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## Seed production and hard seededness of *Trifolium subterraneum* subsp *brachycalycinum*

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# Technical Bulletin

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**Seed production and hard-  
seededness of *Trifolium*  
*subterraneum* subsp. *brachycalycinum***

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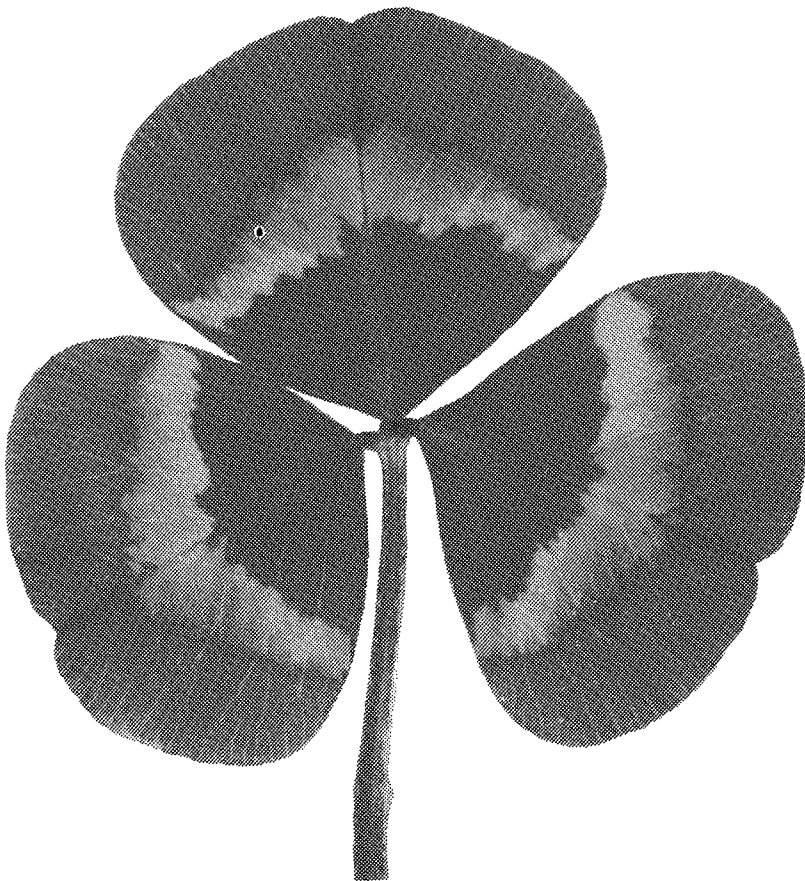
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The distinctive leaf markings of  
Clare, the only cultivar of *Trifolium*  
*subterraneum* subsp. *brachycalycinum*  
grown commercially in Australia.

Most pastures in south-western Australia are based on subterranean clover (*Trifolium subterraneum*) (Rossiter 1978), an annual legume which originates from countries surrounding the Mediterranean Sea and on the Atlantic seaboard of western Europe (Gladstones and Collins 1983). The plant was originally accidentally introduced into Australia by early European settlers, and strains have since become naturalized throughout southern Australia (Donald and Neal Smith 1937; Aitken and Drake 1964; Harrison 1964; Morley and Katznelson 1965; Gladstones 1966, 1967; Gladstones and Collins 1983, 1984). Once the value of subterranean clover as a stock feed and for improving soil organic nitrogen levels was realized, scientists purposefully collected the naturalized Australian strains (see Gladstones and Collins 1983), as well as many strains from the Mediterranean countries and Europe with a view to testing them under Australian conditions and releasing the more promising lines as cultivars (Gladstones and Collins 1983, 1984).

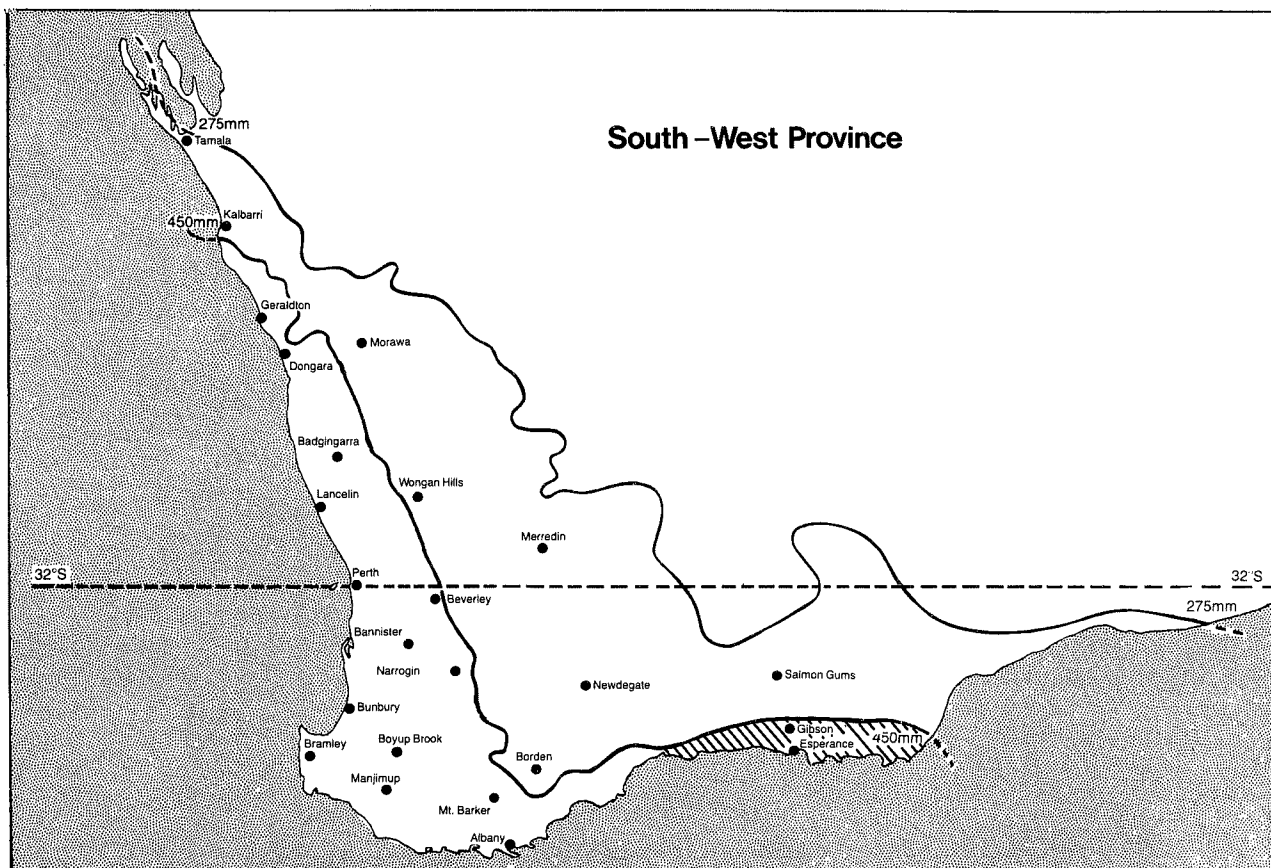
There are three subspecies of subterranean clover (Katznelson and Morley 1965):

