Diversity for redlegged earth mite cotyledon resistance within subterranean clover and annual medics

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Abstract
Redlegged earth mites (RLEM) are a major pest of subterranean clover and annual medics, particularly during seedling establishment. Five new subterranean clover cultivars have recently been released with improved resistance, but all annual medic cultivars are previously reported as susceptible. This paper reports on glasshouse experiments that found wide diversity for RLEM cotyledon resistance among the 97-member core collection of subterranean clover (*Trifolium subterraneum*), representing ~80% of the total diversity within the species, and 48 cultivars. Diversity for resistance was also found among 153 annual medic lines in 11 different *Medicago* species, including 36 cultivars. The detection of new sources of RLEM cotyledon resistance will assist breeders to develop subterranean clovers and annual medics with improved resistance.

Key Words
Redlegged earth mites, subterranean clover, annual medics, pest resistance

Introduction
Redlegged earth mites (RLEM), *Halotydeus destructor*, are found throughout the winter growing-season areas of southern Australia. These mites feed on the upper surface of cotyledons and leaves of annual legumes, including subterranean clover (*Trifolium subterraneum*) and annual medics (*Medicago* spp.), causing high mortality of germinating seedlings, loss of herbage production in winter and spring and a reduction in seed yield (Ridsdill-Smith and Nichols 1998). Insecticides have been the main form of control, but some populations have been found with resistance to some pyrethroid pesticides (Umina 2007).

Genetic variation for RLEM cotyledon resistance has been found in subterranean clover and breeding for resistance to this trait has led to the recent release of cultivars Bindoon, Rosabrook, Narrikup, Tammin and Forbes (Nichols *et al.* 2014; PBR database 2019). However, less than 10% of accessions in the Australian Pastures Genebank have been screened for RLEM resistance and the diversity within subterranean clover is poorly defined. Furthermore, the resistance in the new cultivars is not absolute and high RLEM densities are able to overcome it. Variation for seedling resistance to RLEM in annual medics has been reported by Lake and Howie (1995), but all cultivars were reported as highly susceptible. This paper reports on two glasshouse experiments to examine diversity for RLEM cotyledon resistance among diverse collections of subterranean clover and annual medics, in order to find new sources of resistance for breeding.

Methods
The first experiment, sown on 29 July in 2014, examined diversity within subterranean clover for reaction to RLEM attack. This trial screened the 97-member core collection, which includes cultivars Coolamon (L96) and Urana (L97) (Nichols *et al.* 2013; Kaur *et al.* 2017), and 44 extra cultivars (see Table 1). Cultivar Dalkeith and the RLEM-resistant core collection line L58 (accession DGI007) were used as susceptible and resistant controls, respectively. The second experiment, sown on 20 August in 2015, examined 153 annual medic lines in 11 *Medicago* species, including 36 cultivars and 68 members of the *M. truncatula* core collection (Ellwood *et al.* 2006) (see Table 2), along with the most resistant lines found by Lake and Howie (1995). Twelve subterranean clover cultivars and messina (*Meliotus siculus*) cv. Neptune were also added. L58 (DGI007) subterranean clover and the barrel medic cultivar Jemalong were used as resistant and susceptible controls, respectively.

Seeds were sown into plastic boxes of size 400 mm x 300 mm and 120 mm depth. Six 8 mm diameter holes were drilled into the bottom of each box for drainage. A layer of paper towelling was placed in the bottom of each box before filling them with pasteurised river sand. The sand was wetted and levelled to achieve an even depth of 60 mm. Sticky tape was applied to the top perimeter of each box and Vaseline petroleum jelly was smeared on top of this to prevent RLEM movement between boxes. Both trials used a row-column design with 4 replicates. Sixteen entries were allocated per box in two rows of eight. The resistant and susceptible check varieties were included in randomised positions in each box. Rows of each entry were sown with 0.2 grams of scarified seed to a depth of 5 mm. Sowing furrows were then lightly covered over with sand. Boxes were watered daily prior to introduction of RLEM.

RLEM were collected from the field 8 days after sowing using a Stihl garden Vacblower, modified for suction. A 150 µm mesh cup was used to trap mites. RLEM samples were placed into a stack of sieves, with mesh diameter 710 µm and 375 µm and a solid base. The majority of RLEMs passed through the 710 µm sieve and were retained by the 375 µm sieve. This sieved sample was placed in a plastic container inside a portable refrigerator for transport back to the laboratory. Further sieving was conducted to standardise the sample and eliminate other organisms. The mites applied to seedlings passed through a 600 µm sieve and were retained by a 375 µm sieve. A total of 0.25 grams of RLEM were applied to the centre of each box. RLEM moved rapidly from this position and within 15 minutes were distributed across each box. RLEM feeding damage ratings were conducted 7 days after introduction. Damage was visually scored as the percentage of cotyledon area silvered in each seedling row. AS-REML was used to produce predicted means for percent cotyledon damage with varieties as random effects.

