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Nodulation and growth of pasture legumes with naturalised soil rhizobia 1. Annual *Medicago* spp.

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Abstract. The ability of 11 species of annual medics (*Medicago doliata*, *M. laciniata*, *M. littoralis*, *M. minima*, *M. orbicularis*, *M. polymorpha*, *M. praecox*, *M. rigidula*, *M. rigiduloides*, *M. tornata* and *M. truncatula*) to nodulate and fix nitrogen with naturalised rhizobia from 28 South Australian soils was assessed. The number of rhizobia in the soils was estimated. Medic shoot dry matter production and nodulation were measured, after inoculation of medic seedlings with a soil suspension, in 2 glasshouse experiments.

The number of medic rhizobia ranged from 0.4×10^2 to 1.5×10^6 per gram soil. *Medicago laciniata* was the only medic species tested which was not consistently nodulated by the soil rhizobia. While all the other species formed nodules, they varied widely in their ability to form an effective symbiosis. Symbiotic performance (which indicates how much growth the medic line achieved, when compared to an effective inoculation treatment) of the medic species ranged from 3% (*M. rigiduloides*) to 67% (*M. praecox*). Herald (*M. littoralis*) achieved a symbiotic performance of 49% and it was estimated that this would be insufficient to meet the nitrogen requirements of a Herald-based pasture during early growth. The symbiotic performance of Santiago (*M. polymorpha*) was low (17%) and erratic (from –6 to 72%). The ability of the rhizobia to form an effective symbiosis varied widely also between soil regions. For example, the rhizobia in Riverland soils resulted in only 31% of the shoot dry matter of those in Eyre Peninsula soils, in association with *M. polymorpha*.

There are significant opportunities to improve the symbiotic performance of a number of the species of annual medics examined in this study. Options to improve the effectiveness of the symbiosis of medics with naturalised soil rhizobia are discussed.

Additional keywords: *Rhizobium meliloti*, soil rhizobia, nitrogen fixation.

Introduction

Annual medics (*Medicago* spp.) occur across 20 Mha (Hill and Donald 1998) of Australian soils, mostly on those of alkaline reaction (Cocks *et al*. 1980). They are represented across these soils by numerous species (Andrew and Hely 1960; Amor 1965; Hill and Donald 1998) which may be sown [including *M. littoralis* Rohde ex Lois., *M. truncatula* Gaertn., *M. polymorpha* L., *M. scutellata* (L.) Mill., *M. tornata* (L.) Mill. and *M. murex* Willd.) or occur as naturalised flora (including *M. minima* (L.) Bartalini, *M. polymorpha, M. praecox* DC. and *M. laciniata* (L.) Mill.]. Species distribution tends to be influenced by soil type (Heyn 1963). *Medicago littoralis*, for example, is dominant on the light-textured soils of the Eyre Peninsula of South Australia. Medic pastures are a valued component of ley-farming systems and pastoral grazing zones because of their ability to add up to 100 kg/ha.year of biologically fixed nitrogen to the agricultural system (Peoples and Baldock 1999).

In South Australia, where alkaline soils dominate, seed inoculation with *Rhizobium* is rarely practised when medic pastures are sown because medic rhizobia are known to have become naturalised in most soils. Medic rhizobia are often present in numbers exceeding 2000/g soil (Brockwell *et al*. 1991; Slattery *et al*. 1999) where soil pH is greater than 7. Even when inoculation is practiced, large numbers of soil rhizobia make it difficult for inoculant rhizobia to persist in forming nodules in the long term (Brockwell *et al*. 1982; Slattery and Coventry 1993). They must compete numerically with the soil rhizobia for nodule formation (Ireland and Vincent 1968)

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and overcome an array of stresses during the nodulation process (Dowling and Broughton 1986), to which the soil rhizobia are probably better adapted. In addition, they must be able to persist in the soil in the absence of the host plant (Brockwell *et al*. 1982). Hence, it is likely that most nitrogen fixation by medics occurs in association with naturalised rhizobia in South Australian soils.

To establish whether medic pastures are contributing optimally to replenishing soil nitrogen, it is essential to know whether the naturalised rhizobia are efficient at fixing nitrogen; whether this characteristic varies across geographic regions and whether there is any interaction with the species of medics involved in the symbiosis. This paper seeks to address these questions.

Materials and methods

Two glasshouse experiments were conducted to determine the ability of 12 lines (11 species) of annual medics (Table 1) to nodulate and form an effective symbiosis with 28 sources of naturalised soil rhizobia.

Collection and characterisation of soils

Twenty-nine soil samples were collected from 4 regions of South Australia, namely Eyre Peninsula (EP, n = 8), the Upper Mid-north (UMN, n = 10), the Riverland (RLD, n = 7) and the Upper South-east (USE, n = 4) (Fig. 1, Table 2). Samples from the RLD were collected in December 1994, while the remainder were collected in March and April 1995. All samples were air dried and stored at 4°C before assessment.

