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Development of "best practices" for sampling of an important surface-dwelling soil mite in pastoral landscapes

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Abstract In this study, we analyzed 1145 vacuum samples of redlegged earth mites (RLEM) [*Halotydeus destructor* (Tucker) (Acari: Penthaleidae)] from 18 sampling events at six locations in pastoral landscapes of Western Australia during three growing seasons (2012–2014) (total of 228,299 RLEM individuals). The specific objectives were to determine: (1) presence/absence effects of a range of vegetation characteristics, (2) possible factors influencing RLEM sampling performance during the course of the season and day, (3) effects of size of area sampled and duration of sampling, (4) the spatial structure of RLEM counts in uniform pastoral vegetation, and (5) develop "best practices" regarding field-based vacuum sampling of surface dwelling soil mites in pastoral landscapes. We found that sampling of completely bare ground will lead to very low RLEM counts but spots with sparse vegetation (presence of bare ground) probably increases the presence of microhabitats for mites to shelter in and therefore lead to higher RLEM counts. RLEM counts were positively associated with the height of vegetation, at least up to about 15 cm in height. In early season (May–August), highest RLEM counts will be obtained in the afternoon hours (2–4 pm), whereas in late season sampling (August-November), highest RLEM counts will be obtained around noon. Higher RLEM counts should be expected from spots with grazed/mowed vegetation including cape weed and without presence of grasses and stubble. Variogram analyses of high-resolution data sets suggested that considerable range of spatial autocorrelation should be expected from fields with fairly uniform vegetation, especially if RLEM population densities are high. We are therefore recommending that samples are collected at least 30 m apart, if the objective is to obtain independent (spatially non-correlated) counts. The results from this study may be used to develop effective sampling protocols deployed in field ecology studies of soil surface dwelling mesofauna in pastoral landscapes and other ecosystems.

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Keywords Surface dwelling mites · *Halotydeus destructor* · Pastoral landscapes · Sampling · Soil ecosystems · Integrated pest management

Introduction

Accurate, reliable, cost-effective, and practically feasible sampling methods constitute one of the key elements of field ecology, including ecology of arthropods in agricultural and other anthropogenic systems. That is, field ecology of arthropods involves accurate characterization of spatial patterns of arthropods and other animals, understanding of their associations with vegetation types and micro-climatic conditions, quantification of their responses to anthropogenic interventions and habitat manipulations, and/or estimation of their population densities. The ability to thoroughly investigate any combination of these broadly defined objectives is closely linked to the required effort of gathering large enough data sets at sufficiently high spatial and temporal resolutions. Acknowledgement of the importance of large data sets in field ecology often implies that labor, time, and resources become important constraints, and that optimization of sampling effort is needed. As described by many, including Toews and Nansen (2012), there are essentially two types of sampling methods: (1) indirect sampling, which typically consists of arthropod counts obtained with trapping devices, and (2) direct sampling, which involve arthropod counts directly associated with a unit of area, volume, or weight. Indirect sampling counts are effective when the purpose is to characterize spatial or temporal trends, but they are less reliable as estimators of population densities (Nansen et al. 2001). Direct sampling enables population density estimates, but these sampling methods are often constrained by being quite labor intensive. Soil mites species are distributed differently within soil profiles (Eisenbeis 2010), and here we only consider those mites, which predominantly dwell on the soil surface and vegetation. These surface dwelling soil mites have been sampled based on both direct and indirect sampling methods. Regarding indirect sampling, there are few published studies involving pitfall trapping (Steffen et al. 2012; Wardle et al. 1995). Pitfall trapping with the main purpose of sampling mites has not been widely embraced by either scientists or managers of commercial agricultural systems, as the time investment into setting up traps and analyzing the captures are likely constraints. In addition, there is a lack of studies into relationships between captures and actual population densities. Most field ecology studies of surface dwelling soil mites and other mesofauna are based on direct sampling methods or direct visual inspections, including: (1) counts of surface dwelling soil mites and lucerne flea nymphs [*Sminthurus viridis* L. (Collembola: Sminthuridae)] from soil cores and vegetation (Ridsdill-Smith and Annells 1997; Wallace 1956, 1967), (2) visual inspection of feeding mites (Gaull and Ridsdill-Smith 1996), (3) visual inspection of plant damage (Chapman et al. 2000; Liu and Ridsdill-Smith 2000; Umina and Hoffmann 2004), and (4) vacuum sampling with a modified leaf blower (Arthur et al. 2014; Gaull and Ridsdill-Smith 1996; Liu et al. 2000; Ridsdill-Smith 1997; Ridsdill-Smith and Annells 1997; Ridsdill-Smith et al. 2008; Ridsdill-Smith and Pavri 2000b; Wallace 1972). As indicated by the references above, the most widely used current sampling method of surface dwelling soil mites under field conditions is vacuum sampling with a modified leaf blower. The benefits associated with vacuum sampling were recognized more than 40 years ago by Wallace (1972), who showed that it provides clean and efficient sampling. Umina and Hoffmann (2004, 2005) conducted vacuum sampling of soil mites with each

