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High levels of diversity for seed and forage production exist in *Cullen australasicum*, a potential new perennial forage legume for dry environments in southern Australia

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Abstract. The seed and forage production of a diverse group of the perennial forage legume *Cullen* spp., collected in southern Australia, was assessed with the aim of discovering diversity for exploitation in future breeding programs. Eighty ecotypes were assessed at the Waite Institute in South Australia, using replicated, spaced-plant field trials, between 2008 and 2012. Seed production in collected ecotypes of *Cullen* (Expt 1) ranged from 0 to 485 kg ha^{-1} for windrowed seed yield and from 0 to 790 kg ha^{-1} for total seed yield, which included vacuum-harvested seed from pods that had fallen to the ground. Individual plants were selected for seed production from their original populations, and the seed and fodder production of their progeny was evaluated in a further field experiment (Expt 2). Moderate to high heritability estimates were recorded for seed production traits. Seed production in progeny families ranged from 0 to1 423 kg ha^{-1} and was highly correlated with the number of seeds per inflorescence ($r = 0.85$) and forage yield ($r = 0.59$). Edible biomass, measured using the Adelaide visual appraisal method, ranged from 50 to 906 g dry weight (DW) plant^{-1} in parent ecotypes and from 404 to 1248 g DW plant^{-1} in the selected family progenies. Disease infection with anthracnose (*Colletotrichum trifolii*) caused considerable damage to plants in Expt 1, resulting in the death of all plants of 10 ecotypes, and infection with *Alfalfa mosaic virus* in Expt 2 was linked to the death of 67 individuals. The results are discussed in relation to breeding *C. australasicum* for increased seed yield and disease resistance to overcome these deficiencies as barriers to commercial adoption.

Additional keywords: Bullamon lucerne, domestication, scurf pea, seed production, tall verbine.

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Introduction

New, drought-tolerant perennial forage legumes are being sought to prepare graziers for the onset of climate change, which is predicted to bring about an increased frequency of low rainfall and drought across large parts of southern Australia (Anon. 2007, cited in Hayes *et al*. 2009). Some Australian native legumes are well adapted to the climatic and edaphic conditions of the semi-arid agricultural regions of Australia, and are therefore being considered in the search to discover new, drought-hardy alternatives to the existing commercial legume species (Cocks 2001; Dear *et al*. 2007; Bennett *et al*. 2011). *Cullen* spp., typically semi-herbaceous perennial legumes, are one example of a genus native to Australia that is currently under consideration for use in extensive grazing systems (Cocks 2001; Dear *et al*. 2007; Hayes *et al*. 2009; Suriyagoda *et al*. 2013). Members of the *Cullen* genus are found in Africa, Spain, Portugal, Italy, Asia Minor, and southern Asia, with 25 of the 32 species occurring naturally in Australia (Grimes 1997). Early evaluation of the former taxonomic group, *Psoralea eriantha-patens*, by Skerman (1957), Kerridge and Skerman (1968), and Britten and De Lacy (1979) highlighted the high forage yield potential of this group relative to lucerne (*Medicago sativa* L.) and its value as a fodder for cattle during periods of drought. Recent studies have focussed on the species *C. australasicum* (Schltdl.) J.W. Grimes and confirmed that its forage production (Dear *et al*. 2007; Bennett *et al*. 2012) and persistence (Li *et al*. 2008; Suriyagoda *et al*. 2013) in low-rainfall environments can be similar to exotic perennial pastures such as lucerne. Comparable field performance to that of industry-leading lucerne varieties over 2–3-year periods has also been documented in Australia, in New South Wales (Dear *et al*. 2007;

Hayes *et al*. 2009; Boschma *et al*. 2011) and Western Australia (Bennett *et al*. 2012), leading researchers to conclude that *C. australasicum* is a plant of considerable promise. In terms of potential for commercial development, *C. australasicum* has many agronomic qualities of a valuable forage plant. Its green leaf tissue has a similar digestibility (Dear *et al*. 2007; Bennett *et al*. 2012) and crude protein concentration (Bennett *et al*. 2012) to lucerne; it has the ability to preserve green leafy tissue during the onset of drought (Kerridge and Skerman 1968; Boschma *et al*. 2011); and field observations suggest that it is tolerant to damage from most insect pests (Dear *et al*. 2007; Hayes *et al*. 2009; Bennett *et al*. 2012).

Studies on the breeding system of *C. australasicum* led researchers to conclude that *C. australasicum* is mostly a self-pollinating species, with varying degrees of out-crossing potential among ecotypes (Kroiss *et al*. 2009; Wang *et al*. 2010). This suggests that a variety of breeding strategies could be implemented for plant improvement, from simple ecotype selection to a range of inter- and intra-population breeding methods modified from those used by inbred or out-crossing species. Aspects of *C. australasicum* genetics (or agronomy) that require better understanding before strategic plant breeding and commercialisation of this species can be considered include preference and utilisation by sheep under grazing (Dear *et al*. 2007; Hayes *et al*. 2009) and strategies for improving seed production (Kobelt *et al*. 2011).

The price of seed is a major barrier to the adoption of any new pasture species, and consequently, improved seed production technologies and harvestability traits must be developed to ensure that a viable commercial variety can exist. A major constraint to the seed production of *Cullen* spp. is that large seed losses can occur from low pod retention and uneven ripening of the seed. *Cullen australasicum* flowers prolifically throughout the year following sufficient rainfall (Grimes 1997), making harvest-timing decisions difficult. Nevertheless, seed production from a single accession of *C. australasicum* has been successful, with up to 700 kg ha^{-1} harvested using small-scale commercial machinery (Kobelt *et al*. 2011). However, seed production was not reliable, due to indeterminate flowering and loss of mature seed from the vine resulting from low pod retention. Kobelt *et al*. (2011) found that dry conditions improved the synchrony of flowering and, conversely, that irrigation during flowering promoted an extended flowering period, resulting in lower seeds yields and making it difficult to determine the best timing for harvest. The genetic diversity for seed production potential in *C. australasicum* was unknown before this study.

Here, we predict that variability for seed and forage production traits exists within a collection of *Cullen* spp. sourced from a diverse range of environments in southern Australia. In combination with improved agronomic practices, superior genotypes with desirable 'domestication' characteristics could contribute towards increased seed production and support the commercial success of the species.

