A preliminary evaluation of alternative annual legume species under grazing on the Southern Tablelands of NSW

Richard C. Hayes [1,4], Graeme A. Sandral [1], Richard Simpson[3], Andrew Price[1], Adam Stefanski[3] and Matthew T. Newell [2]

[1] NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Pine Gully Road, Wagga Wagga, NSW 2650
[2] NSW Department of Primary Industries, Cowra Agricultural Research and Advisory Station, Binni Creek Road, Cowra, NSW 2794
[3]CSIRO Agriculture Flagship, GPO Box 1600 Canberra, ACT, 2610
[4] Corresponding author Email: richard.hayes@dpi.nsw.gov.au

Abstract
A field experiment was established at Goulburn, NSW, to identify the likely potential of a range of alternative annual legume cultivars for use in grazing systems on the Southern Tablelands. One cultivar each of arrowleaf clover (*Trifolium vesiculosum*), purple clover (*T. purpureum*), balansa clover (*T. michelianum*), crimson clover (*T. incarnatum*), biserrula (*Biserrula pelecinus*), French serradella (*Ornithopus sativus)* and yellow serradella *(O. compressus)* was compared to subterranean clover *(T. subterraneum) cv. Leura* from 2013-15. All treatments were sown as monocultures at 20kg/ha and left ungrazed during the establishment year. In year two sheep grazed the experiment three times from February-August before stock were again excluded to enable legumes to set seed. Leura subterranean clover (7.0 t/ha) and Dixie crimson clover (6.3 t/ha) were the most productive annual legumes during spring of year 2 and the treatments with the lowest proportion of volunteer weed species (4% and 13%, respectively). By contrast, Mauro biserrula (0.5 t/ha) and Margurita French serradella (1.3 t/ha) were the least productive, with 88% and 75% of total available biomass in these swards composed of volunteer weed species, respectively. Seedling regeneration in year three was negligible in the purple clover, biserrula, French and yellow serradella swards. The poor performance of most alternative annual legume species under relatively lenient management is discussed in the context of introducing alternative annual legume species to permanent grazing systems in cooler more temperate environments. This study highlights the need to develop cultivars of these annual legume species specifically for this new target environment.

Key words
Hard seed; Mediterranean; regeneration; production; competition; weeds

Introduction
A high level of breeding, development and evaluation research has culminated in the commercial release of an unprecedented number of annual pasture legume species and cultivars on the Australian market over the past two decades (Nichols *et al*. 2007; Nichols *et al*. 2012). These alternative legumes were developed and tested in cropping systems and environments to provide forage for livestock and to fix atmospheric nitrogen (N) for subsequent crops. The broad range of cultivars with diverse traits now available provides an opportunity for growers to improve legume production by selecting cultivars suited to their environment and production enterprise. Initial evaluations of most presently commercialised species targeted environments in NSW on the Slopes and Plains (e. g. Dear *et al*. 2003; Boschma *et al*. 2011) with relatively little evidence to date of the performance of these species in higher rainfall Tablelands environments. The Tablelands are often dominated by grazing enterprises and experience cold wet winters, while the soils are frequently shallow and acidic. This study tested the potential adaptation of several alternative annual legumes species developed for cropping systems to an environment in the Southern Tablelands of NSW.

Method
A field experiment was sown near Goulburn using a cone seeder on 21 March 2013. Treatments were randomised in three replicates and included arrowleaf clover cv. Zulu II, purple clover cv. Electra, balansa clover cv. Bolta, crimson clover cv. Dixie, subterranean clover cv. Leura, biserrula cv. Mauro, French serradella cv. Margurita and yellow serradella cv. Santorini, all sown as monocultures in 2.5 ×10 m plots at a seeding rate of 20 kg/ha. The site was fallowed with herbicides for 18 months prior to sowing, and Spinnaker was applied at 70 g/ha as a post sowing pre-emergent selective herbicide. Prior to sowing, the site was limed at 5.0 t/ha and basal nutrients were applied to ensure no nutrients other than N were limiting. The treatments reported here were part of a larger experiment to evaluate the response of pasture species to P nutrition. We

