

# The Impact of Background Rhizobial Populations on Inoculation Response

Jo Slattery<sup>1</sup> and David Pearce<sup>1</sup>

## Abstract

Agronomic programs that target the introduction of legumes into different agro-ecosystems focus initially on the selection of legumes that have the ability to tolerate edaphic constraints that include disease resistance, water stress and tolerance to salinity, acidity and sodicity. Within such selection programs the interaction of the legume with soil *Rhizobium* has largely been ignored. In order to maximise agricultural production, specific programs must link strain selection with plant development together with an understanding of the impact of background populations.

PASTURE and crop legumes have been used extensively in agriculture over the past century in Australia, mainly for maintaining soil fertility. These agricultural soils are often constrained in their ability to sustain productive farming systems due to factors associated with low fertility, sodicity, salinity and extremes of acidity and alkalinity. These same attributes can also have a negative impact on the legume-*Rhizobium* symbiotic relationship reducing the ability of rhizobia to form nodules with optimal N<sub>2</sub>-fixing capacity, thus impeding the continued success of legumes in Australian agricultural systems.

Considerable variation in the soil populations of *Rhizobium* spp has been found in soils throughout Australia, with the range varying from less than 10 to in excess of 10<sup>6</sup> *Rhizobium* bacteria g/m soil (Gibson et al. 1975; Slattery et al. 1999), generally with an average soil population above 1 × 10<sup>4</sup> *Rhizobium* bacteria g/m soil. The size of the soil population is dependent on field history, location of sampling, soil characteristics and the presence of a host plant.

Where there are low (<50 *Rhizobium* bacteria g/m soil) naturalised populations of rhizobia specific to a target legume, the introduction of new strains by seed inoculation is normally successful. On the other hand, inoculation into soils where naturalised rhizobial populations are high (>10<sup>3</sup> *Rhizobium* bacteria g/m soil) introduction of new strains can be difficult

and often unsuccessful (Thies et al. 1991; Brockwell et al. 1995).

The Australian continent provides a large land-mass that constitutes a wide spectrum of climatic conditions and soil environments, in which to maintain a viable agricultural industry. Soil types in the pulse growing regions of southeastern Australia vary from alkaline (NW Victoria and much of South Australia), neutral acidic soils (Wimmera, central and southern Victoria) to highly acidic soils (NE Victoria and southern NSW). This wide variation in soil type has serious implications for *Rhizobium* survival, *Rhizobium* effectiveness and the need for re-inoculation of following pulse crops.

Techniques used for the development of elite inoculant strains have been discussed in an accompanying workshop paper (Slattery and Pearce 2001). The final criteria adopted for the selection of *Rhizobium* strains are the assessment of germplasm for edaphic adaptation and field performance (Howieson et al. 2000; Slattery and Pearce 2001). The capacity of inoculant strains to colonise soils in sufficient quantity to provide effective nodulation is very much dependant on the soil type. Thus, a thorough understanding of the presence of background populations will enable recommendations to be made regarding the need for re-inoculation of pulse legumes.

In this paper, we study the effect of the soil environment on the ability of rhizobia to form nodules and the need for rhizobial inoculation when introducing pulse legumes into different soil environments. A comprehensive soil survey across the pulse legume growing regions of Victoria provide a critical

<sup>1</sup>Rutherglen Research Institute, Department of Natural Resources and Environment, Rutherglen, Victoria 3685, Australia

analysis of the soil chemical and background rhizobial populations under different soil environments. Once the rhizobial status is defined, criteria are needed to identify the requirement for the re-inoculation of pulse crops and background rhizobial survival across these diverse soil types.

## Materials and Methods

### Field survey and soil sampling

Rhizobial populations were monitored and assessed for a range of pulse/crop/pastures. Fifty paddocks were sampled during August 1997, over a wide geographical range for the pulse legume growing regions of Victoria (Rutherglen, Dookie, Elmore, Charlton, Birchip, Horsham and Walpeup regions) (Table 1). *Rhizobium* survival, soil classification and soil chemical characteristics of each paddock were also measured. Ten soil cores (10 cm × 2.5 cm diameter) were collected aseptically (Slattery and Coventry 1993) from each paddock, bulked, thoroughly mixed, passed through a 2 mm sieve to remove stones and large pieces of undecomposed organic matter, then stored at room temperature before chemical and rhizobia analysis.

