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# Controlling redlegged earth mite, *Halotydeus destructor* (Acari: Penthaleidae), with a spring spray in legume pastures

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**Abstract.** The use of a TIMERITE® spring spray to control redlegged earth mite (RLEM), *Halotydeus destructor* (Tucker) (Acari: Penthaleidae), in annual pastures was evaluated on farms across Australia. RLEM populations in autumn in the treatments sprayed in spring 1998 and 1999 were 97% lower in 1999 and 97% in 2000 in the western region (Western Australia), and 93% lower in 1999 and 93% in 2000 in the eastern region (Victoria, New South Wales and South Australia). At sites in the west, control of RLEM resulted in significant increases in subterranean clover seed yield in 1999 and in clover seedling numbers in autumn 1999 and 2000. *Penthaleus major* (blue oat mite) populations in autumn were 60% lower in sprayed treatments, but *Sminthurus viridis* (lucerne flea) populations were not affected. Differences in weather between the west (where there is a hot, dry summer) and the east (where temperature and rainfall regimes are more variable in spring and early summer) seem to cause greater RLEM control and greater benefits in subterranean clover seed yield and seedling numbers with a spring spray in the west.

**Additional keywords:** autumn control, pre-emptive spray, subterranean clover, seedling density, southern Australia.

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## Introduction

Redlegged earth mite (RLEM), *Halotydeus destructor* (Tucker) (Penthaleidae: Acari), is a major pest of pastures (Ridsdill-Smith 1997; Young *et al.*1995) and grain crops (Murray *et al.* 2013) in Australia. It is particularly damaging to seedlings of subterranean clover (*Trifolium subterraneum* L.) in the establishment phase of pastures (Ridsdill-Smith *et al.* 2008; Nichols *et al.* 2014), and to seedlings of canola and lupins in grain crops (Murray *et al.* 2013). When RLEM is controlled in pastures in Western Australia, both clover seed yield (Brennan and Grimm 1992; Ridsdill-Smith *et al.* 2013) and seedling numbers increase (Michael *et al.* 1997; Ridsdill-Smith *et al.* 2013). Pesticides are the major tool for controlling RLEM (Ridsdill-Smith 1997; Ridsdill-Smith *et al.* 2008; Murray *et al.* 2013), but they do not kill winter eggs or oversummering eggs. The proportion of the RLEM population at the egg stage within a population can vary from 25–65% in winter to 100% in summer (Ridsdill-Smith and Annells 1997), and thus a spray will kill only some of the entire population; further RLEM will emerge from surviving eggs. Pesticides are often applied in an *ad hoc* fashion, so that farmers may apply up to four or five sprays in a single season (Umina and Hoffmann 2003). Overuse of synthetic pyrethroid sprays has resulted in the development of resistance to these chemicals in RLEM in Australia (Umina 2007), and there is evidence that resistance to organophosphates may be evolving (P. Umina, pers. comm.). Integrated pest management (IPM) for RLEM is required to reduce use of chemicals, with strategic spraying, cultural control and clover cultivars with resistance to RLEM. Since 1999, three new subterranean clover cultivars have been released with seedling resistance to RLEM (Nichols *et al.* 2014). This resistance is likely to be responsible for improving regeneration density and winter herbage production of subterranean clover, and thus animal production from pastures.

Redlegged earth mites can feed on a wide range of crop and pasture species in the winter, and spend the summer as diapause eggs, protected from desiccation and high temperatures (Ridsdill-Smith and Annells 1997). A particular need exists for effective RLEM control post-sowing and pre-emergence of seedlings (Murray *et al.* 2013). At the seedling stage, RLEM control is recommended in Australia when populations reach 5000 mites $m^{-2}$ in cereals and lower densities in canola and pulses (Ridsdill-Smith *et al.* 2008). Insurance spraying for pest mites and insects is often used to manage the risk of pasture or crop failure. Ridsdill-Smith and Annells (1997) propose that spring control of RLEM in pastures would limit the production of diapause eggs and thus minimise RLEM populations in autumn. The onset of diapause occurs at much the same time each year at a given site for most RLEM. A model has been developed to predict this date, triggered 80% by daylength and 10% by length of growing season, and varies by 6 weeks between sites across Australia

(Ridsdill-Smith *et al*. 2005). Control is recommended 2 weeks earlier, and a database TIMERITE® (www.timerite.com.au) is available for farmers to obtain the optimum spring spray date from the latitude and longitude of their paddock. Initial testing of a spring spray at three farms in the south of Western Australia resulted in 99% RLEM control after the spray and 99% in the following autumn (Ridsdill-Smith *et al*. 2005). A spring spray at five farms in western and central Victoria over 2 years resulted in 70–90% RLEM control the following autumn (Gower *et al*. 2008).

Gower *et al*. (2008) suggest that there may be regional variation in RLEM characteristics that account for the lower control they observed in the east compared with the results of Ridsdill-Smith *et al*. (2005) in the west. In regions in the west with a hot and dry Mediterranean-type climate, RLEM emerge in autumn with rainfall and with mean day temperatures <20.5°C. In the east, they adapt to more varied and uncertain temperature and rainfall regimes in summer and avoid early hatching by not emerging in autumn until mean day temperatures are <16°C (McDonald *et al*. 2015). In this paper, we look for regional differences in the effectiveness of a well-timed spring spray to control RLEM on farms between the western and eastern regions of Australia.

