Improving the nodulation of broad bean on Kangaroo Island's acidic soils

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2 Executive summary

There has been a history of problems with poor nodulation of broad beans on Kangaroo Island. It appears there are two main causes, one being acidic soils, i.e. pH ($CaCl_2$) less than 5 and the other waterlogging. New options are needed, to enable improvement in both the level and consistency of nodulation, which should in turn lead to greater nitrogen fixation inputs and better crop performance.

Three field trials were established on Kangaroo Island in 2015, on highly acidic soils (pH range, in $CaCl_2$, of 4.55 to 4.9). Treatments included two new strains of potentially acid-tolerant rhizobia from SARDI and several products that were applied in-furrow in an attempt to locally increase soil pH.

In each case the trials were managed by the host grower as part of a larger broad bean crop. Trials were sampled for assessment of root nodulation in early spring, for shoot biomass and nitrogen fixation (selected treatments only) in early summer, and for grain yield and quality.

The bean crops suffered significantly from a lack of rainfall after mid-September in the 2015 season, however we have obtained useful, positive results.

Rhizobial strain SRDI954 gave a significantly higher nodulation score than the current commercial strain WSM1455 in both field trials where it was tested. Rhizobial strain Vetch W181 also gave a significantly higher nodulation score in the one trial where it was tested.

Total shoot N was substantially increased by inoculation with acid-tolerant strains Vetch W181 and SRDI954, and the amount of fixed N was almost doubled, from 50 to 60 kg/ha to 95 - 110 kg/ha. Although this result was not statistically significant, it indicates the potential of these new strains of rhizobia to improve both nodulation and N inputs when inoculated onto broad bean in acid soils.

In-furrow treatments Calciprill, CalSap, Pulse Aider and DAP fertilizer (which is not as locally acidifying as MAP) did not improve nodulation.

Strain SRDI954 gave an average 8.5% yield increase over commercial strain WSM1455 in a strip trial, rising to a 14% increase in parts of the trial where there was poor nodulation with the commercial strain.

The results of the third trial showed that seed inoculation with the current commercial strain WSM1455 was considerably more effective than inoculation in furrow, in achieving higher and more uniform nodulation in a highly acidic soil.

Provided the results can be confirmed upon wider field testing, the new potential acid-tolerant strains of rhizobia can potentially be used to improve nodulation of grain legumes in a number of sub-regions in GRDC Southern and Western regions where there are acid soils. These include SW WA, Lower Eyre Peninsula, Southeast of SA, Southwest Victoria, Central Victoria, Gippsland and Tasmania.

Further replicated field trials are under way in 2016 by SARDI (3 sites in SA and Victoria) and University of Adelaide (farmer strip trials on Kangaroo Island).

3 BACKGROUND

There has been a history of poor nodulation of broad beans on Kangaroo Island. It appears there are two main causes, one being acidic soils (pH_{CaCl2} less than 5) and the other waterlogging (Hawthorne, 2012). A survey in 2014 by University of Adelaide (Maarten Ryder and Matt Denton) confirmed that nodulation of broad bean on Kangaroo Island was generally poor and very patchy. Solutions are required to improve both the level and consistency of crop nodulation. This should in turn improve nitrogen fixation and crop performance. In this project, we aimed to investigate options to improve nodulation of broad bean on low pH soils.

In recent years, researchers in SA (Ross Ballard and Liz Farquharson) and WA (Ron Yates), supported by GRDC funding (DAS000128, DAW00221) have been selecting and developing new acid-tolerant strains of rhizobia, but these strains have had limited field evaluation to date. This project aimed to test two new strains of rhizobia, isolated by SARDI (R. Ballard and E. Farquharson, unpublished) for their ability to nodulate broad bean in soils of pH ($CaCl_2$) less than 5 on Kangaroo Island.

Aside from the two rhizobial treatments, we tested agronomic treatments that might increase soil pH locally in the furrow at sowing. Liming soil is a standard practice in many agricultural areas with low soil pH, and Kangaroo Island is no exception. However, lime application can take considerable time (years) to increase the soil pH in the root zone, and suggestions have been made that pH could be increased more quickly and locally for good nodulation, by applying soluble lime products in furrow at sowing. To do this, we used commercial products in furrow including CalSap (soluble Ca) and Calciprill ($CaCO_3$ formulated as a prill) and Pulse Aider which contains CalSap, as well as a garden lime coating on inoculated seed.

A replicated small plot trial (Trial 1) was established, to test two new strains of rhizobia and in-furrow treatments. In addition, a "grower strip trial" (Trial 2) was set up using the grower's equipment, to compare the performance of one of the new rhizobial strains with the current commercial Group F rhizobia which nodulate broad bean. In Trial 1 we collected data on crop emergence, nodulation score and grain yield, and for selected treatments we also determined peak biomass and nitrogen fixation. Similar data were collected for Trial 2.

A third trial (Trial 3) was set up by grower Ben Pontifex to test combinations of freeze-dried rhizobia applied in furrow or on seed with various amendments such as Pulse Aider and CalSap. Recent unpublished work (M. Ryder and J. Rathjen, Adelaide University) had suggested that mixing rhizobia with Pulse Aider (liquid) prior to application in furrow was likely to kill the rhizobia rapidly. For this reason, Trial 3 contained two treatments with different lengths of mixing time for the combination of Pulse Aider and rhizobia and also treatments where rhizobia were applied through a separate liquid line. For Trial 3 we assessed nodulation and the grower generated a yield map of the paddock containing the site.

It should be noted that all crops suffered to some extent from a lack of moisture from mid spring onwards (2015 season), with the plants in Trial 2 being worst affected. We have nevertheless been able to draw some useful conclusions from the trials.

4 METHODS

4.1 TRIAL SITES AND TREATMENTS

Three field trials were established on 8th, 11th & 12th May 2015. All three trials were managed as an integral part of a larger crop, and were subjected to the growers' herbicide and pesticide spray regimes (on all trials) and fertiliser regimes (for Trials 2 and 3, Berry and Pontifex trials).

4.1.1 Trial 1: replicated small plot trial at Michael Mills' property

This trial was established at Michael Mills' property along Timber Creek Road [35°49'3.64"S, 137°21'28.78"E]. Soil pH ($CaCl_2$) was 4.90. The aim of the trial was to investigate the effect of various lime and calcium treatments to increase soil pH locally in the furrow and also to evaluate two new strains of rhizobia from SARDI that may be better suited to acidic soils.

There were 12 treatments and each treatment was replicated four times. The experiment was laid out as a randomized complete block, designed by Paul Eckermann, University of Adelaide Biometry, with dimensions approximately 24 metres by 44 metres. The layout is shown in Figure 1.

