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Prepared by: Alan Humphries, Ross Ballard and Jessica Tan
South Australian Research and Development Institute

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Meat and Livestock Australia Limited
Locked Bag 1961
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Acid tolerant lucerne

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Executive Summary

Lucerne is a perennial pasture legume with high levels of summer production and adaptation to a broad range of agro-ecological environments in southern Australia. The summer production from this plant makes it valuable for our red meat industries, finishing livestock for market, reducing the grow-out period of weaners and increasing ovulation rates in maternal stock.

Lucerne is grown on approximately 3 M ha in south-eastern Australia, but poor tolerance to acidic soils limits its further adoption. Lucerne is ideally established on soils in the pH_{Ca} range of 5.5 to 8.0, and although it is grown on more acidic soils, forage yield and persistence on these soils is often suboptimal.

The primary aim of this research was to define the performance of new lucerne varieties and rhizobia strains selected for improved tolerance to soil acidity across a range of environments in south eastern Australia. Four sites in SA, Vic. and NSW with pH_{Ca} 4.1-4.3 were chosen with contrasting texture, aluminium and fertility, and this combined with a treatment to ameliorate surface pH to varying degrees with lime, was used to generate a range of environments to evaluate the performance of this germplasm. The results of this project have demonstrated that lucerne is more tolerant to highly acidic soils than previously described in literature. Forage production in the first calendar year after sowing ranged from 4 to 12 t/ha under rainfed conditions at three of the sites with soil pH_{Ca} 4.1-4.3. The long term production of lucerne in these environments is however, associated with greater risk because of decreased nodulation and lower persistence at some of the sites compared to the lime treatments.

The addition of 1.2 t/ha lime partially ameliorated the surface soil to pH_{Ca} to 4.6 and increased average forage yield from 8 to 12t/ha/yr, nitrogen fixation by 26%, and plant persistence by 28% (excluding Boralma). The annual production of 12 t/ha had an approximate feed value of 147MJ energy and 2625 kg CP/ha of protein. This would be especially valuable to red meat producers considering that 50% of the growth occurred outside of the traditional winter-spring production period, extending the growing season into summer and autumn. The summer production occurred despite decile 1-3 springs in 2014 and 2015, illustrating the capacity of lucerne to deliver a constant feed supply, which gives confidence for feed budgeting in a variable climate.

The relatively small cost of applying lime (1 t/ha will cost up to $100/ha delivered and spread) will be recouped in the first 12 months of production, with an additional 4 t/ha of growth (valued at $720/ha using $180/t) and as much as 90 kg N/ha fixed (extrapolated from a single cut, valued at $120/ha). The addition of lime is also expected to benefit the production of lucerne and other pastures or crops grown in rotation for up to 10 years.

Poor forage production did occur at Boralma, but we believe that growth at this site was also constrained by subsoil sodicity, poor soil structure, and waterlogging. Lucerne production at this site did not respond to the high lime rate (2.8t/ha), providing evidence that problems with lucerne production were not associated with soil acidity. The poor production at this

site illustrates the need to have a holistic view of the soil and landscape when considering the suitability for lucerne.

The interaction of site and lime treatments was used to create a large number of environments (4 sites x 4 lime treatments = 16 environments) that were used to investigate factors important for determining lucerne performance on acidic soils. Multivariate and principal component analysis identified pH and aluminium concentration as important contributions to variability in forage yield, nodulation and persistence (all sites except Boralma).

A further aim of this research was to compare the performance of two lucerne varieties developed using different methods for improved tolerance to soil acidity. The variety 'SARDI 7 Series 2', selected for on-farm performance in cool temperate environments with acidic soils, was compared to a new variety 'TA37', which was selected directly for traits associated with tolerance to acidic soils (solution culture screening for tolerance to low pH, aluminium toxicity and symbiotic effectiveness). In solution culture the trait-based selected line TA37 has superior root growth with increases of 32, 48 and 32% over SARDI 7 Series 2 at 0,3 and 6uM Al respectively. TA37 also nodulates with rhizobia in more acidic environments, extending nodulation by approximately 0.3 of a pH unit in solution culture. This result extended into field conditions, where TA37 had greater nodulation than SARDI 7 Series 2 between pH_{ca} of 4.1-4.4. However there were few other differences between the varieties detected in the field, each having excellent forage yield and persistence. The results support a recommendation for both TA37 and SARDI 7 Series 2 varieties to be promoted on acidic soils in south eastern Australia.

The new strain of rhizobia produced better nodulation in solution culture than commercial strain RRI128 and improved nodulation most when combined with TA37. The benefits of the new rhizobia strain were greatest below pH_{ca} 4.5, but negligible at pH_{ca} 5.0. SRDI736 also survived at lower number on seed (about 35% of RRI128) which may affect its performance in alkaline soils with substantial populations of rhizobia. All things considered, we propose a revision of the existing recommendation to replace RRI128 with SRDI736. The revised proposal is that rhizobia strain SRDI736 is released commercially as a specialised strain for lucerne on acid soils ($<pH_{ca}$ 5.0) in combination with SARDI 7 Series 2 and TA37.

The recommendation for industry is to continue to advise farmers to grow SARDI 7 Series 2 (because seed is available) in combination with the new rhizobia strain 'SRDI736', on acidic soils. Further research is underway to determine if there are environments where TA37 has an advantage over SARDI 7 Series 2, and the sowing of the two varieties as a mixture is proposed as an additional consideration. Whilst the varieties have shown the potential to be productive, persist and fix nitrogen below pH 4.5 in the field, we recommend that soils below that pH are limed to lift overall lucerne production.

An important output of this research will be to extend the messages to producers so that lucerne production on acidic soils can be optimised and extended into new areas. Heritage Seeds and SARDI gave a total of 16 field days and seminars to over 350 farmers, agronomists and university students at 12 locations throughout the project. Heritage Seeds also have annual field days at Howlong (850 people visits over the last 3 years) and Toowoomba where

messages from this project will continue to be delivered in the future. Finally, an information package will be developed for red meat producers to summarise the results contained in this study. The delivery of this research is expected to increase the confidence of producers growing lucerne, which will have long term benefits for red meat production in Australia.

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1 Background

1.1 Project background

This project evaluated the agronomic performance of lucerne and rhizobia strains developed for improved tolerance to acidic soils. A new plant variety 'TA37', selected for traits directly linked with acidity tolerance in solution culture, was compared to an existing variety 'SARDI 7 Series 2', which was bred for field adaptation to acidic soils. The plant varieties were evaluated in combination with a new rhizobia strain also selected for acidity tolerance, SRDI736, and the existing commercial strain, RRI128.

The project aims to provide information to red meat producers on the performance of lucerne that has been bred for acidic soils, so that educated decisions can be made on where the varieties can be grown. Information gained during this project is expected to improve our understanding of the performance of lucerne on acidic soils with the aim to increase adoption of this valuable plant.

The project focused on evaluating the performance of strain and variety combinations at four field sites that are naturally highly acidic (pH_{Ca} 4.1-4.3 with Al 15-20% of the cation exchange complex). Lime treatments incorporated at the sites were designed to partially amend the acidity stress and create multiple environments at each site, allowing detailed comparisons of the treatments.

An output of the project will be a set of guidelines defining the adaptation of lucerne on acidic soils, written for agronomists and livestock producers.

1.2 History of acidity tolerance in lucerne

Lucerne is a deep-rooted herbaceous perennial legume with high levels of summer production and adaptation to a broad range of agro-ecological environments in southern Australia. The ability of lucerne to extend the growing season of winter-based pasture and respond quickly to rainfall after periods of drought makes it one of the most valuable plants in our feed base. Poor tolerance to acidic soils has long been recognised as a major constraint to lucerne adaptation in Australia (Munns 1965; White 1967). Lucerne is ideally established on soils in the pH_{Ca} range of 5.5 to 8.0 (Stanley et al. 2002), and although it is grown on more acidic soils, lucerne yield and N_2-fixation on these soils is often suboptimal (Simpson *et al.* 1977, Pinkerton and Simpson 1986). Nodulation in lucerne is impaired at pH_{Ca} <5.5 (Munns 1968) and soluble aluminium restricts root growth (Campbell et al. 1988; Munns 1965) and nodulation (Bordeleau and Prevost 1994) in many soils with a pH_{Ca} <5.0. Reductions in nodulation and root growth combine to inhibit establishment, production, nitrogen fixation and survival on very acidic soils.

SARDI has a long history of breeding lucerne for improved adaptation to environments with acidic soils. For over 35 years the SARDI lucerne breeding program has been sowing on-farm trials in environments with acidic soils in southern Australia to evaluate germplasm, and select elite plants from the best populations to develop varieties using recurrent mass selection. The on-farm evaluation trials combine selection for improved performance on

acidic soils with tolerance to cold, wet conditions, competition with grasses, and grazing. The cultivar SARDI 7 Series 2 was released in 2011 because it excelled in the cooler climates of SE Australia, which typically have acidic soils (Fig. 1).

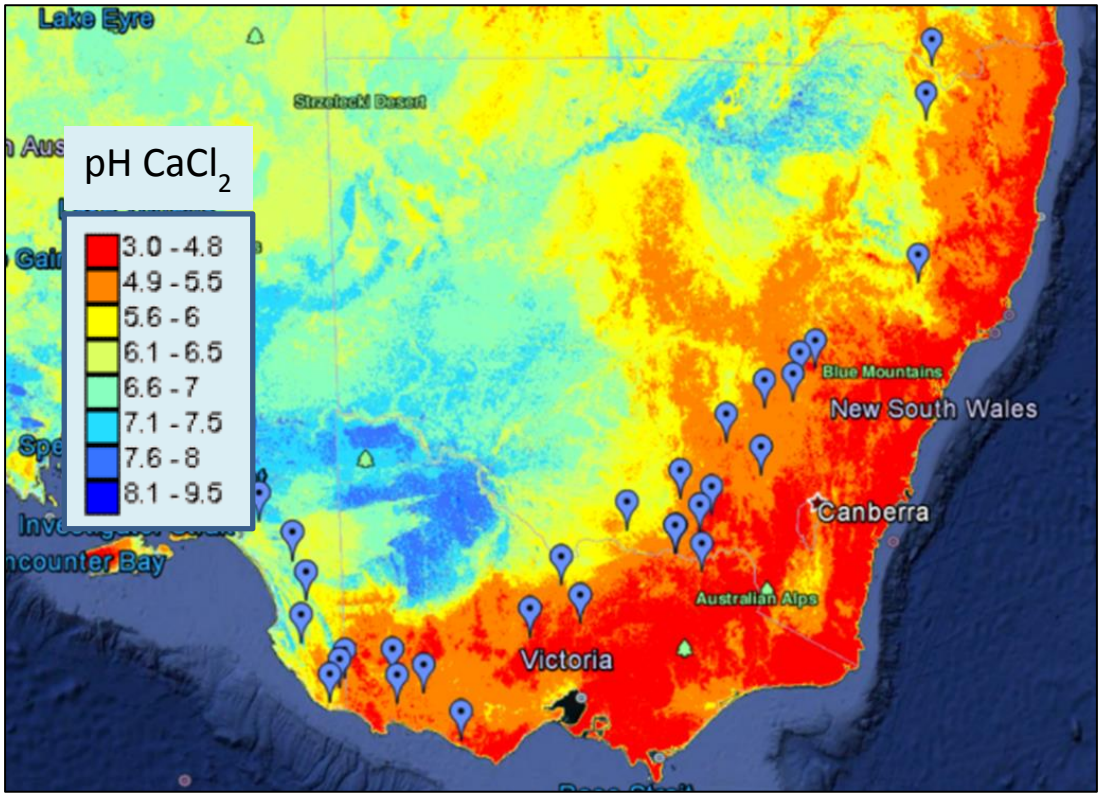


Fig. 1 Interpolated map of surface soil (0-10cm) pH_{Ca} showing locations of SARDI – Heritage Seeds field trials in south-eastern Australia. SARDI 7 Series 2 was released because of excellent performance in the most acidic sites in cooler temperate environments.

In the years 2000-2013, SARDI in collaboration with the FFI CRC has also been selecting lucerne and its rhizobia for traits directly associated with improvements in tolerance to soil acidity. Selection has been based in solution culture for improved root growth and nodulation at low pH and aluminium toxicity. The target for improvement of this new activity is improved adaptation of 0.5 pH unit, which would have a large scale impact in Australia. For example, Robertson (2006) defined the suitability of lucerne to southern Australia with reference to soil pH and rainfall (Fig. 2). There are 11.2 M Ha of acid soils with pH pH_{Ca} 5.0-5.5 with an average annual rainfall of > 450 mm. These environments are currently considered moderately suitable for lucerne and will shift to highly suitable with the introduction of the acid tolerant variety and rhizobia strain.

An acid tolerant lucerne variety is expected to have the greatest impact in areas where lucerne is currently grown at sub-optimum production levels, including the Riverine Plains, Central West and Northern Tablelands of NSW and Victoria and the south-east of South Australia. There is an additional 6.2M Ha in Australia where lucerne is not commonly grown that will shift from low to moderate suitability (soils with a pH_{Ca} of 4.5-5.0).

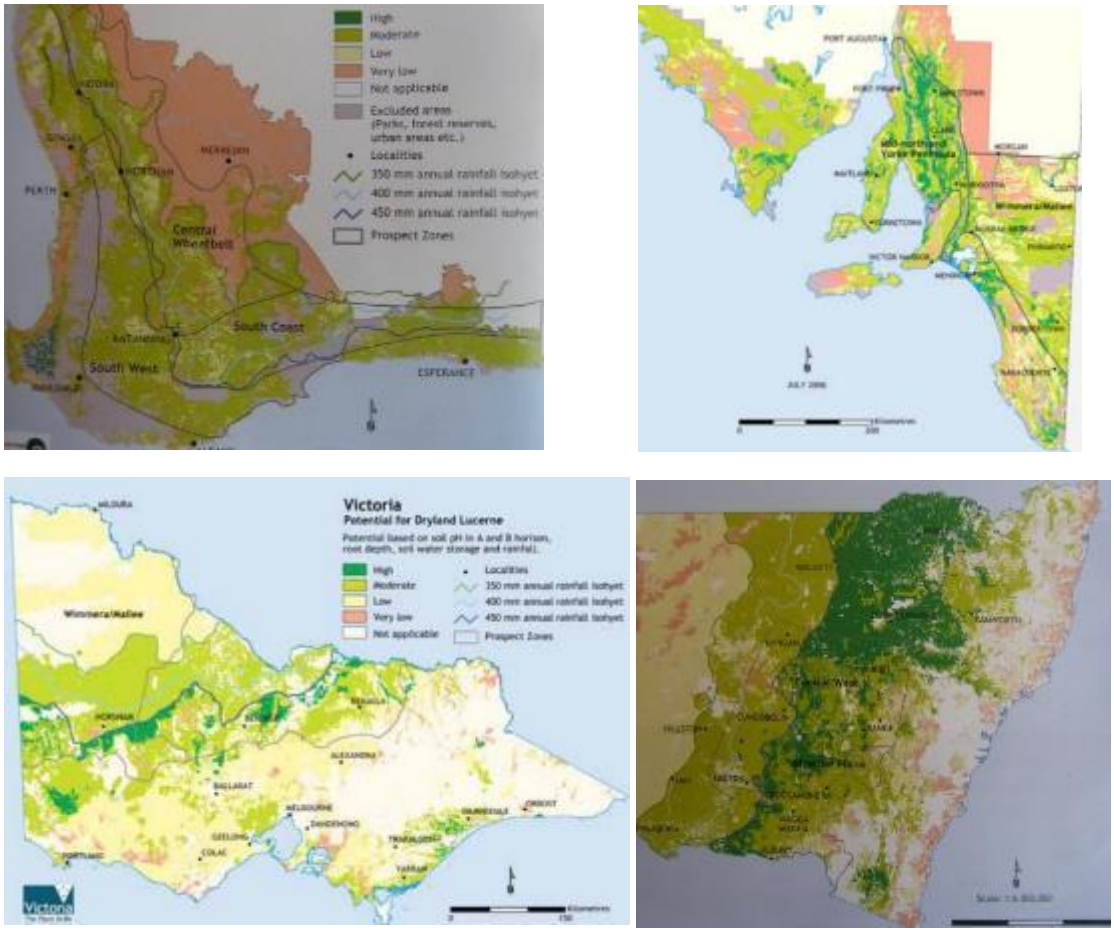


Fig. 2 Suitability Classification of lucerne with reference to soil acidity (Robertson 2006, Lucerne Prospects)

The trait-based plant improvement program (funded by SARDI for 13 years of breeding) has involved six cycles of selection for improved tolerance to aluminium and three cycles of selection for improved capacity to nodulate with rhizobia. The parentage of the line originates from 24 winter active (class 7) SARDI breeders lines with excellent field performance (also used in the breeding of SARDI 7 Series 2). From these lines, 123 selections for improved root length were inter-mated to form half-sib families, which were then subjected to within and between family selection for aluminium tolerance and improved nodulation with rhizobia in acidic solution culture.

Acid tolerant mid-parent lines entered into field trials on commercial farms at eight sites in NSW, Vic. and SA were not significantly different after four years to the highest ranked entries in the trials, 'SARDI Grazer and SARDI 7 Series 2. The good performance of the mid-parents gives encouragement that no special management will be required for this variety, and no penalty for persistence or production will occur on neutral or alkaline soils.

The rhizobia selection (funded by the FFI CRC for 10 years) involved a collection trip in NSW to recover rhizobia from persistent lucerne stands on acidic soils, which were then evaluated in hydroponics and a range of acidic field environments. The two programs have been completed in tandem, so that the final variety, harvested in March 2013, has not been evaluated with the final strain (SRDI736), selected for release in April 2017. Hydroponic

evaluation has shown that the combination of improved plant and rhizobia strain are most successful at improving the performance of the plant. In this project, the final plant/ rhizobia combination was evaluated for the first time in the field against the variety 'SARDI 7 Series 2, which provides the best benchmark for field adaptation to acidic soils.

During this period of investment, several alternatives to growing lucerne on acidic soils have been investigated. A major study funded by the FFI CRC involving the evaluation of 91 perennial legumes and herbs at Barmedman (pH 4.5) and Wallendbeen (pHCa 4.2) NSW, resulted in lucerne achieving the highest yields of all legumes despite its lack of adaptation [including poor nodulation on soils with pH <4.8] to acidic soils (Li et al 2008). There has also been a plant breeding program for Lotus targeting acidic soils, but the lack of adaptation and issues with harvestibility has resulted in the commercial partner only considering international markets for the resulting varieties (R. Salmon Pers. Comm. 2011).

The broad adaptation of lucerne, its acceptance in the market place, and the knowledge surrounding its ability to increase livestock production point clearly to the value of a lucerne variety and rhizobia package with improved adaptation to acidic soils in Australia.

1.3 Systems Benefit

Many environments in southern Australia receiving above 600 mm of average annual rainfall are completely devoid of perennial legumes. This occurs where soils have traditionally been considered too acidic for lucerne and summers too dry and hot for white clover. In these landscapes there is a significant need for perennial legumes that will tolerate both low pH and summer drought. As described in section 1.12 above, there are over 17 m ha at or below a pH of 5.5 in the target areas where lucerne would be readily adopted if a more tolerant variety was available.

The capacity of lucerne to improve livestock production on mixed dryland farming systems in southern Australia

There have been a large number of studies that have shown the benefit of lucerne based pastures for improving meat production in southern Australia. Lucerne based pastures increase growth rates of prime lamb weaners by an average of 17% (Reed et al. 1972, Reeve and Sharkey 1980, Donnelley et al. 1985), and ovulation by 10% (King et al. 2010) in comparison to phalaris and sub clover based pastures. For beef production, the improvement in annual live weight in weaner steers was 20–50 kg/ha (Christian and Shaw 1952, Hamilton 1974, Wolfe et al. 1980). The benefit to red meat [and wool] production is further increased during dry conditions over summer and autumn months, or during periods of drought during the winter growing season (Reeve and Sharkey 1980, Wolfe et al. 1980, Hall et al. 1985, Crawford and Macfarlane 1995). Live weight gain on sheep grazing lucerne in autumn was six times greater than that on grass dominant pasture (Reed et al. 1972) and in another study by Crawford and Macfarlane (1995), lucerne was able to maintain individual wool production at a stocking rate 2.5 times higher than annual pasture, which consequently resulted in much higher wool yields per hectare at high stocking rates.

Results from Evergraze (Robertson *et al.* 2013) show that increasing lucerne to 40% of pasture in the Southern NSW Slopes and Plains improved gross margin by $76/ha or 34%, as

a result of increasing lamb production by 40 kg/ha and decreasing supplementary feeding by 70kg/ha. The results were compared to a system with 20% lucerne and 80% Phalaris / Tall Fescue and would be even greater if the system was being compared to one without any lucerne. The recommendation to increase the percentage of farm sown to lucerne to at least 40% is currently only achievable where constraints from soil acidity are non-limiting.

1.4 Potential scale of adoption

An increased area of 3.0M ha sown to lucerne was reported by Robertson (2006) as being an achievable target, which appears feasible with the availability of a new acid tolerant variety and strain. This increase in area would result in an annual increase in production of 63M kg for lamb and 52M kg for beef in Australia, based on the average increases in production from lucerne reported in literature.*

The value proposition therefore centres on improving the adaptation of lucerne to the 17.4M Ha of acid soils in southern Australia and as a consequence making it available to more farmers (and a higher percentage of their land).