**Table 1.** Number of paddocks and regional location of sites used in the legume field survey.

Regional location	Number of paddocks sampled
Birchip, Southern Mallee, NW Victoria	11
Walpeup, Northern Mallee, NW Victoria	10
Horsham, Woomera, NW Victoria	7
Elmore, Central Victoria	7
Charlton, NC Victoria	5
Dookie, NE Victoria	7
Rutherglen, NE Victoria	3

### Evaluation of nitrogen-fixing effectiveness

Air-dried soil samples collected from each site were used to estimate the symbiotic effectiveness of rhizobial populations. Extreme care was taken in the collection of soil to avoid the sampling of soil from around the plant rhizosphere. The timing of sampling (late August) coincided with seasonal conditions for that year; in particular, the likelihood of an abrupt early ending to the growing season was a key factor in early sampling. The symbiotic effectiveness was tested with 6 legumes—field pea (*Pisum sativum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), vetch (*Vicia sativa*), chickpea (*Cicer arietinum*) and lupin (*Lupinus angustifolius*)—as the plant hosts using the

whole soil inoculation method (Brockwell et al. 1988). This method uses soil rhizobial populations as a means of assessing the N<sub>2</sub>– fixation potential of that soil. Test plants were grown in a semi-sterilised system (washed sand:vermiculite mixture, moistened with N-free nutrient salt solution (Slattery and Pearce 2001)). In this system, the shoots are exposed to the atmosphere while the roots are grown under aseptic microbiological conditions.

Test plant seeds were surface sterilised, germinated on 2% water agar plates at 22°C, prior to planting in 3 cm square seedling tubes. Five days after planting, each seedling was inoculated with a 1 mL soil suspension (10 g soil in 90 mL N-free nutrient salt solution) containing about 10<sup>7</sup> cells of each isolate. The commercial rhizobial strains were used as the positive controls for each species, whilst the uninoculated treatments were either supplemented with nitrogen or without nitrogen. The plants were grown for 4 weeks in a glasshouse (range 12–25°C), with the moisture content of each plant maintained with either sterile distilled water or N-free nutrient solution. All plants were harvested, the roots examined for nodulation, and dry matter (DM) from the whole plant tops was measured after oven drying at 70°C.

### Soil chemical analysis

Soil pH<sub>Ca</sub> (0.01M CaCl<sub>2</sub>) and soil pH<sub>W</sub> 1:5 soil:extractant was measured using an automated system, while Olsen P (0.5 M NaHCO<sub>3</sub>) 1:100 soil:extractant, extractable K (0.5 M NaHCO<sub>3</sub>) 1:100 soil:extractant, total N (Kjeldahl), mineral N (2 M KCL—Kjeldahl) and organic C were determined by standard procedures (Slattery et al. 1999). Soils were identified according to the Australian soil classification (Isbell 1996).

### Experimental sites

In order to make recommendations for farmers on the need for re-inoculation of subsequent legume crops additional treatments (+/– inoculation) were imposed across farmer paddocks. Inoculation response trials for 6 legumes, field pea, faba bean, lentil, vetch, chickpea and lupin, were established at 6 sites in 2000 across the pulse growing regions of northern Victoria. Site establishment and maintenance details are similar to those methods outlined in Slattery and Coventry (1999). At each site, the commercial inoculant was introduced as one of the inoculation treatments in the form of a peat inocula.

### Site management and sowing

Preparation, sowing and maintenance of each site were carried out throughout the growing season.

When needed annual grasses and broadleaf weeds were controlled with the appropriate chemicals according to registered recommendations.

### Field plant sampling

Individual plants were collected 10–12 weeks after sowing for nodulation and plant dry matter measurements. Plant roots were scored for nodulation on a 0–5 scale, based on the nodule number, size, position, distribution and pigmentation of effective nodules on the crown and lateral roots (modification of Corbin et al., 1977). Plant material (tops) was dried in a forced-draught oven at 70°C for 48 hour, then weighed.

### Grain yields

Grain seed yield was determined by mechanical harvesting of the entire plot.

## Results and Discussion

### Evaluation of nitrogen fixing effectiveness

In general, each survey site could be categorised into 7 groups based on soil type and location of site. In this study, we investigated the ability of background rhizobial populations to infect commercially significant cool-season pulse crop species, namely *Rhizobium leguminosarum* bv *viciae*, *Bradyrhizobium* sp. and *Mesorhizobium cicer*.

Results from this soil survey have shown that there was considerable variation in the presence (or survival) of root-nodule bacteria at individual sites within a location or between rhizobial species (Table 2 and Table 3). On some soil types rhizobial strains are present that are capable of infecting the host legume and forming nodules, but at other sites rhizobia are not present in the soil and hence no nodules are formed.