Methods

Eighty populations of *Cullen* spp. (see Supplementary file), assembled by the South Australian Genetic Resource Centre and representing the distribution of this genus throughout Australia, were assessed for a range of morphological traits that relate to seed and forage production. A replicated experiment (Expt 1) was sown in July 2008, and assessment of germplasm was completed on established plants between April 2009 and January 2010. Progeny from individual plants, selected from approximately 2% of the populations, were then advanced in a second experiment (Expt 2) to confirm the heritability of seed production traits. Expt 2 was sown in September 2010, and characterisation of seed production traits and forage yield occurred between December 2011 and January 2012.

Location and soil type

The field site was in the SARDI Genetic Resources field nursery at the Waite Campus, Urrbrae, Adelaide, South Australia (34.97°S, 138.63°E; elevation 110 m). The fine sandy loam at this site is a red-brown earth (Stace *et al*. 1968) of the non-sodic Urrbrae series (Litchfield 1951). The upper 0.10 m contains 18% clay, increasing to 32% in the A2 horizon (Prescott 1931). Soil pH (in $CaCl_2$) was 6.2 and there was negligible calcium carbonate (Grace *et al*. 1995). The site had subsurface drip irrigation, with two lines running 0.5 m apart, 0.2 m beneath each plot, and with drip intervals of 0.5 m. For Expt 1, irrigation was with weekly applications equivalent to 25 mm of rainfall between November and May. No irrigation was used in Expt 2.

Climate

The climate in Adelaide is Mediterranean, characterised by hot, dry summers and a frost-free winter. The Waite Campus has a winter-dominant rainfall, with a long-term (130 years; Glen Osmond weather station) mean of 627 mm. Observations for mean daily minimum and maximum temperatures for Adelaide are shown in Fig. 1. Daylength peaks at 14.3 h in December and falls to 9.7 h in June. Rainfall for both experiments was close to long-term averages, with the exception of July 2009, when monthly rainfall of 131 mm was in the 85th percentile for Adelaide, 47.4 mm above the median rainfall of 83.6 mm. This event caused minor flooding on the experimental site, and waterlogged conditions remained for ~10 days. In November of the same year, the mean maximum temperature of 30.8°C and the mean minimum temperature of 18°C were the highest on record in Adelaide.

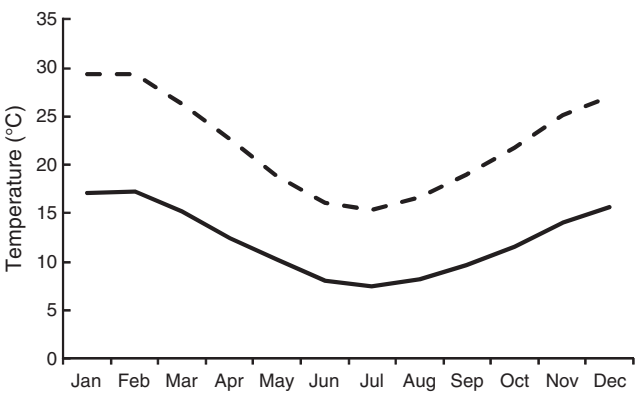

Fig. 1. Long-term (130 years) daily minimum (—) and maximum (- - -) temperatures at Urrbrae (Glen Osmond weather station), South Australia.

Experiment 1: Seed and forage production in a diverse range of Cullen *spp. germplasm*

Germplasm

A map of the native origin of *Cullen* spp. germplasm in Australia used in Expt 1 is shown in Fig. 2. The germplasm represents collections of *C. australasicum*, and ecotypes of closely related species that required their taxonomy to be confirmed in this study, collected at latitudes 23.7–35.3°S, from a diverse range of soils and climates (Supplementary file). Accession SA4966 was used as a standard entry due to its previous evaluation in a range of studies (Dear *et al*. 2007; Li *et al*. 2008; Hayes *et al*. 2009; Bennett *et al*. 2012), including the research by Kobelt *et al*. (2011) to develop harvest technologies for *C. australasicum*.

Experimental design and culture

Eighty ecotypes were evaluated in a randomised grid with 15 columns and 16 rows, using three replications (each with five columns and 16 rows). The ecotypes were germinated in July 2008 in Petri dishes and seedlings were transferred to small, biodegradable pots. The seedlings were grown in a greenhouse until they produced two trifoliate leaves. Ten plants from each accession were transplanted into each plot containing one row, with 30 cm between plants. For calculations of unit area of production, a dimension 1 m by 3 m for each plot was used. The distance between plots was 1.2 m across the length and width directions. No observations were made in the first seedling year, and a herbage cut was used in April 2009 to reduce potential differences that resulted from transplant, recovery, and seedling-year growth.

Experiment 2: Confirmation of seed production traits in selected individual plants

Germplasm

Seed from 42 individual plants displaying a range of seed and forage production traits was harvested from 38 of the populations evaluated in Expt 1 (Tables 1 and 2). A further 15 'families' were included from selections of field evaluation trials, plus seven ecotypes repeated from Expt 1, identified by their good performance in regional row nurseries (unpublished data), and from glasshouse aphid screening (Tables 1 and 2).

Experimental design and culture

The 64 entries were sown in a completely randomised and blocked grid with 64 rows and four columns, with four replications (one replication per column). Seedlings were raised as in Expt 1, and two seedlings from each family were space-planted 0.7 m apart on weed matting in September 2010. Individual plants were considered an experimental unit, and 512 plants (eight plants per line) were successfully established. Seed was harvested from all entries on 7 December 2011.

Evaluation protocols

The measurement protocols for forage yield and seed production components used in Expts 1 and 2 are presented in Table 3. Many of the variables were measured using visual assessment methods from rigorous, well-developed protocols. The use of 1 January as a reference date for flowering date was an arbitrary choice.

