RESEARCH

EVALUATION OF PERENNIAL FORAGE LEGUMES AND HERBS IN SIX MEDITERRANEAN ENVIRONMENTS

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There is an absence of drought tolerant herbaceous perennial forage legume and herb options other than lucerne (*Medicago sativa* L.) for environments with Mediterranean-like climates common in extensive areas of Southern Australia, the Mediterranean basin, and Chile. Therefore, a collection of 174 forage perennial legume and herb entries from 103 species and 32 genera was evaluated for adaptation in a diverse range of Mediterranean climatic environments in Southern Australia. The seasonal rainfall distribution varied from moderately to highly winter dominant with long term average annual rainfall ranging from 318 to 655 mm. The entries were rated for productivity and persistence over 3 yr. The 12 entries identified as the most promising for winter, summer, or all-year round production included *Bituminaria bituminosa* (L.) C.H. Stirt. var. *albomarginata*; *Cichorium intybus* L.; *Cullen australasicum* (Schltdl.) J.W. Grimes; *Dorycnium hirsutum* (L.) Ser.; *Kennedia prostrata* R. Br.; *Lotononis bainesii* Baker, *Lotus pedunculatus* Cav.; *L. corniculatus* L.; *L. cytisoides* L.; *Medicago sativa* subsp. *sativa* L.; *Medicago sativa* subsp. *caerulea* (Less. ex Ledeb.) Schmalh., and *M. sativa* subsp. *falcata* (L.) Arcang. These entries maintained production and persisted for the period of the evaluation, with the exception of *C. intybus* and *L. corniculatus* that declined in persistence over time. The potential role of these species in extensive grazing systems in Mediterranean climatic zones, their attributes and limitations, and current progress in developing them as useful forage plants was discussed.

Key words: *Bituminaria*, *Lotus,* herbage yield, legume persistence, Australian native germplasm.

There is an absence of drought tolerant herbaceous perennial forage legume and herb options other than lucerne (*Medicago sativa* L.) for environments with Mediterranean-like climates common in extensive areas of southern Australia, the Mediterranean basin, and Chile (Dear *et al*., 2003; Li *et al*., 2008). In their extensive review of species adapted to Australian pastures, Gramshaw *et al*. (1989) listed six temperate perennial legumes widely sown in southern Australia; of these, only lucerne has sufficient drought tolerance to withstand the moisture stress and extended droughts typical of Mediterranean-like climates. The greater use of lucerne is in part constrained by poor adaptation to acidic or waterlogged soils and lack of persistence under continuous grazing (Humphries and Auricht, 2001). The other perennial legumes identified by Gramshaw *et al*. (1989) were white clover (*Trifolium repens* L.), strawberry clover (*T. fragiferum* L.), red clover (*T. pratense* L.) and birdsfoot trefoil (*L. corniculatus* L.) and all are adapted to higher rainfall (> 700 mm) regions or localised drainage areas (Gramshaw *et al*., 1989; Reed and Flinn, 1993). Current cultivars of these species lack the drought tolerance necessary to survive in regions that experience pronounced summer droughts (Lolicato, 1993).

While lucerne is clearly a highly drought tolerant temperate perennial legume, it is also highly competitive with annual forage species (Dear and Cocks, 1997), often leading to a monoculture which, when grazed at particular periods, can lead to animal health disorders such as bloat (FitzGerald *et al*., 1980) or red-gut (Gumbrell, 1997).

The case for increasing the diversity of legumes in pastures was made by Oram (1993) and Cocks (2001). The main drivers include better exploitation of diverse ecological niches, buffering against pests and diseases and achieving more sustainable soil management. Alternative perennial legume options have the potential to complement and expand the feed base for grazing livestock and provide fixed soil N and weed control options

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in cropping rotations. In addition, new species should assist in combating soil degradation attributable to deep drainage by using more out-of-season rainfall compared with predominantly winter growing annual pastures and cropping systems and be sufficiently persistent to incrementally dry the soil profile over successive years (Sandral *et al*., 2006; Dear and Ewing, 2008). The need for increased species diversity and the advantages this would convey are relevant not only to Australian pastures but to pastures in similar agroecological environments worldwide (Russi *et al*., 2003).

As the first step in increasing the diversity of perennial forage species Cocks (2001; 2003) and Dear *et al*. (2003) conducted reviews to identify a priority group of species potentially adapted to Mediterranean environments of southern Australia that warranted further evaluation. The Cooperative Research Centre for Plant-based Management of Dryland Salinity (Salinity CRC) subsequently commenced a field screening program using a multi-stage process, as described by Dear and Ewing (2008). The field studies conducted at 10 sites across southern Australia evaluated 45 species from 22 genera and identified a number of perennial legume and herbaceous species whose performance justified more detailed evaluation (Li *et al*., 2008; Reed *et al*., 2008). Based on these prior studies, accessions of the more promising species and an expanded range of previously untested species identified from a desktop review of species adapted to Mediterranean and temperate environments were sourced from collaborating partners/genetic resource centres or by collecting them in their natural environments, together with their associated root nodule bacteria (RNB) (Hughes *et al*., 2008).

The current study tested the production and persistence of a large group of promising herbaceous perennial species and accessions compared with lucerne at a diverse range of sites across southern Australia to identify new or unexploited species that may warrant further development for use in regions with Mediterranean-like environments.

MATERIALS AND METHODS

Site description

Six row nurseries were established across southern Australia at Wagga Wagga and Binalong in New South Wales (NSW), Katanning and Newdegate in Western Australia (WA), and Byawatha and Bealiba in Victoria. All nurseries were sown in 2005, except for Bealiba which was sown in 2006. All six experimental sites were located in non-saline, non-waterlogged areas. The two NSW sites were typical of the medium rainfall crop/pasture zones of the Riverina and Slopes regions respectively of southern NSW. Soils at both sites were acidic, with the Binalong soil containing Al and Mn levels likely to inhibit lucerne growth. The two WA sites were acidic but lucerne is able to perform well and farmers successfully grow it in these soils, most likely due to low Al levels (< 6% in the top 10cm) and negligible Mn. The site in North East Victoria at Byawatha was typical of the high rainfall livestock and crop mixed farming zone. The central Victorian site, Bealiba, was on an acid granitic soil. The soil chemical characteristics at each site are presented in Table 1.

Climatic data

Four of the six sites have a strongly winter-dominant rainfall pattern (Table 2) with the other two sites (Binalong and Wagga Wagga) having a non-seasonal rainfall distribution although the growing season at these sites is also mostly confined to the cooler months due to very high evaporation rates over summer. The Binalong site had the highest long term average annual rainfall (655 mm) and the Newdegate site the lowest

Table 1. Soil chemical analysis at depths of 0-0.10 m and 0.10-0.20 m for each site.