Results

Subterranean clover

There was a wide separation between the resistant check, L58 (DGI007), and the susceptible check, Dalkeith (Table 1). A wide range of damage ratings occurred across the 142 entries, with silvering ranging from 17.8% for cv. Narrikup to several entries with 100%. The three other newly released cultivars with cotyledon resistance, Bindoon, Rosabrook and Tammin, also had low damage scores, while the remaining cultivars, apart from Denmark and Woogenellup, had high damage scores. Also of note were other core collection lines (notably L15, L30, L31, L41, L50, L53, L68, L75 and L93) with low RLEM damage scores.

Table 1. Predicted means (from AS-REML) for percent cotyledon damage of subterranean clover core collection lines and cultivars following glasshouse screening with RLEM. Resistant and susceptible check varieties, L58 (DGI007) and Dalkeith, respectively, are highlighted in bold.

Core collection lines								Cultivars			
Line no	% damage	Line no	% damage	Line no	% damage	Line no	% damage	Cultivar	% damage	Cultivar	% damage
L01	79.2	L25	94.5	L49	94.3	L73	69.7	Antas	93.0	Losa	72.5
L02	98.6	L26	65.6	L50	30.4	L74	43.3	Bacchus Marsh	65.0	Meteora	99.8
L03	96.8	L27	91.9	L51	90.6	L75	22.7	Bindoon	20.4	Mintaro	97.3
L04	97.4	L28	94.7	L52	97.8	L76	96.8	Campeda	51.8	Monti	100.6
L05	93.3	L29	68.9	L53	34.5	L77	96.4	Clare	87.2	Mt Barker	91.3
L06	77.1	L30	32.9	L54	97.8	L78	72.6	Coolamon (L96)	74.5	Nangeela	74.9
L07	72.0	L31	38.2	L55	99.1	L79	97.1	Daliak	98.3	Napier	97.1
L08	99.3	L32	79.2	L56	100.5	L80	70.7	**Dalkeith**	**83.3**	Narrikup	17.8
L09	100.2	L33	96.8	L57	98.7	L81	95.6	Denmark	30.8	Northam	93.2
L10	69.0	L34	87.1	**L58**	**20.8**	L82	87.7	Dinninup	82.7	Nuba	100.3
L11	92.6	L35	60.0	L59	64.1	L83	95.1	Dwalganup	96.6	Nungarin	96.3
L12	76.2	L36	89.0	L60	54.5	L84	99.7	Enfield	79.3	Riverina	97.4
L13	83.4	L37	84.2	L61	65.9	L85	84.1	Esperance	95.6	Rosabrook	36.8
L14	61.3	L38	57.1	L62	96.7	L86	81.8	Geraldton	94.6	Rosedale	95.1
L15	37.0	L39	90.2	L63	75.8	L87	71.4	Gosse	96.0	Tammin	34.7
L16	96.4	L40	61.5	L64	90.0	L88	59.2	Goulburn	93.4	Seaton Park	91.8
L17	96.4	L41	30.5	L65	85.0	L89	72.5	Green Range	97.2	Tallarook	96.9
L18	95.3	L42	83.4	L66	55.0	L90	84.0	Howard	93.4	Trikkala	95.5
L19	82.7	L43	96.9	L67	90.0	L91	97.0	Izmir	87.0	Uniwager	98.9
L20	98.7	L44	81.7	L68	29.7	L92	92.5	Junee	99.7	Urana (L97)	78.5
L21	96.6	L45	45.0	L69	98.5	L93	36.4	Karridale	75.9	Woogenellup	35.6
L22	91.9	L46	65.5	L70	91.6	L94	93.3	Larisa	97.0	Yarloop	100.1
L23	93.3	L47	93.6	L71	98.9	L95	91.7	Leura	91.8	York	76.5
L24	95.6	L48	95.1	L72	89.8						
LSD (*P*=0.05)			21.1								

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Annual medics

Most annual medic lines were very susceptible to RLEM, with a mean cotyledon damage rating of 56.2% (Table 2). There were large differences between and within species. *Medicago rugosa* was the least susceptible (mean of 17.0%), followed by *M. murex* (mean of 22.5%) and *M. polymorpha* (mean of 39.8%). The most susceptible species were *M. orbicularis*, *M. rotata* and *M. rigidula*, each represented by one line.

Table 2. Predicted means (from AS-REML) for percent cotyledon damage of diverse annual medic, subterranean clover and messina lines following glasshouse screening with RLEM. Resistant and susceptible check varieties, L58 (DGI007) subterranean clover and Jemalong barrel medic, respectively, are shown in bold.