The trial consisted of 48 small plots, each ca 1.2 m x 8.5 m, with 3 rows (25.5 metres of row per plot), row spacing 40 cm. The trial was sown using a cone seeder, sowing depth approx 25 – 30 mm (shallow, but not adjustable for deeper sowing).

Treatments are summarized in Table 1. All treatments received DAP (80 kg/ha) at sowing, except for the MAP treatment, which was also added at 80 kg/ha. Cultivar 'Kareema' was sown at 150 kg/ha (approximately 150 seed per plot in 25.5 metres of row, average seed spacing 17 cm). All rhizobia except the granular 'Tag Team' formulation were applied to the seed in peat.

The four in-furrow treatments that were aimed at locally modifying soil pH, or creating more favourable conditions in furrow at sowing were: CalSap (liquid available calcium, from Optima, WA), Pulse Aider (from Injekta Systems, SA), Calciprill (finely ground $CaCO_3$, formulated into a prill with molasses, Omya P/L) and Lime coating on seed, which was done using garden lime.

P fertilizer: MAP (which is locally more acidifying) was used as a comparison with DAP, which was applied to all other treatments.

Molybdenum, which is normally recommended for inoculated legumes on acid soils, was applied to all but one of the treatments.

← **North** **Timber Creek Road**

FENCE LINE

	Row	1	2	3	4
	1	1=SRDI954	2=Pulse Aider	3=CalSap	4=Granular Tag Team
Rep 1	2	5= Nil no rhizobia	6=Rhizobia DAP	7=Urea no rhizobia	8=MAP
	3	9=Rhizobia W181	10=No Mo	11=Calciprill	12=Lime coat
	4	13 =Urea no rhizobia	14=Rhizobia W181	15=Lime coat	16=Nil no rhizobia
Rep 2	5	17=Granular Tag Team	18=Calciprill	19=SRDI954	20=No Mo
	6	21=Pulse Aider	22=MAP	23=Rhizobia DAP	24=CalSap
	7	25=Calciprill	26=Nil no rhizobia	27=Granular Tag Team	28=Rhizobia DAP
Rep 3	8	29=CalSap	30=SRDI954	31=MAP	32=Rhizobia W181
	9	33=Lime coat	34=Urea no rhizobia	35=No Mo	36=Pulse Aider
	10	37=Rhizobia DAP	38=Lime coat	39=Pulse Aider	40=Urea no rhizobia
Rep 4	11	41=MAP	42=CalSap	43=Nil no rhizobia	44=Calciprill
	12	45=No Mo	46=Granular Tag Team	47=Rhizobia W181	48=SRDI954

ca 44 metres N – S, ca 24 metres E - W

Figure 1. **Layout of 48-plot randomized complete block trial (Trial 1, Mills property)**

Treatment details:

1. Nil rhizobia and nil urea + DAP fertilizer 80 kg/ha.
2. Urea 200 kg/ha plus 100 kg/ha top dressing in September (no rhizobia) + DAP
3. Standard commercial rhizobial strain WSM1455 applied in peat (double rate) + MAP fertilizer 80 kg/ha (compared to DAP 80 kg/ha used for all other treatments)
4. Standard commercial rhizobial strain WSM1455 applied in peat (double rate) + DAP
5. CalSap 10L/ha, diluted 1:10 for use (from Michael Eyres, Injekta) + DAP
6. Calciprill 500 kg/ha (Omya P/L) + DAP
7. Pulse Aider 80 L/ha of 1:1 diluted product (Michael Eyres, Injekta) + DAP
8. Lime pelleting peat preparation (treatment 1) on to seed, using garden lime + DAP
9. Tag Team containing rhizobia WSM1455 plus *Pencillium* (2.5 kg/ha, Novozymes)
10. Acid-tolerant rhizobia Vetch W181 in peat (double rate) + DAP
11. Acid-tolerant rhizobia SRDI954 in peat (double rate) + DAP
12. No molybdenum (Mo was added as liquid in furrow at 125 g/ha for all other treatments) + DAP

Table 1. **Summary of treatments, Trial 1**

	TREATMENT	P fertilizer	Rhizobia	Molybdenum
1	Nil	DAP	Nil	Yes
2	High N (urea)	DAP	Nil	Yes
3	Rhizobia	MAP	WSM1455	Yes
4	Rhizobia	DAP	WSM1455	Yes
5	Rhizobia + CalSap	DAP	WSM1455	Yes
6	Rhizobia + Calciprill	DAP	WSM1455	Yes
7	Rhizobia + Pulse Aider	DAP	WSM1455	No
8	Rhizobia + Lime coated seed	DAP	WSM1455	Yes
9	Granular rhizobia (Tag Team)	DAP	WSM1455	Yes
10	Vetch W181	DAP	Vetch W181	Yes
11	SRDI954	DAP	SRDI954	Yes
12	Rhizobia No Molybdenum	DAP	WSM1455	No

Rhizobia were applied by mixing peat formulation with clean rainwater at double the recommended rate (double rate is often used in more challenging situations, such as very acidic soils) and then coating the seed by mixing thoroughly in a 25 litre container with lid. The coated seed was air-dried for 30 minutes and sown within 3 h of treatment (Figure 2).

Figure 2. **Inoculation of bean seed, small plot trial.**

Liquid treatments (CalSap, Pulse Aider, Molybdenum) were applied by hand "in furrow" after sowing, using a 50 ml syringe, applying a measured volume to a single plot row. This method proved to be reasonably reliable and uniform. Molybdenum was applied as 50 ml of 1g/L ammonium molybdate to a 9 metre-long row. Pulse aider was diluted as directed and applied at 30 ml per 9 metre row (equal to 80L per ha of a 1:1 dilution of product). CalSap was diluted as directed and applied at 40 ml per 9 metre row (equal to 100L per ha of a 1:10 dilution of product).

Solid formulations (MAP, DAP and urea fertilizers, Calciprill and Granular "Tag Team") were combined with seed in a ziplock bag, mixed and sown together using a cone seeder.

Trial management: this trial was managed by Michael Mills as part of a regular commercial broad bean crop. Apart from fertilizer, all other treatments (herbicides and pesticides) were applied by the grower.

Late in the season, a number of plots in the centre of the trial, and also an area in the grower's crop to the south of the trial were affected by a foliar disease (symptoms: leaves blackened and plants quite stunted). The pathogen was identified by SARDI scientists Jenny Davidson and Rohan Kimber as probably being a species of *Stemphyllium*. This pathogen is not generally considered to be a major threat, though in this particular case the affected plants may have had a decreased yield due to severe stunting and loss of leaf area.