vacuum sampling event lasting 5–10 s, as preliminary experiments indicated that this sampling time was sufficient to sample >90 % of mites, regardless of time of day and vegetation type. In addition, Umina and Hoffmann (2004, 2005) conducted vacuum sampling in the morning, as it was believed that mites were most active at that time of day. However, these claims have never been studied further. Somewhat surprisingly, we are unaware of any quantitative studies on the performance of vacuum sampling method in field ecology research. With the growing body of research into effects of climate change and into effects of management practices on cultivated ecosystems, we argue that sampling of surface dwelling soil mites deserves further attention. More specifically, it would be valuable to quantify effects of: (1) presence/absence of vegetation characteristics, (2) time of day, (3) size of area sampled, (4) duration of sampling, and (5) a separate, but equally important, aspect is the spatial distribution pattern of soil surface dwelling mesofauna. That is, the range of spatial auto-correlation [often characterized through geostatistical analyses, such as semi-variogram analysis (Isaaks and Srivastava 1989; Liebhold et al. 1993)] can be used to estimate the minimum distance over which samples are taken in order to ensure that samples may be considered spatially independent (non-correlated), and therefore suitable for non-spatial statistical analyses, such as, analysis of variance. Geostatistical analyses of soil fauna has provided detailed insight into responses by taxa and community food webs to soil heterogeneity and into factors that appear to maintain and regulate soil biodiversity (Ettema and Wardle 2002).

Adult herbivorous surface dwelling soil mites, including redlegged earth mites (RLEM) [*Halotydeus destructor* (Tucker) (Acari: Penthaleidae)] and at least three species of oat mites [*Penthaleus major* (Dugés), *P. falcatus* (Qin and Halliday), and *P. tectus* (Robinson and Hoffmann 2001) are approximately 1 mm in size and can reach densities of 60,000 mites per m^2 (Ridsdill-Smith and Pavri 2000a). Different soil mite species show differences in their regional distributions and host plant selection (Robinson and Hoffmann 2001; Weeks and Hoffmann 1999). In Australian cropping systems, the surface dwelling soil mite species are active and bi- or tri-voltine between April and October and show some species-specific variation in timing of diapause and egg production (Umina and Hoffmann 2003) and in regional distribution (Hill et al. 2011). Diapause soil mite eggs hatch in April/May when conditions are optimal after cool temperatures and rainfall (Wallace 1970). In 1991, annual losses in Australia alone to wool, meat, grain, and dairy industries by RLEM damage was estimated to be worth AUS $145–201 million (Ridsdill-Smith 1991), which in 2014-dollars amounts to AUS $258–358 (assuming 78 % inflation, http://fxtop.com/en/inflation-calculator.php) per year. RLEM is considered an establishment pest, as they feed on plants during germination and seedling stages, and they are also economically important pests in other countries, including South Africa and New Zealand (Qin and Halliday 1995).

In this study, we analyzed 1145 vacuum samples (total of 228,299 RLEM individuals) from 18 sampling events at six locations in Western Australia during three growing seasons (2012–2014). The vacuum samples were collected from a range of pastoral landscapes, and the specific objectives were to determine: (1) presence/absence effects of a range of vegetation characteristics, (2) possible factors influencing RLEM sampling performance during the course of the day, (3) effects of size of area sampled and duration of sampling, and 4) the spatial structure of RLEM counts in uniform pastoral vegetation. All of the highlighted factors are critically important for optimized and consistent soil mite sampling and for development of a scientifically sound protocol for estimation of RLEM densities in pastoral field plots. Based on characterization of the importance/influence of these variables, we have developed "best practices" regarding vacuum sampling of RLEM. The

results from this study may be of relevance for sampling of other species of surface dwelling soil mites and of other mesofauna.

Materials and methods

Field sampling and mite counting

In this study, modified leaf blowers were used for vacuum sampling of RLEM (Fig. 1). An 18 cm diameter PVC pipe was mounted on the air inlet and used to suck RLEM individuals from soil and vegetation onto a stainless steel meshed screen (125 μm) at the end of the PVC tube. This stainless steel meshed screen was removed and cleaned between sampling events. Thus, an area of 0.025 m^2 was sampled each time the sampler was placed onto soil and vegetation. The pvc pipe was cut at a 45° angle, so that vacuum can be created by holding the vacuum sampler in that angle. In some of the data sets included in this study, soil mite numbers were very low (0–20 mites per sample), and it was therefore possible to immediately count the RLEM in the plastic ring with a mesh after transfer to a large white plastic container. Regarding RLEM samples with higher numbers, the samples (dust, crop debris, RLEM, etc.) were transferred from the plastic ring with a mesh, using a funnel, to vials with 70 % ethanol for subsequent analysis under stereoscope. In the laboratory, each sample was calibrated to 30 ml 70 % ethanol, stirred with a 5 ml plastic pipette (the tip was cut off to allow suction of plant debris), and a 3 ml subsample (10 % of the total volume) was extracted. The number of RLEM in each subsample was counted and multiplied by 10 to obtain the total mite number per sample. Initial testing of this sub-sampling method indicated that the actual number of RLEM per sample could be estimated with 90–95 % accuracy.