The number of medic rhizobia was estimated in each soil using a plant-infection test consisting of six levels of 10-fold dilutions of the soil, with 3 replicate plants at each level. Approximate 95% confidence limits for each estimate may be calculated by multiplying the estimate by 0.3 and 3.8 (Vincent 1970). Lucerne (*Medicago sativa* L. cv. Trifecta) was used as the trap plant and was inoculated on 16 May 1995. Plants were harvested and assessed for nodulation 35 days after inoculation. Medic rhizobia were not detected in 1 soil from the UMN. This soil was not used in further experiments.

Soil pH (1:5, soil:0.01 mol $CaCl_2$/L), total nitrogen (Kjeldahl), phosphorus (0.5 mol $NaHCO_3$/L) and organic carbon (Walkley/Black) are shown in Table 2. Soil texture is a qualitative estimate based on the cohesion of the soil when moist.

Inoculation and conditions of plant growth

Two glasshouse experiments were completed. In each experiment 6 lines of annual medics (Table 1) were inoculated with 1 mL of an extract of soil in 0.85% NaCl [10:90 (w/v), shaken for 15 min] of each soil sample. Control treatments included uninoculated, mineral nitrogen (1 mL of 150 mmol NH_4NO_3/L at 7-day intervals commencing 18 and 7 days after inoculation in experiments 1 and 2, respectively) and pure rhizobial cultures (WSM826 and WSM688). Strains WSM826 and WSM688 were grown on yeast mannitol agar (Vincent 1970) and were 5 days old at

Table 1. Lines of medic used in experiments

Common medic name	Species	Line	Estimated seed size (mg/seed)	Commercial inoculant recommendation	General comments
Strand	*M. littoralis*	SA8965[A]	3.2	WSM826	Selected for alkaline sands on the Upper Eyre Peninsula, South Australia
Strand	*M. littoralis*	cv. Herald	2.2	WSM826	Prefers neutral/alkaline sandy soils receiving >250 mm annual rainfall
—	*M. rigiduloides*	SA3601	4.2	None	Reputed tolerance to cold (below freezing) conditions
—	*M. rigidula*	SA5618	4.2	None	Reputed tolerance to cold (below freezing) conditions
Wooly burr	*M. minima*	—	1.9	None	Naturalised widely across southern Australia
Small leaf bur	*M. praecox*	—	2.0	None	Naturalised in the mid-north of South Australia
Button	*M. orbicularis*	SA8460	3.9	None	Being investigated for summer-dominant rainfall regions in Queensland
Barrel	*M. truncatula*	cv. Caliph	4.4	WSM688	Prefers alkaline sandy loams and clays receiving >250 mm annual rainfall
Cutleaf	*M. laciniata*	SA11710	0.9	None	Naturalised on clay loams throughout New South Wales
—	*M. doliata*	SA11772	2.4	None	Reputed cold tolerance
Burr	*M. polymorpha*	cv. Santiago	3.4	WSM688	Prefers mildly acidic loams and clays receiving >350 mm annual rainfall
Disc	*M. tornata*	cv. Rivoli	4.5	WSM826	Prefers neutral/alkaline sands receiving >400 mm annual rainfall

[A]Refers to identity of medic line in South Australian Annual Pasture Legume Collection.

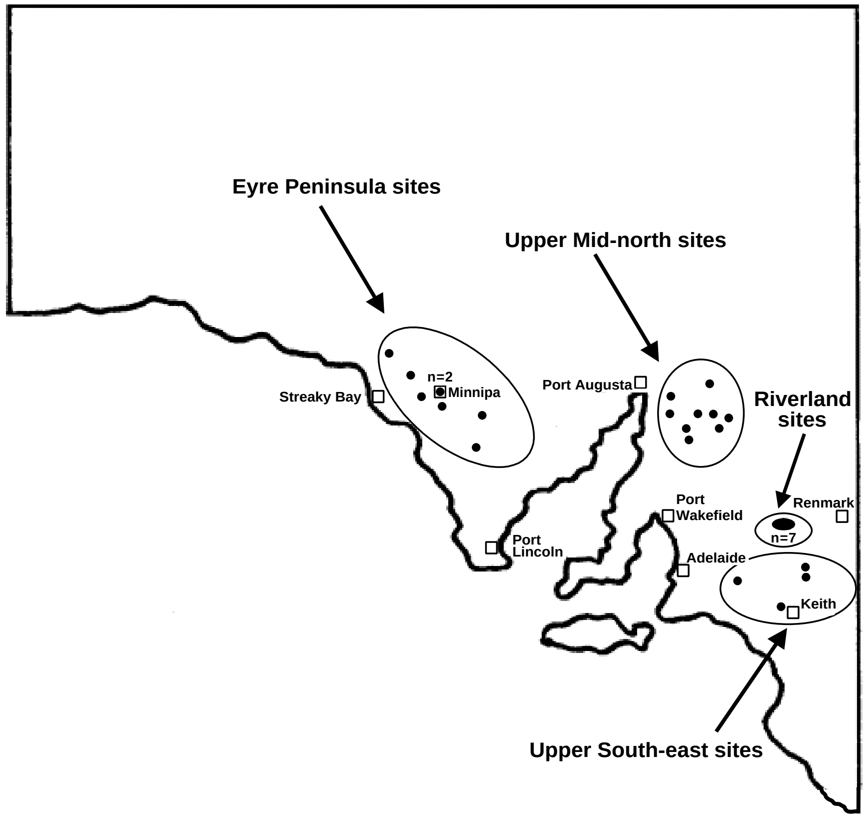

Figure 1. Location of sampling sites in South Australia.

inoculation. Plants were inoculated with 1 mL of pure cultures suspended in quarter-strength nutrient solution (McKnight 1949). Experiments 1 and 2 were inoculated on 7 July and 6 September 1995, respectively.