4.1.2 Trial 2: replicated strip trial at Lloyd and Christine Berry's property

This trial was established on Lloyd and Christine Berry's property, along Wests Road [35°48'54.61"S, 37°28'51.19"E]. Average soil pH ($CaCl_2$) across the site was 4.70 and the two treatments were a new strain of acid-tolerant *Rhizobium* from SARDI (SRDI954) compared to the standard commercial Group F rhizobia (Nodulaid = strain WSM1455, in peat, from BASF). The trial was sown and harvested with the farmer's equipment.

Inoculation: strain SRDI954 in peat (300g) was mixed with 0.5% methyl cellulose (800ml) and 2.4 g of "nodulating trigger" (N/T, *Bacillus subtilis*) was added, because the commercial Nodulaid (WSM1455) was also used together with N/T preparation. Treatments were applied to 'Aquadulce' broad bean seed as the seed moved up through the auger to the bin (a) by hand from a jug for SRDI954 and (b) with a bilge pump for WSM1455. Seed was sown immediately, at 150 kg / ha, with DAP fertilizer at 70 kg / ha.

The trial was sown using Berry's inoculation and seeding equipment, and the same rate of inoculation was used for both of the rhizobia treatments (approx. 2/3 of the recommended rate). Each treatment was replicated four times (Figure 3,4). Strips were 12 metres wide, with average row spacing 35 cm.

Seed of triazine-tolerant canola (10 – 20 seed, cv Thumper) was sown in 1 metre-wide strips in each of the SRDI954 rows (Figure 3), as reference (non-nitrogen-fixing) plant for the measurement of nitrogen fixation by the legume.

Trial management: herbicide and pesticide treatments were applied by the grower.

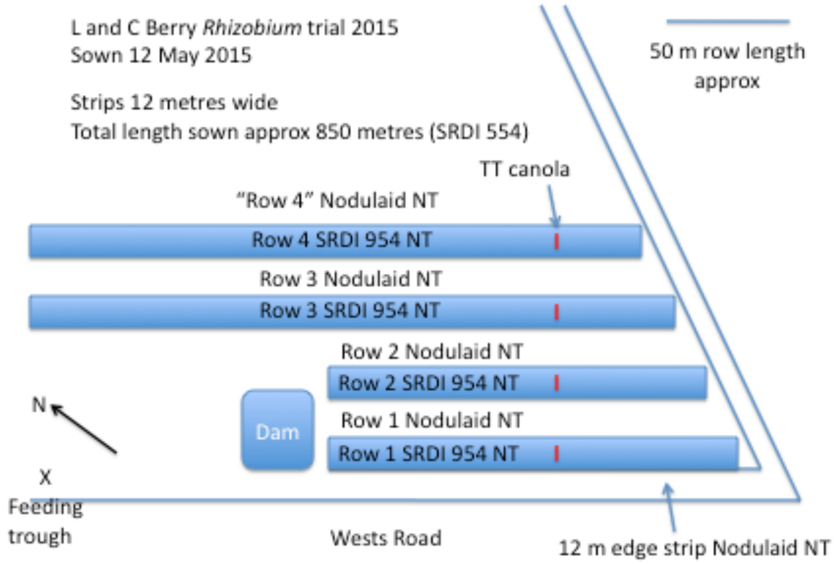

Figure 3. **Trial 2 Layout (L and C Berry property)**
(Nodulaid = commercial strain WSM1455)

Figure 4. **Sowing strip trial, L and C Berry**

4.1.3 Trial 3: strip trial at Ben Pontifex's property

This trial was established by Ben Pontifex at his property on East West Highway 2 [35°53'3.72"S, 137°13'54.36"E]. The soil pH ($CaCl_2$) across the paddock ranged from 4.60 to 5.01). Different combinations of Group F rhizobia (freeze-dried product ("FD") from New Edge Microbials), Alosca granules and FD rhizobia suspended as a liquid preparation were applied in-furrow in 6 treatments, with and without other products (Pulse Aider from Injekta and CalSap from Optima Agriculture) and were compared to a seed coating of FD rhizobia on 'Aquadulce' broad bean.

Treatments for the 9 rows were:
1 Pulse Aider with freeze-dried (FD) rhizobia liquid + Alosca granules
2 Pulse Aider with FD rhizobia applied as a seed coat + Alosca granules
3 Pulse Aider with FD rhizobia applied as a seed coat
4 Pulse Aider liquid / CalSap with FD rhizobia liquid, in furrow through 2 separate lines
5 Pulse Aider liquid / FD rhizobia liquid, in furrow through 2 separate lines
6 Pulse Aider + FD rhizobia liquid, sown 2h after mixing
_________________TRACK across field _________________
7 Pulse Aider + FD rhizobia liquid, sown freshly mixed
8 Pulse Aider + Alosca granules
9 Pulse Aider with FD rhizobia liquid + Alosca granules (as for Row 1)

NOTE:
Rows 1 to 6: history of raised beds
Rows 7 to 9: no raised beds, soil generally wetter in winter.
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Sowing, sampling and harvest dates for the three trials are given in Table 2.

Table 2. **Field trial calendar of events, 2015**

SITE **ACTIVITY**	**Mills** **small plot trial**	**Berry replicated strip trial**	**Pontifex** **strip trial**
Sowing	11 May	12 May	8 May
Sampling for nodulation assessment	3 September (16 weeks)	3 September	4 September
Sampling for biomass and N fixation	12 November (27 weeks)	12 November	n/a
Harvest	1 December	December	December

n/a = not applicable

4.2 Assessing the nodulation of broad bean root systems

In early September 2015, root samples were taken from all three trials and scored for nodulation success. The scoring system, shown in Table 3, was based on that of Corbin et al. (1977). A score of 2.5 or greater is considered "adequate".

4.2.1 Sampling for nodulation assessment

Mills small plot trial: (3 September 2015). Five plants including root systems to about 20 cm deep were sampled from each row of each plot, towards the northern end of the plot (reserving the southern end for yield sampling; Figure 5). Where there were too few plants (e.g. urea treatment) half the plants in the northern half of the plot were taken. Total plants sample: 48 plots x approximately 15 plants = 720.

Samples were washed to remove the bulk of soil and bagged for transport.

Berry strip trial: (3 and 4 September 2015). Plant samples, including root systems to about 20 cm deep, were taken along three transects running perpendicular to the sown strips (Figure 2). One transect was made where triazine-tolerant canola had been sown. A second transect was made 50 m to the north and another 50 m further north again. Transects 1 and 2: at the middle of each 12-metre wide strip, approx 15 plants were taken, 5 at each of three separate places, 5 metres apart. Transect 3: at the middle of each 12-metre strip, 10 plants were taken in two sets of 5 plants, approx three metres apart. The number of plants sampled was at least 160 for each treatment, total at least 320.

Samples were washed to remove the bulk of soil and bagged for transport.

