Between genotype variation of lucerne (*Medicago* spp.) in grazing preference by sheep

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Abstract
Lucerne (*Medicago sativa* L.) is a highly desirable forage species in temperate regions of the world. With the many lucerne cultivars currently available and/or emerging onto the Australian market, producers may find it difficult to determine which cultivar(s) will best suit their needs. An understanding of how these cultivars are selected and grazed by sheep may be useful for producers to make management decisions. It was hypothesised that there will be substantial differences in sheep grazing preference among diverse cultivars. Grazing preference was evaluated on forty-seven entries (commercial cultivars and pre-release lines) at Hamilton, Victoria, in summer, winter and spring of 2017. The plots were rated using a seven-point scoring system (1 = not grazed to 7 = completely grazed) over a 27 h grazing period. The average grazing scores indicated that, with minor qualifications, all lucerne entries evaluated in this study were palatable to sheep.

Key Words
lucerne, grazing, cultivars, Chinese, preference

Introduction
Lucerne (*Medicago sativa* L.) is a highly desirable component of pastures in temperate regions of the world due to its capacity to produce large herbage yields of high nutritional quality (including over summer), an ability to fix nitrogen and a deep root system that provides a high level of drought tolerance (Bittman and McCartney 1994; Irwin *et al.* 2001; Bouton 2012). With the different lucerne cultivars that are currently available or under development in the Australian market, producers may find it difficult to determine which cultivar(s) will best suit their needs in terms of persistence, yield, ground cover and palatability. Measurements such as morphology, chemical analysis or mechanical harvests cannot always predict the palatability of a forage and subsequently the preference of grazing animals for that forage (Baumont 1996; Cougnon *et al.* 2018). In this study, sheep were used to evaluate variation in herbivore preference of lucerne cultivars and pre-release lines. It was hypothesised that there would be differences in sheep grazing preference among lucerne entries.

Methods
Site description
The site consisted of 1 m x 0.6 m plots in 16 rows running down a slope and 12 columns across the slope, at Hamilton in south west Victoria (S37.834, E142.086). Four contiguous rows were assigned to each of four blocks. Thirty-three cultivars and fourteen experimental lines were evaluated, with forty-five entries being represented once in each block, one entry being evaluated in 3 blocks (due to limited seed availability) and the remaining plots (9 in all) being assigned to a standard commercial cultivar (SARDI7). Lucerne entries were randomised to plots within blocks, and between blocks. Soil at the site was a ferric□sodic eutrophic brown chromosol, with pH_{Ca} 5.0 and Olsen P (mg/kg) 20. Lucerne entries ranged in winter dormancy score from 2 to 11. Habits ranged from prostrate to erect. Seven entries evaluated were *M. sativa* subsp. *falcata*, and all other entries were *M. sativa* subsp. *sativa*. Each plot was hand sown, such that each plot consisted of 5 x 1 m drill rows, 15 cm apart. The site was sown on 8 and 9 October 2015. The plots were harvested to 5 cm every six weeks using a mower and, apart for the grazing preference evaluations, were not grazed.

Grazing preference
Grazing preference screening was undertaken in summer (February 2017), winter (June 2017) and spring (November 2017). All animal procedures were approved by DJPR Agriculture Research and Extension Animal Ethics Committee (AEC Approval: 2016-07) and conducted in accordance with Australian code for the care and use of animals for scientific purposes (Anon. 2013). The experimental site was grazed over 2

days by 10 composite ewes (mixed-age) that had previous exposure to grazing lucerne. The grazing preference methodology followed that of Johnston (1988) and Mitchell *et al.* (2001). The sheep entered the plot area at 10 am, the plots were then rated after the sheep had been on the plots for 3 hours after entry (1 pm), then again after 6 hours (4 pm), 24 hours (10 am) and 27 hours (1 pm). The plots were rated using the following scoring system:

1. not grazed;
2. slight grazing of leaves and soft stems (exploratory grazing);
3. light grazing of leaves and soft stems (plot grazed evenly but fair bulk remains);
4. moderate grazing of leaves and soft stems (grazed evenly, but with less bulk than light grazing);
5. moderate grazing of leaves, soft stems and some hard stems;
6. heavy grazing of leaves, soft stems and hard stems (very little remains);
7. completely grazed.

