Effect of rhizobia and other microbial inoculation treatments on field pea

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Key findings

- The Hart field site has a background of pea rhizobia that are numerous, but only moderately effective.
- Inoculation treatment did not affect measured root parameters.
- Some inoculation treatments increased shoot biomass and pod number, but not grain yield or grain N content.
- The N benefit from the extra biomass residues is estimated to be 51 kg N/ha.

Why do the trials?

This trial is part of a broader network of 15 trials sown across South Australia and Victoria, to investigate the potential of inoculation technologies to improve the nitrogen fixation and/or production of field pea. A treatment common to all trials has been a high rate of seed inoculation with rhizobia, to try and overcome any symbiotic constraints at the sites.

How was it done?

Plot size	1.75 m x 10 m	**Fertiliser**	80 kg/ha MAP (10:22) + 2% Zinc
Seeding date	28th May, 2014		Nil post sowing

The trial was arranged in a randomised complete block design with 3 replicates, each comprising an uninoculated control and 6 inoculation treatments. There were four rhizobia treatments (3 strains applied to seed and one applied as a slurry in furrow) and two other microbes suggested to promote plant growth. Rhizobia treatments were applied at approximately 100 fold the rate recommended commercially. Treatments were applied to Kaspa field pea which were sown to achieve a seedling density of 50 plants/m^2. The site was sampled at sowing to determine soil chemistry (analysed by CSBP) and the number and N_2-fixation capacity of the rhizobia present in the soil, using a greenhouse bio-assay.

Six plants were sampled from each plot on 14th August (7 weeks after sowing) and nodule number and nodule dry weight per plant determined. Root health (0 = no damage, 15 = severe damage) of each plant was assessed for symptoms caused by soil borne pathogens and a mean root damage score calculated. An additional ten plant shoots were sampled from each plot on 14th October (late pod fill) and used to estimate shoot biomass, pod number per plant and to estimate the % N derived from fixation (analysis pending). Plots were machine harvested to estimate grain yield and subsamples used for the determination of grain protein (Total N Leco, CSBP).

Results and discussion

Number of rhizobia in the soil, before the trial was sown, exceeded the threshold considered adequate (300 rhizobia/g soil) for prompt legume nodulation. This is often the case for soils with a history of pea cropping (last grown 2012) and neutral to alkaline pH (this site 7.6). The N_2-fixation capacity of the soil rhizobia was moderate (68%) when compared to the commercial inoculant strain SU303 (Table 1).

Table 1. Soil chemistry (0-10 cm), number and N_2-fixation capacity of pea rhizobia at sowing.

Soil pH (0.01M $CaCl_2$)	Soil N Nitrate (mg/kg soil)	Soil N Ammonium (mg/kg soil)	Number of pea rhizobia (per g soil)	Effectiveness of soil rhizobia (% SU303)
7.6	24	2	420	68

In the field trial, un-inoculated plants formed 45 nodules per plant (Table 2), which is about half the median number measured in similar trials across SA and Victoria, and well below the maximum of 151 per plant. Even so, inoculation treatment had no effect on number or mass of nodules (Table 2), or their distribution between the tap and lateral roots (data not shown). Root weight was not affected.

Root damage symptoms attributable to soil-borne disease were very low (root disease score<1.5) at the site and accordingly there was no effect of inoculation treatment on the level of root damage.

Table 2. Nodulation of Kaspa field pea plants at seven weeks after sowing.

Treatment	Nodule Number (number/plant)	Nodule Mass (mg DM/plant)
-Not inoculated	45	7.1
+Rhizobia (Group F WSM1455 on seed)	56	8.2
+Rhizobia (Group F WSM1455 in furrow)	62	8.6
+Rhizobia (Group E SU303 on seed)	54	8.6
+Rhizobia (SARDI strain on seed)	57	8.1
+Microbe B to control soil borne disease	51	7.6
+Microbe X to improve N_2-fixation	47	7.2
	NS	NS

Although there was no obvious effect of inoculation treatment on the roots, maximum shoot (& pod) biomass was significantly increased by two of the rhizobia treatments and microbes B and X (Table 3). Bio-control Microbe B resulted in the greatest increase (+54%) even though there was no indication that soil borne disease was an issue at the site. Rhizobia strain WSM1455 applied in furrow was the most effective of the rhizobia treatments, increasing shoot biomass by 42%.

