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Using morphological traits to identify persistent lucernes for dryland agriculture in NSW, Australia

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Abstract. This paper reports on several studies conducted to better understand the variability between lucerne cultivars and lines, and use this to predict persistence in dryland grazing pastures in eastern Australia. Morphological traits of 20 cultivars/lines were measured in irrigated and dryland spaced plant experiments. Studies were also conducted to describe variation among lucernes in their utilisation of starch and responses to water deficit, pests and diseases. Multiple regression analyses were used to develop simple models where the measured traits could be used to predict persistence of lucerne lines in dryland evaluation experiments.

Although there was significant variation among cultivars/lines in most measured traits, no single trait reliably predicted persistence of cultivars/lines in dryland evaluation experiments. However, variation in persistence at both sites could be explained by models developed by multiple regression using differences in the mean lengths of the longest stems at 10% flower in summer and winter. Persistent lucernes were those that had relatively long stems in summer and short stems in winter. Water use efficiencies, starch utilisation patterns and resistances to pests and diseases of different lucernes provided some improvement to this simple model, but these improvements were not consistent.

Additional keywords: morphological traits, multivariate analysis, NIR, persistence, starch, WUE.

Introduction

Lucerne (*Medicago sativa* L.) is a popular and well adapted forage legume in Australia with significant scope for further expansion (Irwin *et al*. 2001). The main use for lucerne in Australia is in dryland pastures where it can provide major benefits to productivity, profitability and environmental sustainability. Previously, most commercial varieties in Australia have been bred for irrigated haymaking and have shown relatively poor persistence in dryland environments (Williams 1999). This poor persistence of newer cultivars relative to the adapted Australian cultivar Hunter River has limited the rate and degree of lucerne adoption throughout the dryland agricultural zone.

Breeding lucerne to persist in pastures is limited by a relatively poor understanding of the dryland grazing environment and its effects on plant survival. Little is also known of the variation between lucerne varieties in response to these environmental limitations. Furthermore, where certain plant traits are known to influence survival, such as resistances to pests and diseases, little information exists regarding the relative importance of these traits to lucerne persistence. With a lack of such basic information, evaluation and selection for persistence in lucerne is only possible using a slow and costly process of long-term field experiments repeated across several locations.

Persistence *per se*, like 'yield', is a complex character (Pearson and Elling 1961) involving the action and interaction of many component processes, traits, and therefore genes. Donald (1968) argued that by identifying and specifying more simply-inherited component traits in an 'ideotype' framework, breeding towards enhanced persistence could be markedly improved. This approach would increase both the rate and degree of advance in persistence of lucerne in pastures.

This study sought to examine the variation in morphology of lucerne under a range of environmental conditions, and to use this to identify key traits associated with persistence. Successfully identifying such traits would benefit both the breeding and management of superior lucernes for dryland pastures.

Materials and methods

Twelve lucerne commercial cultivars and eight breeding lines from NSW Department of Primary Industries' Lucerne Improvement Program were selected for use in this study on the basis of previously observed differences in winter activity, morphology, pest and disease resistance, productivity and persistence. It was expected that these cultivar/lines would cover the normal range in variability for most traits to be evaluated.

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Lucerne morphological traits

Morphological traits were measured on 20 lucerne cultivar/lines sown in field experiments at Leeton (34.55°S, 146.4°E) and Yanco (34.6°S, 146.4°E) in southern NSW, Australia. An irrigated field experiment was sown spring 1991 at the Leeton Field Station and an additional dryland experiment at Yanco Agricultural Institute during spring 1992. Experiments were arranged using a row-column design with four replicates (Williams 1986). Each plot consisted of 50 spaced plants sown on a 0.30 × 0.30 m grid, in a 5 × 10 plant configuration. The Leeton experiment was flood irrigated every 2–3 weeks (75–100 mm evaporation). The Yanco experiment was irrigated with sprinklers during the first 3 months of establishment.

Data were collected on a range of traits for 12 months following a 3-month establishment period. Plant traits included length of longest stem every 1–2 weeks after cutting until plants of half of the lines reached 10% flower in each season, plant erectness/decumbency (height of the plants in the plot divided by the length of longest stem), herbage dry weight (DW) per plant after each regrowth period (dried at 80°C for 3 days then weighed), leaf–stem DW ratios in autumn and spring (samples dried at 80°C for 3 days, then leaves and stems separated by rolling the plant over a 0.01-m sieve), and crown area [calculated as the area of an elipse using crown length and width (Boschma *et al*. 2003)] in autumn and spring. All measurements were recorded on the inner 24 plants of each plot. In all, there were 24 variables (multiple assessments of above traits) measured over four seasons on a total of 1920 plants in each morphology experiment.

Starch partitioning during regrowth

Greenhouse

Single plants of cultivars CUF101, Aurora and P545 were established in 3 L pots filled with standard commercial potting mix. Pots were watered regularly, fertilised with Aquasol (Hortico Aquasol soluble fertiliser, Yates, Padstow, Australia) and arranged in a row-column design with three replicates. Plants were grown for 2 months before the start of the experiment when all plants were cut to 0.05 m above ground level. Six pots of each cultivar were then progressively sampled 7, 17, 21, 28, 35, 42 and 49 days after cutting. Plant tops were removed, and crowns and roots washed, heat treated for 1–2 min in a 650W domestic microwave to prevent enzymatic degradation of starch (Batten *et al*. 1990), then dried at 50°C for 48 h. The dried roots were ground to 0.5 mm using a cyclone mill (Tecator 1093, Hoganas, Sweden). A subset of 40 samples were analysed for starch concentration using a modified enzymic method. This was based on hydrolysis with heat stable α-amylase and amyloglucosides followed by colourimetric determination of glucose using glucose oxidase (Aman and Hesselman 1984; Blakeney and Matheson 1984; Henry *et al*. 1990). These data were used to construct and validate a calibration curve on a Near Infrared Reflectance Spectrophotometer [NIRS; NIRSystem 6500 (Foss NIRSystems, Inc., Silver Spring, MD, USA); $R^2 = 0.97$, standard error of performance (%) = 1.83].

Field

Seed of all 20 cultivar/lines were hand-broadcast at 5 kg/ha in three replicate plots (1 × 1 m) arranged in a row-column design in the field at Yanco during October 1993. After 3 months establishment, all shoot growth was removed by mowing to 0.05 m from ground level and plants allowed to regrow. Five plants were subsequently removed from each plot after 0, 13, 20, 27, 33 and 42 days regrowth. Samples of crown and root material were trimmed to 0.10 m in length, washed, heat treated in a microwave, then oven-dried as described above. Ground samples were weighed and analysed for starch concentration using NIRS.