Fig. 1 Vacuum sampling of RLEM

Field locations

The field data included in this study were collected by six different people. It is possible that people sampled with different efficiency, but this aspect was not included in this study. Vacuum sampling of RLEM was conducted in six pastoral landscapes in Western Australia. Each of the sampling locations is briefly described.

Albany (61 samples) is located in the southern part of Western Australia and was sampled once. The sampling location was a fairly uniform pasture grazed by sheep. The main purpose of the sampling was to characterize the spatial distribution pattern of soil mite counts within a 30 × 30 m grid. The vacuum samples were collected at known grid points (measured to the nearest m) with a minimum distance apart of 1 m at haphazardly selected grid locations. All samples were collected July 4, 2012 in the afternoon.

Boyup Brook (18 samples) is located in the south-west of Western Australia and was sampled once. The main objective with this sampling event was to assess the importance of sampled area (effect of how many consecutive times the vacuum sampler was placed on top of soil and vegetation at adjacent spots—see description below) and effect of different pastoral plants. All samples were collected September 2, 2013 in the afternoon.

Capel (438 samples) is located in the South Western region of Western Australia and was sampled twice. The sampling location was a highly, uniformly grazed pasture with ryegrass (*Lolium perenne* L) as the dominant species. The main purpose of the sampling was to characterize the spatial distribution pattern of soil mite counts within two adjacent 50 × 50 m grids with a minimum distance apart of 1 m at haphazardly selected grid locations and about 100 samples collected at each combination of sampling event and sampling grids. Both fields were sampled on two separate dates (June 24 and July 9, 2014), and soil mite sampling was conducted throughout the day.

Pingelly (140 samples) is a UWA field station in the central agricultural region of Western Australia. Grazed pasture was sampled at three events at this location, and the main sampling purpose was to obtain soil mite samples from different pastoral plants. Soil mite samples were collected on three dates (September 20, October 3 and 17, 2013) throughout the day from this field location.

Shenton Park (176 samples) is a UWA field station near Perth in Western Australia, and we sampled continuous and uniform grassy areas with varying levels of mowing (simulated grazing). A total of six sampling events were conducted at this location, and the main sampling purposes were to assess effect of vegetation type, time of sampling, duration of sampling, and area sampled (see details below). Soil mite samples were collected on six dates (September 11, 13, 16, 17, 30, and October 2, 2013) throughout the day from this field location.

York (312 samples) is located in the central agricultural region of Western Australia and was sampled four times. The sampled location consisted of patchy vegetation, and the main purpose of this sampling was to obtain soil mite counts from different distinctly separated patches of uniform host plant composition. Soil mite samples were collected on three dates (July 13, 20, and August 17, 2012) throughout the day from this field location.

Data preparation

Although sampled spots were fairly small (0.025 m^2), accurate quantifications of plant species within the sampled spots was considered challenging. Consequently, all vegetation characteristics were assessed on a dichotomous basis (presence = 1 and absence = 0): (1)

presence/absence of vegetation, (2) vegetation grazed/mowed (yes/no), (3) presence/absence of decaying vegetation (some samples were collected from spots without any vegetation), (4) presence/absence of bare ground (some samples were collected from spots with partial bare ground but vegetation was also present), (5) presence/absence of wheat (*Triticum aestivum* L.) stubble, (6) presence/absence of cape weed (*Arctotheca calendula* (L.) Levyns), (7) presence/absence of clover (*Trifolium* spp.), and (8) presence/absence of grass (Graminae). Time of day (hour) was rounded to closest whole hour to create hourly intervals. Area sampled denoted the number of times the vacuum sampler was placed on top of vegetation and soil at spots immediately adjacent to one another. Thus, the total area sampled was equivalent to the number of adjacent spots times 0.025 m^2, and we sampled from 1 (sampled area = 0.025 m^2), 3 (sampled area = 0.075 m^2), 5 (sampled area = 0.125 m^2), or 10 (sampled area = 0.250 m^2) adjacent spots. Duration of sampling was measured in seconds and denoted how long the vacuum sampler was held on top of vegetation and soil at each spot, and we sampled for 1, 3, 5, 10, or 20 s at each spot. Vegetation height was divided into 5-cm intervals: 0–5, 5–10, 10–15, 15–20 cm, and above 25 cm.