Pod number per plant was increased by inoculation treatments, except rhizobia strain SU303. Microbe B was most effective, increasing pod number by 51%, compared to the un-inoculated treatment (Table 3).

Improvements in shoot biomass and pod number did not translate to increased grain yield or grain N content (Table 3). Accordingly, harvest index (HI) was lower in the inoculated treatments, except for treatment SU303.

Overall, responses to inoculation at this site were inconsistent; there were no measured effects on the roots, significant effects on biomass, pod number and harvest index, but no effect on grain yield or quality.

The lack of effect of rhizobial inoculation on nodule number and mass is consistent with the results at 13 other sites, where responses have usually been small or absent when rhizobia were already present at reasonable number in the soil. However, these measures do not indicate which strains of rhizobia occupy the nodules and in particular if displacement of the less effective naturalised soil rhizobia has occurred. It is plausible that shoot biomass and pod number responses were due to a shift in nodule occupancy, but this remains to be confirmed using nodules collected from the trial.

Table 3. Shoot & pod biomass, pod number, grain yield, harvest index and grain protein.

Treatment	Shoot & pod biomass (g/plant)	Pod number (#/plant)	Grain yield (kg/ha)	Harvest index (%)	Grain N (%)
-Not inoculated	15	8.0	1897	34	3.67
+Rhizobia (Group F WSM1455 on seed)	18	10.6*	1837	26*	3.63
+Rhizobia (Group F WSM1455 in furrow)	21*	10.3*	2017	26*	3.64
+Rhizobia (Group E SU303 on seed)	15	8.5	1883	33	3.61
+Rhizobia (SARDI strain on seed)	21*	10.9*	1777	23*	3.76
+Microbe B to control soil borne disease	23*	12.0*	1973	23*	3.73
+Microbe X to improve N_2-fixation	19*	10.4*	1953	27*	3.67
LSD	4	1.9	NS	6	NS

**Significantly different from un-inoculated control*

The poor correlation (*P* = 0.49, R^2 = 0.09) between biomass and grain yield may indicate water or some other limitation to grain production. Biomass estimates were high and HI lower than expected, indicating the main effect of inoculation was on plant growth rather than yield.

Nitrogen supply, from fixation by the naturalised soil rhizobia and soil N reserves, was adequate to meet grain yield requirements at Hart. Grain yields have similarly been unresponsive to inoculation with rhizobia (Group F WSM1455 applied to seed at 100 times recommended rate) across the broader data set (14 sites). However, across these sites highly significant (P<0.01) increases in grain N concentration and amount provide evidence of inoculation benefits, other than to grain yield.

At this site, we estimate (assuming 2.3% N in residual herbage) the N benefit from the extra biomass residues to be 51 kg/ha for the best rhizobia treatment, noting that very high rates of inoculation were used. The proportion of this N derived from fixation is still to be determined.

So far, microbes B and X have been tested at two other sites, also in 2014. They increased shoot biomass and pod number by approximately 30% overall (multi-site analysis) however, as was the case at Hart, had no effect on grain yield. Whilst the biomass responses are encouraging, further work is needed to validate and understand the basis of the responses.

Summary & implications

At sites where substantial populations of pea rhizobia reside in the soil, even very high rates of inoculation with rhizobia have failed to improve grain yield. However, other benefits to grain N content have been measured and show that potential for symbiotic improvement exists. Capturing this potential will probably be contingent on the provision of better inoculants that increase the number of rhizobia delivered.

Inoculation of field pea with rhizobia is still strongly recommended if there has been no previous history of a rhizobia host crop (pea, bean, lentil, vetch) or if soil pH is less than 6.0. In these situations, there is a strong likelihood of response to inoculation for nodulation, biomass production, N_2-fixation and grain yield.

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Photo: Field pea trial at Hart in 2014