Assessing the interaction between acid-tolerant strains of rhizobia and faba bean – Tamworth 2022.

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Key points

- Several high-performing (acid-tolerant) rhizobia strains have been recently tested for their ability to nodulate pulse crops. Most tests have been conducted in acidic soils, with a small number in alkaline soils.
- The nodulation ability of 2 high-performing strains (SRDI969 and WSM4643) on faba bean was tested at Tamworth in 2022. These strains were compared with the current Australian commercial inoculant group E/F strain, WSM1455
- Where rhizobia were applied to seed not treated with fungicide (common practice for faba bean), roots inoculated with SRDI969 and WSM4643 had nodule numbers equal to or higher than seed inoculated with the commercial strain. Where seed was inoculated with SRDI969, root nodule numbers were significantly higher than the commercial strain.
- No yield increases were recorded for seed inoculated with SRDI969 and WSM4643, compared with WSM1455 inoculation.
- The National Rhizobium Steering Committee (NRSC) has endorsed a strain change from WSM1455 to SRDI969 for faba bean inoculation. The new strain is available to growers for the 2024 season.

Keywords: faba bean, rhizobia, inoculation

Introduction

Pulses play an important role in Australian farming systems due to their ability to fix their own nitrogen (N), increase soil N levels and act as disease breaks for cereal crops. This ability of pulses to fix N and subsequently contribute to soil N depends on the association that pulses form with symbiotic, N-fixing bacteria or rhizobia. There are several soil physiochemical conditions and management practices that affect rhizobia survival. Rhizobia survival and effectiveness in the soil can be limited by soil texture, soil moisture, genetic stability and soil acidity (Rigg et al. 2020). Most of the currently available commercial rhizobia strains are sensitive to acidic soil and are generally more persistent and effective in neutral to alkaline soils, similar to where they were originally isolated from. For example, the current Australian commercial Group E/F strain (WSM1455), recommended for faba bean and lentil inoculation, was isolated from soil in Greece with a pH_{Ca} of 8.0. This strain shows a significant decline in its ability to nodulate plants where soil pH drops below 6.0, with plants generally inadequately nodulated in soils with a pH <5.0 (Yates et al. 2016; Ballard et al. 2019). Such limitations often constrain the use of pulses in cropping rotations to regions with alkaline soil.

Experiments have been conducted in recent years Australia wide to evaluate the performance of high-performing, acid-tolerant rhizobia strains short-listed by the South Australian Research and Development Institute (SARDI) and the Centre for

Rhizobium Studies (CRS), Murdoch University, Western Australia (WA). These strains (SRDI969 and WSM4643, respectively) were isolated from acidic soil (pH_{Ca} 4.5–5.5) and have been assessed in experiments conducted in WA, South Australia (SA) and New South Wales (NSW). The aim of this research was to deliver strains to industry that increase grain legume adaptation and production, especially in acidic soil. However, experiments were also conducted in non-acidic soil in northern NSW to assess these strains' performance in different environments. Experiments have also been conducted at Tamworth to assess the associations that selected high-performing rhizobia strains formed with lentil and field pea in 2020 (Mukasa Mugerwa and Formann 2020) and 2021. At Tamworth in 2022, an experiment was conducted where faba bean was inoculated with the high-performing rhizobia strains and the rhizobium-legume association was assessed. This was then compared with inoculation with the current commercial group E/F strain. The study also looked at different inoculation methods and the potential effects of fungicide seed treatments on rhizobia.

Site details

Location	Tamworth Agricultural Institute, Tamworth, NSW, 2340. 31° 15'24. 1" S; 150° 98' 37.5" E.
Paddock history	• 2020 – fallow. • 2021 – barley.
Soil type	Brown-red vertosol.
Soil characteristics	Presented in Table 1.
Fertiliser	120 kg/ha single superphosphate (8.8% P, 11% S, 19% Ca) banded below the seed at sowing.
Soil moisture	117.5 mm, plant available water (PAWC) (0–120 cm) at sowing.
Rainfall	Tamworth 2022 rainfall: 980 mm (long-term average 630 mm). In crop rainfall: 565 mm.
Experiment design	• Randomised complete block design. • Three replicates of each treatment, 12 m plots. • Five plant rows at 33 cm spacings.
Plant population	Target 25 plants/m^2.
Rhizobia levels	Soil at the site had low levels of group E/F rhizobia (Table 1).
Variety	PBA Warda⍉, a large-seeded, early flowering variety whose uniform seed size and colour make it acceptable for the human consumption market. It is moderately resistant to rust and moderately tolerant to *Bean leafroll virus* (BLRV). It is well-adapted to the northern NSW and southern QLD growing conditions.
Sowing date	29 April 2022.
Crop management	• Fungicide: chlorothalonil 720 (720 g/L chlorothalonil) at 2 L/ha, applied 6 October 2022. Veritas®Optis (370 g/L tebuconazole + 222 were made following chocolate spot detection.