The sheep were removed from the plot area at the end of the 27-hour rating and all plots were slashed to a height of 5 cm to remove residual dry matter.

Statistical analysis

For each plot, on each occasion of grazing assessment, an average grazing assessment score was calculated for 24 h and 27 h grazing. The average grazing scores were modelled using a residual maximum likelihood (REML) mixed model. Model terms examined included random entry effects, characteristics related to the source of each entry, and experimental site effects (block, row, column, autoregressive correlation structure between plots etc.). Individual entries that did not reasonably fit into the general genetic variation of other entries were modelled as an indicator (yes/no) factor for that entry. Fixed effects were tested for inclusion/exclusion in the final model using Wald F tests and, other than the main effect of entry, random effects and plot error terms (e.g. row and column auto-correlation) were tested using χ^2 change in deviance tests. The random effect of entry was tested using a 'one-sided' χ^2 change in deviance tests. In this test, the χ^2 value was multiplied by minus one if the estimated between entry variance was negative. The asymptotic χ^2 approximation can still be used under the null hypothesis ($(\sigma_{Cultivar})^2 = 0$), but with critical points appropriately adjusted. Fixed block effects were *a priori* included in each model, to reflect the randomisation of entries to plots within blocks. A random entry effect was included in the model whenever the estimated between-entry variance was positive even without reasonable statistical significance, but not included in the final model if the best estimate of between-entry variance was 'negative'. Statistical analyses were carried out using GenStat 18 (VSN International 2017).

At each assessment the final model included a fixed block effect and an autoregressive plot correlation structure, with extra measurement (nugget) error in November. Including the plot correlation structure allowed greater precision in the estimation of the amount of genetic variation between entries (Table 1). In addition, the June assessment model included a fixed effect for country of origin, the interaction of block and country of origin and an indicator of whether the entry was KI Creepa. KI Creepa has a prostrate growth habit. Random between-entry variation was included in the model for the February assessment (although the between-entry variance was not statistically significant (P =0.46) the variance was estimated to be positive) and the November assessment (the between-entry variance was statistically significant (P =0.03) and the variance was estimated to be positive), but not the June assessment (the between-entry variance was not statistically significant (P =0.99) and the variance was estimated to be 'negative').

Table 1. Terms included in final models for average grazing scores. The × symbol indicates the term is included in model.

	February	June	November
Fixed effects			
Block[a]	×	×	×
Country of origin (Australia vs China)	-	×	-
Block by country of origin interaction	-	×	-
Indicator of KI Creepa (KI Creepa vs all other entries)	-	×	-
Random Entry effect[b]	×	-	×
Plot error terms			
Autoregressive row effect within columns	×	-	×
Autoregressive column effect within rows	×	×	×
Measurement (nugget) effect	-	-	×

[a] Block effects are *a priori* included in model, in accordance with design constraint.

[b] A cultivar effect is included in model, provided that the estimate of cultivar variance is greater than 0.

Results and Discussion

Averaging over the four blocks at the June assessment, Chinese origin cultivars had lower average grazing scores than Australian origin cultivars (Figure 1). However, the difference varied down the slope of the experimental site, with greater disparity in the block at the highest elevation (1.2 units of grazing score; se = 0.33) and negligible difference in the block at the lowest elevation (-0.4 units of grazing score; se = 0.34).