- subspecies *subterraneum* (S types), which include most of the Australian clover cultivars such as: Nungarin, Northam, Dwalganup, Geraldton, Daliak, Dalkieth, Seaton Park, Woogenellup, Esperance, June, Green Range, Karridale and Mt Barker.
- subspecies *yanninicum* (Y types), the present Australian cultivars are Yarloop, Trikkala, Larisa and Meteora.
- subspecies *brachycalycinum* (B types), Clare is the only present Australian cultivar.

S types have proved to be successful in the > 400 mm/a average rainfall areas of south-western Australia, and Y types in the > 500 mm/a areas. B types have not been as intensively researched, but Clare is successful on the red-brown earth soils in South Australia (Cocks and Phillips 1979). Light has been found to inhibit the development of seed in subterranean clover burrs (Taylor 1979), and they must bury their burrs to produce seed, either by actively pushing burrs into the soil or by finding suitable dark habitats for the developing burrs (Katznelson and Zohary 1970; Katznelson 1974).

## Introduction

S, Y and B types



The South-West Province of Western Australia, as determined by the 275 mm isohyet, (Johnston 1983) showing the research area near Esperance.

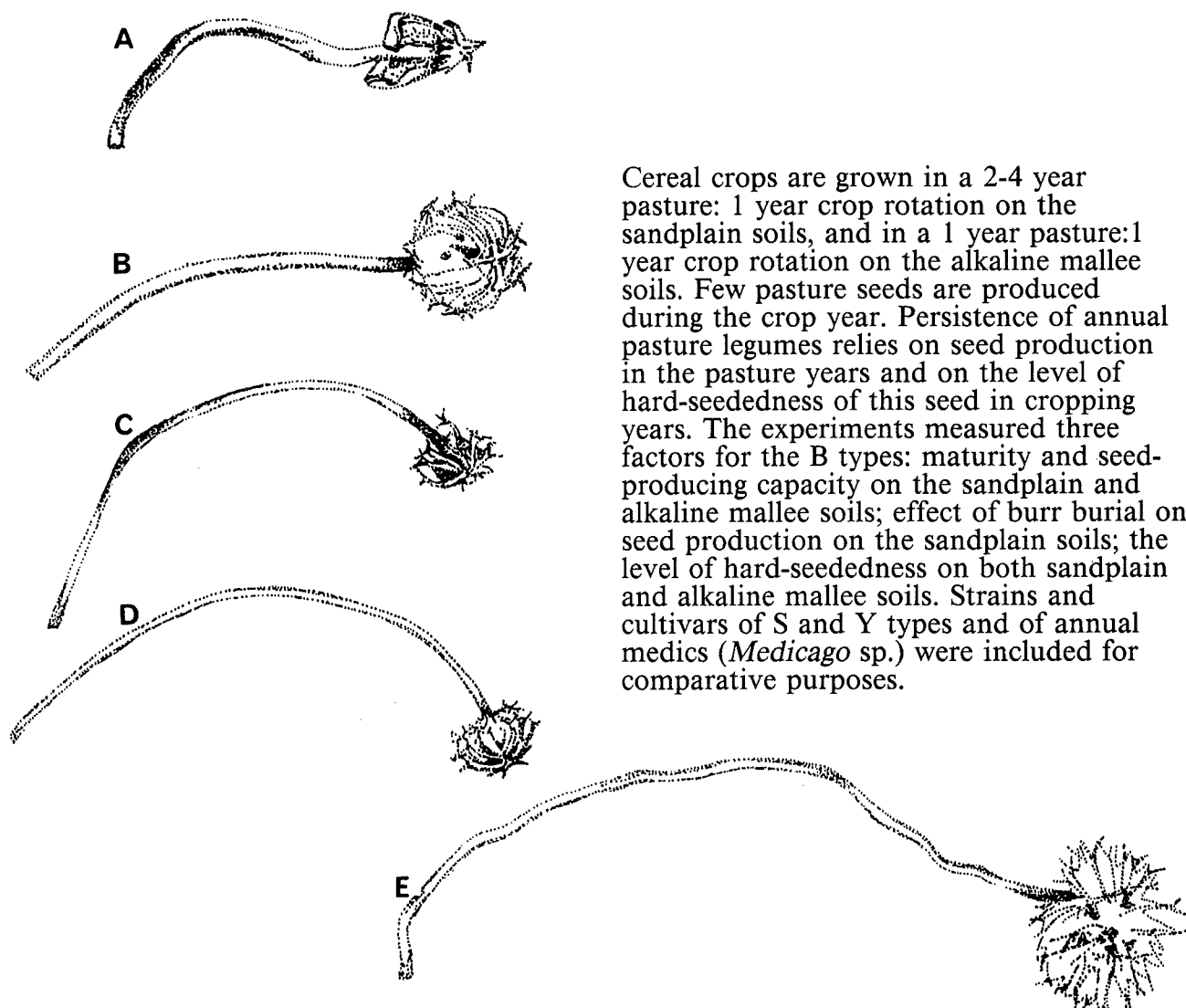
S and Y types are found growing naturally on neutral to moderately acidic soils (Katznelson 1974; Gladstones and Collins 1983). The Y types are more tolerant to waterlogging (Francis and Devitt 1969). S and Y types are often sown as a mixture in areas receiving > 500 mm/a average rainfall such as cvs Esperance and Trikkala near Esperance.

