Herbage production, nitrogen fixation and water use efficiency of ten annual pasture legumes grown with and without lime on an acid soil

Belinda Hackney[1], Brian Dear[1], Mark Peoples[2], Gabrielle Dyce[1] and Craig Rodham[1]

[1]EH Graham Centre for Agricultural Innovation (NSW Department of Primary Industries and Charles Sturt University), Pine Gully Rd, Wagga Wagga NSW 2650
Email: belinda.hackney@dpi.nsw.gov.au
[2]CSIRO Plant Industries, GPO Box 1600, Canberra ACT 2601

Abstract

Herbage yield and nitrogen (N) fixation of ten temperate annual legumes, was measured when grown with and without lime on an acid soil. N-fixation of *O. compressus* and *O. sativus* was significantly lower on limed and unlimed treatments (average 3.4 kg N/tDM) compared to *T. subterraneum* (average of 18 kg N/tDM). *T. michelanium*, *T. purpureum* and *T. vesiculosum* fixed a similar amount of N to *T. subterraneum* in unlimed treatments but *T. vesiculosum* fixed significantly more N (28 kg N/t DM) than all other legumes in limed soils. Herbage production of *T. purpureum* and *T. vesiculosum* was very high, particularly in limed treatments (>9.5t/ha) and consequently the total quantity of N fixed was significantly higher (>220 kgN/ha) than for all other species (20-131 kgN/ha) except *T. michelanium*. Water use efficiency (WUE) of the two highest N-fixing legumes was amongst the highest of all species. The results indicate some new annual pasture legumes can fix large quantities of N and use growing season rainfall more efficiently than *T. subterraneum*, particularly where lime has been used to amend soil acidity.

Keywords

Trifolium, Ornithpus, Biserrula, Medicago

Introduction

Subterranean clover (*Trifolium subterraneum*) is the dominant annual legume species used in pasture and cropping systems in southern New South Wales (NSW) (Dear *et al*. 2003). Recently a wide range of new annual legumes have been developed and released for use in Australian farming systems (Nichols *et al*. 2007). Many of the new annual legumes have significant agronomic advantages over subterranean clover including higher production, improved pest tolerance, and better tolerance of difficult soil conditions including acidity and water logging (Nutt and Loi 2002, Nichols *et al*. 2007).

Use of pasture legumes in cropping rotations can increase soil N for the following crop and act as a 'disease break' in the cropping rotation (Dear *et al*. 2003). Pasture legumes also provide high quality feed either utilised directly or conserved for use in times of feed shortage. Farmers in central and southern NSW have indicated they will be increasing their use of pastures in their farming systems, particularly for reducing reliance on inorganic N sources for crops, but also as a means of increasing fodder conservation for improved drought preparedness (Hackney *et al*. 2008). Many of the annual pasture legumes developed in the past 15 years are well suited to this multi-purpose role.

Soil acidity affects 13.7 million hectares of agricultural land in NSW (Scott *et al*. 2000) with many soils also acidic at depth (Helyar *et al*. 1990). Pasture legumes used in farming systems with acid soils must cope with acidity and its associated toxicities *per se*, or demonstrate response to amelioration of soil acidity to justify farmers shifting their focus from a subterranean clover-based farming system to use of alternative legumes. The aim of this experiment was to assess N-fixation and growth (including efficiency of growth) of a range of annual pasture legume species in an acid soil with high levels of exchangeable aluminium (Al) and manganese (Mn), with and without use of lime, when grown as a one-year forage legume crop.

Materials and methods

The site was located at Binalong, NSW (34.671?S, 148.632?E, 480m a.s.l) where long-term average annual rainfall is 620 mm. In the year of the experiment, annual rainfall was 547 mm and growing season rainfall (GSR) 373 mm. GSR is the rainfall recorded from the autumn break until peak herbage production measurement in spring. The autumn break occurred on the 25^{th} May 2004, and peak spring herbage production was measured on 23 November 2004. Wheat was sown at the site in the previous three years.