Plants were grown in a shaded glasshouse in 20 by 150 mm glass tubes containing vermiculite moistened with a nitrogen-free nutrient solution (McKnight 1949), and were watered with sterile distilled water as required. The roots of the plants were contained within the tube under bacteriologically-controlled conditions, the shoots were exposed to the air. The average ambient day/night temperature was 24/20°C. Plants were harvested 35 days after inoculation. Shoots were dried at 60°C for 72 h to determine dry matter (DM). In order to overcome differences that seed weight and other inherent characteristics have on the absolute shoot DM production of the different medic species, we have calculated symbiotic performance (SP). Symbiotic performance indicates the level of potential growth achieved by each medic species. It enables comparison between species and was calculated as follows:

$$\frac{SDM_{inoc} - SDM_{uninoc}}{SDM_{binoc} - SDM_{uninoc}} \times 100$$

where SDM_{inoc} is the mean shoot DM of the treatment, SDM_{uninoc} is the shoot DM of the uninoculated treatment and SDM_{binoc} is the shoot DM of the best inoculation treatment. The presence or absence of nodules on the plants was recorded. The colour of the nodules (red or white) was also recorded.

Statistical analyses

Both experiments were set up in a completely randomised block design with 8 replicates. Shoot DM was square-root transformed before analysis of variance was conducted. All references to significance in shoot DM in the text imply statistical significance at $P = 0.05$.

Results

Number of rhizobia

The most probable number of rhizobia/g soil ranged from 0.4×10^2 to 1.5×10^6 (Table 2). Numbers were highest in the EP soils (mean 2.1×10^5, range 2.3×10^3–1.5×10^6) and lowest in the USE soils (mean 1.3×10^2, range 0.4×10^2–2.3×10^2). There was no significant correlation between the number of rhizobia in the soil and any of the measured soil factors.

Experiment 1

Both strains WSM826 and WSM688 failed to significantly increase the shoot DM of *M. littoralis*

Table 2. Location, rhizobial and chemical characteristics of soils
EP, Eyre Peninsula; UMN, Upper Mid-north; USE, Upper South-east; RLD, Riverland

Soil	Map reference	Number of rhizobia/g soil	pH ($CaCl_2$)	N (mg/kg)	P (mg/kg)	Organic C (%)	Texture
EP 1	33°09´S, 135°43´E	2300	6.9	20	30	0.8	Sandy loam
EP 2	33°35´S, 135°39´E	36 700	6.4	32	83	1.2	Loam
EP 3	32°52´S, 135°09´E	23 000	7.7	21	36	0.8	Loam
EP 4	32°52´S, 135°09´E	42 400	7.5	23	41	1.2	Loam
EP 5	32°24´S, 134°29´E	23 000	7.5	16	32	0.8	Loam
EP 6	32°34´S, 134°43´E	1 490 000	7.7	18	26	1.0	Loam
EP 7	32°47´S, 134°53´E	23 000	7.7	13	26	1.6	Sandy loam
EP 8	32°56´S, 135°05´E	14 700	7.6	21	27	0.9	Loam
UMN 1	33°02´S, 138°24´E	91 900	7.6	2	26	1.4	Sandy loam
UMN 2	32°52´S, 138°24´E	9180	7.0	17	35	1.0	Clay loam
UMN 3	32°45´S, 138°24´E	23 000	7.7	19	58	1.1	Loam
UMN 4	32°49´S, 138°14´E	2300	6.8	3	40	1.7	Loam
UMN 5	32°37´S, 138°14´E	14 700	7.6	13	13	0.9	Loam
UMN 6	32°30´S, 138°27´E	42 300	7.5	2	20	1.3	Loam
UMN 7	32°40´S, 138°39´E	42 300	7.4	36	15	1.0	Loam
UMN 8	32°49´S, 138°46´E	1440	7.0	10	21	0.7	Loam
UMN 9	33°01´S, 138°38´E	9180	6.8	2	28	2.0	Loam
USE 1	35°20´S, 140°36´E	230	6.7	23	32	0.8	Sand
USE 2	36°01´S, 140°06´E	90	6.8	11	17	1.4	Sand
USE 3	35°29´S, 139°57´E	150	6.3	15	19	0.8	Sand
USE 4	35°16´S, 140°29´E	40	6.1	10	29	0.8	Sand
RLD 1	34°15´S, 140°05´E	5880	7.6	10	9	0.8	Loam
RLD 2	34°10´S, 139°48´E	3680	6.5	4	6	0.3	Sand
RLD 3	34°15´S, 140°00´E	36 700	7.6	6	11	0.8	Loam
RLD 4	34°18´S, 140°11´E	4240	7.7	3	16	0.3	Sand
RLD 5	34°13´S, 140°07´E	42 400	7.6	6	14	0.6	Sandy loam
RLD 6	34°18´S, 140°16´E	9180	7.6	5	4	0.5	Sand
RLD 7	34°16´S, 140°00´E	9180	7.5	5	18	0.4	Loam

line SA8965 and *M. rigiduloides* (E. Small) line SA3601, compared with the uninoculated treatment (Table 3) but significantly improved the shoot DM of the other medic lines.