State	New South Wales				Western Australia				Victoria			
Site Location	Wagga Wagga Wagga Wagga Agricultural Institute		Binalong Private landholder		Katanning Great Southern Agricultural Research Institute		Newdegate Newdegate Research Station		Byawatha Private landholder		Bealiba Private landholder	
Latitude, °S	35°05’		34°36’		33°42’		33°06’		36°10’		36°49’	
Longitude, °E	147°35’		148°40’		117°37’		118°49’		146°54’		143°35’	
Elevation, m.a.s.l.	220		588		309		330		250		260	
Average rainfall, mm	527		655		428		318		625		469	
Australian soil classification	Red Kandosol		Yellow Kandosol		Grey Sodosol		Yellow Chromosol		Red Dermosol		Kurosols	
Soil depth, m	0-0.10	0.10-0.20	0-0.10	0.10-0.20	0-0.10	0.10-0.20	0-0.10	0.10-0.20	0-0.10	0.10-0.20	0-0.10	0.10-0.20
pH ($CaCl_2$)	4.4	4.6	4.1	4.0	4.6	4.4	4.9	4.7	5.1	4.4	4.4	4.6
EC, $\mu s\ cm^{-1}$	6.8	7.1	4.3	2.3	4.0	2.3	2.7	1.5	48	47	0.0	0.0
Colwell P, mg kg^{-1}	45	18	170	80	36	24	29	33	28	23	13.9	8.0
	Exchangeable cations ($cmol_{(+)}\ kg^{-1}$ soil)											
Al	0.28	0.12	0.38	0.46	0.23	0.32	0.02	0.06	0.02	0.19	0.18	0.09
Mn	0.39	0.26	0.57	0.47	0.02	0.02	0.02	0.02	0.44	0.15	0.03	0.01
Ca	3.47	4.00	1.82	0.64	2.88	1.49	1.95	0.92	2.38	0.79	2.31	1.25
K	1.11	1.03	0.81	0.60	0.10	0.05	0.18	0.12	0.69	0.46	2.14	1.80
Mg	0.97	1.25	0.26	0.12	0.43	0.33	0.48	0.33	0.16	0.13	0.83	0.73
Na	0.02	0.02	0.01	0.01	0.30	0.15	0.09	0.03	0.10	0.10	0.27	0.18
Total	6.24	6.68	3.85	2.30	3.96	2.36	2.74	1.48	3.79	1.82	5.76	4.06
Al, %	4.5	1.8	9.9	20.0	5.8	13.6	0.7	4.1	0.5	10.4	3.1	2.2

EC: Electrical conductivity.

Table 2. Monthly rainfall for 2005-2008 and 30 yr long term average annual rainfall (LAR) for Wagga Wagga, Binalong, Katanning, Newdegate, Byawatha and Bealiba (Bureau of Meteorology, 2009).

State	Site	Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Total
NSW	Wagga Wagga	2005	18.9	41.0	7.2	17.6	5.2	65.5	51.3	60.6	81.9	62.8	23.0	40.9	475.9
		2006	43.2	4.2	14.0	6.8	26.4	63.6	48.6	6.0	21.4	4.8	26.3	7.0	272.3
		2007	19.1	38.2	23.1	43.2	50.0	18.9	39.7	12.9	5.7	22.8	41.3	67.4	382.3
		2008	53.8	47.2	24.8	20.1	11.8	32.0	51.5	24.0	28.2	16.6	48.9	54.6	413.5
		LAR	37.0	35.6	33.9	37.0	47.7	48.5	55.5	51.8	52.5	47.1	40.5	39.8	527.0
	Binalong	2005	50.8	47.6	20.4	5.0	0.6	113.4	120.2	88.2	115.8	76.6	69.2	20.8	728.6
		2006	50.3	3.4	15.8	15.3	11.2	52.1	60.8	15.2	40.0	0.0	15.8	11.3	291.2
		2007	21.4	26.9	57.9	66.1	59.6	49.2	47.3	16.0	9.6	24.1	126.4	94.8	599.3
		2008	55.1	39.7	44.0	34.7	13.4	47.6	34.7	50.4	20.0	49.0	75.2	57.1	520.9
		LAR	43.1	36.2	45.3	45.6	53.4	62.9	74.6	63.3	62.8	57.4	58.2	52.3	655.1
WA	Katanning	2005	1.0	0.8	36.6	84.8	162.4	66.6	28.2	39.8	50.0	36.0	35.8	12.8	554.8
		2006	80.8	2.2	1.0	35.8	14.6	17.6	57.6	60.6	22.2	13.8	8.4	1.8	316.4
		2007	20.2	1.0	7.6	50.2	18.4	43.4	87.2	60.4	49.4	52.2	2.2	38.8	431.0
		2008	0.2	0.6	3.6	64.2	49.0	64.0	96.0	8.6	38.6	50.0	26.0	20.8	421.6
		LAR	26.0	15.0	12.0	59.0	61.0	48.0	67.0	42.0	40.0	38.0	16.0	18.0	442.0
	Newdegate	2005	0.0	3.0	32.4	18.6	70.0	65.6	14.6	31.4	39.4	39.0	16.0	5.4	335.4
		2006	151.4	12.0	31.2	28.2	4.0	16.2	32.2	66.0	28.2	16.2	28.8	13.4	427.8
		2007	29.2	0.0	0.2	33.8	18.2	43.2	64.0	32.2	23.6	38.6	0.4	47.4	330.8
		2008	0.2	25.8	3.4	50.6	51.2	44.6	91.4	11.6	61.0	89.6	36.0	63.0	528.4
		LAR	23.0	14.0	15.0	20.0	38.0	41.0	46.0	38.0	31.0	21.0	17.0	14.0	318.0
Victoria	Byawatha	2005	45.0	182.5	12.5	8.5	6.0	122.5	73.5	101.5	76.0	101.0	73.5	46.5	849.0
		2006	9.5	14.5	21.5	34.5	26.5	32.5	57.0	22.5	33.5	1.5	44.0	4.0	301.5
		2007	16.8	34.8	52.5	40.8	111.0	49.5	74.0	11.5	13.3	20.5	38.0	49.0	511.5
		2008	59.0	29.0	22.5	20.0	44.0	18.5	97.5	53.8	25.5	9.5	82.5	53.8	515.5
		LAR	43.1	38.0	35.1	43.2	57.2	59.3	67.4	67.2	60.5	58.8	50.2	40.7	619.5
	Bealiba	2005	32.6	72.6	3.8	21.4	12.6	84.9	30.2	56.8	41.8	46.6	32.6	53.8	489.7
		2006	33.2	2.2	12.4	32.0	26.4	23.2	69.3	15.0	28.2	0.8	13.8	18.4	274.9
		2007	60.4	14.6	20.8	34.8	87.4	27.6	40.2	8.6	26.2	5.0	56.2	27.2	409.0
		2008	16.0	8.8	21.0	8.2	28.4	23.6	55.8	29.2	17.0	5.8	61.1	57.2	332.1
		LAR	31.0	23.0	27.0	35.0	47.0	45.0	48.0	52.0	47.0	45.0	37.0	32.0	469.0

NSW: New South Wales; WA: Western Australia.

(318 mm). Summers at all sites are typically hot with average maximum temperatures above 29 °C (Table 3). Winters are mild to cool at all sites with minimum average temperatures above 0 °C, however the more elevated sites can experience up to 30 d of frosts per year with absolute minimum temperatures in the coldest day of the coldest month of -7.5 °C at Byawatha in June 2006.

Seasonal conditions at the sites during the experimental period showed large variation in rainfall from year to year as is common in southern Australia. Some locations such as the Wagga Wagga, Byawatha and Bealiba sites also experienced prolonged periods of below average rainfall (Table 2) which severely drought stressed the germplasm with annual rainfall in some years being only 49-58% of the long term average.

Selection of genera and species

A total of 174 legume and herb entries from 103 species in 32 genera were evaluated. These entries were sourced from the *Trifolium* Genetic Resource Centre at the Department of Agriculture and Food, Western Australia (DAFWA), Perth, WA or the South Australian Research and Development Institute (SARDI), Adelaide, South Australia (Hughes *et al*., 2008) and include cultivars, selected lines and some composites of a few entries within a species (Appendix 1). Lucerne was selected as the common control species across all sites as it is the most drought hardy and widely grown of all the available perennial legumes.

The risk of introducing new weeds to the Australian environment is well recognised and all entries new to Australia underwent appropriate quarantine assessments

Table 3. Mean monthly maximum and minimum temperatures (°C) for 2005-2008 at each site.