## Materials and methods

### Selection of sites

Pasture sites of 4 ha were selected in 1998 on 18 farms in the south of Western Australia (western region) and 10 farms in Victoria and New South Wales (eastern region). Each site was divided into two equal experimental plots of 2 ha; one plot was sprayed on the TIMERITE® spray date for that location and the other plot was left unsprayed as a control (Table 1). These plots are large enough to prevent RLEM from moving from one treatment to another (Weeks *et al*. 2000). Gower *et al*. (2008) found no evidence that RLEM adults or diapause eggs were carried by wind into 2 m by 2 m enclosures in a treated plot and confounding measurements of the impact of spraying. In 1999, 16 of these farms were given a second spring spray in the western region, and 10 farms a second spring spray in the eastern region (Victoria, New South Wales and South Australia) (Table 2). A wider range of sites was used in developing the TIMERITE® model (Ridsdill-Smith *et al*. 2005), but drought conditions in New South Wales and difficulty in accessing sites in Tasmania for sampling restricted the use of all of these areas in this study. In 2002, a further nine farms were selected in South Australia and Victoria to expand the range and number of samples in the eastern region (Table 3). Site locations ranged from 29° to 38°S and 115° to 149°E, with spray dates between 4 September and 18 October (Tables 1–3).

The sites were selected with help from farmers for uniform pasture and the presence of RLEM. All pasture sites had a subterranean clover legume content (estimated as a proportion of groundcover) of 30–80% and there was evidence of RLEM activity (confirmed with a vacuum sample or by observation of RLEM feeding damage on the clover). Stakes, natural markers or a tape tied to the fence were used to mark the boundaries. All pastures were well established at the start of the study, and no new subterranean clover seed was sown during the study. In the western region, the dominant pasture species were subterranean clover, annual ryegrass (*Lolium rigidum* Gaudin), barley grass (*Hordeum leporinum* Link) and capeweed (*Arctotheca calendula* (L.) Levyns). In the eastern region, as well as subterranean clover, legumes included barrel medic (*Medicago truncatula* (Gaertn.) and white clover (*T. repens* L.), and the grasses included perennial ryegrass (*L. perenne* L.), cocksfoot (*Dactylis glomerata* L.) and Yorkshire fog grass (*Holcus lanatus* L.). Sprayed and unsprayed areas were not fenced off and were subject to normal grazing management by using sheep or cattle at stocking rates typical for the areas.

**Table 1. Sampling sites in spring 1998 and autumn 1999**

All sites were sprayed in spring on the recommended TIMERITE® day

| West ($n = 18$ sites) | | | | East ($n = 10$ sites) | | | |
|---|---|---|---|---|---|---|---|
| State | Site | Coordinates | Spray date | State | Site | Coordinates | Spray date |
| Western Australia | Cunderdin | 117°14′E, 31°39′S | 4 Sept. | Victoria | Mitiamo | 144°14′E, 36°13′S | 16 Sept. |
| | Corrigin | 117°46′E, 32°22′S | 8 Sept. | | Wonwondah | 142°24′E, 36°88′S | 27 Sept. |
| | Bindi Bindi | 116°39′E, 30°72′S | 9 Sept. | | Dookie | 145°41′E, 36°20′S | 1 Oct. |
| | Eneabba | 115°32′E, 29°47′S | 11 Sept. | | Natte Yallock | 143°28′E, 36°57′S | 1 Oct. |
| | Walebing | 116°13′E, 30°42′S | 12 Sept. | New South Wales | Bendick-Murrell | 148°27′E, 34°10′S | 10 Oct. |
| | Badgingarra | 115°22′E, 30°34′S | 18 Sept. | Victoria | Ararat | 142°56′E, 37°17′S | 13 Oct. |
| | Williams M | 116°53′E, 33°02′S | 18 Sept. | | Violet Town | 145°44′E, 36°38′S | 13 Oct. |
| | Wandering | 116°40′E, 32°41′S | 19 Sept. | | Winchelsea | 143°59′E, 38°15′S | 15 Oct. |
| | Brookton | 116°46′E, 32°24′S | 20 Sept. | | Maldon | 144°04′E, 36°59′S | 18 Oct. |
| | Arthur River | 116°35′E, 33°45′S | 21 Sept. | | Bobinawarrah | 146°30′E, 36°31′S | 18 Oct. |
| | Williams S | 116°43′E, 32°54′S | 21 Sept. | | | | |
| | Kojonup B | 117°09′E, 33°58′S | 21 Sept. | | | | |
| | Bakers Hill | 116°26′E, 31°45′S | 21 Sept. | | | | |
| | Kojonup Y | 117°02′E, 33°58′S | 22 Sept. | | | | |
| | Cranbrook | 117°33′E, 34°18′S | 28 Sept. | | | | |
| | Carbarup T | 117°41′E, 34°32′S | 5 Oct. | | | | |
| | Mt Barker | 117°48′E, 34°38′S | 5 Oct. | | | | |
| | Porongurup | 118°01′E, 34°42′S | 5 Oct. | | | | |

**Table 2. Sampling sites in spring 1999 and autumn 2000**
All sites were sprayed in spring on the recommended TIMERITE® day