Across all medic hosts, soil rhizobia from the USE performed worst, producing, on average, only 7 mg more DM than the uninoculated treatments. Nodulation (data not shown) associated with the USE soils was erratic with 31% of plants failing to form nodules. Soil rhizobia from EP, the UMN and the RLD generally performed better, producing 17, 14 and 15 mg more DM, respectively, than the uninoculated treatments. Only 6% of plants (mainly the UMN 4 treatment) inoculated with these soils failed to form nodules.

Medic lines varied in their ability to form an effective symbiosis with the soil rhizobia. Most sources of soil rhizobia increased the shoot DM of *M. littoralis*, *M. rigidula* (L.) and *M. praecox* compared with the uninoculated treatments. Only 14 sources of soil rhizobia increased the shoot DM of *M. minima*. None of the soil rhizobia increased the shoot DM of *M. rigiduloides*, even though 79% of the soil-inoculated plants were nodulated. Symbiotic performance (SP) provides a measure of how much of the potential plant growth was achieved, relative to the best inoculation treatment. Symbiotic performance of the medic lines with soil rhizobia ranged from 3 ± 11% (mean ± s.d.) for *M. rigiduloides* to 41 ± 19% for *M. minima*, 49 ± 25% for *M. littoralis* cv. Herald, 58 ± 25% for *M. littoralis* line SA8965, 65 ± 22% for *M. rigidula* and 67 ± 20% for *M. praecox*.

Experiment 2

Strain WSM826 failed to significantly increase shoot DM of *M. laciniata*, *M. polymorpha* and *M. tornata*

Table 3. Effect of inoculation treatment on the shoot dry matter of six lines of medic

Transformed means are shown in parentheses and should be used for statistical comparisons
For the transformed means, the l.s.d. for comparison of inoculation treatment is 0.37; medic line is 0.16 and interaction is 0.91
EP, Eyre Peninsula; UMN, Upper Mid-north; USE, Upper South-east; RLD, Riverland