Response to water deficit

Single plants of all 20 cultivar/lines were grown in a greenhouse for 4 months in polystyrene cups (0.08 m diameter, 0.20 m deep) filled with 3 : 1 coarse sand and peat moss. Temperature averaged 27°C (ranging 15–30°C) and daylength extended to 18 h. Cups were arranged in a row-column design with five replicates. Permanent wilting point and field capacity were measured using a pressure plate and used to calculate 30% plant available water content (PAWC, similar to Hattendorf *et al*. 1988).

At the start of the experiment, plant tops were removed, each cup watered to field capacity and plastic lids fitted to minimise evaporation from the soil surface with a small hole added for the plant stem. Cups were weighed every 1–2 days thereafter, with water added only when 1 of a subset of 40 cups measured 30% PAWC. The volume of water added to each cup was 40% of that required to return the heaviest cup of the subset to field capacity. Additional cups without plants, covered with a lid, were used to measure evaporation so that plant water use could be determined.

Plants were harvested twice. At the first harvest, when 50% of plants had flowers (25 days regrowth), all cups were weighed and length of the longest stem recorded. Leaf and stem were separated and DW recorded for each plant. The second harvest was at the second flowering (43 days regrowth) when cups were re-weighed and whole plants harvested. Measurements on whole plants included leaf, stem and root DW, length of longest stem and total leaf area. These data were then used to calculate water use efficiency (WUE, mg DW/mm water used), and leaf–stem and root–shoot DW ratios. Data from both harvests were combined to calculate total herbage DW, total water use efficiency (TWUE, mg DW/mm water used) and leaf–stem DW ratio.

Pest and disease resistance

Relative resistances of cultivar/lines to phytophthora root rot (*Phytophthora megasperma* f.sp. *medicaginis*), anthracnose (*Colletotrichum trifolii*) and spotted alfalfa aphid (*Therioaphis trifolii*) were measured on seedlings in the greenhouse using Northern American Alfalfa Improvement Conference approved procedures (Fox *et al*. 2001).

Lucerne persistence in the field

All 20 cultivar/lines were sown for field evaluation in dryland experiments in September 1988 at Yanco and Tamworth, NSW (31°09′S, 150°59′E). Plots (5 × 2 m) were sown by hand-broadcasting seed at a rate of 2 kg/ha in a nearest neighbour design with four replicates. Frequency was monitored every 6 to 18 months in each experiment ~7 days after cutting/grazing using two fixed quadrats per plot. Each quadrat was 1 × 1 m

and divided into 100 cells (each 0.1 × 0.1 m). The number of cells containing a portion of a live lucerne plant was recorded (presence) and the proportion of occupied cells used to estimate frequency of occurrence (Brown 1954). Each experiment was fertilised annually in early spring with 22 kg/ha phosphorus and 27.5 kg/ha sulfur and routinely mown (for herbage DW assessments) then grazed by sheep when most plots had reached 10% flower.

Statistical analyses

All data were analysed using GENSTAT 5 (Genstat 5 Committee 1987). Data from row-column design experiments were analysed using the REML directive within GENSTAT 5.

Model to predict persistence

Correlations were calculated between persistence measures (i.e. frequency) from the dryland experiments at Yanco and Tamworth and all measured variables (where significant differences existed among lines) from the above studies. Multivariate analyses (Draper and Smith 1981) were used to identify which measured traits were associated with differences in persistence in the dryland field experiments. Regression equations were developed to predict persistence at each measurement date, *viz*. for the Yanco experiment after 16, 33, 52 and 67 months and the Tamworth experiment after 15, 22, 27, 33, 40, 46 and 57 months.

Results

Summer and winter temperatures were similar at Tamworth and Yanco. Tamworth has a higher average annual rainfall (674 mm) with summer dominance compared to Yanco (422 mm) which has a near even rainfall distribution throughout the year. Leeton and Yanco are in the same locality with similar rainfall and temperatures. Temperature and rainfall data for Tamworth and Yanco from 1988 until 1993 are in Fig. 1.

Total rainfall for September and October 1988 when the long-term dryland experiments were sown was much higher at Tamworth (127 mm) than Yanco (57 mm). Rainfall at Tamworth was above average most months in 1990 and below average 3 to 7 months of most other years. At Yanco, rainfall was below average 4–10 months a year, including eight consecutive months from October 1990 to May 1991.

Rainfall at Yanco was above average in the months following establishment (spring 1992) of the dryland morphology experiment. Rainfall was below average during the summer (February–March 1993) and autumn assessment periods (May 1993) and twice the long-term average in July 1993 before the winter assessment. Rainfall in November 1993 during the spring assessment was average.

Lucerne morphological traits

Lucerne cultivar/lines differed in the majority of their morphological traits at both Leeton and Yanco (Table 1). All plant stem length and herbage mass values were higher at the irrigated Leeton morphology experiment than those at the dryland Yanco morphology experiment, except the first stem length assessment in autumn (13–15 days regrowth) and the

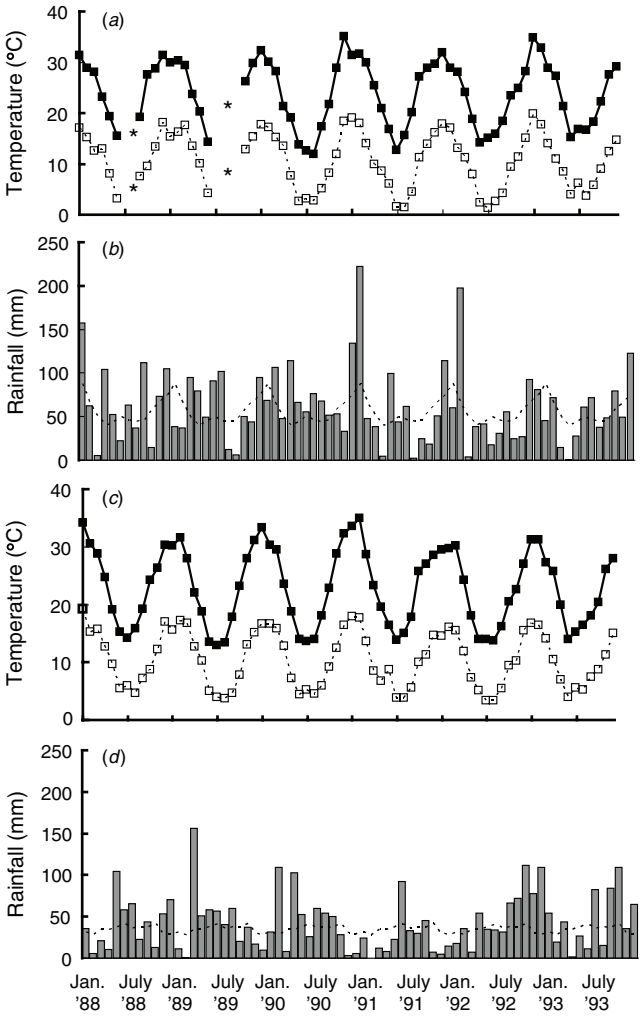

Fig. 1. (*a*, *c*) Monthly temperature range (°C) and (*b*, *d*) monthly rainfall (mm) at (*a*, *b*) Tamworth Agricultural Institute and (*c*, *d*) Yanco Agricultural Institute, 1988–1993. Monthly maximum temperature (■); monthly minimum temperature (□); missing values (⋆); actual rainfall (shaded bars); long-term average (broken line).