Site	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Total
						Maximum temperature							
Wagga	34.6	32.1	29.9	24.7	19.6	16.5	13.9	15.8	20.0	25.4	28.6	30.9	24.3
Binalong	31.6	29.6	27.3	22.5	17.6	13.5	12.0	14.2	18.1	23.4	25.9	28.1	22.0
Katanning	27.9	28.8	27.3	20.9	18.1	15.1	14.1	14.9	16.3	19.8	24.3	26.2	21.2
Newdegate	28.9	29.8	28.1	22.0	19.2	15.7	14.8	15.9	17.8	21.9	25.6	27.3	22.3
Byawatha	32.9	29.9	28.5	22.9	18.1	14.8	12.9	15.0	18.5	23.3	26.8	30.1	22.8
Bealiba	30.4	28.8	27.5	22.1	17.1	14.2	12.9	15.1	18.1	22.4	25.9	27.6	21.8
						Minimum temperature							
Wagga	18.1	16.7	14.8	10.1	6.6	5.2	4.0	4.0	5.0	9.0	13.7	14.7	10.1
Binalong	16.2	14.9	12.5	7.5	4.0	3.1	2.3	2.1	4.2	7.5	11.3	13.0	8.2
Katanning	12.9	13.7	12.7	9.9	8.6	5.6	5.5	5.7	6.0	7.1	9.6	10.9	9.0
Newdegate	13.3	14.2	13.0	9.8	7.8	4.9	4.7	4.3	5.4	7.5	10.2	11.3	8.9
Byawatha	15.7	13.9	10.9	6.2	4.2	2.1	2.5	1.8	2.7	5.4	10.6	11.4	7.3
Bealiba	15.5	12.9	12.2	7.3	5.3	6.5	3.8	2.9	4.9	7.1	10.2	11.3	8.3

Source: Bureau of Meteorology, 2009.

by the Australian Quarantine and Inspection Service (AQIS), the Western Australian Quarantine and Inspection Service (WAQIS), and the internal weed risk assessment scheme of the Salinity CRC (Stone *et al*., 2008).

A total of 102 entries were sown at Wagga Wagga and Binalong, 95 at Katanning and Newdegate, 94 at Byawatha, and 53 at Bealiba. A paucity of seed of some lines meant that not all could be sown at all sites although there were 54 entries in common at the first five sites sown. Some entries were only sown at the later sown Bealiba site as they had not passed through quarantine in time for sowing at the other sites. The entries were selected based on (a) site characteristics of their native environments (Hughes *et al*., 2008); (b) previous performance in other marginal environments (Real *et al*., 2005); (c) performance during seed increase at the SA and WA Genetic Resource Centres (Hughes *et al*., 2008); (d) previous field performance in WA (G. Moore, 2004, Department of Agriculture and Food Western Australia, unpublished data), and (e) relative performance in previous field studies (Li *et al*., 2008).

Each entry was sown into single 1 m long rows (plots), replicated three times, and separated by buffer pathways 1 m apart at Byawatha, Wagga and Binalong, 2 m at Katanning and Newdegate and at 0.5 m at Bealiba. Each plot was sown as a monocrop with 1 g of viable seed. Even though there was a diverse range of seed sizes within the evaluated entries, 1 g of seed allowed a full 1 m row cover for all species. Leguminous entries were inoculated with the appropriate strain of root nodule bacteria (RNB), and lime pelleted prior to sowing. Appropriate RNB were provided by the Centre for *Rhizobium* Studies (CRS), Murdoch University, WA; SARDI, SA or the Department of Primary Industries, Victoria (Howieson *et al*., 2000). For species where an effective RNB has yet to be identified, a diverse mixture of strains was used that offered the best probability of forming an effective symbiosis. The list of strains is provided in Appendix 1.

Experimental design

A row and column design was used to restrict spatial repetition of treatments and reduce the number of treatment neighbouring concurrences in rows and columns (Cullis *et al*., 2006). All temperate and Mediterranean entries were sown in winter while subtropical or tropical entries were sown in spring to accommodate different temperature requirements at germination. At each site, winter and spring experiments were adjacent to each other. All entries at Bealiba were sown in winter only.

Site management and measurements

The experiments were kept weed-free by spraying the buffers with a non-selective herbicide (glyphosate, 510 g a.i. L^{-1}; Nufarm Australia Ltd., Laverton North, Victoria, Australia). At the Katanning and Newdegate experimental sites weed growth in the buffer pathways were controlled by mowing when required. Weeds within the rows or close by were hand-weeded to avoid potential herbicide damage to the sown species. Starter fertilizer (15% N, 13% P, 10% S) was applied to the Wagga Wagga and Binalong sites at sowing at 2 g per row which equated to approximately 160 kg ha^{-1}. Single superphosphate (8.8% P, 11% S) was applied at a similar rate prior to spring in the second year to all treatments at both sites. Single superphosphate was applied at 125 kg ha^{-1} once in early spring 2005 at Byawatha and at 200 kg ha^{-1} at sowing and each autumn (2007, 2008) at Bealiba. Both the Katanning and Newdegate experimental sites were hand topdressed with 150 kg ha^{-1} of single superphosphate and potash mixed in a ratio of 3:2 in early autumn each year.

Herbage production. All entries were assessed visually with a score of 10 as the highest and 1 as the lowest herbage DM yield at each measurement at each site. At Newdegate and Katanning all plant material from each plot was harvested, bagged, oven-dried at 60 °C for 48 h, and then weighed to obtain herbage DM yield per row. At Wagga Wagga and Binalong, the visual DM scores were converted into herbage DM yield with appropriate calibration equations ($R^2 > 0.90$) derived from 10 representative plots at each assessment. No herbage yield calibrations were taken at Byawatha and Bealiba. Herbage production was assessed once every 3-mo, normally at the end of each season at each site. No assessment was carried out in some seasons at some sites if there was negligible growth. There were up to 13 assessments of herbage yield at each site over 3 yr. For all sites in NSW and Victoria, plots were hand cut to 5 cm height above ground level immediately after each assessment. The Wagga Wagga site was crashed-grazed in 1 day by sheep on two occasions to avoid any differential grazing due to animal selection.

Plant persistence. Basal frequency was assessed as plant persistence using a 0.2 × 1.0 m quadrant with 50 × 50 mm grids in each autumn shortly after the initial 'breaking' rains from year 2 onwards at all sites except at Byawatha and Bealiba. Basal frequency is presented as the percentage of squares containing at least one sown perennial plant base.

Data analysis

ASReml-R (Butler *et al*., 2009) was used to fit a linear mixed model to each of the response variables of DM yields, visual scores, and the square root of the basal frequency. The DM yields were also transformed by log10(x+1) to improve the normality assumptions and stabilize the variance. The winter and spring experiments were analyzed separately because the nature of the entries were mostly different for the two different sowings.

Since the number of entries is very unbalanced between the sites, we used the linear mixed model approach to predict the means for each entry (BLUPS) even though the entries may not be present at all of the sites. The linear mixed model takes the form of a simple nested repeated-measures model (assuming 'equal-correlation between times') as in Cochran and Cox (1957) for multi-environment trials. The model is nested as follows: *SITES/REP/PLOTS/TIMES* with *ENTRIES* allocated at the *PLOTS* at random.