Of the 50 paddocks surveyed 33% of the paddocks had sufficient background populations of *Rhizobium leguminosarum* bv *viciae* for infection of

the faba bean host, 54% for lentils, 55% for field pea, and 66% had sufficient populations for infection of the vetch host plant (Table 2). At 17 sites, no *Rhizobium leguminosarum* bv *viciae* populations were detected on either host plant identifying the importance of understanding your paddock history when sowing a legume crop. The presence of chickpea rhizobia, *Mesorhizobium cicer* was very low with only 7% of paddocks surveyed having sufficient background populations for the infection of chickpea plants. In the case of *Bradyrhizobium* sp, 38% of paddocks had background populations with numbers sufficiently high enough for effective nodulation of the lupin plant.

When the data from the survey paddocks were assessed across each location the presence of background root-nodule bacteria could be related to soil type and pH. The major *Rhizobium leguminosarum* bv *viciae* pulse growing regions in Victoria are the Birchip and Horsham regions. Background rhizobial populations in these regions are present and in the past, rhizobial inoculation has not been recommended, however this survey suggests that 30% of faba bean legume crops have inadequate nodulation leading to a reduction in crop yields. The presence of background chickpea rhizobia was lower in the Birchip regions (10%) compared to a value of 67% in the Horsham region (Table 3).

*Rhizobium leguminosarum* bv *viciae* populations at the Elmore, Dookie and Rutherglen locations are extremely low (Table 3). Soil pH<sub>Ca</sub> for these sites are also low (Table 5), suggesting a strong correlation between soil pH and rhizobial persistence (Figure 1). Poor rhizobial persistence indicates a need for inoculation of pulse legumes when sowing into acidic soils. In contrast to this for the alkaline soils there are usually high background rhizobial populations and the inoculation of pulses is not always necessary. Pulse production in Australia is increasing rapidly as farmers appreciate the financial and rotational benefits that pulses provide (Siddique and Sykes 1997) but when sowing pulse legumes the response of pulse production to inoculation must be clearly

**Table 2.** Mean values for background root-nodule bacteria encountered in a soil survey of paddocks (n=50) in the pulse growing regions of Victoria using the whole soil inoculation technique as the method of assessment.

	Host plant					
	Field pea	Faba bean	Lentil	Vetch	Chickpea	Lupin
Mean value (n=50)	55 <sup>A</sup>	33	54	66	7	38

<sup>A</sup>Number refers to the % of paddocks exhibiting infective soil root-nodule bacteria as recorded by the effective nodulation of the host plants.

**Table 3.** Presence of soil root-nodule bacteria collected from 50 paddocks across 7 locations in the pulse growing regions of Victoria using the whole soil inoculation technique as the method of assessment.

Location	Soil classification	Host					
		Field pea	Faba bean	Lentil	Vetch	Chickpea	Lupin
Birchip	Calcarosol	100 <sup>A</sup>	67	100	100	10	33
Walpeup	Calcarosol	71	0	56	78	0	14
Horsham	Sodosol	86	71	100	100	67	13
Elmore	Dermosol	0	0	0	14	0	57
Charlton	Dermosol	67	100	40	80	0	33
Dookie	Ferosol	14	0	14	14	0	71
Rutherglen	Kurosols	0	0	0	0	0	33