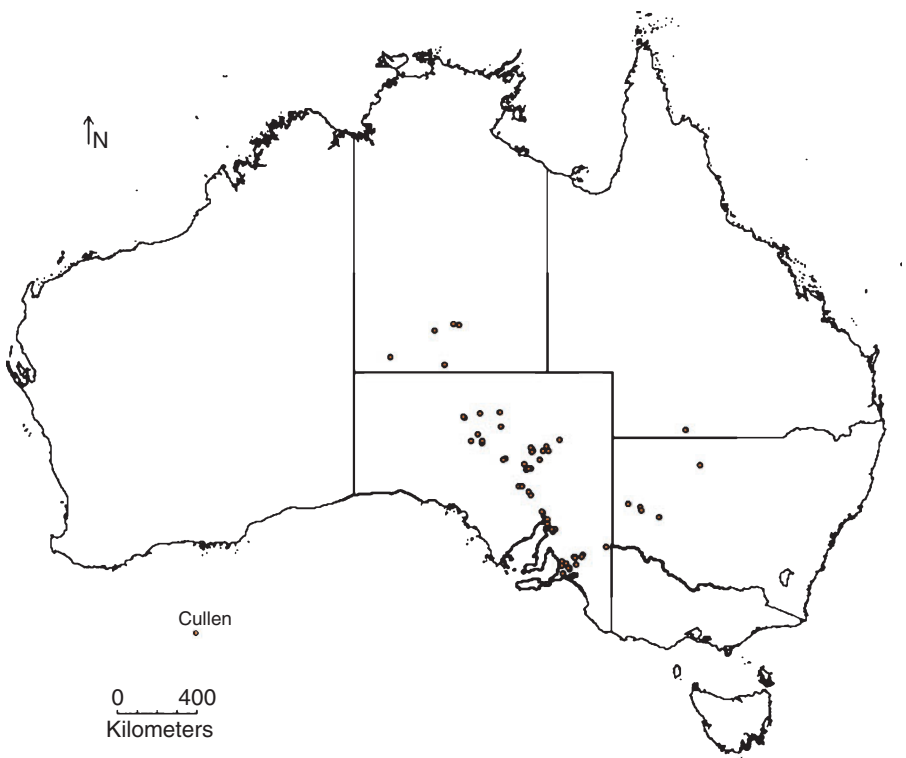

Fig. 2. Origins of *Cullen* spp. ecotypes used in Expt 1 (developed from GPS coordinates of collection sites).

Table 1. Description of *Cullen australasicum* and *C. pallidum* entries used in Expt 2 to identify seed and forage yield production traits

Parent SA/breeders line	No. of entries	Type	Selection
		C. australasicum	
Selections from ecotypes in Table 2	42	Family	Individual plant selections in Expt 1, showing a diverse range seed and forage production traits
PA29-31	14	Family	Progeny from individual field selections from Barmedman, NSW[A]
Recruiter	1	Family	Demonstrated capacity to recruit near established lucerne plants at Barmedman, NSW
SA41020, SA42566, SA42858	3	Accession	Good performance in regional row nurseries
SA45391, SA42564, SA4966	3	Accession	Repeated from Expt 1
		C. pallidum	
SA42722	1	Accession	Bluegreen aphid resistant (*Acyrthosiphon kondoi* Shinji) population[A]

[A]Hayes *et al*. (2009).

Statistical analyses

Means of fixed entry effects for each response variate (edible biomass, canopy density, flowering intensity, anthracnose resistance, days from flowering to harvest, days to harvest, and seed yield in Expt 1; and forage yield, seeds per inflorescence, and seed yield in Expt 2) were calculated using spatial linear mixed models performed by GENSTAT 15 (Lawes Agricultural Trust 2012). Diagnostic plots of sample variograms and residuals were used in conjunction with REML log-likelihood ratios and Wald tests to fit new models that compartmentalised and removed random and fixed effects of variation (Smith *et al.* 2005). Estimates of genetic variance were made using entry as a component of the random model. Heritability was calculated using the equation $h^2 = Yg/(Yg + Ye)$, where Yg is genotypic variance and Ye is residual variance ($Yg + Ye$ = phenotypic variance; Mather and Jinks 1982). Correlation analysis was used to determine linear relationships between forage and seed yield components.

Results

Experiment 1

Edible biomass for *Cullen* ecotypes ranged from 70 to 420 g dry weight (DW) plant^{-1} with a mean of 251 g DW plant^{-1}. Accession SA42965 had the highest autumn edible biomass (420 g DW plant^{-1}) and canopy density (5.0), whereas accession SA4966 had the highest spring edible biomass (906 g DW plant^{-1}). Accession SA42965 was also the last entry to flower (269 days) but had less than the median of days to harvest (351 days *v.* the median value of 368 days). Heritability for edible biomass was high, with estimates of 0.68 for autumn and 0.43 for spring. Anthracnose (caused by *Colletotrichum trifolii*) had a severe impact on plant survival, resulting in the death of 14 of the ecotypes before any measurements on seed production. The remaining entries expressed low to moderate symptoms for this disease and received damage scores of 0.8–2.9.

Cullen pallidum entry SA44108 had moderately high autumn (281 g DW plant^{-1}) and spring (260 g DW plant^{-1}) edible biomass but a low seed yield (31 g DW plant^{-1}). The cross between *C. pallidum* and *C. australasicum*, SA42596, also had moderately high edible biomass (303 g DW plant^{-1} for autumn and 283 g DW plant^{-1} for spring) and a low seed yield (16 kg ha^{-1}). The *C. discolor*, *C. discolor* × *australasicum*, and *C. graveolens* entries displayed relatively low forage yield (35–280 g DW plant^{-1}) and seed yield (86–151 kg ha^{-1}).

Seed production for *Cullen* ecotypes was highly variable, with 0–790 kg ha^{-1} harvested from the windrow and ground (Table 4). Within *C. australasicum*, the highest total seed yield of 790 kg ha^{-1} was produced by SA42564 and the highest seed yield, harvested directly from the windrow of 485 kg ha^{-1}, was produced by SA42766. There was significant variation for pod retention, with the percentage of total seed harvested from the vine ranging from 0 to 99% (Table 4). Accession SA42772 had the highest ranking for number of inflorescences (23.4 out of a possible visual score of 30), and produced a high total seed yield of 594 kg ha^{-1}. Genetic variance for seed production was large relative to residual variance, representing a high percentage of the phenotypic variance, or heritability ($h^2 = 0.53$ for windrow-harvested seed yield and 0.77 for total seed yield).