| West ($n = 16$ sites) | | | | East ($n = 10$ sites) | | | |
|---|---|---|---|---|---|---|---|
| State | Site | Coordinates | Spray date | State | Site | Coordinates | Spray date |
| Western Australia | Cunderdin | 117°14′E, 31°39′S | 4 Sept. | Victoria | Mitiamo | 144°14′E, 36°13′S | 16 Sept. |
| | Bindi Bindi | 116°39′E, 30°72′S | 9 Sept. | New South Wales | Savernake | 146°03′E, 35°44′S | 23 Sept. |
| | Eneabba | 115°32′E, 29°47′S | 11 Sept. | Victoria | Wonwondah | 142°24′E, 36°88′S | 27 Sept. |
| | Walebing | 116°13′E, 30°42′S | 12 Sept. | | Natte Yallock | 143°28′E, 36°57′S | 1 Oct. |
| | Badgingarra | 115°22′E, 30°34′S | 18 Sept. | South Australia | Naracoorte | 140°50′E, 37°10′S | 2 Oct. |
| | Williams M | 116°53′E, 33°02′S | 18 Sept. | New South Wales | Bendick- Murrell | 148°27′E, 34°10′S | 10 Oct. |
| | Wandering | 116°40′E, 32°41′S | 19 Sept. | Victoria | Ararat | 142°56′E, 37°17′S | 13 Oct. |
| | Arthur River | 116°35′E, 33°45′S | 21 Sept. | | Violet Town | 145°44′E, 36°38′S | 13 Oct. |
| | Williams S | 116°43′E, 32°54′S | 21 Sept. | | Maldon | 144°04′E, 36°59′S | 18 Oct. |
| | Bakers Hill | 116°26′E, 31°45′S | 21 Sept. | | Bobinawarrah | 146°30′E, 36°31′S | 18 Oct. |
| | Kojonup Y | 117°02′E, 33°58′S | 22 Sept. | | | | |
| | Carbarup I | 117°45′E, 34°33′S | 27 Sept. | | | | |
| | Cranbrook | 117°33′E, 34°18′S | 28 Sept. | | | | |
| | Carbarup T | 117°41′E, 34°32′S | 5 Oct. | | | | |
| | Mt Barker | 117°48′E, 34°38′S | 5 Oct. | | | | |
| | Porongurup | 118°01′E, 34°42′S | 5 Oct. | | | | |

**Table 3. Sampling sites in the east in spring 2002 and autumn 2003**
All sites were sprayed in spring on the recommended TIMERITE® day; $n = 9$ sites

| State | Site | Coordinates | Spray date |
|---|---|---|---|
| South Australia | Koppio | 135°53′E, 34°26′S | 19 Sept. |
| | Willalooka | 140°21′E, 36°24′S | 25 Sept. |
| | Clare | 138°55′E, 33°43′S | 26 Sept. |
| | Kongorong 1 | 140°33′E, 37°54′S | 6 Oct. |
| | Kongorong 2 | 140°33′E, 37°54′S | 6 Oct. |
| Victoria | Lismore | 143°12′E, 37°34′S | 13 Oct. |
| | Hexham | 142°42′E, 38°01′S | 14 Oct. |
| | Hamilton 1 | 141°48′E, 37°52′S | 16 Oct. |
| | Hamilton 2 | 141°46′E, 37°54′S | 16 Oct. |

### *Sampling for mites*

Abundance of RLEM was measured by using a modified, petrol-driven garden vacuum (Ryobi Sweeper Vac RSV 1100A MK11). The mouth of the vacuum, covering 109.4 cm$^2$, was placed in position for ~5 s, and the contents were transferred to a vial containing 70% ethanol (Ridsdill-Smith and Pavri 2000). A more accurate method to sample RLEM populations uses a steel corer 10 cm in diameter to collect plant cores with soil 2–3 cm deep, and the mites are tapped into a collecting tube (Ridsdill-Smith and Annells 1997), but this is time consuming and is not feasible when so many samples are required. Gower *et al*. (2008) report that vacuum sampling overestimates the density of earth mites, and that counts from vacuum samples collected within a frame correlated better with the core counts. We took 20 vacuum samples of mites in a straight-line transect at 10-m intervals in the middle of each sprayed and unsprayed treatment plot. RLEM populations in pastures appear to occupy a continuous surface in space, and although aggregations are found at all scales, nested analyses showed high densities to be generally of the order of 80 by 160 m, which move around during the season through population processes (Ridsdill-Smith *et al*. (2013). We considered transects 190 m long would include both these high and low large-scale population patches.

A Leica dissecting microscope at 12-fold magnification was used to identify and count RLEM and other plant pest species. Blue oat mite (BOM), *Penthaleus major* (Duges) (Acari: Penthaleidae), which can be separated from RLEM by the position of the anus, was counted in all samples. In 1999, 2000, 2002 and 2003, counts were also made of lucerne flea (LF), *Sminthurus viridis* (L.) (Collembola: Sminthuridae) (immature globular collembola are included here as LF). A single mean (from the 20 samples) for RLEM, BOM and LF abundance was estimated for each treatment and site and expressed as numbers m$^{-2}$. Three further mite species identified were a herbivore, Bryobia mite, *Bryobia praetiosa* (Koch) (Acari: Tetranychidae), a predator of LF, *Bdellodes lapidaria* (Kramer) (Acari: Bdellidae) (Roberts *et al.* 2011*a*), and a fungivorous mite, *Tyrophagus* sp. (Acari: Acaridae). Their numbers were consistently low and were not included in further analyses.

Spraying was always carried out on the date recommended for that location by TIMERITE®, and at each site, a 2-ha plot was sprayed with an organophosphate insecticide by the farmer using their own equipment, with an adjacent 2-ha plot remaining unsprayed as the control. The same treatment plots and sites were sprayed in 1998 and 1999. Most farmers used omethoate (Le-Mat® supplied by Bayer Australia Ltd, Pymble, NSW) at the label rate of 100 mL ha$^{-1}$ for RLEM control. A few farmers in Western Australia used dimethoate at 100–200 mL ha$^{-1}$ (above the label rate) in situations where they were already using it for pest control at other times on their farm. In Western Australia in 1998, records were collected for RLEM control at three sites where dimethoate was used and 11 sites where omethoate was used, in order to compare them. At all sites from New South Wales and Victoria where the chemical used was recorded, it was omethoate. No other pesticide was applied on or near the experimental area during this study.