Line	% damage	Line	% damage	Line	% damage	Line	% damage
Medicago truncatula		*M. truncatula*		*M. italica*		*M. polymorpha*	
APG1316[a]	58.9	APG14163[a]	68.4	Murrayland[b]	84.3	APG9615	22.8
APG1489[a]	61.3	APG15268[a]	68.0	Rivoli[b]	61.7	APG17978	37.2
APG1502[a]	66.5	APG15951[a]	49.9	Swani[b]	32.7	APG17991	32.5
APG2168[a]	49.9	APG17590[a]	59.5	Toreador[b]	86.1	APG18010	41.5
APG2193[a]	28.6	APG18176	39.4	Tornafield[b]	47.2	APG18678	5.1
APG2203[a]	55.2	APG18254	40.1	*M. littoralis*		APG22540	39.8
APG2204[a]	49.9	APG18346[a]	63.8	APG2381	80.2	APG24204	49.3
APG2260	33.3	APG18532[a]	60.4	APG2490	22.8	APG25943	26.7
APG3047[a]	56.4	APG18543[a]	56.8	Angel[b]	73.1	Cavalier[b]	85.4
APG3054[a]	54.4	APG19961	33.8	Harbinger[b]	89.1	Circle Valley[b]	60.2
APG3116[a]	62.4	APG19964[a]	80.9	Harbinger AR[b]	92.2	Santiago[b]	37.1
APG3562[a]	48.2	APG21362[a]	50.8	Herald[b]	90.2	Scimitar[b]	58.5
APG3646	53.6	APG23859[a]	71.6	Jaguar[b]	90.1	Serena[b]	39.5
APG3916[a]	41.1	APG24576[a]	17.1	Pildappa	85.4	Z2100	56.1
APG4087[a]	56.9	APG24714[a]	65.3	PM2	85.9	*M. rigidula*	
APG4482	54.3	APG24968[a]	40.8	PM250	73.4	Laramie	85.5
APG4564	56.1	APG25898[a]	78.7	*M. murex*		*M. rotata*	
APG4971	49.5	APG25915[a]	61.8	APG23208	9.6	Highlander	88.0
APG2840[a]	40.9	APG25941[a]	80.5	Zodiac[b]	35.3	*M. rugosa*	
APG6088[a]	56.8	APG27062[a]	34.2	*M. sphaerocarpos*		Paragosa[b]	17.3
APG7627	46.4	APG27137[a]	69.7	Orion[b]	31.5	Paraponto[b]	18.2
APG7749[a]	73.7	APG27138[a]	71.1	*M. orbicularis*		Sapo[b]	15.4
APG8105[a]	66.7	APG27774[a]	50.2	Bindaroo[b]	97.9	*M. scutellata*	
APG8454	89.7	APG27882[a]	55.9	*M. polymorpha*		Essex[b]	89.1
APG8496[a]	73.9	APG28064[a]	42.0	APG412	50.5	Kelson[b]	80.4
APG8625[a]	67.8	APG28375[a]	53.8	APG2606	48.6	Robinson[b]	80.5
APG8626[a]	81.5	APG28712[a]	75.5	APG2772	56.5	Sair[b]	74.7
APG8642[a]	64.4	43 Mt[a]	79.9	APG3577	30.4	Sava[b]	79.4
APG8916[a]	82.1	Borung[a,b]	66.6	APG3609	60.5	*Trifolium subterraneum*	
APG8935[a]	68.2	Caliph[a,b]	57.0	APG4042	50.7	**L58 (DGI007)**	**8.3**
APG9138[a]	82.0	Cyfield[b]	72.3	APG4169	13.3	Bindoon[b]	15.6
APG9141[a]	68.1	Cyprus[b]	59.8	APG5508	45.3	Dalkeith[b]	57.0
APG9356[a]	81.2	**Jemalong[b]**	**70.8**	APG5709	19.3	Narrikup[b]	19.3
APG9357[a]	65.9	Jester[b]	71.3	APG5723	4.0	Rosabrook[b]	13.8
APG9388	62.0	Lucas[a]	63.5	APG5727	20.0	Tammin[b]	19.4
APG9456[a]	44.4	Mogul[b]	62.7	APG6336	24.0	Gosse[b]	73.6
APG9596[a]	74.0	Parabinga[b]	45.2	APG6337	28.5	Riverina[b]	73.7
APG9693[a]	51.7	Sanza	69.7	APG7489	67.7	Rouse[b]	69.2
APG9700[a]	68.8	Sephi[b]	52.6	APG7751	59.3	Yanco[b]	48.2
APG9712[a]	73.6	Sultan-SU[b]	58.3	APG8134	50.1	Clare[b]	44.1
APG9728[a]	87.3	Z2504	69.0	APG8193	29.9	Lofty[b]	46.0
APG9866[a]	67.2	*M. arabica*		APG8234	13.4	Mawson[b]	64.2
APG9876[a]	57.0	APG8746	84.7	APG8902	21.6	*Melilotus siculus*	
APG10406[a]	37.0	APG8774	79.7	APG9611	33.3	Neptune[b]	61.1
APG10481[a]	48.1	APG36809	35.5	APG9614	55.0		
LSD (*P*=0.05)	11.1						