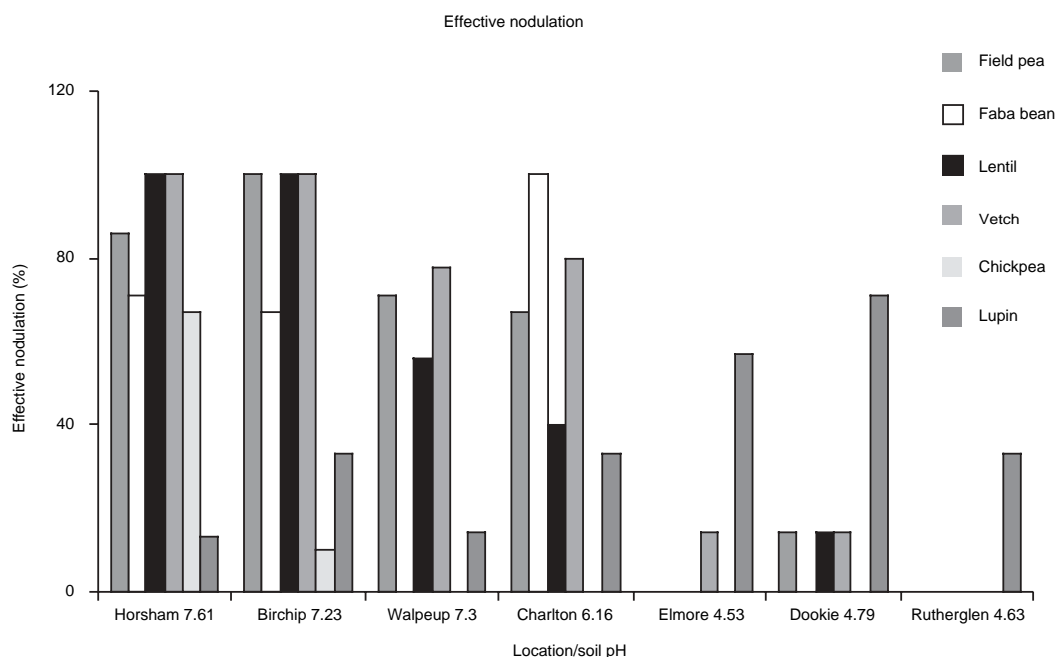
<sup>A</sup>Number refers to the % of paddocks exhibiting infective soil root-nodule bacteria as recorded by the effective nodulation of the host plants.

understood. To further increase pulse production, inoculation of the crop is essential especially where the naturalised rhizobial populations are low.

With the exception of the Horsham location, the extremely low *Mesorhizobium cicer* population again highlights the need for inoculation when chickpea crops are introduced into a paddock (Table 3). The chickpea-*Rhizobium* symbiosis is highly specific and extensive studies have demonstrated the uniqueness of the chickpea rhizobia with 99% of the chickpea

isolates nodulating only the original host plant and not species belonging to the Fabaceae and Mimosaceae families (Gaur and Sen 1979).

*Bradyrhizobium* sp populations were present in all soil types and varied between 12 and 71% across the different locations (Table 3). With the exception of the Dookie location where 71% of sites had sufficient population numbers, inoculation of future lupin crops is necessary at the other locations. Nonetheless, *Bradyrhizobium lupini* does not occur naturally



**Figure 1.** Relationship between effective nodulation of six legume species on soil pH<sub>Ca</sub> at seven locations.

in the soils of north-east Victoria, and it is essential that lupin seed be inoculated when first growing lupins (Slattery and Coventry 1989).

### Soil chemical parameters

Values for the means and range of concentration of soil properties at the Birchip sites are shown in Table 4. Soil pH for the 8 paddocks ranged from 6.83 to 8.83, with 63% of the sites being highly alkaline ( $pH_w > 8.0$ ). Soil  $pH_{Ca}$  for the 8 paddocks ranged from 6.06 to 8.03. Soil organic C ranged from 0.40 to 1.62%; the mean value of 1.1% is fairly low and can be explained by intense cereal rotation preventing a build up of organic matter. The mean total soil N value is low (0.06%), indicating potential for improvement in crop yields, if nitrogen were supplied, in fact 63% of the sites had a total soil N value  $< 0.06\%$ . There was a ten fold difference in maximum and minimum values of extractable P, with 75% of sites having a low Olsen P value of  $< 17$  mg/kg and would benefit from additional applied superphosphate. With the exception of one property the soils at the Birchip sites were classified as Calcarosols derived from limestone with calcium carbonate throughout the profile (Isbell 1996).

**Table 4.** Mean values and ranges for soil properties (n=8) encountered in a survey of the Birchip pulse growing regions of Victoria.

Soil Properties	Mean	Min. of range	Max. of range
$pH_w$	8.09	6.83	8.83
$pH_{Ca}$	7.23	6.06	8.03
Organic carbon (%)	1.15	0.40	1.62
Total N (%)	0.06	0.01	0.09
Olsen P (mg P/kg)	15.6	4.5	46.6
Extractable K (mg K/kg)	420	168	618

Values for the means and range of concentration of soil properties at the Charlton, Dookie, Elmore, Horsham and Rutherglen sites vary according to the region and the different soil types (data not shown). A summary showing soils classification and soil  $pH_{Ca}$  for the 7 sites identified the similarities and differences between soil types and regions (Table 5). Soil  $pH_{Ca}$  at the Charlton and Elmore sites has declined compared with previous data with  $pH_{Ca}$  values ranging from 5.78 to 6.77 at Charlton and from 4.41 to 4.71 at the Elmore sites. The major soil classification for the Charlton and Elmore sites is a Dermosol; these soils tend to form surface crusting if not managed appropriately (Isbell 1996). Soil  $pH_{Ca}$  in the Dookie region were lower than for the other regions with  $pH_{Ca}$  ranging from 4.42 to 5.29 and

soils classified as Ferrosols, containing a high iron content and a distinctive chocolate brown colour (Isbell 1996).