length of longest stem in winter (10% flower after 35–46 days regrowth) where differences between the two experiments were small. Variation in measured traits between the two experiments was highest during the summer assessments when the Yanco morphology experiment received only 20 mm rainfall during the 5-week summer assessment period. Variation in length of the longest stem was highest in autumn and winter for the irrigated Leeton morphology experiment ($P < 0.001$). In both morphology experiments, the length of longest stems measured in autumn and winter were highly correlated ($P < 0.001$, Table 2) and reflected the continuous range in winter dormancy among cultivar/lines from the winter-dormant P545 to the highly non-dormant (winter-active) CUF101 (Table 1). Significant correlations among traits indicated that winter-dormant lucernes (short stem lengths in autumn/winter) had slower rates of regrowth (shorter stem lengths) than winter-active types in all seasons ($P < 0.001$). Winter-dormant types also had lower herbage DW in summer, autumn and winter ($P < 0.001$),

Table 1. Variation among lucerne cultivar/lines for morphological traits measured on (*a*) an irrigation experiment at Leeton, NSW, Australia, in 1992, and (*b*) a dryland experiment at Yanco, NSW, Australia, in 1993; also pest and disease resistance in a greenhouse and late autumn-winter dormancy level

Plant traits measured are stem length (SL, m), herbage mass (HM, g), crown area (CA, $\times 10^{-4}$ m^2), leaf-stem dry weight ratio (LSR), plant erectness (ER), tap root score (TR), lateral root score (LR), crown depth (CD, m), cross-sectional area of tap root (TA, $\times 10^{-4}$ m^2), anthracnose resistance (ANT, %), phytophthora root rot resistance (PRR, %) and spotted alfalfa aphid resistance (SAA, %). All traits significant $P < 0.001$, except tap root score ($P < 0.01$), lateral root score ($P > 0.05$) and cross-sectional area of tap root ($P < 0.05$). Anthracnose, phytophthora root rot and spotted alfalfa aphid resistance are means of multiple tests. The dormancy levels are highly winter active (HWA), winter active (WA), semi-winter dormant (SD) and winter dormant (WD)

Season:	Summer				Autumn					Winter			Spring							
Trait:	SL1	SL2	SL3	HM1	CA1	SL4	SL5	SL6	HM2	SL7	ER7	HM3	CA2	ER2	LSR2	HM4	ANT	PRR	SAA	Dormancy
Date:	6 Feb	20 Feb	5 Mar	7 Mar	28 Apr	7 May	14 May	27 May	27 May	18 Aug	18 Aug	27 Aug	9 Nov	18 Nov	1 Dec	1 Dec				level
Days after cutting:	7	21	35	37	4	13	20	33	33	46	46	55	5	14	27	27				
Cultivar/line																				
Aquarius	0.152	0.492	0.723	39.3	33.9	0.127	0.237	0.333	17.2	0.216	0.888	18.7	69.8	0.893	1.3	19.4	14.5	46.0	33.4	WA
Aurora	0.119	0.430	0.652	37.8	35.3	0.104	0.191	0.273	13.5	0.180	0.859	15.8	71.3	0.881	1.2	18.3	32.3	31.9	67.5	WA
CUF101	0.157	0.463	0.663	39.9	32.2	0.168	0.300	0.394	17.9	0.283	0.952	20.0	56.6	0.917	1.2	17.8	8.6	21.1	46.0	HWA
Genesis	0.125	0.456	0.684	37.5	31.5	0.109	0.199	0.284	15.0	0.195	0.848	17.5	69.0	0.876	1.3	19.0	27.1	30.3	45.4	WA
Hunter River	0.118	0.422	0.652	38.1	28.9	0.085	0.152	0.231	11.1	0.140	0.839	11.8	59.3	0.863	1.6	14.2	11.3	9.3	0.8	SD
P545	0.095	0.347	0.543	35.3	29.9	0.054	0.087	0.133	10.4	0.075	0.681	10.4	71.4	0.848	1.7	17.7	9.1	31.2	35.0	WD
P577	0.141	0.444	0.663	39.7	25.6	0.108	0.203	0.289	15.1	0.201	0.905	16.9	51.5	0.891	1.5	15.3	19.1	11.0	52.5	HWA
P581	0.140	0.423	0.603	37.7	31.6	0.077	0.137	0.197	11.9	0.130	0.740	14.8	72.6	0.867	1.4	18.3	13.6	33.3	37.7	SD
P5929	0.169	0.481	0.690	38.9	31.4	0.167	0.291	0.382	17.0	0.280	0.949	18.5	51.7	0.923	1.1	17.6	15.6	32.9	48.4	HWA
Trifecta	0.141	0.424	0.636	36.6	28.8	0.103	0.193	0.290	12.4	0.182	0.897	13.7	56.8	0.890	1.5	14.1	36.3	28.2	46.2	WA
WL605	0.136	0.473	0.692	39.0	28.2	0.161	0.281	0.371	16.1	0.273	0.929	19.6	57.8	0.913	1.1	19.0	8.6	29.9	47.3	HWA
WLSS	0.116	0.408	0.616	35.1	30.9	0.095	0.181	0.252	13.8	0.139	0.771	14.2	78.0	0.887	1.3	19.2	6.9	18.1	39.3	SD
Y8401	0.116	0.413	0.619	34.9	35.7	0.084	0.136	0.190	11.5	0.111	0.699	11.0	81.4	0.816	1.5	17.1	8.2	45.9	24.7	WD
Y8402	0.131	0.486	0.716	39.2	32.2	0.124	0.223	0.322	14.7	0.234	0.931	17.4	62.9	0.881	1.3	17.1	4.0	42.0	54.4	WA
Y8512	0.111	0.436	0.666	37.3	32.1	0.106	0.189	0.277	13.7	0.167	0.822	15.3	74.5	0.887	1.3	17.3	35.8	31.6	39.1	SD
Y8606	0.109	0.415	0.669	37.7	31.3	0.098	0.176	0.273	12.7	0.161	0.715	14.7	74.1	0.843	1.4	17.3	6.3	20.3	49.2	WA
Y8609	0.110	0.417	0.640	38.2	32.8	0.065	0.113	0.172	11.8	0.111	0.631	12.6	98.6	0.722	1.5	17.7	7.6	25.7	45.8	WD
Y8616	0.135	0.457	0.700	40.5	31.2	0.088	0.151	0.239	14.0	0.148	0.695	15.0	84.0	0.858	1.5	17.6	11.3	21.7	45.3	SD
Y8622	0.117	0.429	0.648	38.6	35.3	0.087	0.164	0.261	15.3	0.143	0.643	16.0	92.4	0.819	1.5	20.9	25.1	32.8	74.7	SD
Y8625	0.123	0.461	0.685	39.7	35.0	0.095	0.172	0.254	14.0	0.157	0.809	16.0	78.6	0.869	1.3	17.9	24.6	19.6	36.5	SD
l.s.d. ($P = 0.01$)	0.022	0.030	0.044	3.9	5.4	0.016	0.026	0.032	2.8	0.022	0.058	3.2	13.8	0.037	0.2	3.2	–	–	–	

