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# Symbiotic performance of pasture legumes with naturalised soil rhizobia

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**Summary** - Most Australian soils contain large and diverse populations of naturalised rhizobia for the pasture genera of *Medicago* and *Trifolium*. This paper examines the performance of several pasture legumes with these soil rhizobia. The selection of medics and clovers, better able to form an effective symbiosis with soil rhizobia, is proposed as a way of significantly improving nitrogen fixation in the field.

**Key-words:** *Rhizobium*, *Medicago*, *Trifolium*, pasture

***Résumé*** - *La plupart des sols d'Australie contient des populations importantes et variées de rhizobium naturalisés dans les pâturages de* Medicago *et de* Trifolium. *Cet article présente les performances de plusieurs légumineuses en association avec ces rhizobium. Une sélection des trèfles et medics les plus efficaces en association avec ces rhizobium est proposée dans le but d'améliorer de façon significative la fixation d'azote au champ.*

***Mots-clés:*** Rhizobium, Medicago, Trifolium, *pâturage*

## Introduction

The legume component of pastures in southern Australia is dominated by the genera *Trifolium* and *Medicago*. Traditionally, efforts to improve the level nitrogen fixation by these pasture genera have focused on the selection and introduction of better inoculant rhizobia. Improved rhizobia have been selected for high nitrogen fixation (Gibson, 1962), adaptation to specific soil conditions such as acidity (Howieson and Ewing, 1986) and for competitive ability (Brockwell *et al.*, 1982; Hebb *et al.*, 1998).

On most occasions inoculant rhizobia have to compete with substantial numbers (>100 $g^{-1}$ soil) of naturalised soil rhizobia. Where the inoculation practice is poor, conditions are unfavourable to inoculant survival, or the inoculant strain competes poorly, it is likely to have limited impact. Even if an inoculant strain is successful in the year of sowing, in subsequent years its potency is often lessened due to declining persistence (Brockwell *et al.*, 1982; Slattery and Coventry, 1993) or 'genetic drift' of the rhizobial population following the exchange of genetic material with other soil bacteria (Sullivan and Ronson, 1998). Nitrogen fixation associated with soil rhizobia is often less than optimal (Bowman *et al.*, 1998; Quigley *et al.*, 1997).

An alternative approach to improve the symbiosis is to select plants which form an effective symbiosis with the soil rhizobia. As well as having the potential to significantly improve the symbiosis in the field, this approach would largely eliminate the need for seed inoculation with rhizobia. This would provide farmers with the flexibility to sow pasture seed well in advance of opening rains, without fear of inoculant mortality.

This paper provides an overview of the occurrence of rhizobia in Australian soils which nodulate *Trifolium* and *Medicago* spp. It highlights differences between pasture legumes in their ability to form an effective symbiosis with these naturalised soil rhizobia. Opportunities for improving the symbiosis through the selection of plant material are briefly explored.

**Occurrence and diversity of soil rhizobia**

For *Trifolium* and *Medicago* species, rhizobia have naturalised in most areas where the host legume occurs, except for medic rhizobia, which may be absent on soils of pH $<6.0_{Ca}$, even if the host is present (Brockwell *et al.*, 1991). Recent surveys of Australian soils indicate that rhizobial number generally exceeds 500 $g^{-1}$ soil (Table 1).

Table 1. Number of *Trifolium* and *Medicago* rhizobia in Australian soils.

| Legume | No. soils | Mean no. Rhizobia ($g^{-1}$ soil ) | Rhizobia absent (no. soils) | Source |
|---|---|---|---|---|
| *Medicago spp.* | 29 | 69, 100 | 1 | Ballard unpub. Data |
| *Medicago spp.* | 84 | 900 | 16 | Brockwell *et al.*, 1991 |
| *Medicago spp.* | 141 | 32, 000 | 0 | Slattery *et al.*, 1999 |
| *T. subterraneum* | 61 | 5300 | 5 | Denton *et al.*, 2000 |
| *T. michelianum* | 43 | 165,300 | 6 | Ballard unpub. Data |
| *T. repens* | 72 | 245,000 | 0 | Riffkin *et al.*, 1999 |
| *T. subterraneum* | 9 | 18,300 | 0 | Unkovich and Pate, 1998 |