**Table 5.** Summary of soil pH and soil classification data across 7 locations in the pulse growing regions of Victoria.

Location	Soil classification	Mean $pH_{Ca}$	Min. of range	Max. of range
Birchip	Calcarosol	7.23	6.06	8.03
Walpeup	Calcarosol	7.30	5.93	8.05
Horsham	Sodosol	7.61	7.40	7.70
Elmore	Dermosol	4.53	4.43	4.71
Charlton	Dermosol	6.16	5.78	6.77
Dookie	Ferrosol	4.79	4.42	5.29
Rutherglen	Kurosols	4.63	4.21	4.92

Results from this survey have shown that soil factors influence the legume-*Rhizobium* symbiotic relationship (Figure 1). The growth and survival of *Rhizobium* spp in soil environments can be affected by a combination of factors including acidity (including toxicities of Al), salinity, alkalinity (including high concentrations of Ca and B), soil temperature, moisture, fertility (including nutrient deficiencies), and soil structure. Much of the area affected by soil acidity is naturally acidic, though agricultural practice is further acidifying the soil. Acidity factors (high Al, low Ca and low  $PO_4^-$ ) have a direct impact on either rhizobia growth and persistence, or nodule initiation and  $N_2$ -fixation effectiveness (Coventry and Evans 1989). On acid soils the populations of rhizobia can be low in subterranean clover-based pasture (Coventry and Hirth 1992) or poorly nodulated in medic-based pasture (Howieson and Ewing 1986).

Soil acidity limits *Rhizobium* survival and persistence in soils, and the subsequent root colonisation, infection and nodule activity (Brockwell et al. 1991). The correct soil pH is crucial for the survival of *Rhizobium* spp, and in adverse soil pH environment strains of rhizobia differs in their ability to infect the host plant (Brockwell et al. 1995).

This soil survey identified considerable variation in both soil type and the survival of effective *Rhizobium* strains. However, we need to understand the implications that these factors have for farmers regarding the need for re-inoculation when sowing into a paddock with a previous history of legumes. If there is sufficient effective rhizobia, is there a need to re-inoculate? This question is especially important in Australian systems where intensified cropping is often accompanied by an increased reliance on chemicals for weed, pest and disease control; these chemicals are used as seed dressings or in pre- and post-emergent crop situations. To answer

the question of re-inoculation of subsequent legume crops additional treatments (+/- inoculation) were imposed on these same farmer paddocks.

### Farmer inoculation response trials

Following the assessment of survey data, a selection of paddocks were sown with inoculated and non-inoculated legumes in 2000. These trials were established to verify *Rhizobium* survival in the soils and then make recommendation for farmers regarding the need for re-inoculation of a paddock based on soil type and background *Rhizobium* populations. Inoculation trials were sown at six sites across the Victorian Mallee (alkaline soils, high background populations), southern Mallee to north-east Victoria (acidic soils, low background populations). Selection criteria for each site was based on soil type, soil pH, location and rainfall (Table 6).

**Table 6.** Location, soil pH and background rhizobial populations for farmer inoculation response trials (2000).

Site location	Soil type	Soil pH <sub>Ca</sub>	Background populations
Bakers-Rutherglen, NE Vic	Kurosols	4.60	Low
Ferriers-Birchip, NW Vic	Calcarosol	7.60	High
Smith-Birchip, NW Vic	Calcarosol	7.78	High
Corbetts-Walpeup, NW Vic	Calcarosol	8.80	High
Nihill-Walpeup, NW Vic	Calcarosol	7.29	High
Pohlner-Walpeup, NW Vic	Calcarosol	7.16	High

On the acidic kurosol clay soils at Rutherglen the nodulation derived from the background rhizobial population varied between plant species (Table 7). For lupins, there was a limited response to inoculation, which supports the survey findings for the 6 district paddocks in that 33% of the paddocks had background *Bradyrhizobium lupini* in numbers sufficient for effective nodulation of lupins. Adequate nodulation occurred in the nil treatment of field pea, lentil and vetch. However, with inoculation nodulation and grain yields were further increased thus highlighting the need, and additional benefits of inoculation when growing pulse legumes in these acidic soils.