Inoculation treatment	Shoot dry matter (mg/plant)						
	M. littoralis SA8965	*M. littoralis* cv. Herald	*M. rigiduloides* SA3601	*M. rigidula* SA5618	*M. minima*	*M. praecox*	Overall mean (all lines)
Uninoculated	5 (2.1)	8 (2.6)	11 (3.3)	11 (3.3)	3 (1.6)	3 (1.8)	7 (2.5)
Nitrogen	16 (3.9)	20 (4.5)	29 (5.2)	30 (5.4)	7 (2.6)	12 (3.4)	19 (4.2)
WSM826	6 (2.3)	37 (6.0)	11 (3.3)	60 (7.7)	8 (2.8)	20 (4.4)	24 (4.4)
WSM688	6 (2.4)	32 (5.6)	9 (2.9)	59 (7.6)	7 (2.7)	18 (4.3)	22 (4.2)
EP 1	35 (5.9)	34 (5.7)	7 (2.6)	65 (8.0)	13 (3.5)	17 (4.1)	28 (5.0)
EP 2	8 (2.8)	21 (4.3)	11 (3.3)	49 (7.0)	6 (2.3)	16 (3.9)	19 (3.9)
EP 3	24 (4.8)	29 (5.3)	11 (3.2)	69 (8.3)	8 (2.7)	17 (4.0)	26 (4.7)
EP 4	25 (4.9)	22 (4.6)	12 (3.4)	65 (8.0)	7 (2.6)	20 (4.4)	25 (4.7)
EP 5	24 (4.8)	25 (5.0)	13 (3.5)	61 (7.8)	7 (2.6)	16 (4.0)	24 (4.6)
EP 6	26 (5.1)	21 (4.5)	13 (3.5)	58 (7.6)	6 (2.4)	17 (4.0)	23 (4.5)
EP 7	24 (4.8)	27 (5.1)	11 (3.2)	51 (7.1)	9 (2.9)	14 (3.6)	22 (4.4)
EP 8	31 (5.6)	24 (4.8)	11 (3.2)	53 (7.0)	7 (2.6)	14 (3.7)	23 (4.5)
UMN 1	21 (4.5)	25 (5.0)	13 (3.5)	62 (7.8)	7 (2.7)	10 (3.0)	23 (4.4)
UMN 2	18 (4.0)	23 (4.7)	16 (3.9)	45 (6.7)	6 (2.5)	18 (4.1)	21 (4.3)
UMN 3	23 (4.8)	32 (5.6)	13 (3.6)	58 (7.6)	7 (2.5)	17 (4.1)	25 (4.7)
UMN 4	7 (2.6)	5 (2.1)	10 (3.1)	22 (4.5)	5 (2.1)	12 (3.4)	10 (3.0)
UMN 5	28 (5.3)	29 (5.4)	11 (3.3)	49 (6.9)	8 (2.7)	14 (3.7)	23 (4.5)
UMN 6	34 (5.8)	30 (5.4)	10 (3.1)	61 (7.8)	12 (3.5)	16 (3.9)	27 (4.9)
UMN 7	22 (4.5)	32 (5.5)	11 (3.2)	45 (6.5)	8 (2.8)	20 (4.3)	22 (4.5)
UMN 8	22 (4.5)	18 (3.9)	13 (3.6)	38 (6.1)	8 (2.8)	19 (4.3)	20 (4.2)
UMN 9	21 (4.4)	25 (5.0)	15 (3.8)	38 (6.0)	7 (2.6)	21 (4.5)	21 (4.4)
USE 1	6 (2.4)	11 (3.1)	10 (3.1)	24 (4.8)	4 (1.9)	7 (2.4)	10 (3.0)
USE 2	20 (4.4)	18 (4.1)	10 (3.2)	37 (5.9)	6 (2.4)	10 (3.1)	17 (3.9)
USE 3	13 (3.3)	7 (2.7)	13 (3.6)	39 (6.0)	6 (2.4)	10 (3.0)	15 (3.5)
USE 4	11 (3.1)	13 (3.4)	10 (3.2)	26 (4.9)	6 (2.4)	8 (2.7)	12 (3.3)
RLD 1	25 (4.9)	20 (4.4)	13 (3.5)	57 (7.5)	5 (2.2)	12 (3.4)	22 (4.3)
RLD 2	24 (4.8)	19 (4.2)	14 (3.6)	51 (7.1)	6 (2.4)	15 (3.8)	22 (4.3)
RLD 3	27 (5.1)	20 (4.4)	12 (3.4)	46 (6.8)	6 (2.4)	15 (3.8)	20 (4.3)
RLD 4	30 (5.4)	23 (4.7)	11 (3.4)	52 (7.2)	6 (2.3)	16 (3.9)	23 (4.5)
RLD 5	25 (4.9)	25 (4.9)	10 (3.1)	59 (7.7)	7 (2.6)	16 (4.0)	24 (4.5)
RLD 6	29 (5.4)	26 (4.9)	12 (3.4)	38 (6.1)	9 (2.8)	14 (3.7)	21 (4.4)
RLD 7	25 (4.9)	19 (4.3)	9 (2.9)	50 (7.0)	6 (2.3)	18 (4.0)	21 (4.2)
Mean							
Soils only	22 (4.6)	22 (4.5)	12 (3.3)	49 (6.8)	7 (2.6)	15 (3.7)	21 (4.3)
All treatments	21 (4.3)	23 (4.6)	12 (3.4)	48 (6.7)	7 (2.5)	15 (3.7)	21 (4.2)

above the uninoculated treatments, but increased shoot DM of the remaining medic lines significantly (Table 4). Strain WSM688 increased the shoot DM significantly with all medic lines except *M. laciniata*.

Generally, soil rhizobia resulted in at least a partially effective symbiosis with all the medic species, except *M. laciniata*. Only 39% of *M. laciniata* plants formed nodules (data not shown) and only 6 of these plants appeared to have functional (red) nodules. Medic species inoculated with soils from the USE produced on average only 3 mg more DM than the uninoculated treatments, with 73% (*M. laciniata* excluded) of plants forming nodules. Soil rhizobia from the EP, UMN and RLD produced 35, 25 and 21 mg more shoot DM,

Table 4. Effect of inoculation treatment on the shoot dry matter of six lines of medic

Transformed means are shown in parentheses and should be used for statistical comparisons

For transformed means, the l.s.d. for comparison of inoculation treatments is 0.56; medic line is 0.24 and interaction is 1.36