Pontifex strip trial: (4 September 2105). Plant samples, including root systems to about 20 cm deep, were taken in four East – West transects. There were 9 treatment strips (rows). Six rows were to the east of the track, an area which had previously been formed into raised beds. Three rows were to the west of the track, not on raised beds and the soil was noticeably wetter in this area at sampling time.

For each transect and row, 10 plants (2 x 5 plants, taken 3 metres apart). Total plants sampled: 4 transects x 9 rows x 10 plants = at least 360 plants.

Samples were washed to remove the bulk of soil and bagged for transport.

4.2.2 Scoring root nodulation

Root samples were washed several times to remove adhering soil, rinsed clean and scored according to number and position of nodules on crown/tap root and on lateral roots, on a 0 to 5 scale based on that of Corbin et al 1977 (originally developed for chickpea, Table 3).

Figure 5. **Sampling the small plot trial for root nodulation assessment, Sept 2015.**

4.3 Collection of samples for nitrogen fixation and crop biomass determination

On 12th November 2015, plant samples were taken from the Mills small plot trial and Berry strip trial for total biomass determination and for measurement of nitrogen fixation.

Mills small plot trial: Owing to financial limitations on the project, only the 5 treatments of greatest interest were sampled at the small plot trial. Plots sampled were: 3 treatments which had the highest nodulation = SRDI954, Vetch W181 and Granular "Tag Team", and 2 controls = nil rhizobia and WSM1455 (standard commercial strain). Above-ground parts of three plants were sampled from each row of the selected plots (total 9 plants per plot). Shoots (stem + leaf + pod) were oven-dried for up to 1 week at 60C. Non-nitrogen-fixing plants were also sampled from the trial area, including one sample of grasses and another of broad-leafed weeds. These samples were also oven-dried at 60C prior to processing.

Berry strip trial: Above-ground parts of plants were taken along a single transect, in line with the sown triazine-tolerant canola_(Figure 2). Ten plants were taken from each of the 8 rows, with single plants being sampled at 3-metre intervals along the centre of the 12-metre wide rows. The shoots of sown triazine-tolerant canola plants (non-N-fixing) were taken from Rows 1, 3, 5 and 7 and combined. Soil samples were taken from each row for soil pH measurement. Plant shoot samples were dried at 60C for up to one week and then weighed.

Table 3. **Scoring the nodulation of broad bean roots on a 0-5 scale based on the system of Corbin et al. (1977).**

Nodulation score	Nodules on crown and tap root	Nodules on lateral roots
0	0	0
0.5	0	0 - 5
1	0	5 - 10
1.5	0	More than 10
2	Few (10 to 20)	0
2.5	Few	Few
3	Few	Many (more than 30)
4	Many (more than 20)	Few
5	Many	Many

Sample processing

As a first step in processing for nitrogen fixation measurement, samples were macerated very effectively using an electric mulcher, which was thoroughly cleaned in between samples using a compressed air line. As a second step, subsamples were taken and ground in a Wylie Mill, then further subsamples were ground to a fine powder using a Retch Mill. Samples were prepared for analysis and sent to the UC Davis Stable Isotope Facility, Davis, California for measurement of ^{15}N, ^{14}N and total nitrogen. Calculation of fixed nitrogen was based on the method of Unkovich et al (1994).

4.4 HARVEST AND ASSESSMENT OF SEED QUALITY

Trials were harvested in late November/early December; the Mills trial by hand and the others by commercial harvesters.

The Mills small plot trial was hand-harvested (2 metre length of plot, equals 6 metres of row). The harvested pods were dried at 60C for at least 4 days and then hand threshed and winnowed before obtaining grain weights.

The Berry and Pontifex trials were harvested using the grower's own harvester.

Seed was graded and quality (size distribution and protein was assessed by Dennis Jamieson, KI Pure Grain site manager, Kangaroo Island).

4.5 STATISTICAL ANALYSIS OF DATA

Data collected from Trial 1 (Randomized complete block design, 12 treatments, 4 replicates) was analysed using Genstat software (VSN International Ltd, UK).

Data from Trial 2 (two treatments, 4 replicates) was analysed using a t-Test with the Microsoft Excel Data Analysis package, after checking for equality of variance.

5 RESULTS

5.1 TRIAL 1. TESTING ACID-TOLERANT RHIZOBIA AND IN-FURROW TREATMENTS INCLUDING LIME

Replicated small plot trial (Michael Mills property)

Crop emergence in this trial was patchy, due to the limited capabilities of the cone-seeder, in terms of sowing depth (shallow, at 25 to 30 mm, instead of the desired 50 mm or greater). This patchiness was not expected to affect the results for nodulation. However, patchy emergence was expected to possibly influence biomass and yield data later in the season, owing to variation in plant competition effects between rows and plots that were unrelated to experimental treatment. This point is raised again later in the report, in relation to the biomass, yield and nitrogen fixation results.

The limitations of the cone seeder also meant that we applied urea together in close proximity to the seed and DAP. This placement of urea caused an inhibitory effect and a very low emergence for this treatment, which was originally designed to give the yield for a bean crop that was not limited by N.

5.1.1 Nodulation

The nodulation scores for the 12 treatments plus the surrounding bean crop are shown in Table 4. It was clear that nodulation with both of the potential acid-tolerant strains of rhizobia SRDI954 (mean score 3.3) and Vetch W181 (mean score 3.2), was superior to that achieved with the commercial strain (WSM1455, mean score 1.5) and being greater than 2.5 would be considered good. The result for improved nodulation with both SRDI954 and Vetch W181 was statistically significant, compared to "Rhizobia + DAP", the standard treatment. In addition to the higher average nodulation scores, inoculation with SRDI954 and Vetch W181 gave much more consistent nodulation than for the commercial strain (data not shown).

The granular formulation (Tag Team, Novozymes) also gave a good level of nodulation (mean score 2.7) and the lime pelleting treatment, gave a slightly higher mean nodulation score than the remaining treatments, though neither of these results were statistically better than the current commercial strain. Plants sampled from all other treatments had mean nodulation scores less than 2, which is not considered adequate, and nodulation in these treatments was extremely variable, with many plants having few nodules. Plants sampled from the bean crop surrounding the trial scored almost 2, which was better than for many of the in-furrow treatments in the trial. The surrounding crop received strain WSM1455 both as a seed inoculant and as granular in furrow inoculant.

There was a background of rhizobia in the soil, as indicated by the nodulation observed in the two "nil added rhizobia" treatments (nil and urea); however, the nodulation scores were very poor in these two treatments. Preliminary greenhouse tests in summer, early 2015, had indicated that bean rhizobia were below the limit of detection in this soil. Nevertheless, some rhizobia were clearly present, perhaps surviving on organic matter or on the decaying roots of vetch, which can occur in these fields (and vetch rhizobia can also nodulate bean).