Numbers of soil rhizobia of this order present a numerical obstacle to the introduction and persistence of inoculant rhizobia (Brockwell and Bottomley, 1995; Slattery and Coventry, 1993). Theis *et al.* (1991) have shown that inoculation responses decline dramatically as the number of soil rhizobia increases from 10 to 50 $g^{-1}$ soil.

Surveys, ranging from detailed molecular investigations (Hebb *et al.*, 1998) through to investigations of plant growth following inoculation with soil extracts (Quigley *et al.*, 1997; Denton *et al.*, 1999), all point to a high degree of diversity amongst soil rhizobia. This diversity may best be managed, to achieve consistently high levels of nitrogen fixation, through the careful selection of plant host.

**Role of the host plant**

Some pasture legume species are able to form an effective symbiosis with different sources of soil rhizobia (Figure 1). For example, *Medicago sativa* (lucerne) is able to achieve a high level of symbiotic performance (SP) with most sources of soil rhizobia (mean = 84 %). Indeed, lucerne is generally considered a rhizobially promiscuous plant (Bowman *et al.*, 1998). In contrast, *Medicago rigiduloidies* fails to establish a symbiosis of any consequence (mean = 4 %) with any source of soil rhizobia. Species such *Medicago polymorpha* are intermediate (mean = 29 %) and characterised by a highly variable SP (-8 to 100 %). Other *Medicago* spp. are equally variable in their ability to form an effective symbiosis with soil rhizobia (Figure 2). It is possible that the agronomic potential of some species may be overlooked in environments where the growth of the species is limited by poor SP with soil rhizobia. This may be the case for *M. polymorpha* on some South Australian soils.

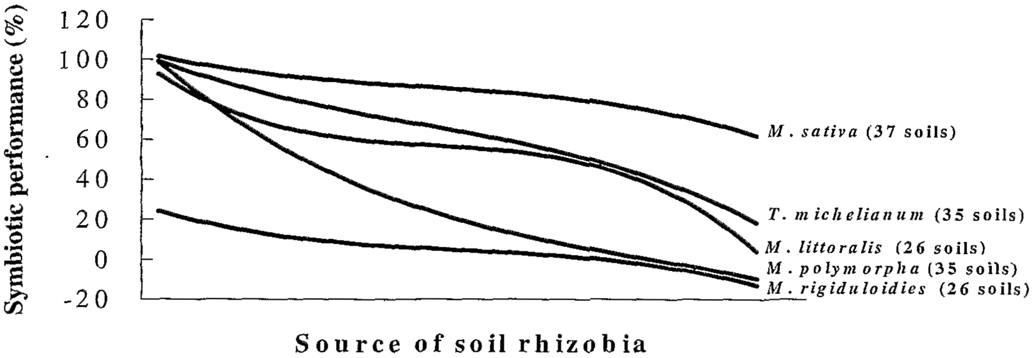


**Figure 1.** Pattern of symbiotic performance (SP = shoot dry matter of plants inoculated with soil rhizobia expressed as a percentage of an effective inoculation treatment) of *Trifolium* and *Medicago* spp. nodulated by naturalised rhizobia in Australian soils. Adapted from 4 surveys and based on soils containing >100 rhizobia $g^{-1}$.