EP, Eyre Peninsula; UMN, Upper Mid-north; USE, Upper South-east; RLD, Riverland

Inoculation treatment	Shoot dry matter (mg/plant) *M. orbicularis* SA8460	*M. truncatula* cv. Caliph	*M. laciniata* SA11710	*M. doliata* SA17722	*M. polymorpha* cv. Santiago	*M. tornata* cv. Rivoli	Overall mean (all lines)
Uninoculated	17 (4.1)	30 (5.3)	9 (2.9)	34 (5.8)	13 (3.4)	19 (4.3)	20 (4.3)
Nitrogen	67 (8.1)	100 (10.0)	57 (7.4)	60 (7.6)	114 (10.6)	109 (10.4)	85 (9.0)
WSM826	61 (7.6)	126 (11.2)	9 (2.8)	60 (7.7)	12 (3.5)	19 (4.1)	48 (6.1)
WSM688	45 (6.6)	142 (11.9)	7 (2.5)	60 (7.7)	75 (8.6)	48 (6.8)	63 (7.3)
EP 1	52 (7.0)	81 (8.8)	10 (2.9)	55 (7.4)	16 (3.9)	87 (9.2)	50 (6.5)
EP 2	51 (6.9)	108 (10.2)	9 (2.8)	65 (8.0)	63 (7.6)	77 (8.7)	62 (7.4)
EP 3	54 (7.3)	84 (9.1)	16 (3.6)	59 (7.6)	45 (6.6)	119 (10.9)	63 (7.5)
EP 4	58 (7.5)	63 (7.8)	13 (3.2)	66 (8.1)	61 (7.7)	85 (9.1)	57 (7.2)
EP 5	47 (6.7)	82 (8.8)	13 (3.3)	64 (8.0)	18 (4.1)	92 (9.5)	53 (6.8)
EP 6	48 (6.9)	62 (7.8)	5 (2.2)	65 (8.0)	30 (5.1)	94 (9.6)	51 (6.6)
EP 7	65 (8.0)	42 (6.1)	14 (3.5)	45 (6.7)	41 (6.0)	98 (9.8)	51 (6.7)
EP 8	71 (8.3)	40 (6.2)	15 (3.7)	47 (6.8)	37 (5.7)	87 (9.2)	49 (6.6)
UMN 1	59 (7.6)	54 (7.2)	16 (3.8)	50 (7.1)	71 (8.3)	93 (9.6)	57 (7.3)
UMN 2	48 (6.8)	44 (6.1)	14 (3.7)	55 (7.4)	61 (7.6)	56 (7.3)	46 (6.5)
UMN 3	43 (6.4)	33 (5.7)	12 (3.3)	56 (7.4)	20 (4.3)	83 (9.0)	41 (6.0)
UMN 4	26 (4.8)	17 (4.1)	18 (4.1)	29 (5.3)	21 (4.4)	13 (3.6)	21 (4.4)
UMN 5	64 (7.9)	46 (6.4)	13 (3.5)	51 (7.1)	40 (6.0)	91 (9.5)	51 (6.7)
UMN 6	74 (8.4)	43 (6.4)	15 (3.7)	44 (6.6)	29 (5.1)	97 (9.8)	50 (6.7)
UMN 7	57 (7.4)	46 (6.6)	14 (3.6)	50 (7.0)	32 (5.5)	87 (9.3)	48 (6.6)
UMN 8	43 (6.4)	40 (5.9)	19 (4.1)	49 (6.9)	19 (4.2)	69 (8.2)	40 (6.0)
UMN 9	47 (6.7)	40 (6.1)	8 (2.6)	50 (7.0)	86 (9.2)	75 (8.6)	51 (6.7)
USE 1	21 (4.4)	16 (3.9)	5 (2.1)	24 (4.5)	7 (2.7)	20 (4.3)	16 (3.6)
USE 2	16 (3.9)	25 (4.8)	8 (2.6)	58 (7.6)	14 (3.5)	74 (8.6)	33 (5.2)
USE 3	25 (4.7)	22 (4.4)	8 (2.4)	33 (5.4)	7 (2.7)	34 (5.3)	21 (4.2)
USE 4	19 (4.3)	17 (4.0)	8 (2.6)	27 (4.9)	29 (5.3)	28 (5.0)	21 (4.3)
RLD 1	47 (6.6)	40 (6.1)	8 (2.7)	52 (7.2)	10 (3.1)	80 (8.9)	40 (5.8)
RLD 2	54 (7.2)	51 (7.1)	8 (2.5)	43 (6.3)	11 (3.1)	59 (7.3)	38 (5.6)
RLD 3	42 (6.4)	45 (6.5)	6 (2.3)	48 (6.8)	11 (3.2)	79 (8.9)	38 (5.7)
RLD 4	56 (7.2)	36 (5.9)	9 (2.8)	47 (6.8)	10 (3.1)	75 (8.5)	39 (5.7)
RLD 5	63 (7.8)	51 (6.9)	6 (2.2)	58 (7.6)	10 (3.1)	86 (9.0)	46 (6.1)
RLD 6	40 (6.3)	34 (5.6)	20 (4.1)	64 (8.0)	18 (4.0)	91 (9.5)	45 (6.2)
RLD 7	30 (5.3)	29 (5.3)	16 (3.5)	56 (7.5)	16 (3.9)	84 (8.9)	39 (5.7)
Mean							
Soils only	47 (6.6)	46 (6.4)	12 (3.1)	50 (7.0)	30 (5.0)	75 (8.4)	43 (6.1)
All treatments	47 (6.6)	53 (6.8)	13 (3.2)	51 (7.0)	33 (5.2)	72 (8.1)	45 (6.2)

respectively, than the uninoculated treatments, with 97% of plants (*M. laciniata* excluded) forming nodules.

Medic lines varied in their ability to form an effective symbiosis with the soil rhizobia. Symbiotic performance of the medic lines ranged from 6 ± 9% (mean ± s.d.) for *M. laciniata*, to 14 ± 20% for *M. truncatula*, 17 ± 21% for *M. polymorpha*, 51 ± 36% for *M. doliata* Carmign., 53 ± 27% for *M. orbicularis* (L.) Bartal. and 57 ± 25% for *M. tornata*.