(*Continued next page*)

Table 1. (*continued*)

Season:	Summer				Autumn						Winter			Spring					Roots			
Trait:	SL1	SL2	SL3	HM1	CA1	SL4	SL5	SL6	LSR1	HM2	SL7	ER7	HM3	CA2	SL9	ER9	LSR2	HM4	TR	LR	CD	TA
Date:	12 Feb	17 Feb	10 Mar	11 Mar	4 May	12 May	20 May	31 May	31 May	31 May	30 Aug	30 Aug	1 Sep	11 Nov	6 Dec	6 Dec	7 Dec	7 Dec				
Days after cutting:	7	12	33	34	7	15	23	34	34	34	35	35	37	7	39	39	40	40				
Cultivar/line																						
Aquarius	0.034	0.083	0.218	3.3	29.4	0.127	0.150	0.174	2.3	5.1	0.219	0.649	8.5	51.1	0.384	0.790	1.1	11.7	2.5	2.8	0.790	0.036
Aurora	0.040	0.083	0.202	3.4	27.9	0.107	0.125	0.144	2.9	4.7	0.198	0.713	7.9	48.4	0.353	0.803	1.3	11.0	2.7	2.9	0.805	0.038
CUF101	0.053	0.131	0.265	4.2	28.7	0.158	0.189	0.221	2.0	5.9	0.270	0.860	8.4	41.8	0.455	0.835	1.0	13.7	3.2	2.8	0.887	0.041
Genesis	0.037	0.086	0.242	3.7	31.7	0.123	0.151	0.177	2.5	5.2	0.218	0.736	9.2	52.8	0.409	0.809	1.2	12.8	3.0	2.5	0.842	0.043
Hunter River	0.039	0.092	0.280	4.4	29.0	0.109	0.132	0.155	2.6	4.5	0.191	0.559	8.4	51.0	0.420	0.747	1.2	13.8	2.8	2.9	0.919	0.036
P545	0.032	0.072	0.176	3.1	30.2	0.075	0.091	0.099	3.3	4.1	0.154	0.513	7.9	52.4	0.349	0.743	1.2	11.6	2.9	2.6	0.788	0.034
P577	0.039	0.093	0.244	3.6	26.2	0.131	0.157	0.180	2.5	6.1	0.227	0.793	8.5	46.5	0.396	0.795	1.1	12.3	2.9	3.2	0.776	0.032
P581	0.037	0.079	0.187	3.1	31.1	0.099	0.112	0.129	3.2	4.7	0.185	0.548	8.3	50.2	0.363	0.788	1.2	12.0	3.2	2.4	0.859	0.038
P5929	0.048	0.113	0.248	4.5	30.4	0.147	0.174	0.202	2.3	7.2	0.249	0.828	9.4	46.4	0.442	0.825	1.0	14.0	2.6	2.7	0.835	0.037
Trifecta	0.036	0.079	0.224	3.3	28.2	0.117	0.139	0.164	2.9	4.6	0.204	0.708	7.8	47.7	0.386	0.806	1.1	12.2	3.1	2.6	0.807	0.032
WL605	0.048	0.116	0.251	4.2	27.8	0.154	0.182	0.214	2.1	6.7	0.263	0.844	9.1	42.3	0.419	0.812	1.0	13.0	3.5	2.8	0.815	0.039
WLSS	0.031	0.071	0.183	3.0	27.6	0.087	0.099	0.108	3.9	3.4	0.156	0.589	7.0	50.0	0.343	0.746	1.4	11.0	2.8	2.8	0.744	0.031
Y8401	0.033	0.068	0.188	2.9	31.6	0.092	0.112	0.126	2.4	4.4	0.176	0.526	8.0	53.2	0.356	0.757	1.1	12.8	3.0	2.8	0.808	0.034
Y8402	0.038	0.089	0.227	3.5	27.3	0.132	0.154	0.182	2.4	5.6	0.219	0.787	8.7	49.6	0.392	0.819	1.1	11.6	2.9	2.9	0.765	0.035
Y8512	0.035	0.080	0.206	3.4	29.9	0.103	0.121	0.140	2.9	4.8	0.199	0.609	8.5	55.2	0.354	0.728	1.3	10.5	2.8	2.5	0.833	0.039
Y8606	0.036	0.081	0.221	3.3	30.3	0.113	0.136	0.162	2.9	4.8	0.197	0.604	7.4	48.3	0.393	0.728	1.2	12.9	2.8	3.9	0.823	0.038
Y8616	0.037	0.075	0.212	3.5	31.2	0.126	0.151	0.180	3.7	5.5	0.193	0.516	9.0	52.2	0.418	0.750	1.3	15.7	2.9	2.8	0.852	0.032
Y8622	0.038	0.080	0.195	3.0	32.2	0.109	0.129	0.147	2.6	4.4	0.192	0.450	9.1	55.9	0.369	0.719	1.4	13.0	3.5	2.8	0.760	0.030
Y8625	0.033	0.079	0.195	3.1	28.1	0.096	0.109	0.125	2.8	4.0	0.184	0.562	8.0	46.1	0.350	0.775	1.2	10.3	2.9	2.9	0.763	0.033
Y9203	0.034	0.068	0.162	2.7	34.0	0.080	0.093	0.109	2.8	4.2	0.174	0.415	8.3	56.1	0.358	0.682	1.3	12.3	2.3	3.0	0.763	0.037
l.s.d. ($P = 0.01$)	0.007	0.015	0.030	0.8	4.9	0.020	0.024	0.027	1.6	1.4	0.021	0.068	1.4	6.8	0.034	0.046	0.1	2.5	0.6	n.s.	0.082	
l.s.d. ($P = 0.05$)																						0.006