Ten annual legumes were sown on 24 May 2004 into a prepared seed-bed, plots 2 m x 8 m, in a randomised split-plot design replicated 3 times. Soil at the site was a sodosol (Isbell 2002). Prior to sowing, soil was collected from each replicate for analysis of pH and exchangeable cations. Analysis showed soils were acidic with high levels of exchangeable Al and Mn. Plots within replicates were split with lime (2.5t/ha) applied to half of the plot. Soil pH and exchangeable cations were analysed from each replicate (unlimed and limed) 12 months after lime application (Table 1). Annual legumes sown and their seeding rate were *Ornithopus compressus* cv. Avila (10kg/ha), *O. sativus* cv. Erica (10 kg/ha), *Biserrula pelecinus* cv. Mauro (7 kg/ha), *Trifolium vesiculosum* cv. Zulu II (8kg/ha), *T. michelanium* cv. Bolta (7 kg/ha), *T. glanduliferum* cv. Prima (7 kg/ha), *Medicago murex* cv. Zodiac (10 kg/ha), *T. purpureum* cv Electra (7kg/ha), *T. hirtum* cv. Hykon (10 kg/ha), and *T. subterraneum* cv. Goulburn (10kg/ha). Seeding rates were the typical rates used for each species grown as a one-year forage legume break crop. Prior to sowing seed of each species was inoculated and lime pelleted using the appropriate commercial rhizobium group. Molybdenum fortified single superphosphate (8.8 % P, 11% S, 0.05% Mo) was applied at 230 kg/ha with seed at sowing. Trifluralin (2 L/ha) was applied and incorporated prior to sowing. Clethodim and alpha-cypermethrin were applied at 2L/ha and 100 mL/ha to control annual grass weeds and red-legged earth mites respectively on 29 July 2004.

Peak spring biomass was assessed by a calibrated visual scoring system. Scores were calibrated by scoring 10, 0.25m^2 quadrats across the scoring range (0-50), cutting the herbage in the quadrats and drying it at 70^oC for 48 hours to determine dry weight. Herbage samples were collected from each plot (limed and unlimed) for analysis of total N content and the proportion of herbage N derived from N-fixation using the natural abundance technique (Unkovich *et al*. 1994, Dear *et al*. 1999). Annual ryegrass (*Lolium rigidum)* was used as the non-N fixing reference plant. WUE was calculated by dividing peak spring herbage yield by the GSR.

Table 1. Average soil pH, exchangeable Al and Mn12 months after experiment commencement in unlimed and limed (2.5 t lime/ha) treatments at Binalong NSW.

Treatment	Depth cm	pH_{CaCl2}	Exchangeable Al %	Exchangeable Mn cmol/kg
Unlimed	0-10	4.2	18	0.21
Unlimed	10-20	4.0	38	0.30
Limed	0-10	5.3	1	0
Limed	10-20	4.1	36	0.28

A spatial regular grid model was fitted using Genstat 10. Species and lime treatment were fitted as fixed effects and replicate as a random effect.

Results

Only four species, *T. hirtum*, *B. pelecinus*, *M. murex* and *T. subterraneum*, showed no significant increase in herbage production with addition of lime. Five species, *O. compressus*, *T. michelanium*, *T. purpureum*, *O. sativus* and *T. vesiculosum*, were as productive as *T. subterraneum* without lime addition and all except *T. michelanium* were more productive than *T. subterraneum* where lime was applied (Table 2).

No species x lime effect on tissue N content was found, but there was a significant effect of species alone with *T. glanduliferum* having the lowest tissue N content and *T. vesiculosum* the highest. All other species except *O. sativus* and *T. hirtum* had similar tissue N concentration to *T. subterraneum*. A significant species x lime effect on the proportion of tissue N fixed was found with the use of lime significantly increasing the proportion of tissue N fixed in *T. subterraneum*, *T. hirtum*, *B. pelecinus*, *M. murex* and *T. vesiculosum*. The proportion of tissue N fixed in *O. compressus* and *O. sativus* was not affected by lime addition and the lowest of all species in the limed treatment and amongst the lowest in the unlimed treatment.