Both S and Y types actively bury their burrs (Katznelson and Morley 1965; Katznelson and Zohary 1970). The flower stalk or peduncle bends (reflexes) towards the soil surface, thickens and elongates and helps push the developing burr into the soil. The developing burr structure also helps to anchor the burr into the soil. Though seed production is markedly improved when burrs are buried (Quinlivan and Francis 1971; Collins *et al.* 1976) and seeds are produced in the dark (Taylor 1979), some

S types (such as Daliak) are capable of producing reasonable quantities of seed in unburied burrs (Quinlivan and Francis 1971; Collins *et al.* 1976).

The earliest maturing subterranean clover strains belong to the S and B types. B types are different to S and Y types in many ways and may in fact be a different species (Katznelson 1974; Gladstones and Collins 1983). B strains are found growing naturally on neutral to moderately alkaline soils (Katznelson 1974; Gladstones and Collins 1983). The B types do not appear to actively bury burrs, but passively find dark habitats. The flowers and burrs are produced on long, thin sarmentous peduncles which meander about until they find a suitably dark habitat in which the burrs complete their seed development (Katznelson and Morley 1965; Katznelson and Zohary 1970). Such habitats are in cracks, under rocks or within loose stones.





Cereal crops are grown in a 2-4 year pasture: 1 year crop rotation on the sandplain soils, and in a 1 year pasture:1 year crop rotation on the alkaline mallee soils. Few pasture seeds are produced during the crop year. Persistence of annual pasture legumes relies on seed production in the pasture years and on the level of hard-seededness of this seed in cropping years. The experiments measured three factors for the B types: maturity and seed-producing capacity on the sandplain and alkaline mallee soils; effect of burr burial on seed production on the sandplain soils; the level of hard-seededness on both sandplain and alkaline mallee soils. Strains and cultivars of S and Y types and of annual medics (*Medicago* sp.) were included for comparative purposes.

### Research on B types at Esperance

The B types in the National Subterranean Clover Collection in Perth include many early-maturing strains (B.J. Quinlivan and C.J.B. Sykes, personal communication) which have not been extensively tested in south-western Australia. Some of these B types have been assessed for their potential as pasture legumes on the two major soil types namely, the sandplain and alkaline mallee soils found in the farming areas near Esperance and the results are reported here.

The sandplain soils are adjacent to the coast. They are neutral to moderately acidic, sandy surfaced soils on which both S and Y types have been very successful (Quinlivan and Francis 1976). Mallee soils are found to the north and are neutral to alkaline. Some are heavy brown clays, but most are sandy surfaced over light yellow clay (duplex). Annual medics grow and persist successfully on these soils, with Cyprus barrel medic being the most widely sown on farms.

The peduncle is important in burr burial. The strong, thick peduncles A and B are from varieties of *Trifolium subterraneum*, which bury readily. C and D are from varieties (also *T. subterraneum*) with poor burying ability, while variety E (*T. brachycalycinum*) normally does not bury any burrs.

## Materials and methods

All experiments were on newly cleared soil.

### Maturity and seed yield

There were two experiments in 1979, one on sandplain soil near Condingup, 55 km east of Esperance and the other on alkaline mallee soil at Mt Ney, 65 km north-east of Esperance. In 1979, all species (table 1) were sown at 50 kg/ha and the plots were not grazed or defoliated. Plots were 1 x 10 m and surrounded by 2 m buffers. The design was a randomized block with three replications. Seed was inoculated and lime pelleted the day before sowing, using *Rhizobium trifolii* strain WU 95 for S, Y and B types, and on the alkaline mallee soils *Rhizobium meliloti* strains U 45 and SU 47 was used for the annual medics. The lime pelleted seed was mixed with fertilizer just before sowing, and hand-broadcast onto the soil surface. The fertilizer applied was: 450 kg/ha superphosphate No. 1 (9.6% P; 12% S; 23% Ca; 0.6% Cu; 0.3% Zn and 400 ppm Mo); 450 kg/ha superphosphate (9.6% P; 12% S; 23% Ca); and 100 kg/ha potassium chloride (50% K). After sowing, the plots were lightly hand-raked to cover the seed with soil. The plots were sprayed with 2 L/ha DDT after sowing to prevent damage by red-legged earthmite (*Halotydeus destructor*), and again during flowering to prevent the larvae of *Heliothis punctiger* from eating developing seed. During flowering, blue-green aphids (*Acyrtosiphon kondoi*) were controlled by applying 150 g/ha pirimicarb once a fortnight.

The time interval from sowing to appearance of first flowers (one flower/dm<sup>2</sup>) was recorded for each strain. Seed yields were measured just after all the plots had dried off by collecting burrs in random 10 x 100 cm quadrats. Both buried and unburied burrs were collected by digging the soil up to a depth of 5 cm within each quadrat and sieving out the burrs. The seed was removed from the burrs by threshing and was then weighed.

Encouraged by the 1979 results for seed yield of the B types, larger plots (2 x 40 m, surrounded by 2 m wide buffers) were sown on both soil types in 1981 adjacent to the 1979 experiments. Seed was sown as previously described at a sowing rate of 8 kg/ha. The plots were grazed with 4 dry sheep/ha. The stock were removed at the commencement of flowering. Other details are as for the 1979 experiments.

An ancillary experiment was sown in 1981 at the Mt Ney site adjacent to the main 1981 experiment. This experiment was not grazed. The experimental design was a randomized block with three replications and comprised two annual medic cultivars and 7 B type strains (table 3), which were sown at 8 kg/ha in plots 2 x 10 m.

Table 1.