Table 2. Simple correlation coefficients between the length of longest stem at 10% flower in autumn, and winter in the Leeton and Yanco (NSW, Australia) experiments and the main morphology traits measured

All traits significant ($P < 0.001$), except where indicated; n.s., not significant

Season	Trait	Leeton		Yanco	
		Autumn	Winter	Autumn	Winter
Summer	Stem length				
	(1 week)	0.75	0.78	0.82	0.88
	(2.5 weeks)	0.80	0.81	0.84	0.91
	(10% flower)	0.71	0.68	0.83	0.77
	Herbage dry matter	0.54	0.57	0.78	0.76
Autumn	Crown area	–0.13 n.s.	–0.19 n.s.	–0.31 n.s.	–0.32 n.s.
	Stem length				
	(2 weeks)	0.97	0.98	0.99	0.96
	(3 weeks)	0.99	0.98	1.00	0.95
	(10% flower)	1.00	0.97	1.00	0.95
	Leaf-stem ratio	–0.86	–0.82	–0.74	–0.79
	Herbage dry matter	0.78	0.77	0.88	0.89
Winter	Stem length (10% flower)	0.97	1.00	0.95	1.00
	Canopy erectness	0.83	0.86	0.80	0.85
	Herbage dry matter	0.90	0.90	0.60	0.59
Spring	Stem length (2.5 weeks)	0.90	0.92	0.84	0.90
	Canopy erectness	0.72	0.70	0.64	0.74
	Herbage dry matter	0.09 n.s.	0.05 n.s.	–0.07 n.s.	–0.04 n.s.
Summer	Crown area	–0.63	–0.68	–0.62	–0.67
	Crown growth rate				
	(Sowing – late autumn)			–0.62	–0.69
	(Sowing – late spring)			–0.62	–0.67

higher leaf–stem DW ratios ($P < 0.001$), larger crown areas in the summer following the seedling year ($P < 0.001$) and higher crown growth rates from sowing to autumn and from sowing to spring ($P < 0.001$; Tables 1 and 2). Herbage DW in spring was one of the few traits not correlated with winter dormancy.

Starch partitioning during regrowth

Greenhouse

After defoliation, starch concentration declined and was recorded lowest 17 days ($P < 0.001$) after cutting. Starch concentration then increased to a maximum, recorded 35 days after cutting ($P < 0.001$). Starch concentration at each assessment is in Fig. 2. Starch concentration of the cultivars varied (main effect, $P < 0.01$) with CUF101 (12.1%) having a lower concentration than Aurora and P545 (15.0 and 15.8%, respectively), but their response over time was similar ($P > 0.05$; data not shown).

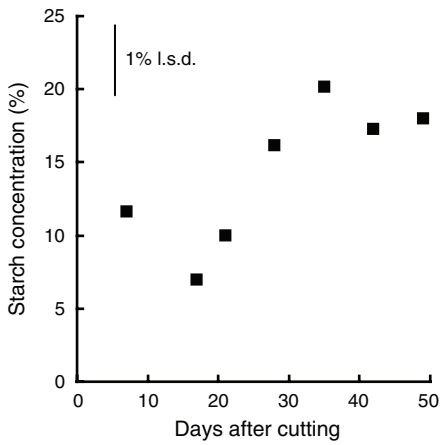

Fig. 2. Response of starch concentration in lucerne crown and roots grown in a greenhouse following cutting ($P < 0.001$). Data for the three cultivars are averaged.

Field

Changes in starch concentration with regrowth followed the same trend as that recorded in the greenhouse. After defoliation, starch concentration declined for 13 days ($P < 0.001$) before increasing, reaching a maximum concentration after 42 days. Starch concentration returned to initial levels ~32 days after cutting (main effect, Fig. 3*a*). Again, starch levels varied among the 20 cultivar/lines (main effect, $P < 0.001$), with all responding similarly over time ($P > 0.05$; data not shown). Line Y9203 had the highest starch concentration (25.2%, Table 3) and was not significantly different to seven other cultivar/lines. The lowest concentration was in Hunter River at 18.3%.

Defoliation and the subsequent regrowth also affected root DW ($P < 0.001$; data not shown). During the first 3 weeks after

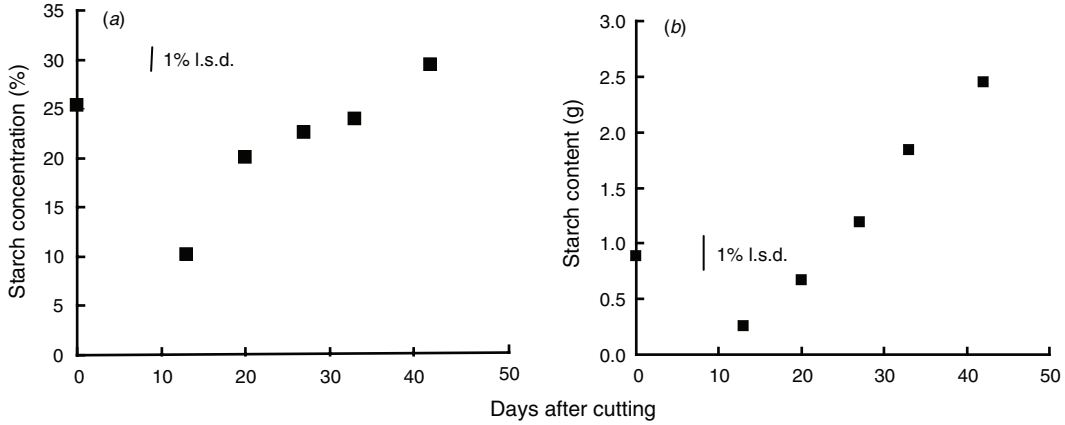

Fig. 3. Starch (*a*) concentration and (*b*) content in field gown lucerne crown and roots during regrowth after cutting ($P < 0.001$). Data are averaged over the cultivar/lines assessed.

Table 3. Starch concentration in lucerne crown and roots in the field ($P < 0.001$)
Data are averaged over the six assessment times

Cultivar/line	Starch concentration (%)
Y9203	25.2
P5929	24.2
Y8512	23.6
P581	23.5
Y8616	23.0
P577	22.7
Y8622	22.1
P545	22.0
Genesis	22.0
CUF 101	22.0
Aurora	21.9
WLSS	21.8
Y8402	21.7
Y8625	21.4
Y8401	21.3
Aquarius	21.2
WL605	20.9
Y8606	20.6
Trifecta	19.7
Hunter River	18.3
l.s.d. ($P = 0.01$)	3.18

defoliation ($P > 0.05$), root DW did not change significantly then increased until ~33 days after defoliation ($P < 0.001$) when it again slowed until the final assessment (42 days after cutting, $P > 0.05$). In contrast, the starch content of the crowns and roots of the young plants declined for ~2 weeks after cutting, then increased ($P < 0.001$; Fig. 3*b*). There were no cultivar/line differences for either root DW or starch content (main effect, $P > 0.05$) and their response over time was similar ($P > 0.05$).