Visible growth differences were observed late in the growing season for lentils. Nonetheless, these differences could not be related to increased grain yields as the farmers' sheep grazed the lentil crop prior to harvest. Inoculation of faba bean was vital in this paddock as shown by the increase in yield from 0.34 t/ha for the nil treatment to 4.4 t/ha after inoculation. Even though background rhizobial populations were present the response to inoculation was enormous. This farmer routinely inoculates his legume seed but now understands the reasons why it is necessary. Poor persistence of *Rhizobium leguminosarum* bv *viciae* in acidic soils was also demonstrated and was shown by a low nodulation score and poor plant growth, however in another study some strains were found to be more tolerant of acidic soils than other strains examined (Carter et al. 1995). Frey and Blum (1994) also give an example of soil pH affecting *Rhizobium* survival and legume growth.

A visual response to inoculation was also obtained for the chickpea crop with an increase in yields from 0.47 to 2.37 t/ha. In general, chickpea crops are not

**Table 7.** Nodule number, nodule score, shoot dry weight (g/pl), root dry weight (g/pl) and grain yield (t/ha) for the farmer inoculation response trial at the Rutherglen site (2000).

Legume	Rhizobia Strain	Nodule no./plant	Nodule score	Root DM (g/plant)	Top DM (g/plant)	Yield (t/ha)
Lupin	Nil	16.4	2.64	0.63	1.80	0.71
	WU425	19.9	3.18	0.50	1.91	0.98
Faba bean	Nil	10.2	2.64	1.39	4.33	0.34
	WSM1274	34.5	4.21	4.21	18.50	4.41
Field pea	Nil	43.1	4.07	0.55	6.28	2.15
	SU303	53.7	4.27	0.46	7.43	2.87
Lentil	Nil	10.1	2.21	0.11	0.89	0.15 <sup>A</sup>
	WSM1274	21.6	3.19	0.11	0.86	0.12 <sup>A</sup>
Vetch	Nil	33.6	3.45	0.66	5.44	7.87 <sup>B</sup>
	SU303	64.1	4.81	0.51	7.09	8.07 <sup>B</sup>
Chickpea	Nil	1.62	0.62	0.88	3.13	0.47
	CC1192	23.5	4.02	2.20	3.78	2.37

<sup>A</sup> Crop grazed by sheep, reduced grain yield

<sup>B</sup> Vetch dry matter t/ha, vetch cut prior to grain harvest

usually grown on acidic soils, especially where the soil pH is only 4.6. However, when the growing seasonal rainfall is below average and crop disease is controlled, chickpea yields of 2.37 t/ha are an economic option for farmers in northeast Victoria. The chickpea-*Rhizobium* symbiosis is highly specific and, as a consequence, rhizobial survival in the soil and growth in the rhizosphere is most important. Rai (1991) in a study involving the symbiosis between chickpea and *Rhizobium leguminosarum* bv *cicer* strains for adaptation to acidic soils demonstrated that only 5% of the strains examined were found to be suitable for nodulation and growth in these strongly acidic soil environments.

On the alkaline calcarosol soils, the response to inoculation was not visually evident. At the Ferrier-Birchip site (Table 8), background populations under lupins were low; inoculation significantly increased nodulation and improved grain yields. However, as a consequence of the high background populations of *Rhizobium leguminosarum* bv *viciae* inoculation did not improve the nodulation of faba bean, field pea, lentil or vetch. Inoculation with SU303 improved the yields compared to the nil treatment. In the case of lentils, grain yields for WSM1455 and WSM1483 were higher than WSM1274. For chickpeas, background populations were low and inoculation significantly increased nodulation and spring dry matter production. The

low district growing season rainfall is able to explain the low grain yields at this site.

The inoculation response trials provided us with field evidence that supports the field survey data. Soil pH, paddock history, disease control and rainfall all contribute to successful crops and grain yields. The presence of background rhizobia is not always sufficient in itself, to ensure optimal N<sub>2</sub>-fixing capacity in the host legume as the effectiveness of strains to fix N within naturalised populations can vary considerably. It is usually accepted that it is difficult to introduce superior strains (by inoculation) when there is a high background population of indigenous and well-adapted rhizobia, and nodules occupied by the inoculant strain may decline in the years following inoculation (Unkovich and Pate 1998; Slattery and Coventry 1999). Soil surveys in high pH areas of southeast Australia show that the lack of effective *Rhizobium* may limit the performance of annual medics (Ballard and Charman 1996; Slattery et al. 1999). In these areas, the naturalised medic *Rhizobium* populations are generally high (>10<sup>4</sup> *Rhizobium* bacteria g/m soil), but the annual medic species frequently do not achieve an effective symbiosis with the naturalised rhizobia (Ballard and Charman 1996; Slattery et al. 1999). The reasons for this poor effectiveness of naturalised medic populations are uncertain at this stage but may be related to soil chemical characteristics at high pH. It is clear that to obtain optimum N<sub>2</sub>-fixation for a range of