1979 results from ungrazed plots of S and B types and annual medics sown in May 1979. M is the number of days from sowing to appearance of first flowers, and SY the seed yields. Numbers are Commonwealth Plant Introduction numbers given to each strain. The experiment near Condingup was sown May 22, 1979, and near Mt Ney May 23, 1979. The season started May 22, 1979, and seedlings emerged 5-7 days later.

	Condingup (sandplain soil)		Mt Ney (alkaline mallee soil)	
	M	SY	M	SY
	(days)	(kg/ha)	(days)	(kg/ha)
<i>Medicago truncatula</i>				
cv. Cyprus	Not sown		89	730
cv. Jemalong	Not sown		100	480
<i>Medicago littoralis</i>				
cv. Harbinger	Not sown	93	690	
<i>Trifolium subterraneum</i>				
S types				
cv. Nungarin	92	1050	95	70
cv. Daliak	98	850	Not sown	
cv. Seaton Park	105	680	Not sown	
cv. Woogenellup	108	320	Not sown	
56894D	99	610	100	270
56911	99	390	113	170
B types				
cv. Clare	125	230	119	80
19451	101	520	105	380
24417	88	100	100	550
25308B	101	310	104	290
28095	101	170	100	280
28096	114	770	104	240
28102	99	190	100	610
33242	99	14	100	390
33245	101	130	104	590
33248	99	110	100	510
56897A	101	370	113	170
69970A	101	420	119	70
69972	110	560	104	290
69973	114	460	104	470
69976	99	660	100	280
69981	101	120	113	220
69988	99	250	100	310
70058A	120	200	119	60
70058B	120	230	119	160
70082A	120	390	119	160
70094B	101	430	104	350
70099	120	320	119	160
70100	114	480	104	390
70124B	114	310	104	490
*Q 081	114	100	100	480
*Q 085	114	90	100	240
l.s.d. (P > 0.05)		106		83

\* Collected in Israel by B.J. Quinlivan.

Table 2.

1981 results from grazed plots of S and B types and annual medics sown in May 1981. M is the number of days from sowing to appearance of first flowers, and SY the seed yields. The plots were grazed with 4 adult dry sheep/ha from a week after emergence until the earliest strain started flowing. Numbers are Commonwealth Plant Introduction numbers given to each strain. The experiment at Condingup was sown May 18, 1981, and near Mt Ney on May 24, 1981. The season started on May 30, 1981, and seedlings emerged 5-7 days later.

	Condingup (sandplain soil)		Mt Ney (alkaline mallee soil)	
	M	SY	M	SY
	(days)	(kg/ha)	(days)	(kg/ha)
<i>Medicago truncatula</i>				
cv. Cyprus	Not sown		85	1050
cv. Jemalong	Not sown		91	506
<i>Medicago littoralis</i>				
cv. Harbinger	Not sown		89	881
<i>Trifolium subterraneum</i>				
S types				
cv. Nungarin	87	987	Not sown	
cv. Daliak	95	950	Not sown	
cv. Seaton Park	102	701	Not sown	
56894D	98	772	Not sown	
56911	98	403	Not sown	
B types				
cv. Clare	122	30	117	15
19451	99	2	95	0
24417	85	3	82	5
25308B	95	17	97	10
28095	95	11	99	5
28096	111	5	107	3
28102	93	12	91	0
33242	93	0	91	0
33245	95	13	93	17
33248	93	27	91	7
56897A	95	3	93	3
69970A	97	12	91	16
69972	105	6	97	3
69973	109	0	97	32
69976	95	32	93	5
69981	97	15	95	19
69988	93	2	89	41
70058A	113	47	105	21
70058B	113	5	103	4
70082A	111	18	107	11
70094B	97	0	91	8
70099	117	15	109	4
70100	107	24	101	0
70124B	109	5	103	10
*Q 081	105	0	101	2
*Q 085	105	2	101	0
l.s.d. (P < 0.05)	71		64	

\* Collected in Israel by B. J. Quinlivan.



Table 3.

Results for the 1981 ancillary experiment at Mt Ney. M is the number of days from sowing to appearance of first flowers, and SY the seed yields. Numbers are Commonwealth Plant Introduction numbers given to each strain. Seed (8 kg/ha) was sown on May 24, 1981. The season started on May 30, 1981, and seedlings emerged 5-7 days later.

	M (days)	SY (kg/ha)
<i>Medicago trunculata</i>		
cv. Cyprus	85	1283
<i>Medicago littoralis</i>		
cv. Harbinger	89	976
<i>Trifolium subterraneum</i>		
B types		
cv. Clare	117	105
25308B	97	406
28102	91	824
33245	93	708
69973	97	649
70124B	103	670
*Q 081	101	552
l.s.d. (P < 0.05)		87

\* Collected in Israel by B.J. Quinlivan.

### Burr burial

This experiment was sown in 1982 near Condingup, 60 km east of Esperance. The design was a split-plot randomized block with three replications. Strains were the main plots (table 4) and burr-burial treatments the subplots. Seed of S and B types were sown in two parallel 2 m rows which were 30 cm apart. Before the development of runners, a sheet of asbestos cement (1 m x 25 cm) was placed between the two rows in one half of each plot. Burrs which developed over the asbestos could not bury. In the second half of each plot, burrs which developed over the sand between the two rows were covered with 1.5 cm depth of sand to ensure that they were buried. The 7 g of seed used for each plot was inoculated with *Rhizobium trifolii* strain WU 95 and lime pelleted the day before sowing. Fertilizer was hand-broadcast onto the area and comprised: 900 kg/ha superphosphate (9.6% P; 11% S; 23% Ca); 100 kg/ha potassium chloride (50% K); 10 kg/ha copper sulphate (27% Cu); 2 kg/ha zinc oxide (80% Zn); 0.5 kg/ha molybdenum trioxide (67% Mo); 0.5 kg/ha cobalt sulphate (21% Co); 3 kg/ha sodium borate (11% boron). Insects were controlled as described earlier.

The plants were defoliated with pasture shears to a height of about 4 cm every 2 weeks until the earliest strain flowered.

Table 4. Seed yields at Esperance in the burr burial experiment.