	• Herbicide: Roundup Ultra®MAX (glyphosate 570 g/L) at 2 L/ha + Sharpen® (700 g/kg saflufenacil) at 34 g/ha applied 4 November 2022.
Harvest date	24 November 2022.

Table 1 Soil characteristics (0–30 cm depth) at Tamworth in 2022.

Characteristic	Depth	
	0–10 cm	10–30 cm
Arbuscular mycorrhizal fungi (AMF) Test A (kDNA/g soil) *	65	–
AMF Test B (kDNA/g soil) *	33	–
Rhizobia group E/F#	0	–
Ammonium N (mg/kg)	4	2
Nitrate N (mg/kg)	6	2
Phosphorus Colwell (mg/kg)	31	7
Potassium Colwell (mg/kg)	634	333
pH ($CaCl_2$)	6.8	6.8
Organic carbon (%)	1.10	0.52

* Predicta B

\# Predicta rNod

Treatments

- PBA Warda⦶ was grown with 16 treatments (Table 2).

Table 2 Treatments established at Tamworth, 2022.

Rhizobia strain	Rhizobia inoculation method	Fungicide treatment	Treatment reference* (in figures)
Nil	In-furrow	No	In-furrow −pickel
		Yes	In-furrow +pickel
	Seed-coat	No	Seed −pickel
		Yes	Seed +pickel
WSM1455 (current commercial Group F strain)	In-furrow	No	In-furrow −pickel
		Yes	In-furrow +pickel
	Seed-coat	No	Seed −pickel
		Yes	Seed +pickel
SRDI969	In-furrow	No	In-furrow −pickel
		Yes	In-furrow +pickel
	Seed-coat	No	Seed −pickel
		Yes	Seed +pickel
WSM4643	In-furrow	No	In-furrow −pickel
		Yes	In-furrow +pickel
	Seed-coat	No	Seed −pickel
		Yes	Seed +pickel

* Pickel refers to P-Pickel T® fungicide seed dressing;
−pickel = absence on seed; +pickel = presence on seed

- Fungicide treatments: seed was treated with P-Pickel T® (360 g/L thiram, 200 g/L thiabenazole) 24 hours before sowing.

- **Rhizobia strains**: WSM1455 (current commercial Group F strain), SRDI969 and WSM4643 were supplied by the Australian Inoculants Research Group (AIRG) as peat-based products.
- **Seed inoculation**: a peat-based slurry was prepared early on the day of sowing. The seed was allowed to dry at 4 °C before being sown on the same day.
- **In-furrow inoculation**: a liquid formulation was prepared from the peat-based inoculum early on the day of sowing and stored at 4 °C before being applied in-furrow (alongside the seed) at sowing.
- **Liquid formulations**: applied at rates so plots receiving rhizobia in-furrow received the same rhizobia number as plots sown with rhizobia-treated seed.

Measurements

Roots from each treatment were assessed for nodulation 14 weeks post-sowing. Intact plant roots were carefully extracted from the soil, washed and the number of nodules on each plant counted. Ten plants/plot were assessed.

Above-ground plant biomass was measured at flowering, 16 weeks post-sowing. Individual plants were cut at the crown, and the material dried at 50 °C for 48 hours before being weighed.

Grain yield from each plot was measured with a Kingaroy Engineering Works plot harvester on 24 November 2022.