This project aimed to define the level of tolerance of lucerne to acidic soils so that clear recommendations can be made on where the acid tolerant lucerne varieties can be grown. This will avoid damage to adoption of new varieties that can be caused by over-confidence in the technology and failures resulting from sowing the plant in the wrong environment. The differences in production (yield and quality) will provide data for modelling to show how changes in lucerne's tolerance to acidic soils result in changes to whole farm profitability.

*Assuming the land is split evenly for beef and lamb production. For lamb: 10dse/ha, 25kg dressed carcass weight, 17% increase in production (Reed et al. 1972, Reeve and Sharkey 1980, Donnelley et al. 1985), and for beef: 15dse/ha and 35kg/ha increase production from weaner steers (Christian and Shaw 1952, Hamilton 1974, Wolfe et al. 1980)

This project evaluated the performance of lucerne on highly acidic soils with a view to test and demonstrate the potential value of the cultivar improvements to red meat producers in south-eastern Australia.

A set of guidelines defining the adaptation of lucerne on acidic soils, written for agronomists and livestock producers will be developed as an output of this research.

2 Project Objectives

1. Develop a detailed understanding of the improvement in adaptation of lucerne on acidic soils, separating the individual stresses of pH and aluminium toxicity.

2. Develop an information package for red meat producers, which describes the adaptation of lucerne on acidic soils, and to what extent this new variety and strain extend the production of lucerne onto acidic soils.

3 Part 1. Evaluation of aluminium tolerance and nodulation at low pH in solution culture

3.1 Experiment 1 - Root elongation of 20 lucerne genotypes in presence of Al

The root elongation of 20 lucerne lines was measured at four concentrations of aluminium at pH 4.5 in a solution culture experiment.

3.1.1 Materials and methods

3.1.1.1 Experimental design.

The experimental treatments comprised:

1. Four Al levels (0, 3, 4, & 6 uM) at pH 4.5, plus 0 Al at pH 7.0 (non acidic control)
2. Twenty lucerne genotypes

    a. Eleven commercial varieties in winter activity class 7; cvv. Aurora, Force 7, Genesis, Haymaster 7, L70, Q75, Quadrella, SARDI 7, SF714QL, Stamina GT6, Titan 7.
    b. SARDI 7 Series 2 (syn S7s2 or SARDI7s2) developed for improved field adaptation to acidic soils
    c. SARDI trait-based acid tolerant line TA37 (syn SARDI AT7), proposed trait-based acid tolerant line selected in solution culture for improved tolerance to low pH and aluminium toxicity).
    d. Five progenitor lines of TA37 with varying cycles of recurrent selection, indicated in parenthesis; TA16-18 (2) TA22 (3), TA28 (4), TA33 (5), TA36 (6) and TA37 (6)
    e. Two breeding lines with winter activity classes 5 and 9 that have had two cycles of trait-based selection for improved tolerance to aluminium toxicity in solution culture (AT5-TA34 and AT9-TA35).

Lucerne seedlings were grown in hydroponic solution consisting of 120 L of 1 mM $CaCl_2$ solution pumped through 15 L containers with floating seed holders (plate 1). No other nutrients were added. Lucerne seeds were surface sterilised and pre-germinated in petri dishes, before being planted with a uniform 5 mm radical length into the seed holders. Four hundred seedlings (20 seedlings × 20 lucerne lines) were planted in each 15 L container.

The hydroponic solutions were maintained at pH 4.5 with daily adjustments using 0.1 M HCl throughout the experiment. Al concentration of the solution in each 15 L container was adjusted to the desired treatment level using $AlCl_3.6H_2O$ on day 3 of the experiment. All plant selection had previously been undertaken at 3 uM Al.

Root length of individual seedlings was measured approximately 14 days after the addition of Al and a mean root length for each lucerne genotype calculated.

The experiment was run twice (two replicates per run) and data pooled to provide four replicates for analysis.

3.1.1.2 Statistical analysis

To perform the analyses assuming continuous data, the scored data were transformed to continuous in the model fitting process by averaging the scores where multiple measurements were taken from within each experimental unit (For example, 20 measurements of root length to form an experimental unit for 'root length'). Means of fixed cultivar effects were calculated using spatial linear mixed models performed by Genstat 11 (Lawes Agricultural Trust, Rothamsted). The fixed effects were 'variety x strain'. Diagnostic plots of sample variograms and residuals were used in conjunction with REML log-likelihood ratios and Wald tests to fit new models that compartmentalized and removed random and fixed effects of variation (Smith et al. 2005).

3.1.2 Results

Root length of TA37 (55 mm) was 48% greater than that of SARDI 7 Series 2 (29 mm) at 3 uM Al (Table 1 and Fig. 3). Root length decreased with increasing aluminium concentration, but the relative improved tolerance of TA37 was still evident (+32%) at 6 uM Al, which is double the concentration used to select the tolerant line.

Some increase in root length of TA37 in the absence of Al at pH 4.5 (Table 1). It suggests improved tolerance of the selected material to low pH, independent of AL concentration.

The progenitor lines and acid tolerant winter activity class 5 and 9 breeding lines also showed improved tolerance to aluminium (Fig. 3), and root growth improved with their number of cycles of selection.

None of the commercial varieties tested showed significant tolerance to Al in solution culture (Fig 3).

Table 1. Root growth of TA37 (syn SARDI AT7) and 12 other winter active commercial lucerne varieties sold in Australia at neutral (7.0) and acidic pH (4.5) at four levels of aluminium (0, 3, 4, 6 uM).

Organ/Plant Part: Context	TA37	Aurora	Force 7	Genesis	Haymaster 7	L70	Q75	Quadrella	SARDI 7	SARDI 7 s2	SF714QL	Stamina GT6	Titan 7
Root: Growth (pH 7.0, Aluminium = 0, cm)													
Mean	84	77	78	80	74	83	80	81	80	80	74	72	77
Std. Deviation	8.2	3.9	5.5	8.47	8.6	10.3	8.1	4.0	4.5	2.0	9	11.4	8.6
Lsd/sig	13	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Root: Growth (pH 4.5, Aluminium = 0, cm)													
Mean	58.8	37.9	47.5	42.4	39.2	46.2	39.6	45.2	38.7	40.3	46.8	42.7	40.3
Std. Deviation	7.8	7.3	8.2	5.6	6.6	18.6	3	10.4	6.2	6.0	6.6	7.7	5.4
Lsd/sig	6.9	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01
Root: Growth (pH 4.5, Aluminium =3µM, cm)													
Mean	55.4	25.7	32.7	25.2	28.9	23.3	20.9	27.8	22.4	29.1	31.2	26.5	21.7
Std. Deviation	23.9	7.5	9.3	7.1	9.5	9.2	5.8	9.9	6.5	11.8	9.6	11.6	10.6
Lsd/sig	6.9	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01
Root: Growth (pH 4.5, Aluminium = 4µM, cm)													
Mean	41.6	22.3	23.7	23.4	22.2	16.1	16.9	21.8	21.4	20.9	24.8	21.2	17.9
Std. Deviation	9.8	6.0	3.6	3.7	4.4	3.6	2.6	5.0	6.5	5.5	3.4	5.4	5.1
Lsd/sig	6.9	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01
Root: Growth (pH 4.5, Aluminium = 6µM, cm)													
Mean	30.6	21.2	23.5	23.4	22.1	16.1	16.5	21.8	21.0	18.6	24.8	21.2	17.9
Std. Deviation	15.3	5.8	5.7	2.4	2.6	4.2	2.3	2.8	5.1	5.5	3.6	5.4	5.3
Lsd/sig	6.91	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01

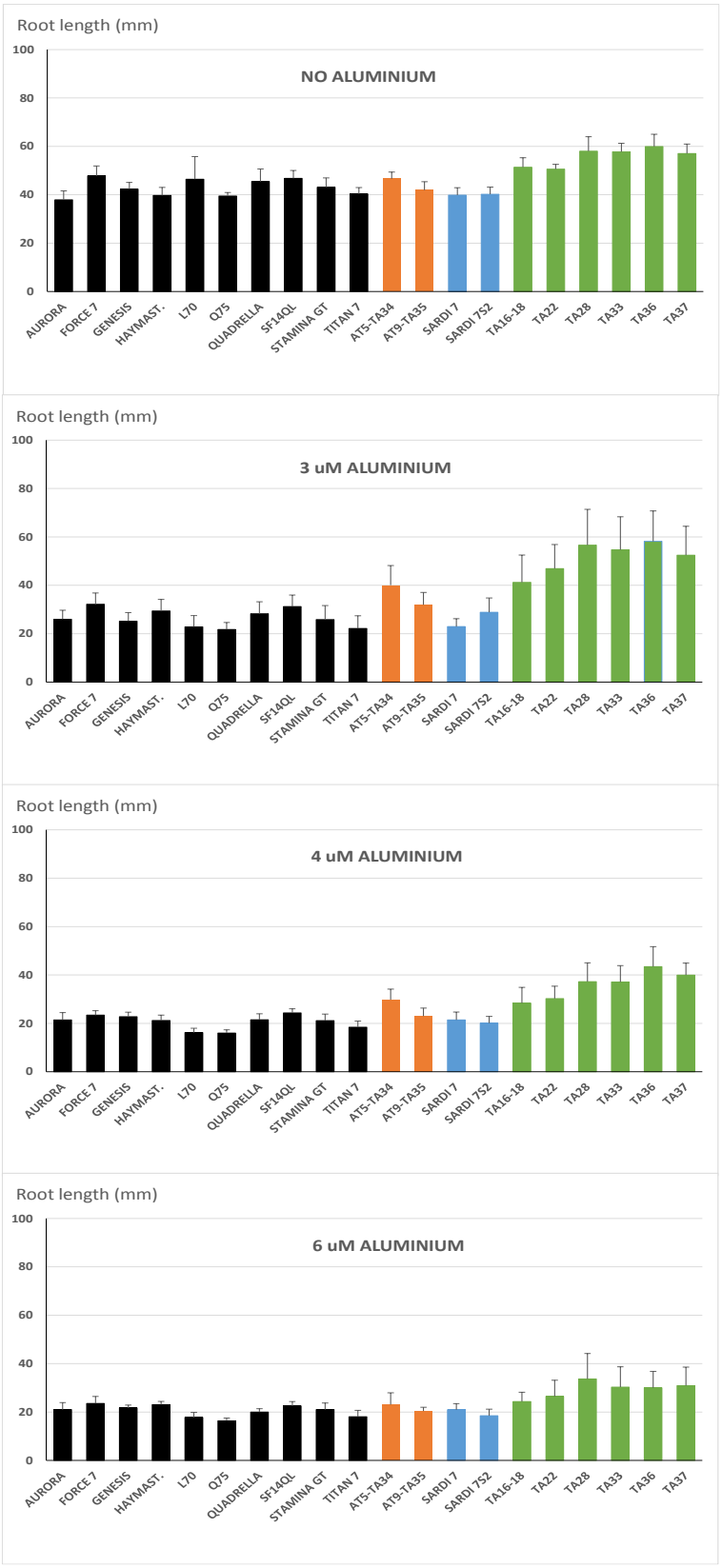


Fig. 3 Root length of twenty lucerne genotypes growing in solution culture at pH 4.5 and at four levels of Al. TA37 (syn, SARDI AT7) and its progenitors are shown in green, SARDI 7 and SARDI7 S2 in blue, Activity 5 and 9 selections in orange and commercial non-SARDI varieties in black. Bars indicate standard error. Lsd for comparing between lucerne's at 3 uM Al is 12 (*P* = 0.05, n = 4).

3.2 Experiment 2 - Nodulation of plant and rhizobia combinations at low pH

Two strains of rhizobia were assessed for their ability to form nodules on two genotypes of lucerne growing in aerated solution cultures (without Al).

3.2.1 Materials and Methods

3.2.1.1 Experimental design.

The experiment comprised the treatments:

i) Five pH levels (4.5, 4.7, 4.9, 5.1 & 5.3).
ii) Two strains of rhizobia (acid tolerant strain SRDI736 and commercial strain RRI128).
iii) Two lucerne lines, TA37 (syn SARDI AT7) and commercial variety SARDI 7 series 2.

The experiment was arranged in a randomised block design with rhizobia and pH as the main treatments (each applied to an individual pail of nutrient solution) and lucerne genotype as sub-treatments (split within a pail). There were 3 replicates.

Each experimental unit comprised a 25 L pail containing one-quarter strength N- and Al-free nutrient solution (McKnight 1949), aerated and mixed using an aquarium pump (see Appendix 2). The pH of the nutrient solution in each pail was monitored daily and adjusted as needed with the addition of 0.1 mol/L NaOH or HCl.

Surface sterilised seeds of the two lucerne's were pre-germinated and seedlings of uniform size were sown into holes in a plastic lid floating on the nutrient solution. Fifty seedlings of each lucerne genotype were planted in each pail. Rhizobia were added the day after the seedlings were sown, to give a final concentration of ~10^5 cells/mL in each pail.

Plants were harvested 10 days after rhizobial inoculation and nodule number counted. Shoot and root dry weights were determined.

3.2.1.2 Statistical analysis

Analysis was performed using the statistical software R (R Core Team 2015, version 3.1.3, https://www.r-project.org/). For each of the measured variables (percentage nodulation (seedling and mature), number of nodules per nodulated plant (seedling and mature), sum of nodules (seedling and mature), density, yield, cumulative yield), summary statistics (means and standard deviations) were calculated for each combination of site, variety and strain. The distribution of the results were also graphed using side-by-side boxplots for each categorical variable and over time for density and yield measurements. Analysis of variance and mixed effects models with a random effect for site and fixed effects of variety, strain, lime and soil pH were used to assess significant treatment effects (R package 'nlme'). Multivariate correlation analysis was performed using the R package 'corrplot' to calculate and graphically depict the pairwise correlations between all the measured variables as well as soil pH and aluminium. Finally, principal component analysis (R package 'factoextra') was

used to identify the internal structure of the high-dimensional data in a way which best explains the variance in the data, using a set of values of linearly uncorrelated variables called principal components. Note that the multivariate analysis with aluminium was performed at the treatment level, using averages of the measured variables. A significance level of 5% was used to assess regression models and significance tests.

3.2.2 Results

Nodulation of the commercial standard combination (SARDI 7s2 with rhizobia RRI128) declined rapidly below pH 5.1, with nodulation absent at pH 4.7 (Fig. 4). At this same pH, 86% of plants in the acid tolerant combination (TA37 with rhizobia SRDI736) had nodules (Fig. 4). Nodulated plants also had more nodules. Across the pH range of 4.7 to 5.3, the acid tolerant combination increased nodule number by 138% on average (Fig. 5).

Both rhizobia strain and lucerne genotype contributed to the improvements in nodulation, with the combination of the two producing greatest benefit.

At pH 4.5, nodulation in all treatments was negligible indicating that this pH level provides the lower boundary for lucerne nodulation in the solution culture system. The acid tolerant combination provides satisfactory nodulation (86% of plants producing 3.8 nodules at pH 4.7) very close to the lower boundary.

At pH 5.3, nodulation (both percent and number) in the current commercial combination (SARDI 7s2 with RRI128) was lower than in other treatments, indicating some sensitivity of that symbiosis even at the highest pH level.

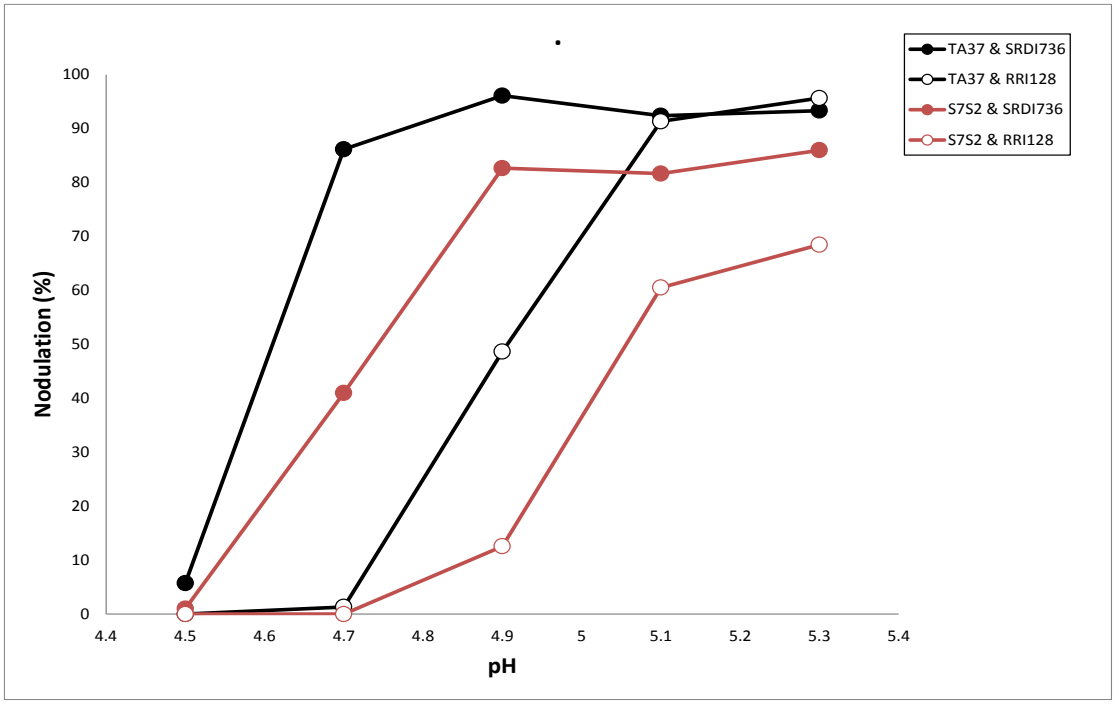


Fig. 4 Effect of rhizobia and lucerne genotype on the percentage of lucerne seedlings (S7S2 and TA37) forming nodules in solution culture in the pH range of 4.5 to 5.3), 10 days after inoculation.

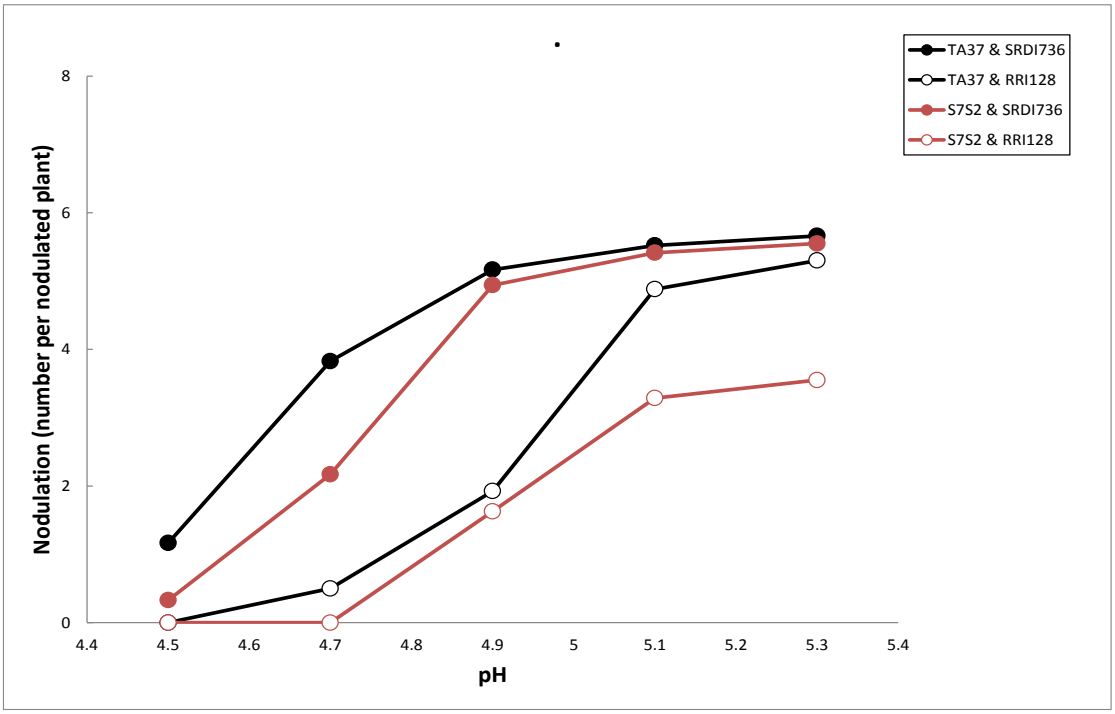


Fig. 5 Effect of rhizobia and lucerne genotype on the number of nodules on lucerne seedlings (S7S2 & TA37) growing in solution cultures in the pH range 4.5 to 5.3), 10 days after inoculation.

3.3 Discussion of solution culture experiments

Both solution culture experiments demonstrate that substantial and significant progress has been made in improving the acidity and/or Al tolerance of lucerne.

Root elongation at 3 uM Al was increased by 82%. The combination of TA37 lucerne (selected for both root elongation and improved nodulation) and new rhizobia strain SRDI736 also increased nodulation from 0% (for S7S2+RRI128) to 86% at pH 4.7. Although the combination has not been specifically selected for Al tolerance because symbiotic impacts occur at pH levels higher than where Al is soluble and problematic, in supplementary experiments (data not shown) the new plant rhizobia combination has also been shown to form significantly more nodules at 8 uM Al.

At pH 4.5, nodulation in all treatments was negligible indicating the lower boundary for lucerne nodulation in the solution culture system. The acid tolerant combination of TA37 and SRDI736 provided satisfactory nodulation (86% of plants with 3.8 nodules at pH 4.7) near this lower boundary, indicating most of the potential gain for nodulation at low pH has been captured in the activity 5 material.