Sampling for RLEM each year was carried out pre-spray in spring and in the following autumn, to measure impact. The pre-spray sample was collected from sprayed and unsprayed treatment plots between late August and early October,

~10 days before the spray dates. On three occasions in spring, a single RLEM sample was taken in error from the unsprayed plots after the spray date, and these sites were excluded from analyses in that year (set). The autumn sample was collected on a day that varied between 22 April and 6 July about 2 weeks after RLEM emergence for that site. Mite cohorts emerge at different times depending on local weather at different sites and from different patches within sites (Ridsdill-Smith and Annells 1997; McDonald *et al*. 2015). At five sites, no autumn sample was obtained; for one site, the farm was withdrawn from the trial at the end of the previous year; two had been ploughed up; and on two sites, no RLEM were found at the time of the visit because of a false break of season or drought. These sites were excluded from analyses in that year (set).

### Sampling for plants

Seed yield of subterranean clover was measured in sprayed and unsprayed treatment plots after the plants had senesced when the soil was dry, using methods described in Ridsdill-Smith and Annells (1997). In December 1998 and 1999, at the centre of each treatment area, 20 soil cores 2–3 cm deep were taken in a transect at 10-m intervals, using a steel corer of 10 cm diameter, with a surface area of 78.5 $cm^2$. The soil from each core was placed in a plastic bag for later analysis. Soil was sieved through a 0.3-mm-aperture mesh sieve, retaining subterranean clover burrs and loose seeds. Subterranean clover seed is large and can be easily distinguished from other pasture species. In the laboratory, loose seed was counted first, and the burrs were separated into those assumed produced that year (new burrs) and those assumed produced in an earlier year (old burrs). Seed was removed from burrs by hand-threshing over a ribbed rubber mat by using a small cork block. In 1998, new seed, old seed and loose seed were each counted and weighed as a measure of seed yield from 18 sites in the west and eight sites in the east. However, separation of loose seed by age proved difficult, especially from sites in the east, and this separation by age was dropped in 1999. The mean total weight of subterranean clover seed per core per treatment from 20 samples was used as the measure of seed yield at each site and expressed as kg $ha^{-1}$.

Subterranean clover seedling densities were assessed in autumn, 3–4 weeks after germination. A soil core containing seedlings was removed by using the steel corer with a surface area of 78.5 $cm^2$. The seedlings were counted in the field when numbers were low while the corer was still in the ground. When numbers were higher, they were counted after breaking up the soil core onto a plastic tray. At the centre of each treatment area, 30 cores were taken in a transect at 10-m intervals. The mean count of seedlings per core per treatment from 30 samples was used as a measure of seedling density at each site and expressed as number plants $dm^{-2}$.

### Statistical analyses

A key aim of this study was to compare regions, and one mean from each site was used as the replicate. There were three sets of data, each set consisting of RLEM sampled in sprayed and unsprayed treatment plots pre-spray in spring and again following mite emergence in autumn. The first set was sampled pre-spray in spring 1998 and in autumn 1999 at sites in the west and in the east. The second set used the same sites sprayed again and sampled pre-spray in spring 1999 and autumn 2000. The third set used new sites in South Australia and Victoria sampled pre-spray in spring 2002 and autumn 2003. The RLEM, BOM and LF counts for samples between sites were variable, and the mean sample for each species from each site, expressed as numbers $m^{-2}$, was $\log_{10}$-transformed to normalise the data. Two-way analyses of variance (ANOVA) were used for the 1998 and 1999 sets of data to test the effects of spray (sprayed and unsprayed), region (west and east) and the spray × region interaction, on the RLEM populations in autumn, using the pre-spray spring data from sprayed and unsprayed treatments as covariates. This tests whether populations in sprayed and unsprayed plots in autumn were influenced by populations in sprayed and unsprayed plots the previous spring. Populations of BOM and LF were analysed in the same way; LF were not counted in the spring 1998 sampling. Seed yield (kg $ha^{-1}$) and seedling numbers (plants $dm^{-2}$) were also $\log_{10}$-transformed to normalise the data before 2-way analysis of variance to test the effects of spray, region, year and the spray × region interaction. For each sampling occasion, the untransformed means were plotted to compare spray treatment effects, and separate analyses of variance were carried out for each sample occasion. All analyses were carried out using Statistix version 12.5 (Analytical Software, Tallahassee, FL, USA).

## Results

### Abundance of RLEM

Populations of RLEM in unsprayed treatments were high but varied between seasons, years and regions (Table 4). The spring spray resulted in significantly lower RLEM populations in the sprayed treatments; in autumn 1999, populations at sprayed sites in the west were 97% lower than in unsprayed treatments and in the east 93% lower (Table 5, Fig. 1). In the sprayed treatments in autumn, there were 829 RLEM $m^{-2}$ in the west and 434 $m^{-2}$ in the east (Fig. 1). There was no significant difference in RLEM control in autumn 1998 in the western region between the sites where omethoate and dimethoate were used (ANOVA on $\log_{10}$-transformed RLEM numbers comparing chemicals: *F*-ratio = 0.887, d.f. = 1,25 (n.s.); for spray treatment: *F*-ratio = 45.7, d.f. = 1,25 ($P < 0.0001$)).