report results from only the legume treatments at the highest P level which received triple superphosphate (20% P and 1.5% S) at sowing applied at a rate calculated to deliver 80 kg P/ha to ensure P was not limiting. All legume seed was inoculated with appropriate rhizobia groups (Table 1) and lime pelleted prior to sowing. An area around the trial site of approximately 2 ha was sown to various grasses and legumes which was later grazed in common with the experimental plots, to ensure a more realistic grazing pressure and to provide space away from the plots for stock water and camp sites.

Table 1. Approximate percentage of hard seed, required inoculum group and recommended rainfall required for annual legume cultivars included in the field experiment (adapted from Lattimore and McCormick 2012)**.**

Species	Cultivar	Hard seed levels (%)	Inoculum Group	annual rainfall (mm)
Arrowleaf clover	Zulu II	80	C	>400
Balansa clover	Bolta	90	C	>550
Biserrula	Mauro	95	WSM 1497	>400
Crimson clover	Dixie	<10	C	>450
French serradella	Margurita	85	S	>400
Purple clover	Electra	80	C	>550
Subterranean clover	Leura	<10	C	>700
Yellow serradella	Santorini	90	S	>400

Estimates of seedling establishment were made on 5 July by counting the numbers of seedlings in two 0.15 m^2 quadrats placed randomly in each plot. An assessment of first year biomass was made on 15 October 2013 by cutting herbage in one 0.1 m^2 quadrat per plot, drying at 60ºC for 48 hours and weighing. Stock were excluded from the trial plots until February 2014 to ensure all species had adequate opportunity to set seed, at which time 50 merino wethers (weighing approximately 60 kg) grazed the 2 ha site for two weeks. The site was grazed again in May for two weeks by the same mob of sheep, and finally in August for another fortnight before being removed on 1 September to enable the legumes to set seed at the end of year two. Plots were scored on 1 September for the percentage cover of the sown legume species by laying a 0.24 m^2 quadrat (divided into 380 squares, each 25 × 25 mm) in a representative area of each plot and counting the number of squares that contained the base of a sown legume. An assessment of aboveground biomass was taken in November 2014 and March 2015 by cutting herbage in one 0.1 m^2 quadrat per plot, sorting sown species from weeds, drying at 60ºC for 48 hours and weighing each component. Seedling regeneration was assessed on 4 May 2015 by counting the number of seedlings in a 1 m × 1m quadrat. ANOVA was conducted in Genstat and treatment differences were reported at $P < 5\%$.

Results

Rainfall received at the site during the establishment year was below average for all months post sowing (March 2013), other than June which received more than three times the monthly average (Table 2). The spring and summer of year 1 was dry with very low pasture growth equivalent to short term drought conditions. Breaking rains were received on 14 February 2014 followed by twice the monthly average in March. With generally well-timed rainfall events throughout the year including 145 mm rainfall in August, 2014 was one of the best pasture-growth years in three decades.

Table 2. Monthly rainfall (mm) recorded near the experimental site (Bureau of Meteorology station 070147) 2013-15 compared to the long term (55 year) median and average for that location

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2013	74.6	77.2	92.8	15.0	28.0	182.6	34.8	21.2	34.0	28.8	56.2	24.2	669.4
2014	13.0	113.8	114.8	57.6	42.2	51.8	17.4	145.8	40.0	50.0	18.2	154.4	819.0
2015	156.6	33.2	18.2	104.4	-	-	-	-	-	-	-	-	-
Mean	69.8	64.9	60.4	51.3	49.4	58.9	46.8	60.7	56.1	67.3	67.4	67.6	718.2
Median	54.2	52.4	48.0	34.2	37.4	48.7	41.0	51.0	47.0	60.0	64.0	53.8	702.8