The tripartite approach of selection for root elongation and nodulation in the plant and acidity tolerance in the rhizobia has worked well. The improved selection for nodulation at low pH should improve the adaptation of lucerne to acidic soils.

4 Part 2. Evaluating the tolerance of lucerne to highly acidic soils in the field

The agronomic performance of lucerne breeder's line TA37 selected in solution culture for trait-based tolerance to acidity is compared to the best existing cultivar SARDI 7 Series 2 for acidic soils, in combination with the acid tolerant rhizobia SRDI736 and current commercial strain RRI128 at four locations in South Australia, Victoria and New South Wales.

4.1 Methodology

4.1.1 Trial site selection and experimental design

4.1.1.1 Sites

Acid tolerant lucerne evaluation sites were sown at Tooperang SA in 2013 and at Pewsey Vale SA, Holbrook NSW and Boralma Vic. in 2014. The sites were selected on the basis of being highly acidic, in the range of pH_{Ca} 4.1-4.3, and with moderate levels of aluminium (10-15 mg/kg Al $CaCl_2$ and between 10 and 20% Al of the CEC). Additional considerations included paddock history (recent fallowing or cropping for weed control) and involvement of farmer producer groups.

4.1.1.2 Experimental design

Each site was sown in two experiments; the first was sown without lime addition and comprised a complete factorial design with two factors: plant genotype and strain genotype, and five replicates. The second experiment at each site investigated the impact of increasing soil pH with 3 rates of lime on a combination of the plant and rhizobia genotypes.

Experiment 1 design (Full factorial trials)

Plant genotype, two levels: SARDI AT7 and SARDI 7 Series 2

Rhizobia genotype, three levels: SRDI736 (experimental), RRI128 (commercial), nil

Replications 5

Experiment 2 design

Plant_strain genotype, 4 levels: S7s2_nil, S7s2_RRI128, S7s2_SRDI736 and TA37_SRDI736

Lime rate, 3 levels: low (600*, 700 kg/ha), medium (1200*/1400 kg/ha), and high (2400*, 2800 kg/ha).

*lower application rate for Tooperang where the lighter texture soil was expected to have a lower pH buffering capacity.

Experimental designs were randomised using the program "Digger", which prepares the experiment for spatial analysis by reducing the likelihood of the same treatment combination occurring multiple times in the same row or column.

4.1.1.3 Seed preparation and sowing

All seed was surface sterilised in ethanol for 30 seconds and allowed to dry before inoculation. The fungicide Apron was made into a slurry and applied to seed bulks at rate of 200g apron/100 kg seed. Peat inoculants of each rhizobia strain were prepared at SARDI. Inoculant slurries were prepared immediately before inoculation as 6.25 g peat in 25 ml 1.5% methylcellulose sticker, and applied at approximately double the recommended rate of 1250g per 25kg of seed and pelleted with fine lime. Seed was inoculated close to sowing (usually the day before) , and the number of rhizobia surviving on the seed estimated on the day of sowing.

The trials were hand sown into 1.2 x 5 m plots in furrows created by a cone seeder with narrow tynes. In order to reduce contamination, all of the nil treatment seed was sown first, followed by the commercial strain RR128 and lastly the acid tolerant strain SRDI736. Seed was incorporated with a clean rake.

4.1.1.4 Measurements

Soil measurements.

During the establishment stage of the first year (lucerne 3-5 leaf stage), soil in the 0-10cm was sampled from 3 cores in the centre of each plot in order understand the spatial variation of pH across each experiment. From these samples, soil was bulked into each treatment for chemical analysis.

Final soil pH and chemistry was also measured at the conclusion of the experiment, by combining cores from each plot into a bulk for each treatment.

All samples were air dried and sent to CSBP for analysis.

Plant and rhizobia measurements.

Plant assessments focussed on establishment, nodulation, yield and persistence. Details for each of the plant and rhizobia measurements are listed in Table 2.

Table 2. Details of plant and rhizobia measurements made on acid tolerant lucerne trials

Measurement	Timing	Details
Rhizobia on seed	At sowing	50 seed were washed in 50 ml sterile water and the number of rhizobia per seed determined.
Nodulation	Seedling 3-5 leaves	Destructive harvest of 20 seedlings carefully removed from the northern end of each plot. Plants washed and assessed for % nodulation, and nodulation per nodulated plant. Root and shoot weights also recorded.
Density	Seedling	Plants counted in 0.75m2 area at southern edge of plot. Quadrat location marked with a peg and cattle tag
Forage Yield	Mature plant, 8 times	2.5m^2 from centre of plots cut with hedge trimmer to reduce soil movement. Weighed, subsampled and oven dried to calculate dry weight yield.

Nodulation	Mature plant	Destructive harvest of 10 plants dug from the northern end of each plot. Plants washed and assessed for % nodulation, and nodulation per nodulated plant.
Density (Persistence)	Mature plant, bi-annual	Plants counted in 1m2 area at southern edge of plot. Quadrat location marked with a peg and cattle tag, OR total number of crowns per plot counted (thin plots)
N-Fixation	Spring year 2	Nitrogen from N-fixation assessed using ^{15}N natural abundance technique on Spring year 2 forage sample
Saprophytic colonisation	Year 2	Five soil cores to 10 cm depth and approximately 10 cm from each lucerne crown collected from each plot and assessed for rhizobia density using the Most Probable Number plant bioassay.

4.1.1.5 Statistical Analysis

To perform the analyses assuming continuous data, the scored data were transformed to continuous in the model fitting process by averaging the scores where multiple measurements were taken from within each experimental unit (For example, 20 measurements of nodulation to form an experimental unit for %nodulation). Means of fixed cultivar effects were calculated using spatial linear mixed models performed by Genstat 11 (Lawes Agricultural Trust, Rothamsted). The fixed effects were 'variety x strain' + soil pH, with soil pH was used as a covariate for each plot. Diagnostic plots of sample variograms and residuals were used in conjunction with REML log-likelihood ratios and Wald tests to fit new models that compartmentalised and removed random and fixed effects of variation (Smith et al. 2005).

4.2 Results

4.2.1 Soil Description

Tooperang. The site has a strongly acidic duplex soil with a light textured sand (0-10cm pH_{Ca} 4.2, Al 10% CEC) over a bleached B horizon (10-20cm, pH_{Ca} 4.5, Al 41% CEC) above a clay layer at approximately 30 cm (20-30cm pH_{Ca} 4.5, Al 11%, Table 1). The paddock has been sown with ryegrass for hay for at least the last three years, and has a history of P fertiliser. The site would be considered too acidic to grow lucerne, and therefore the greater differences would be expected in the second part of this experiment following amelioration of soil acidity with different amounts of lime.

Pewsey Vale. A highly acidic loam soil with low 0-10 pH_{Ca} 4.1. The site has been fallowed for 12 months following a degraded phalaris pasture and a very good history of single superphosphate application. Aluminium is 16% of the CEC in the 0-10cm, but decreases with depth and increasing pH, reducing to 5% CEC in the 10-20cm. The very low pH and high aluminium make this soil unsuitable for lucerne recommendation.

Holbrook. The site was previously a degraded phalaris-based permanent pasture with little fertiliser inputs. The soil is a strongly acidic red-brown earth (0-10cm pH 4.3, Al 13% CEC, Table 1). In addition to high levels of aluminium, this site also has Mn toxicity (140 mg/kg DTPA Mn in 0-10cm). With no history of lime, this soil would be considered too acidic to be recommended for lucerne.

Boralma. The soil is a pale clay with $pH_{(Ca)}$ and Al (% CEC) close to the target levels of pH 4.4 and 10%. The pale clay indicates a history of waterlogging, and the lack of structure or oxygen in this soil may be an additional limiting factor. Lime will benefit both pH and soil structure, indicating that the lime treatments will be important at this site, and that it will be a robust test of lucerne acidity tolerance (Table 1).

Spatial variation in pH and Al level (varying with depth and across blocks) has been measured and will be important to explaining variation in plant performance across each site.

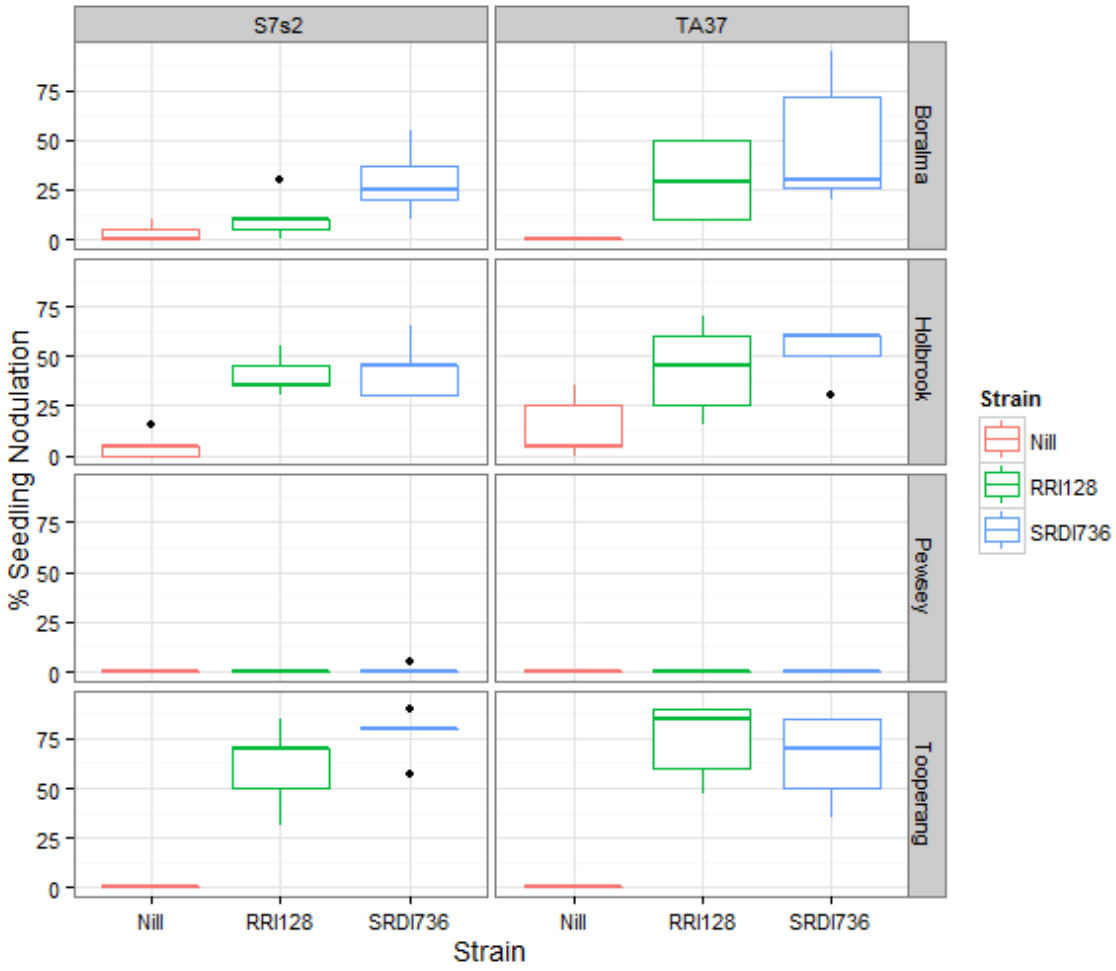
4.2.2 Nodulation in the absence of soil applied lime

4.2.2.1 % Seedling Nodulation

Nodulation was constrained across all sites and treatments, typically with fewer than 75% of plants nodulated. Even so, the variates '% nodulation', 'number of effective nodules per m^2 and 'number of nodules per nodulated plant;' were all trending towards being significant for improved nodulation with strain SRDI736 compared to RRI128 at the 5% probability level, and were significant at the 10% level (Fig. 6). There was also a trend for SARDI AT7 having higher nodulation (significant at the 10% level) with commercial strain compared to SARDI seven series 2 at Boralma, Tooperang and Holbrook.

The means and variability of % seedling nodulation for each cultivar and strain treatment are shown in Fig. 6. No nodulation was found at Pewsey Vale in the first spring after sowing, thought to be related to unfavourable surface soil moisture conditions for germination in the first two weeks after sowing.

The equal or better nodulation by new rhizobia strain SRDI736 occurred despite it always having lower numbers of rhizobia (mean 29,000 cfu/seed) than commercial strain RRI128 (mean 83,000 cfu/seed) on seed at sowing. This is consistent with previous experiments and has not so far limited the potency of the strain in nodule formation on acidic soils.



Means of percentage seedling nodulation for site, variety and strains.

		Site			
Variety	**Strain**	**Boralma**	**Holbrook**	**Pewsey**	**Tooperang**
S7s2	**Nil**	3.0	5.0	0 (0)	0
	RRI128	11.0	40.0	0 (0)	61.2
	SRDI736	29.5	43.0	0.8 (2.0)	77.4
TA37	**Nil**	0	14.0	0 (0)	0
	RRI128	29.9	43.0	0 (0)	74.4
	SRDI736	48.4	52.0	0 (0)	65.0

Fig. 6 Percentage seedling nodulation SARDI 7S2 and TA37 with rhizobia treatments nil (no rhizobia control), RRI128 (current commercial strain) and SRDI736 (acid tolerant strain) at Pewsey Vale, Tooperang, Holbrook and Boralma evaluation sites.

Given the level of soil acidity (pH 4.1-4.3), the % nodulation achieved exceeded expectations with both strains. Observations of plant roots indicated a loss of most fine roots and nodules over summer, providing the conditions to test the persistence of rhizobia in the soil and their capacity to re-nodulate established plants. We therefore expected improved discrimination between the strains of rhizobia given re-nodulation by the commercial strain will not be assisted by the lime applied to seed as it was in year 1.

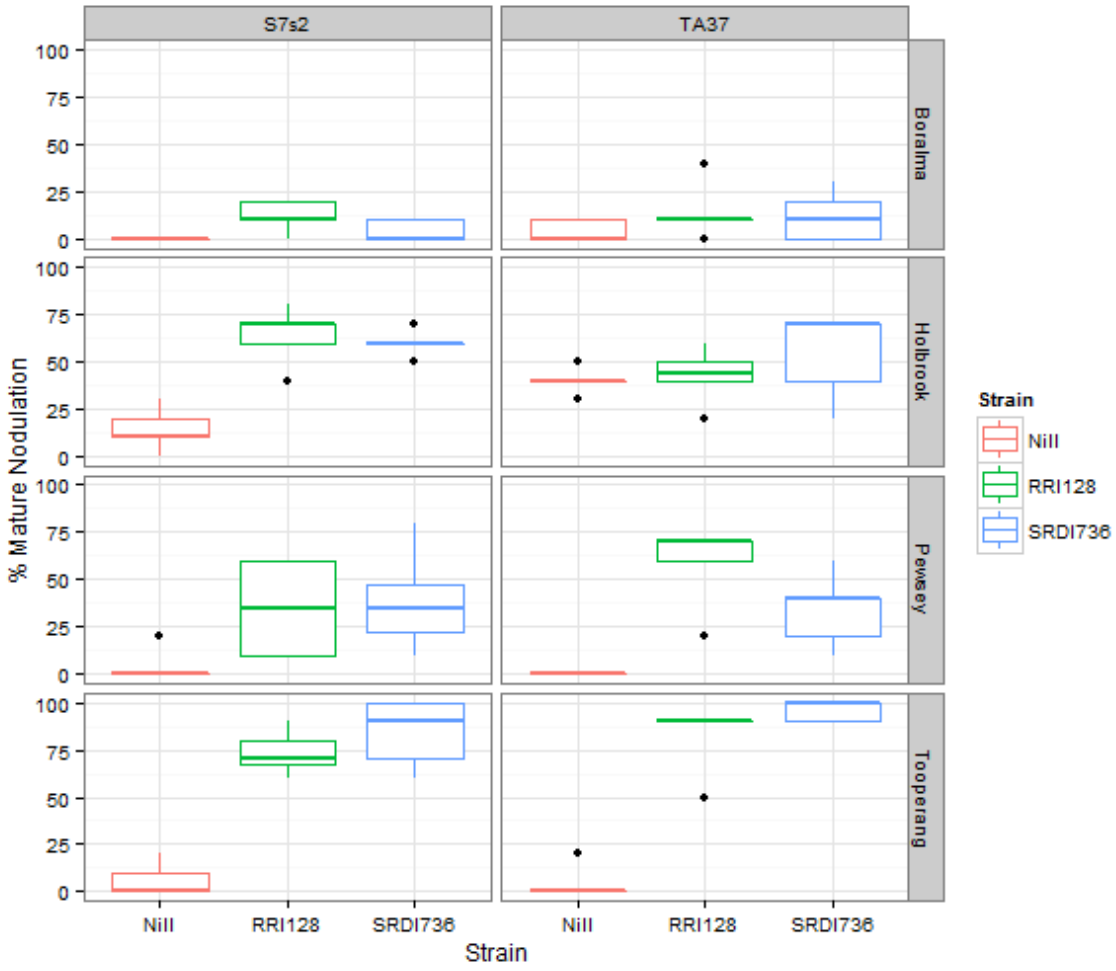
The results indicate that each of the sites are highly suitable for Experiment 2, where different lime treatments will partially alleviate the acidity stress, creating a range of topsoil pH. The lime treatments will enable the development of a pH response curve which will better define the pH range where the plant and rhizobia genotypes have greatest impact.

4.2.2.2 Mature Nodulation

The nodulation of established plants in late autumn following dry and hot summer conditions typical of these environments is shown in Fig.7. This measurement of nodulation provides an indication of the rhizobia's saprophytic competence in the soil/root rhizosphere over summer, and their capacity to re-nodulate new fine roots when the plant begins to re-grow. The percentage nodulation in mature plants increased from the measurement taken on seedlings at all sites with the exception of Boralma, including the detection of nodulation where it was previously absent at the seedling stage at Pewsey Vale. The number of nodules per nodulated plant also increased dramatically at these three sites, from an average of 1.6 in year 1 to 20 nodules per nodulated plant in year 2. The increase in nodulation over time in all treatments indicates that any benefit arising from improved nodulation at the seedling stage is likely to diminish over time.

The interaction between site and variety was significant, with TA37 having higher nodulation than SARDI 7 Series 2 at Pewsey Vale and Tooperang, but ranked lower at Holbrook. The lower average nodulation in TA37 at Holbrook was due to the low nodulation with RRI128, as the median nodulation with SRDI736 was ranked higher for SARDI AT7 than SARDI 7 Series 2 (Fig.7).

Rhizobia strains differed significantly only at Tooperang where SRDI736 was better than RRI128 for percentage plants nodulated (90 v. 77), nodule number per nodulated plant (30 v. 19) and sum of all nodules (279 v. 147).



Means of mature plant nodulation for site, variety and strains.

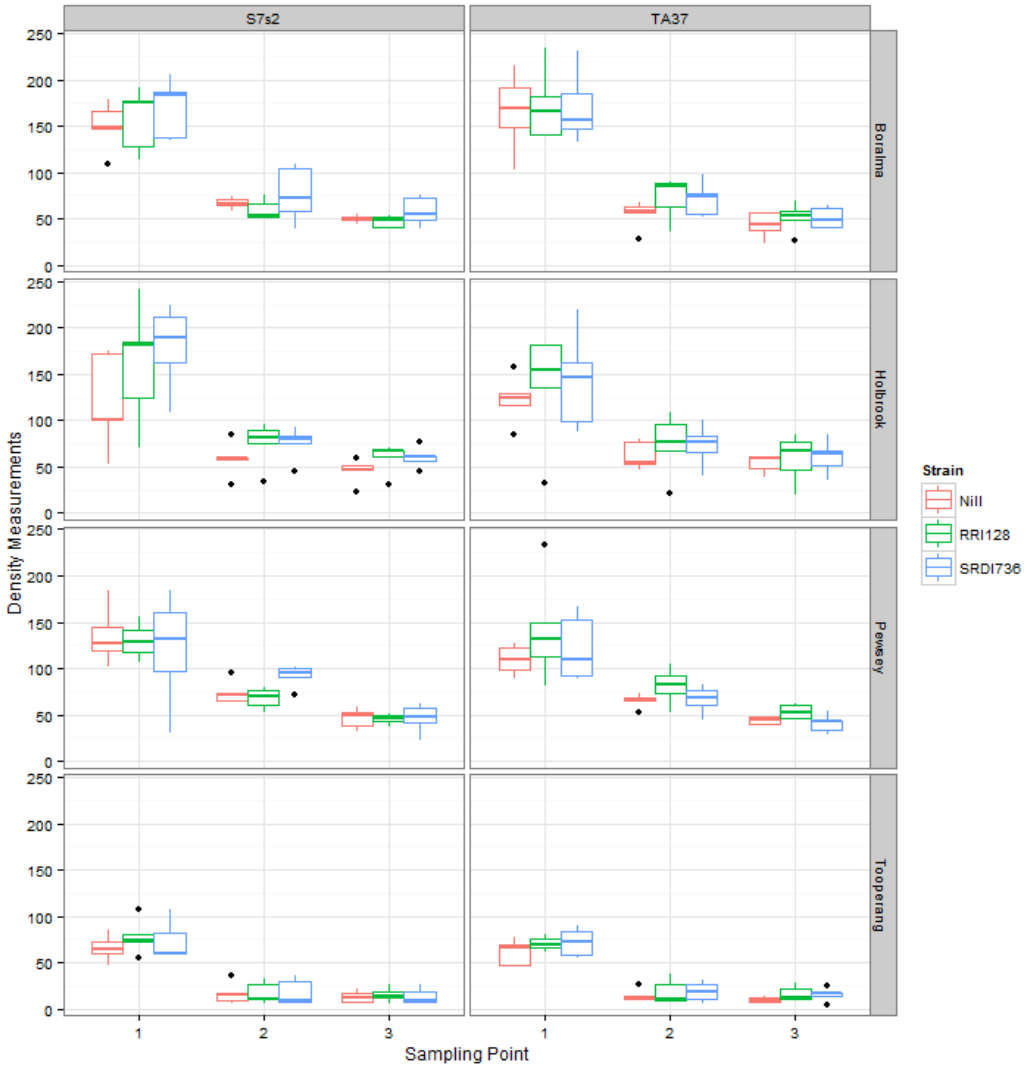
		Site			
Variety	**Strain**	**Boralma**	**Holbrook**	**Pewsey**	**Tooperang**
S7s2	**Nil**	0	14.0	4.0	6.0
	RRI128	12.0	64.0	35.0	73.4
	SRDI736	4.0	60.0	38.3	84.0
TA37	**Nil**	4.0	40.0	0	4.0
	RRI128	14.0	42.8	58.0	82.0
	SRDI736	12.0	54.0	34.0	96.0
Fprob (var.strain.site)			ns		
Average (in absence of nil treatment)					
S7s2		8.0	62.0	36.7	78.7
TA37		13.0	48.4	46.0	89.0
Fprob (var. site)			0.04		

Fig 7. Percentage mature plant nodulation SARDI 7S2 and TA37 with rhizobia treatments nil (no rhizobia control), RRI128 (current commercial strain) and SRDI736 (acid tolerant strain) at Pewsey Vale, Tooperang, Holbrook and Boralma evaluation sites.