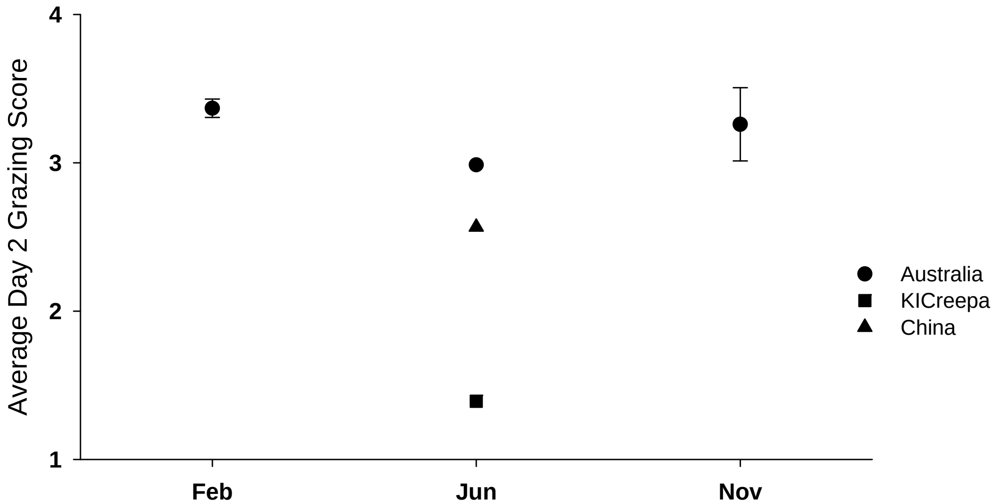

Figure 1. The between entry variation in average grazing scores for lucerne entries originating from either Australia or China. The 'error bars' represent the estimated 95% range in genetic variation between entries from the same region (i.e. 95% of cultivars will be between the top and bottom end of the error bars). Where there is no error bar it is estimated that there is no between entry variation. No separate values for China or KI Creepa, at an assessment (February and November), indicates that the best model has no systematic difference between country of origin and that KI Creepa is within the general variation observed between entries. The values for Australia and China, at the June assessment, are an average effect over the four blocks.

The systematic difference between Chinese and Australian cultivars was not observed at the February or November assessments. KI Creepa was minimally grazed at the June assessment (score of 1.4) but was not systematically different to other cultivars at the February or November assessments (Figure 1). The parent material of KI Creepa was collected from surviving plants from a 40-year-old stand of cv. Cancreep on King Island (Nichols *et al.* 2012). The trial contained 15 other entries that were also scored as the same habit class, with three of these the same subspecies as KI Creepa (*M. sativa* subsp. *falcata*). The reason for the difference in grazing preference for KI Creepa is unknown.

There was no evidence of a systematic difference between source of lucerne entry within a country (including the SARDI experimental accession source), or the subspecies of lucerne (*M. sativa* subsp. *falcata* vs *M. sativa* subsp. *sativa*). The two main subspecies (*sativa and falcata)*, have very different growth characteristics, having originated from the Middle East and Eurasia respectively (Julier *et al.* 1995). The sub species *sativa* is characterised by an erect growth habit, purple flowers, a tap root, poor winter resilience and low dormancy while the sub species *falcata* is characterised by a more prostrate growth habit, yellow flowers, good winter resilience and winter dormancy (Julier *et al.* 1995). These differences did not appear to impact on the grazing preference of the sheep.

Apart from KI Creepa, the genetic variation observed at the June assessment between entries in average grazing scores within the same country of origin is small or not apparent (

Table 2, Figure 1). There was evidence of some genetic variation between entries at the November assessment ($P = 0.03$), but even in this case the estimated 95% genetic range in average grazing scores is only about 0.5 ($\approx 4 \times 0.13$, from

Table 2) scoring units.

Table 2. Estimates of entry variance and entry standard deviation, average grazing scores, for entries sourced from the same country (estimates exclude KI Creepa at June assessment). A negative estimate of entries variance is presented for June, that is obtained from an analysis that adds an unconstrained (i.e. allows negative and positive variance) entry term to the final model. The term can then be meaningfully assessed with respect to its standard error.

Assessment	Estimate of entry variance (σ_C^2)	Standard error of σ_C^2	Entry standard deviation used in graphs	Standard error of entry standard deviation
February	0.001	0.0085	0.03	0.134
June	-0.07	0.015	0	-
December	0.016	0.0111	0.13	0.044

Conclusion

Our hypothesis that there would be substantial differences in sheep grazing preference among the lucerne entries was rejected on the evidence from this small plot trial. We found sheep did not differentiate between lucerne entries in this study. Although the entries varied in some traits, such as seasonality of growth and plant habit, limited differences in grazing preference were observed. The small differences in palatability between lucerne entries might indicate that a mixture of lucerne lines with different growth habits (some prostrate and some upright) could be sown together to address the issue of bare ground within lucerne stands.

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