Bulletin of Entomological Research (2010) **100**, 175–183
doi:10.1017/S0007485309006981

Field-scale dispersal of *Aphodius* dung beetles (Coleoptera: Scarabaeidae) in response to avermectin treatments on pastured cattle

L. Webb[1]*†, D.J. Beaumont[2], R.G. Nager[3] and D.I. McCracken[1]

[1]Land Economy & Environment Research Group, Scottish Agricultural College, Auchincruive, Ayr, KA6 5HW, UK: [2]Reserves Ecology, RSPB Scotland, 25 Ravelston Terrace, Edinburgh, EH4 3TP, UK: [3]Division of Environmental & Evolutionary Biology, Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, UK

Abstract

Very few studies have examined, at the field scale, the potential for faecal residues in the dung of avermectin-treated cattle to affect dung-breeding insects. The current study examined populations of dung beetles (Scarabaeidae: *Aphodius*) using pitfall traps baited with dung from untreated cattle on 26 fields across eight farms in southwest Scotland. The fields were grazed either by untreated cattle or by cattle treated with an avermectin product, i.e. doramectin or ivermectin. During the two-year study, significantly more beetles were trapped in fields grazed by treated cattle ($n = 9377$ beetles) than in fields where cattle remained untreated ($n = 2483$ beetles). Additional trials showed that beetles preferentially colonised dung of untreated versus doramectin-treated cattle. This may explain the higher captures of beetles in traps baited with dung of untreated cattle, which were located in fields of treated cattle. Given that *Aphodius* beetles avoided dung of treated cattle in the current study, the potential harmful effects of avermectin residues in cattle dung could be reduced through livestock management practices that maximise the availability of dung from untreated livestock in areas where avermectins are being used.

Keywords: attraction, colonisation, doramectin, avermectin, Scarabaeidae

(Accepted 2 April 2009)

Introduction

The use of avermectin veterinary drugs in livestock farming systems and the potential consequences for non-target dung insects has been generating environmental concern for the last two decades (e.g. Wall & Strong, 1987; McCracken, 1993; Hutton & Giller, 2003; review in Floate *et al.*, 2005). Since the 1980s, avermectin (such as ivermectin and doramectin) and milbemycin (moxidectin) products have been on the animal health market for the control of internal (e.g. lung worm, stomach worm) and external (e.g. ticks, mites) parasites. Following treatment of an animal with an avermectin, active residues of the drug and its metabolites are excreted in the faeces (Campbell, 1985; Halley *et al.*, 1989), and subsequent exposure to avermectin residues can be harmful for the survival and development of

---

*Author for correspondence
Fax: +44 141 331 9080
E-mail: lisa.webb@rspb.org.uk
† Current address: South & West Scotland Regional Office, RSPB Scotland, 10 Park Quadrant, Glasgow, G3 6BS, UK.

various dung insect species (e.g. Wardhaugh & Rodriguez-Menendez, 1988; Krüger & Scholtz, 1997; Dadour *et al*., 2000). Highest concentrations of residues are excreted within several days of treatment, but residues can be excreted for up to several months post-treatment (Herd *et al*., 1996; Toutain *et al*., 1997; Floate, 1998; Suarez *et al*., 2003; Floate *et al*., 2008).

The overall objective of the current study was to assess the abundance and dispersal of dung beetles (Coleoptera: Scarabaeidae) in response to use of avermectin treatments on pastured cattle. This was achieved by both a field-scale study on livestock farms in southwest Scotland and by a series of four smaller-scale colonisation trials. To our knowledge, no other field-scale studies have been performed in temperate climates although such studies have been performed in South Africa (Krüger & Scholtz, 1998a,b; Kryger *et al*., 2005). The smaller-scale studies were performed to identify factors that could potentially be important in influencing field-scale dispersal, such as avermectin treatment, dung moisture content and resource availability (Barth, 1993; Wratten & Forbes, 1995; Finn *et al*., 1998) in order to better understand and complement the results from the field-scale study.

Methods

Study sites and species

The field-scale study was carried out on seven dairy farms and one beef and sheep farm in Ayrshire, southwest Scotland between late April and mid-July in both 2002 and 2003. This sampling period was selected because treated livestock are grazing fields at this time, and it encompasses the main dung insect breeding season. Prior to sampling, a questionnaire survey of livestock farmers in southwest Scotland ascertained that doramectin and ivermectin (both administered as a pour-on) were the most commonly used avermectins in that region (Webb, 2004). Consequently, study farms using these treatments were selected to be representative of the treatment strategies in the wider area.

Over the two years, sampling was conducted in 14 fields grazed by cattle treated with doramectin (Dectomax™; $n=12$ fields) or ivermectin (Ivomec®, Noromectin®; $n=2$ fields) applied topically at the manufacturer's recommended dose rate of 500 μg per kg body weight. A further 12 fields were grazed by cattle that had received no endectocide treatment during the study period and for at least six months prior. A subset of the fields (six 'untreated' and six 'treated') was sampled in both years while all of the other fields were sampled during only one of the two study years. For a map showing the spatial distribution and Ordnance Survey grid reference of study fields, see fig. S1 and table S1 in Supplementary Material, respectively.

The treated fields in this study were grazed by young livestock, which, because they are more susceptible to parasite infections than older animals, were dosed with an anthelmintic at turnout and thereafter to afford them protection throughout the grazing season. These cattle in treated fields were grazed permanently in the same field for the duration of the season. The untreated fields were grazed by dairy cows, which remain untreated due to acquired parasite immunity and avoidance of drug residues in the milk supply. The untreated dairy cattle were grazed rotationally through several fields on the farms.

On farms in the study area, cattle are typically housed over winter before being turned out to grass in April or May for approximately six months. The time of turnout and the number of months spent out at grass is dependent on weather and the growth and quality of grass. For example, cattle may be periodically returned to housing during particularly wet periods. In two-thirds of the treated fields, cattle were treated at turnout, i.e. within four days before or after turnout in late April or early May, followed by a second dose some eight weeks later. Cattle in the remaining treated fields were dosed within 14 days following turnout and given a second dose some eight weeks later. This variation in timings of treatments reflects the reality of doing studies incorporating a range of commercial farms, but such variation was accounted for within the statistical analyses (see 'Method: Data analyses').