The relationships between seed and forage production traits are presented in a correlation matrix (Table 5). Windrow-harvested seed yield was positively correlated with total seed yield ($r = 0.85$), autumn edible biomass ($r = 0.40$), and spring edible biomass ($r = 0.55$). Autumn edible biomass was highly correlated with spring edible biomass ($r = 0.62$). Anthracnose damage had a high negative correlation with autumn edible biomass ($r = –0.45$) and spring edible biomass ($r = –0.46$), and a high positive correlation with the number of days to harvest ($r = 0.52$). Anthracnose damage score was negatively correlated with windrow-harvested seed yield ($r = –0.32$) but not total seed yield. Windrow-harvested seed yield was negatively correlated with the number of days to harvest ($r = –0.32$) but not with the number of days to flowering or the number of days between first flower and harvest.

Experiment 2

Seed yield of progeny families ranged from 128 to 1423 kg ha^{-1}, and was highly correlated ($r = 0.59$) with forage yield, which ranged from 404 to 1248 g DW plant^{-1} (Fig. 3*a*, Table 2). Individual plants grew to up to 2.5 m high and 1.2 m^2 in area, such that the plants with the highest forage yield produced up to 10.4 t DW ha^{-1} (based on the distribution of spaced plants). Seed yield per plant was also highly correlated with the number of seeds per inflorescence ($r = 0.82$; Fig. 3*b*), and heritability for

Table 2. Seed and forage production of progeny lines selected from Expt 1 and used in Expt 2
n.d., Missing data resulting from death of all individuals in the line before seed was harvested

Species	Parent (SA)	Line	Seed yield (kg ha^{-1})	Forage yield (g plant^{-1})	No. of seeds per peduncle
C. australasicum	–	SA 41020	n.d.	n.d.	n.d.
C. australasicum	–	SA 42564	169	643	13
C. australasicum	–	SA 42566	n.d.	n.d.	n.d.
C. australasicum	–	SA 42722	n.d.	n.d.	n.d.
C. australasicum	–	SA 42858	332	955	19
C. australasicum	–	SA 45391	321	534	17
C. australasicum	42690	101/1	n.d.	n.d.	n.d.
C. australasicum	45573	104/1	580	404	37
C. australasicum	4966	107/1	991	1040	47
C. australasicum	42965	111/1	649	750	22
C. australasicum	42603	115/1	1166	1048	60
C. australasicum	45493	135/1	897	821	48
C. australasicum	44381	155/1	787	695	39
C. australasicum	44381	155/2	747	841	30
C. australasicum	42766	156/1	421	825	26
C. australasicum	45562	160/1	423	774	22
C. australasicum	45496	165/1	692	811	25
C. australasicum	41272	169/1	780	484	48
C. australasicum	45576	173/1	683	848	39
C. australasicum	45576	173/2	663	632	34
C. australasicum	45574	175/1	326	508	19
C. australasicum	45574	201/1	397	561	35
C. australasicum	45562	206/1	647	586	39
C. australasicum	44783	220/1	1124	1059	45
C. australasicum	45572	226/1	565	609	39
C. australasicum	42766	232/1	421	410	34
C. australasicum	4966	236/1	463	809	28
C. australasicum	44381	249/1	792	773	38
C. australasicum	45563	259/1	379	590	21
C. australasicum	45576	269/1	631	912	31
C. australasicum	44775	271/1	n.d.	n.d.	n.d.
C. australasicum	45561	277/1	425	612	19
C. australasicum	42781	278/1	360	626	14
C. australasicum	4685	304/1	400	752	24
C. australasicum	45574	313/1	924	658	42
C. australasicum	45562	324/1	286	539	20
C. australasicum	45575	328/1	1094	998	48
C. australasicum	42603	334/1	789	663	45
C. australasicum	45496	341/1	823	774	53
C. australasicum	4966	343/1	846	1100	44
C. australasicum	44775	358/1	492	441	28
C. australasicum	44775	358/2	306	547	22
C. discolor	45388	359/1	353	591	24
C. australasicum	45493	362/1	813	798	53
C. australasicum	45493	362/2	554	729	30
C. australasicum	45573	372/1	519	471	34
C. australasicum	45426	378/1	631	432	40
C. australasicum	44381	379/1	760	785	59
C. australasicum	45469	402/1	651	678	54
C. australasicum	4966	pa29/1	696	1103	36
C. australasicum	4966	pa29/2	1423	1151	57
C. australasicum	4966	pa29/3	266	504	24
C. australasicum	4966	pa29/4	882	928	38
C. australasicum	4966	pa29/5a	526	837	26
C. australasicum	4966	pa29/5b	802	1248	29
C. australasicum	4685	pa30/1	128	515	10
C. australasicum	4685	pa30/2	n.d.	n.d.	n.d.
C. australasicum	4685	pa30/3	959	1178	37

(*Continued next page*)

Table 2. (*continued*)

Species	Parent (SA)	Line	Seed yield (kg ha^{-1})	Forage yield (g plant^{-1})	No. of seeds per peduncle
C. australasicum	4685	pa30/4	629	1174	32
C. australasicum	4685	pa30/5	674	1022	38
C. australasicum	41020	pa31/1	423	517	40
C. australasicum	41020	pa31/2	986	693	49
C. australasicum	41020	pa31/3	875	747	51
C. australasicum	Unknown	Recruiter	375	906	19
Av. l.s.d. ($P = 0.05$)			640	751	36
F-prob.			0.004	0.008	<0.002
$h^2 = Vg/(Vg + Ve)$			0.31	0.32	0.35

Table 3. Measurements used in the evaluation of *Cullen* spp. germplasm at the SARDI Genetic Resource Centre