In this experiment, there were large and significant interactions between soil rhizobia and the line of medic. This can be clearly seen when comparing shoot DM of *M. tornata* with that of *M. polymorpha*, where both are inoculated with the soil rhizobia from the RLD. With

M. tornata, these rhizobia resulted in a 60 mg increase in shoot DM compared with the uninoculated treatment (67% of the best treatment, EP 3). By comparison, none of the soil rhizobia from the RLD significantly increased shoot DM of *M. polymorpha*, even though all but 1 of the plants formed nodules (white in colour).

Discussion

Number and effectiveness of rhizobia in soils

Medic rhizobia were found in all but 1 of the 29 soils collected. In the 28 soils used in these experiments (except for 2 of the USE soils), there were more than 100 medic rhizobia per gram of dry soil. Since about 50 rhizobia/cm^3 soil are delivered via the inoculation of medic seed at sowing, it is reasonable to assume that the number of rhizobia in nearly all the surveyed soils is sufficient to allow prompt nodulation in the field, of medics that are commercially grown in South Australia (mainly *M. littoralis*, *M. truncatula* and *M. polymorpha*). The only species that consistently failed to nodulate with the naturalised rhizobia was *M. laciniata*, which is known to have highly specific rhizobial requirements (Brockwell and Hely 1966). While there was a large variation in the number of rhizobia/g soil, with the lowest numbers occurring in the USE soils, there was no significant correlation with any of the measured soil factors. While soil pH (<6.5) is generally considered to be a main determinant of the number of medic rhizobia in a soil (Howieson and Ewing 1986; Brockwell *et al*. 1991), even this parameter was poorly correlated with rhizobial number (r = 0.048) in this study. This is probably because the pH of most of the soils exceeded 6.5 (mean pH 7.3). It is likely, that the frequency of annual medics in the pasture accounts for part of the variation in the number of rhizobia (Hely and Brockwell 1962); however, this factor was not measured.

While the number of rhizobia in the soils appears to be sufficient to enable prompt nodulation in the field, the inoculation technique used in these experiments, where a 10-fold dilution is used to extract the rhizobia from the soil, may cause reductions in shoot growth due to delayed nodulation in soils that contain <100 rhizobia/g soil (Brockwell *et al*. 1988). Across all plant hosts, the number of rhizobia in the soil accounted for 45% of the variation in shoot DM in experiment 1 and 52% of the variation in experiment 2. However, this association declines to 15 and 36% for experiments 1 and 2, respectively, if the USE soils (2 contain <100 rhizobia/g) are excluded. Slattery *et al*. (1999) have undertaken a comprehensive survey of soils in this region and shown that, where the number of rhizobia in the soil is adequate, they are able to form a moderately effective symbiosis with both strand (*M. littoralis*) and barrel (*M. truncatula*) medics.

Number of rhizobia in the soil aside, there is nonetheless evidence that soils from different regions support populations of medic rhizobia that vary greatly in their inherent effectiveness with some species of medic. This is clear on comparing soils from the RLD and EP regions with *M. polymorpha*. While all the soils from these regions contained >2000 rhizobia/g, nodules resulting from the RLD soils, although numerous, were always ineffective (white) with *M. polymorpha*. By comparison, nodules resulting from inoculation with EP soils were generally effective (red) and resulted in greater increases in shoot DM (39 mg with the EP soils compared with 12 mg with the RLD soils). Even within a geographic region, large differences in nitrogen fixation ability occurred between populations of soil rhizobia. For example, while the rhizobia from EP 2 were able to form a highly effective symbiosis (63-mg increase in DM and presence of red nodules) with *M. polymorpha* those from EP 1 were clearly ineffective (16-mg increase in DM and presence of white nodules). These 2 sites are about 45 km apart. Our results support findings of other studies in that populations of soil rhizobia vary widely in their effectiveness with *Medicago* spp. (Barber 1980; Bowman *et al*. 1998; Materon 1991). Genetic studies (Eardly *et al*. 1990; Hartman and Armarger 1991) have also shown that natural populations of rhizobia vary widely in their composition, and some (Rome *et al*. 1996) have proposed that divergence within medic rhizobia is sufficient to merit separation of the group into 2 species. The practical implication of this diversity is that the medic plant is destined to be nodulated by different rhizobia, possibly on a paddock-to-paddock basis. Those medics that are capable of good and consistent levels of nitrogen fixation with diverse strains of rhizobia are likely to be of greatest benefit to agriculture.

Performance of the medic species

It is clear from these experiments that different species of annual medic vary widely in their capacity to form an effective symbiosis with naturalised populations of medic rhizobia. At the extremes, *M. rigiduloides* was prolifically nodulated, but failed to fix nitrogen with any of the sources of soil rhizobia. The closely related *M. rigidula* (Small 1990) on the other hand, averaged 65% of its potential shoot growth across all the naturalised populations of rhizobia.