We limited our study to dung beetles in the genus *Aphodius*, which are the most specious genus in temperate climates (e.g. Hutton & Giller, 2003). Among *Aphodius*, there are two larval feeding strategies (Gittings & Giller, 1997): (i) coprophagous larvae that feed exclusively on dung (guild 1); and (ii) saprophagous larvae that feed on plant roots and decaying vegetation (guild 2). Hence, larvae of guild 1 *Aphodius* will have greater exposure to avermectins than guild 2 larvae; and guild 1 adults could be expected to be relatively more selective when colonising dung, in terms of its potential suitability for larval development, than guild 2 adults. Thus, *Aphodius* were split into the two guilds according to larval feeding strategy prior to data analyses.

Environmental and management variables

We took into account many of the environmental variables known to be important for *Aphodius* populations, e.g. weather (Finn *et al*., 1998), seasonal factors (Hanski, 1980a; Gittings & Giller, 1997), soil type (Ryan *et al*., 1978) and agricultural management practices (Hutton & Giller, 2003).

Climate data were obtained from the weather station at the Scottish Agricultural College, Auchincruive, which was situated within 1–16 km of the study fields. Total rainfall (mm), sunshine hours and the mean maximum and minimum temperatures were calculated for each individual trapping period. Aspect, altitude, size of field, field boundary type (fence, gappy hedge, well-established hedge, woodland), surrounding land use and avermectin use were recorded for each field. Sward height was measured ten times in the area around the traps, using the direct method (Stewart *et al*., 2001), and mean sward height was calculated for each trapping period.

The availability of fresh dung within a study field in each trapping period was gauged using a 'dung index'. This was the product of the number of days that cattle grazed a field in a particular trap period and the number of cattle, corrected for field size, to give an index of the number of dung pats that would be deposited per unit area per trapping period. An 'avermectin index' was calculated to estimate the proportion of land within a 400 m radius of the centre of the study field that was grazed by avermectin-treated livestock. The 400 m radius was selected because it encompasses the maximum area travelled by many dung-breeding insects (Smith & Wall, 1998; Roslin, 2000). Similarly, the 'pasture index' estimated the percentage area of grazed pasture within a 400 m radius around the study field to reflect potential availability of livestock dung in surrounding fields.

Larval development of some guild 2 *Aphodius* species takes place in the soil below dung pats (Gittings & Giller, 1997), and soil type can influence dung beetle abundance (Ryan *et al*., 1978). To compare general soil quality between fields, six soil cores (10 cm length × 6.5 cm diameter) were taken from the area around the traps in each study field and sent for laboratory analysis to determine soil pH, moisture and organic matter. Cores were taken once in January 2003 and once in August 2003, with the former taken to reflect 2002 study fields and the latter taken to reflect 2003 study fields. The temporal difference in sampling meant that comparisons of soil characteristics between years were treated with caution; however, comparisons were made between fields for each year.

An index of management intensity was calculated for each study field. Management information was collected through interview of farmers who were asked about management practices in the fields: sward type, age (time since re-seeding), soil disturbance, cutting regime, grazing intensity, inorganic and organic fertiliser input and herbicide use. A score between 0 and 3 was assigned to each factor and summed to give an overall management intensity score (MIS) between 0–24, with 24 being the most intensively managed (Downie *et al*., 2000).

Dung beetle sampling – the field-scale study

Adult dung beetles were sampled using dung-baited pitfall traps. Traps were 1-l plastic containers (11.5 cm diameter) sunk flush with the ground and filled with approximately 3 cm depth of 70% monopropylene glycol (to act as a killing agent and preservative). Wire mesh was secured over the traps to support dung baits. Baits were formed using a hemispherical mould (6 cm diameter and 3 cm depth) and were placed on the centre of the mesh. Dung was collected on one occasion in both 2002 and 2003 from grass silage-fed housed cattle at each study farm in April, prior to their receiving any avermectin treatment. Dung from each farm was mixed thoroughly and stored in a sealed container at 4°C. It was observed that dung remained attractive to dung insects after being kept in cold storage for up to six months (Webb, 2004). In order to maintain good biosecurity (reflecting concerns following the outbreak of Foot and Mouth Disease in 2001), dung from different farms was kept in separate containers and used as bait only on farms from where it had been collected. Eight pitfall traps were set in two grids of four in each field, with traps spaced approximately 8 m apart within each grid. Grids were set in a central position at each end of the field, away from margins to counteract possible edge effects. Traps were emptied and re-baited every 7–10 days in 2002 and every 14 days in 2003, thus giving a maximum of nine and six collections per field over the sampling season in 2002 and 2003, respectively. In each year, trapping took place in the same fields for the duration of each sampling season. These sampling intervals were selected as the best compromise between sampling a sufficient number of fields and the required sampling effort. Variability in the duration of trapping periods, between five and 20 days, did not affect the composition of species trapped in dung-baited pitfall traps (Webb, 2004). On each sampling date, two traps from each of the two grids in each study field were selected, and the individuals of each *Aphodius* species were counted and summed across those four traps.

Colonisation trials

Four separate trials were conducted in an area of grassland, on the farm at the Scottish Agricultural College, which was ungrazed by livestock but within approximately 200 m of grazing sheep and cattle. Dung beetles were sampled using dung-baited pitfall traps of the design described above. For all experiments, dung was collected from housed Holstein-Friesian cattle fed on grass silage. Dung was collected from the same cattle five days prior to, and two days after, treatment with a doramectin pour-on at the recommended dose of 500 μg per kg bodyweight. The two-day post-treatment collection was selected because relatively high concentrations of residues are excreted in dung within this period following treatment (Toutain *et al*., 1997); and, in addition, it enabled dung to be collected from housed animals before they were turned out to grass. Dung was mixed thoroughly to homogenise it and was stored in a sealed container at 4°C until use. The specific details for each colonisation trial are given below.