Species	Seed yield (g/m <sup>2</sup> ) <sup>A</sup>		No of burrs/m <sup>2</sup>		No. of seeds/burr		Seed weight	
	Unburied	Buried	Unburied	Buried	Unburied	Buried	Unburied	Buried
<i>Trifolium subterraneum</i>								
subsp. <i>subterraneum</i>								
cv. Esperance	98.8	140.0	4800	6965	3.4	3.6	5.2	6.6
subsp. <i>yanninicum</i>								
cv. Trikkala	4.8	81.2	592	2684	2.8	2.9	5.1	9.5
subsp. <i>brachycalycinum</i>								
cv. Clare	41.2	118.4	1943	4095	2.9	3.5	5.3	9.1
CPI 19451B	78.4	199.6	3081	6314	3.0	3.4	8.7	10.7
CPI 25308B	51.6	126.0	1539	4320	2.8	2.9	7.3	11.6
CPI 28096	13.6	104.5	935	4580	2.9	3.1	5.0	9.8
CPI 33245	20.4	150.8	1732	4808	2.7	3.2	7.4	12.7
CPI 69976	7.6	70.9	884	8964	2.5	3.4	5.7	9.9
CPI 69988	4.8	173.3	332	6436	2.8	3.0	5.4	10.6
CPI 70082A	19.6	187.3	1952	5848	2.7	2.9	5.5	10.0
CPI 70100	24.4	81.2	1220	3751	2.7	3.0	5.1	9.2
CPI 70124B	42.1	210.4	1268	7924	2.6	3.2	5.6	12.0
l.s.d. (P < 0.05)								
Main treatments	30.7		956		0.7		0.8	
Subtreatments	7.6		358		0.1		.3	

A Data for seed yield were obtained from a different quadrat to that used for the other measurements.

B CPI, Commonwealth Plant Introduction number.

When the plants had dried off, burrs were collected from two 15 x 40 cm quadrats between the two rows, one from over the asbestos and one from over the sand. These samples were used to measure the number of burrs, seed per burr and seed weight. The seed was removed from the burrs by hand. In another quadrat (15 x 40 cm) from each half of each plot, seed was removed from the burrs by hand and used to determine seed yield.

#### Hard-seed studies

Hard-seeds of B and S types and of annual medics were grown near Esperance for three consecutive years (1981 to 1983). The pattern of softening of these hard-seeds was measured in an oven which was programmed to increase slowly from 15°C to 60°C and back to 15°C in 24 hours. This temperature fluctuation simulates the temperature fluctuations encountered at the soil surface in the field during summer in most of south-western Australia (Quinlivan 1961) and is used as a means of comparing the hard-seededness of annual legumes.

In 1981 the seed was grown near Mt Ney, in 1982 near Wittenoom Hills, and in 1983 at Neridup. The sites were respectively 65, 53 and 30 km north-east of Esperance. The experiments were on alkaline mallee soils in 1981 and 1982, and on sandplain soil in 1983. The experiments were not grazed or defoliated.

The design was a completely randomized block of 19 treatments in 1981, 20 treatments in 1982, and 18 treatments in 1983 with three replications. The species and strains are listed in tables 5, 6 and 7. Inoculated and lime-pelleted seed was hand-sown, mixed with fertilizer, in plots 2 x 10 m, with 2 m wide buffers around each plot. *Rhizobium trifolii* strain WU 95 was used to inoculate both subspp. of *T. subterraneum*, *Rhizobium meliloti* U 45 and SU 47 for *M. truncatula* and *M. littoralis*, and *Rhizobium meliloti* strain NA 2290 for *M. polymorpha*. Seed was sown at 50 kg/ha. The fertilizer applied to the soil surface by hand at seeding was: 900 kg/ha superphosphate, 100 kg/ha potassium chloride, 10 kg/ha copper sulphate, 2 kg/ha zinc oxide, 0.5 kg/ha molybdenum trioxide, 0.5 kg/ha cobalt sulphate, and (Neridup only) 3 kg/ha sodium borate. Insects were controlled as described earlier.

Burrs were collected from the plots in early December after the plants had dried off. Burrs of S and B types were collected by

sieving soil to a depth of 4 cm. Most burrs of the S types were buried. In contrast, burrs of the B types were on the soil surface. Burrs of the annual medics were all on the soil surface. Seed was removed from the burrs by hand. Random samples of 200 seeds from each plot were placed in small plastic ice-cube trays and placed in an alternating temperature oven programmed to produce a diurnal temperature fluctuation of 15/60°C and the percentage swollen (soft) seed determined about once a month using a method similar to that described by Taylor and Palmer (1979). The seed was soaked after removal from the oven in water for 48 h in a germination cabinet maintained at a constant 15°C after which the water was removed and the number of soft-seeds counted. The remaining seeds were returned to the oven for about one month before again determining the number of soft-seeds.

When measurement of hard-seededness began, further subsamples of seed were selected for seed moisture contents determined after 7 d in the oven by oven-drying at 105°C for 48 h. The percentage seed moisture content was calculated on a dry weight basis.

The number of soft-seeds counted each time were expressed as a percentage of the total number of seeds for the sample (200). These data were transformed, using an arcsine  $\sqrt{x}$  transformation before statistical analysis.