Name	Descriptor	Expt no.	Date measured	Description
Edible BA, Edible BS	Edible biomass autumn, Edible biomass spring	1, 2	July (Expt 1 only), December	Adelaide method: a subsample of forage considered to be edible (non-woody) is hand-held as a reference unit and the number of units in each plot is assessed visually. Yield per plant is quantified using correlation between the sample and the representative plant scored in each plot (Andrew *et al*. 1979; Gholinejad *et al*. 2012)
Canopy D	Canopy density	1	August	Visual assessment of forage density scored 1–5, where 1 is thin density and 5 is dense
Days Flwr	Days to first flower	1	At first single flower	Number of days from 1 January to the emergence of the first flower
No. Inf	Number of individual flowers	1	12, 19, 26 Oct. 2009	Visual assessment scored 1–10, where 1 is no flowers and 10 is very large number of flowers. Value is sum of three measurements taken 7 days apart
Anth	Anthracnose damage	1	August	Visual assessment scored 1–10, where 1 is no damage and 10 is dead plant
Days F–H	Days to harvest from first flowering	1	At harvest	Number of days from the appearance of first flowers to harvest date
Days H	Days to harvest	1	At harvest	Number of days from 1 January to harvest date visually determined when ≥30% of pods contain mature seed.
Wind Sd	Windrow seed weight		At harvest	Yield of seed cleaned from windrowed forage (kg ha^{-1}). Forage cut by hand-shears and dried for 3 days before threshing using small-scale, commercial equipment
Grnd Sd	Ground seed weight		At harvest	Yield of vacuum-harvested seed from the ground of the whole plot (kg ha^{-1}). Seed is vacuum-harvested from the ground 1 week after windrowed seed yield is harvested
Total Sd	Total seed weight	1, 2	At harvest	Windrowed seed plus ground seed weight.
Seeds/inflorescence	Seed per inflorescence	1, 2	At harvest (Dec. 2011)	Number of seeds retained on a mature reproductive stem at harvest, measured on selected individuals in Expt 1, and all progeny in Expt 2

this trait was moderate ($h^2 = 0.35$). The seed yield of progenies harvested in Expt 2 was positively correlated with the seed yield of their parent accession in Expt 1 ($r = 0.30$, Fig. 3*c*), and the number of seeds per inflorescence in these progeny rows was highly correlated with that of their parents in Expt 1 ($r = 0.51$, Fig. 3*d*).

Alfalfa mosaic virus (AMV, genus *Alfamovirus*, family *Bromoviridae*) significantly reduced the forage and seed production of individual plants in Expt 2. Of the 564 individuals, 133 displayed severe yellow mosaic and leaf distortion symptoms, similar to those observed by Nair *et al*. (2009), and in 67 of these individuals the distortion was followed by stunting, necrosis, and death (all 167 diseased plants were treated as missing values in the statistical analysis). There was no significant effect of variety on plant damage, which appeared to be randomly distributed through the experiment (data not shown).

Discussion

Selecting/breeding Cullen australasicum *with high seed yield*

Seed production in *C. australasicum* is very diverse, with mechanically harvestable seed yield from windrows of 0−485 kg ha^{-1} in Expt 1, and 128−1 423 kg ha^{-1} in Expt 2. The ratio of genetic to environmental variance for seed production was moderate to high ($h^2 = 0.50-0.77$), indicating that this trait would respond to improvement from selection and breeding. Levels of seed production in this experiment were similar to those achieved by Kobelt *et al*. (2011), where up to 700 kg ha^{-1} of windrow-harvested seed was produced. The results show that high levels of seed production, ~1 t ha^{-1}, can be produced under rain-fed conditions.

Seed production in Expt 2, under rain-fed conditions, was higher than the seed yields achieved in Expt 1, where rainfall was

Table 4. Assessment of seed production traits of a diverse range of *Cullen* spp. for the entries in Expt 1

Edible BA, Autumn edible biomass; Canopy D, canopy density; Days Flwr, no. of days from 1 January to first flower; No. Inf, no. of inflorescences; Edible BS, spring edible biomass; Anth, anthracnose damage; Days F–H, no. of days from flowering to harvest; Days H, no. of days from 1 January to harvest; Wind Sd, seed yield harvested from windrow; Grnd Sd, seed yield harvested from the ground; Total Sd = Grnd Sd + Wind Sd; %Harv, percentage of total seed harvested from windrow. n.d., Missing data resulting from death of the accession before seed was harvested; n.h., not ground harvested