Application of lime did not increase the calculated N fixed/t DM for either *Ornithopus* spp. but did increase fixation by *B. pelecinus*, though all three species were inferior to *T. subterraneum* in unlimed and limed treatments. Use of lime also significantly increased N fixed/t DM for *T. hirtum*, *M. murex* and *T.vesiculosum*. *T. vesiculosum* fixed significantly more N/t DM in the limed treatment compared to *T. subterraneum*.

Where no lime was added, calculated total N fixed per hectare was highest for *T. subterraneum*, *T. purpureum*, *T. vesiculosum* and *T. michelanium*. Except for *T. subterraneum*, addition of lime significantly increased total N fix/ha of all these species with *T. vesiculosum* having the highest N fix/ha overall. Total N fix/ha was also significantly increased with the use of lime on *T. hirtum*, *B. pelecinus*, *T. glanduliferum* and *M. murex*. Total N fix/ha of *O. compressus* and *O. sativus* was not affected by lime addition and was consistently low in the un-limed and limed treatments.

Where no lime was applied, no species showed significantly higher WUE than *T. subterraneum*. However, *T. hirtum*, *B. pelecinus*, *T. glanduliferum* and *M. murex* had significantly lower WUE. Where lime was applied *O. compressus*, *T, purpureum*, *O. sativus* and *T. vesiculosum* were significantly more efficient in utilising growing season rainfall compared to *T. subterraneum*. Use of lime also significantly increased the WUE of *T. glanduliferum* to a level where it was comparable to *T. subterraneum*.

Table 2. Herbage yield, tissue-N concentration, proportion of tissue-N fixed, N-fixation (kg N/t DM and kg N/ha) and WUE of 10 annual pasture legumes grown as a one year forage crop at Binalong NSW in 2004.

	Tissue N (%)	Proportion of tissue N fixed (%)		Herbage yield (kg DM/ha)		N fixed kg N/t DM		Total N fixed kgN/ha		Water use efficiency kg DM/mm GSR	
Botanical name		-L	+L	-L	+L	-L	+L	-L	+L	-L	+L
O. compressus	3.06^{c}	10.7^{ab}	8.20^{a}	5831^{defg}	9439^{hij}	3.06^{a}	2.17^{a}	17.1^{a}	20.1^{a}	14.1^{cde}	25.5^{ij}
T. michelanium	3.04^{c}	67.6^{f}	70.4^{f}	5507^{cdef}	7847^{ghi}	19.5^{fghij}	22.1^{ij}	108^{cd}	177^{ef}	15.1^{cdef}	20.7^{ghi}

T. purpureum	3.04^{c}	58.3^{ef}	71.6^{f}	7481^{fgh}	10189^{j}	17.3^{fghi}	21.4^{hij}	130de	222^{fg}	19.8^{fg}	27.0^{j}
O. sativus	2.76^{b}	23.4^{bc}	11.5^{ab}	5935^{efg}	9269^{hij}	5.4^{ab}	3.11^{a}	34.2^{a}	34.0^{a}	19.9^{fgh}	25.1^{hij}
T. subterraneum	3.19^{c}	44.9^{d}	59.7^{ef}	7457^{fgh}	6081^{efg}	16.6^{efgh}	19.6^{ghij}	134de	131de	16.7^{efg}	15.8^{defg}
T. hirtum	2.70^{b}	32.1^{cd}	65.6^{f}	3558^{abc}	5146^{cde}	9.06^{bc}	18.8^{fghij}	32.6^{a}	90.7^{cd}	11.3^{bcd}	14.7^{cdef}
B. pelecinus	3.65^{d}	5.53^{a}	41.9^{d}	3860^{abcd}	4800^{bcde}	3.57^{a}	14.5^{def}	13.2^{a}	68.3^{bc}	10.5^{bc}	13.2^{cde}
T. glanduliferum	2.34^{a}	49.6^{e}	60.4^{ef}	2740^{a}	5955^{efg}	11.7^{cde}	14.6^{defg}	32.9^{a}	89.9^{bcd}	7.41^{ab}	15.7^{cdefg}
M. murex	3.14^{c}	43.5^{d}	66.4^{f}	2892^{ab}	3443^{abc}	11.2^{cd}	22.8^{j}	15.9^{a}	60.9^{abc}	4.72^{a}	7.4^{ab}
T.vesiculosum	3.90^{e}	41.3^{d}	66.5^{f}	6457^{efg}	9803^{ij}	16.0^{defg}	28.2^{k}	124de	277^{g}	16.3^{defg}	26.9^{j}
LSD $_{(5\%)}$ interaction	ns[1]	13.8		2066		5.10		55.0		5.21	
LSD $_{(5\%)}$ species	0.23										