4.2.3 Density

Lucerne had excellent establishment density on the four highly acidic sites with median density ranging from 70−180 plants/m^2 (Fig. 8). Density decline over the next 12 months (sampling points 1 and2, are at age 3 and 12 months) was in line with expectations in each environment, before stabilsing between sampling points 2 and 3 (12 to 24 months). Density at Tooperang was lower than the other sites, which also expected due its lighter texture soil, which has lower soil water storage and capacity to support plant density.

Inoculation treatment was significant (P = .01), with the nil treatment having lower plant density than rhizobia strains RRI128 and SRDI736. This is an important result as it demonstrates the importance of establishing an effective symbiosis for maintaining stand persistence. There were no differences in establishment density or persistence of the plant and rhizobia genotypes over the 27 months of the experiment.



Means and standard deviations of final plant density (den. 3) for site, variety and strains.

Variety	Strain	Boralma	Holbrook	Pewsey	Tooperang	Average
S7s2	**Nil**	49.9	45.5	46.6	13.0	38.8
	RRI128	46.9	59.2	45.3	15.4	41.7
	SRDI736	58.4	60.0	46.5	13.8	44.7
TA37	**Nil**	44.0	53.3	43.6	9.8	37.8
	RRI128	51.2	59.2	52.8	16.4	44.9
	SRDI736	50.9	60.5	40.4	15.8	41.9
Average		51.5	56.4	45.9	14.0	41.6

Fig. 8 Plant density over time (sampling points 1,2 and 3 represent initial plant density, after 12 and 24 months) of SARDI 7S2 and TA37 with rhizobia treatments nil (no rhizobia control), RRI128 (current commercial strain) and SRDI736 (acid tolerant strain) at Pewsey Vale, Tooperang, Holbrook and Boralma evaluation sites.

4.2.4 Forage Yield

The two lucerne varieties had excellent forage yield at three of the four sites (Pewsey Vale, Tooperang, and Holbrook) with between 11 and 15 t/ha produced at these sites in their first full calendar year as a mature plant. The production curve of the two varieties was similar throughout the year (Fig. 9). This was to be expected as they are both winter active class 7 varieties. However spring production of SARDI 7 Series 2 appears to be better in some environments, notably at Tooperang and Boralma (Fig. 9). In 2015, SARDI 7 Series 2 produced 14.4 t/ha at Tooperang, compared with 11.8 t/ha from SARDI AT7.

At each site lucerne is producing 50-60% of its forage yield over summer and autumn (i.e. for Tooperang, Fig. 10). This shows that lucerne is still able to supply valuable out of season production on acidic soils, despite potential limitations to its root growth. The decline in density at the conclusion of the experiment is related to a soil water deficit at each site following dry spring and summer conditions.

The forage yield for each rhizobia x plant treatment is shown in the box plot, Fig.11. Main effects of rhizobia and variety treatments were not significantly different across the four sites.

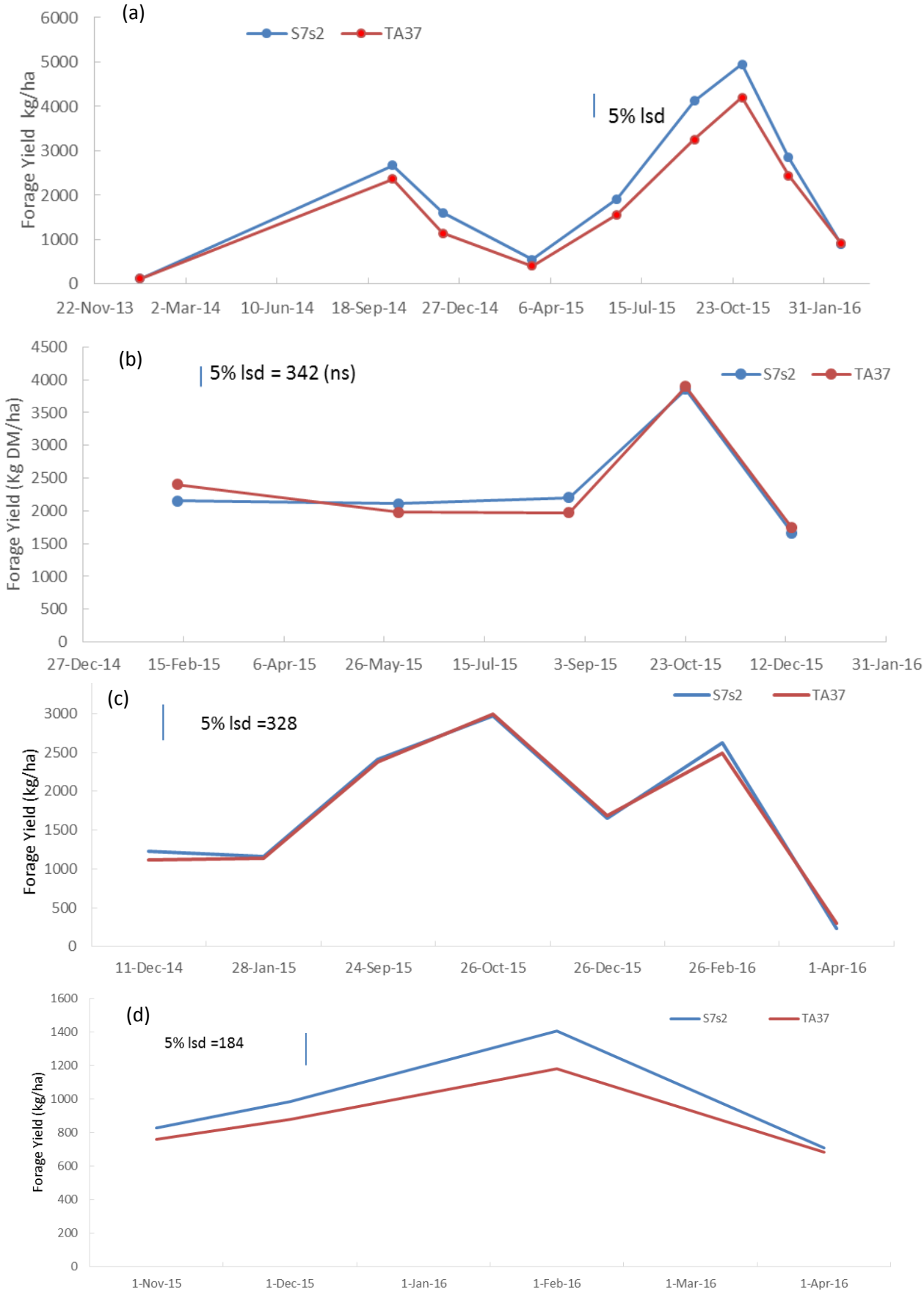


Fig. 9 Seasonal yield production at (a) Tooperang, (b) Pewsey Vale, (c) Holbrook and (d) Boralma. Average forage yields in the first calendar year were 4026 kg/ha (14510 kg/ha in 2015) at Tooperang, 11990 kg/ha at Pewsey Vale, 8189 kg/ha at Holbrook, 1725kg/ha at Boralma (2 cuts from November only)

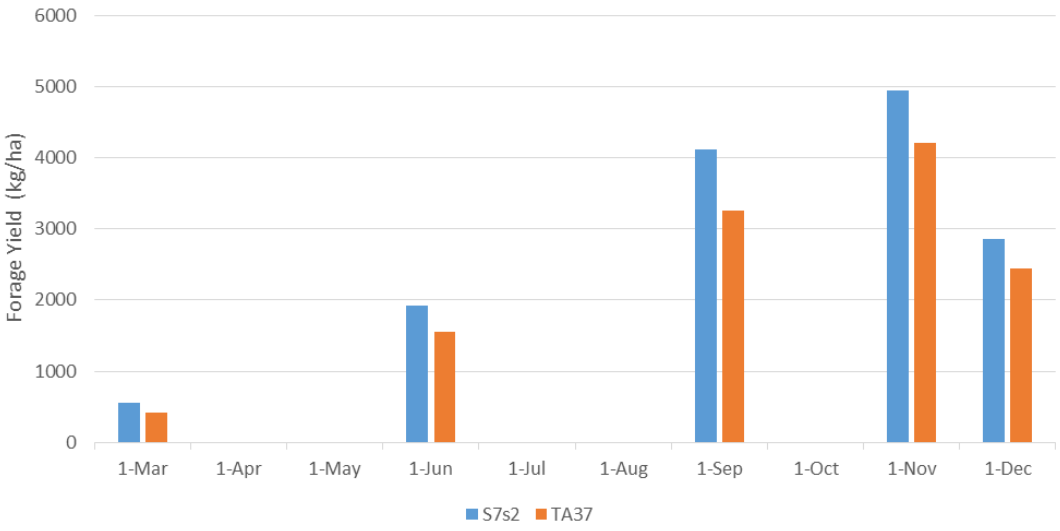


Fig. 10 Lucerne still extends the growing season and gives summer feed at Tooperang. Decile 1-2 spring inducing a soil water deficit from mid-September. S7s2 produced 14.4 t/ha in 2015 versus 11.8 t/ha from TA37.

The forage yield for each variety and strain treatment is shown in Fig. 11. The nil strain treatment had significantly lower yield than RRI128 and SRDI736, which again illustrates the importance of an effective symbiosis for maintaining a productive lucerne stand.

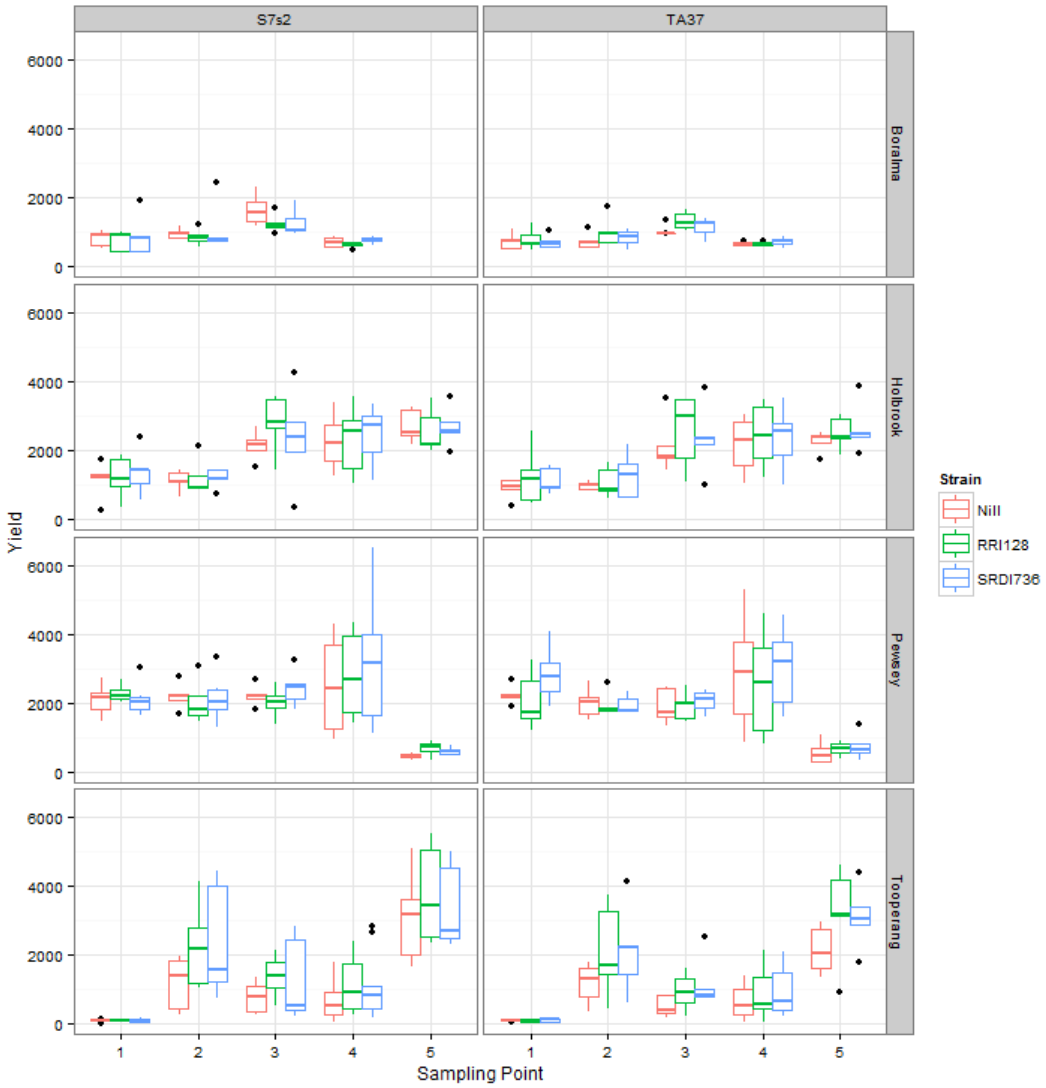


Fig 11. Lucerne forage yield over time of SARDI 7S2 and TA37 with rhizobia treatments nil (no rhizobia control), RRI128 (current commercial strain) and SRDI736 (acid tolerant strain) at Pewsey Vale, Tooperang, Holbrook and Boralma evaluation sites.

4.2.5 Nitrogen Fixation

Nitrogen fixation of the lucerne re-growth after cutting was measured by the ^{15}N natural abundance method at all sites, on a single forage cut late in 2015.

Results for N content (%) and N fixed (% and kg/ha) are shown in Table 3. Across all sites, lucerne inoculated with SRDI736 fixed 36 kg/ha N, compared to 29 kg/ha when inoculated with RRI128, but these values were not significantly different (Table 3). In the multisite analysis the only significant difference between the inoculant strains was positive for N content at Boralma. There was no significant of effect of lucerne line on any of the N measures (individual lucerne genotype data not shown).

Because there were large differences in lucerne production at the different sites, statistical comparisons have also been made at the individual sites and are summarised in Table 6.

Lucerne re-growth was vigorous at Pewsey Vale (>3500 kg/ha) and there were significant effects of inoculation treatment at this site. Rhizobia strain SRDI736 performed better than strain RRI128. Across the range of N measures, SRDI736 performed better than RRI128 for % N fixed (39 v. 32), ^{15}N delta (6.2 v. 7.1), showed a trend of improvement for kg/ha N fixed (42 v. 33) and produced similar percent N content. There was no significant effect of lucerne line.

Even though lucerne production was low (966 kg/ha) at Boralma, SARDI 7 lucerne inoculated with SRDI736 fixed more of its N (41%), compared to when inoculated with RRI128 (12%).

There were no significant treatment effects on plant N at Holbrook or Tooperang.

Table 3. Main effect of rhizobia strain on measures contributing to the amount of nitrogen fixed (kg/ha) at the 4 field sites.

Site	Lucerne growth (kg/ha)		N content (%)		N fixed (%)		N fixed (kg/ha)	
	RRI 128	SRDI736	RRI 128	SRDI736	RRI 128	SRDI736	RRI 128	SRDI736
Pewsey Vale	3703	4166	2.8	2.6	32	39[b]	33	42
Tooperang	4803	4769	2.3	2.6	69	62	74	82
Holbrook	1587	1842	2.1	2.1	57	69	20	26
Boralma	952	981	2.6	2.8[a]	25	36	8	12
All sites	2534	2736	2.4	2.5	44	50	29	36

[a]Paired values significantly different in multi-site analysis or [b]in single site analysis.

4.2.6 Amending the acidity stress of lucerne with lime

4.2.6.1 Impact of lime on soil pH

The application and cultivation of lime with a rotary hoe was successful at increasing soil pH in the 0-10cm. Although differences in pH were not measured at the low lime application rate, increases in Ca (meq/100g) were measured at all sites.

Tooperang: The low lime treatment had negligible effect on soil pH, but the 1400 and 2800 kg/ha rates increased soil pH in the 0-10cm from 4.3 to 4.5 and 4.7 respectively. No response to lime was measured below the 0-10cm zone.

Pewsey Vale: The low lime had a small or negligible effect, lifting pH by 0.1 of a unit (from 4.2 to 4.3). Further increases in 0-10 pHCa were made with 1400kg.ha (4.4) and 2800 kg/ha (pH 4.7).

Holbrook: The lime treatments were measured to have only a small effect on soil pH, increasing pH_{Ca} by less than 0.3 pH unit across all lime rates and soil layers. The concentration of aluminium (%CEC) indicates that lime did have an effect at this site, declining from 18 to 10 and 11 with increasing lime rates. The concentration of Mn has also been decreased with the application of lime.

Boralma. The low lime treatment had a substantial effect on soil pH, increasing pH from 4.3 to 4.7. The higher lime rates increased soil pH in the 0-10cm further to 5.0 and 5.2 respectively. A response to lime was measured below the 0-10cm zone with similar increases

in pH at 10-20cm and 20-30cm. Aluminium was reduced from 23-33 to 7% of the CEC in the 0-10 and 10-20cm soil layers with the addition of 700 and 1400kg/ha lime. Sodium as a percentage of the CEC and the Na absorption ratios for Boralma indicate that the surface soil is close to being classified as sodic. The subsoil (>40cm) at Boralma is sodic (data not shown).

4.2.6.2 Impact of pH on seedling nodulation

An analysis of seedling nodulation versus pH (factor = variety*pH) shows an improvement in nodulation in TA37 x SRDI736 compared with SARDI 7 S2 x RRI128 in soils with pH 4.1-4.4 (Fig. 12). Nodulation of SARDI 7 S2 with RRI128 was constrained below pH 4.8 (80% nodulation), whereas TA37 x SRDI736 achieved the same level of nodulation at pH 4.3. The difference between the two variety/strain combinations increased as pH decreased further. These results are consistent with the findings of the solution culture experiment reported in section 3.2.

The equal or better nodulation by new rhizobia strain SRDI736 once again occurred despite it always having lower numbers of rhizobia on seed (mean 25,000 cfu/seed) compared to the commercial strain RRI128 (mean 68,000 cfu/seed).

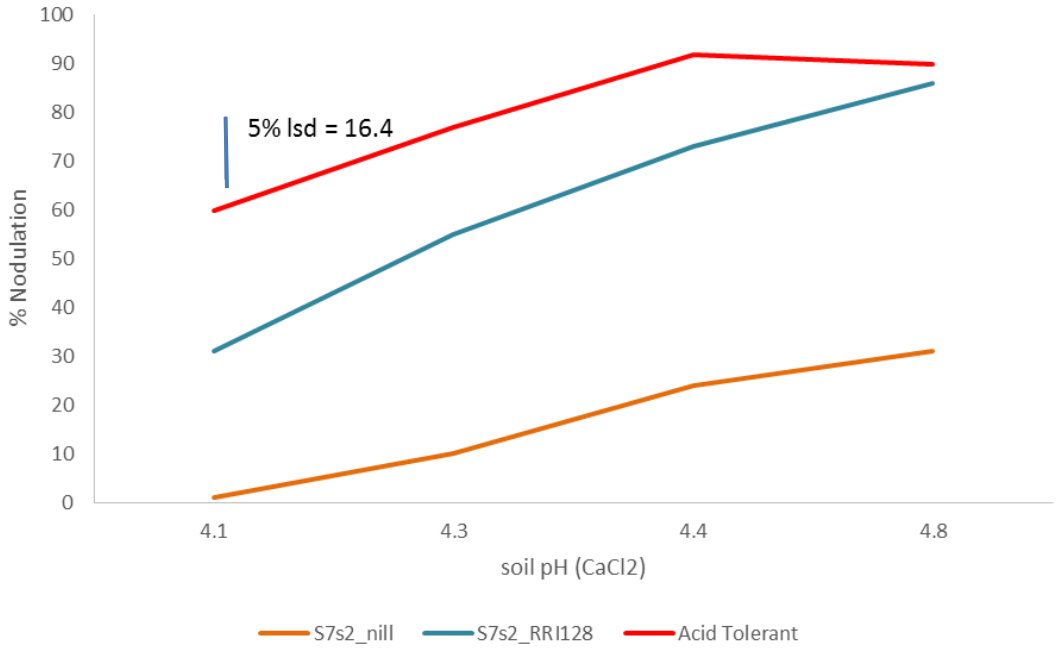


Fig. 12 Analysis of percentage seedling nodulation in SARDI 7S2 x nil rhizobia (S7S2_nill), SARDI 7S2 x RRI128 (current commercial cultivar and strain) and TA37 x SRDI736 (acid tolerant combination of variety and strain) by soil pH averaged across Pewsey Vale, Tooperang, Holbrook and Boralma evaluation sites.