Statistical analysis

Several thousand RLEM individuals may occur within a single vacuum sample, and the highest count was 4160 in a single sample. Consequently, all RLEM counts were $\log_{10}(y+1)$ transformed prior to statistical analysis. All statistical analyses were performed with PC-SAS 9.4 (SAS Institute, Cary, NC, USA). Due to the unbalanced numbers of RLEM counts from different locations and types of vegetation, we used generalized linear models (proc glm) to examine average differences in RLEM counts in response to vegetation height classes and each of the dichotomous variables and to examine differences in RLEM counts collected during the day. In all generalized linear models, statistical results were based on type III weighted squares of means to account for the unbalanced nature of the data set and to adjust for all effects (including interactions) listed in each model statement. Semi-variogram analysis (proc variogram) was used to characterize the spatial structure of five soil mite count data sets (collected at locations in Albany and Capel). This analytical approach has been described in other spatial studies of arthropods (Liebhold et al. 1993; Mankin et al. 2014; Nansen et al. 2003, 2009), and a detailed description is also available in Nansen (2012). In all five data sets subjected to semi-variogram analysis, we chose a lag distance = 2 m, and in analyses of data sets from the Capel location, we calculated semi-variance in 15 lag distance intervals, while it was only calculated in 6 lag distance intervals for the Albany data set. The difference in number of lag distance intervals was based on differences in size of sampling spaces (Capel = 2500 m^2 and Albany = 900 m^2). Similar to Nansen et al. (2014a, b), we used the following non-linear regression fit to model the semi-variance as a function of lag distance:

$$F(D) = a + b \times [1 - e^{(-c \times D)}] \quad (1)$$

in which a, b and c are fitted parameters, and *F(D)* is the semi-variance at each lag distance interval, D. In geostatistical terms (Isaaks and Srivastava 1989; Liebhold et al. 1993), "a" denotes the "nugget" or the intercept with the y-axis, "b" is a relative estimate of the "sill" or the level of spatial variance at which the "range" is reached, and "c" is a relative estimate of the "range", which is the lag distance at which paired observations are no longer spatially autocorrelated. We conducted a principal component analysis (proc

princomp) to investigate the relationship between average RLEM counts, variogram coeffiecients (derived from Eq. 1), and average presence of clover, grass, and cape weed at the sampled locations in the five spatial data sets.

Results

Figure 2a shows the frequency distribution of the 1145 soil mite samples included in this study. A total of 228,299 RLEM individuals were counted, and the highest count in a single sample was 4160, or the equivalent of more than 150,000 RLEM individuals per m^2. At all locations except Albany, we obtained soil mite counts of zero, and both average and highest soil mite counts per location varied considerably across the six field locations (Fig. 2b). The within and among location heterogeneity in sampling of RLEM underscore that numerous environmental and practical variables (and potentially complex interactions amongst them) are likely affecting obtained RLEM counts.

Seasonal and diurnal trends and effects of sampling effort

The average RLEM counts for the 17 data sets included in this study were plotted as a function of day number of the year (1–365) (Fig. 3a). After accounting for effects of data being unbalanced, it was seen that average RLEM counts acquired in 2012 and 2014 in early season (days 160–250) were significantly lower than those sampled in late season (days 250–320) ($F_{1,1143} = 431.84$, $P < 0.001$). This important seasonal trend was incorporated into all the subsequent statistical analyses.

Regarding size of sampled area (how many 0.025 m^2 spots being sampled) and duration of each sampling, we were concerned about possible loss of suction if a sampled area was too large and/or sampling time too long, as soil and debris may clog the sampling filter. After accounting for seasonal variation, we found that sampled area (number of sampled spots) did not have a significant effect on average RLEM counts ($F_{3,1140} = 1.96$, $P = 0.12$). However, there was a significant and positive effect of sampling time on average RLEM counts ($F_{4,1139} = 6.80$, $P < 0.001$) (Fig. 3b).

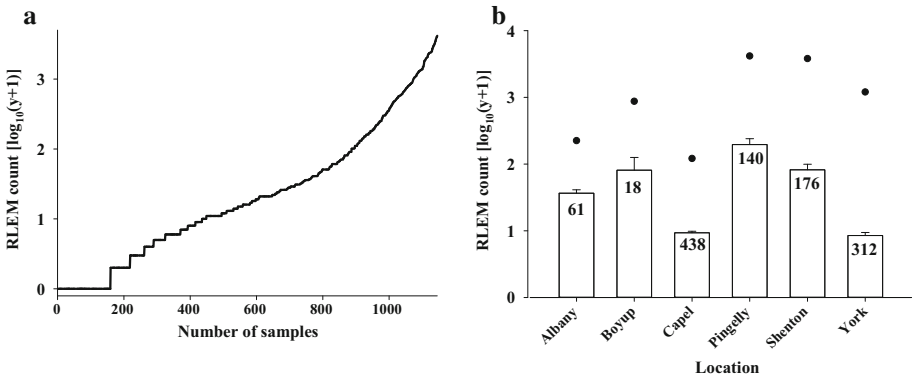

Fig. 2 Frequency distribution (**a**) and average (SE) and maximum (*black dots*) RLEM counts for each of the six field locations (**b**) of the 1145 vacuum samples included in this study