Species	Accession	Edible BA ($g\ plant^{-1}$)	Canopy D (1–5)	Days Flwr (days)	No. Inf (1–10)	Edible BS ($g\ plant^{-1}$)	Anth (1–10)	Days F–H (days)	Days H	Wind Sd ($kg\ ha^{-1}$)	Grnd Sd	Total Sd	%Harv
C. australasicum	4685	413	5.0	251	12.5	554	1.9	100	350	193	207	400	48
C. australasicum	4966	327	4.3	228	11.3	906	2.9	130	359	171	194	365	47
C. australasicum	41020	303	4.1	237	15.3	600	1.7	118	358	157	2	159	99
C. australasicum	41272	303	4.4	262	14.2	377	1.6	90	352	280	200	480	58
C. australasicum	42564	233	3.2	253	9.9	354	3.6	142	395	367	423	790	46
C. australasicum	42566	233	3.2	260	16.5	64	3.5	125	383	49	n.h.	49	n.h.
C. australasicum	42567	70	1.9	246	13.4	57	2.0	132	377	17	10	27	63
C. australasicum	42603	303	4.6	249	17.5	620	1.5	96	347	419	95	514	82
C. australasicum	42690	327	4.0	233	14.4	337	2.1	172	403	60	61	121	50
C. australasicum	42723	257	3.8	237	10.6	96	3.3	117	353	89	n.h.	89	n.h.
C. australasicum	42726	350	3.6	240	19.3	219	2.8	107	349	94	n.h.	94	n.h.
C. australasicum	42733	187	3.3	239	19.1	204	1.5	107	348	61	2	63	97
C. australasicum	42736	233	3.5	238	17.3	175	3.5	115	354	92	3	95	97
C. australasicum	42741	210	3.3	236	17.3	252	3.2	191	429	143	n.h.	143	n.h.
C. australasicum	42745	233	3.7	247	14.9	187	3.4	140	390	187	208	395	47
C. australasicum	42749	187	3.4	242	n.d.	n.d.	7.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	42751	187	3.3	244	14.3	406	3.4	129	371	114	21	135	84
C. australasicum	42762	210	3.4	239	15.8	188	4.0	94	334	35	14	49	71
C. australasicum	42766	280	3.9	241	19.0	383	1.4	144	384	485	174	659	74
C. australasicum	42772	233	3.3	228	23.4	435	1.9	128	353	371	223	594	62
C. australasicum	42778	280	3.4	240	17.3	186	2.5	133	369	103	n.h.	103	n.h.
C. australasicum	42781	216	3.1	242	14.4	189	2.2	123	365	63	65	128	49
C. australasicum	42791	175	2.1	238	8.4	56	4.7	151	389	56	n.h.	56	n.h.
C. australasicum	42808	210	2.9	235	n.d.	n.d.	9.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	42825	216	2.6	247	12.8	163	4.1	135	385	74	8	82	90
C. australasicum	42851	93	2.4	228	11.0	99	5.1	196	425	58	2	60	97
C. australasicum	42858	210	4.0	241	14.5	73	1.7	148	393	39	13	52	75
C. australasicum	42866	163	2.9	237	12.6	51	1.3	173	408	107	22	129	83
C. australasicum	42883	203	3.1	n.d.	n.d.	n.d.	8.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	42885	303	4.7	262	9.5	239	1.4	100	363	193	5	198	97
C. australasicum	42965	420	5.0	269	8.0	431	2.2	85	351	44	47	91	48
C. australasicum	44100	233	3.7	247	13.8	277	2.8	143	386	118	8	126	94
C. australasicum	44101	216	2.7	236	16.5	158	2.6	138	375	27	39	66	41
C. australasicum	44228	280	3.0	233	18.8	143	5.7	155	385	63	n.h.	63	n.h.
C. australasicum	44239	117	2.0	234	n.d.	n.d.	8.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	44246	163	3.6	233	n.d.	n.d.	8.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	44276	280	3.7	251	17.1	362	2.7	89	343	104	50	154	68
C. australasicum	44286	373	3.9	238	15.5	393	2.1	160	396	65	n.h.	65	n.h.
C. australasicum	44341	251	3.0	247	11.2	120	4.8	179	428	57	295	352	16
C. australasicum	44373	163	3.0	n.d.	n.d.	n.d.	8.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	44378	181	2.6	237	18.8	88	4.6	145	386	40	n.h.	40	n.h.
C. australasicum	44380	327	4.0	241	20.1	510	2.9	96	338	187	7	194	96
C. australasicum	44381	280	4.0	241	15.8	799	1.4	100	343	144	12	156	92
C. australasicum	44383	257	3.4	244	18.8	270	4.2	122	365	223	n.h.	223	n.h.
C. australasicum	44388	420	4.0	265	8.2	353	1.3	93	362	0	45	45	0
C. australasicum	44468	233	4.3	257	n.d.	n.d.	9.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	44775	303	5.0	269	8.5	584	1.5	110	376	22	9	31	71
C. australasicum	44783	350	4.0	269	6.0	767	0.7	78	343	429	n.h.	429	n.h.
C. australasicum	45385	117	3.3	259	11.3	84	4.3	113	374	74	4	78	95
C. australasicum	45387	70	2.7	257	11.8	50	4.1	190	443	4	21	25	16
C. australasicum	45391	187	2.7	251	14.3	99	4.8	141	400	15	420	435	3
C. australasicum	45426	233	3.6	243	13.0	378	2.3	135	383	43	10	53	81
C. australasicum	45429	280	3.8	247	15.5	205	1.6	124	370	79	n.h.	79	n.h.
C. australasicum	45446	163	2.7	237	n.d.	n.d.	9.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. australasicum	45462	397	4.3	254	14.4	102	2.2	113	369	86	9	95	91

(*Continued next page*)

Table 4. (*continued*)

Species	Accession	Edible BA (g plant^{-1})	Canopy D (1–5)	Days Flwr (days)	No. Inf (1–10)	Edible BS (g plant^{-1})	Anth (1–10)	Days F–H (days)	Days H	Wind Sd	Grnd Sd (kg ha^{-1})	Total Sd	%Harv
C. australasicum	45493	350	4.0	250	16.5	357	1.6	89	338	334	6	340	98
C. australasicum	45496	233	3.7	243	15.7	570	1.6	93	333	78	8	86	91
C. australasicum	45561	233	4.3	265	12.9	258	0.8	89	354	42	n.h.	42	n.h.
C. australasicum	45562	303	5.0	249	14.8	466	0.9	88	341	197	n.h.	197	n.h.
C. australasicum	45563	257	4.6	250	15.0	390	2.5	107	356	18	n.h.	18	n.h.
C. australasicum	45572	350	4.4	254	13.6	363	1.3	71	331	76	n.h.	76	n.h.
C. australasicum	45573	280	4.3	244	17.5	451	1.3	94	338	116	n.h.	116	n.h.
C. australasicum	45574	327	5.0	262	14.9	514	1.8	72	338	238	19	257	93
C. australasicum	45575	303	4.7	259	14.2	355	2.0	78	338	93	5	98	95
C. australasicum	45576	233	4.4	259	12.5	538	2.6	83	342	160	n.h.	160	n.h.
C. discolor	42973	35	0.9	268	12.5	76	0.3	78	350	10	141	151	7
C. discolor × *australasicum*	42974	35	1.1	265	17.0	98	2.1	97	363	32	54	86	37
C. discolor × *australasicum*	45388	93	2.5	242	7.5	106	5.3	169	409	51	75	126	40
C. graveolens	44393	280	4.0	249	2.9	76	4.7	112	360	0	50	50	0
C. graveolens	45433	35	0.9	287	13.0	20	0.0	83	368	0	7	7	0
C. graveolens	45531	n.d.	n.d.	n.d.	n.d.	n.d.	9.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C. pallidum	41740	35	1.0	278	14.3	69	3.1	157	435	8	37	45	18
C. pallidum	42722	70	1.9	265	8.1	37	9.8	159	427	27	106	133	20
C. pallidum	44108	281	3.3	259	12.5	260	6.0	133	392	65	48	113	58
C. pallidum	44112	181	1.9	245	18.6	209	5.5	189	431	49	16	65	75
C. pallidum	44385	58	1.0	268	9.0	81	7.6	105	374	5	68	73	7
C. pallidum	44387	7	1.1	278	13.3	57	10.1	142	418	9	1	10	90
C. pallidum	45390	70	1.1	291	3.5	58	9.6	101	385	2	83	85	2
C. pallidum × *australasicum*	42596	303	3.3	242	17.0	283	2.0	172	413	9	46	55	16
Av. s.e.d.		63	0.4	7.3	2.7	122	1.2	18	18	108	32	140	n.h.
F-prob.		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.h.	n.h.
l.s.d. ($P = 0.05$)		119	0.8	13.8	5.1	232	2.2	35	34	205	61	266	

supplemented with irrigation. The change in management practice followed a recommendation by Kobelt *et al*. (2011) that reducing soil moisture during flowering improved the synchrony of flowering, pod set, and maturation, resulting in improved seed yields. In this study, the improved management in Expt 2 is confirmed by the *y*-intercept of 550 kg ha^{-1} on the trend line in Fig. 3*c*, which compares with the seed production of related plants in each experiment. In faba beans (*Vicia faba* L.), a water shortage during flowering can increase seed yield by 20−60%, and water supply patterns can account for >90% of the variation in seed yield (Grashoff 1990). Forage yield, as a surrogate measure of water use, explained 75−85% of seed yield (Grashoff 1990). In this study, windrow-harvestable seed yield was also highly defined by spring forage yield, with 55% of the variation attributable to this measurement (Table 5).