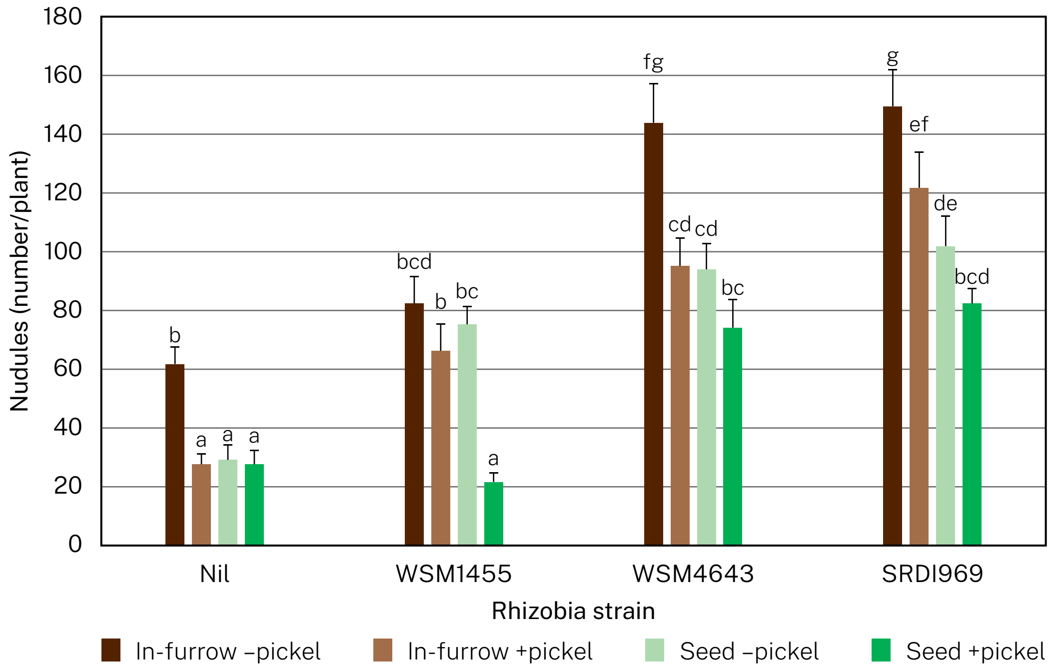
Data were analysed using analysis of variance (ANOVA) with Genstat for Windows, 19th edition. Treatment means were compared using the Fisher's Least Significant Difference (l.s.d.) method, with significant differences accepted at $P<0.05$.

Figure 1 Faba bean growing on vertosol soil with a series of rhizobial and fungicide treatments at Tamworth in 2022.

Results

Root nodulation

- Nodule numbers/plant increased where faba bean was inoculated with rhizobia (Figure 2). Where faba bean was uninoculated (nil rhizobia), the average number of nodules/plant across treatments was 37. This increased to 62 nodules/plant where faba bean was inoculated with current commercial group F strain WSM1455, and then to 102 and 114 nodules/plant where faba bean was inoculated with the high-performing strains WSM4643 and SRDI969, respectively (averaged across treatments).



Letters indicate significant differences between treatments.
l.s.d. (*P*<0.05) = ???). Vertical bars represent standard error.

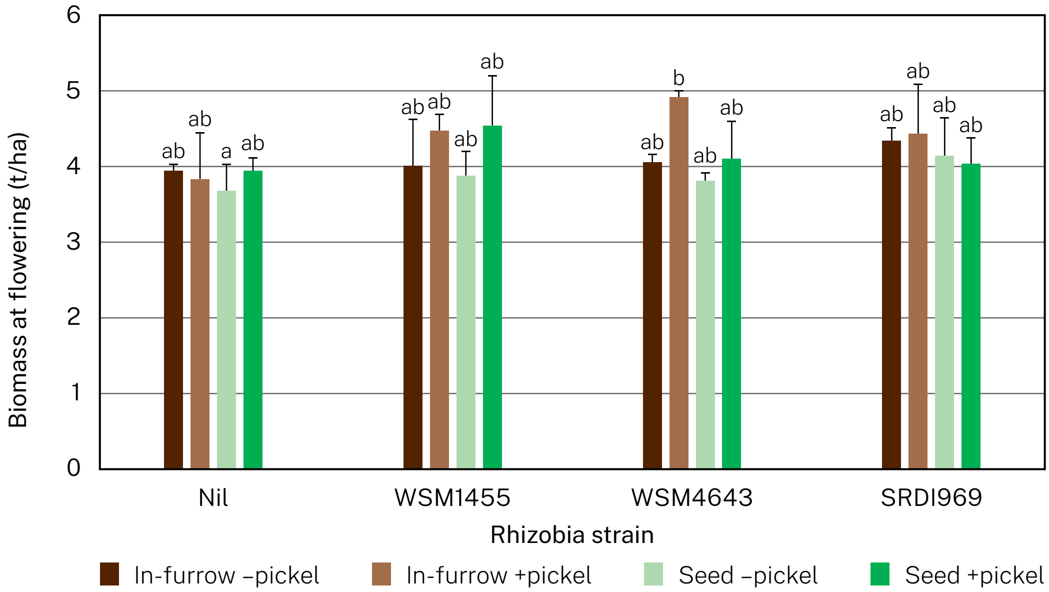
Figure 2 Average number of nodules per faba bean plant root with a series of rhizobial and fungicide treatments at Tamworth in 2022.

