by sampling five 1 m sections of rows per treatment. Grain yield was determined by mechanical harvesting of the entire plots with a plot harvester. Four replicates were used to measure nitrogen fixation.

Statistical Analysis

Analyses of variance (ANOVA) were conducted for individual experiments (site and crop), for nodule number, nitrogen content, N_2 fixed and grain yield using GenStat 11 software. Histograms of residuals and graphs of residuals verses fitted values were used to check distributional normality assumptions. Data were log-transformed where necessary.

Results

At Mininera, there was greater crown nodulation from seed-applied peat-slurry inoculants and greater nodule mass on lateral roots in granular- or liquid-inoculant treatments (data not shown). Here, peat-slurry inoculation provided the best nodulation of lupin and faba bean; legumes inoculated with peat injected into furrows or with granular inoculants had less nodule mass per plant (data not shown). At the Rutherglen site nodulation did not differ among treatments, as the soil rhizobial populations were sufficiently high that all treatments were well nodulated (data not shown).

At the Mininera site, faba bean inoculated with peat or with peat injected into furrows fixed more N_2 than faba bean inoculated with granules or uninoculated treatments ($P<0.001$; Fig. 1). Lupin was responsive to all inoculants. At the Rutherglen site a larger soil rhizobial population masked any response to inoculants. Wheat grown as the succeeding crop after wheat produced significantly less grain than wheat grown following either lupin (3.3 t/ha cf. 4.6-5.5 t/ha at Mininera) (3.4 t/ha cf. 4.6-4.9 t/ha at Rutherglen) or faba bean (3.0 t/ha cf. 3.7-4.3 t/ha at Rutherglen) (3.5 t/ha cf. 4.9-5.4 t/ha at Mininera), presumably due to lower soil N availability (data not shown) as cereal root diseases or other issues were not evident.

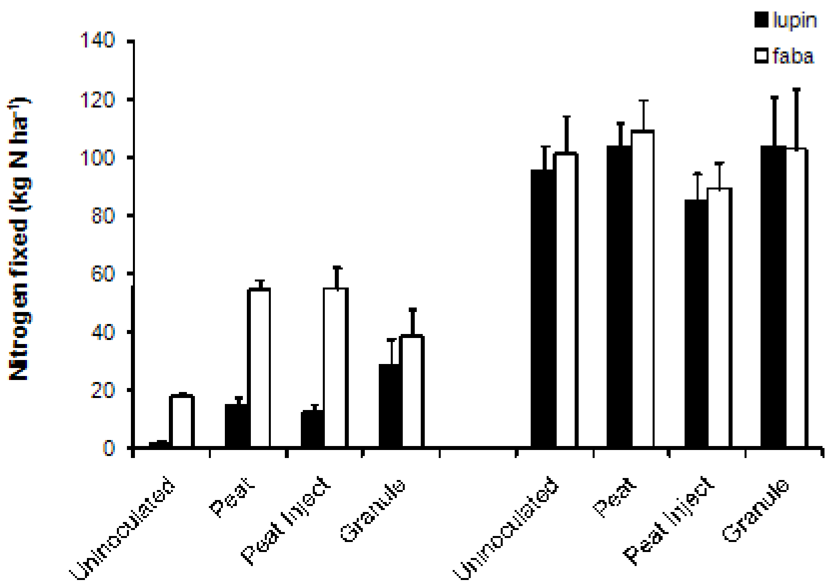

Figure 1. The influence of rhizobial inoculation using different inoculants on the amount of fixed N for faba bean and lupin grown at Mininera and Rutherglen, Victoria. Treatments on the left of the figure are from the Mininera site, those on the right are from Rutherglen. Standard errors are indicated for treatments (n=4).

Increasing the amount of inoculant on seed increased the total nodule number on faba bean at Mininera (Table 1). Here, better nodulation increased fixed nitrogen in faba bean shoots at peak biomass (Fig. 2). Although rhizobia were not detected by the most probable number estimate, some nodulation occurred in the uninoculated plots (Table 1) which appeared to contribute a small amount of fixed N_2 (Fig. 2). Increasing inoculation rates increased the proportion of N derived from fixation such that at 100 times inoculation rate there was >100 kg N_2 fixed in shoot material (Fig. 2).

Table 1. Nodule numbers, peak shoot biomass and grain yield of faba bean at Mininera following inoculation with zero to one hundred times the normal inoculation rate (7.9 log rhizobia/seed). Standard errors of the means are shown in brackets (n=6). Data within a column that do not share the same letter are significantly different.