Cullen spp. has the potential to be a high-yielding forage plant for medium-rainfall environments in southern Australia. In this study, mature plant edible biomass in second-year stands of up to 1248 g DW plant^{-1} (equal to ~10.4 t DW ha^{-1}) was measured on plants growing 2.5 m high. The heritability of edible biomass was moderate to high (0.68 in Expt 1 and 0.32 in Expt 2), indicating that forage production should also respond to improvement from selection and breeding, but sources of environmental error (possibly associated with AMV infection in Expt 2) need to be minimised. Accession SA4966 had the highest edible spring biomass in this study, and was previously ranked in the top three ecotypes of *Cullen* spp. for high spring leaf (Bennett *et al*. 2012) and total forage (Hayes *et al*. (2009) production in experiments evaluating diverse collections of *Cullen* spp. in low–medium-rainfall environments. This accession also performed well relative to lucerne in national evaluation trials at five locations (Li *et al*. 2008), and in two additional sites in the low–medium-rainfall wheatbelt of southern New South Wales (Dear *et al*. 2007). A selection from SA4966, denoted pa29/5b, was also the highest yielding line in Expt 2, indicating that this line should feature prominently in future breeding activities.

Cullen pallidum, *C. discolor*, and *C. graveolens* ecotypes produced less seed and forage than *C. australasicum* ecotypes assessed in this study, and had higher levels of damage from anthracnose. The superior productivity of *C. australasicum* is supported by the findings of Hayes *et al*. (2009) and Bennett *et al*. (2012), which compared the yield and persistence of *Cullen* spp. ecotypes in low-rainfall environments. Despite their relatively low field production, *C. pallidum* and *C. discolor* can form inter-specific hybrids with *C. australasicum* and may, therefore, add value to future breeding programs by contributing genes for specific traits.

For the future development of *Cullen* as a forage plant, our results indicate that selection for forage yield will often result in increased seed production, due to the positive relationship found between these traits in both experiments (Table 5, Fig. 3*a*). Seed-production traits recorded moderate to high heritability, and

Table 5. Correlation matrix of forage and seed production traits of *Cullen australasicum*

Edible BA, Autumn edible biomass; Days Flwr, no. of days from 1 January to first flower; No. Inf, no. of inflorescences; Edible BS, spring edible biomass; Anth, anthracnose damage; Days F–H, no. of days from flowering to harvest; Days H,, no. of days from 1 January to harvest; Wind Sd, seed yield harvested from windrow; Grnd Sd, seed yield harvested from the ground; Total Sd, Grnd Sd + Wind Sd. Italicised values are significant at $P = 0.05$; and values italicised and bold are significant at $P = 0.01$ for 78 pairs of data

	Edible BA	Days Flwr	No. Inf	Edible BS	Anth	Days F–H	Days H	Wind Sd	Grnd Sd
Days Flwr	*–0.23*	–							
No. Inf av.	0.13	***–0.48***	–						
Edible BS	***0.62***	–0.16	0.12	–					
Anth	***–0.45***	–0.03	*–0.29*	***–0.46***	–				
Days F–H	***–0.31***	***–0.42***	0.05	***–0.39***	***0.4***	–			
Days H	***–0.47***	–0.01	–0.16	***–0.52***	***0.52***	***0.91***	–		
Wind Sd	***0.4***	–0.17	*0.25*	***0.55***	***–0.32***	–0.21	***–0.32***	–	
Grnd Sd	0.08	–0.05	–0.04	0.07	0.08	0.14	0.14	***0.36***	–
Total Sd	*0.27*	–0.11	0.12	***0.4***	–0.16	–0.06	–0.12	***0.85***	***0.79***

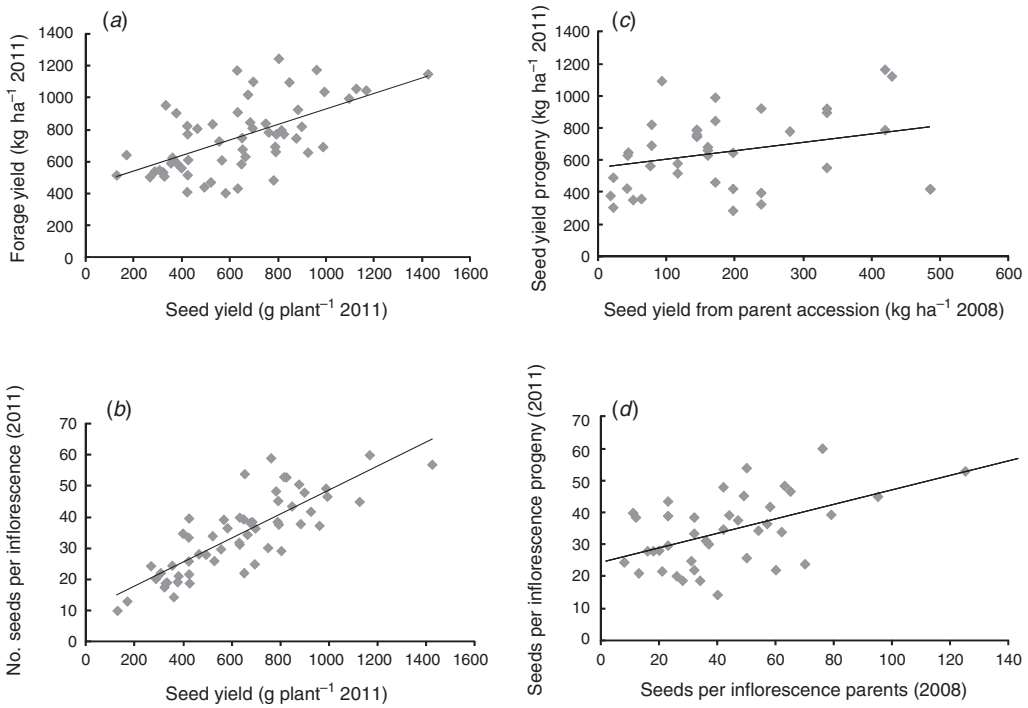

Fig. 3. Relationship between seed yield (per plant and per inflorescence) and forage yield harvested in Expts 1 and 2, comparing: (*a*) seed yield in 2011 with forage yield in 2011, $r = 0.59$; (*b*) seed yield in 2011 with the number of seeds per inflorescence in 2011, $r = 0.82$; (*c*) seed yield of the parent accession in 2009 with the seed yield of progeny selected from a single plant from within the accession in 2011, $r = 0.30$; and (*d*) number of seeds per inflorescence of individual parents in 2009 with that of their progeny in 2011, linear regression: $y = 0.23x + 24$, $r = 0.51$.