**Table 5. Percentage of soft-seed in samples of seed collected from Mt Ney in 1981 and stored in an alternating temperature oven (15/60°C, one cycle/day). Figures in parenthesis are the standard errors of the mean, n = 3.**

Species and strain*	Storage period—days											
	7	39	67	101	129	163	207	241	275	303	330	389
	Soft-seed at each storage period—per cent											
	%	%	%	%	%	%	%	%	%	%	%	%
<i>Medicago truncatula</i>												
cv. Cyprus	3 (2)	3 (2)	3 (2)	6 (2)	12 (1)	27 (4)	39 (2)	50 (1)	59 (2)	61 (2)	63 (2)	66 (2)
cv. Jemalong	3 (1)	3 (1)	4 (1)	8 (2)	10 (2)	17 (4)	35 (5)	42 (6)	50 (6)	53 (7)	55 (6)	58 (5)
<i>Medicago littoralis</i>												
cv. Harbinger	4 (1)	5 (1)	6 (1)	7 (1)	8 (2)	15 (4)	26 (3)	40 (2)	41 (2)	50 (3)	53 (3)	55 (3)
<i>Trifolium subterraneum</i> subsp. <i>subterraneum</i>												
cv. Nungarin	15 (2)	18 (3)	24 (3)	41 (2)	53 (3)	73 (3)	76 (3)	85 (2)	86 (2)	87 (2)	88 (2)	89 (2)
cv. Daliak	24 (4)	29 (2)	35 (2)	47 (5)	61 (5)	73 (5)	82 (5)	82 (6)	83 (6)	84 (6)	86 (4)	88 (3)
56894D	21 (1)	25 (1)	28 (2)	48 (4)	65 (3)	77 (4)	86 (4)	88 (4)	90 (4)	91 (4)	92 (3)	95 (1)
56911	45 (4)	53 (3)	60 (3)	72 (2)	85 (2)	92 (1)	98 (1)	100 (0)				
<i>Trifolium subterraneum</i> subsp. <i>brachycalycinum</i>												
cv. Clare	49 (4)	61 (2)	68 (1)	89 (1)	92 (1)	96 (1)	98 (1)	100 (1)	100 (0)			
24417	16 (2)	19 (2)	21 (1)	36 (3)	40 (3)	57 (2)	68 (5)	79 (1)	81 (1)	88 (1)	98 (2)	100 (0)
25308B	24 (1)	29 (1)	40 (2)	53 (2)	62 (1)	76 (2)	83 (2)	85 (2)	91 (4)	93 (4)	94 (3)	95 (2)
28096	18 (2)	23 (3)	26 (2)	36 (1)	45 (1)	58 (1)	64 (1)	68 (3)	70 (4)	72 (4)	74 (4)	75 (5)
28102	17 (4)	21 (3)	29 (4)	48 (5)	56 (6)	67 (7)	74 (7)	80 (6)	84 (7)	85 (6)	86 (5)	86 (5)
33245	12 (1)	14 (1)	18 (1)	34 (1)	38 (2)	60 (3)	65 (4)	66 (3)	75 (4)	75 (6)	76 (6)	76 (6)
33248	11 (1)	19 (2)	27 (1)	45 (3)	55 (4)	62 (5)	85 (5)	91 (1)	95 (2)	98 (2)	100 (0)	
69972	26 (1)	34 (1)	41 (2)	66 (6)	71 (6)	88 (2)	92 (2)	97 (3)	98 (2)	100 (1)	100 (0)	
69973A	23 (2)	32 (1)	39 (3)	67 (3)	80 (4)	90 (2)	91 (3)	96 (1)	99 (1)	100 (0)		
69976	30 (3)	34 (3)	46 (3)	66 (4)	82 (3)	95 (2)	100 (0)					
700100	15 (3)	17 (3)	23 (3)	34 (2)	81 (3)	93 (4)	100 (0)					
70124B	11 (4)	18 (4)	25 (3)	49 (3)	63 (1)	81 (1)	90 (1)	92 (2)	97 (1)	97 (1)	98 (1)	99 (1)

\* Numbers are CPI (Commonwealth Plant Introduction) numbers

**Table 6. Percentage of soft seed in samples of seed collected from Wittenoom Hills in 1982 and stored in an alternating temperature oven (15/60°C, one cycle/day). Figures in parenthesis are the standard errors of the mean, n = 3.**

Species and strain*	Storage period-days										
	7	35	65	93	126	156	189	219	247	282	308
	Soft-seed at each storage period—per cent										
	%	%	%	%	%	%	%	%	%	%	%
<i>Medicago truncatula</i>											
cv. Cyprus	9 (2)	11 (2)	13 (1)	21 (1)	34 (2)	42 (2)	52 (1)	57 (2)	62 (2)	70 (2)	71 (2)
cv. Jemalong	5 (1)	6 (1)	6 (1)	17 (2)	28 (1)	36 (1)	39 (3)	50 (1)	56 (1)	57 (1)	59 (1)
<i>Medicago littoralis</i>											
cv. Harbinger	4 (2)	6 (2)	9 (2)	17 (1)	28 (1)	38 (3)	51 (1)	51 (1)	58 (1)	61 (1)	62 (1)
<i>Medicago polymorpha</i>											
cv. Serena	6 (1)	8 (1)	9 (1)	10 (1)	13 (1)	26 (2)	29 (1)	32 (2)	36 (2)	37 (2)	37 (2)
<i>Trifolium subterraneum</i> subsp. <i>subterraneum</i>											
cv. Nungarin	11 (1)	13 (1)	29 (2)	56 (3)	76 (4)	81 (4)	87 (4)	90 (4)	91 (4)	92 (3)	94 (3)
cv. Daliak	2 (1)	4 (1)	25 (3)	78 (2)	91 (3)	95 (2)	98 (2)	98 (2)	100 (0)		
<i>Trifolium subterraneum</i> subsp. <i>brachycalycinum</i>											
cv. Clare	18 (3)	26 (3)	63 (6)	89 (5)	94 (3)	97 (2)	100 (0)				
24417	9 (1)	11 (1)	18 (3)	28 (3)	40 (4)	53 (7)	64 (10)	68 (12)	70 (12)	72 (11)	74 (11)
25308B	8 (2)	8 (2)	23 (4)	43 (4)	64 (5)	78 (5)	84 (4)	90 (4)	91 (3)	93 (3)	94 (3)
28096	5 (1)	6 (2)	10 (2)	23 (3)	38 (5)	48 (3)	56 (6)	62 (5)	64 (5)	69 (4)	73 (5)
28102	10 (1)	12 (1)	23 (4)	42 (8)	64 (8)	71 (6)	76 (5)	83 (4)	85 (4)	88 (3)	90 (3)
33245	8 (3)	9 (3)	25 (3)	43 (5)	59 (6)	70 (8)	77 (6)	85 (5)	87 (5)	90 (4)	93 (4)
33248	10 (2)	13 (2)	30 (1)	60 (2)	76 (3)	86 (4)	92 (3)	95 (3)	96 (2)	97 (2)	97 (2)
69972	12 (1)	14 (1)	39 (4)	66 (9)	88 (5)	90 (5)	95 (3)	97 (2)	98 (2)	99 (1)	100 (0)
69973A	10 (3)	16 (4)	52 (5)	80 (6)	91 (3)	97 (3)	100 (1)	100 (0)			
69976	6 (1)	9 (4)	45 (4)	77 (9)	86 (8)	92 (6)	97 (5)	100 (0)			
70099	5 (1)	16 (1)	18 (1)	70 (4)	86 (4)	95 (1)	99 (1)	100 (1)	100 (0)		
70100	10 (3)	13 (2)	32 (3)	63 (3)	84 (3)	97 (1)	99 (1)	99 (1)	100 (0)		
70124B	9 (1)	11 (1)	27 (3)	64 (5)	77 (6)	83 (6)	92 (3)	94 (3)	97 (1)	100 (0)	
Q081	10 (2)	16 (1)	30 (4)	54 (6)	74 (6)	84 (5)	89 (4)	95 (4)	95 (4)	96 (3)	97 (3)