The mean pre-spray populations were not significantly different in the unsprayed and sprayed treatments in spring 1998, 1999 or 2002 (Fig. 1). During winter 1999, the RLEM populations recovered and they were not significantly different in sprayed and unsprayed treatments in spring 1999 in the west or the east. Following a second spray in spring 1999, the RLEM populations in autumn 2000 in the sprayed treatments were again 97% of those in the unsprayed treatments in the west and 93% in the east (Table 5, Fig. 1). There were 276 RLEM $m^{-2}$ in the sprayed treatment in the west and 235 $m^{-2}$ in the east. Abundance in the unsprayed treatments was consistently high, but the populations were relatively lower in autumn than in the previous spring on all occasions except autumn 1999 in the west (Table 4). The covariance was significant in autumn 1999 when the population in the unsprayed treatment was low in the east but remained high in the west. In autumn 2000 when the covariance was significant, the population in the unsprayed treatments was lower in the east than in the west in both

**Table 4. Herbivorous mesofauna (redlegged earth mite, RLEM; blue oat mite, BOM; lucerne flea, LF) (mean number $m^{-2}$ ±s.e.) sampled in the unsprayed treatments of pastures**

| Region | Species | Spring 1998 | Autumn 1999 | Spring 1999 | Autumn 2000 |
|---|---|---|---|---|---|
| West | RLEM | 23439 ± 6167 | 32112 ± 8536 | 23277 ± 4891 | 10307 ± 3307 |
| | BOM | 547 ± 135 | 733 ± 406 | 789 ± 216 | 310 ± 65 |
| | LF | – | 296 ± 89 | 405 ± 244 | 795 ± 280 |
| East | RLEM | 21506 ± 7133 | 6448 ± 2804 | 13728 ± 6069 | 3214 ± 1314 |
| | BOM | 778 ± 248 | 378 ± 146 | 662 ± 489 | 484 ± 143 |
| | LF | – | 318 ± 100 | 571 ± 325 | 606 ± 283 |
| | | Spring 2002 | Autumn 2003 | | |
| East | RLEM | 30091 ± 10280 | 10663 ± 3067 | | |
| | BOM | 6846 ± 1542 | 3113 ± 1228 | | |
| | LF | 6601 ± 5041 | 1510 ± 505 | | |

**Table 5. Mean-squares for analyses of variance on numbers of redlegged earth mite, blue oat mite and lucerne flea ($log_{10}$-transformed) across sites in southern Australia**

Two ha of the experimental plot was sprayed in spring and 2 ha was left unsprayed. Regions: sites separated into those in west (Western Australia) and east (Victoria, New South Wales, South Australia). Three sets of data were analysed for spring (pre-spray) 1998–autumn 1999, spring (pre-spray) 1999–autumn 2000, and spring (pre-spray) 2002–autumn 2003 (south coastal east region). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

| Sample date (*n* = no. sites) | Spray (d.f.) | Region (d.f.) | Spray × region (d.f.) | Covariance with spring numbers (d.f.) | Error (d.f.) |
|---|---|---|---|---|---|
| | | | *Redlegged earth mite* | | |
| Autumn 1999 (28) | 23.43 (1)*** | 2.40 (1)* | 1.04 (1) | 3.40 (1)** | 0.43 (51) |
| Autumn 2000 (26) | 13.85 (1)*** | 0.09 (1) | 0.51 (1) | 2.03 (1)* | 0.36 (47) |
| Autumn 2003 (9) | 12.07 (1)** | – | – | 0.0078 (1) | 0.563 (15) |
| | | | *Blue oat mite* | | |
| Autumn 1999 (28) | 0.35 (1) | 0.07 (1) | 0.29 (1) | 0.05 (1) | 0.348 (39) |
| Autumn 2000 (26) | 0.711 (1) | 0.062 (1) | 0.114 (1) | 0.851 (1) | 0.251 (38) |
| Autumn 2003 (9) | 4.86 (1)* | – | – | 0.035 (1) | 0.464 (15) |
| | | | *Lucerne flea* | | |
| Autumn 1999 (28) | 0.828 (1) | 0.090 (1) | 0.042 (1) | – | 0.356 (44) |
| Autumn 2000 (26) | 1.032 (1) | 1.216 (1) | 0.075 (1) | 1.031 (1) | 0.407 (35) |
| Autumn 2003 (9) | 0.54 (1) | – | – | 0.028 (1) | 0.355 (15) |

spring and autumn, but fell in autumn 2000 more in the east than in the west (Tables 4 and 5).

The RLEM populations in autumn 2003 were significantly lower (92%) in the sprayed than in the unsprayed treatment, but there was no significant covariate (Table 5, Fig. 1). In autumn 2003 in the sprayed treatment, there were 649 mites $m^{-2}$.

### Abundance of BOM and LF

Pasture communities were dominated by RLEM. In the west in 1999 and 2000 (no LF were counted in spring 1998), RLEM comprised 94% of the population in unsprayed treatments, and in the east in 1999, 2000, 2002 and 2003, RLEM comprised 79% (Table 4). At sites in the west, BOM and LF each made up 3% of the population in these years, whereas in the east, BOM made up 12% and LF 9% (Table 4). When abundance is compared for each species separately on each sampling occasion, there were significant reductions with the spray treatment in BOM populations in autumn 1999 (70% reduction) and autumn 2000 (60%) in the west but not in the east, whereas in autumn 2003, there was a significant reduction in BOM (87%) in the east (Fig. 2). There were no significant effects of the spray treatment on LF populations.

### Subterranean clover seed yield and seedling density

Seed yields for sprayed and unsprayed treatments were significantly higher in the western region than the east (Table 6). Seed yield in unsprayed treatments at sites in the west (505 kg $ha^{-1}$) was twice that of sites in the east (216 kg $ha^{-1}$). When effect of the spray treatment was tested separately for each sampling occasion, there was a significant increase in yield in summer 1999 at sites in the west, but not in 1998 or in the east (Fig. 3). In 1998 when seed age was estimated, most seeds in the west were new in both sprayed (87%) and unsprayed (86%) treatments. In 1998, only 59% of the seeds in the east were new in sprayed treatments and 47% in unsprayed treatments. The proportion of new seed was thus lower in the east, and at these sites there was no increase in total seed yield with the spray treatment.