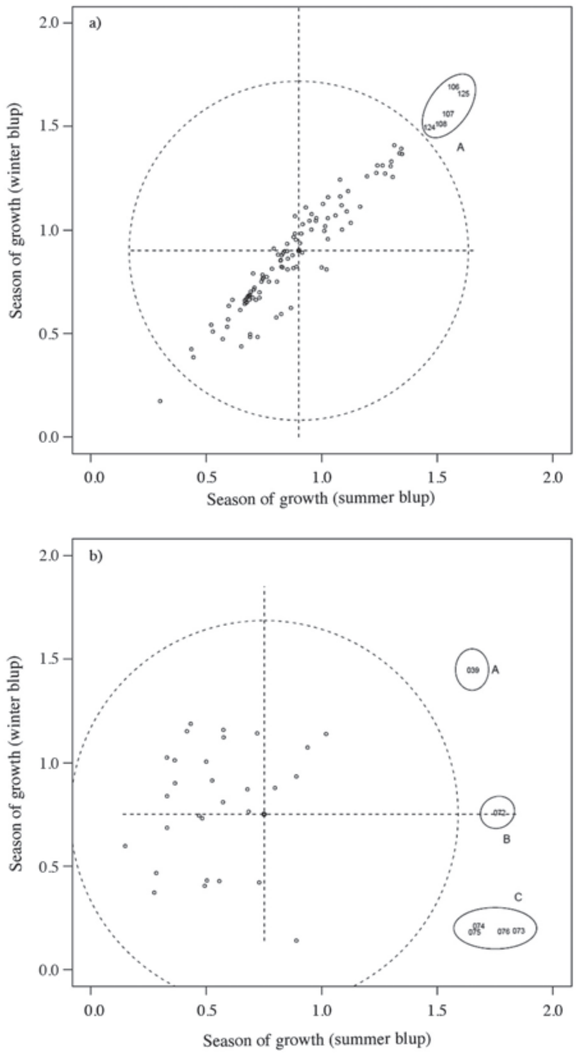
We considered the entries as RANDOM so therefore the means for the ENTRIES are called BLUPS or 'adjusted means'. Since the nature of the ENTRIES was quite unbalanced across the sites, the adjusted means would be expected to be shrunken towards the mean of all of the *ENTRIES* across all of the *SITES*. The only fixed factor was *TIMES* since the *SITE* has different entries it was considered as random.

We looked at the response over time but we noticed that the seasonality comparison of Winter vs. Summer as a strong component of this time response. An aspect of the time response was to consider a comparison between the winter and summer performance since this explained most of the variability in the time responses.

The summary of the responses was displayed with a graph (a 2-dimensional plot of effects) between the comparison over time of a "Winter" vs. "Summer" BLUP or adjusted mean. The graph shows a quadrant of responses where the response in the top-right hand corner represented the better entries compared to the ones in the bottom left-hand corner where the entries went to a zero end point were poor performers.

The averages of the standard errors of the BLUPS for the entries were used to construct approximate 95% confidence interval for the mean of the BLUPS to indicate a separation of the performance of the entries relative to the mean of the entries.

For persistence data, there were only three annual time points of measurements so the *TIMES* factor was subjected to a simple linear trend or slope comparison across the *TIMES*. We examined the slopes and final basal frequency in a similar way to the above measurements but only summarised in the manuscript results without graphical display.



Entry numbers outside the 95% confidence interval correspond to the codes given in Appendix 1.
Entries inside the 95% confidence interval or in the left lower quadrant were represented with a circle.

Figure 1. Herbage dry matter yield (BLUPS) with a 95% confidence interval (dashed) about the mean for (a) winter sown experiments and (b) spring sown experiments.

RESULTS

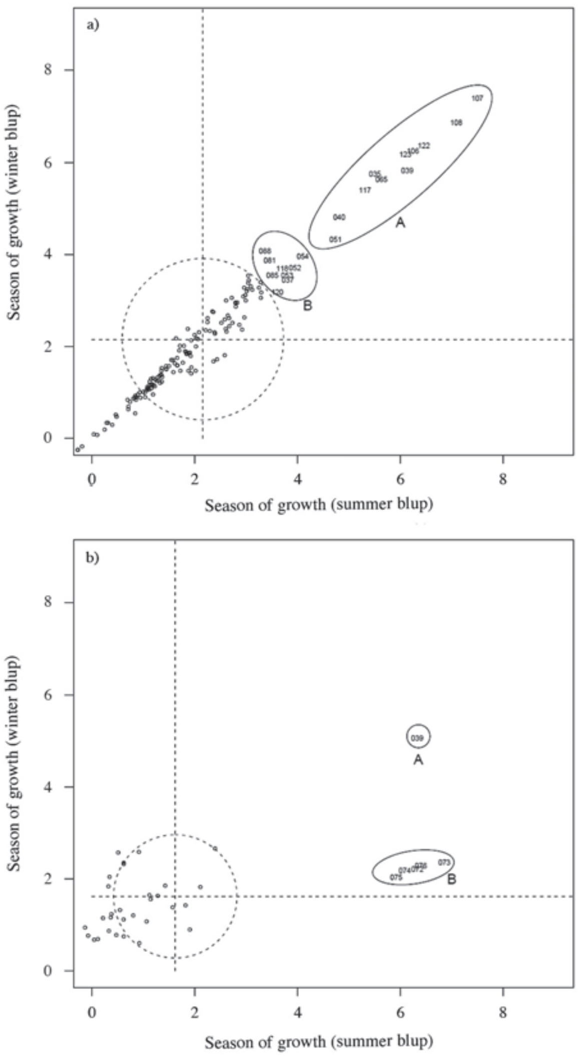
Herbage yield

Despite the different seasonal and soil conditions, the various entries had a similar performance across sites with no significant G × E interaction in herbage yields when determined by either herbage DM yield or visual DM scores. The only significant term in the model was *Entry* ($P \leq 0.05$). There were no other significant interactions between any other terms in the model.

Herbage DM yield. A pair-wise plot of the BLUPS of the summer vs. winter BLUPs for the log transformed DM cuts is presented in Figures 1a and 1b for the winter and spring experiments. For winter sown experiments, only one cluster of entries was formed outside the 95% confidence interval central ellipse around the grand mean with the best summer and winter performance. It consists of five entries corresponding to two species, *M. sativa* subsp. *sativa* (codes 106, 107, and 108) and *M. sativa* subsp. *falcata* L. Arcang. (124 and 125). For spring sown experiments, cluster A had the best summer and winter performance. It consisted of only entry, *C. australasicum* (Schltdl.) J.W. Grimes (39). Cluster B had a very good summer production and an average winter production and consisted of *Lotononis bainesii* Baker (72). Cluster C had a very good summer production and a low winter production and consisted of 4 entries of *L. bainesii* (73, 74, 75, and 76).

Herbage visual DM scores. A pair-wise plot of the BLUPS for the effects of the visual DM scores for summer and winter across the six sites is presented in Figure 2a for the winter sown experiments and Figure 2b for the spring sown experiments. An ellipse representing the 95% confidence interval from the grand mean was also plotted.

For winter sown experiments, Cluster A had the best summer and winter production. It consisted of 11 entries corresponding to six species with entry code in brackets as follows: *Bituminaria bituminosa* var. *albomarginata* C.H. Stirt (35); *Cullen australasicum* (39 and 40); *Dorycnium hirsutum* (L.) Ser. (51); *Kennedia prostrata* R. Br. (65); *M. sativa* subsp. *sativa* (106, 107, 108, and 117) and *M. sativa* subsp. *falcata* (122 and 123). Cluster B production in summer and winter was medium. It consisted of nine entries corresponding to seven species, *C. intybus* L. (37); *D. hirsutum* (52, 53, and 54); *L. pedunculatus* Cav. (81); *L. corniculatus* L. (85); *L. cytisoides* L. (88); *M. sativa* subsp. *sativa* (118); *M. sativa* subsp. *caerulea* (Less. ex Ledeb.) Schmalh (120).



The solid ellipses A and B indicate 2 diverse clusters.
Entry numbers outside the 95% confidence interval correspond to the codes given in Appendix 1.
Entries inside the 95% confidence interval or in the left lower quadrant were represented with a circle.

Figure 2. Herbage visual dry matter scores (BLUPS) with a 95% confidence interval (dashed) about the mean for (a) winter sown experiments and (b) spring sown experiments.

For spring sown experiments, Cluster A had the highest summer and winter production. It consisted of only one entry of *C. australasicum* (39). Cluster B had a very good summer production and close to average winter production. It consists of five entries of *L. bainesii* (72, 73, 74, 75, and 76).