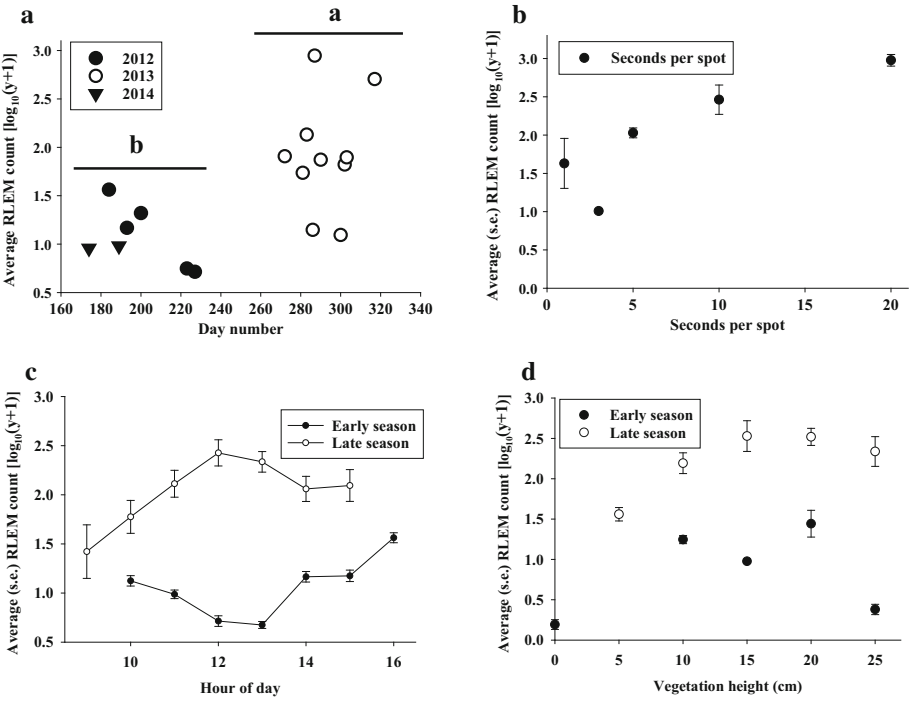

Fig. 3 Average RLEM counts per sample as a function of day number with data being divided into early and late seasons (**a**). Average RLEM count (SE) in response to sampling time (seconds) at each spot (**b**). Diurnal trends (**c**) and effect of vegetation height (**d**) on RLEM counts in early and late season sampling. *Letters* denote significant difference at the 0.05-level

After accounting for the seasonal effect and sampling time, there was a significant effect of hour of sampling on RLEM counts ($F_{7,1132} = 11.55$, $P < 0.001$), but the diurnal effect was also significantly different between early and late season sampling ($F_{7,1137} = 8.19$, $P < 0.001$) (Fig. 3c). It was seen that in early season sampling, RLEM counts peaked

Table 1 Analyses of variance (type III sum of squares) of eight dichotomous vegetation variables

Average counts of RLEM (number of counts) in samples in which the variable was either absent or present

Variable	Absence	Presence	F	*P*
Significant variables				
Cape weed	123.2 (869)	439.2 (276)	104.76	<0.001
Grazed/Mowed	155.1 (907)	368.0 (238)	87.05	<0.001
Stubble	216.8 (1039)	28.5 (106)	48.74	0.001
Vegetation	2.0 (51)	208.6 (1094)	21.17	<0.001
Bare ground	148.4 (990)	525.3 (155)	15.44	<0.001
Grass	385.5 (553)	25.6 (592)	4.04	0.045
Non-significant variables				
Decaying	207.4 (1082)	61.2 (63)	2.80	0.095
Clover	180.9 (660)	224.5 (485)	0.86	0.36

around noon, while in late season sampling the highest counts were obtained in the afternoon.

Effects of vegetation

Due to the results described above, the subsequent analyses of vegetation variables were conducted with effects of season, sampling time, and hour of day as co-variables. The effect of vegetation height was highly significant ($F_{5,1127} = 32.32$, $P < 0.001$) and similar in the two season periods with highest counts in vegetation with a height between 10 and 20 cm (Fig. 3d). The relative effect of eight dichotomous vegetation variables was therefore assessed after also including vegetation height (in addition to season, sampling time, and hour of day) as a co-variable (Table 1). In descending order of significant effects, it is seen that significantly higher RLEM counts were obtained from spots with presence of cape weed, with mowed/grazed vegetation and absence of grass. In addition, very low RLEM counts were obtained from spots with no vegetation, but presence of bare ground (sparse vegetation had a positive effect on RLEM counts. Finally, absence of grass also had a positive effect on RLEM counts. Presence of decaying vegetation and clover did not have a significant effect on RLEM counts. Individuals were sampled on spots with completely bare ground versus on spots with vegetation present. However, presence of bare ground within the sampled area (compared to complete vegetation coverage) had a significantly positive effect on average RLEM counts.