Unsprayed treatments had, on average, 7.3 seedlings $dm^{-2}$ at sites in the west in 1999 and 2000, compared with 5.0 seedlings $dm^{-2}$ at sites in the east in 1999, 2000 and 2003. There

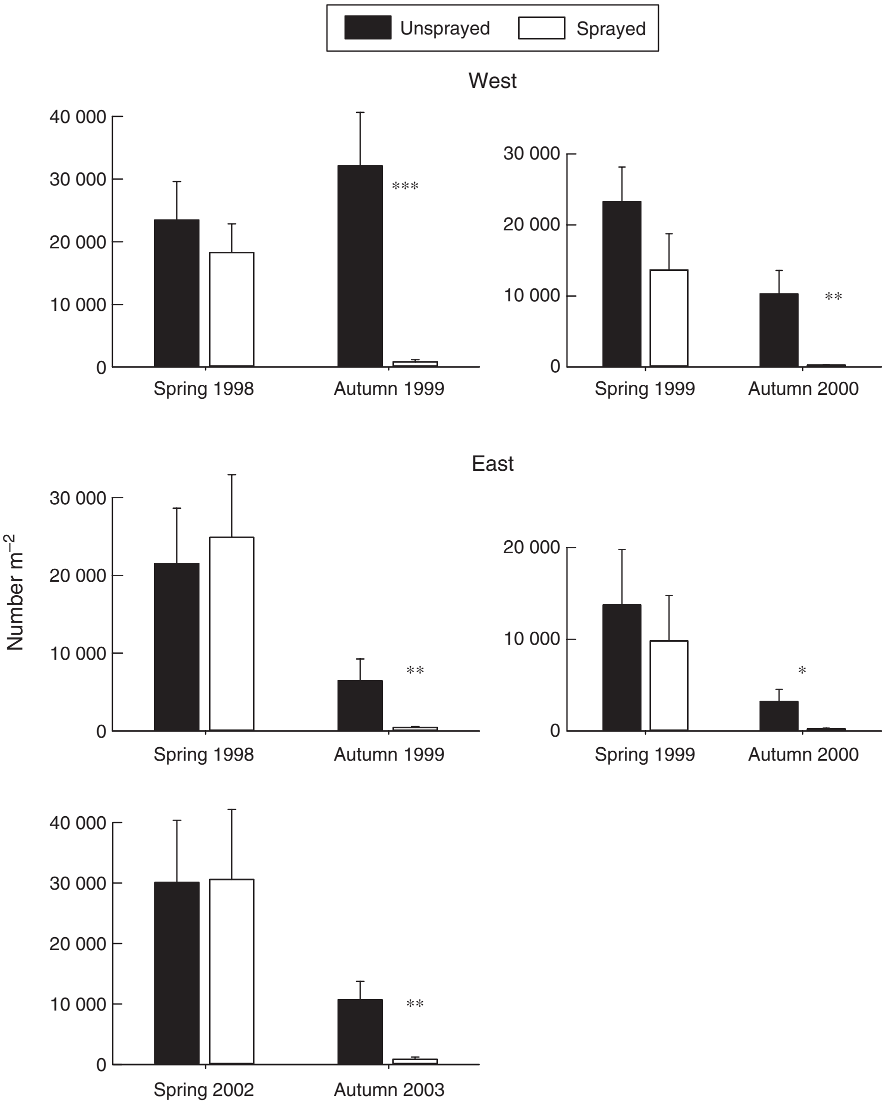

**Fig. 1.** Comparison between the sprayed (black bars) and unsprayed (white bars) treatments for numbers of *Halotydeus destructor* $m^{-2}$ (untransformed mean ± s.e.). Analyses of variance on untransformed data to compare spray effects in spring and autumn for 1998–99, 1999–2000 and 2002–03. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

were significantly more seedlings in sprayed treatments than unsprayed treatments, in the west in both 1999 and 2000 (Table 6). In the separate analyses for each sampling occasion, the spray treatment resulted in a significant increase in seedling numbers for both years (autumn 1999 and 2000) in the west and no significant increase for the sites in the east (Fig. 3).

## Discussion

A single TIMERITE® spring spray resulted in effective control in autumn despite differences in the size of the unsprayed populations. RLEM numbers in the sprayed treatment were, on average, 97% lower in the west and 93% lower in the east than in the unsprayed treatments. Most of the sites that had been sprayed (85%) had <1000 RLEM $m^{-2}$, the lower economic threshold reported for mite control, and the largest number surviving in a sprayed treatment was 5758 RLEM $m^{-2}$, which still represents 95% control for this site (there were 112 056 RLEM $m^{-2}$ in the unsprayed treatment). The results confirm that a spring spray on the TIMERITE® date gives effective RLEM control in autumn across Australia. There was no evidence from our limited data that a well-timed spring spray with dimethoate was less effective than omethoate. The greater level of control reported in the western region mirrors reports for three sites in the west (99%) by Ridsdill-Smith *et al.* (2005). A lower level of control in the east was also noted by Gower *et al.* (2008), who reported control from 74% in 2004 to 89% in 2005 for five sites in the east (western and central Victoria).