Inoculation treatment	Nodule number (per plant)			Peak shoot biomass (t/ha)			Grain yield (t/ha)		
0 (uninoculated)	0.1	(0.1)	e	6.0	(0.4)	b	1.6	(0.1)	d
0.001 N	4	(1.6)	d	6.6	(0.3)	ab	2.0	(0.1)	cd
0.01 N	6	(2.3)	d	7.0	(0.4)	ab	2.2	(0.2)	bc
0.1 N	12	(1.6)	c	7.3	(0.7)	ab	2.5	(0.2)	ab
Normal	22	(2.2)	b	6.5	(0.3)	ab	2.4	(0.2)	abc
10N	23	(2.3)	b	7.7	(0.3)	a	2.6	(0.2)	a
100N	28	(2.6)	a	7.4	(0.6)	a	2.6	(0.1)	a

Peak shoot biomass generally increased with inoculation rate, and grain yields of these plots increased from 1.6 to 2.6 t/ha, indicating the value of inoculation for this system (Table 1). Grain N concentration increased in response to greater inoculation rate (data not shown). At the Rutherglen site, the large number of rhizobia resident in the soil reduced the value of inoculation and responses to inoculation were not observed in terms of nodulation nor grain yield. However, here, the highest rate of inoculum (100 times normal inoculation) produced significantly greater N_2 fixation compared with the uninoculated treatment (data not shown).

Discussion

Inoculation of faba bean with liquid inoculants provided similar N_2 fixation to that of plants inoculated with the industry standard peat slurry, however, nodulation of faba bean with granular inoculants did not increase N_2 fixation compared with uninoculated controls. Lupins were responsive to inoculation, but fixed less nitrogen than faba bean. In previous studies, soil-applied inoculant application produced greater seed yields than seed-applied inoculants, an advantage that was presumed to be from greater lateral root nodules (Kyei-Boahen et al. 2002; Gan et al. 2005); this advantage was not observed in our study. Granules do not appear to provide significant benefits compared with standard peat inoculation when legume crops are sown into moist soil. Although granules are predicted to enhance survival of rhizobia (Herridge 2008) it has not been demonstrated that granules promote the survival of rhizobia and improve the growth of legumes sown in dry soil. The influence of the size of the soil rhizobial population was previously shown to influence the effectiveness of soil-applied inoculants (Denton et al. 2009). In the present study, the value of inoculation was reduced at the site with a larger soil rhizobia population. At the site with a small population of soil rhizobia, increasing the rate of inoculation increased legume N_2 fixation and N accumulation. Increases in N_2 fixation corresponded with a one tonne greater grain production, indicating the importance of effective inoculation strategies in providing nitrogen for use in legume crop production and grain quality, and to increase soil N for succeeding crops.

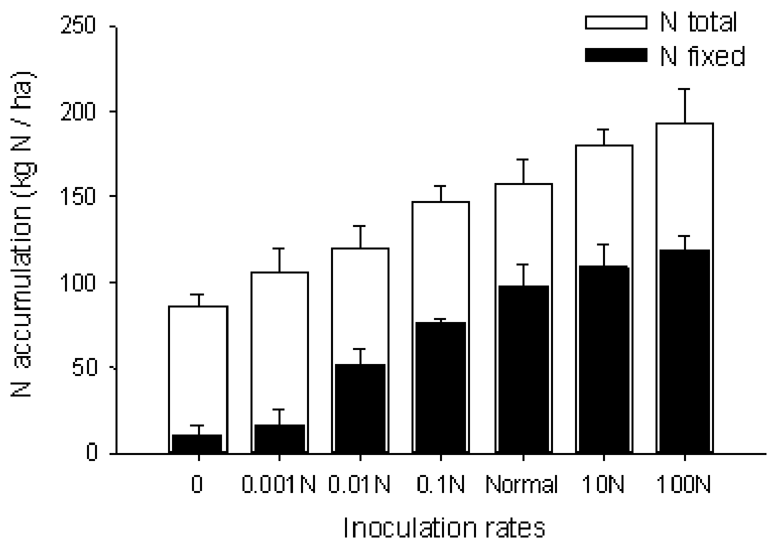

Figure 2. The influence of rhizobial inoculation rate on total shoot N and fixed shoot N for faba bean grown at Mininera, Victoria. Inoculation rates include no inoculation (0), to one hundred times normal rate of inoculation (7.9 log rhizobia / seed). Bars are treatment means with standard error (n=4).

Conclusion

Where soil rhizobia were low, legumes inoculated with liquid inoculants fixed a similar amount of N_2 to those with standard peat slurry inoculants; faba bean inoculated with granules fixed less N_2. Increasing the seed inoculation rates improved nitrogen fixation and grain yields but the increase was dependent on soil rhizobial population. These studies highlight the importance of establishing effective symbioses to maximise legume growth, N_2 fixation and the nitrogen benefits of using grain legumes in cropping systems.

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