Trial 1

The aim of this trial was to determine whether *Aphodius* dung beetles exhibited a preference to, or an avoidance of, dung from doramectin-treated cattle. In the first phase of the trial, traps were set in two grids of 3 × 2, with traps within each grid spaced 1.5 m apart (fig. S2). The two grids were established approximately 70 m apart. All six traps in one grid were baited with dung from untreated cattle, and the six in the other grid were baited with dung from treated animals. In May 2003, traps were set and the contents collected after five days. To exclude any bias in the location of traps, each grid of traps was then re-baited with the alternate dung type, and the trap contents were collected after a further five days. After checking for no significant differences between the two trapping periods, collections from the two trap periods were pooled to increase sample size, and thus data from twelve untreated traps and twelve treated traps were analysed.

Trial 2

The experimental set-up was as described for trial 1 with the exception that, within each grid of six traps, traps were baited alternately with 'treated' and 'untreated' dung to determine whether *Aphodius* made a choice between the two dung types at a scale of 1.5 m (fig. S3). In June 2003, traps were set and emptied after five days and then re-baited with the same dung type and collected after a further five days. Trap collections from the two trap periods were pooled, as for trial 1, prior to analyses.

Trial 3

The aim of this trial was to assess the combined influence of moisture content and doramectin treatment on dung colonisation by *Aphodius*. Dung was homogenised and water was mixed into one lot of treated and untreated dung to increase moisture content. Water was added until the dung reached a consistency that was extremely moist but still could be supported on the mesh of the pitfall trap. Three

Table 1. List of environmental and management variables included in mixed model analyses of *Aphodius* abundance data. The recorded ranges of continuous variables across 'treated' and 'untreated' fields are given in parentheses. Note that the negative number of days in the 'days post-turnout' category refers to the fact that some sampling occurred prior to cattle being turned out to pasture.

Variable	Level or Range
Farm	8 levels
Field	26 levels
Year	2 levels: 2002, 2003
Avermectin treatment	2 levels: 1, untreated; 2, treated
Seasonality	Sampling date: days from 1 April (23–112 days)
Days post-turnout	Days since cattle were put out to pasture (−2 to 89 days)
Sward height	(3–30 cm)
Rainfall	mm per trap period (0.2–67.4 mm)
Sun	hours per trap period (37–167 hours)
Maximum temperature	Mean max. temp. per trap period (10.0–21.1°C)
Minimum temperature	Mean min. temp. per trap period (3.4–13.8°C)
Dung index	Density per ha per trapping period (0–197 pats ha^{-1})
Field size	(Range 2.0–8.3 ha) (Mean size ± SE = 4.59 ± 0.32 ha)
Soil pH	(pH 5.0–6.7)
Soil Moisture	(20.7–59.8%)
Soil Organic content	% loss on ignition (6.3–24.0%)
Grazing System	2 levels: rotation or permanent grazing
Management Intensity Score	(Score 7–17)
Avermectin index	(0–72%)
Pasture index	(27.6–88.5%)
Habitat characteristic score	Ordination of aspect, altitude, field boundary, pasture index

samples (approximately 20 g each) of each of the four dung types were taken and dried in an oven to constant weight to determine moisture content. Eight replicate traps baited with each of the four dung types were set out in an 8 × 4 grid, with traps spaced approximately 5 m apart, and each of the four dung types were used in a repeated alternating sequence (fig. S4). Traps were collected in May 2004 after six days exposure.

Trial 4

The availability of suitable dung in the vicinity of pitfall traps could influence the number of *Aphodius* trapped. This trial investigated whether the number of beetles caught in pitfall traps baited with dung was affected by the presence of fresh dung pats within 1 m of those traps. Two 4 × 2 grids of eight traps were set up and all traps were baited with untreated dung. The two grids were approximately 70 m apart, and individual traps within a grid were spaced at 1.5 m (fig. S5). In order to simulate a field that was permanently stocked with cattle, six 'artificial' pats of approximately 20 cm diameter were formed from the collected untreated dung and placed within 1 m of the traps at one grid. At the other grid, the baited pitfall traps had no dung surrounding them (to simulate the intermittent periods where no dung would be deposited in a rotationally grazed field). Traps were set in May 2004 and collected after seven days.

Data analyses

Potential effects of avermectins on *Aphodius* population size at different times of the season were investigated using general linearised mixed models in SAS (SAS Institute, 2001). We used the GLIMMIX macro with a Poisson error, log link function and a Satterthwaite correction to derive the degrees of freedom for the denominator. Abundance data from the field samples were corrected to a standard period of ten days to allow comparisons between trapping periods of different duration. 'Field' and 'farm' were included as random factors, and sampling date was included as a repeated measure. The autoregression of order 1 covariance structure was used because it assumes that measures further apart in time are less correlated than measures closer together (Littell *et al.*, 1996). The Akaike's Information Criterion was used to support selection of the best covariance structure and model (Littell *et al.*, 1996). Ordination was used to simplify and reduce the number of habitat parameters introduced into the model (Fox, 2004; Rushton *et al.*, 2004). Altitude, aspect, field boundary type and 'pasture index' data were ordinated using detrended correspondence analysis to generate a 'habitat' score based on the first two axes scores for each site and, hence, reflecting the degree of similarity between sites with regard to these characteristics. All environmental and management variables (table 1) were tested independently for significance at the $P < 0.05$ level. Those variables that were significant were then added to a multivariate model using a step-wise procedure, whereby variables were retained in the model if they were significant and any variables that were no longer significant were removed. To consider non-linear relationships, the quadratics of all continuous variables and interactions of interest were tested and retained in the model if significant. Only the final models are presented in this paper.

The Mann-Whitney test was used to compare *Aphodius* abundance in trials 1, 2 and 4. Data were collated across two trap periods in those trials; therefore, patterns of significance were checked for consistency across the two trap periods to rule out variation due to the time of trapping or trap location. For trial 3, data were square-root transformed to normalise and then analysed using a two-way analysis of variance to assess the significance of treatment, moisture level and the interaction between the two.