From the analysis of both herbage DM yield and visual DM scores of the winter and spring experiments, 12 entries were identified as the most promising for either their winter, summer, or all-year round production as follows, *B. bituminosa* var. *albomarginata* (35); *C. intybus* (37); *C. australasicum* (39); *D. hirsutum* (51); *K. prostrata* (65); *L. bainesii* (72); *L. pedunculatus* (81); *L. corniculatus* (85); *L. cytisoides* (88); *M. sativa* subsp. *sativa* (107); *M. sativa* subsp. *caerulea* (120) and *M. sativa* subsp. *falcata* (123). The identified 12 entries maintained production for the entire experimental period, with the exception of *C. intybus* and *L. corniculatus* which declined in production over time.

Basal frequency

For the winter and spring sown experiments there was a significant G × E interaction for basal frequency assessed shortly after the "breaking" rains in years 2, 3, and 4. Therefore, results are presented for Wagga Wagga, Binalong, Katanning, and Newdegate separately.

For the winter sown experiments, 19, 1, 28, and 27 entries had above average basal frequency at Binalong, Wagga Wagga, Katanning, and Newdegate, respectively. Overall *M. sativa* subsp. *falcata* (122) was the only entry better than average at all sites. At Binalong, Katanning, and Newdegate, the following entries had superior persistence, *C. australasicum* (39); *D. graecum* Ser. (48), *D. hirsutum* (50, 52, 53, and 54); *D. pentaphyllum* Scop. (55 and 57); *L. pedunculatus* (81); *L. cytisoides* (89); *M. sativa* subsp. *sativa* (106, 107, and 108); *M. sativa* subsp. *caerulea* (120 and 121) and *M. sativa* subsp. *falcata* (122). At Katanning and Newdegate, the following entries had high basal frequency: *D. graecum* (49), *D. hirsutum* (51); *D. pentaphyllum* (56); *L. creticus* (87); *L. cytisoides* (88); *L. tenuis* Willd. (93) and *T. tumens* M. Bieb (171). *Lotus corniculatus* (92) had high basal frequency at Binalong and Newdegate. *Trifolium ambiguum* M. Bieb (158) was superior at the higher rainfall Binalong site whereas *Lotus corniculatus* (82) and *T. physodes* M. Bieb. (167, 168, and 169) performed best at Katanning. *Argyrolobium uniflorum* Jaub. & Spach. (9 and 10); *H. boutignyanum* Alleiz. (63) and *M. suffruticosa* DC (124) had superior persistence at Newdegate.

For the spring sown experiments, 6, 8, 7, and 8 entries had better than average persistence at Wagga Wagga, Binalong, Katanning, and Newdegate, respectively. *Lotononis bainesii* (72 to 76) was within the better than average group at these four sites. *Glycine canescens* F.J. Herm. (60) was within this group at Katanning and Newdegate, *G. tabacina* Benth. (61) and *Cullen tenax* J.W. Grimes(44) at Newdegate, *C. tenax* (45) at Binalong, *Cullen australasicum* (39) at Wagga Wagga and Binalong (not sown in spring at Katanning and Newdegate), and *Astragalus cicer* L. (15) at Binalong and *Desmanthus acuminatus* Benth. (46) at Katanning.

DISCUSSION

The 3-yr evaluation at six sites in southern Australia identified a set of 10 priority perennial forage legumes (*B. bituminosa* var. *albomarginata*; *C. australasicum*; *D. hirsutum*; *K. prostrata*; *L. bainesii*; *L. pedunculatus*; *L. cytisoides*; *M. sativa* subsp. *sativa*; *M. sativa* subsp. *Caerulea*, and *M. sativa* subsp. *falcata*) with suitable adaptation, performance, and persistence.

Six of the top 10 entries identified in the current study (*C. australasicum*, *D. hirsutum*, *L. cytisoides*, *L. pedunculatus*, *M. sativa* subsp. *sativa* and *M. sativa* subsp. *caerulea*) were also ranked in the top 10 entries by Li *et al*. (2008), based on their forage yield in the waterlogged soil and/or acid soil environments and in environments with less soil constraints. The good performance of *C. australasicum* at a number of sites in the current study (cluster A in both winter and spring sown experiments, Figures 2a, 2b) and those of Li *et al*. (2008) supports recent findings by Hayes *et al*. (2009) that found accessions of this species were the most promising of the four *Cullen* species they evaluated, with persistence in grazed swards equivalent to lucerne. The lower palatability of *C. australasicum* observed in some field studies (Hayes *et al*., 2009) suggests it could have a niche in lower rainfall regions where set stocking and large paddock sizes restrict the ability to implement rotational grazing. *Cullen australasicum* performed well in both winter and spring sown experiments in the current study suggesting it establishes readily in cooler conditions despite being most active in the warmer months.

The appearance of the two perennial *Medicago* subspecies *sativa* and *caerulea* in the top groups (cluster A and B) is supported by a recent study by Li *et al*. (2010b) of a diverse range of germplasm from the *M. sativa* complex in similar environments to the current study. They found that subspecies *sativa* was superior in terms of both persistence and productivity in less moisture stressed environments but unselected accessions of subspecies *caerulea* demonstrated persistence equivalent to the control lucerne cultivar Sceptre in drier environments (Li *et al*., 2010b). *Medicago sativa* subsp. *caerulea* is found in the drier regions of the natural distribution of the *M. sativa* complex (Small, 2003) and hence this subspecies is the most likely to yield highly drought tolerant germplasm and should be further exploited for semi arid Mediterranean environments. A third member of the *M. sativa* complex, *M. sativa* subsp. *falcata* also fell within the best performing species group in the current study. This species occupies the more northern range of the *M. sativa* complex in its natural environment and is regarded as very cold and drought hardy and more able to tolerate acid soils (Small, 2003). However the recent study by Li *et al*. (2010b) at three locations in eastern Australia found the *falcata* subspecies to be far less productive than subspecies *sativa*, *caerulea* and *varia*.

Lotononis bainseii was another species in the high priority cluster B group (Figure 2b) in the spring sown experiments. *Lotononis bainseii* had superior basal frequency scores at four sites reflecting the ability of this stoloniferous species to cover the ground surface. The use of this subtropical species has, to date, been restricted in southern Australia by unreliable establishment from seed and very specific seed bed and temperature requirements for emergence (Blumenthal and Hilder, 1989). Poor seedling establishment has also been a major factor in reducing adoption of the species in Uruguay (D. Real, 2008 personal communication). This species requires warm temperatures for growth (> 9 ºC) and hence its productivity relies on sufficient rainfall in late spring and early summer when temperatures are most suitable for its growth. Its requirement for warmer temperatures for growth is reflected by its superior DM scores during summer in the current study (cluster B, Figure 2b).

The *Lotus* genus had a number of species listed within the high priority cluster B group (Figure 2a), namely *L. corniculatus*, *L. pedunculatus* and *L. cytisoides*. Since this study commenced, significant progress has been reported in breeding new cultivars of *L. corniculatus* with greatly improved flowering and seed production at lower latitudes similar to the experimental sites of the current study (D. Real, 2007 personal communication).

The identification of *B. bituminosa* var. *albomarginata* (35) in the current study as a species of high potential (Cluster A, Figure 2a) demonstrates that it is still possible to identify new prospective species that offer valuable adaptive characteristics that can be exploited. Early studies conducted in the Canary Islands (Mendez *et al*., 2006) and in Italy (Pagnotta *et al*., 2003) also showed the promising potential of this species in Mediterranean environments. It demonstrates a high level of drought and grazing tolerance in its native environments in the Mediterranean basin and the Canary Islands (Gutman *et al*., 2000; Muñoz *et al*., 2000). It is reported to be tolerant of grazing by cattle (Sternberg *et al*., 2006) and goats (Ventura *et al*., 2000). There is substantial genetic variation within the species with *B. bituminosa* var. *albomarginata* found in coastal semi arid environments (average annual rainfall 150-300 mm) but other varieties, such as var. *crassiuscula*, are found in high elevation (1700-2200 m) sub humid 500 mm rainfall environments (Muñoz *et al*., 2000; Real *et al*., 2009). An important attribute of this species is that unlike lucerne it retains its leaves when moisture stressed, therefore providing valuable feed over summer (Real and Verbyla, 2010).