Nodulation of broad bean grown in acidic soils on Kangaroo Island with the current commercial strain of rhizobia has, in the past and in this field trial, been characterized by the formation of few nodules, often located on the lateral roots (Figure 6A). While the few nodules that form on a root system can grow very large (more than 1 cm in diameter) and may therefore be able to fix a substantial amount of nitrogen, the fact that there are so few nodules means that there is little margin for error. If seasonal conditions are somewhat adverse, with waterlogged soil, or if there is weevil attack, plants with few nodules could easily be reduced to having no nodules. In addition, early nodulation around the crown of the plant is considered advantageous, enabling early nitrogen fixation.

Inoculation with the potential acid-tolerant strains SARDI954 and Vetch W181 led to much greater nodulation around the crown of the plant, and larger nodule numbers (Figure 6B): the pattern of nodulation was similar to that seen with adequate nodulation on higher pH soils.

Table 4. **Nodulation of 'Kareema' broad bean in response to different rhizobial strains and in-furrow treatments (0 to 5 scale).**

Treatment	**Mean nodulation score**	**Statistical significance**	**% of plants with score 2.5 or more**	**Statistical significance**
SRDI954 strain + DAP	3.28	a	84.5	ab
Vetch W181 strain + DAP	3.17	ab	87.4	a
Granular Tag Team + DAP	2.72	abc	66.0	abc
Rhizobia* + DAP + Lime pellet	2.30	abcd	61.2	abcd
Rhizobia + DAP + Calciprill	1.86	bcde	41.3	abcd
Rhizobia + DAP + Pulse Aider	1.81	cde	34.4	abcd
Rhizobia + DAP, no molybdenum	1.72	cde	37.1	abcd
Rhizobia + DAP + CalSap	1.66	cde	36.3	abcd
Rhizobia + MAP	1.57	cde	28.3	bcd
Rhizobia + DAP	**1.46**	**cde**	**30.1**	**abcd**
Nil rhizobia + DAP	1.22	de	17.0	c
Nil rhizobia + DAP + urea	0.87	e	3.6	d
Mills bean crop surrounding trial	*1.96*	*(n/a)*		

* "Rhizobia" = current commercial strain WSM1455 applied to seed in peat at double rate (standard treatment **in bold**); all treatments received DAP except one which received MAP; all treatments except one received molybdenum in furrow; treatments followed by the same letter are not significantly different at P = 0.05. n/a = not analysed.

Figure 6A, B. **Nodulation Score 1 (Left) Score 4 (Right)**

5.1.2 Peak biomass and nitrogen fixation

The peak biomass of plants sampled from selected treatments in November 2015 in shown in Figure 7. There are no significant differences among these treatments, and there was considerable variation between replicates for all treatments. Mean values for plants from the granular Tag Team treatment were 50% higher than for WSM1455 peat (standard commercial rhizobia); mean biomass for SRDI954 treatment was 22% higher and for Vetch W181 was 14% higher. These data are to some extent confounded by the problem of poor and patchy emergence, leading to uneven plant competition effects that appeared to be unrelated to inoculation treatment. The plant density for the granular Tag Team treatment was lower, hence plants were larger through decreased competition.

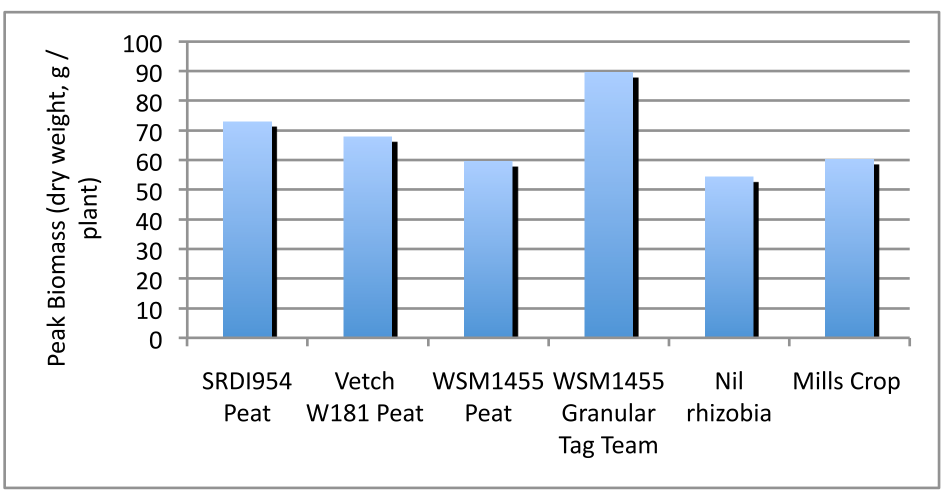

Figure 7: **Peak plant biomass of 'Kareema' broad bean in response to selected treatments compared to commercial strain WSM1455 in peat. (Differences not significant at P = 0.05).**

Shoot nitrogen and amount of fixed nitrogen in shoots, for selected treatments, is shown in Table 5. Data is presented in kg/ha based on dry weight data and plant emergence counts. Despite lack of statistical significance, the trends suggest that inoculation with the new strains of rhizobia increased the amount of fixed nitrogen (almost double) compared to the standard strain WSM1455.

Table 5. **Nitrogen fixed by plants sampled from selected treatments**

Treatment	**Shoot Nitrogen (kg/ha)***	**Fixed Nitrogen (kg/ha)****
No rhizobia	78.8	58.5
WSM1455	61.0	52.2
TagTeam	92.9	70.7
Vetch W181	124.7	110.5
SRDI954	117.0	95.4

4 replicates, * Treatment effect P = 0.23, ** Treatment effect P = 0.22

5.1.3 Grain yield and quality

Yield data for this trial are unfortunately confounded by a poor and rather patchy emergence (discussed above), which led to variation in plant competition between plots that was unrelated to treatment. Prior to the hand harvest, the most uniform 2-metre section of the plots was chosen, to be harvested for yield. These were plants growing at the southern end of each plot.

Yield data are presented but are highly variable and difficult to interpret in relation to inoculation and in-furrow treatments. There were no significant differences between grain yields for different treatments (P = 0.31, Table 6). Yields ranged from 0.44 t/ha (Urea) to 1.86 t/ha (lime coating of seed plus rhizobia). Many treatments yielded in the range 1.2 to 1.4 t/ha: these were Calciprill, Pulse Aider, Vetch W181, Granular Tag Team, CalSap, No Molybednum and SRDI954.

Seed quality data are presented in Figures 8A (size distribution) and 8B (grain protein). Data were collected on samples consisting of all four replicates bulked together, and no statistical analysis has therefore been possible. There does not appear to be a clear relationship between nodulation score and seed size.

Grain protein ranged from 24.5% to 28%. There was no clear relationship between grain protein and shoot N or fixed N for the 5 treatments that were assessed for N fixation (Table 5).