A final general linear model was conducted, in which the dichotomous vegetation variables were included together with the variables accounting for season, sampling time, and hour of day. In this general linear model, we also included interactions between the dichotomous vegetation variables and season and hour of day. After excluding all variables and interactions without significant contributions, we obtained a highly significant model

Table 2 Generalized linear model of redlegged earth mite counts

Source	DF	Type III SS	F	*P*
Linear variables				
Early season (yes/no)	1	11.20	36.57	<0.0001
Height class	5	33.25	21.72	<0.0001
Bare ground (presence/absence)	1	5.08	16.58	<0.0001
Dry matter (presence/absence)	1	4.71	15.40	<0.0001
Hour of day	7	10.96	5.12	<0.0001
Interactions				
Mown/grazed × hour of day	6	35.08	19.10	<0.0001
Grass × early season	1	2.70	8.81	0.0031
Bare ground × hour of day	6	15.70	8.55	<0.0001
Dry matter × hour of day	3	7.32	7.97	<0.0001
Grass × hour of day	6	10.38	5.65	<0.0001
Height class × hour of day	24	36.39	4.95	<0.0001
Cape weed × hour of day	7	9.67	4.51	<0.0001
Height class × early season	3	3.23	3.52	0.015

with five variables and eight interactions ($R^2 = 0.671$, $F_{74,1070} = 29.45$, $P < 0.001$), which explained around 67 % of the total variance in RLEM counts (Table 2).

Spatial auto-correlation of RLEM counts

As indicated by the analyses presented above, there are numerous variables greatly impacting RLEM counts obtained from vacuum sampling, so characterization of the spatial auto-correlation (spatial structure) would only be meaningful in pastures with fairly homogenous vegetation within the sampled area and with all samples being collected the same way (all samples representing RLEM from 0.025 m^2 and sampling duration equal to 3 s). Consequently, only RLEM counts from Albany (one field sampled once) and Capel (two fields sampled twice) were included in this part of the study, and average counts varied between 6.1 and 51.9 among these data sets (Fig. 4a) and results from variogram analyses are presented in Table 3. The spatial distribution patterns of RLEM counts in the two Capel fields sampled on two separate dates showed that (Fig. 4b): (1) the spatial variance was higher on day 1 compared to day 2 and there was considerable difference between the two fields, (2) on day 2, the spatial distribution patterns were very similar for the two fields, and (3) in three of the four data sets, there was no indication of spatial dependence (auto-correlation) reaching an asymptote within 50 m. This suggests that the RLEM counts were spatially auto-correlated beyond 50 m apart. Regarding the Albany field, an asymptote of spatial autocorrelation was reached (denoted the "range") at a lag-distance of about 10 m.

We conducted principal component analysis of average RLEM counts, variogram parameters (a, b, and c), and average presence of clover, grass, and cape weed at the sampled locations in the five data sets (Fig. 5). About 80 % of the variance (PCA1 = 59.2 % and PCA2 = 21.7 %) was explained by the two principal axes, and positive associations between vegetation characteristics and variogram parameters were identified.

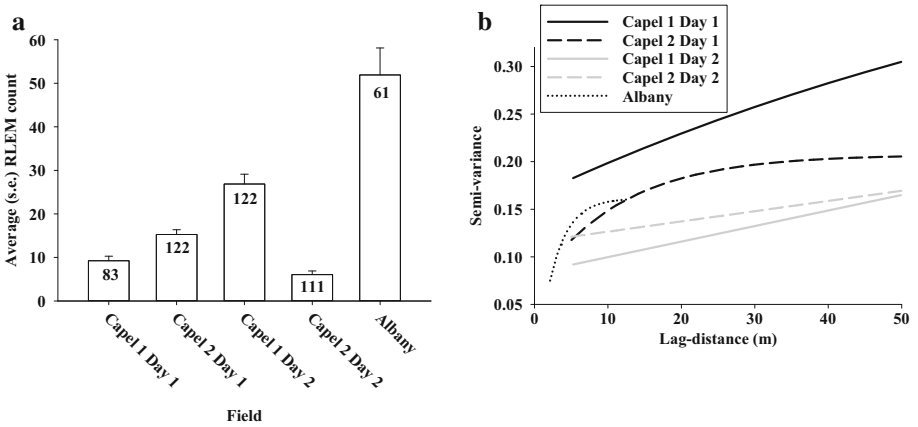

Fig. 4 Average RLEM counts (SE) per sample for each of the five spatial data sets (**a**) and their corresponding semi-variograms (derived based on Eq. 1)

Table 3 Basic statistics and variogram parameters from five spatial data sets

Variogram parameters				Regression fit	
Data set	*a*	*b*	*c*	Adj R^2	F
Capel 1 day 1	0.1602 (0.0139)	0.2946 (0.2345)	0.0134 (0.0155)	0.75	34.21***
Capel 2 day 1	0.0834 (0.0088)	22.1148 (4616.8567)	7.40E−05 (0.0155)	0.84	58.29***
Capel 1 day 2	0.0844 (0.0278)	0.1252 (0.0224)	0.0689 (0.0279)	0.7	26.33***
Capel 2 day 2	0.1158 (0.0121)	14.8097 (6760.0856)	7.28E−05 (0.0333)	0.53	12.89***
Albany	0.015 (0.0412)	0.1491 (0.0338)	0.2948 (0.1303)	0.91	25.54*