The strand medic cv. Herald, a significant medic species in South Australian farming systems, achieved 49% of the SP of the best inoculation treatment

(WSM826). The question remains as to whether this level of SP is likely to meet the requirements of the plant for fixed nitrogen in the field. A pasture of the cv. Herald, 5 weeks after germination, could be expected to produce 200 kg/ha of above-ground DM, in excess of seed reserves, at a density of 600 plants/m^2 (A. Lake pers. com.). Such a pasture would have an above-ground nitrogen requirement of 1.20 mg N/plant, based on a nitrogen content of 3.6% (Butler 1988). In the glasshouse experiment, Herald medic reliant on soil rhizobia (USE and UMN 4 soils excluded) was estimated (assuming 3.6% nitrogen in DM) to have fixed 0.60 mg N/plant shoot, after 5 weeks growth in conditions considered optimal for nodulation and nitrogen fixation. This amount of fixed nitrogen would only meet 50% of the above-ground nitrogen requirement of a Herald pasture. This deficit could be expected to increase later in the season as pasture growth rates increase and soil nitrogen is depleted (Cocks 1980). Hence, even though Herald medic fixes nitrogen with most sources of soil rhizobia, there is clearly room for improvement (at least to the level of strain WSM826) with a good chance that these improvements would be translated to increased DM production in the field.

Nationally, burr medic (*M. polymorpha*) is of significance, being estimated to occur on over 8 Mha of Australian soils (Hill and Donald 1998). The poor SP of Santiago burr medic with the rhizobia in South Australian soils (17% of nitrogen treatment), may partly explain the sporadic performance of the species in medic evaluation trials in this state (J. Howie pers. com.). The SP of the species was erratic with the soil rhizobia from the EP (range 3–50%) and UMN (range 6–72%) and failed to form an effective symbiosis with any of the rhizobia from the RLD. This is a species with many valuable agronomic characters which may not be realised, in many situations, due to its erratic symbiotic properties.

Although the barrel medic Caliph was equally as poor as Santiago, it differed from the burr medic in that the nodules resulting from the soil rhizobia rarely appeared to be non-functional (only 4 plants had white nodules). It is possible that the poor performance of this species may have been due to delayed nodulation (34 plants were noted to have <5 red nodules) in the testing system, rather than ineffectiveness of the symbiosis *per se*. Although this requires validation, Ewing and Robson (1990) have also found that this species tends to form fewer nodules when grown in solution culture.

One further implication of this study is that high levels of nitrogen fixation may have little bearing on the proliferation of a legume species in an environment. There was no significant relationship between SP and the seed density (G. Sweeney unpublished data) of *M. polymorpha* (r = 0.25, P = 0.52) or *M. minima* (r = 0.23, P = 0.55) at the UMN sites. Bowman *et al*. (1998) concluded similarly for *M. polymorpha*.

Performance with strains WSM826, WSM688 and nitrogen

The degree of specificity for rhizobia within the genus *Medicago* is reinforced by the differential performance of the medic lines with WSM826 and WSM688. While both strains of rhizobia failed with SA8965, they were effective with Herald, even though both medics are *M. littoralis*. This dichotomy indicates that selection within this legume species for better N fixation is possible. The failure of WSM826 with Rivoli (*M. tornata*) reflects an error at inoculation. Subsequent testing (data not shown) indicates that this association is highly effective.

Commencement of the nitrogen treatment 18 days after inoculation in the first experiment did not allow plants to achieve the full potential of their growth; however, it was still useful in defining highly ineffective symbioses, such as that of *M. rigiduloides*.

Improving the symbiosis

Nitrogen fixation is a primary purpose of pastures in Australian farming systems. Ironically, this characteristic is seldom directly considered in the selection and recommendation of pasture species even though it has the potential to profoundly effect pasture growth in the field. This study has shown that the naturalised soil rhizobia are likely to limit the growth of some species of medic.

However, it remains difficult to displace naturalised soil rhizobia and it would appear optimistic to expect a single inoculant strain, selected for high levels of nitrogen fixation, to persist in a range of soils and farming systems. The differential performance of the soil rhizobia between sampling sites in this study suggests that this has not happened naturally. This being the case, plant selection should be considered as a way of improving the efficiency of the symbiosis.

It may be possible to select within a medic species, for improved nitrogen fixation. Others have had some success with this approach (Barnes *et al*. 1984; Provorov and Simarov 1990) and we also have some evidence showing variation between lines of burr medic (unpublished data) in this regard. However, in our experience, intra-species selection is less likely to overcome a totally ineffective symbiosis, such as that which occurs between the burr medic Santiago and the RLD soils. In this instance, the a solution may lie in the choice of medic species. For example, the strand medic cv. Herald is clearly the preferred option over Santiago for the RLD region. While the SP of *M. rigidula* was both good and consistent (mean SP of 65%, SP <40% with only 3 soils), other agronomic characteristics make this species poorly suited to South Australian farming systems. Its value is as source of material for breeding programs seeking to develop plants which are efficient at nitrogen fixation with soil rhizobia.

If plant species are to be matched to localities, based on their efficiency of nitrogen fixation, regional maps detailing the SP of key medic species with soil rhizobia will need to be developed. While an onerous task, this information would clearly be useful in assisting with pasture legume recommendations and could be used to optimise the symbiosis in the field.

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