Saprophytic competence (ability of the strains to persist and colonise the soil) was also estimated in the 2nd year plots for the no lime and lowest lime treatments. The lower limit of the MPN assay used is approximately 1 rhizobia/g soil. Lucerne rhizobia were detected in 13 of the 72 assays. None were detected at Boralma or Pewsey Vale. At Holbrook, SRDI736 was detected twice and RRI128 once. At Tooperang, SRDI736 was detected four times and

RRI128 six times. The implication of these results is that soil colonisation by the strains has been negligible and that re-inoculation is mandatory on these soil even where lucerne has previously been grown.

4.2.6.3 Impact of lime on nitrogen fixation

Nitrogen fixation of the lucerne re-growth after cutting was measured by the ^{15}N natural abundance method at all sites, on a single forage cut late in 2015.

Across all sites, there were consistent and positive responses of lucerne regrowth and N_2-fixation to the application of lime and the practice of inoculation. Overall (averaged across all treatments and sites) the addition of lime and consequent mean increase in pH (from 4.3 to 5.0) increased the amount of nitrogen fixed from 32 to 51 kg/ha (+59%). This accrued from increased DM production (2225 to 2835 kg/ha, +27%), increased N content (2.6 to 2.7%) and an increase in the percentage of N derived from symbiotic fixation (35 to 52%). Responses were greatest at the lowest lime rate, where the amount of N fixed increased from 16 to 38 kg/ha (+138%).

Because there were large differences in the amount of N fixed at the different sites, treatment comparisons at the individual sites were explored, but in concert with the multi-site analysis no additional improvement from either the new lucerne line (TA37) or new strain of rhizobia (SRDI736) were measured. The extent of the variation in the amount of nitrogen fixed at the different sites, and effect of lime rate is shown in Table 4.

Table 4. Effect of inoculation treatment and lime rate on the amount of nitrogen fixed (kg/ha) by SARDI 7 Series 2 at the four sites.

	Low lime rate (600 to 700 kg/ha)		Mod. lime rate (1200 to 1400 kg/ha)		High lime rate (2400 to 2800 kg/ha)		Mean of lime rates	
	RRI 128	SRDI736	RRI 128	SRDI736	RRI 128	SRDI736	RRI 128	SRDI736
Pewsey Vale	103	131	126	125	135	117	121	124
Tooperang	23	23	49	49	53	36	42	37
Holbrook	7	10	13	12	27	33	16	18
Boralma	2	1	3	2	3	4	2	2
Mean of sites	34	43	48	47	54	47	45	46
Site × rhizobia	39		48		51		46	

At Holbrook and Boralma the amounts of fixed N were low due to both low dry matter production (<500 kg/ha) and a low percentage N fixation (22% at Holbrook and 16% at Boralma).

Positive response to both lime application and inoculation indicate that, in general, the field sites provided the conditions needed to demonstrate better N_2-fixation resulting from improved acidity tolerance. Having said this, low dry matter production at two of the sites combined with obvious spatial variation at all sites is likely to have limited the potential of some responses.

Even so, the two lucerne lines (SARDI 7 and TA37) were always similar with regard to their nitrogen content, percentage and amount of N fixed and also in the way they responded to increasing pH with the application of lime.

Whilst no significant N fixation benefits between rhizobia strain were evident in the lime trials, to some extent this can be interpreted as showing that RRI128 performed better with the addition of lime. The implication here is that any advantage of SRDI736 is more likely when soil pH is less than 4.5 (Fig. 13).

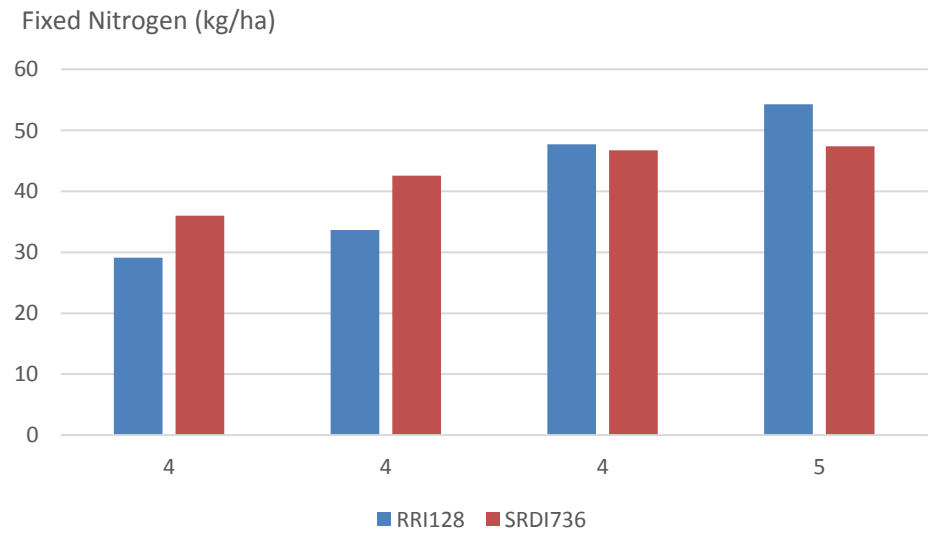


Fig. 13 Effect of rhizobia strain on the relationship between the soil pH (0.01 M $CaCl_2$) and the amount of nitrogen fixed in a late-spring forage cut. Data are means of rhizobia and lime trials. A decrease in pH from 4.54 to 4.26 decreased average N fixation by 47% with strain RRI128, compared with 23% in SRDI736.

The results provide some insight into the relationship between the amounts of N fixed and soil pH. With strain RRI128, the amount of N fixed declined by 47% between pH 5.02 and 4.26 (R^2 =0.998), compared with 23% in SRDI736 (an average increase of 35%).

4.2.6.4 Impact of lime on forage yield

Lime increased forage yield at all sites, with the high rate increasing production on average by 22% or between 700kg and 2800kg over 24 months. The biggest responses of forage yield to lime were at Tooperang, Holbrook and Pewsey Vale.

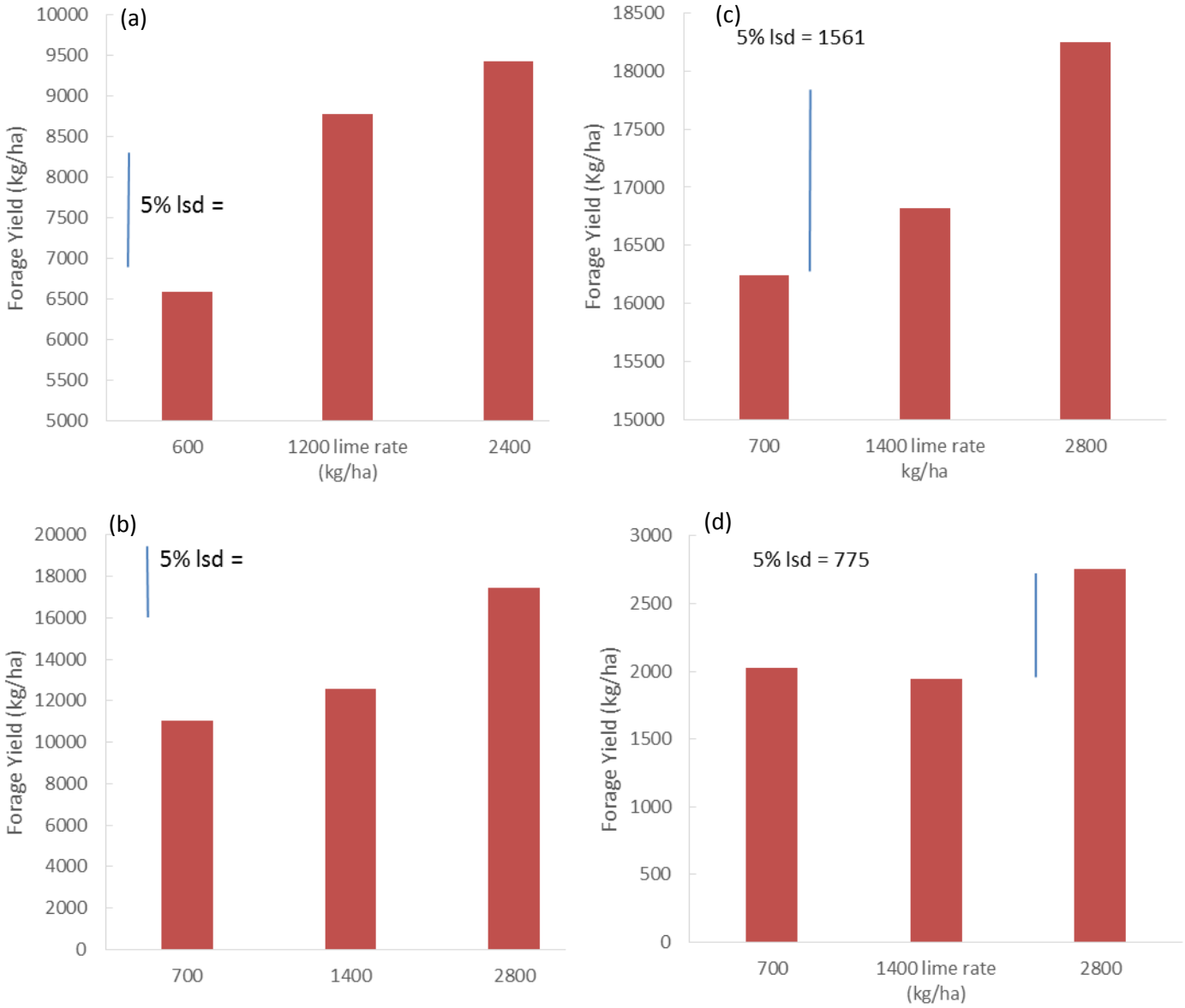


Fig. 14 Lime increases cumulative production at Tooperang (a), Holbrook (b), Pewsey Vale (c), and Boralma (d). Average yields across sites a-c were 11290kg/ha for low lime, 12700kg/ha for mid lime and 15000 kg/ha for high lime (data is for first 15 months after establishment and includes approximately 1 t/ha cut in Dec2014).

4.2.6.5 Impact of lime on lucerne persistence

The impact of lime on final density (a measure of persistence) was only positive at Tooperang and Holbrook. The benefit was greatest at Holbrook, with persistence approximately doubling with every increase in 1400kg/ha of lime from the base level of 700kh/ha. At Tooperang, there was a benefit of lime for the first 1200kg/ha but no further increase in persistence with additional lime application. There was little or no clear trend for applying lime at Pewsey Vale or Boralma. Despite this, Pewsey Vale and Boralma had the highest average plant densities at the conclusion of the experiment (Fig. 15).

The results indicate that a minimum of 1.2t/ha is used to ameliorate highly acidic sands, and that around 2.5t/ha it's used on heavier soils. The Boralma site is an exception to this rule, and it highlights the need to examine all of the soil characteristics (such as a lack of structure and history of waterlogging), when considering the suitability of lucerne.

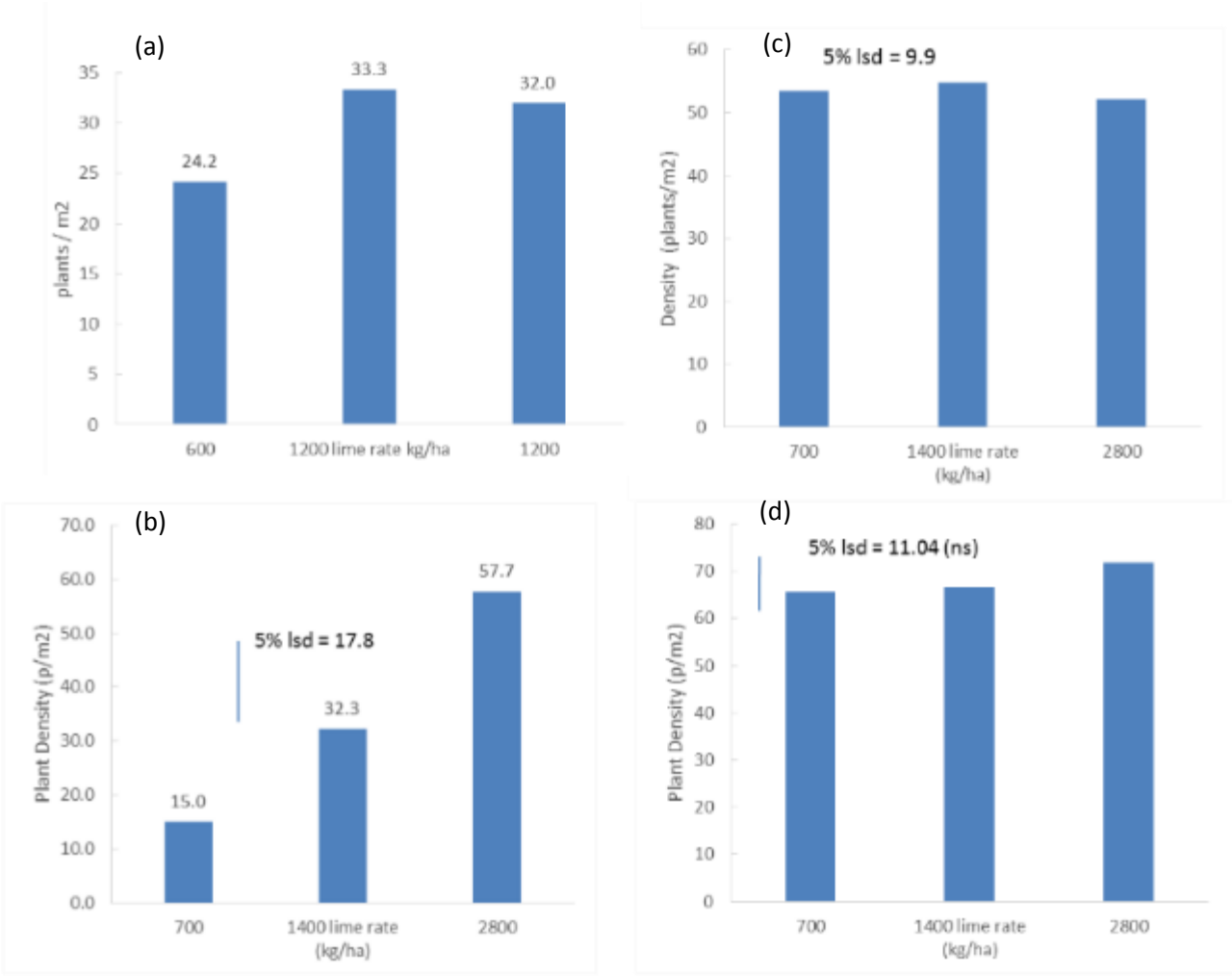


Fig. 15 Lime increases persistence of lucerne at Tooperang (a) and Holbrook (b), but had no impact at Pewsey Vale (c) or Boralma (d).

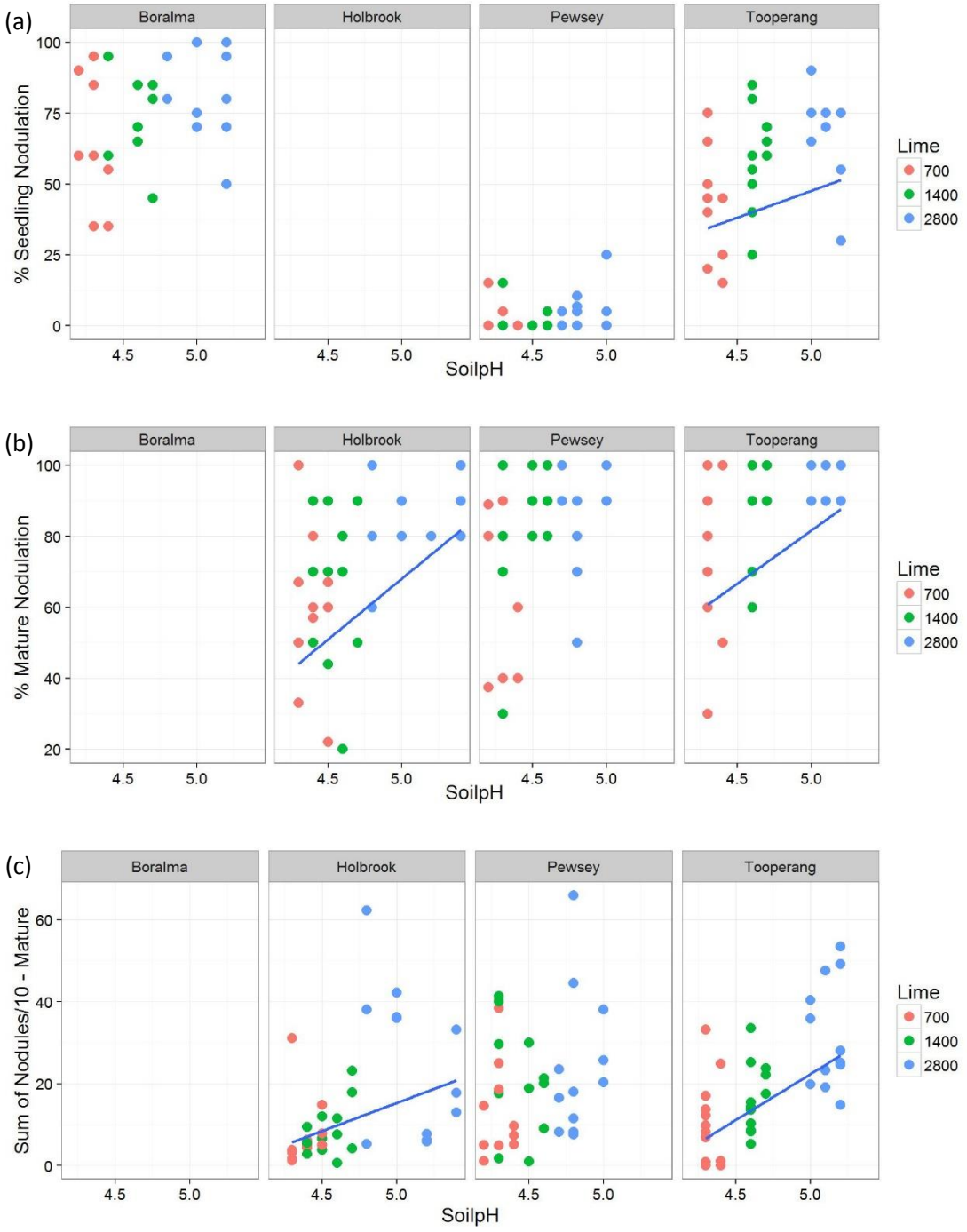
4.2.7 Correlation analyses and linkage of traits

Nodulation was positively correlated with soil pH at Boralma and Tooperang at the seedling stage (Fig. 16a) and at all sites at the mature plant stage (Fig. 16 b, c). Mature plant nodulation percentage was generally adequate (>80%) at pH_{Ca} 4.5 but quickly decreased with increasing acidity below this level. Whilst the number of nodules per nodulated plant increased with pH above pH_{Ca} 4.5 at Holbrook and Tooperang (from an average of 15 across all site to >25), the amount of nodulation on plants pH_{Ca} 4.5 is also considered satisfactory.

The results of this project suggest that soils with pH_{Ca} ≥4.5 do not substantially constrain the nodulation of SARDI Seven S2 or TA37 inoculated with strains RRI128 or SRDI736.