All treatments established successfully with a high level of variability in initial seedling density, but above-ground biomass in spring of year 1was similar across treatments (Table 3). On 1 September 2014, regenerated subterranean clover swards had achieved almost complete groundcover with crimson clover the only other species to occupy more than 50% of the sown area. The aboveground biomass of subterranean and crimson clovers at the end of spring in year two (2014) was double that of the next most productive legume, arrowleaf clover. All other legume species yielded less than arrowleaf clover, although differences with balansa and purple clovers were not significant (P=0.05). Weed biomass in year 2 was significantly less in the subterranean clover and crimson clover treatments, with legume DM yields higher in swards of those species. Significant rains in December/January (Table 2) prompted an early germination of some annual legume as well as weed species. Again, there was significantly less weed biomass in the subterranean clover and crimson clover swards at the end of summer 2014/15, corresponding with higher levels of legume biomass (Table 3).

Table 3. Seedling density (plants/m2), legume cover (%), legume and weed dry matter (DM; t/ha) of 8 annual legume genotypes sampled between 2013-15.

Sampling	Arrowleaf clover	Balansa clover	Biserrula	Crimson clover	French serradella	Purple clover	Subterranean clover	Yellow serradella	l.s.d. (P=0.05)
				Year 1; 2013					
Seedling establishment	97	270	294	77	192	152	173	117	(P=0.07)
Legume DM	5.4	3.4	5.5	4.6	5.2	3.6	4.2	4.0	(P=0.21)
				Year 2; 2014					
Legume cover (%)	11.7	28.3	10.7	59.7	25.1	16.5	92.2	38.0	14.02
Legume DM	3.0	1.7	0.5	6.3	1.3	2.0	7.0	1.6	1.35
Weed DM	3.6	3.6	4.3	1.1	3.8	2.8	0.3	3.6	1.86
				Year 3; 2015					
Legume DM	0.0	0.1	0.0	2.8	0.0	0.0	0.8	0.0	1.01
Weed DM	4.0	3.5	3.0	0.7	3.0	5.5	1.2	4.6	2.29
Seedling regeneration	101	124	1	263	2	5	573	15	144.7

The final density of regenerating annual legumes in year 3 was significantly greater in the subterranean clover sward compared to all other species (Table 3). Sampled during the first week of May, the seedlings of subterranean and crimson clovers were so large that individual plants were difficult to distinguish, reflecting the fact that they had been growing from as early as late December 2014. A small number of crimson clover flowers were evident at the time of sampling, indicating the mild autumn conditions the swards had experienced and the relative age of the seedlings. By contrast, seedlings of other species were at a very early stage of development. Density of biserrula, purple clover, French and yellow serradella was negligible in autumn of year 3.

Discussion

It is not possible in a preliminary experiment such as this to categorically attribute poor performance of a cultivar to any one factor. However, there are some obvious factors which were likely to have influenced the results. Seasonal conditions did not favour the harder seeded cultivars. The experimental period experienced one relatively dry summer (2013/14 - until mid-February) in the 12 months post-sowing, and one relatively wet summer in 2014/15 (Table 1). In both instances, the softer seeded cultivars were able to germinate on February and December rains, respectively, and compete with weed species. The ability to utilise opportunistic summer rainfall would seem to be an important adaptive trait for legumes in environments such as this which typically experience cold winter conditions that supress pasture growth and which receive over 50% of average annual rainfall between October and March (Table 2). Both Leura subterranean clover and Dixie crimson clover were able to respond to summer rainfall and compete effectively with weeds. Both species also happened to be those with the lowest levels of hard seed used in this experiment (Table 1). Even though Zulu II arrowleaf clover and Bolta balansa clover had over 100 seedlings/m^2 regenerate in May 2015 (year 3), those young seedlings were emerging amongst a dense mat of well-developed weeds such as sorrel (*Rumex acetosa*) which emerged on summer rainfall, and given the smaller plant size, their ability to out-compete the established weeds in the longer term seems unlikely.