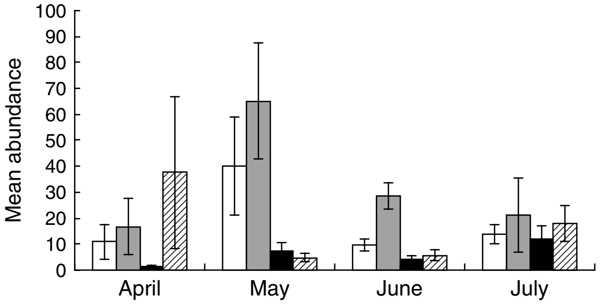

Fig. 1. Mean number (±SE) of guild 1 *Aphodius* beetles trapped in treated and untreated fields in each sample month in 2002 and 2003 (□, untreated 2002; ▒, treated 2002; ■, untreated 2003; ▨, treated 2003).

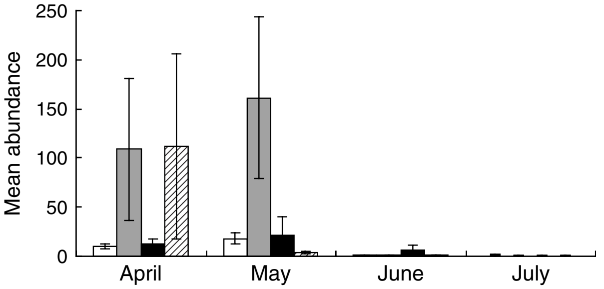

Fig. 2. Mean number (±SE) of guild 2 *Aphodius* beetles trapped in treated and untreated fields in each sample month in 2002 and 2003 (□, untreated 2002; ▒, treated 2002; ■, untreated 2003; ▨, treated 2003).

Results

Dung beetle abundance in grazed pastures

Guild 1 species

Over the two study years for guild 1 species, 1552 and 3534 beetles were collected in fields grazed by untreated and treated cattle, respectively. Guild 1 species comprised *Aphodius depressus* (Kugelann) (80%), *A. rufipes* (L.) (13%) plus *A. ater* (Degeer), *A. fimetarius* (L.), *A. fossor* (L.), *A. lapponum* Gyllenhal, *A. pusillus* (Herbst), and *A. rufus* (Moll) (7% combined).

The April to July sampling period in 2003 was warmer (mean maximum temperature 16.1°C) and drier (230.5 mm total rainfall) than in 2002 (14.3°C and 312 mm, respectively). More guild 1 beetles were recorded in 2002 than in 2003 and in fields grazed by treated cattle versus untreated cattle (fig. 1, table 2). The abundance of guild 1 beetles peaked in late April and early May, then subsequently declined. There was a significant non-linear relationship between rainfall and guild 1 abundance. Total rainfall in individual trapping periods ranged from 0.2–67.4 mm, and numbers of guild 1 *Aphodius* were lowest in trapping periods with 30–42 mm of rainfall.

Table 2. Generalised linear mixed model (see methods) describing the variation in *Aphodius* guild 1 abundance in fields containing avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003. All interactions were non-significant.

Variable	Estimate	SE	Test statistics	*P*
***Aphodius* guild 1**				
Field			$Z = 1.06$	0.145
Farm			$Z = 1.06$	0.145
Year	1.209	0.225	$F_{1,122} = 28.99$	< 0.0001
Seasonality	−0.079	0.023	$F_{1,142} = 11.42$	0.0009
Seasonality^2	0.0005	0.0002	$F_{1,141} = 8.69$	0.0037
Rainfall	−0.104	0.017	$F_{1,220} = 36.96$	< 0.0001
Rainfall^2	0.001	0.0003	$F_{1,219} = 24.51$	< 0.0001
Avermectin treatment	−0.873	0.29	$F_{1,16.1} = 9.12$	0.008
Intercept	6.665	0.762		

Guild 2 species

Of the two species belonging to guild 2 (*Aphodius prodromus* and *A. sphacelatus*), 931 individuals were trapped in untreated fields and 5843 individuals were trapped in fields grazed by treated cattle. *Aphodius prodromus* was trapped more frequently (77.8% of the total catch of guild 2 species) than *A. sphacelatus* (22.2%). Guild 2 beetles were trapped in higher numbers in 2002, had their highest abundance in April and May and then decreased continuously as the sampling season progressed (fig. 2, table 3). There was a significant interaction between year and avermectin treatment. In 2002, guild 2 individuals were recorded more often in treated than in untreated fields ($F_{1,43.9} = 7.27$, $P = 0.01$), whereas in 2003 there was no difference between treated and untreated fields ($F_{1,22.9} = 0.97$, $P = 0.34$).

Although dung density was not significant in either of the models, it is notable that trapping periods in 2003 had significantly higher dung density indices (mean ± SE, 51.7 ± 2.8, $n = 120$ trapping periods) than those in 2002 (32.5 ± 1.7, $n = 123$ trapping periods; $F_{1,217} = 11.21$, $P = 0.001$). There was no significant difference in dung density between fields containing untreated or avermectin-treated cattle in 2002 ($F_{1,14.2} = 0.2$, $P = 0.66$) or in 2003 ($F_{1,18.8} = 1.6$, $P = 0.22$).

The variables 'farm' and 'field', which were included as random effects in both guild 1 and guild 2 models were not significant in either. This indicates that there was no significant level of variation in dung beetle abundance between individual farms or individual fields.

Table 3. Generalised linear mixed model (see methods) describing the variation in *Aphodius* guild 2 abundance in fields containing avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003. All interactions were non-significant.

Variable	Estimate	SE	Test statistics	*P*
***Aphodius* guild 2**				
Field			$Z = 1.53$	0.063
Farm			$Z = 0.9$	0.183
Year	2.22	0.3	$F_{1,123} = 35.98$	< 0.0001
Seasonality	−0.113	0.006	$F_{1,120} = 316.29$	< 0.0001
Avermectin treatment	−0.475	0.632	$F_{1,25.3} = 4.4$	0.046
Year ∗ avermectin treatment	−1.267	0.525	$F_{1,123} = 5.82$	0.017
Intercept	7.516	0.611		

Table 4. Mean abundance (±SE) of *Aphodius* beetles in pitfall traps: baited with dung from treated and untreated cattle at 70 m apart (trial 1) and 1.5 m apart (trial 2); baited with dung of different moisture levels from treated and untreated cattle (trial 3); baited with untreated dung and surrounded either by dung pats (present) or no dung pats (absent) (trial 4).