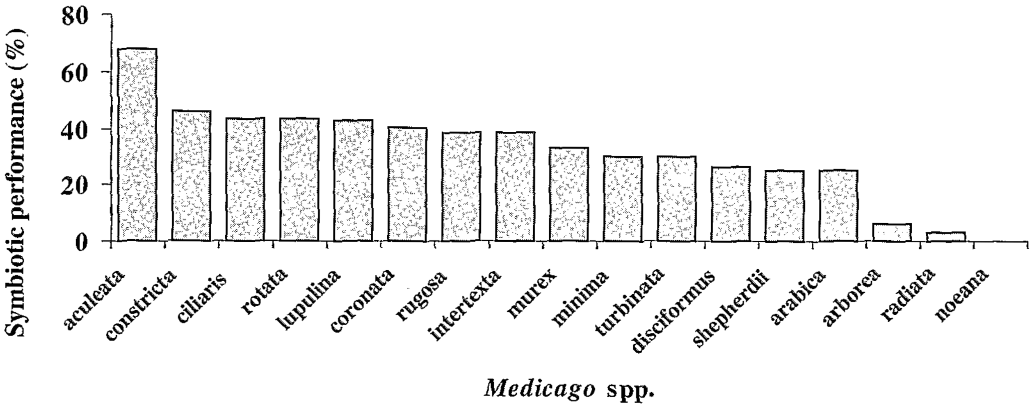


**Figure 2.** Symbiotic performance of 17 *Medicago* spp. inoculated with a soil from South Australia containing 42, 400 medic rhizobia $g^{-1}$.

***Plant selection to improve symbiotic performance***

Given the constraints to the introduction of new inoculant strains, we propose plant selection as a means for improving pasture legume symbioses in the field.

A plant which forms a highly effective association with all sources of soil rhizobia is desirable. Lucerne largely meets this criterion and any selection for improved SP would be difficult. However, for species such as *M. polymorpha* and to a lesser extent *Medicago littoralis* and

*Trifolium michelianum*, significant improvements in N-fixation appear possible. Preliminary screening efforts for *M. polymorpha* and *M. littoralis* (Table 2) shows considerable intra-species variability for this characteristic.

For *M. polymorpha* we have recorded a 7-fold variation in the ability of plant lines to form an effective symbiosis when inoculated with a rhizobially moderate (SP of 22 % for Santiago) soil. Lines with a symbiotic ratio (SR) exceeding 7 occurred at a frequency of 1.5 %. Similar levels of variation have been recorded for *M. littoralis* (across 3 soils), although in this instance, there appears less scope to improve the performance of the commercial cultivar Herald (SR = 5.9).

Table 2. Number of *M. polymorpha* and *M. littoralis* lines in each of 5 symbiotic ratio (SR) classes. SR is the ratio of shoot dry matter (after inoculation with soil rhizobia):shoot dry matter of an uninoculated treatment. A high SR ratio indicates a more effective symbiosis. SR of control treatments; *M. polymorpha* cv. Santiago = 3.4, *M. littoralis* cv. Herald = 5.9.

| Legume species | No. lines | Symbiotic ratio class | | | | |
|---|---|---|---|---|---|---|
| | | <1 | 1 to 3 | >3 to 5 | >5 to 7 | >7 |
| *M. polymorpha* | 202 | 14 | 157 | 27 | 1 | 3 |
| *M. littoralis* | 107 | 2 | 42 | 43 | 17 | 3 |

Whether the high SR lines have any agronomic merit, or the trait can be transferred into existing cultivars, remains to be seen. At the very least, an opportunity exists to consider the performance of pasture lines with soil rhizobia before they are released to the farming community. Species such as *Trifolium glanduliferum* and *Trigonella balansae* are currently being evaluated by Australian pasture programs and are good candidates for this approach.

## Conclusions

Given the obstacles to the introduction of inoculant rhizobia, plant selection appears a promising avenue for improving the effectiveness of the symbiosis in the field. There is ample evidence that pasture legumes vary at an inter and intra species level, in their ability to form an effective symbiosis with soil rhizobia. Efforts are currently under-way to find pasture legumes that form an effective symbiosis across a range of soil rhizobia.

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