**Table 8.** Nodule number, nodule score, shoot dry weight (g/pl), root dry weight (g/pl) and grain yield (t/ha) for the farmer inoculation response trial at the Ferriers-Birchip site (2000).

Legume	Rhizobia Strain	Nodule no./plant	Nodule score	Root DM (g/plant)	Top DM (g/plant)	Yield (t/ha)
Lupin	Nil	0.1	0.10	0.33	1.80	0.74
	WU425	6.1	2.37	0.40	1.62	0.81
Faba bean	Nil	23.6	4.31	0.99	6.01	0.26
	WSM1274	37.9	4.25	0.78	5.15	0.59
	SU303	25.5	4.02	0.92	5.61	0.73
	RRI294	20.2	3.69	0.65	4.09	0.27
	RRI339	20.2	3.95	0.89	5.48	0.46
Field pea	Nil	30.8	3.95	0.40	6.57	0.78
	SU303	24.1	4.15	0.28	6.19	0.92
	RRI294	22.4	4.18	0.28	6.57	0.81
	RRI339	22.3	4.04	0.23	5.92	0.81
Lentil	Nil	24.1	4.24	0.09	1.03	0.83
	WSM1274	18.8	3.57	0.10	1.09	1.01
	WSM1455	24.6	4.02	0.09	1.03	1.17
	WSM1483	21.0	3.51	0.09	1.00	1.35
Vetch	Nil	20.5	4.19	0.17	2.40	1.76
	SU303	13.5	3.36	0.12	2.14	1.81
Chickpea	Nil	0.19	0.19	0.23	1.30	NH
	CC1192	4.00	2.02	0.26	1.61	NH

NH– not harvested, cut and removed due to Ascochyta damage.

soil environments the soil chemical status and background rhizobial populations must first be investigated. Only then can rational conclusions be made regarding the effectiveness of new strains for specific soil conditions.

### Conclusion

Cropping systems in Australia are intensifying with longer periods of the crop phase used in a rotation. Associated with this intensification is the higher input of chemicals, increased reliance on N-fertiliser, less tillage and more flexibility in sowing management. Within these changed systems there still exists a need for productive pulse crops, not only as economic crops in their own right, but to provide options for weed and disease management. However, these pulse crops are reliant on optimal nitrogen fixation and it is unlikely that intensified cropping systems will be sustainable in the long term without periods of highly productive legume-based pastures. Pasture input may be required for weed management, organic matter conservation and in some situations for managing a rising subsoil watertable. Pasture quality for either livestock or forage enterprises is dependent on a significant and productive legume component within the pasture and will also require optimal nitrogen fixation to achieve maximum production. In association with the improvement in legume productivity and subsequent improvements in soil fertility, there is an on-going need to develop more effective rhizobial microflora.

### Acknowledgments

We acknowledge the financial support of the Grains Research and Development Corporation (GRDC) to undertake this work. The financial support provided to the senior author by the Australian Centre for International Agriculture Research (ACIAR) to attend this workshop is gratefully appreciated.