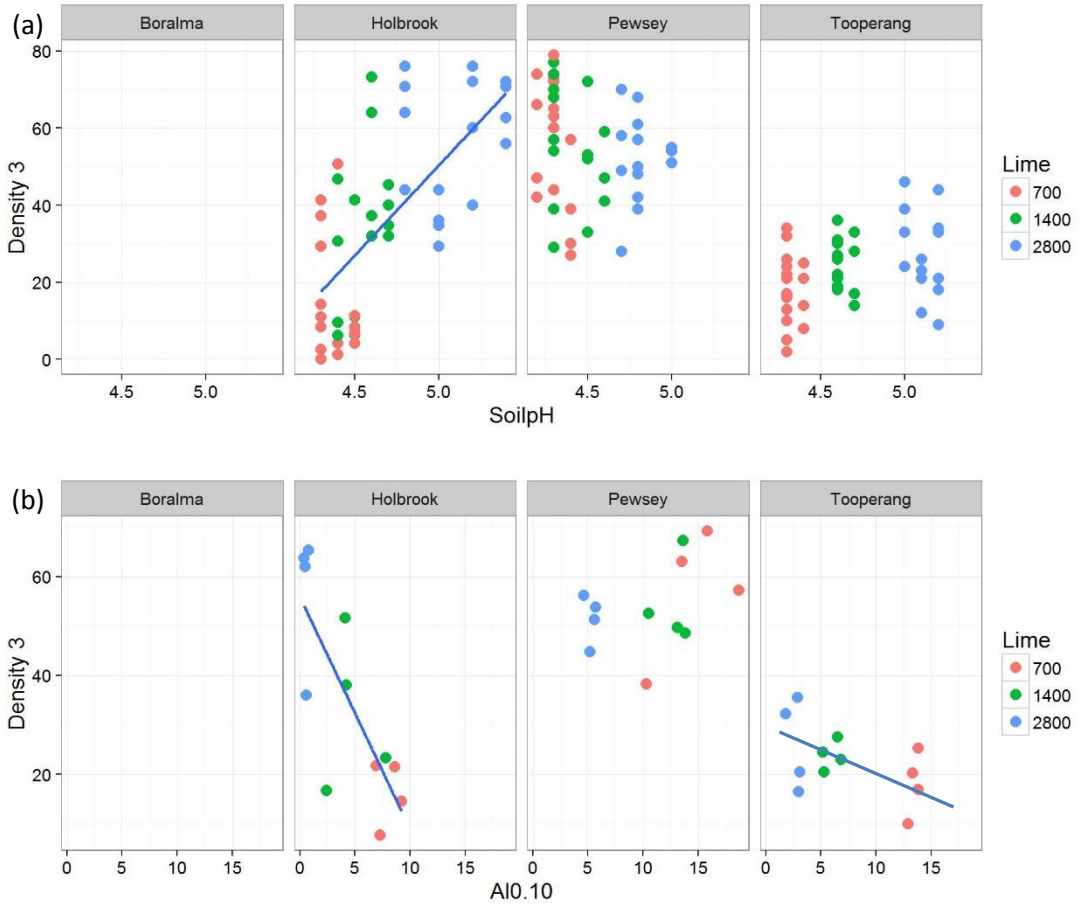
4.2.1 Density 3 versus Soil pH and aluminium

Final plant density (density 3, 27 months after sowing) was positively correlated with 0-10 cm soil pH at Holbrook and Tooperang, but not at Pewsey Vale (Fig. 17). The slope of the graph was much greater at Holbrook, indicating a greater reliance on lime at this site. The spread of the data indicates that a pH_{Ca} of around 4.5 is critical for ensuring persistence in SARDI Seven Series 2 and TA37 lucerne varieties.



	% Seedling nodulation (a)			% Mature nodulation (b)			NNP- Mature (c)		
Site	**Bor**	**Pew**	**Toop**	**Hol**	**Pew**	**Toop**	**Hol**	**Pew**	**Toop**
Slope	17.25	6.05	25.2	30.6	22.9	24.3	169	122	262.6
P-value	0.08	0.11	0.01	0.002	0.09	0.004	0.01	0.23	<0.001
R-value	0.3	0.26	0.42	0.49	0.28	0.48	0.4	0.2	0.65

Fig. 16 Seedling (a) and mature plant nodulation (b,c) is positively correlated with soil pH at Boralma (Bor), Pewsey Vale (Pew), Tooperang (Toop) and Holbrook (Hol). Seedling nodulation at Holbrook and mature plant nodulation at Boralma were not measured.

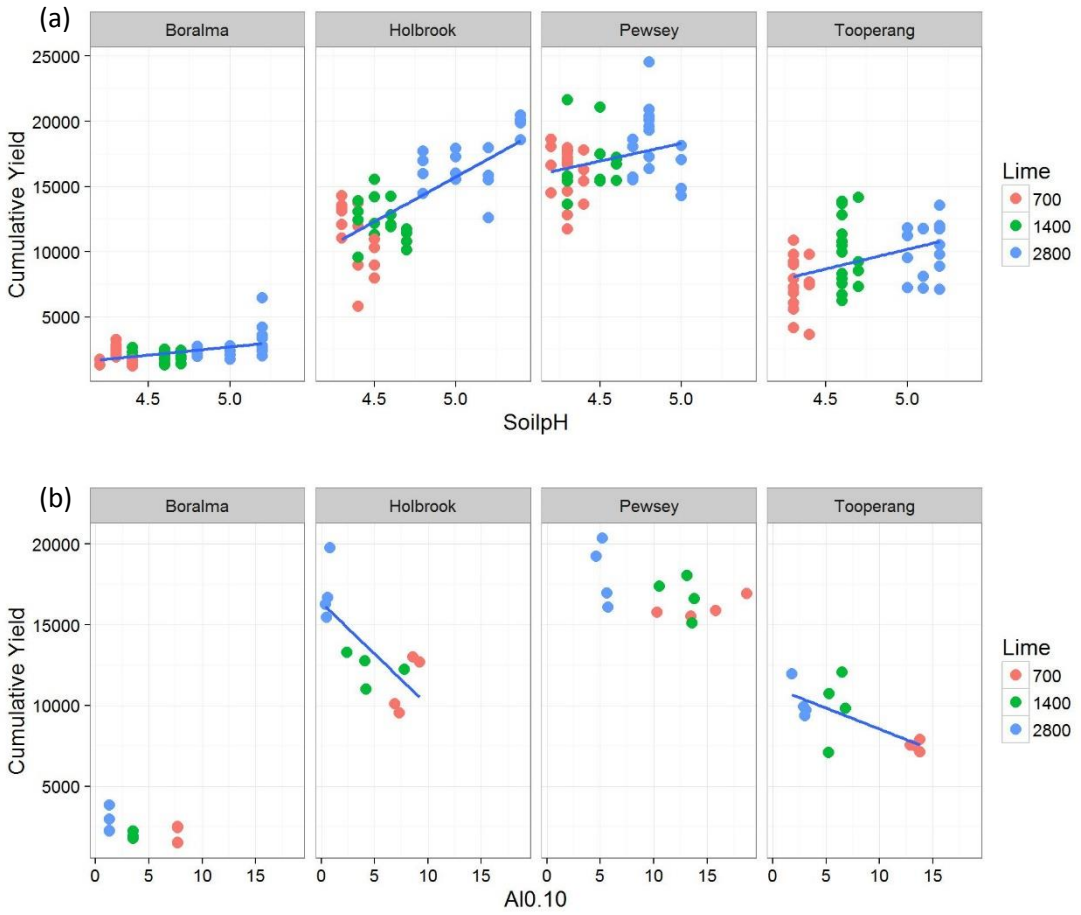


	soil pH 0-10 x density			aluminium 0-10 x density		
Site	**Holbrook**	**Pewsey**	**Tooperang**	**Holbrook**	**Pewsey**	**Tooperang**
Slope	-4.7	0.83	-0.74	46.8	-12.8	8.1
P-value	0.003	0.17	0.1	<0.001	0.12	0.05
R value	0.77	0.42	0.50	0.68	0.22	0.28

Fig. 17 Lucerne persistence (density after 3 years) is positively correlated with soil pH (a) and negatively correlated with soil aluminium (b) at Holbrook and Tooperang. No correlation was found between soil pH or aluminium and plant persistence at Pewsey Vale (Boralma site not old enough).

4.2.2 Cumulative forage yield versus Soil pH and aluminium

Cumulative forage yield was positively correlated with 0-10 soil pH at all sites, with the slope of the relationship between 12476 and 68773 kg/ha per unit of pH (Fig. 18). Although forage yield was considered good to excellent (with the exception of Boralma) in the absence of lime, the positive correlation with pH shows that increases in forage yield are available (particularly at Holbrook) by ameliorating the acidity stress.



	soil pH_{Ca} 0-10 x forage yield				Aluminium 0-10 x forage yield			
Site	**Bor**	**Hol**	**Pew**	**Toop**	**Bor**	**Hol**	**Pew**	**Toop**
Slope	1247	6873	2729	3003	-111.8	-636.7	-172.9	-259.8
P-value	0.0007	<0.001	0.05	0.006	0.12	0.006	0.09	0.01
R value	0.48	0.72	0.28	0.40	0.47	0.73	0.51	0.69

Fig. 18. Cumulative yield of SARDI 7 Series 2 and TA37 is positively correlated with (a) soil pH at at Boralma (Bor), Holbrook (Hol), Pewsey Vale (Pew) and Tooperang (Toop) and negatively correlated with (b) soil aluminium (0-10cm) at Holbrook and Tooperang.

4.2.3 Correlation of soil pH and soil aluminium 0-10

Aluminium concentration as a percentage of the cation exchange was highly, negatively correlated with pH (Fig. 19).

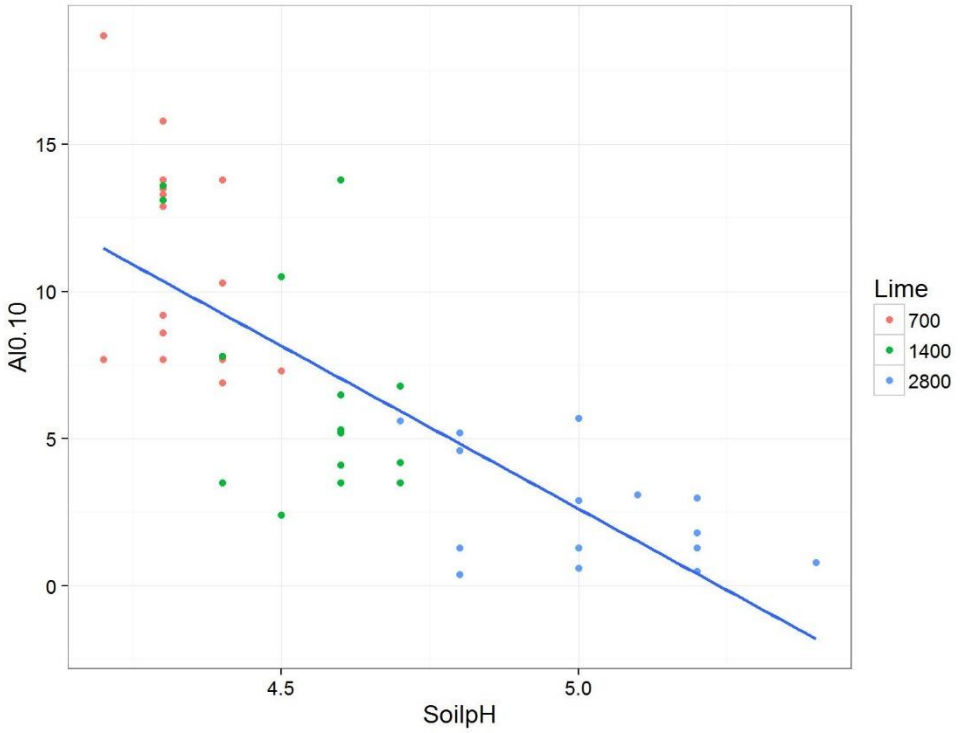


Fig. 19. Aluminium concentration as a percentage of the cation exchange is highly negatively correlated with soil pH. Data is pooled across sites from the lime treatments at Tooperang, Pewsey Vale, Holbrook and Boralma. (Fprob <0.001, r = 0.77).

4.2.4 Correlation analysis - Summary

A summary of the correlation of traits measured in the lime experiments at Tooperang and Holbrook is shown in Fig. 20. The analysis shows that several of the traits are closely linked (like % nodulation, number of nodules per nodulated plant, and sum nodules) are similarly correlated with the soil properties, pH, aluminium, and the depths of aluminium measurement.

The analysis shows that pH (+ve) and aluminium (-ve) are correlated with nodulation, persistence (density3) and cumulative forage yield. The 0-10 cm Aluminium has the strongest correlation with these traits at Holbrook, whereas the 10-20cm is more important for density and cumulative yield at Tooperang, probably reflecting the different distribution of aluminium in these two soils (Al as a percentage of the CEC was much higher in the 10-20cm than in the 0-10cm profile at Tooperang)

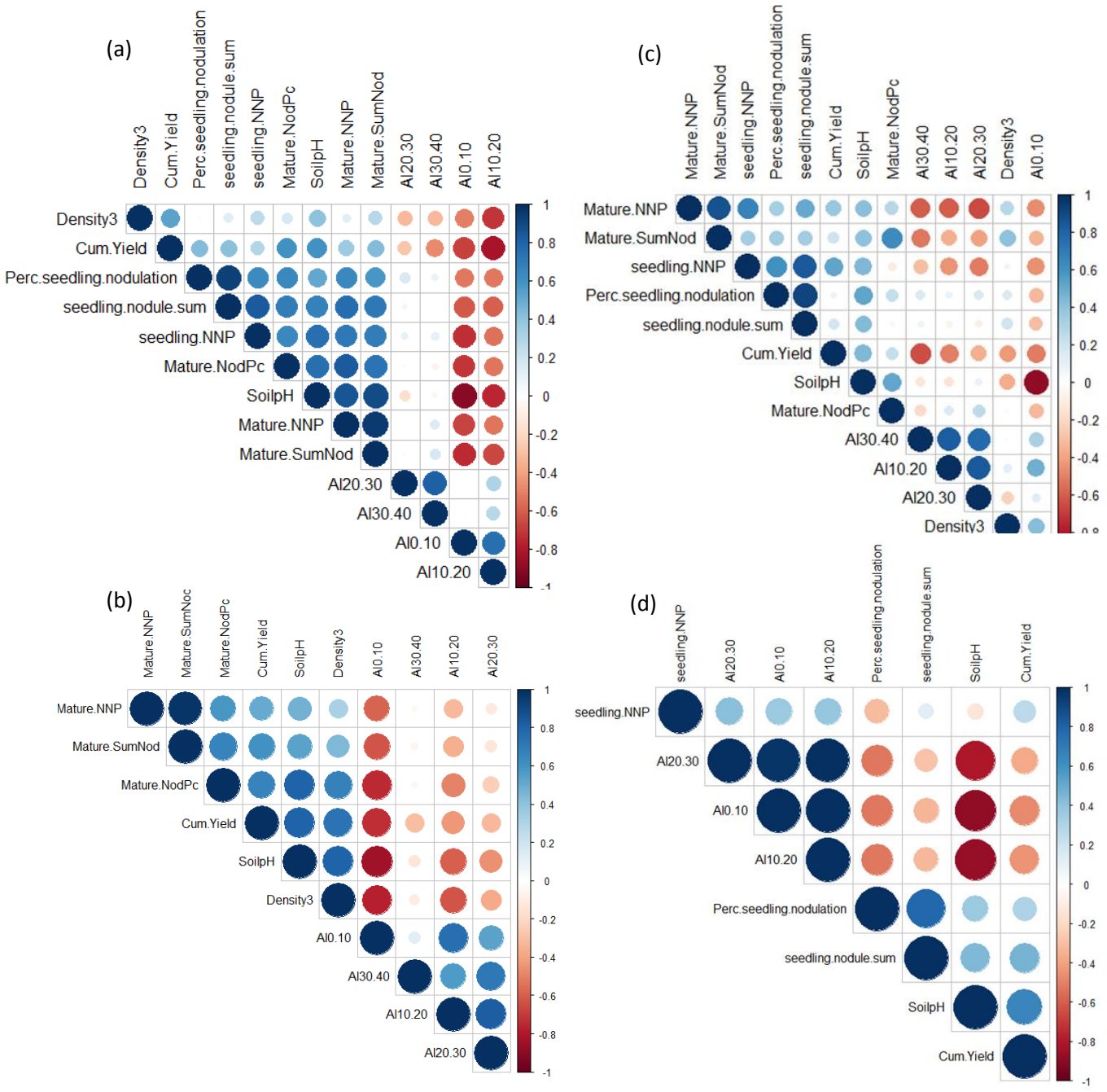


Fig. 20 Correlation analysis of main traits measured at Tooperang (a), Holbrook (b), Pewsey Vale (c), and Boralma (d). The most highly correlated traits are represented by the larger, darker circles (for example soil pH is highly positively correlated with mature nodulation percentage), where blue is positive and red is a negative correlation. NNP =number of nodulated plants, Cum. = cumulative, Perc and Pc = percentage, Al = aluminium at depths 0-10 (0.10), 10-20, 20-30 and 30-40.

A summary of correlations between the three main measurements of % mature nodulation, density3 and cumulative yield and soil pH and soil aluminium are shown in Table 5. Increasing soil pH through use of lime to remove acidity stress (concentration of H^+ and aluminium toxicity) increases plant density, cumulative yield and % mature nodulation.

Table 5. The impact of soil pH and aluminium on density, yield and nodulation

	Density3		Cumulative Yield		% Mature Nodulation	
	Soil pH	Aluminium	Soil pH	Aluminium	Soil pH	Aluminium
Boralma	-	-	Positive	Negative	-	-
Holbrook	Positive	Negative	Positive	Negative	Positive	Negative
Pewsey	Not significant	Not significant	Positive	Negative	Positive	Negative
Tooperang	Positive	Negative	Positive	Negative	Positive	Negative

4.2.5 Principal Component Analysis

The PCA analysis showed that 72% of the data variability measured is controlled by two groups of variation (Fig. 21). The first dimension explains 45% of the variability, and shows that the measurements of seedling nodulation are very different to mature nodulation, density and yield (Fig. 22). The second dimension explains an additional 27% variability, and pulls apart density and yield from the measurements of mature nodulation. The information shows that measurements of density and yield report very similar information, which is encouraging given that lucerne breeders focus on measuring plant density over time as the driver for lucerne production. The different measures of seedling and mature nodulation are also clustered, indicating that one of the measurements from each of these groups could also be used to adequately explain the variation in this study (Fig. 22). The length of the vectors on this graph also show that the traits measured are important for explaining the variability in the data (Fig. 22).

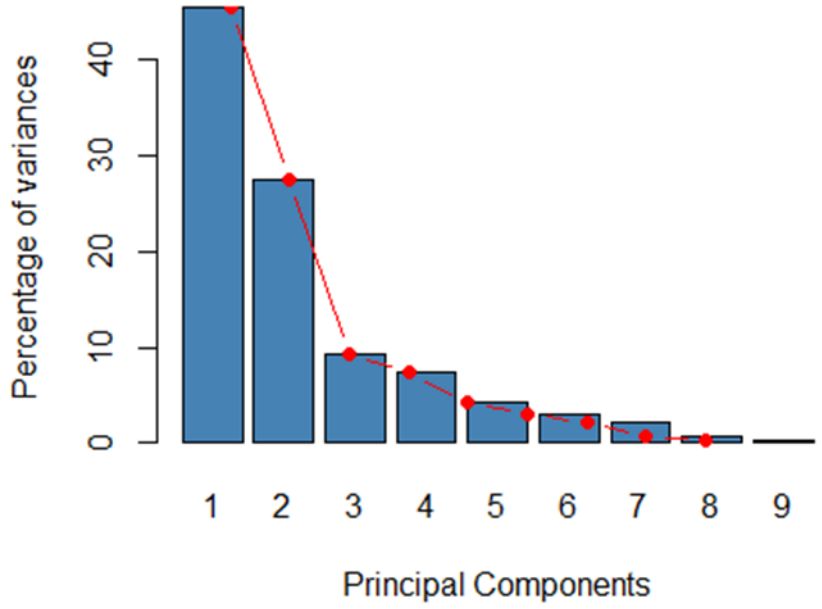


Fig. 21 Principal components derived from the data and their percentage of variances. Seventy percent of the variability measured is controlled by two groups of variation.

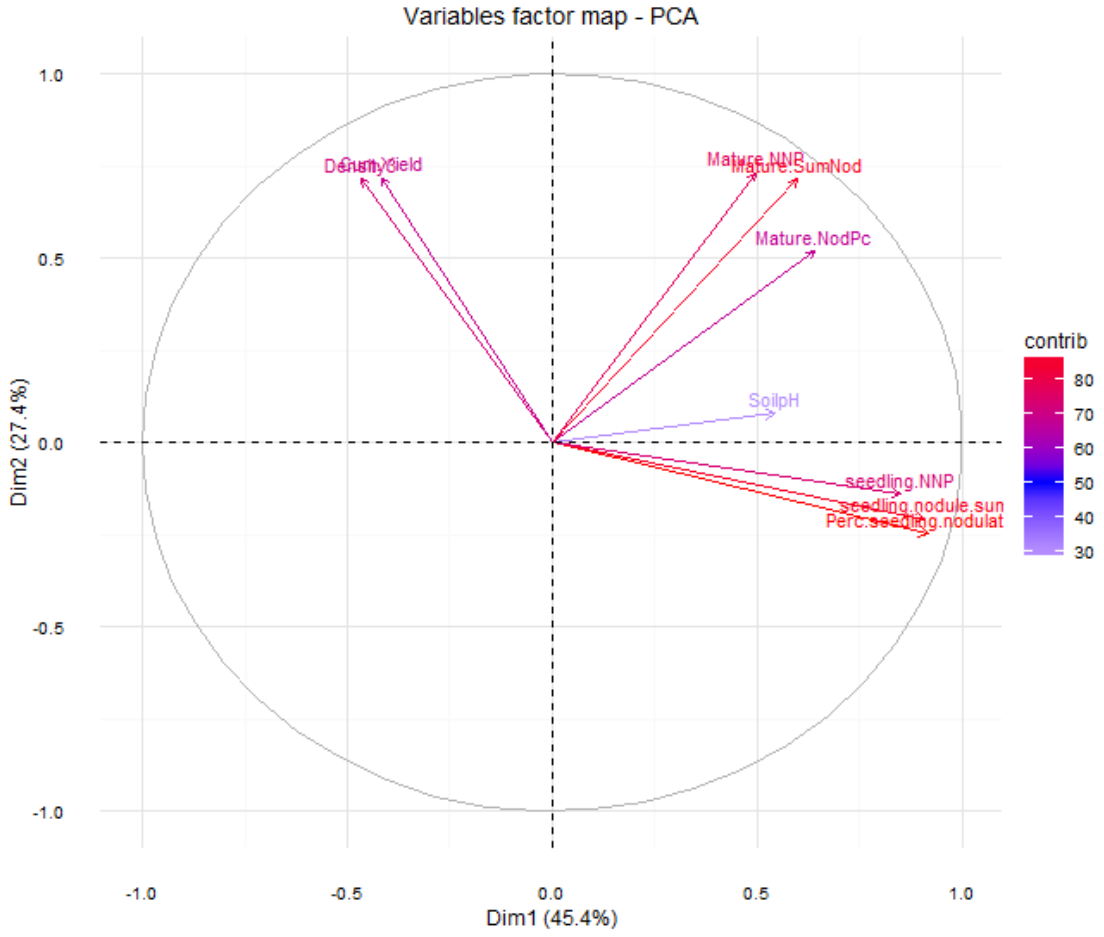


Fig. 22 Principal component analysis vector map showing relatedness of traits

5 Discussion

5.1 Tolerance of lucerne to acidic soils

The primary aim of this research was to define the performance of lucerne and rhizobia selected for improved tolerance to soil acidity across a range of environments with acid soils in south eastern Australia. Four sites in SA, Vic. and NSW with pH_{Ca} 4.1-4.3 were chosen with contrasting texture, aluminium and fertility, and this combined with a treatment to ameliorate surface pH to varying degrees with lime, was used to generate a range of environments to evaluate the performance of this germplasm. The results of this project demonstrate that lucerne is more tolerant to highly acidic soils than previously described in literature. Annual forage production of 8-12 t/ha was measured under rainfed conditions at three of the sites with soil pH_{Ca} 4.1-4.3, which is considered to be a high yield for these environments. Poor forage production did occur at Boralma, but we believe that growth at this site was also constrained by subsoil sodicity, poor soil structure, and waterlogging.