The remaining two leguminous species in the top group were *D. hirsutum* and *K. prostrata*. *Dorycnium hirsutum* originates in the Mediterranean region and is distributed from the Canary Islands to the Balkans (Bennett, 2003). High levels of condensed tannins and low reliability of establishment are two of the current limitations of *Dorycnium* species identified by Bell *et al*. (2008). Less is known about the Australian native perennial legume *K. prostrata*. Recent studies have found it to be more productive under low P conditions than lucerne (Pang *et al*., 2010), achieving maximum growth at 12 mg P kg^{-1} soil compared to an optimum of 24 mg P kg^{-1} for lucerne. This low P requirement most likely reflects its adaptation to the low P status of soils in its native environment and a root distribution that enhances P acquisition (Denton *et al*., 2006). The attractiveness of this species as a forage may be limited by the quality of its herbage. Analysis of herbage quality of the related species *K. prorepens* found high levels of condensed tannins (10-18 g kg^{-1} DM) and relatively low crude protein (12%) and *in vitro* digestibility (55%) values (Robertson *et al*., 2007). *Kennedia prostrata* was only evaluated at one site, Bealiba, and its potential cannot be properly assessed until its performance is determined at a greater range of sites and herbage quality is better documented.

Cichorium intybus was the only non leguminous species to fall within the priority groups (cluster B, Figure 2a) in the current study. This species has performed well in less stressful New Zealand environments (Li and Kemp, 2005) and in a number of recent studies in southern Australia (Li *et al*., 2008; Reed *et al*., 2008; Li *et al*., 2010a), but it is relatively short lived in drier environments. The productivity declined progressively in the current study. Its poor performance in drier environments is not unexpected given that no cultivars of this species have been developed specifically for low rainfall environments. Further selection to exploit the large genetic diversity within this species is warranted for increased persistence in lower rainfall environments.

For forage legumes to perform to their potential under field conditions, an effective symbiosis is essential to provide adequate biologically fixed N to the plant (Howieson *et al*., 2000). All leguminous species evaluated were inoculated with either commercial or experimental inoculants that were the best inoculant with the information available at the time of sowing. It is acknowledged that some non commercialized species may not have highly effective RNB available as until they demonstrate significant agronomic potential it is difficult to justify the intensive research required to identify superior inoculants. The lack of well adapted RNB for non domesticated species is always a restriction when evaluating wild or non commercial species.

A second group of 15 entries (*A. uniflorum*, *A. cicer*, *C. tenax*, *D. acuminatus*, *D. graecum*, *D. pentaphyllum*, *G. canescens*, *G. tabacina*, *H. boutignyanum*, *L. corniculatus*, *L. tenuis*, *M. suffruticosa*, *T. ambiguum*, *T. physodes*, and *T. tumens*) was identified for their good persistence across sites or at particular sites. Although this group may lack productivity, their persistence, often under severely drought stressed growing conditions, warrants further selection to identify more productive genotypes as the majority of these have undergone little agronomic selection.

For any of these selected species to be successful on a commercial scale there are several other characteristics that need to be considered in their development. As a minimum they would be required to i) have a broad soil and climatic adaptation to help promote a large seed market; ii) have a reliable seed establishment and the necessary seedling vigour; iii) have high seed yields and can be cost effectively harvested with a final seed cost that can compete favourably with alternative existing forage options; iv) have good grazing tolerance; v) have good seasonal forage quality especially outside the annual growing season when it is most valuable to support an animal industry; vi) have no adverse effect on animal health or risks (such as bloat) and be managed effectively with grazing. Some of the more promising species identified are being studied further by the Future Farm Industries Cooperative Research Centre (previously Salinity CRC) as new forage perennial pasture options for farmers in Mediterranean climatic zones.

CONCLUSIONS

The evaluation of a broad range of species supported the hypothesis that there are herbaceous perennials not currently utilised in Australia that are sufficiently persistent and productive under Australian field conditions to warrant further development for use in Mediterranean-like climate agriculture. The promising species identified based on herbage production were *B. bituminosa* var. *albomarginata*, *C. australasicum*, *D. hirsutum*, *K. prostrate*, *L. bainesii*, *L. pedunculatus*, *L. cytisoides*, *M. sativa* subsp. *sativa*, *M. sativa* subsp. *caerula*, and *M. sativa* subsp. *falcata*. These species require further study to exploit their potential development as new forage perennial pasture options.

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Evaluación de leguminosas y hierbas forrajeras perennes en seis medioambientes mediterráneos. Existe una escasez de leguminosas y hierbas perennes herbáceas además de alfalfa (*Medicago sativa* L.) tolerantes a sequía para ambientes con clima mediterráneo como los que se encuentran en el Sur de Australia, el Mediterráneo y Chile. Por lo tanto, una colección de 174 leguminosas perennes y hierbas correspondientes a 103 especies y 32 géneros fue evaluada por su adaptación a un diverso rango de climas mediterráneos en el Sur de Australia. La distribución de las precipitaciones en los sitios experimentales varían desde moderada a altamente invernales, con un promedio de precipitaciones anuales de 318 mm a 655 mm. Las accesiones fueron evaluadas por su productividad y persistencia en un período de 3 años. Las 12 accesiones identificadas como las más promisorias por su producción invernal, estival o anual fueron *Bituminaria bituminosa* (L.) C.H. Stirt. var. *albomarginata*; *Cichorium intybus* L.; *Cullen australasicum* (Schltdl.) J.W. Grimes; *Dorycnium hirsutum* (L.) Ser.; *Kennedia prostrata* R. Br.; *Lotononis bainesii* Baker, *Lotus pedunculatus* Cav.; *L. corniculatus* L.; *L. cytisoides* L.; *Medicago sativa* subsp. *sativa* L.; *Medicago sativa* subsp. *caerulea* (Less. ex Ledeb.) Schmalh., y *M. sativa* subsp. *falcata* (L.) Arcang. Estas accesiones mantuvieron la productividad y persistencia durante el período de evaluación, con la excepción de *C. intybus* y *L. corniculatus* que no persistieron. El rol potencial de estas especies en sistemas de producción extensivos en zonas de clima mediterráneo, sus ventajas y desventajas y el estado actual de desarrollo de las mismas es presentado en este trabajo.

Palabras clave: *Bituminaria*, *Lotus*, rendimiento de forraje, persistencia de leguminosas, germoplasma nativo australiano.

Appendix 1. Perennial legumes evaluated at Wagga Wagga (1) and Binalong (2), New South Wales; Katanning (3) and Newdegate (4), Western Australia and Byawatha (5) and Bealiba (6), Victoria from 2005 to 2008. Entry was sown in winter (W) or spring (S) and inoculated with appropriate root nodule bacteria (RNB).