	Guild 1	Guild 2	Dung moisture (mean ± SE)
Trial 1			
Treated dung	1.42 ± 0.58	5.83 ± 1.27	
Untreated dung	7.5 ± 1.25	95.1 ± 20.8	
Trial 2			
Treated dung	1.42 ± 0.58	0.92 ± 0.45	
Untreated dung	2.83 ± 0.87	4.83 ± 1.02	
Trial 3			
Treated 'moist' dung	1.5 ± 0.38	9.5 ± 2.20	89.3 ± 0.2%
Treated 'dry' dung	1.0 ± 0.27	9.88 ± 2.91	85.2 ± 0.3%
Untreated 'moist' dung	1.75 ± 0.49	21.13 ± 3.95	88.3 ± 0.2%
Untreated 'dry' dung	3.0 ± 0.46	27 ± 4.63	85.1 ± 0.2%
Trial 4			
Dung absent	46.5 ± 4.2	842 ± 105	
Dung present	43.5 ± 10.1	484 ± 163	

Colonisation trials

The abundance of each dung beetle guild caught in traps in trials 1 and 2 are summarised (table 4). When traps were 70 m apart, untreated dung attracted significantly more guild 1 (Mann-Whitney, $n=24$, $P<0.001$) and guild 2 individuals (Mann Whitney, $n=24$, $P<0.0001$) than treated dung. In traps spaced 1.5 m apart, significantly more guild 2 beetles were attracted to untreated dung (Mann Whitney, $n=24$, $P<0.001$), but this difference was not significant for guild 1 beetles (Mann Whitney, $n=24$, $P>0.05$).

Numbers of *Aphodius* in traps baited with dung of varying treatment and moisture level were compared in trial 3 (table 4). 'Moist' dung had a higher moisture content ($88.8+0.2\%$) than 'dry' dung ($85.2+0.3\%$; $F_{1,8}=248.97$, $P<0.001$) irrespective of avermectin treatment ($F_{1,8}=4.83$, $P=0.06$; table 4 for percentage moisture). For *Aphodius* guild 1, significantly more individuals were attracted to traps baited with dung from untreated cattle than to dung from doramectin-treated cattle ($F_{1,28}=6.58$, $P=0.02$) but the effect of dung moisture was not significant ($F_{1,28}=0.68$, $P=0.42$). However, the interaction between moisture and treatment was close to significance ($F_{1,28}=4.05$, $P=0.054$) as more beetles were attracted to 'dry' untreated dung than to any other dung type. This difference was driven by the colonisation behaviour of *A. ater*, which was more attracted to traps baited with untreated 'dry' dung than to all other dung types (Kruskal Wallis test: $H=8.67$, $df=3$, $P=0.03$). Guild 2 *Aphodius* were more attracted to untreated dung than to treated dung ($F_{1,28}=18.27$, $P<0.001$), regardless of moisture level ($F_{1,28}=0.31$, $P=0.58$).

In trial 4, traps with dung pats present trapped similar numbers of guild 1 beetles as traps where dung pats were absent (Mann Whitney, $n=16$, $P=0.67$). There was a non-significant trend towards higher numbers of guild 2 *Aphodius* in traps where dung pats were absent than in those where dung pats were present (Mann Whitney, $n=16$, $P=0.07$; table 4).

Discussion

Colonisation trials – relative attractiveness of dung

These trials indicated that *Aphodius* beetles belonging to each guild preferred to colonise dung from untreated cattle rather than doramectin-treated cattle. Guild 2 beetles were relatively more attracted to untreated dung regardless of moisture content, while guild 1 species may select dung on the basis of both moisture content and treatment.

Our results have shown that *Aphodius* dung beetles can discriminate between dung from untreated cattle and cattle treated with a doramectin pour-on at a spatial scale of at least 70 m. This preference for dung from untreated cattle was evident even when dung type was alternated between trapping grids; and, therefore, the observation was not a consequence of trap location. The ability to distinguish between dung from treated and untreated cattle at such a scale has implications for the movements of beetles between natural cattle-grazed pastures. Dung beetles tend to remain in the pasture in which they emerge rather than dispersing large distances (Roslin, 2000). Therefore, *Aphodius* beetles are unlikely to disperse further than to adjacent pastures provided there is a suitable dung resource to colonise nearby. Our results suggest that, where fields grazed by untreated cattle surround a pasture grazed by avermectin-treated cattle, emergent beetles from the 'treated' pasture may disperse to colonise dung in the adjacent 'untreated' fields. However, if a pasture of treated cattle is surrounded by fields also grazed by treated cattle or by land with no dung resource, e.g. arable cropping, then the insects may have little choice but to colonise dung with avermectin residues.

Our study also indicates that dung beetles can select between dung from untreated cattle and dung from doramectin-treated cattle at a spatial scale of 1.5 m, whereby higher numbers of guild 2 beetles occurred in traps baited with untreated dung. Previous research has shown that *Aphodius* beetles can distinguish between dung from untreated and avermectin-treated cattle at 3.5 m (Holter *et al.*, 1993a) and 3 m (Floate, 2007), and our findings indicate that they can do this at an even finer scale.

Other studies on the attractiveness of dung from avermectin-treated cattle to insects have yielded mixed results. As in our research, some studies have found untreated dung to be more attractive to *Aphodius* beetles than dung containing ivermectin residues (Holter *et al.*, 1993b), while others have shown that dung beetles preferred dung from avermectin-treated livestock than from untreated livestock (Wardhaugh & Mahon, 1991; Floate, 1998). Floate (2007) found seven cases where *Aphodius* were more attracted to dung from doramectin-treated animals compared to two cases where *Aphodius* preferred untreated dung. These conflicting observations cannot be attributed to differences in study methodologies. It has been proposed that differences in attraction could be due to changes in cattle diet (Barth, 1993; Floate, 1998). However, this can be excluded as a contributory factor in our study because cattle were fed on the same silage diet throughout the dung collection process. Differences in the attractiveness of dung could be due to a change in dung quality caused by avermectin treatment rather than simply an avoidance of, or attraction to, the avermectin compound. For example, Wratten & Forbes (1995) suggested that avermectin treatment may result in dung with lower moisture content due to the alleviation of worm-induced diarrhoea and Wardhaugh & Mahon (1991) suggested that changes in the gut flora of livestock following avermectin treatment could alter dung quality. Our study indicated that moisture was not critical for the attraction of guild 2 species, as there was an overwhelming preference by *A. prodromus* and *A. sphacelatus* for untreated dung regardless of moisture content. As these two species oviposit in soil (Gittings & Giller, 1997), they do not have the same constraint as dung-ovipositing species in terms of eggs drowning in very moist dung. Both moisture and treatment were important for guild 1 beetles, whereby more individuals were attracted to drier untreated dung than to the drier treated dung. This difference was mainly due to the colonisation behaviour of *A. ater*, a species known to favour dry dung for oviposition (Hirschberger & Degro, 1996; Gittings & Giller, 1997, 1998).