Numbers within main columns followed by the same letter are not significantly different at p=0.05. [1]ns=not significiant

Discussion

This experiment found on strongly acid soils with high levels of exchangeable Al and Mn, several annual legumes produced similar quantities of herbage and fixed similar amounts of N as *T. subterraneum*. With addition of lime, *T. vesiculosum*, and *T. purpureum* were superior to *T. subterraneum* in herbage production, WUE and N-fixation/ha (*T. vesiculosum* was also superior on a kg N-fixed /t DM basis).

In this experiment *T. subterraneum* showed no increase in herbage production and total N-fixation with use of lime. *T. subterraneum* responses to lime have frequently been reported (e.g. Scott and Cullis 1992), however, many other studies have shown little impact of lime on production (e.g Evans *et al*. 1990). The herbage yield increases in response to lime in this experiment for *T. michelanium*, *T. glanduliferum* and *T. vesiculosum* concur with previous studies (Evans *et al*. 1990, Hayes 2003, Cripps *et* *al*. 1988), and indicate greater suppression of growth of these species in strongly acid soils compared to *T. subterraneum.*

Herbage production and WUE of *O. compressus* and *O. sativus* was amongst the highest of all species in this experiment. However, N-fixation in both unlimed and limed plots was poor, yet *Ornithopus* showed no indication of N-deficiency and tissue N-content was similar to *T. subterraneum*. These results suggest that both *Ornithopus* spp*.* may have been scavenging rather than fixing N. Low N-fixation may also have been due to ineffective nodulation due perhaps to poor survival of rhizobia in this soil or low viability of rhizobia used in the inoculation process. Other experiments (e.g. Ovalle *et al*. (2006)), reported relatively high N-fixation of *O. compressus* (91 kg N/ha). Herbage production increases of 61% and 32% were recorded for *O. compressus* and *O. sativus* respectively with addition of lime. The magnitude of these increases were surprising given the reputation of *Ornithopus* spp. for acid soil tolerance. However other studies (e.g. Michalk 1994) reported significant increases in herbage production of *Ornithopus* spp. with the use of lime. *Ornithopus* spp. are known to be sensitive to Mn (Jenkins 2004) and exchangable Mn levels at this site were above the threshold level (0.20 cmol/kg) where yield reductions in sensitive species are known to occur (Bromfield *et al*. 1983). Bromfield *et al*. (1983) additionally reported that the threshold level for yield suppression by Mn was lower (0.10 cmol/kg) where conditions of high exchangeable Al also occurred. At this site exchangeable Mn in the unlimed treatment and in the limed treatment at 10-20cm was above both threshold levels and exchangeable Al was also high. While no indications of Mn toxicity were observed on the unlimed plots, there may still have been suppression of production and perhaps of N-fixation.

While N-fixation *Ornithopus* spp. was unaltered by lime addition, N-fixation of *B. pelecinus* (kgN/t DM and kg N/ha) increased significantly with liming but was still inferior to *T. subterraneum*. Poor N-fixation but high total tissue N in the unlimed treatments suggest *B. pelecinus* was scavenging rather than fixing N. Poor N-fixation may have been due to the same factors suggested for *Ornithopus* spp. *B. pelecinus* has significant potential in Australian farming systems due to its adaptation potential and ease of seed harvest (Nichols *et al*. 2007). However, on the basis of this research further clarification of its ability to fix N is warranted.