Code	Genera/Species	Entry	1	2	3	4	5	6	Time of sowing	RNB
1	*Adesmia* sp1	SA33599	1	1	1	1	1		W	Mix 7
2	*A. mucronata* Hook. & Arn.	SA33602	1	1			1		W	Mix 7
3	*A. muricata* (Jacq.) DC	SA22024	1	1	1	1	1		W	Mix 7
4	*A. punctata* (Poir.) DC	SA33605	1	1	1	1	1		W	Mix 7
5	*Adesmia* sp2	SA33593	1	1			1		W	Mix 7
6	*Aeschynomene falcata* (Poir.) DC	ATF2196	1	1	1	1	1		S	Mix 7
7	*Anthyllis vulneraria* L.	SA35244	1	1	1	1	1		W	Mix 7
8	*A. vulneraria* L.	SA36132			1	1			W	Mix 7
9	*Argyrolobium uniflorum* Jaub. & Spach.	WA123410	1	1	1	1	1		W	Mix 7
10	*A. uniflorum* Jaub. & Spach.	WA12345			1	1			W	Mix 7
11	*Astragalus adsurgens* Pall.	SA17720	1	1			1		S	Mix 1
12	*A. aleppicus* Boiss.	SA33904	1	1			1		S	Mix 1
13	*A. atropilosulus* Bunge	SA32263	1	1			1		W	Mix 1
14	*A. cicer* L.	SA38090			1	1			W	Mix 1
15	*A. cicer* L.	SA38091	1	1	1	1	1		W + S	Mix 1
16	*A. crotalariae* A. Gray	SA33691	1	1			1		S	Mix 1
17	*A. galegiformis* L.	SA33820	1	1			1	1	W + S	Mix 1
18	*A. glycyphyllos* L.	SA38093	1	1	1	1	1		W + S	Mix 1
19	*A. nothoxys* A. Gray	SA33698	1	1			1		W	Mix 1
20	*A. onobrychis* L.	SA12667	1	1			1		W	Mix 1
21	*A. palaestinus* Eig.	SA30484	1	1			1		W	Mix 1
22	*A. refractus* Boiss. & Buhse	SA34026	1	1			1	1	W + S	Mix 1
23	*A. siliquosus* Boiss.	SA34038	1	1	1	1	1		W	Mix 1
24	*A. siliquosus* Boiss.	SA34039			1	1			W	Mix 1
25	*A. sinicus* L.	SA33929	1	1			1	1	W + S	Mix 1
26	*Astragalus* sp11	Composite1	1	1			1		S	Mix 1
27	*Astragalus* sp9	Composite2	1	1			1	1	W + S	Mix 1
28	*A. stipulosus* Boriss.	SA34045	1	1			1		W	Mix 1
29	*A. suberosus* Banks & Sol.	Composite3	1	1			1		S	Mix 1
30	*A. suberosus* Banks & Sol.	SA16094			1	1			W	Mix 1
31	*A. suberosus* Banks & Sol.	SA16095			1	1			W	Mix 1
32	*A. thurberi* A. Gray	SA33700	1	1			1		W	Mix 1
33	*A. wootonii* E. Sheld	SA33701	1	1			1		W	Mix 1
34	*Bituminaria bituminosa* (L.) C.H. Stirt	SA36033	1	1			1		W	CB 2080
35	*B. bituminosa* var. *albomarginata* C.H. Stirt	PNF22A15						1	W	CB 2080
36	*B. bituminosa* var. *albomarginata* C.H. Stirt	PNF33A2						1	W	CB 2080
37	*Cichorium intybus*[1] L.	Puna						1	W	NA
38	*Coronilla grandiflora* Willd.	SA33705	1	1			1		W	CC1107/CC401
39	*Cullen australasicum* (Schltdl.) J.W. Grimes	SA4966	1	1	1	1	1		W + S	Mix 2
40	*C. australasicum* (Schltdl.) J.W. Grimes	SA42965						1	W	Mix 2
41	*C. parvum* J.W. Grimes	SA40356						1	W	Mix 2
42	*C. patens* J.W. Grimes	CB02CS						1	W	Mix 2
43	*C. patens* J.W. Grimes	SA42565						1	W	Mix 2
44	*C. tenax* J.W. Grimes	NIND008	1	1	1	1	1	1	W + S	Mix 2

Continuation Appendix 1.

Code	Genera/Species	Entry	1	2	3	4	5	6	Time of sowing	RNB
45	*C. tenax* J.W. Grimes	SA35778	1	1	1	1	1	1	W + S	Mix 2
46	*Desmanthus acuminatus* Benth.	78383	1	1	1	1	1	1	W + S	CB3126
47	*D. virgatus* Willd.	85177	1	1	1	1	1	1	W + S	CB3126
48	*D. graecum* Ser.	SA35667			1	1			W	CC856
49	*D. graecum* Ser.	SA36069	1	1	1	1	1	1	W	CC856
50	*D. hirsutum* (L.) Ser.	9314			1	1			W	CC856
51	*D. hirsutum* (L.) Ser.	AL3343	1	1	1	1	1	1	W	CC856
52	*D. hirsutum* (L.) Ser.	AL4283			1	1			W	CC856
53	*D. hirsutum* (L.) Ser.	AL4598			1	1			W	CC856
54	*D. hirsutum* (L.) Ser.	SA36131			1	1			W	CC856
55	*D. pentaphyllum* Scop.	SA33723			1	1			W	CC856
56	*D. pentaphyllum* Scop.	SA33721	1	1	1	1	1	1	W	CC856
57	*D. pentaphyllum* Scop.	Tas1273			1	1			W	CC856
58	*Galega officinalis* L.	PI251825	1	1	1	1	1	1	W	WSM2699
59	*G. officinalis* L.	VIR28855	1	1	1	1	1		W	WSM2699
60	*Glycine canescens* F.J. Herm.	Composite4	1	1	1	1	1		S	CC1604/13
61	*G. tabacina* Benth.	NIND004	1	1	1	1	1		S	CC1604/13
62	*Glycyrrhiza ancanthocarpa* J.M. Black	SA9536	1	1	1	1	1		S	Mix 7
63	*Hedysarum boutignyanum* Alleiz.	SA13265	1	1	1	1	1		W	WSM1592
64	*Kennedia prorepens* F. Muell.	KIMS001	1	1	1	1	1		W	Mix 7
65	*K. prostrata* R.Br.	HR001						1	W	Mix 7
66	*Lespedeza juncea* Pers.	AL2180			1	1			W	CC497/8
67	*L. juncea* Pers.	AL489			1	1			W	CC497/8
68	*L. juncea* Pers.	AUGRAZIER			1	1			W	CC497/8
69	*L. juncea* Pers.	AULOTAN	1	1	1	1	1	1	W	CC497/8
70	*L. juncea* Pers.	INTERSTATE			1	1			W	CC497/8
71	*L. juncea* Pers.	SA22026						1	W	CC497/8
72	*Lotononis bainesii* Baker	B63101	1	1	1	1	1	1	W + S	CB376
73	*L. bainesii* Baker	B662S1			1	1			S	CB376
74	*L. bainesii* Baker	CPI49182			1	1			S	CB376
75	*L. bainesii* Baker	Miles			1	1			S	CB376
76	*L. bainesii* Baker	PI224983			1	1			S	CB376
77	*Lotus aegaeus* Boiss.	SA37124	1	1			1	1	W	Mix 3
78	*Lotus australis* Andrews	SA17132	1	1	1	1	1		W	Mix 3
79	*L. australis* Andrews	SA33610			1	1		1	W	Mix 3
80	*L. collinus* Heldr.	SA34060	1	1	1	1	1	1	W	Mix 3
81	*L. pedunculatus* Cav.	SA12952			1	1			W	Mix 3
82	*L. corniculatus* L.	SA32863	1	1	1	1	1		W	Mix 3
83	*L. corniculatus* L.	SA25270	1	1	1	1	1		W	Mix 3
84	*L. corniculatus* L.	Goldie						1	W	Mix 3
85	*L. corniculatus* L.	San Gabriel						1	W	Mix 3
86	*L. creticus* L.	SA2317			1	1			W	Mix 3
87	*L. creticus* L.	SA37654	1	1	1	1	1	1	W	Mix 3
88	*L. cytisoides* L.	SA12951	1	1	1	1	1	1	W	Mix 3
89	*L. cytisoides* L.	SA27513			1	1			W	Mix 3
90	*L. discolor* E. Mey.	SA38069	1	1			1		W	Mix 3
91	*L. gebelia* Vent.	SA13750	1	1	1	1	1		W	Mix 3
92	*L. corniculatus* L.	SA833			1	1			W	Mix 3
93	*L. tenuis* Willd.	SA25285	1	1	1	1	1		W	Mix 3
94	*L. krylovii* Schischkin & Serg.	SA37754	1	1			1		W	Mix 3
95	*L. palustris* Vell.	SA37668	1	1			1	1	W	Mix 3
96	*L. pedunculatus* Cav.	SA37149	1	1	1	1	1		W	Mix 3
97	*L. pedunculatus* Cav.	SA 26851	1	1	1	1	1		W	Mix 3
98	*L. pedunculatus* Cav.	Maku						1	W	Mix 3
99	*L. pedunculatus* Cav.	Sharnae						1	W	Mix 3
100	*L. preslii* Ten.	SA 37962	1	1			1	1	W	Mix 3
101	*Lotus* sp1	SA 34094	1	1			1		W	Mix 3
102	*Lotus* sp2	SA38011	1	1			1		W	Mix 3
103	*Medicago lupulina* L.	SA38557	1	1	1	1	1		W	Mix 4
104	*M. marina* L.	SA2525	1	1			1		W	Mix 4
105	*M. papillosa* Boiss.	SA32531	1	1			1		W	Mix 4
106	*M. sativa* subsp. *sativa* L.	Sceptre	1	1	1	1	1	1	W	Mix 4
107	*M. sativa* subsp. *sativa* L.	SA38082			1	1			W	Mix 4
108	*M. sativa* subsp. *sativa* L.	SA41650			1	1			W	Mix 4
109	*M. sativa* subsp. *varia* Martyn	Cancreep						1	W	Mix 4
110	*M. sativa* subsp. *caerulea* (Less. ex Ledeb.) Schmalh.	Jindera						1	W	Mix 4
111	*M. sativa* subsp. *sativa* L.	SA43484						1	W	Mix 4
112	*M. sativa* subsp. *sativa* L.	SA43485						1	W	Mix 4
113	*M. sativa* subsp. *sativa* L.	SA43486						1	W	Mix 4
114	*M. sativa* subsp. *sativa* L.	SA43492						1	W	Mix 4
115	*M. sativa* subsp. *sativa* L.	SA43493						1	W	Mix 4
116	*M. sativa* subsp. *sativa* L.	SA43497						1	W	Mix 4
117	*M. sativa* subsp. *sativa* L.	SA44979						1	W	Mix 4
118	*M. sativa* subsp. *sativa* L.	SARDI10						1	W	Mix 4
119	*M. sativa* subsp. *sativa* L.	Zanjani						1	W	Mix 4