Response to water deficit

There was significant variation among the 20 lucerne cultivar/lines in all plant traits measured (Table 4), except leaf DW (harvest 1), leaf area (harvest 2), root DW (harvest 2) and root–shoot ratio (harvest 2 and total). There was also significant variation in WUE of the 20 cultivar/lines during both regrowth periods and overall ($P < 0.05$; Table 4). As there was no difference in the water used, the variation among lines in WUE was due to differences in herbage mass as indicated by correlation between WUE and total herbage DW at each harvest ($r = 0.72$–0.80).

Pest and disease resistance

Cultivar/lines differed in their relative resistance to anthracnose ($P < 0.01$), phytophthora root rot ($P < 0.01$) and spotted alfalfa aphids ($P < 0.01$) in separate tests in the greenhouse (Table 1). These differences among cultivar/lines in pest and disease resistances were not associated with differences in winter activity.

Lucerne persistence in the field

In the dryland experiments, the response in plant frequency through time at the two experiments was different. At Tamworth, 15 months after sowing average cultivar/line, frequency was 58% and then declined almost linearly until the final assessment when the average frequency was 28%. The range in plant frequency values increased during the experiment to be greatest 27 months after sowing then declining to be less than the first assessment 57 months after sowing. At Yanco, average plant frequency following establishment was initially low (30%) due to low rainfall (Fig. 1), but was relatively stable throughout the experiment, increasing to a maximum of 38% (52 months after sowing), then declined to 28% at the final assessment (Fig. 4). The range in frequency was lowest following establishment, increasing to be highest 52 months after sowing.

Plant frequency of cultivar/lines at Yanco after 52 months was highly correlated with frequency at Tamworth after 46 months ($r = 0.89$, $P < 0.01$). Although the time of the assessment at the two experiments varied by 6 months, it was at these times that average frequency was most similar (i.e. 38% at Yanco and 32% at Tamworth). Most cultivar/lines had higher frequencies

Table 4. Morphology traits of lucerne lines measured in a water deficit experiment conducted in a greenhouse
Traits measured are stem length (SL, m), plant herbage mass (HM, g), leaf-stem ratio (LSR), plant water use efficiency (WUE, mg DW/mm water used), root dry weight (RDW, g) and root–shoot ratio (RSR). n.s., Not significant

	Harvest 1				Harvest 2						Total		
	SL1	HM1	LSR1	WUE1	SL2	HM2	LSR2	RDW	RSR	WUE2	HM3	LSR3	WUE3
Y8512	0.24	0.29	2.36	1.05	0.29	0.33	1.18	0.93	2.84	1.81	0.66	1.49	1.49
Trifecta	0.29	0.31	1.73	1.28	0.26	0.33	1.36	1.04	3.35	1.70	0.69	1.45	1.61
CUF101	0.25	0.33	2.72	1.17	0.25	0.30	1.46	0.99	3.31	1.85	0.62	1.70	1.39
Y8401	0.25	0.36	2.21	1.41	0.24	0.34	1.40	1.04	3.23	1.89	0.74	1.73	1.72
Y8606	0.21	0.28	2.99	1.08	0.20	0.30	1.56	1.04	3.59	1.63	0.63	1.79	1.45
Aquarius	0.26	0.27	2.46	1.03	0.23	0.29	1.48	0.83	2.98	1.66	0.58	1.65	1.35
WL605	0.25	0.25	1.79	1.04	0.25	0.31	1.35	0.96	3.34	1.48	0.61	1.54	1.35
Y9203	0.26	0.29	1.75	1.08	0.22	0.31	1.67	1.01	3.33	1.72	0.61	1.62	1.43
Aurora	0.25	0.29	2.02	1.23	0.25	0.34	1.67	0.90	2.56	2.04	0.67	1.83	1.60
Hunter River	0.25	0.26	1.95	1.07	0.22	0.28	1.35	1.02	3.62	1.50	0.60	1.45	1.40
Genesis	0.24	0.27	2.16	1.15	0.22	0.27	1.61	0.86	3.12	0.75	0.61	1.75	1.41
P581	0.24	0.28	2.30	1.11	0.25	0.27	1.41	0.78	3.06	1.37	0.56	1.69	1.30
P5929	0.24	0.27	2.01	1.02	0.23	0.30	1.49	1.03	3.77	1.81	0.59	1.69	1.38
P545	0.25	0.29	1.74	1.12	0.25	0.36	1.35	1.06	2.99	2.11	0.72	1.40	1.68
Y8402	0.25	0.29	1.95	1.07	0.24	0.28	1.53	0.87	3.42	1.59	0.58	1.60	1.39
Y8622	0.24	0.27	2.50	1.14	0.24	0.31	1.48	0.83	2.84	1.59	0.61	1.75	1.40
WLSS	0.25	0.31	2.21	1.18	0.27	0.31	1.45	0.87	3.17	1.75	0.64	1.70	1.48
P577	0.22	0.29	2.30	1.20	0.26	0.34	1.33	1.04	3.16	1.75	0.72	1.57	1.59
Y8625	0.27	0.30	2.22	1.20	0.28	0.30	1.13	0.96	3.18	1.58	0.61	1.43	1.42
Y8616	0.24	0.31	2.30	1.25	0.30	0.34	1.14	0.98	3.09	1.61	0.69	1.47	1.57
l.s.d. ($P = 0.05$)	0.03	0.07	0.70	0.18	0.04	0.05	0.33	n.s.	n.s.	0.60	0.10	n.s.	0.22

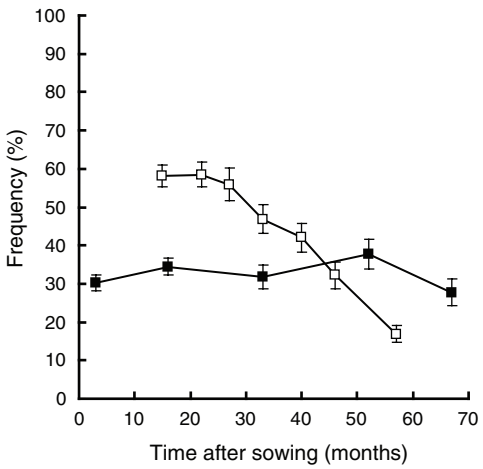

Fig. 4. Decline in the persistence (plant frequency, %) of lucerne in dryland experiments sown at Yanco (■) and Tamworth (□) in September 1988. Bars indicate the 95% confidence interval at each assessment.