Hence, our colonisation trials suggest that doramectin treatment indirectly reduced the suitability of dung for *Aphodius* beetles. The mechanism by which avermectins alter the attractiveness of dung remains unclear. We speculate that the quality of dung as a feeding resource is reduced following treatment. Adult beetles feed on the energy-rich bacteria that are abundant in fresh dung (Hirschberger, 1999); therefore, a significant reduction or change in the bacterial assemblage could potentially make the dung less nutritious and less desirable. Alternatively, the attractiveness of dung to beetles may be lower due to reduced activity of pre-colonising species. Larvae of dung-breeding flies aerate dung during their development, thus making it more suitable for beetle colonisation (e.g. Suarez *et al*., 2003). Avermectin residues in dung can kill or impair fly larvae (e.g. Fincher, 1992; Floate *et al*., 2001) and *Scathophaga stercoraria* L. (Diptera: Scathophagidae) larvae may undergo developmental stress in dung from treated cattle (e.g. Strong & James, 1993; Webb *et al*., 2007). Therefore, dung fly larval activity within individual dung pats may be impeded.

The results from trial 4 indicated that abundance of dung beetles in traps did not differ with surrounding dung density. However, there was a non-significant trend for more guild 2 beetles in traps without alternative dung pats present, suggesting a possible 'dilution effect' whereby fewer individuals were trapped when there were greater quantities of suitable dung nearby.

It is notable that there was large variation in numbers of beetles trapped in the colonisation trials where mean abundance (±SE) ranged from 0.92±0.45 to 842±105 individuals. The reason for the observed higher abundance of *Aphodius* in trial 4 compared to the other three trials is unclear, as it could not be attributed to location of traps, sample year or season. It is possible that, because a greater quantity of dung (i.e. the artificially formed pats) was present around the pitfall traps, relatively more dung beetles were attracted into the vicinity of the traps. Results from the colonisation trials are discussed below in the context of our wider field study on avermectin effects on *Aphodius* populations.

Field-scale study

These results show that fields containing avermectin-treated livestock had significantly higher numbers of *Aphodius* dung beetles recorded in baited pitfall traps than fields containing untreated livestock. This difference in abundance could not be attributed to habitat characteristics of the field, such as aspect, boundary type, sward height or dung density, or wider landscape factors, such as availability of grazed pasture. We propose that increased trapping of *Aphodius* in fields grazed by treated livestock could have been due to differences between pastures in terms of avermectin treatment and/or grazing regime.

Avermectin treatment

The presence of avermectin residues in dung, in a pasture situation, could have affected *Aphodius* abundance via the 'attraction/repellency effect' discussed above. All baits on the pitfall traps were formed from dung from untreated cattle. Hence, inflated numbers of beetles may have occurred in traps in treated fields if beetles were avoiding the naturally occurring dung from avermectin-treated cattle in those fields.

Livestock grazing regime

Average dung density did not differ significantly between treated and untreated fields and was not a significant factor in the models. However, the 'pattern' of dung deposition differed between treated and untreated fields, whereby the former were grazed permanently by cattle while the latter were grazed rotationally by cattle at a relatively higher stocking density. As dung beetles may follow the movements of cattle around pastures (Finn *et al*., 1998), it cannot be ruled out that the lower abundance of *Aphodius* in untreated fields in this study was due to beetles migrating from a field as cattle were periodically rotated. Conversely, we might expect fewer beetles to occur in traps in permanently grazed fields because a constant fresh supply of natural dung would be available for beetles to colonise. However, if the freshly deposited dung in those fields was unattractive then one could expect more beetles to be attracted to the untreated dung on traps in these fields. This would explain our observation of more beetles being trapped in treated fields.

Variation due to environmental factors

Abundance of *Aphodius* changed significantly between the two sample years. It is not uncommon for *Aphodius* abundance to vary naturally between years (Finn *et al*., 1999). Duration of trapping period varied between years in this study because, for logistical reasons, trapping periods ranged from 7–10 days in 2002 but were increased to 14 days in 2003. Therefore, we must be cautious when comparing abundance between years.

The model shows that the relationship between guild 1 *Aphodius* and rainfall was not straightforward, as insect activity was highest during trapping periods with close to no rainfall and in trapping periods with most rainfall. Heightened beetle activity during low rainfall could be explained by a negative correlation between rainfall and sunshine, as beetle activity is positively correlated with radiant energy (Lobo *et al*., 1998). Moreover, insects may have been more active during periods of low rainfall because heavy rain can impair activity (Finn *et al*., 1998). Conversely, rainy weather and a lack of sunshine can optimise pat colonisation by preventing formation of a hard impenetrable crust on dung. It was noted that fields sampled in 2002 became extremely waterlogged because of high rainfall in June and July (personal observation). The dung beetles that occurred in high numbers in the spring of 2002 could have been expected to breed successfully in dung at that time. However, disintegration of pats and flooding could have impaired the subsequent development of larvae throughout the period of high rainfall in that year.