[a]Member of the *M. truncatula* core collection; [b]Cultivar

Most annual medic cultivars were highly susceptible, with a mean cotyledon damage rating of 65.0%. However, the three *M. rugosa* cultivars, Paragosa, Sapo and Paraponto, were not significantly different to the resistant check, DGI007. Several other annual medic lines also had cotyledon damage ratings no different to the resistant control, including *M. murex* line APG23208, *M. polymorpha* lines APG5723, APG18678, APG4169, APG8234 and APG5709 and *M. truncatula* line APG24576. The subterranean clover cultivars Rosabrook, Bindoon, Narrikup and Tammin were also no different to the resistant check, while the other new cultivars (Yanco, Rouse, Mawson and Lofty) and *Melilotus siculus* cv. Neptune were susceptible.

Discussion

The wide diversity of RLEM cotyledon damage ratings among the subterranean clover lines examined confirmed previous findings, with the majority of cultivars being susceptible and the newly released cultivars Bindoon Rosabrook, Narrikup and Tammin having resistance. Of note were ten core collection lines with low RLEM damage scores. The continuous distribution of RLEM cotyledon resistance in the germplasm suggests the trait is multi-genic (Nichols *et al.* 2014); it is not known whether the resistant lines in this study have the same or different genes for resistance. If different genes are present, intercrossing could be conducted to pyramid complementary genes to produce cultivars with greater and more durable resistance. Further genetic analyses are required to identify the different genes involved in resistance.

The finding that most annual medics, including the cultivars, are highly susceptible to RLEM supports the results of Lake and Howie (1995). However, low cotyledon feeding damage scores in the *M. rugosa* cultivars Paragosa, Sapo and Paraponto have not been previously reported. The observation that *M. murex* has lines with resistance also supports the results of Lake and Howie (1995). The identification of some lines with low RLEM feeding damage ratings among the widely sown annual medics, *M. truncatula* and *M. polymorpha*, suggests the potential to develop cultivars of these species with improved RLEM resistance. However, further work is required to understand the genetics of this trait.

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References

Ellwood SR, D' Souza NK, Kamphuis LG, Burgess TI, Nair RM and Oliver RP (2006). SSR analysis of the *Medicago truncatula* SARDI core collection reveals substantial diversity and unusual genotype dispersal throughout the Mediterranean basin. Theoretical Applied Genetics 112, 977–983.

Kaur P, Appels R, Bayer PE, Keeble-Gagnere G, Wang J, Hideki H, Kenta S, Vercoe PE, Stefanova K, Durmic Z, Nichols P, Revell C, Isobe S, Edwards D and Erskine W (2017). Climate clever clovers: New paradigm to reduce the environmental footprint of ruminants by breeding low methanogenic forages utilising haplotype variation. Frontiers in Plant Science https://doi.org/10.3389/fpls.2017.01463. Accessed 3 March 2019.

Lake AHW and Howie JH (1995) Selection for redlegged earth mite resistance in annual Medicago species. Plant Protection Quarterly 10, 45-46.

Nichols PGH, Foster KJ, Piano E, Pecetti L, Kaur P, Ghamkhar K and Collins WJ (2013). Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. Crop and Pasture Science 64, 312–346.

Nichols PGH, Jones RAC, Ridsdill-Smith TJ and Barbetti MJ (2014). Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 2. Breeding for disease and pest resistance. Crop and Pasture Science 65, 1207–1229.

PBR database (2019). Plant Breeders Rights database. IP Australia, Commonwealth Government of Australia. Available at: http://www.ipaustralia.gov.au/get-the-right-ip/plant-breeders-rights. Accessed 3 March 2019.

Ridsdill-Smith TJ and Nichols PGH (1998). Development of pasture legumes resistant to redlegged earth mite. Proceedings of the 6th Australasian Applied Entomology Research Conference', Brisbane, Australia. (Eds MP Zalucki, RAI Drew, GG White), Vol. 2: 382-389. (Horticultural Research & Development Corporation: Brisbane).

Umina PA (2007). Pyrethroid resistance discovered in a major agricultural pest in southern Australian: the redlegged earth mite *Halotydeus destructor* (Acari: Penthaleidae). Pest Management Science 63, 1185–1190.