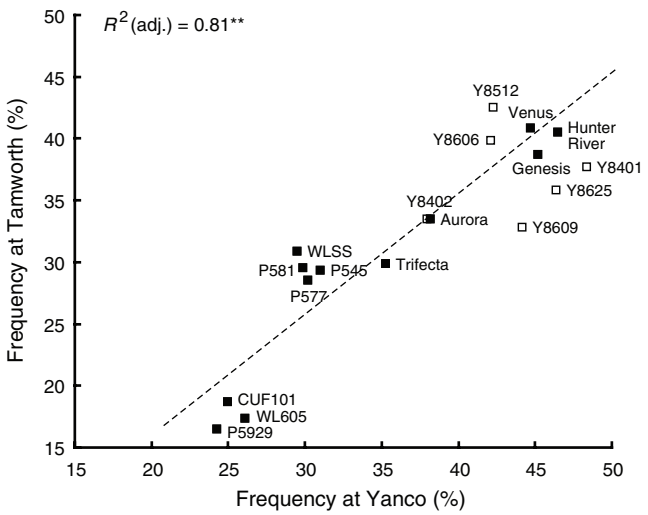

Fig. 5. The association between the persistence of commercial cultivars (■) and breeding lines (□) of lucerne in the Yanco and Tamworth field experiments after 52 and 46 months, respectively. The linear relationship is indicated by a dashed line.

at Yanco than Tamworth while Y8512, Y8606, WL Southern Special (WLSS), P581, P545 and P577 performed similarly at both sites (Fig. 5).

Developing a persistence model

Although there were significant differences among lucerne cultivar/lines in their morphology traits described in this paper, no single trait reliably predicted persistence, as reflected by plant frequency, after ~4 years in the dryland field experiments at both Yanco and Tamworth.

Variation among cultivar/lines in their persistence (%P) at Yanco and Tamworth could, however, be explained using functions based on differences between the length of the longest stems in summer [5 weeks regrowth (10% flower), LSS] and winter [7 weeks regrowth (10% flower), LSW] measured

in the irrigated experiment at Leeton (i.e. SL3 and SL7, Table 1):

$$\%\text{P (Yanco, 52 months)} = 2.07(\pm 0.31) * \text{LSS} - 1.75(\pm 0.21) * \text{LSW} - 67.39\ (\pm 17.95)$$
$$(R^2\text{adj.} = 0.79;\ P < 0.001)$$

$$\%\text{P (Tamworth, 46 months)} = 1.89(\pm 0.37) * \text{LSS} - 1.82(\pm 0.26) * \text{LSW} - 49.55\ (\pm 20.94)$$
$$(R^2\text{adj.} = 0.75;\ P < 0.001)$$

Models developed using multiple traits, from the Yanco morphology experiment were less successful than those from the irrigated Leeton experiment in predicting lucerne frequency after ~4 years. The most successful models developed using data from the Yanco experiment included crown area, and rate of regrowth and length of longest stems in summer. Although these models were significant ($P < 0.01$), they explained fewer differences among the cultivar/lines for frequency in the dryland experiments at Tamworth and Yanco.

WUE at the first harvest of the water deficit experiment was the only physiological trait correlated with lucerne plant frequency ($P < 0.05$). Its inclusion into the stem length models improved success in predicting plant frequency at Yanco after 33 and 52 months (Table 5).

Concentration of starch in the crown and uppermost roots of field grown plants (13 days regrowth) also improved models developed to predict persistence at Yanco after 16 and 52 months, and Tamworth after 40 months. Change in starch concentration during regrowth also improved the model for frequency data from the Tamworth experiment after 22, 27, and 33 months (Table 5).

Table 5. Coefficients of determination (R^2) for regression models to predict persistence of lucerne in dryland experiments at Yanco and Tamworth using stem lengths alone, or best fit models using stem lengths with a combination of traits from all experiments

All models are significant at $P < 0.001$, except '*' where $P < 0.05$. Traits other than stem lengths are anthracnose resistance (ANT), % starch in roots after 13 days regrowth (% Starch), spotted alfalfa aphid resistance (SAA), cross-sectional area of tap root (TA), water use efficiency (WUE), % change in root starch concentration from initial to 13 days regrowth (% Starch change)

Location	Time after sowing (months)	Stem length models (R^2)	Best fit models R^2	Best fit models Traits other than stem lengths
Yanco	16	65.2	83.7	ANT, % Starch, SAA
	33	72.3	88.1	TA, WUE
	52	79.1	88.6	% Starch, TA, WUE
	67	70.5	70.5	–
Tamworth	15	52.4	52.4	–
	22	58.2	72.4	% Starch change
	27	80.5	84.2	% Starch change
	33	69.0	75.6	% Starch change
	40	71.4	77.5	% Starch
	46	74.0	74.0	–
	57	27.3*	27.3*	–

Resistance to anthracnose and spotted aphids explained a significant proportion of the variation in persistence at Yanco after 16 months when incorporated in a model with a starch trait and the stem length traits (Table 5).

Although significant, the models were least successful in predicating frequency in young (e.g. <22 months) and older thin stands (e.g. >57 months) when variation among lines was least. For all other occasions, models based on two simple measures of stem length under irrigation at Leeton were highly predictive of plant survival in mature dryland experiments at both Yanco and Tamworth. Comparisons of observed and predicted frequency of lines at Yanco after 52 months and Tamworth after 46 months from the stem length models indicate the range in frequency of the evaluation experiments.

Discussion

Measured traits

Lucerne populations can vary in a range of traits, the greatest differences usually being in traits such as winter-dormancy, plant height and herbage DW (e.g. Lorenzetti *et al*. 1972). In the current study, large and significant differences in these traits were also found. Significant decreases in the lengths of the longest stem in late autumn and winter in response to decreasing photoperiods and lower temperatures is termed 'winter-dormancy'. In our study, autumn stem lengths, a surrogate for growth rate or winter-activity, were highly correlated with winter stem lengths. In northern latitudes, true expression of winter-dormancy aids survival during extreme winter conditions. In the dryland agricultural zone of eastern Australia, extreme winter conditions rarely exist and winter-dormancy can be determined by growth rate in either late autumn or winter.

The continuous range in winter-dormancy from the very winter-dormant P545 to the highly non-dormant or highly winter-active CUF101 as measured in this study covers much of the published range in expression of this trait. However, previous reports have documented few differences between the morphology and productivity of dormant and non-dormant cultivars during spring and summer. In this study, increasing winter-dormancy was associated with greater leaf–stem DW ratios and larger crowns. Furthermore, in contrast to popular conception, winter-dormant lucernes were shown to be less 'summer-active' than highly winter-active lucernes with slower rates of regrowth in both summer and spring. Forage yield in spring was one of the few traits not associated with the degree of winter-dormancy.