Three of the four species which failed to regenerate adequately in year 3, biserrula, French and yellow serradella, were all species with a requirement for species of root nodule bacteria unlikely to exist in the background population at this site (see Table 1). Whilst N-fixation efficiency and rhizobia populations were not monitored in the current experiment, strain-host incompatibility with background rhizobium populations cannot be discounted as a factor contributing to the poor performance of these species. Biserrula is known for its poor capacity to nodulate with a range of commonly available species of root nodule bacteria (Howieson *et a*l. 1995) and the negative effects of naturalised soil rhizobia on the formation of a successful symbiosis has even been recorded with *Trifolium* species such as balansa clover (Ballard et al. 2002). The site used for the present experiment had a history of subterranean and naturalised annual clovers for at least 4 decades prior to the establishment of the experiment and was, in this respect, similar to many Tablelands environments. This deserves further research.

The performance of French serradella may have been improved in the current study had we sown the softer seeded cultivar, Cadiz. There are few alternative cultivars of the other species with lower levels of hard seed which we suspect would assist their use and persistence in the Southern Tablelands. Over 30 cultivars of subterranean clover currently exist on the Australian market (Lattimore and McCormick 2012), providing a range of traits available to growers to cope with aspect, soil and climatic conditions. The lack of cultivar choice of the alternative legume species limits the ability to use a mixture of cultivars to guard against natural variability expected in a commercial permanent pasture situation.

Our results document the apparent failure of several new annual legume species under seemingly favourable seasonal conditions in this high rainfall environment, presenting a cautionary message to farmers and advisors to check the adaptation of new annual legume cultivars before planting them on a commercial scale. The results are perhaps unsurprising given that many of the cultivars of the alternative species were developed for lower rainfall and more Mediterranean climates. Their failure to persist at this site should not necessarily be interpreted as an inadequacy of the whole species, but rather a reflection of the fact that commercial cultivars of these species have not yet been developed for higher rainfall more temperate environments, highlighting a significant opportunity for future cultivar development. Subterranean clover is a species of Mediterranean origin, but has undergone decades of improvement which has led to the development of cultivars such as Leura which was selected for tablelands environments (Nichols et al. 2013). No doubt its disease resistance characteristics, its later maturing habit and low levels of hard seed have contributed to the superior performance of cv. Leura in this experiment.

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References

Ballard RA, Craig AD & Charman N (2002). Nodulation and growth of pasture legumes with naturalised soil rhizobia.2. Balansa clover (Trifolium michelianum Savi). *Australian Journal of Experimental Agriculture* **42** (7): 939-944.

Boschma SP, Crocker GJ, Lodge GM & Harden S (2011). Evaluation of pasture legumes in northern New South Wales, Australia. *New Zealand Journal of Agricultural Research* **54** (3): 203-213.

Dear BS, Sandral GA, Peoples MB, Wilson BCD, Taylor JN & Rodham CA (2003). Growth, seed set and nitrogen fixation of 28 annual legume species on 3 Vertosol soils in southern New South Wales. *Australian Journal of Experimental Agriculture* **43** (9): 1101-1115.

Howieson J, Loi A & Carr S (1995). *Biserrula pelecinus* L. - a legume pasture species with potential for acid, duplex soils which is nodulated by unique root-nodule bacteria. *Australian Journal of Agricultural Research* **46** (5): 997-1009.

Lattimore MA & McCormick L (2012).Pasture varieties used in New South Wales 2012-13. New South Wales: Published jointly by NSW Department of Primary Industries and the Grassland Society of NSW Inc.

Nichols PGH, Foster KJ, Piano E, Pecetti L, Kaur P, Ghamkhar K & Collins WJ (2013). Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. *Crop and Pasture Science* **64** (4): 312-346.

Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ & You MP (2007). New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research* **104** (1-3): 10-23.

Nichols PGH, Revell CK, Humphries AW, Howie JH, Hall EJ, Sandral GA, Ghamkhar K & Harris CA (2012). Temperate pasture legumes in Australia—their history, current use, and future prospects. *Crop and Pasture Science 63* (9): 691-725.