Seasonality was significant for guild 1 beetles although there was no distinct seasonal pattern presumably because the two most common species have phenologies that 'balanced' each other. The most abundant species of that guild, *A. depressus*, peaked in abundance in May 2002, while *A. rufipes* was typically most abundant in June and July (White, 1960; Holter, 1979; Gittings & Giller, 1997). Seasonality was an important factor for the guild 2 species, *A. prodromus* and *A. sphacelatus*, which were trapped in highest numbers in April and May. This seasonal pattern is typical of these two species (Wassmer, 1994; Gittings & Giller, 1997).

Management recommendations

Dung beetles' avoidance of dung from doramectin-treated cattle in a pasture environment could minimise any negative effects associated with exposure to residues if alternative avermectin-free dung is available for them to colonise. Thus, any management practice that maximises the availability of doramectin- and ivermectin-free dung in the landscape could be beneficial for dung insects. On dairy farms, adjacent fields often hold the same livestock cohort, i.e. younger treated cattle are often grazed together in a cluster of adjacent fields, while untreated milking cows are rotationally grazed through fields that are often situated together, close to the milking parlour (Webb, 2004). Where possible, young stock undergoing avermectin treatment should be grazed in fields beside untreated milking cows to enable dung beetles to disperse between fields to locate the more attractive dung from untreated cattle. This could be achieved either at an individual farm level or by taking into account the locations of treated and untreated animals on neighbouring farms. Livestock managers should also consider (consulting a veterinary surgeon as necessary) whether all animals require to be dosed or whether older animals have acquired immunity. It is recommended that the precautionary principle be retained in areas managed for nature conservation, especially where there are likely to be populations of dung invertebrates or dependent wildlife of high conservation value. In such areas, a wormer from an alternative anthelmintic class (e.g. benzimidazoles) or a less toxic avermectin, such as moxidectin (Floate *et al*., 2001, 2002) should be used where possible. If a doramectin or ivermectin product must be used, then altering the time of treatment so that it is outside the main dung insect breeding period could be beneficial, i.e. restricting use to autumn or when livestock are housed.

Acknowledgements

This research was carried out as part of a PhD studentship funded by the Royal Society for the Protection of Birds and the Scottish Agricultural College Dr D. MacLagan Trust Fund. The Scottish Agricultural College receives financial support from the Scottish Government Rural and Environment Research and Analysis Directorate (RERAD).

Supplementary material

The online table and figures can be viewed at http://journals.cambridge.org/ber

References

Barth, D. (1993) Importance of methodology in the interpretation of factors affecting degradation of dung. *Veterinary Parasitology* **48**, 99–108.

Campbell, W.C. (1985) Ivermectin: an update. *Parasitology Today* **1**, 10–16.

Dadour, I.R., Cook, D.F. & Hennessy, D. (2000) Reproduction and Survival of the Dung Beetle *Onthophagus binodis* (Coleoptera: Scarabaeidae) Exposed to Abamectin and Doramectin Residues in Cattle Dung. *Environmental Entomology* **29**, 1116–1122.

Downie, I.S., Ribera, I., McCracken, D.I., Wilson, W.L., Foster, G.N., Waterhouse, A., Abernethy, V.J. & Murphy, K.J. (2000) Modelling populations of *Erigone atra* and *E. dentipalpis* (Araneae: Linyphiidae) across an agricultural gradient in Scotland. *Agriculture, Ecosystems and Environment* **80**, 15–28.

Fincher, G.T. (1992) Injectable Ivermectin for Cattle: Effects on Some Dung-Inhabiting Insects. *Environmental Entomology* **21**, 871–876.

Finn, J.A., Gittings, T. & Giller, P.S. (1998) *Aphodius* dung beetle assemblage stability at different spatial and temporal scales. *Applied Soil Ecology* **10**, 27–36.

Finn, J.A., Gittings, T. & Giller, P.S. (1999) Spatial and temporal variation in species composition of dung beetle assemblages in southern Ireland. *Ecological Entomology* **24**, 24–36.

Floate, K.D. (1998) Does a repellent effect contribute to reduced levels of insect activity in dung from cattle treated with ivermectin? *Bulletin of Entomological Research* **88**, 291–297.

Floate, K.D. (2007) Endectocide residues affect insect attraction to dung from treated cattle: implications for toxicity tests. *Medical and Veterinary Entomology* **21**, 312–322.

Floate, K.D., Spooner, R.W. & Colwell, D.D. (2001) Larvicidal activity of endectocides against pest flies in the dung of treated cattle. *Medical and Veterinary Entomology* **15**, 117–120.

Floate, K.D., Colwell, D.D. & Fox, A.S. (2002) Reductions of non-pest insects in dung of cattle treated with endectocides: a comparison of four products. *Bulletin of Entomological Research* **92**, 471–481.

Floate, K.D., Wardhaugh, K.G., Boxall, A.B.A. & Sherratt, T.N. (2005) Fecal residues of veterinary pesticides: Nontarget effects in the pasture environment. *Annual Review of Entomology* **50**, 153–179.

Floate, K.D., Bouchard, P., Holroyd, G.L., Poulin, R.G. & Wellicome, T.I. (2008) Does doramectin use on cattle indirectly affect the endangered Burrowing Owl? *Rangeland Ecology and Management* **61**, 543–553.

Fox, A.D. (2004) Has Danish agriculture maintained farmland bird populations? *Journal of Applied Ecology* **41**, 427–439.

Gittings, T. & Giller, P.S. (1997) Life history traits and resource utilisation in an assemblage of north temperate *Aphodius* dung beetles (Coleoptera: Scarabaeidae). *Ecography* **20**, 55–66.

Gittings, T. & Giller, P.S. (1998) Resource quality and the colonisation and succession of coprophagous dung beetles. *Ecography* **21**, 581–592.

Halley, B.A., Nessel, R.J. & Lu, A.Y.H. (1989) Environmental Aspects of Ivermectin Usage in Livestock: General Considerations. pp. 162–172 *in* Campbell, W.C. (Ed.) *Ivermectin and Abamectin*. New York, USA, Springer-Verlag.

Hanski, I. (1980a) The community of coprophagous beetles (Coleoptera, Scarabaeidae and Hydrophilidae) in northern Europe. *Annales Entomologici Fennici* **46**, 57–73.