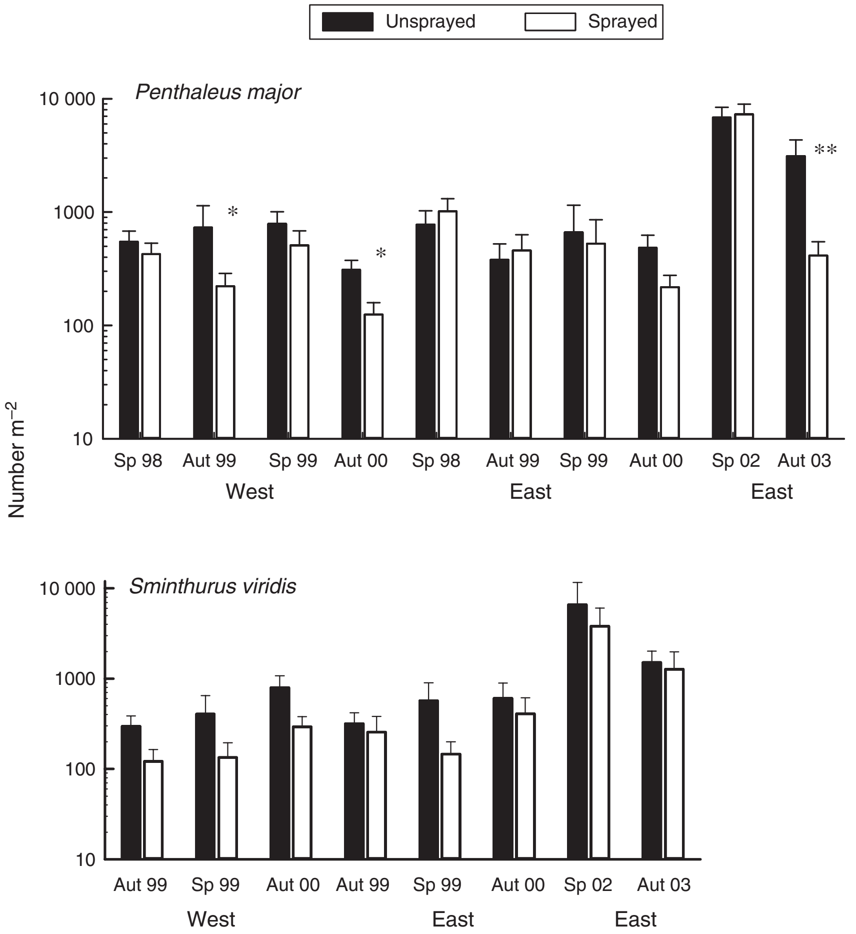

**Fig. 2.** Comparison between the sprayed (black bars) and unsprayed (white bars) treatments for numbers of *Penthaleus major* and *Sminthurus viridis* m$^{-2}$ (untransformed mean ± s.e. plotted with $\log_{10} y$-axis). Analyses of variance on transformed data to compare spray effects in spring and autumn for 1998–99, 1999–2000 and 2002–03. $*P < 0.05$; $**P < 0.01$.

In developing the TIMERITE® model, 81% of the observed dates for onset of 90% summer-diapause egg production are within 1 week of the predicted dates. However, at higher rainfall sites in the east region of Australia, the results are more variable with only 67% of the observed dates being within 1 week of the predicted 90% diapause dates (Ridsdill-Smith *et al*. 2005). At these higher rainfall sites in the east, TIMERITE® spray dates were 14–19 October (T. J. Ridsdill-Smith, C. Pavri, unpubl. data). No sites in the west were in this range, but about one-third of the sites in the east were, as were those reported by Gower *et al*. (2008) (spray dates 12–16 October). The more variable dates for onset of diapause observed at sites that are cooler and wetter in the spring and early summer may account for the slightly lower levels of control evident in the east in our study and in the study of Gower *et al*. (2008).

An ecological study of RLEM showed that more eggs are laid at the site where populations are higher (Ridsdill-Smith and Annells 1997). It is suggested that higher rainfall at this site resulted in greater pasture resources for RLEM and they lay more eggs, demonstrating the opportunistic adaptations of RLEM that make it such a successful pest. The populations controlled in autumn 1999 in this study had largely recovered by spring 1999, and Gower *et al*. (2008) came to the same conclusion. RLEM control is effective only for one season and a spring spray would therefore be required for each season when autumn control is needed. Because the spring spray killed 97% of the whole RLEM population at sites in the west, there is clearly scope to select for resistance to chemicals applied at this one time of year. We think that the risk of resistance developing can be reduced by not spraying the same population (site) every year and, if using several sprays a year, by making sure the TIMERITE® spring spray uses different chemical actions from those used earlier in the year.

Large populations of 20 000–30 000 RLEM m$^{-2}$ are common at pasture sites in Australia. If pastures remain green in spring after the production of diapause eggs is triggered, RLEM females can continue feeding for several weeks, accumulating diapause eggs before they die, but if pastures senesce early, fewer diapause eggs are produced. Even when very high numbers of diapause eggs are counted in December, it does not necessarily result in large populations of RLEM in autumn

**Table 6. Mean-squares for analyses of variance on seed yield ($\log_{10}$-transformed) for summer 1998 and summer 1999, and on numbers of seedlings ($\log_{10}$-transformed) for autumn 1999, autumn 2000 and autumn 2003**

Same sites were sampled in 1998, 1999 and 2000. $^{*}P < 0.05$; $^{**}P < 0.01$

| Season | Year and region ($n$ = no. sites) | Spray (d.f.) | Error (d.f.) |
|---|---|---|---|
| | *Seed yield (log kg ha$^{-1}$)* | | |
| Summer | 1998 West (17) | 0.220 (1) | 0.130 (34) |
| | 1999 West (15) | 0.531 (1)** | 0.061 (28) |
| | 1998 East (10) | 0.004 (1) | 0.091 (18) |
| | 1999 East (10) | 0.004 (1) | 0.091 (18) |
| | *Seedlings (log no. of seedlings dm$^{-2}$)* | | |
| Autumn | 1999 West (18) | 1.300 (1)** | 0.139 (34) |
| | 2000 West (16) | 1.294 (1)* | 0.186 (30) |
| | 1999 East (9) | 0.140 (1) | 0.163 (16) |
| | 2000 East (10) | 0.098 (1) | 0.173 (18) |
| | 2003 East (9) | 0.174 (1) | 0.167 (16) |