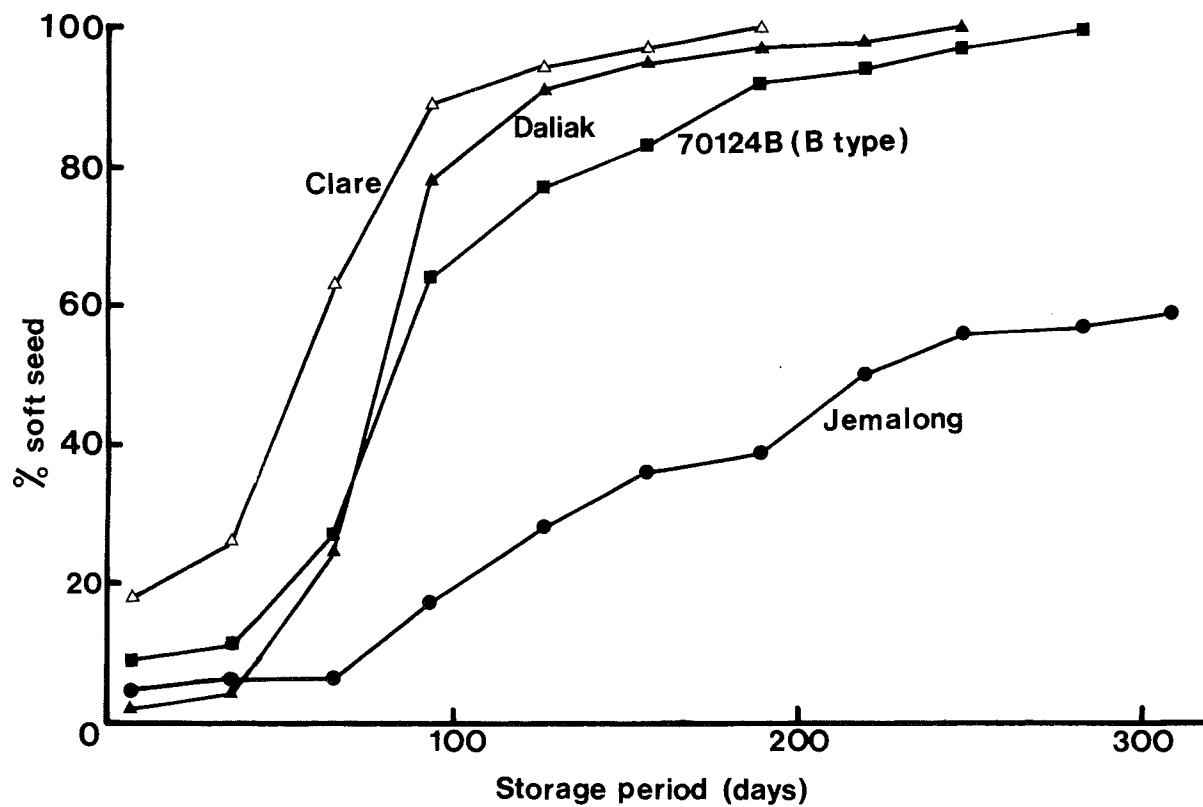
\* All numbers are CPI (Commonwealth Plant Introduction) numbers, except Q 081, which was collected by B.J. Quinlivan in Israel

Table 7. Percentage of soft-seed in samples of seed collected from Neridup in 1983 and stored in an alternating temperature oven (15/60°C, one cycle/day). Figures in parenthesis are the standard errors of the mean, n = 3.