### References

- Ballard, R. and Charman, N. 1996. Are the *Rhizobium* in Australian soils limiting the performance of annual medics? In 'Farming Systems Developments'. Proceedings of the workshop on farming development systems of southern Australia, 26-28 March 1996, 107-108.
- Brockwell, J., Bottomley, P.J. and Thies, J.E. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. *Plant and Soil*, 174: 143-80.
- Brockwell, J., Holliday, R.A. and Pilka, A. 1988. Evaluation of the symbiotic nitrogen-fixing potential of soils by direct microscopic means. *Plant and Soil*, 108: 163-70.
- Brockwell, J., Pilka, A. and Holliday, R.A. 1991. Soil pH is a major determinant of the numbers of naturally occurring *Rhizobium meliloti* in non-cultivated soils in central New South Wales. *Australian Journal of Experimental Agriculture*, 31: 211-219.
- Carter, J.M., Tieman, J.S. and Gibson, A.H. 1995. Competitiveness and persistence of strains of rhizobia for faba bean in acid and alkaline soils. *Soil Biology and Biochemistry*, 27: 617-623.
- Corbin, E.J., Brockwell, J. and Gault, R.R. 1977. Nodulation studies on chickpea (*Cicer arietinum*). *Australian Journal Experimental Agriculture & Animal Husbandry*, 17: 126-134.
- Coventry, D.R. and Evans, J. 1989. Symbiotic nitrogen fixation and soil acidity. In: Robson, A.D. ed. *Soil Acidity and Plant Growth*. Academic Press, Sydney, 103-137.
- Coventry, D.R. and Hirth, J.R. 1992. Effects of tillage and lime on *Rhizobium trifolii* populations and survival in wheat-subterranean clover rotation in southeastern Australia. *Soil and Tillage Research*, 25: 67-74.
- Frey, S.D. and Blum, L.K. 1994. Effect of pH on competition for nodule occupancy by type I and type II strains of *Rhizobium leguminosarum* bv *phaseoli*. *Plant and Soil*, 163: 157-64.
- Gaur, Y.D. and Sen, A.N. 1979. Cross inoculation group specificity in *Cicer* rhizobium symbiosis. *New Phytology*, 83: 745-754.
- Gibson, A.H., Curnow, B.C., Bergersen, F.J., Brockwell, J. and Robinson, A.C. 1975. Studies of field populations of *Rhizobium*: effectiveness of strains of *Rhizobium trifolii* associated with *Trifolium subterraneum* L. pastures in south-eastern Australia. *Soil Biology and Biochemistry*, 7: 95-102.
- Howieson, J.G., Malden, J., Yates, R.J. and O'Hara, G.W. 2000. Techniques for the selection and development of elite inoculant strains of *Rhizobium leguminosarum* in southern Australia. *Symbiosis*, 28: 33-48.
- Howieson, J.G. and Ewing, M.A. 1986. Acid tolerance in the *Rhizobium meliloti*-*Medicago* symbiosis. *Australian Journal of Agricultural Research*, 37: 55-64.
- Isbell, R.F. 1996. *The Australian Soil Classification*, CSIRO Publishing, Australia.
- Rai, R. 1991. Effects of soil acidity factors on interaction of chickpea (*Cicer arietinum* L.) genotypes and *Rhizobium* strains: Symbiotic N-fixation, grain quality and grain yield in acid soils. In: Wright, R.J., Baliger, V.C. and Murrmann, R.P. ed. *Plant-soil interactions at low pH*. Proceedings of the 2<sup>nd</sup> international symposium on plant-soil interactions at low pH. (Kluwer Academic Publishers, Netherlands) 597-601.
- Siddique, K.H.M. and Sykes, J. 1997. Pulse production in Australia past, present and future. *Australian Journal of Experimental Agriculture*, 37: 103-11.
- Slattery, J.F. and Coventry, D.R. 1989. Populations of *Rhizobium lupini* in soils used for cereal-lupin rotations in north-east Victoria. *Soil Biology and Biochemistry*, 21: 1009-1010.
- Slattery, J.F. and Coventry, D.R. 1993. Variations of soil populations of *Rhizobium leguminosarum* bv *trifolii* and the occurrence of inoculant rhizobia in nodules of subterranean clover after pasture renovation in north-eastern Victoria. *Soil Biology and Biochemistry*, 25: 1725-1730.

- Slattery, J.F. and Coventry, D.R. 1999. Persistence of introduced strains of *Rhizobium leguminosarum* by *trifolii* in acidic soils of north-eastern Victoria. *Australian Journal of Experimental Agriculture*, 39: 829–837.
- Slattery, J.F., Slattery, W.J. and Carmody, B.C. 1999. Influence of soil chemical characteristics on medic rhizobia in the alkaline soils of south eastern Australia. In: Martinez and Hernandez ed. *Highlights of Nitrogen Fixation Research*. (Kluwer Academic/Plenum Publishers, New York) 243–249.
- Thies, J.E., Singleton, P.W. and Bohlool, B. 1991. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field grown legumes. *Applied and Environmental Microbiology*, 57: 19–28.
- Unkovich, M.J. and Pate, J.S. 1998. Symbiotic effectiveness and tolerance to early season nitrate in indigenous populations of subterranean clover rhizobia from SW Australian pastures. *Soil Biology and Biochemistry*, 30: 1435–1443.