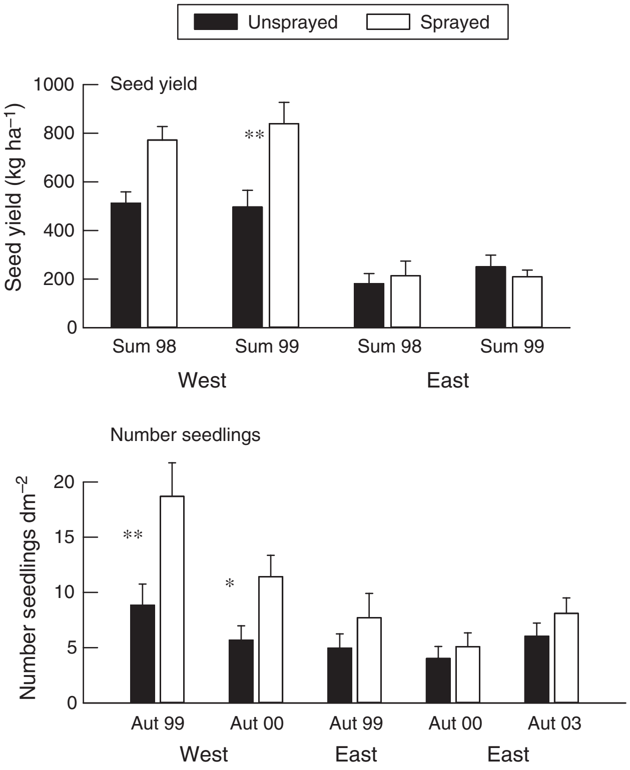

**Fig. 3.** Comparison between sprayed (black bars) and unsprayed (white bars) treatments on subterranean clover seed yield (kg ha$^{-1}$) in summer and numbers of seedlings (number dm$^{-2}$) in autumn (untransformed means ± s.e.). Analyses of variance on untransformed data to compare spray effects for each region and each year of sampling. $^{*}P < 0.05$; $^{**}P < 0.01$.

(Ridsdill-Smith and Annells 1997). Significant covariate effects in autumn 1999 and 2000 seemed to be associated with substantial falls in the numbers of mites between spring and autumn in unsprayed treatments, especially in the east. Aestivating RLEM eggs that are artificially moistened in December show much greater mortality than aestivating eggs that are moistened in January (Annells and Ridsdill-Smith 1991). The Mediterranean climate in the west has hot, dry summers, whereas in the east, the temperature and rainfall regimes in spring and early summer tend to be more variable (www.bom.gov.au/iwk/climate_zones). This may cause greater mortality of oversummering RLEM eggs at sites in the east than sites in the west, and cause the lower populations observed in autumn than in spring, and especially in the east.

In autumn, the BOM populations on average were 60% lower in the sprayed than the unsprayed treatments, and the effects were significant on three occasions. Both BOM (Umina and Hoffmann 2003) and LF (Wallace 1968; Roberts *et al.* 2011*b*) have a summer diapause, but they start producing diapause eggs earlier in the winter and spring than do RLEM (Ridsdill-Smith *et al.* 2005), and would be less affected by the TIMERITE® spring spray. BOM and LF were most abundant at sites in the east in 2002–03. RLEM is reported to prefer sandy soils (Ridsdill-Smith 1997), whereas LF prefers fine-textured soils (Roberts *et al.* 2011*b*). In the present study, we do not have sufficient data to identify the factors affecting the abundance of these species.

The seedbank for subterranean clover was greater at sites in the west than in the east, and the proportion of new seed was greater. The increase in seed yield with the spring spray treatment was significant in 1999 (69% greater) in the west but not on other occasions. It is likely that subterranean clover seed yield was lower in the east because the summer rainfall causes some germination and subsequent death of seedlings when weather becomes hot and dry again; for example, mortality is greater at Tamworth, NSW, where there is more summer rainfall, than at Forbes, NSW, which is drier (Blumenthal and Ison 1994). Numbers of seedlings depend on the number germinating and the number killed by RLEM feeding after germinating. Seedling numbers were more than 100% greater with RLEM control at sites in the west in 1999 and 2000, but not in the east. The methods used here were developed by several authors in the west to measure seedling density by sampling 3–4 weeks after germination, and these methods may not be appropriate in the east where there may be false breaks of season more often. Different measures of plant production should be developed to look at plant density in the east, perhaps by sampling a little later in the winter.

Differences in weather between the west (where there is a hot, dry summer) and the east (where there are more variable temperature and rainfall regimes in the spring and early summer) seem to cause lower RLEM control with a spring spray in eastern Australia. In addition, benefits as measured by clover seed yield and seedling numbers appear to be greater in the west than the east of Australia. However, RLEM is considered a severe establishment pest to crops and pastures in both regions (Ridsdill-Smith *et al.* 2008; Murray *et al.* 2013), and TIMERITE® provides a tool that can be used to control populations for all regions in autumn. The methods developed in the west to measure benefits of RLEM control with subterranean clover seedling densities may need to be modified to improve measurement of benefits of control in pastures in the east. Factors influencing population ecology, and whether spring sprays can cause genetic adaptation by RLEM, will require detailed studies.

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