Based on Eq. 1, we calculated variogram parameters, "*a*", "*b*", and "*c*" and conducted a non-linear regression fit of RLEM counts

* $P < 0.05$, *** $P < 0.001$

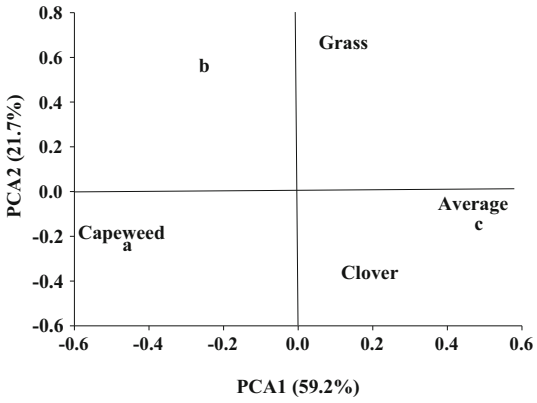

Fig. 5 Principal component analysis of average RLEM counts, three variogram parameters (*a*, *b*, and *c*) (see Fig. 4) and three vegetation characteristics (presence of clover, grass and cape weed) from the five spatial data sets

Discussion

Probably the single most important difference between conventional pest management and integrated pest management (IPM) of arthropods in agricultural systems is that in IPM, the decision of whether action/intervention (i.e. spraying pesticides or release of natural enemies) is warranted or not is based on: (1) knowledge about the relationship between pest density and yield losses, and (2) the ability to accurately estimate the pest population density (Nansen and Meikle 2011; Nansen and Ridsdill-Smith 2013). Thus, sampling needs to be both practically feasible and provide a reliable estimate of pest populations in order to enable implementation of IPM. It is also important to emphasize the growing interest in and acknowledgement of the importance of the soil surface dwelling mesofauna and their food webs, especially in response to research into climate change (Bokhorst et al. 2012; Sundqvist et al. 2014). This study represents the first quantitative assessment of the performance of vacuum sampling of an economically important soil surface dwelling mite in response to a wide range of environmental and practical variables.

Sampling methods

Total counts of arthropods from soil cores (Ridsdill-Smith and Annells 1997; Wallace 1956, 1967) is undoubtedly the most accurate and complete assessment method, but it is also highly time-consuming and labor intensive and therefore not practically feasible on a large scale. Visual inspection of plant damage may be considered a useful tool in detection of presence of crop-feeding RLEM (Pavri 2007). However, feeding damage is obviously cumulative and may therefore over-estimate a population of RLEM, if the actual density decreases. A series of studies have investigated the feasibility of visual inspection of RLEM. Arthur et al. (2014) found that visual inspection in a 0.05 m^2 (15 × 30 cm) area with early-stage canola (*Brassica napus* L.) provided higher population densities than those detected by vacuum-based sampling. Ridsdill-Smith and Pavri (2000b) observed three times more RLEM feeding on sub clover (*Trifolium subterraneum* L.) than on cape weed in the field, but twice as many RLEM were vacuum sampled from cape weed compared to sub clover. Thus, the authors found considerable discrepancies between observations of feeding RLEM and concurrent counts obtained with vacuum sampling. Visual inspection of feeding RLEM is likely to be influenced by vegetation (Gaull and Ridsdill-Smith 1996), and may be a challenge in pastoral crops, in which canopy visibility is reduced compared to an early-stage canola crop. In pastures and broad-leafed weed plants, Ridsdill-Smith and Pavri (2000b) demonstrated that RLEM mainly feed on the adaxial surface of leaves (and therefore are visible from above), but only move onto plants to feed for short periods, and that RLEM individuals were predominantly found on the soil surface. In dense vegetation, visual inspection of RLEM may be less consistent/accurate and also time-consuming. Gaull and Ridsdill-Smith (1996) made observations of RLEM feeding on the adaxial side of leaves of clovers and cape weed and showed that, on average, there were 1022 mites per m^2 feeding, while concurrent vacuum sampling indicated average RLEM densities of 10530 mites per m^2. Thus only about 10 % of the population appeared to be feeding at any one time, meaning that counts of feeding RLEM on the upper canopy would likely underestimate the RLEM density.