A further aim of this research was to compare the performance of two lucerne varieties developed using different methods for improved tolerance to soil acidity. The variety 'SARDI 7 Series 2', selected for on-farm performance in cool temperate environments with acidic soils, was compared to a new variety 'TA37', which was selected directly for traits associated with tolerance to acidic soils (solution culture screening for tolerance to low pH, aluminium toxicity and symbiotic effectiveness). In solution culture the trait-based selected line TA37 has superior root growth with increases of 32, 48 and 32% over SARDI 7 Series 2 at 0,3 and 6uM Al respectively. TA37 also nodulates with rhizobia in more acidic environments, extending nodulation by approximately 0.3 of a pH unit in solution culture. This result extended into field conditions, where TA37 had greater nodulation than SARDI 7 Series 2 between pH_{ca} of 4.1-4.3. However there were few other differences between the varieties detected in the field, each having excellent forage yield and persistence. The results support a recommendation for both TA37 and SARDI 7 Series 2 varieties to be promoted on acidic soils in south eastern Australia.

There are several explanations for the difference observed between the acidity tolerance of SARDI 7 Series 2 measured under greenhouse and field conditions. The first is that the level of improvement in aluminium tolerance exhibited by TA37 is not biologically significant. Improved tolerance in the range of 3-6uM Al might be inadequate if tolerance in the order of >30 uM Al is required. However, the solution culture experiments show that TA37 has improved root growth compared to SARDI 7 Series 2 and other varieties, even in the absence of aluminium, which you may expect to translate to acidic soils that have low aluminium. We can assume that root growth of both varieties was negatively affected by pH and or aluminium at Holbrook and Tooperang, given the large response in forage yield to lime and increased soil pH. Experiments on earlier generations of the trait-based selections selected under solution culture and evaluated in greenhouse pot experiments using field soils indicated improvements in root growth and or forage production (Humphries *et al. 2009* and Hayes *et al.* 2011). These studies revealed improvements in root growth of 0-225% in a soil from Warrnambool Victoria (Humphries et al. 2009) and 30-40% (Hayes et al. 2012) in a soil from Binalong New South Wales, when compared to parent variety SARDI 7.

Another possibility to explain the differences between solution culture and field experiments could be the impact of different soil chemistry including alternative complexes of aluminium, other toxic nutrients common to acid soils such as Mn (Hayes *et al.* 2012), or deficiencies in macro or micronutrients related to their lower availability at low pH. In particular, Mn is likely to have had an influence on production at Holbrook, with soil levels of DTPA extractable Mn in the range of 80-140 mg/kg. The field selected cultivar SARDI 7 Series 2 has had five generations of selection for improved performance in acidic soils, and thus some indirect selection for the combination of all of these 'field' traits. The correlation analysis in this study did however confirm that aluminium concentration was particularly important at explaining the decline in production and persistence at Holbrook (Fig. 18), and was also an influence at Pewsey Vale and Tooperang. The complex nature of the physical and chemical impediments to production on acidic soils make it very difficult to further isolate individual traits that contribute to the tolerance of lucerne on acidic soils.

Lastly it is also possible that the spatial variation at each site, and the differences between sites, had made it difficult to capture the benefit in tolerance found in TA37.

5.2 Nodulation of lucerne with rhizobia and nitrogen fixation in acidic soils

The lucerne rhizobia symbiosis was also more tolerant to soil acidity in the field than in solution culture, the latter test indicating no nodulation was likely at or below pH 4.5. In the field, significant levels of nodulation and nitrogen fixation were measured in soil with pH_{Ca} >4.1. Nodulation and nitrogen fixation were inhibited at this level of soil acidity, but became adequate at pH_{Ca} >4.5 (60-80% nodulation and >10 nodules per nodulated plant, Fig. 12 and 16) and unlimited around pH 5.0.

The project measured trends (significant at the 10% level) for improved seedling nodulation in TA37 in combination with the strain SRDI736 compared to SARDI 7 Series 2 and RRI128 at all of the sites where seedling nodulation occurred (Tooperang, Holbrook and Boralma). Percentage plant nodulation and the number of nodules per nodulated plant increased with time in all treatments associated with the larger mature plants and possibly the death of un-nodulated seedlings. Providing an indication of rhizobia persistence and their ability to re-nodulate, mature plant nodulation also indicated benefits of the new inoculant strain. A multivariate analysis with plant genotype x strain x pH showed that the new plant and strain combination had significantly higher nodulation between pH_{Ca} 4.1 - 4.4 (Fig. 12).The results of improved seedling nodulation and mature plant nodulation confirm observations from Ballard *et al.* (2005) and Charman *et al.* (2008) that there is considerable potential to improve nodulation of lucerne at low pH by changing the inoculant strain. A commercial release of rhizobia strain SRDI736 as a specialised strain for acid soils (<pH_{ca} 5.0) in combination with TA37 and SARDI 7 Series 2 is supported by the results of this research.

Whilst there were no differences found in nitrogen fixation between plant varieties (using a mature spring second year cut), trends for improved N fixation from the new rhizobia strain were found across all sites, with the improvement largest between pH 4.26 (no lime) and 4.33 (Fig. 13). The decline in N-fixation in RRI128 from pH 5.02 to 4.26 was 47% in RRI128 (54 to 29 kg/ha/cut) and only 23% in SARDI736 (48 to 37 kg/ha/cut). This result suggests that the

new strain is more effective at fixing nitrogen in very acidic soils, with this result supported by evidence of improved mature plant nodulation.

Forage yields of 4-12 t/ha at Tooperang, Holbrook and Pewsey Vale can be shown by extrapolation of N content and % N fixed from a single cut to a full year (using 2.1-2.8% N and 32-69% N-fixation from Table 3 in the absence of lime), to produce 52-228 kg/N/ha by N fixation. The amount of N fixation reported here is lower than the 221-389 kg N ha-yr (with 65-96% of 10-13t/ha shoot N from N fixation) fixed by lucerne in a soil with moderate acidity at Ginninderra (soil pH_W 6.5, Gault *et al.* 1995). The lower levels of N from N-fixation contribute to this difference, and suggest that in the poorest scenario lucerne may struggle to provide enough N for growth in highly acidic soils.

The capacity of lucerne to achieve close to 50% of its N from N fixation at pH_{Ca} 4.5 suggests that the symbiosis is functional at this level of soil acidity. This also underpins our conclusion that long-term production of lucerne on these soils is possible. Both the he symbiosis and production are increasingly constrained at pH_{Ca} <4.5resulting in us to conclude that this is the lowest pH where lucerne should be broadly recommended. Although the performance of lucerne at lower pH was sometimes quite acceptable in this study, the benefits and relatively low cost of adding lime should not be ignored.

5.3 Impact of ameliorating surface pH with lime on lucerne's performance on acidic soils

Lime increased forage yield and nitrogen fixation at all sites, and had a significant impact on persistence at Tooperang and Holbrook. The high lime rate increased production on average by 22% or between 700-2800 kg/ha over 24 months. Assuming a cost of $100/ha to deliver and spread 1.2 t/ha of lime, the payback period for spreading lime at Pewsey Vale, Tooperang and Holbrook is under one year where the value of the fodder is >$120/t (which it easily exceeds).

Forage production of lucerne with the high lime rate at Pewsey Vale (18.5 t/ha) is similar to the 20.9 t/ha measured in an ideal environment (pH 6.5_{Ca}) and under more intensive management at the Waite Institute (Humphries *et al* 2016). This result shows that if surface pH is ameliorated, the acidic subsoil has minimal impact and production can approach non-limiting conditions.

Lime application has been associated with increased yield, nodulation, crude protein content and nitrogen fixation in studies by Simpson *et al.* (1977), Gault *et al.* (1995), Grewal and Williams (2003) and Mullen *et al. (2006)*. The impact of lime on ameliorating surface pH is related to the concentration of H^+ ions (pH, as shown in the response of root growth to solution pH in Table 1 and Fig. 3), but also to the addition of Ca, an essential plant nutrient that is particularly high in lucerne foliage. Whilst not measured in this study, the addition of lime has previously been shown to significantly increase Ca and decrease Al concentration in lucerne shoots (Grewal and Williams 2003).

The moderate lime rate also accounted for 16 kg/ha or 26% more nitrogen fixation in the single forage cut, which is both critical for the long-term production of the lucerne stand,

and has physical value that can be estimated. The moderate lime rate could account for as much as an additional 90 kg/ha of N, and with a current value of $680/t N (based on $310/t for Urea), the return of the high lime rate for N fertility alone can be estimated at $60/ha/year.

The capacity to grow lucerne on deep acidic soils with only the surface soil ameliorated with lime as demonstrated in this study is supported by Mullen et al. (2006). In their study at Tomingley in western NSW, lime application on a highly acidic soil (pHCa 4.4, 0–10 cm depth) improved the establishment, persistence and production of lucerne over a 6 year duration, even when the subsurface soil (10–30 cm depth) was equally acidic, and moderate acidity extended to 75 cm into the soil profile.

The impact of lime on lucerne production has also been demonstrated to be long lasting, with rates up to 2t/ha of lime increasing lucerne production for at least six (Mullen et al. 2006) to nine (Simpson et al. 1977) years. Whilst the performance of lucerne on highly acidic soils in this experiment is encouraging, the results support the surface application of lime to improve the productivity of lucerne as part of an integrated strategy together with variety and rhizobia strain selection to improve lucerne production on acidic soils.

5.4 Implications for industry and recommendations for additional research

The recommendation for industry is to continue to advise farmers to grow SARDI 7 Series 2 (because seed is available) in combination with the new rhizobia strain 'SRDI736', on acidic soils. Further research is underway to determine if there are environments where TA37 has an advantage over SARDI 7 Series 2, and the sowing of the two varieties as a mixture is proposed as an additional consideration. Whilst the varieties have shown the potential to be productive, persist and fix nitrogen below pH 4.5 in the field, we recommend that soils below that pH are limed to lift overall lucerne production.

Suggestions for future research include

1. The requirement to incorporate lime. Will surface applied lime that is not incorporated benefit nodulation? Many producers have zero till systems to reduce weeds, erosion and to stop bringing stones to the surface.
2. Sowing times to improve establishment on acidic soils. Whilst spring sowing lucerne is often practiced, reduced root growth combined with a higher probability of low spring rainfall due to climate change may combine to make this practice less successful. Autumn sowing can be practiced if the break of the season is early enough to allow sowing in April and weeds have been controlled in previous years. An alternative to spring sowing may be late winter (July-mid August) depending on the rainfall and soil type. It is suggested to explore this sowing time, in combination with easy modifications of machinery to target the placement of seed outside of the furrow (and on the rise) to provide drainage and avoid fungal problems of dampening off associated with wet soil.
3. Future breeding for lucerne on acidic soils should include field selection into each cycle of a greenhouse based screening system to refine plant selections for field-

based traits such as drought and grazing tolerance. Genotypes can be replicated by evaluating half-sib families across multiple environments to estimate GxE interactions.

4. Selections from both of the varieties at the four field sites in this project (together with other breeding trials sown on highly acidic sites), should be used to develop the next generation acid tolerant lucerne variety.

5.5 Extension messages

Heritage Seeds and SARDI gave a total of 16 field days and seminars to over 350 farmers, agronomists and students at 12 locations throughout the project to provide information on this research and the practical opportunities and limitations of growing lucerne on acidic soils. Heritage Seeds also have annual field days at Howlong (with 850 site visits over the last 3 years) and Toowoomba where messages from this project will continue to be delivered.

The main message from this project;

SARDI 7 Series 2 and TA37 lucerne varieties can grow very well on very acidic soils, but applying lime to ameliorate the surface pH will improve production and persistence, and is economically viable. SARDI 7 Series 2 and TA37 are recommended for $pH_{Ca} \geq 4.5$. Farmers should be encouraged to start with a relatively small area, to test the production of lucerne and learn from costly mistakes on a small area. Lucerne should be managed with rotational grazing, as reducing additional stresses such as overgrazing, competition with other species and waterlogging are likely to be important where lucerne is grown under sub-optimal conditions. Despite these cautions, lucerne can be extremely valuable for red meat producers, intensifying livestock production by extending the growing season of the feedbase into summer and autumn. The results show that ameliorating surface pH is sufficient on most soils to achieve excellent production. Advice should be sought on managing livestock production from lucerne in order to maximise livestock production and achieve the best fit within your whole farm system.

5.6 Improvements for future research

Were the objectives met and what could be improved? By 15th March 2016, the objectives were:

1. Developed a detailed understanding of the improvement in adaptation of lucerne on acidic soils, separating the individual stresses of pH and aluminium toxicity.

This objective was successfully met, the project has provided detailed investigations into the growth of lucerne in acidic soils through;

1. Experiments in solution culture to separate the impacts of pH and aluminium stress
2. The use of 4 sites and 16 site x lime treatments to create an array of environments with different levels of acidity stress that were used investigate the impact of ameliorating surface pH with lime on the production of lucerne.
3. Multi-variate and PCA analysis to look at the contribution of soil traits and observations on lucerne production.

2. Developed an information package for red meat producers which describe the adaptation of lucerne on acidic soils, and to what extent this new variety and strain extend the production of lucerne onto acidic soils.

This objective has been partially met, with an information package continuing to be developed in the next six months from the results in this final report. Red meat producers were actively engaged in this project, with 350 farmers, agronomists and students attending 16 information sessions and field days in 2015/16.

6 Conclusions/Recommendations

- SARDI 7 Series 2 and TA37 lucerne varieties are more tolerant to highly acidic soils than expected, and should be recommend on soils with pH_{Ca} ≥4.5. These varieties have excellent yield and persistence potential, and form an effective and persistent symbiosis with rhizobia. Use of these varieties is possible at even lower soil pH, but both production and N fixation can be improved with moderate applications of lime.
- The use of at least 1 t/ha of lime to ameliorate surface pH is recommended. Additional gains in production on most acid soils can be expected with rates up to 2 t/ha. Amelioration of surface soil pH is sufficient in most cases to achieve excellent production of SARDI 7 Series 2 and TA37 lucerne varieties on soils that are acidic at depth. Physical and chemical constraints to subsoil root growth such as sodicity, salinity, waterlogging or impenetrable subsoil are likely to reduce the suitability of lucerne on any soil.
- The commercial release of rhizobia strain SRDI736 is supported by this research, as a special strain for inoculation with SARDI 7 Series 2 and TA37 on acidic soils. Additional research is required to understand the symbiosis of this strain with other lucerne varieties and in soils with neutral pH.
- Lucerne should be promoted as a viable option on acidic soils to boost red meat productivity. Lucerne can produce substantial (5-9 t/ha) forage with high nutritive value in summer and autumn, extending the growing season and reducing both time to slaughter and the cost of production.

7 Key Messages for producers

They key message for producers is to reconsider the use of lucerne on acidic soils where they have previously been advised that it will not grow Producers should have a recent soil test and use lime to increase soil pH_{Ca} >4.5. Higher applications of lime may be important where the concentration of Aluminium as a percentage of the cation exchange is above 15%.

Why grow lucerne on acidic soils?

Lucerne is a highly productive and nutritious perennial legume that can intensify red meat production. In southern Australia, forage yields of 10-20 t/ha can be expected under rainfed conditions with soils pH≥4.5, with approximately half of this production occurring in summer and autumn.

The major reason to grow lucerne is to improve the production efficiency of a red meat production enterprise. Grazing systems can be as simple as a two paddock rotation, but the performance of lucerne (yield and persistence) and forage utilisation will increase with more intensive grazing (being maximised with cell or techno grazing). Whilst beyond the scope of this project, it is important to understand the reasons to grow lucerne with respect to different finishing and production systems;

Prime lamb production targets that utilise lucerne

1. Aim to finish late lambs that would fail to meet market specifications in a traditional winter pasture. Split lambing times (autumn and spring lambing) to produce lambs out of season.
2. Aim to grow out young breeding ewe lambs so that they can be successfully joined at 12 months.
3. Use lucerne to quickly regain weight on ewes after weaning and flush ovulation. Investigate the possibility of developing a system with three lamb cycles every 2 years in favourable environments.
4. Use lucerne to reduce supplementary feeding

Beef production system targets that utilise lucerne

1. Use the extended growing season from lucerne to reduce the grow-out phase that occurs on senesced pastures from November to May, associated with increases in carcass size and loss of condition. Reduce the time to market on yearling stock and improve meat quality.
2. Use lucerne to reduce supplementary feeding costs with direct grazing and or silage production.
3. Use lucerne to grow out young breeding stock, or improve condition on cows after weaning.
4. Incorporate multiple lucerne paddocks into the grazing rotation to avoid transitions from dry pasture to green lucerne, and consider using chicory as a non-bloating companion species.

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9 Appendix

9.1 Appendix 1 - Soil chemical properties at Tooperang, Pewsey Vale, Holbrook and Boralma

Table A1. Baseline soil chemical properties at the four experimental research sites, Tooperang, Pewsey Vale (SA), Holbrook (NSW) and Boralma (Vic).