Figure 3 Well-nodulated faba bean root at Tamworth in 2022.

- Within treatments, the average number of nodules/plant inoculated with the high-performing strains WSM4643 and SRDI969 was higher than that of plants inoculated with commercial group F strain WSM1455. These increases were significant, apart from where plants were inoculated with rhizobia as a seed coat without fungicide (Figure 2).
- The highest average number of nodules/plant (150 nodules) was from the SRDI969 treatment, applied in-furrow with no fungicide. This was closely followed by 144 nodules/plant for the WSM4643 treatment applied in-furrow with no fungicide.

Biomass at flowering

- The average biomass at flowering (above-ground dry matter) across all treatments was 4.14 t/ha (Figure 4).
- The highest flowering biomass recorded was from the WSM4643 treatment in-furrow plus fungicide (4.92 t/ha).
- Biomass at flowering was similar across treatments.

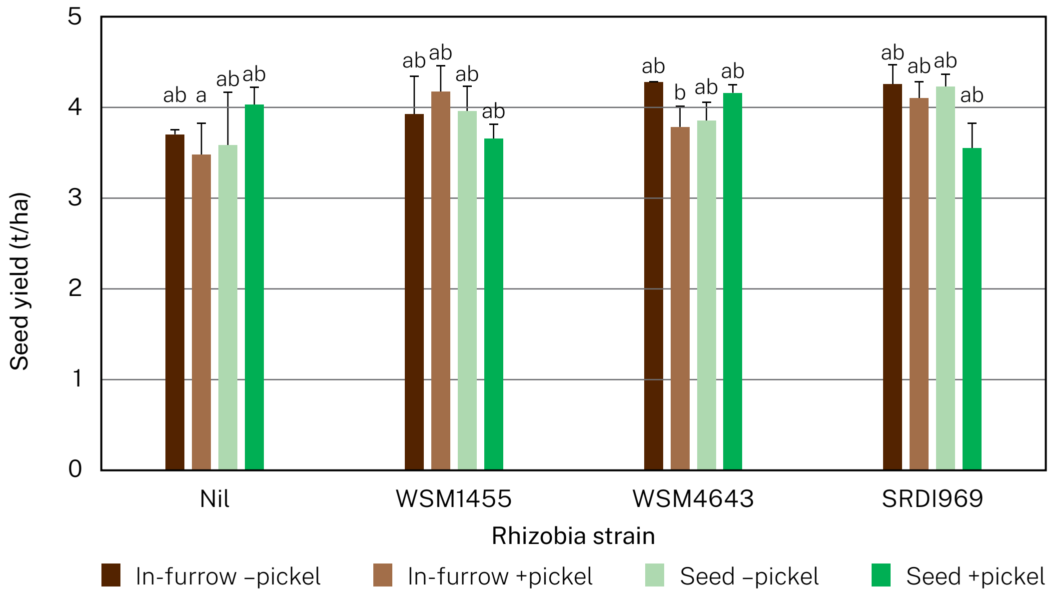


Letters indicate significant differences between treatments.
l.s.d. (*P*<0.05) = 1.1 t/ha). Vertical bars represent standard error.

Figure 4 Above-ground biomass of flowering faba bean plants with a series of rhizobial and fungicide treatments at Tamworth in 2022.

Grain yield

- The average yield across all treatments was 3.92 t/ha (Figure 5).
- The highest yield was from the WSM4643 treatment as a seed coat without fungicide (4.27 t/ha).
- Yield across most treatments was not significantly different. Only the yield of the WSM4643 seed coat treatment without fungicide was significantly higher than the Nil treatment in-furrow with fungicide yield.