Foutz *et al*. (1976) suggested that morphological traits were more reliable than physiological traits as indicators of lucerne productivity. The current study showed that morphological traits can also be reliable indicators of lucerne persistence. However, the success of summer and winter stem lengths as predictors of persistence was at least partly due to their associations with physiological criteria. For example, highly winter-active cultivars with long stems in winter generally had lower water use efficiencies and were generally more exploitive of root starch reserves during regrowth than cultivars with shorter stems in winter. This less conservative approach towards using valuable water and energy reserves by highly winter-active

plants may adversely affect their persistence when challenged by inappropriate grazing and/or drought in spring and summer compared with less winter-active plants which can use the dormancy mechanism to maintain reserves and/or escape severe stress. Severe water deficit may also require varieties to cease shoot growth and become dormant rather than chasing declining water levels to maintain productivity. Although dormancy was not assessed in our study, changes in lucerne morphology in response to water stress were apparent in the water deficit experiment. Water deficits reduce plant growth rates and change the pattern of growth (Kramer 1962; Carter and Sheaffer 1983). Morphological changes associated with water stress often include changes in cell wall thickness, leaf thickness, the degree of cutinisation and lignification, leaf area and leaf–stem DW ratios (Kramer 1962; Carter and Sheaffer 1983).

Models to predict persistence

This study has shown that morphological traits measured on irrigated plants in their seedling year can be successfully used to predict persistence of mature dryland stands. Broadly-adapted cultivars bred in Australia for Australian conditions such as Hunter River, Aurora, Trifecta and Genesis generally maintained the same relative persistence at the two locations (Fig. 5). These same cultivars also tended to persist better than predicted based on their stem lengths in summer and winter compared with cultivars bred overseas such as WL605, P5929, P581, and WLSS. This suggested that the Australian-bred cultivars and lines possessed additional traits which were not present in the overseas-bred material, thus highlighting the advantages of breeding and evaluating lucernes in Australia for Australian conditions.

Our models also highlighted that lucerne persistence was not a simple function of winter dormancy. Regression coefficients consistently indicated that persistent cultivars in these dryland experiments were not only those that were relatively dormant in winter, but also those that had the greatest variation between summer and winter stem lengths. Neither the highly winter-active nor winter-dormant types were the most persistent; rather it was those lines with high summer activity and some winter dormancy. Also, in general, the stem length models were least successful when there were few differences in persistence among lucerne cultivar/lines, such as in young stands or when very few plants survived towards the end of experiments.

Stem length data from the dryland Yanco experiment were less successful in models to predict lucerne persistence than those from the irrigated Leeton experiment. Irrigation allowed the lines to develop more fully, thereby maximising differences in their expression of morphological traits, particularly during the hot summer months when rainfall is low in south-eastern Australia. Reduced variation among lines was also noted in the water deficit experiment.

Models based on relatively simple criteria such as stem length should be more robust than those based on more highly complex traits. Therefore for application in breeding programs, stem length models may be potentially more useful in predicting persistence of new material in dryland evaluation experiments throughout eastern temperate Australia. Once validated, simple models also provide selection and/or evaluation criteria that are non-destructive and that can be easily and quickly measured in lucerne breeding programs that traditionally need to screen large numbers of plants both within and among populations. Potentially, this could reduce time and resources spent on developing inappropriate lines (i.e. lines with both short stems in summer and long stems in winter) and reduce the need for intensive and long-term field evaluation of early generation breeding material. However, it is important to note that the measurement of stem lengths would not negate the need for limited field experiments, ideally under irrigated conditions, to measure the stem length traits. Validation of these models is also required before their application in a breeding program.

Lucerne varieties and breeding lines normally consist of highly heterogeneous populations of plants. The current study aimed to investigate differences among a large number of lucerne lines, highlighting the variability that existed between lines rather than within lines for the measured traits. Other more detailed studies of physiological criteria and the mechanisms of stress tolerance have been conducted using lucerne clones to reduce the variation that exists naturally in a lucerne population (e.g. Cole *et al*. 1970). In our study, traits were expressed as means so that they could be associated with the persistence of the same populations in the field. Models based on individual plant type rather than population type may potentially be more useful in a breeding program where selection is normally practiced on a plant basis. For example, Smith *et al*. (1989) suggested that improved grazing tolerance in lucerne plants was associated with deep set and broad crowns, subsurface budding, prolific and asynchronous budding over extended periods, maintenance of root starch concentration, drought resistance, pest and disease resistance and slow recovery after cutting.

Inclusion of traits other than stem lengths to the models

Resistance to common pests and diseases is known to be important for lucerne persistence, evident by the annihilation of cv. Hunter River in 1976 by the spotted alfalfa aphid. However, this study generally showed no direct association between resistance to any one disease or pest and the persistence of lines in dryland field experiments. More recent studies have also shown that increasing pest and disease resistance had no consistent benefits to lucerne persistence or productivity across a range of fifteen dryland experiments (Williams 2004). This suggests that other adaptive traits in lucerne are more important in conditioning success under dryland conditions than relative responses to pests and diseases. It also suggests that the incidence and/or effects of pests and diseases under dryland conditions may be relatively minor. In contrast, a study in the Australian sub-tropics, a more humid environment, showed that lucerne diseases did play a significant role in determining plant persistence under irrigated conditions (Lowe *et al*. 1985).

Interestingly, resistance to pests and diseases significantly improved the prediction of persistence in establishing stands at Yanco when included in the stem length models. It is known that lucerne seedlings are much more susceptible to damage from pests and diseases than mature plants, and the Yanco models suggest that the effects of pests and diseases on plant frequency is most pronounced during establishment (<16 months, Table 5).

Once stands had matured, WUE and the level of starch reserves (root related traits) became consistently more important

in improving the predictive power of the stem length models for Yanco. In contrast, only the extent of the decline in starch reserves 2 weeks after defoliation were added to the 'best-fit' models for Tamworth. Starch accumulation in roots is the primary source of energy for initial regrowth after defoliation (Heichel *et al*. 1988). Other work suggested that lucerne cultivar/lines vary in the amount of starch they use in the initial regrowth period (S. P. Boschma and R. W. Williams, unpubl. data). Our current study showed that the greater the reduction in starch reserves during regrowth, the poorer the persistence of lines in the field at Tamworth. Together, these suggest that lines which are less dependent on starch reserves to produce new leaf following defoliation are likely to have better persistence under conditions similar to those at Tamworth. However, further investigation is required to confirm this.

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