Diurnal and seasonal trends

Arthur et al. (2014) found that visual assessments were impacted by the operator, sampling date and time of day. The results from this study strongly support the claim that diurnal patterns are affected by seasonal trends (Fig. 3a), so that, irrespectively of all other variables, RLEM sampling are consistently higher in late season sampling compared to early season sampling. Ridsdill-Smith and Annells (1997) sampled two large (2548 m^2) field sites for 27 weeks during consecutive seasons and highlighted important seasonal trends. Moreover, the authors showed that there were clearly identifiable peaks in counts each year, and these peaks were directly linked to predicted emergence of winter eggs. In addition, distinct diurnal trends with important differences between early and late season sampling were identified in this study. We are unaware of data describing the diurnal activity of other surface dwelling soil mites, so it is not possible to discuss whether these species would show the same diurnal trends as RLEM counts suggested. Arthur et al. (2014) found that RLEM counts from visual inspection showed no clear association with ambient temperature, relative humidity, wind speed, cloud cover or soil surface conditions. Similarly, Gaull and Ridsdill-Smith (1996) conducted visual inspections of RLEM in the morning and afternoon on four separate dates and found no significant effect of time of

day. The significant diurnal trends identified in this study is likely explained by the sample size involved, and because counts were obtained throughout the day. In early season sampling, we obtained highest RLEM counts in the afternoon, while highest counts during late season sampling were obtained around noon (Fig. 3c).

Spatial trends

We analyzed fairly large data sets (61–122 samples per field), and variogram analyses revealed that only one of the four data sets from Capel had "range" (spatial-autocorrelation) within the maximum lag-distance of 50 m (Table 3). In other words, RLEM counts appear to be spatially auto-correlated within quite large distances, when the vegetation is very uniform. The Albany data set was different from the Capel data sets in two important ways: (1) data was collected at a higher spatial resolution and smaller total area, (2) a few sampling spots involved presence of bare soil, and (3) the vegetation was more diverse. The principal component analysis revealed that vegetation characteristics affect the variogram parameters, and therefore the spatial structure of RLEM sampling data, in quite different ways, as (Fig. 5): (1) presence of cape weed appears to increase the nugget, (2) presence of grass and absence of clover tend to increase the sill, (3) the range increases in absence of cape weed, and (4) there was a positive association between average RLEM counts and the range. The latter suggesting that at high RLEM population density means counts are spatially auto-correlated over wide spatial distances (probably more than 30 m). Thus, there may be practical concerns associated with small-plot trials, in which RLEM data are collected from replicated plots under treatment regimes (i.e. miticide applications). If plots are, 10 × 10 m (or even smaller), then our spatial analysis that observations may be spatially auto-correlated and therefore not admissible for conventional analyses of variance or similar means comparisons.

Ettema and Wardle (2002) reviewed the literature on the spatial (horizontal) distribution of soil fauna and highlighted a study of Collembola showing spatial dependency of sampling beyond 200 m (Fromm et al. 1993). On a 10 ha scale, Ridsdill-Smith et al. (2013) analyzed the spatial distribution pattern of RLEM counts based on 252 vacuum samples collected 20 m apart from grazed pasture over a 3 year period at Waroona, Western Australia in the 1950s [raw data from the study by Wallace (1967)]. The authors identified significant spatial aggregations and using hierarchical mean square analysis that were generally of the order of 80 × 160 m. Based on Mantel correlations, the authors also identified travelling waves of high populations, so the high density patches were not spatially fixed over time. Importantly, the spatial study by Ridsdill-Smith et al. (2013) corroborate the current findings that RLEM counts show spatial aggregation and that spatial considerations are important when developing sampling programs for RLEM.

Best sampling practices

When developing sampling programs and using them in field ecology, the overall goal is to obtain the most accurate estimation of the actual population density. This means that selection of both sampling method (selection of equipment) and sampling protocol (when, where, and how to sample) should attempt to reduce/minimize the effects of temporal, spatial and ecological variables. As all sampling programs are influenced by these variables, it is important to characterize and quantify their relative importance and use this knowledge to standardize sampling so that obtained counts are as directly comparable as possible. We propose the following "best practices" recommendations regarding vacuum

sampling of RLEM. Sampling time affects RLEM counts, so it is important to maintain consistent duration of sampling from each spot. Sampling of completely bare ground will lead to very low RLEM counts but spots with sparse vegetation (presence of bare ground) probably increases the presence of microhabitats for mites to shelter in and therefore lead to higher RLEM counts. RLEM counts were positively associated with the height of vegetation, at least up to about 15 cm in height. In early season (May–August), highest RLEM counts will be obtained in the afternoon hours (2–4 pm), while in late season sampling (August–November), highest RLEM counts will be obtained around noon. Higher RLEM counts should be expected from spots with grazed/mowed vegetation including cape weed and without presence of grasses and stubble. Variogram analyses of high-resolution data sets suggested that considerable range of spatial autocorrelation should be expected from fields with fairly uniform vegetation, especially if RLEM population densities are high. We are therefore recommending that samples are collected at least 30 m apart, if the objective is to obtain independent (spatially non-correlated) counts. The results from this study may be used to develop effective sampling protocols deployed in field ecology studies of soil surface dwelling mesofauna in pastoral landscapes and other ecosystems.

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