Continuation Appendix 1.

Code	Genera/Species	Entry	1	2	3	4	5	6	Time of sowing	RNB
120	*M. sativa* subsp. *caerulea* Ledeb.	SA36317	1	1	1	1	1		W	Mix 4
121	*M. sativa* subsp. *caerulea* Ledeb.	SA38052			1	1			W	Mix 4
122	*M. sativa* subsp. *falcata* (L.) Arcang.	SA32090			1	1			W	Mix 4
123	*M. sativa* subsp. *falcata* (L.) Arcang	SA32091	1	1	1	1	1		W	Mix 4
124	*M. suffruticosa* DC	SA6529	1	1	1	1	1		W	Mix 4
125	*Melilotus polonicus* Pall.	SA36977	1	1			1		W	Mix 4
126	*Onobrychis arenaria* DC	SA34674	1	1					W	Mix 5
127	*O. arenaria* DC	SA34700					1		W	Mix 5
128	*O. armena* Boiss & A. Huet	SA32454	1	1					W	Mix 5
129	*O. armena* Boiss & A. Huet	SA32455					1		W	Mix 5
130	*O. inermis* Steven	Composite5	1	1					W	Mix 5
131	*O. tanaitica* Spreng.	SA38891	1	1	1	1			W	Mix 5
132	*O. tanaitica* Spreng.	SA38956					1		W	Mix 5
133	*O. tanaitica* Spreng.	PA39						1	W	Mix 5
134	*O. viciifolia* Scop.	SA33874	1	1	1	1	1		W	Mix 5
135	*O. viciifolia* Scop.	Othello						1	W	Mix 5
136	*Ononis intermedia* sensu Auct.	SA38737	1				1		W	Mix 7
137	*Ptilotus polystachyus*[1] F. Muell.	Merredin		1					W	NA
138	*Sanguisorba minor*[1] Scop.	8969	1	1	1	1	1		W	NA
139	*S. minor*[1] Scop.	SA25861	1	1	1	1	1		W	NA
140	*S. minor*[1] Scop.	SA32463						1	W	NA
141	*Securigera varia* Lassen	Tas1322	1	1					W	Mix 7
142	*S. securidaca* Degen & Dorfl.	Composite6	1	1					W	Mix 7
143	*Stylosanthes guianensis* Sw.	11493	1	1	1	1	1		S	Mix 7
144	*S. mexicana* Taub.	87484	1	1	1	1	1		S	Mix 7
145	*S. scabra* Vogel	ATF3076	1	1	1	1	1		S	Mix 7
146	*S. scabra* Vogel	ATF3077			1	1			S	Mix 7
147	*Swainsona beasleyana* F. Muell	KIMS004					1	1	W + S	Mix 7
148	*S. canescens* F. Muell	KIMS003	1	1	1	1	1		S	Mix 7
149	*S. colutoides* F. Muell	NIND006	1	1	1	1	1		S	Mix 7
150	*S.a colutoides* F. Muell	SA41398						1	W	Mix 7
151	*S. cyclocarpa* F. Muell	KIMS002					1		S	Mix 7
152	*S. paradoxa* W. Fitzg.	KIMS002	1	1	1	1			S	Mix 7
153	*S. purpurea* Joy Thomps.	KIMS004	1	1	1	1			S	Mix 7
154	*Swainsona* sp.	NF023	1	1	1	1	1		S	Mix 7
155	*S. swainsoides* J.M. Black	NIND005	1	1			1	1	W + S	Mix 7
156	*Tephrosia grandiflora* Pers.	930029	1	1	1	1	1		W	Mix 7
157	*Tetragonolobus maritimus* Roth.	SA32512	1	1			1		W	Mix 7
158	*Trifolium ambiguum* M. Bieb.	Tas38904	1	1					W	Mix 6
159	*T. burchellianum* Ser.	AZ4520	1	1	1	1	1		W	Mix 6
160	*T. fragiferum* L.	Palestine						1	W	Mix 6
161	*T. fragiferum* L.	SA38076						1	W	Mix 6
162	*T. hybridum* L.	AB302	1	1	1	1	1		W	Mix 6
163	*T. hybridum* L.	SA33368			1	1			W	Mix 6
164	*T. ochroleucum* Huds.	AZ4257			1	1			W	Mix 6
165	*T. ochroleucum* Huds.	Composite7	1	1	1	1	1		W	Mix 6
166	*T. ochroleucum* Huds.	Tas433J502	1	1					W	Mix 6
167	*T. physodes* M. Bieb.	AZ4336	1	1	1	1	1		W	Mix 6
168	*T. physodes* M. Bieb.	124667			1	1			W	Mix 6
169	*T. physodes* M. Bieb.	124669			1	1			W	Mix 6
170	*T. pratense* L.	Tas1732	1	1					W	Mix 6
171	*T. tumens* M. Bieb.	AZ3048			1	1			W	Mix 6
172	*T. tumens* M. Bieb.	SA16758	1	1	1	1	1		W	Mix 6
173	*T. tumens* M. Bieb.	Tas2568	1	1					W	Mix 6
174	*Vicia cracca* L.	SA38058	1	1			1		W	SU303
	Total number of entries	174	102	102	95	95	94	53		

[1]Non-leguminous species.
Mix 1: NA1001; SRDI229; SRDI267; WSM1333; WSM1344; WSM1816; and WSM2193.
Mix 2: SRDI1482; SRDI 1483; SRDI1504; SRDI1507; SRDI1508; and SRDI1520.
Mix 3: CC829; CC856; SRDI110; SU343; and WSM1293.
Mix 4: M49; RRI128; SRDI291; and WSM1115.
Mix 5: CC1099; CC1107; and CC1108.
Mix 6: CC2483; TA1; and WSM409.
Mix 7: mix of all inoculants

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