Site	*Depth*	NH4	$N0_3$	P Colwell	K Colwell	S	pH ($CaCl_2$)	pH (H_2O)	DTPA Mn	Exc. Al	Exc. Ca	Exc. Mg	Exc. K	Exc. Na	CEC total	Al CEC
		mg/ kg	mg/ kg	mg/kg	mg/kg	mg/ kg	pH	pH	mg/kg	meq/ 100g	meq/ 100g	meq/ 100g	meq/ 100g	meq/ 100g	meq/ 100g	%
Tooperang	0 to 10	13	5	89.4	91	6	4.2	5.2	10.7	0.4	2.5	0.3	0.2	0.1	3.5	11
	>10-20	3	1	13	29	4	4.5	5.3	3.8	0.3	0.3	0.1	0.1	0.0	0.7	41
	>20-30	6	3	3	146	12	4.5	5.4	2.2	0.9	3.5	1.8	0.4	0.1	6.7	14
Pewsey Vale	0 to 10	4	61	88	116	9	4.1	4.7	10.7	0.8	1.9	0.3	0.2	0.1	3.3	24
	>10-20	M	12	51	94	4	4.3	4.9	3.8	0.6	0.9	0.2	0.2	0.0	2.0	33
	>20-30	1	11	33	86	4	4.4	5.0	2.2	0.6	0.8	0.2	0.2	0.0	1.7	32
Holbrook	0 to 10	11	42	48	323	16	4.3	5.0	140	0.8	4.4	0.9	0.8	0.1	7.0	12
	>10-20	3	14	11	216	12	4.6	5.4	101	0.3	4.5	1.3	0.5	0.1	6.6	5
	>20-30	1	20	6	177	11	5.1	5.8	45	0.1	5.4	2.0	0.4	0.1	8.1	1
Boralma	0 to 10	15	46	15	319	8	4.3	5.1	100	0.8	2.1	0.6	0.8	0.2	4.3	16
	>10-20	2	6	6	189	4	4.7	5.7	60	0.6	2.8	0.6	0.4	0.1	4.5	18
	>20-30	2	4	4	151	2	5	6.1	24	0.3	3.7	1.8	0.3	0.3	6.4	5

Table A2. Description of soil characteristics at Tooperang, Pewsey Vale, and Boralma in Experiment 2, 12 months following lime Treatments (0-10cm). Soil samples taken in September 2013 (Tooperang) and September 2014 (other sites).

site	lime	depth	NH3	NO3	P	K	S	pHCa	pHW	Mn	ExAL	ExCa	ExMg	ExP	ExNa	CEC total	Al%CEC
Tooperang	600	0 to 10	3	20	62	79	5	4.3	4.9	2.0	0.4	2.1	0.3	0.2	0.1	3.1	13.5
Tooperang	600	10-20	*	4	24	42	2	4.3	4.8	0.6	0.4	0.6	0.1	0.1	0.0	1.2	37.0
Tooperang	600	20-30	*	3	9	42	2	4.6	5.2	0.8	0.3	0.7	0.2	0.1	0.0	1.3	29.6
Tooperang	600	30-40	2	8	4	105	11	4.6	5.5	0.8	0.4	3.8	2.2	0.3	0.2	6.8	6.1
Tooperang	1200	0 to 10	4	26	69	85	5	4.6	5.3	2.0	0.2	2.9	0.5	0.2	0.1	3.9	6.0
Tooperang	1200	10-20	*	6	33	46	3	4.3	5.0	1.1	0.4	0.9	0.1	0.1	0.0	1.5	26.8
Tooperang	1200	20-30	*	3	12	34	2	4.5	5.1	0.7	0.3	0.5	0.1	0.1	0.0	1.0	32.4
Tooperang	1200	30-40	2	8	5	95	12	4.8	5.5	0.8	0.4	4.1	2.2	0.2	0.2	7.2	6.3
Tooperang	2400	0 to 10	3	24	68	82	5	5.1	5.7	1.6	0.1	3.6	0.5	0.2	0.1	4.5	2.7
Tooperang	2400	10-20	*	5	29	44	2	4.5	5.2	0.8	0.3	1.0	0.2	0.1	0.0	1.5	20.2
Tooperang	2400	20-30	*	3	12	33	2	4.6	5.3	0.8	0.3	0.6	0.1	0.1	0.0	1.1	27.1
Tooperang	2400	30-40	2	8	4	102	12	4.7	5.5	1.1	0.4	4.2	2.3	0.3	0.2	7.3	5.9
Pewsey Vale	700	0 to 10	2	76	94	105	13	4.3	4.9	9.1	0.6	2.6	0.5	0.2	0.1	4.0	14.6
Pewsey Vale	700	10-20	*	13	56	95	5	4.4	5.0	3.8	0.6	1.0	0.2	0.2	0.0	2.1	30.3
Pewsey Vale	700	20-30	*	9	34	77	4	4.6	5.1	2.2	0.5	0.6	0.2	0.2	0.0	1.5	31.9
Pewsey Vale	700	30-40	*	10	23	70	4	4.6	5.1	1.9	0.5	0.6	0.2	0.2	0.0	1.5	30.2
Pewsey Vale	1400	0 to 10	*	82	92	104	13	4.4	5.0	7.5	0.5	2.9	0.6	0.2	0.1	4.3	12.8
Pewsey Vale	1400	10-20	*	14	66	88	5	4.4	4.9	4.1	0.6	1.0	0.2	0.2	0.0	2.1	30.1
Pewsey Vale	1400	20-30	*	10	47	76	4	4.5	5.0	2.4	0.5	0.7	0.2	0.2	0.0	1.7	30.6
Pewsey Vale	1400	30-40	*	10	30	65	4	4.6	5.1	1.8	0.5	0.7	0.2	0.2	0.0	1.6	28.8
Pewsey Vale	2800	0 to 10	*	83	91	99	14	4.8	5.4	4.5	0.3	4.0	0.7	0.2	0.1	5.3	5.3
Pewsey Vale	2800	10-20	*	14	59	83	5	4.5	5.0	3.8	0.6	1.2	0.3	0.2	0.0	2.3	27.4
Pewsey Vale	2800	20-30	*	11	38	79	5	4.5	5.1	2.4	0.5	0.8	0.2	0.2	0.0	1.8	30.7
Pewsey Vale	2800	30-40	*	12	25	71	5	4.6	5.1	2.1	0.5	0.8	0.3	0.2	0.0	1.8	27.3

site	lime	depth	NH3	NO3	P	K	S	pHCa	pHW	Mn	ExAL	ExCa	ExMg	ExP	ExNa	CEC total	Al%CEC
Holbrook	700	0 to 10	11	96	48	314	14	4.4	4.9	125	0.6	5.3	0.9	0.7	0.1	7.6	8.0
Holbrook	700	10-20	7	64	26	244	12	4.5	5.0	112	0.4	4.7	1.0	0.6	0.0	6.7	6.2
Holbrook	700	20-30	2	33	13	199	10	4.6	5.3	71	0.3	4.4	1.3	0.5	0.1	6.6	4.9
Holbrook	700	30-40	*	31	6	163	9	5.1	5.7	31	0.2	5.2	2.1	0.4	0.1	8.0	2.0
Holbrook	1400	0 to 10	7	108	45	300	16	4.6	5.1	102	0.4	6.2	1.0	0.7	0.1	8.4	4.6
Holbrook	1400	10-20	4	71	23	234	14	4.9	5.4	79	0.2	6.4	1.7	0.6	0.1	8.9	1.8
Holbrook	1400	20-30	3	58	20	211	13	4.9	5.4	74	0.1	6.0	1.8	0.5	0.1	8.5	1.6
Holbrook	1400	30-40	*	34	8	164	12	5.0	5.7	48	0.2	6.0	2.6	0.4	0.1	9.2	1.7
Holbrook	2800	0 to 10	3	95	49	293	17	5.1	5.7	116	0.1	8.6	0.9	0.7	0.1	10.3	0.6
Holbrook	2800	10-20	2	61	26	226	14	5.1	5.6	88	0.1	6.5	1.0	0.5	0.1	8.2	0.8
Holbrook	2800	20-30	*	30	10	167	12	4.8	5.5	63	0.1	4.8	1.4	0.4	0.1	6.8	2.0
Holbrook	2800	30-40	*	32	5	134	12	5.4	5.9	28	0.2	5.5	2.4	0.3	0.1	8.5	1.8
Boralma	700	0 to 10	3	67	19	307	10	4.3	4.9	50	0.6	2.7	0.7	0.8	0.2	5.0	12.8
Boralma	700	10-20	3	38	7	177	5	4.2	4.9	46	0.8	1.4	0.7	0.4	0.1	3.4	22.6
Boralma	700	20-30	2	18	5	143	3	4.4	5.5	19	0.4	2.4	1.8	0.3	0.3	5.2	7.1
Boralma	700	30-40	1	11	7	156	3	4.8	6.1	12	0.3	4.1	4.1	0.4	0.7	9.6	3.3
Boralma	1400	0 to 10	6	51	17	301	9	4.6	5.3	42	0.3	3.1	0.7	0.8	0.1	5.0	5.9
Boralma	1400	10-20	1	30	5	163	5	4.2	4.9	43	0.7	1.3	0.7	0.4	0.1	3.3	22.3
Boralma	1400	20-30	1	17	4	131	3	4.3	5.4	24	0.6	2.0	1.8	0.3	0.3	5.0	12.0
Boralma	1400	30-40	2	11	7	141	3	4.7	6.0	14	0.6	3.3	4.1	0.4	0.8	9.2	6.5
Boralma	2800	0 to 10	3	61	21	308	11	5.1	5.6	37	0.1	4.8	0.7	0.8	0.1	6.5	1.4
Boralma	2800	10-20	1	35	7	179	5	4.3	5.0	40	0.7	1.5	0.7	0.4	0.1	3.4	20.5
Boralma	2800	20-30	2	19	4	148	3	4.7	5.9	17	0.3	2.8	1.9	0.4	0.3	5.7	5.6
Boralma	2800	30-40	1	12	5	159	3	5.1	6.3	9	0.3	4.3	3.9	0.4	0.7	9.7	3.0

Final soil pH measurements

Table A3. Soil chemical properties at the conclusion of the experiments at four experimental research sites, Tooperang, Pewsey Vale (SA), Holbrook (NSW) and Boralma (Vic). Data includes impact of lime treatments at each site. Soil samples taken in March 2016.

Name	Lime	Depth	NH4	N03	P Col	K Col	S	pH (CaCl2)	pH (H2O)	DTPA Mn	Exc. Al	Exc. Ca	Exc. Mg	Exc. K	Exc. Na	CEC Total	AL CEC	NA CEC	SAR
			mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	pH	pH	mg/Kg	meq/100g					%	%	%	
Tooperang	0	0-10	15	13	63	48	7.4	4.2	5.1	1.7	0.4	1.3	0.2	0.1	0.1	2.2	19	2.8	0.07
Tooperang	0	10-20	< 1	1	22	20	2.4	4.3	5.1	0.6	0.4	0.2	0.0	0.1	0.0	0.7	53	1.5	0.03
Tooperang	0	20-30	< 1	1	6	66	4.7	4.6	5.6	0.9	0.5	1.7	1.0	0.2	0.1	3.5	16	2.3	0.07
Tooperang	600	0-10	7	8	54	44	7.8	4.2	5.2	2.0	0.3	1.5	0.2	0.1	0.1	2.2	16	3.1	0.08
Tooperang	600	10-20	< 1	< 1	15	23	2.3	4.4	5.2	0.8	0.4	0.3	0.1	0.1	0.0	0.9	50	1.2	0.02
Tooperang	600	20-30	< 1	< 1	5	35	3.2	4.6	5.6	0.9	0.4	1.0	0.4	0.1	0.0	1.9	23	1.6	0.04
Tooperang	1200	0-10	5	11	59	32	8	4.5	5.4	2.5	0.2	2.2	0.4	0.1	0.1	2.9	8	2.0	0.05
Tooperang	1200	10-20	< 1	< 1	18	18	2	4.4	5.3	1.0	0.3	0.3	0.1	0.1	0.0	0.8	42	1.3	0.02
Tooperang	1200	20-30	1	< 1	6	36	2.9	4.7	5.7	1.1	0.3	1.1	0.4	0.1	0.0	1.9	17	2.1	0.05
Tooperang	2400	0-10	6	12	47	25	7.1	4.7	5.6	1.7	0.2	2.4	0.4	0.1	0.1	3.1	5	1.9	0.05
Tooperang	2400	10-20	1	1	23	21	1.9	4.4	5.3	0.9	0.3	0.4	0.1	0.1	0.0	0.9	38	1.1	0.02
Tooperang	2400	20-30	< 1	< 1	8	42	2.8	4.6	5.6	0.9	0.4	0.7	0.3	0.1	0.0	1.6	26	1.3	0.03
P.Vale	0	0-10	6	8	87	76	9.7	4.2	5	9.6	0.6	1.5	0.2	0.2	0.1	2.6	25	1.9	0.05
P.Vale	0	10-20	1	1	46	67	2.8	4.5	5.4	2.9	0.5	0.7	0.1	0.2	0.0	1.5	33	1.3	0.03
P.Vale	0	20-30	< 1	1	29	69	2.5	4.5	5.4	1.9	0.5	0.6	0.1	0.2	0.0	1.4	37	1.5	0.03
P.Vale	700	0-10	7	9	89	69	12.3	4.3	5.1	9.1	0.5	2.1	0.4	0.2	0.1	3.2	17	2.2	0.06
P.Vale	700	10-20	< 1	2	45	67	3.2	4.4	5.4	2.9	0.5	0.7	0.2	0.2	0.0	1.6	30	1.9	0.04
P.Vale	700	20-30	< 1	1	33	76	2.1	4.6	5.5	1.6	0.4	0.6	0.2	0.2	0.0	1.3	29	1.6	0.03
P.Vale	1400	0-10	4	9	70	61	7.5	4.6	5.5	5.1	0.3	2.5	0.4	0.1	0.1	3.4	8	1.8	0.05
P.Vale	1400	10-20	< 1	1	43	70	2.9	4.4	5.3	2.8	0.5	0.7	0.2	0.2	0.0	1.6	32	1.9	0.05
P.Vale	1400	20-30	< 1	1	32	69	2.7	4.5	5.5	1.9	0.5	0.6	0.2	0.2	0.0	1.5	32	2.1	0.05
P.Vale	2800	0-10	3	18	82	71	10.5	4.7	5.4	5.2	0.3	2.8	0.5	0.2	0.1	3.8	8	1.6	0.05

P.Vale	2800	10-20	< 1	3	63	93	3.5	4.4	5.3	3.4	0.6	0.8	0.2	0.2	0.0	1.8	32	1.7	0.04
P.Vale	2800	20-30	1	2	48	75	2.5	4.6	5.5	1.8	0.4	0.6	0.2	0.2	0.0	1.4	29	2.2	0.05
Holbrook	0	0-10	7	22	38	171	11.1	4.3	5	108	1.0	3.2	0.7	0.4	0.1	5.3	18	1.3	0.05
Holbrook	0	10-20	2	5	13	79	9.7	4.6	5.5	72	0.4	3.3	0.9	0.2	0.1	4.8	7	1.2	0.04
Holbrook	0	20-30	2	2	5	78	14.5	5.1	5.9	37	0.1	4.1	1.5	0.2	0.1	5.9	2	1.2	0.04
Holbrook	700	0-10	6	21	33	284	11.9	4.4	5.2	109	0.7	5.0	0.8	0.7	0.0	7.4	10	0.5	0.02
Holbrook	700	10-20	3	8	6	185	9	4.6	5.4	72	0.3	3.9	0.9	0.5	0.0	5.7	6	0.7	0.03
Holbrook	700	20-30	2	3	4	181	11.7	5.6	6.4	29	0.1	4.9	1.5	0.5	0.1	7.1	2	0.8	0.03
Holbrook	1400	0-10	5	26	35	260	13.3	4.3	5	113	0.8	4.6	0.9	0.7	0.0	7.0	11	0.4	0.02
Holbrook	1400	10-20	3	6	11	243	13.8	4.6	5.5	32	0.3	6.9	2.6	0.6	0.1	10.5	3	0.6	0.03
Holbrook	1400	20-30	2	2	5	218	11.8	5.1	6.1	8.9	0.1	8.0	3.9	0.6	0.1	12.6	1	0.7	0.04
Holbrook	2800	0-10	11	20	26	289	12.5	4.4	5.1	112	0.7	4.2	0.6	0.7	0.0	6.2	11	0.3	0.01
Holbrook	2800	10-20	3	6	7	196	10.3	4.5	5.4	66	0.5	3.8	0.8	0.5	0.0	5.6	8	0.5	0.02
Holbrook	2800	20-30	1	2	3	158	15.3	5.4	6.2	25	0.1	5.8	1.6	0.4	0.1	8.0	1	0.9	0.04
Boralma	0	0-10	17	41	14	283	11.8	4.3	4.9	101	0.8	1.4	0.5	0.7	0.1	3.6	23	3.6	0.13
Boralma	0	10-20	2	6	4	161	4.4	4.5	5.4	82	0.9	0.8	0.5	0.4	0.1	2.6	33	3.5	0.11
Boralma	0	20-30	1	6	4	166	2.8	4.5	5.8	33	0.6	1.5	1.4	0.4	0.2	4.1	14	5.8	0.20
Boralma	700	0-10	11	68	9	225	10.6	4.7	5.3	102	0.3	2.5	0.8	0.5	0.3	4.4	7	6.8	0.23
Boralma	700	10-20	3	20	5	108	5.3	4.8	5.5	58	0.3	1.8	1.0	0.3	0.2	3.6	7	6.4	0.19
Boralma	700	20-30	1	10	5	74	3.4	5	6.1	10.9	0.2	3.1	2.9	0.2	0.5	6.9	3	7.5	0.30
Boralma	1400	0-10	13	79	16	257	11.8	4.8	5.5	107	0.2	3.2	0.8	0.7	0.4	5.2	3	7.1	0.26
Boralma	1400	10-20	2	12	3	106	4	5	6	51	0.1	1.7	0.9	0.3	0.3	3.2	4	8.3	0.24
Boralma	1400	20-30	< 1	12	4	118	3.4	6.3	7	6.0	0.1	5.5	3.3	0.3	1.0	10.2	1	9.8	0.48
Boralma	2800	0-10	5	39	22	355	11.4	5	5.8	72.2	0.1	4.5	0.7	0.9	0.1	6.3	1	1.3	0.05
Boralma	2800	10-20	< 1	5	4	175	3.2	5.3	6.3	28.6	0.1	2.1	0.8	0.4	0.1	3.5	3	2.6	0.08
Boralma	2800	20-30	< 1	5	3	156	2	5.9	6.7	6.2	0.0	3.8	2.0	0.4	0.3	6.6	1	4.9	0.19

9.2 **Appendix 2 - Images/ Plates**

Plate 1 – Equipment used in the solution culture root elongation experiment

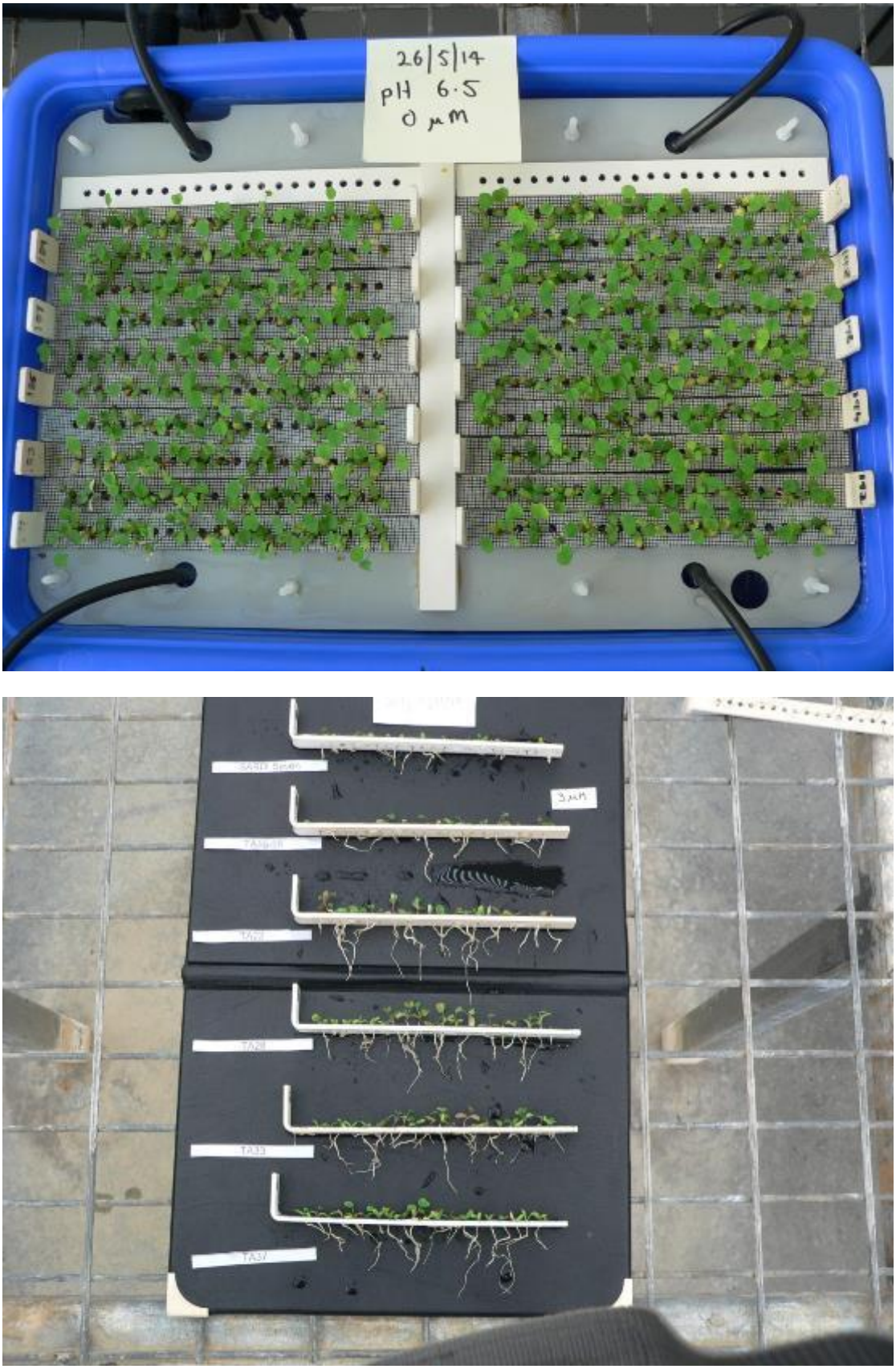

Plate 2. Overview of the root elongation test (top) and variation in root length of SARDI 7 and TA37, at pH 4.5 with 3 uM Al (bottom).

Plate 3. Overview of the nodulation test (top) and of the level of nodulation produced by the acid tolerant rhizobia strain SRDI 736 at pH 4.7 (bottom) at 10 days after inoculation. Roots are longer in this experiment because the solution contained nutrients and the plants were grown for three weeks.

Plate 4. Comparison of root growth, nodulation and shoot growth in SARDI 7 Series 2 (left) and TA37 grown in acidic solution culture. The modified (-N) 0.25 strength McKnights solution was maintained at pH4.8 for three days and then adjusted to pH4.5 before adding 35uM AlCl3. Photo was taken 28 days after germination.

Plate 5. Soil profile at Tooperang showing light sand over contrasting clay at approximately 30cm.

Plate 6. Tooperang trial without lime, production in 23 December 2015

Plate 7. Symptoms of nitrogen deficiency (yellowing) were intermittent in nil plots, photo taken 23 December 2015

Plate 8. Tooperang trial, fence removed and grazed.

Plate 9. Pewsey Vale spring production in 2015 following decile 1 rainfall (not senesced perennial pastures in background)

Plate 10. Collecting winter forage at Pewsey Vale, 2 June 2015

Plate 11. Hereford bulls give freshly cut SARDI 7 series 2 the tick of approval (and pushing their weight around when we weren't cutting the plots quickly enough!)

Plate 12. Lucerne production at Pewsey Vale on 15 April 2016 following dry summer conditions

Plate 13. Sowing the Boralma lime trial

Plate 14. Forage production was very slow at Boralma, yield first measured in Nov 2015 fourteen months after sowing.

Plate 15. Boralma field trial at February 2016 following summer drought

Plate 16. Late autumn production at Holbrook, on 15 May 2015

Plate 17. Holbrook lucerne trial – out of season summer growth during very dry conditions. Lucerne can extend the growing season.