Table 6. **Grain yield (t/ha), ranked in descending order.**
(Mean nodulation scores from Table 4 are also presented).

Treatment	Grain Yield (t/ha)	Mean nodulation score
Rhizobia + DAP + Lime pellet	1.86	2.30
Rhizobia + DAP + Calciprill	1.44	1.86
Rhizobia + DAP + Pulse Aider	1.38	1.81
Vetch W181 strain + DAP	1.37	3.17
Granular Tag Team + DAP	1.33	2.72
Rhizobia + DAP + CalSap	1.32	1.66
Rhizobia + DAP, no molybdenum	1.28	1.72
SRDI954 strain + DAP	1.17	3.28
Rhizobia + MAP	1.01	1.57
Rhizobia + DAP	**0.79**	**1.46**
Nil rhizobia + DAP	0.78	1.22
Nil rhizobia + DAP + urea	0.44	0.87
Mills bean crop surrounding trial for comparison	*1.68*	*1.96*

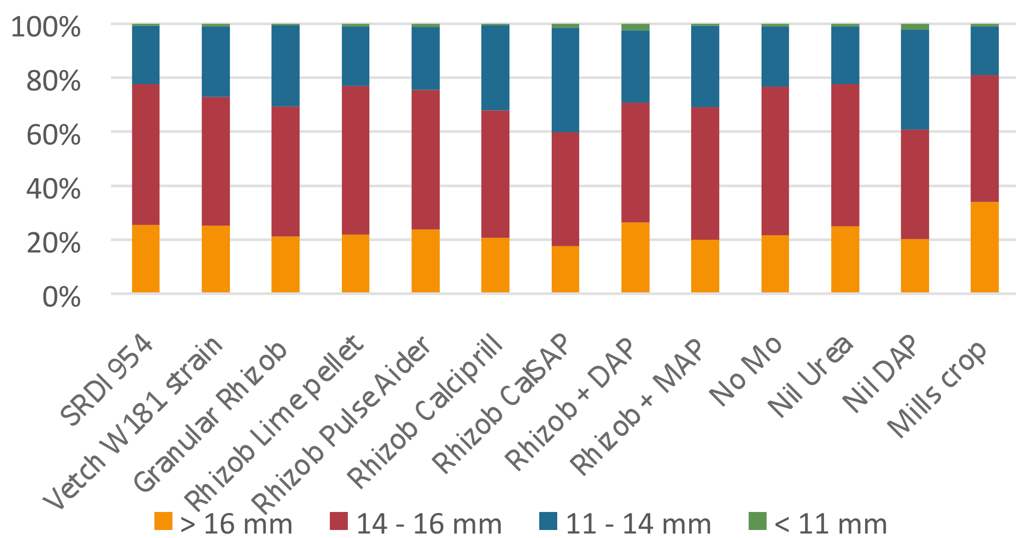

Figure 8A**. Seed size distribution; treatments in decreasing order of nodulation score (L to R); Crop surrounding trial included as comparison. Data are single values from bulked samples.**

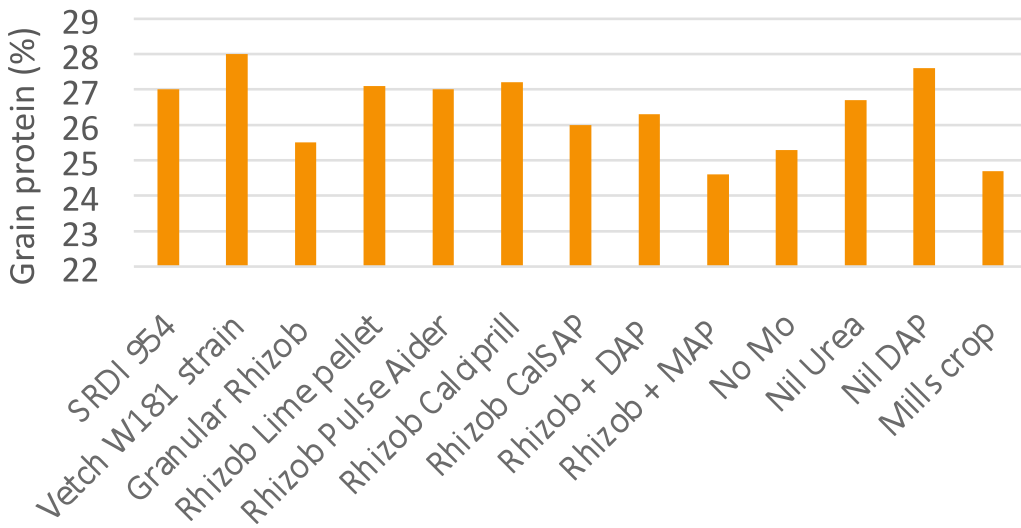

Figure 8B. **Grain protein; treatments in decreasing order of nodulation score (L to R); Crop surrounding trial included for comparison. (Data are single values from bulked samples).**

5.2 TRIAL 2. TESTING ACID-TOLERANT RHIZOBIA

Replicated strip trial, Lloyd and Christine Berry's property

5.2.1 Nodulation

The mean nodulation scores across the entire trial were 3.58 for SRDI954 N/T (considered very good), which was significantly better than the 2.50 score achieved with commercial strain WSM1455 N/T (P = 0.042, one-tailed T-test). The mean 2.5 nodulation score with WSM1455 is considered adequate. A more detailed examination of the results (Figure 9) shows a gradient in nodulation score across the field with the commercial strain, from poor nodulation in Replicates 1 and 2 to good nodulation in Replicates 3 and 4, whereas the acid-tolerant strain SRDI954 performed well across the whole trial, Replicates 1 - 4.

This result is possibly due to the small but definite increase in soil pH ($CaCl_2$) across the trial site from 4.45 – 4.65 at the edge (Replicate 1; see Figure 2) to 4.75 – 4.85 in the middle of the field (Replicate 4). The pH gradient and the corresponding change in nodulation score with WSM1455 (Figure 5) suggests that the commercial rhizobial strain does not nodulate broad bean well below pH 4.6 or 4.7 but can perform adequately when pH is 4.8 or higher in this soil type.