Letters indicate significant differences between treatments.
l.s.d. (*P*<0.05) = 0.78 t/ha). Vertical bars represent standard error.

Figure 5 The seed yield of faba bean with a series of rhizobial and fungicide treatments at Tamworth in 2022.

Conclusions

This experiment compared the performance of 2 select high-performing strains of rhizobia against the current commercial Group F strain, WSM1455. Treatments included rhizobia applied in-furrow versus seed inoculated, as well as treatments where seed was treated with fungicide. Root nodulation was used to measure the high-performing strain's ability to effectively nodulate faba bean roots. Nodulation was also used to assess the strength of the rhizobium-legume association. Faba bean inoculated with the high-performing strains WSM4643 and SRDI969 had nodule numbers equal to or higher than plants inoculated with the current commercial Group F rhizobia strain (Figure 2). This was the case across all treatments.

The highest numbers of nodules/plant were recorded where the high-performing strain was applied in-furrow, compared with seed-coat inoculation. Whether inoculation was in-furrow or as a seed-coat, nodule numbers were reduced in the presence of fungicide (P-Pickel T®). This increased when inoculum was applied as a seed-coat. Applying inoculum to seed treated with fungicide would have resulted in the greatest direct exposure of rhizobia to fungicide (both applied to seed) and most likely contributed to reduced nodule numbers compared with the in-furrow treatments. By reducing the direct exposure of rhizobia to fungicide, increased root nodulation can be achieved, as fungicides can be toxic to rhizobia (Inoculant and Seed Treatment Fact Sheet, 2022).

Treating seed with fungicides protects crops from diseases. However, treating seed is not common practice with faba bean in the northern region. Growers generally inoculate their seed with rhizobia using a seed-applied peat slurry. As such, the main conclusions from this experiment will be drawn from the treatment where rhizobia was applied to seed with no fungicide treatment. Here, root nodulation was significantly higher with SRDI969 than WSM1455 (Figure 2). Both SRDI969 and WSM4643 also appeared to be more tolerant of fungicide seed treatments. Where P-Pickel T® was applied to seed, plants inoculated with the high-performing strains had significantly higher numbers of nodules than plants inoculated with WSM1455. Root nodulation was significantly reduced when WSM1455 was applied to fungicide treated seed.

Increases in root nodulation did not translate to increases in above-ground biomass (Figure 4) or grain yield (Figure 5) at Tamworth in 2022. Biomass and yields were similar across treatments, with an average yield of 3.92 t/ha across all treatments. This relatively high yield reflected the above-average rainfall at Tamworth in 2022 (in-crop rainfall 565 mm, long-term average 630 mm), with faba bean yields generally ranging between 2 and 4 t/ha (Matthews and Marcellos 2003; Raymond et al. 2016).

The field experiment at Tamworth formed part of a larger study conducted across the country in recent years to evaluate the performance of select high-performing strains of rhizobia. Following the analysis of trial results conducted across the Northern Growing Region, the NRSC voted to endorse a strain change from WSM1455 to SRDI969 for faba bean inoculation. Results from Tamworth in 2022 support this endorsement. Future studies will assess the performance of high-performing strains such as SRDI969 applied as a granular inoculant. Granular inoculums might provide growers with an option where seed can be treated with fungicide and rhizobia separated from the seed-applied fungicide.

The new acid-tolerant strain, SRDI969, is available from commercial inoculant suppliers for the 2024 season.

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Acknowledgements This experiment was part of a GRDC-funded project, DAN1901-002RTX, Increasing the effectiveness of N fixation in pulses through improved rhizobial strains in the GRDC Northern region, led by Belinda Hackney (NSW DPI Wagga Wagga). Assistance provided by Jessica Rigg (NSW DPI Menangle) and the staff at the Australian Inoculants Research Group (AIRG) is gratefully acknowledged. Assistance provided by additional technical and farm staff from the Tamworth Agricultural Institute (NSW DPI Tamworth) is also gratefully acknowledged. We would like to thank Penny Heuston, Carey Martin and Amanda Warren-Smith for their constructive comments in the development of this report.

Published by NSW Department of Primary Industries, a part of the Department of Regional New South Wales.

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