Species and strain*	Storage period-days									
	7	35	68	98	124	159	189	222	252	280
	Soft-seed at each storage period-per cent									
	%	%	%	%	%	%	%	%	%	%
<i>Medicago truncatula</i>										
cv. Cyprus	7 (1)	8 (1)	12 (1)	13 (1)	22 (1)	41 (1)	49 (1)	53 (1)	56 (1)	57 (1)
cv. Jemalong	6 (1)	8 (1)	11 (1)	14 (1)	20 (2)	28 (1)	34 (1)	37 (1)	39 (1)	40 (2)
<i>Medicago littoralis</i>										
cv. Harbinger	12 (1)	15 (2)	16 (1)	18 (1)	24 (1)	36 (2)	42 (2)	47 (2)	48 (2)	49 (2)
<i>Medicago polymorpha</i>										
cv. Serena	1 (1)	2 (1)	3 (1)	3 (1)	4 (1)	6 (1)	13 (2)	19 (2)	20 (2)	24 (1)
cv. Circle Valley	1 (1)	1 (1)	1 (0)	1 (1)	4 (2)	7 (2)	13 (2)	18 (1)	20 (2)	22 (2)
<i>Medicago scutellata</i>										
cv. Robinson	2 (1)	3 (1)	7 (2)	7 (2)	12 (3)	16 (2)	19 (2)	22 (2)	23 (2)	24 (2)
cv. Sava	1 (1)	1 (1)	2 (1)	4 (2)	6 (2)	10 (2)	13 (3)	17 (2)	20 (3)	22 (2)
<i>Medicago tornata</i>										
cv. Tornafeld	3 (2)	4 (1)	7 (1)	11 (1)	28 (3)	53 (2)	65 (2)	67 (2)	70 (3)	71 (3)
Saleq	2 (1)	2 (1)	4 (1)	6 (1)	10 (1)	34 (2)	46 (3)	52 (4)	55 (3)	56 (3)
<i>Trifolium subterraneum</i> subsp. <i>subterraneum</i>										
cv. Nungarin	7 (0)	12 (2)	33 (4)	57 (0)	67 (1)	73 (3)	76 (3)	78 (2)	80 (3)	81 (2)
cv. Daliak	10 (1)	16 (2)	4 (5)	71 (6)	80 (7)	86 (5)	89 (4)	92 (3)	95 (3)	96 (2)
<i>Trifolium subterraneum</i> subsp. <i>brachycalycinum</i>										
1945I	28 (3)	34 (4)	49 (6)	67 (5)	69 (5)	79 (8)	81 (5)	83 (4)	86 (3)	88 (2)
25308B	14 (3)	27 (8)	54 (10)	72 (5)	78 (5)	82 (6)	85 (6)	87 (7)	90 (6)	91 (4)
28095	19 (1)	24 (2)	40 (1)	54 (2)	63 (2)	68 (3)	70 (2)	74 (2)	81 (1)	85 (1)
28096	9 (2)	12 (3)	15 (3)	19 (4)	22 (3)	29 (1)	36 (0)	41 (1)	43 (1)	45 (1)
69988	16 (6)	25 (8)	49 (5)	70 (5)	77 (3)	80 (4)	82 (3)	85 (1)	84 (2)	87 (1)
70082B	29 (5)	42 (9)	69 (7)	84 (4)	89 (3)	93 (3)	95 (4)	95 (3)	96 (3)	97 (2)
Q 081	8 (2)	11 (1)	28 (3)	37 (1)	43 (3)	50 (2)	53 (2)	55 (2)	58 (2)	60 (3)

\* Numbers are CPI (Commonwealth Plant Introduction) numbers, except Q 081, which was collected by B.J. Quinlivan in Israel

Figure 1: Percentage soft-seeds measured for seed of S and B types and of annual medics grown in the field in 1982 at Wittenoom Hills, 53 km north-east of Esperance, after different storage periods in a diurnally alternating temperature oven (15/60°C, one cycle/day).





## Results and discussion

In 1979, the B types produced dense herbage by spring and some strains also produced many burrs and reasonable seed yields within the darkness of the canopies (table 1). Most of the B type burrs were found on the soil surface, whereas most of the burrs of the S type were buried.

The 1981 main experiments were grazed with sheep up to the commencement of flowering. The S types buried most of their burrs and produced between 400 and 1000 kg seed/ha (table 2). Annual medics did not bury their burrs and produced between 500 and 1000 kg seed/ha. By contrast, the flowers of the B types produced very few burrs and little seed (between 0 and 50 kg/ha). Pieces of wood and stones were scattered on the plots. Most of the seed of the B types were produced when the burrs were able to meander under this debris and were able to produce seed in these dark habitats. The seed yields of the B types were largely dictated by the amount of this debris on individual plots.

The 1981 ancillary experiment near Mt Ney was not grazed and the B types produced a large canopy of leaves by spring. Burrs developed satisfactorily in the darkness of these canopies and produced reasonable seed yields (table 3). The seed yields of the B types in the ungrazed ancillary experiment are in stark contrast to the poor seed yields produced by the same strains in the grazed main experiment sown with the same amount of seed on an adjacent site.

These experiments suggest that the B types were incapable of burying their burrs and so did not produce seed. Evidently the burrs must seek out suitable dark habitats to produce burrs and seed on both soil types on the Esperance Plain.

Prevention of burr burial reduced seed yields of the S types by between 30 and 94%, depending on strain, and of B types by between 60 and 97%, these reductions being the result of fewer burrs, numbers of seeds and weight per seed (table 4). The experiment confirmed the importance of burr burial for seed production in both S and B types.

A research problem on B types on sandy surface soils in south-western Australia is that wind-blown sand can sometimes bury developing burrs. This has led to conflicting results about the ability of B types to bury burrs and produce seed on these soils.

The plots were not grazed or defoliated and, as was noted in the 1979 maturity and seed yield experiments and in the 1981 ancillary experiment, the B types produced seed in burrs which were developed in the darkness under the canopy of leaves.

The rate of softening of hard-seeds of the B and S types were similar, which was much more rapid than that measured for annual medics (figure 1; tables 5, 6 and 7). The S types generally fail to persist in the <400 mm/a average rainfall areas under intense cropping systems (Ewing 1983). Such systems are common on the alkaline soils near Esperance and annual medics persist successfully on these soils even in the marginal rainfall areas. B types are therefore unlikely to be successful, persistent, self-regenerating annual legumes on the alkaline mallee soils near Esperance because of inadequate levels of hard-seededness.

In their natural Mediterranean habitat, B types are found growing and persisting on self-mulching and cracking soils. Neither the sandplain nor the alkaline mallee soils near Esperance are self-mulching or cracking. My experiments have demonstrated that B types will produce seed under a dense ungrazed canopy, but fail to produce seed in a grazed sward and fail to bury their burrs on the sandplain soils. Taylor (1979) demonstrated that an inhibitory light factor operates to prevent seed production in both B and S types. Thus, self-mulching or cracking soils must be essential for the persistence of B types under continuous grazing, otherwise B types cannot bury their burrs and grazing exposes developing burrs and seed to light and little or no seed is produced. B types may produce seed equally well on acid or alkaline self-mulching or cracking soils.

B types cannot bury burrs on the two major soil types near Esperance and, when grazed before flowering, failed to produce seed. They are thus unlikely to be successful pasture legumes on these soils. Moreover, they are unlikely to persist under the intense cropping systems encountered, particularly on the alkaline mallee soils, because of inadequate levels of hard-seededness.

## **Conclusion**

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