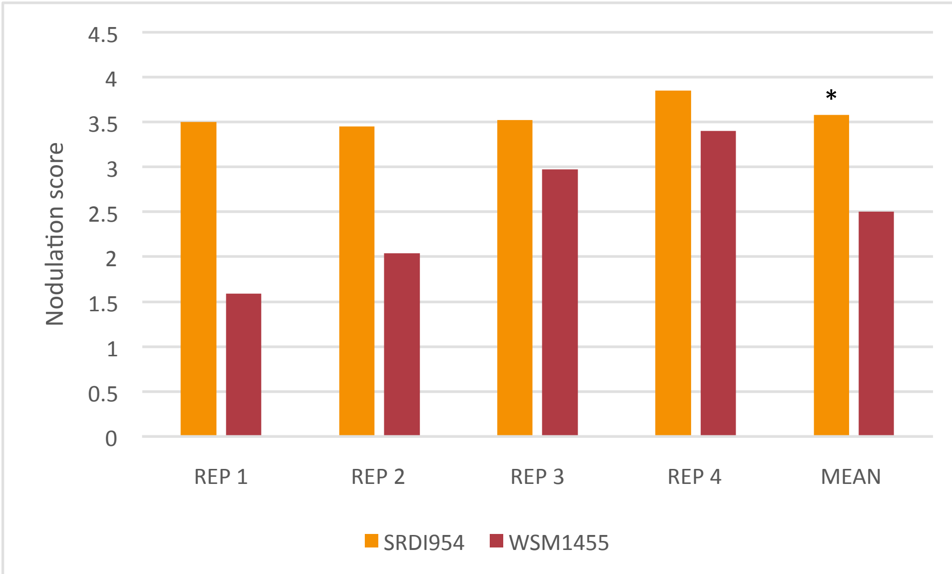

Figure 9. **Nodulation of 'Aquadulce' broad bean inoculated with *Rhizobium* strain SRDI954 or WSM1455 in a strip trial at L and C Berry's property (0 to 5 scale)**
* SRDI954 significantly greater than WSM1455 (t Test)

5.2.2 Peak biomass and nitrogen fixation

Table 7 shows that, despite lack of statistical significance, plants inoculated with SRDI954 had 37% higher shoot N content and fixed 28% more nitrogen per ha than plants inoculated with standard commercial strain WSM1455.

Table 7. **Nitrogen fixed by plants inoculated with rhizobial strain WSM1455 or SRDI954.**

Treatment	**Shoot Nitrogen (kg/ha)***	**Fixed Nitrogen (kg/ha)****
WSM1455 N/T	44.7	32.6
SRDI954 N/T	56.4	44.2

4 replicates, * Treatment effect P = 0.34, ** Treatment effect P = 0.28

5.2.3 Grain yield

The average bean yield was 8.45% higher in the SRDI954 inoculated rows than for the commercial strain WSM1455. This difference was not statistically significant (P = 0.29). Figure 10 show the trends across the trial, with SRDI954 having a higher yield than WSM1455 except in Replicate 4, where both strains were also equally good at nodulating the plants (Figure 4) and gave a similar yield.

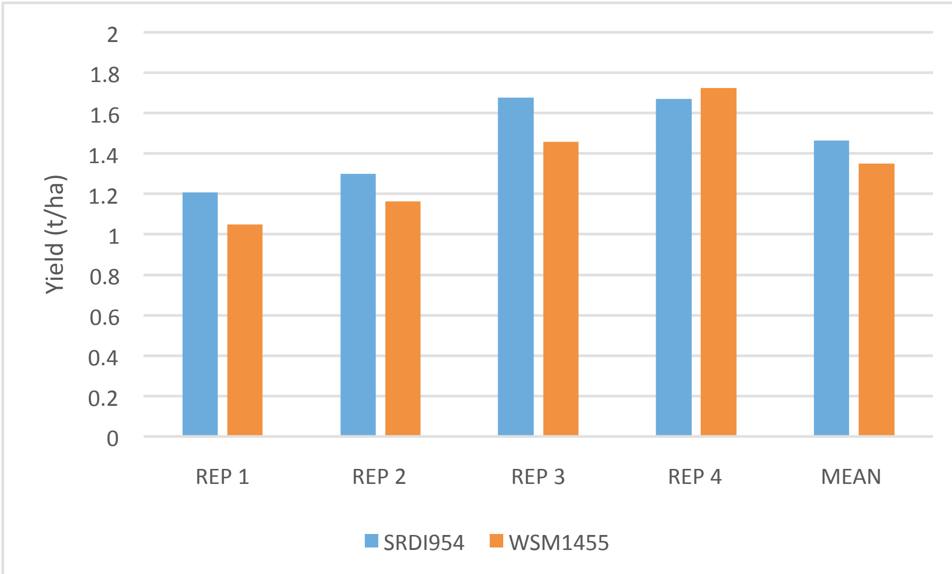

Figure 10. **Yield of 'Aquadulce' broad bean across the trial at L and C Berry**

The seed size distribution is shown in Figure 11. Results for SRDI954 and WSM1455 treatment were very similar, with a small increase in both the 14-16 mm and 11-14 mm seed size categories (1.5% each) for SRDI954.

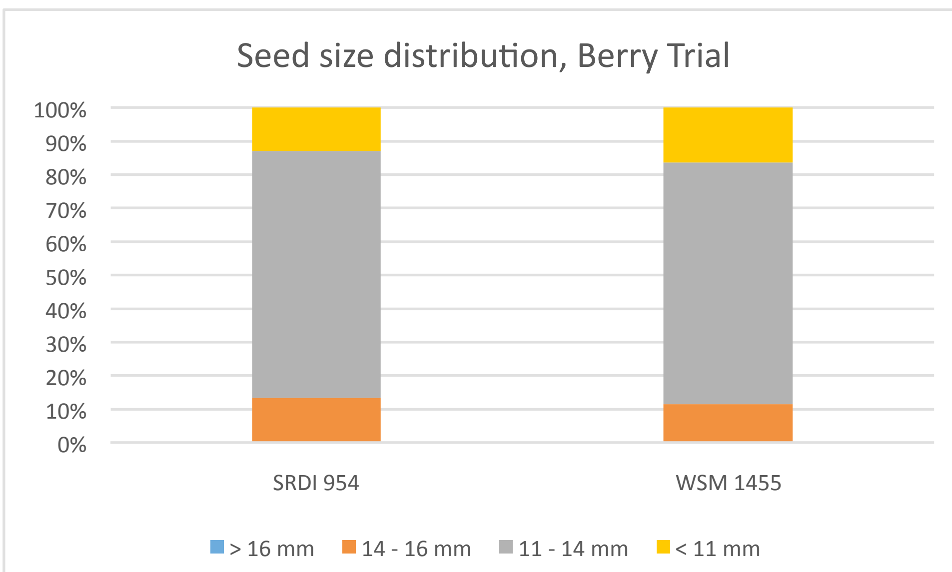

Figure 11**. Seed size distribution of bulked samples.**

5.3 Trial 3. Testing rhizobia with additives and liquids applied in furrow and on seed.

Unreplicated strip trial, Ben Pontifex's property.