Of the annual legume species evaluated in this experiment, *T. vesiculosum*, *T. purpureum* and *T. michelanium* had a significant advantage over *T. subterraneum* in herbage production, N fixation and WUE when used as a high density legume break crop on acid soils with lime amendment. Rising inorganic N costs and decreased reliability of growing season rainfall mean producers need to choose legumes for cropping rotations that can fulfil a number of roles. While other legumes such as *Ornithopus* spp. appear well suited to fulfilling the high productivity, high WUE criteria, further research is required to better define their N fixation capability. Additional research is also needed to better define the N-fixing capability of *B. pelecinus*.

References

Bromfield SM, Cumming RW, David DJ, Williams CH (1983) The assessment of available manganese and aluminium status in acid soils under subterranean clover pastures of various ages. *Australian Journal of Experimental Agriculture and Animal Husbandry* 28: 192-200

Cripps RW, Young JL, Bell TL, Leonard AT (1988) Effects of lime and potassium application on arrowleaf clover, crimson clover and coastal bermudagrass yields. *Journal of Production Agriculture* 1: 309-313

Dear BS, Cocks PS, Peoples MB, Swan, AD, Smith AB (1999) Nitrogen fixation by subterranean clover growing in pure culture and in mixtures with varying densities of lucerne or phalaris. *Australian Journal of Agricultural Research* 50. 1047-1058

Dear BS, Sandral GA, Peoples MB, Wilson BCD, Taylor JN, Rodham CA (2003) Growth and nitrogen fixation of 28 annual legume species on 3 vertisol soils in southern NSW. *Australian Journal of Experimental Agriculture* 43: 1101-1115

Evans J, Dear B, O'Connor GE (1990) Influence of an acid soil on herbage yield and nodulation of five annual pasture legumes. *Australian Journal of Experimental Agriculture* 30: 55-60

Hackney BF, Dear BS, Li G, Rodham CA, Tidd J (2008) Current and future use of pasture legumes in central and southern NSW – results of a farmer survey. *Proceedings 14th Australian Society of Agronomy Conference* (In press)

Hayes R (2003) Performance of four annual legume species grown in mixtures on an acid soil. Honours thesis, Charles Sturt University, Wagga Wagga NSW, Australia

Helyar KR, Cregan PD, Godyn DL (1990) Soil acidity in New South Wales, current pH values and estimates of acidification rates. *Australian Journal of Soil Research* 28: 523-37

Isbell RF (2002) The Australian Soil Classification Revised Edition. CSIRO Publishing, Melbourne.

Jenkins J (2004). Perennial pastures, their place in the rotation, NSW Agriculture, Orange NSW, Australia.

Michalk DL (1994) Serradella research in New South Wales. In "Alternative pasture legumes 1993'. (Eds DL Michalk, AD Craig, WJ Collins) pp. 55-60. Department of Primary Industries, South Australia, Technical Report 219.

Nichols PGH, Loi A, Nutt BJ, Evans PM. *et al*. (2007) New annual and short-lived perennial pasture legumes for Australian agriculture – 15 years of revolution. Field Crops Research 104: 10-23

Nutt BJ, Loi A (2002) Prima gland clover. Farmnote no 4/2002, Department of Agriculture Western Australia.

Ovalle MC, Urquiaga S, Del Pozo AL, Zagal EV, Arredondo S (2006) Nitrogen fixation in six forage legumes in Mediterranean central Chile. *Acta Agriculturae Scandinavica Section B – Plant and Soil Science*, 56: 277-83

Scott BJ and Cullis BR (1992) Subterranean clover pasture responses to lime application on the acid soils of southern NSW. *Australian Journal of Experimental Agriculture* 32: 1051-1059

Scott B, Schuman B, Fenton G (2000). Acid soil action – 1997-2000. NSW Agriculture.

Unkovich MJ, Pate JS, Sanford P, Armstrong EL (1994) Potential precision of the 15N natural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. *Australian Journal of Agricultural Research* 45. 119-132.