selection for the number of mature seeds per inflorescence at harvest provides a quick and easy proxy for seed yield (Fig. 3*b*). The number of mature seeds per inflorescence is influenced strongly by the capacity of the plant to retain mature pods on the vine. The timing of the harvest in Expt 2 was successful in capturing variation for the number of mature seeds per inflorescence, and similar techniques have been used by breeders to exploit variation in pod retention and harvestable seed yield of lupin (*Lupinus angustifolius* L.; French and Buirchell 2005) and lentil (*Lens culinaris* Medik; Erskine 1985). The identification of germplasm with high seed yield in this study suggests that the only major hurdle in the development of *Cullen* as a commercial species is now likely to be associated with selection for disease resistance or its management through improved agronomy.

Potential influence of diseases on commercial development

Cullen was severely damaged by anthracnose in Expt 1, as previously reported by Nair *et al*. (2010). The variation in damage scores for anthracnose, and the high estimate of heritability for this score ($h^2 = 0.75$), suggest that disease infection was uniform

across the site, and that damage can be reduced through plant selection. These observations for plant resistance or tolerance should be confirmed in a further study using a controlled environment. The Waite Campus received rainfall well above average and minor flooding in July 2009, and this is likely to have increased the severity of anthracnose infection. No visual symptoms of anthracnose damage were observed in the following experiment, which also had a recent history of lucerne production and therefore a likely source of inoculum. It is therefore likely that this impediment to seed production can be overcome with either the use of resistant lines and/or the use of seed production ground with low levels of anthracnose inoculum.

The severe yellow mosaic and leaf distortion symptoms identified in Expt 2 are most likely associated with AMV, as reported by Nair *et al*. (2009), but further work is required to ascertain whether AMV was the sole causal agent of the mosaic disease, and whether the stunting, necrosis, and death in many of the plants displaying these symptoms was caused by a secondary agent. Intolerance to AMV is a threat to the use of this species in Australia, given the severe damage caused in Expt 2 and the widespread use of annual *Medicago* and *Trifolium* pastures, lucerne, and crop legumes that can provide sources of inoculum for transmission of the virus (Latham and Jones 2001*a*, 2001*b*). A lucerne nursery was directly adjacent to Expt 2, and infection from this area with sap-sucking insects (Hiruki and Hampton 1990) may have increased the rate of infection above what is likely to occur in commercial production. Other factors, such as sowing infected seed stock (Latham and Jones 2001*b*) and infection with machinery (Hiruki and Hampton 1990), may also account for the source and rapid spread of AMV infection in Expt 2, but transmission of AMV through mechanical inoculation or seed has not been observed in preliminary experiments (Nair *et al*. 2009). Although previous reports have identified a high incidence of AMV infection (~80%; Nair *et al*. 2009), this is the first report of infected plants becoming stunted, necrotic, and dead. Plant death from AMV infection is uncommon in other pasture legumes, but does occur in biserrula (*Biserrula pelecinus* L.), and other plants, such as French serradella (*Ornithopus sativus* Brot.), are also considered susceptible and highly sensitive (Latham and Jones 2001*b*). Selection for resistance or tolerance to AMV has been found in many other legumes species (Latham and Jones 2001*b*) and would clearly need to be considered in any future genetic improvement strategy for *Cullen*. Aphid resistance is likely to contribute in reducing AMV infection (Garran and Gibbs 1982), but aphid-resistant *Cullen pallidum* accession SA42722 (Hayes *et al*. 2009) had all eight of its plants killed by AMV in Expt 2. Another strategy that may contribute to a solution is to develop a *Cullen* variety that has the capacity to regenerate through seedling recruitment, providing that seed transmission is practically insignificant.

The concept of developing a *Cullen* variety with the capacity to recruit seedlings under favourable conditions is supported by observations of natural recruitment in arid environments. Although the low level of pod retention observed within most Cullen ecotypes is a negative trait for commercial seed production of *Cullen*, it has undoubtedly contributed to the success of the species in arid environments. Seed dispersal, combined with high levels of hard seededness, are necessary adaptations of plants to environments with highly variable rainfall patterns (Crawford *et al*. 1989; Real *et al*. 2012), and there is some potential to mimic the ley farming system success of annual medics (*Medicago* spp.), whereby these traits are critical factors to the success of the farming system (Crawford *et al*. 1989). The breeder's line 'Recruiter', evaluated in Expt 2, is a field selection identified for its ability to recruit seedlings at Barmedman, New South Wales. The Recruiter line had high forage production (906 g DW plant^{-1}), but the compromise in seed yield (375 kg ha^{-1}) and low level of pod retention (19 seeds per inflorescence) may not represent an acceptable balance for commercial production. Further studies are required to show whether this or other *Cullen* germplasm may be successful in a system based on self-regeneration via seedling recruitment.

Conclusions

High commercial seed yields of >1 t ha^{-1}, directly harvested from a windrow, are achievable for *C. australasicum* using a combination of the best genetics and seed production management practices. This should ensure that the production of low-cost seed is not a limiting factor to the adoption of this species as a forage plant in low-input systems. Further research is required to investigate possible cultural or genetic strategies to reduce AMV infection or damage before this species can be considered a viable forage option. Overall, the high fodder and seed production potential demonstrated in this study indicates that *C. australasicum* may emerge as a viable, drought-tolerant alternative for graziers in semi-arid to medium-rainfall environments of southern Australia.

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