Nodulation assessment (Table 8) showed that the highest average scores were obtained for the two treatments where rhizobia were applied directly to the seed (shown in **bold)**, which had mean scores of 2.2 and 1.9. However these average nodulation scores are not considered adequate. The same two treatments also gave the highest proportion of plants with nodulation scores of 2.5 or greater ("adequate nodulation"), being more than 30%, and the lowest proportion of plants with zero nodules (0%). All other treatments led to poor nodulation.

Alosca granules, mixing rhizobia with Pulse Aider for shorter or longer times, and inoculating liquid rhizobia through a separate line did not lead to adequate nodulation in this trial.

Yield data from this trial were difficult to interpret from a yield map owing to the very large "natural" variation in yield across the site, the lack of replication of treatments and differences in site history across the trial (e.g. raised beds in rows 1 to 6 versus no raised beds in rows 7 to 9). It was not possible to discern any effects of treatments on yield from the yield map.

The main message from this trial is that for the best chance of nodulation success, rhizobia should be applied to seed, if using the current commercial strain of rhizobia.

Table 8. **Nodulation scores for inoculation trial at the Pontifex property**

Row	Treatment	Nodulation score (0 - 5 scale)	% plants with zero nodules	% plants rating 2.5 or better ("adequate")
1	Pulse Aider with FD rhizobia liquid + Alosca granules	1.54	9.6	25.6
2	**Pulse Aider with FD rhizobia seed coat + Alosca granules**	**2.17**	**0.0**	**37.1**
3	**Pulse Aider with FD rhizobia seed coat**	**1.89**	**0.0**	**33.3**
4	Pulse Aider liquid / CalSap FD rhizobia liquid, 2 separate lines	1.35	10.0	17.4
5	Pulse Aider liquid / FD rhizobia liquid, 2 separate lines	0.99	21.6	9.6
6	Pulse Aider + FD rhizobia liquid, 2 h after mixing	1.00	23.5	8.3
7	Pulse Aider + FD rhizobia liquid, freshly mixed	0.85	48.8	13.6
8	Pulse Aider + Alosca granules	0.96	13.7	2.3
9	Pulse Aider with FD rhizobia liquid + Alosca granules	1.11	8.9	13.6

6 DISCUSSION

The results of Trials 1 and 2, taken together, show that inoculation with new strains of rhizobia that are more acid-tolerant offers the best opportunity to improve the nodulation of broad bean on Kangaroo Island. Strain SRDI954 significantly improved nodulation score in both trials where it was tested, and strain Vetch W181 significantly improved nodulation in the one trial where it was tested.

Inoculation with the current commercial strain WSM1455 resulted in poor nodulation (mean nodulation score less than 2.5) in Trial 1 (small plot trial). Treatments that were designed to increase soil pH locally in furrow were not effective in improving nodulation by WSM1455. Granular “Tag Team” and lime coating of seed slightly increased nodulation but this was not significant. In Trial 2, the commercial strain WSM1455 performed well in sections of the trial where soil pH was 4.8 or higher. On the other hand, where soil pH was less than 4.7 WSM1455 performed poorly, whereas strain SRDI954 nodulated well across the entire trial, regardless of soil pH.

Did the improved nodulation with new strains SRDI954 and Vetch W181 lead to an increase in nitrogen fixation?

In Trial 1, inoculation with acid-tolerant strains Vetch W181 and SRDI954 led to substantially greater amounts of N fixation: almost doubled from around 50 – 60 kg /ha to 95 – 110 kg /ha. Although these increases were not statistically significant, the result indicates that there is potential to obtain both better nodulation and greater amounts of N fixed by inoculating with new strains of rhizobia. This will be of benefit both to the current legume crop and also future crops, through release of fixed N from stubble breakdown.

New rhizobia strains SRDI954 and Vetch W181 were selected for improved nodulation of pea in acid conditions in the laboratory and greenhouse (R. Ballard and E. Farquharson, unpublished). The improved nodulation results obtained in 2015 on Kangaroo Island show that this can be extended to broad bean grown in the field. The results of these trials are potentially of benefit to growers of broad bean, faba bean or pea in acid soils in other regions including WA, lower Eyre Peninsula, the Southeast of SA, Southwestern Victoria, central Victoria and Gippsland.

Yield data for Trial 1 were to some extent confounded by poor and patchy emergence and dry spring, even though the most uniform subsections of the trial were harvested for yield. Most yields were between 1.2 – 1.4 t/ha and are likely to have been water-limited (there was very low rainfall from mid-September onwards) rather than N-limited.

In Trial 2, inoculation with SRDI954 led to an 8.5% yield increase over treatment with WSM1455 (not statistically significant). As with the nodulation scores, SRDI954-treated rows out-yielded WSM1455 treatments at the edge of the field, but not towards the middle of the field where nodulation scores were very similar. Overall yields were again low, due largely to lack of mid to late season rainfall.

This work is being followed up in 2016 by SARDI (GRDC project DAS000128). SARDI are conducting replicated field trials on Kangaroo Island, Eyre Peninsula and at Hamilton in Victoria to test a larger cohort of potential acid-tolerant rhizobia on faba bean and field pea. In addition M Ryder has co-ordinated three growers on Kangaroo Island who are comparing SRDI954 and the commercial inoculant strain WSM1455 on broad bean in strip trials (GRDC project UA000138).

The main conclusion from Trial 3 was that seed inoculation with the current commercial strain WSM1455 was considerably more effective than inoculation in furrow, in achieving higher and more uniform nodulation in a highly acidic soil.

7 CONCLUSIONS

- Rhizobial strain SRDI954 consistently gave a significantly higher nodulation score than the current commercial strain WSM1455 in two field trials. Rhizobial strain Vetch W181 also gave a significantly higher nodulation score in the one trial where it was tested (Mills small plot trial).

- Granular Tag Team formulation (Rhizobium plus a phosphorus-solubilising *Penicillium*) performed adequately in the small plot trial.

- Calciprill, CalSap, Pulse Aider and DAP fertilizer (which is not as locally acidifying as MAP) did not improve nodulation.

- Lime pelleting slightly increased the nodulation score. The pelleting method could be improved, however given the success of new strains of rhizobia, it may not be worthwhile pursuing the lime pelleting option.

- Total shoot N was substantially increased by inoculation with acid-tolerant rhizobia strains (Vetch W181 and SRDI954) and fixed N was almost doubled, to around 100 kg/ha. Although this result was not statistically significant, it indicates the potential of these new strains to improve both nodulation and N inputs in acid soils and supports the need for further replicated field trials.

- Strain SRDI954 gave an average 8.5% yield increase in Trial 2 at Berry's property (rising to 14% where there was poor nodulation with the commercial strain).

- The results of Trial 3 showed that seed inoculation with the current commercial strain WSM1455 was considerably more effective than inoculation in furrow, in achieving higher and more uniform nodulation in a highly acidic soil.

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