Herd, R.P., Sams, R.A. & Ashcraft, S.M. (1996) Persistence of Ivermectin in Plasma and Faeces Following Treatment of Cows with Ivermectin Sustained-release, Pour-on or Injectable Formulations. *International Journal for Parasitology* **26**, 1087–1093.

Hirschberger, P. (1999) Larval population density affects female weight and fecundity in the dung beetle *Aphodius ater*. *Ecological Entomology* **24**, 316–322.

Hirschberger, P. & Degro, H.N. (1996) Oviposition of the dung beetle *Aphodius ater* in relation to the abundance of yellow dungfly larvae (*Scatophaga stercoraria*). *Ecological Entomology* **21**, 352–357.

Holter, P. (1979) Abundance and reproductive strategy of the dung beetle *Aphodius rufipes* (L.) (Scarabaeidae). *Ecological Entomology* **4**, 317–326.

Holter, P., Sommer, C., Grønvold, J. & Madsen, M. (1993a) Effects of ivermectin treatment on the attraction of dung beetles (Coleoptera: Scarabaeidae and Hydrophilidae) to cow pats. *Bulletin of Entomological Research* **83**, 53–58.

Holter, P., Sommer, C. & Grønvold, J. (1993b) Attractiveness of dung from ivermectin-treated cattle to Danish and afrotropical scarabaeid dung beetles. *Veterinary Parasitology* **48**, 159–169.

Hutton, S.A. & Giller, P.S. (2003) The effects of intensification of agriculture on northern temperate dung beetle communities. *Journal of Applied Ecology* **40**, 994–1007.

Krüger, K. & Scholtz, C.H. (1997) Lethal and sublethal effects of ivermectin on the dung-breeding beetles *Euoniticellus intermedius* (Reiche) and *Onitis alexis* Klug (Coleoptera, Scarabaeidae). *Agriculture, Ecosystems and Environment* **61**, 123–131.

Krüger, K. & Scholtz, C.H. (1998a) Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under drought conditions. *Acta Oecologica – International Journal of Ecology* **19**, 425–438.

Krüger, K. & Scholtz, C.H. (1998b) Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. II. Impact of ivermectin under high-rainfall conditions. *Acta Oecologica – International Journal of Ecology* **19**, 439–451.

Kryger, U., Deschodt, C. & Scholtz, C.H. (2005) Effects of fluazuron and ivermectin treatment of cattle on the structure of dung beetle communities. *Agriculture, Ecosystems and Environment* **105**, 649–656

Littell, R.C., Milliken, G.A., Stroup, W.W. & Wolfinger, R.D. (1996) *SAS® System for Mixed Models*. Cary, NC, USA, SAS Institute Inc.

Lobo, J.M., Lumaret, J.P. & Jay-Robert, P. (1998) Sampling dung beetles in the French Mediterranean area: effects of abiotic factors and farm practices. *Pedobiologia* **42**, 252–266.

McCracken, D.I. (1993) The potential for avermectins to affect wildlife. *Veterinary Parasitology* **48**, 273–280.

Roslin, T. (2000) Dung beetle movements at two spatial scales. *Oikos* **91**, 323–335.

Rushton, S.P., Ormerod, S.J. & Kerby, G. (2004) New paradigms for modelling species distributions? *Journal of Applied Ecology* **41**, 193–200.

Ryan, M.F., Clare, N. & Cole, A.J. (1978) A comparative study of coprophilic beetles in pasture over a peat and a mineral soil. *Scientific Proceedings of the Royal Dublin Society, Series A* **6**, 215–221.

SAS Institute (2001) *SAS® /STAT Release 8.2*. Cary, NC, USA, SAS Institute Inc.

Smith, K.E. & Wall, R. (1998) Estimates of population density and dispersal in the blowfly *Lucilia sericata* (Diptera: Calliphoridae). *Bulletin of Entomological Research* **88**, 65–73.

Stewart, K.E.J., Bourn, N.A.D. & Thomas, J.A. (2001) An evaluation of three quick methods commonly used to assess sward height in ecology. *Journal of Applied Ecology* **38**, 1148–1154.

Strong, L. & James, S. (1993) Some effects of ivermectin on the yellow dung fly, *Scatophaga stercoraria*. *Veterinary Parasitology* **48**, 181–191.

Suarez, V.H., Lifschitz, A.L., Sallovitz, J.M. & Lanusse, C.E. (2003) Effects of ivermectin and doramectin faecal residues on the invertebrate colonisation of cattle dung. *Journal of Applied Entomology* **127**, 481–488.

Toutain, P.L., Upson, D.W., Terhune, T.N. & McKenzie, M.E. (1997) Comparative pharmacokinetics of doramectin and ivermectin in cattle. *Veterinary Parasitology* **72**, 3–8.

Wall, R. & Strong, L. (1987) Environmental consequences of treating cattle with the antiparasitic drug ivermectin. *Nature* **327**, 418–421.

Wardhaugh, K.G. & Mahon, R.J. (1991) Avermectin residues in sheep and cattle dung and their effects on dung-beetle (Coleoptera: Scarabaeidae) colonization and dung burial. *Bulletin of Entomological Research* **81**, 333–339.

Wardhaugh, K.G. & Rodriguez-Menendez, H. (1988) The effects of the antiparasitic drug, ivermectin, on the development and survival of the dung-breeding fly, *Orthelia cornicina* (F.) and the scarabaeine dung beetles, *Copris hispanus* L., *Bubas bubalus* (Oliver) and *Onitis belial* F. *Journal of Applied Entomology* **106**, 381–389.

Wassmer, T. (1994) Seasonality of coprophagous beetles in the Kaiserstuhl area near Freiburg (SW-Germany) including the winter months. *Acta Oecologia* **15**, 607–631.

Webb, L. (2004) The impact of avermectin usage on the ecology of dung insect communities and the potential implications for foraging birds. PhD thesis, University of Glasgow, Glasgow, Scotland, UK.

Webb, L., Beaumont, D.J., Nager, R.G. & McCracken, D.I. (2007) Effects of avermectin residues in cattle dung on yellow dung fly (*Scathophaga stercoraria* L.) populations in grazed pastures. *Bulletin of Entomological Research* **97**, 129–138.

White, E. (1960) The natural history of some species of *Aphodius* (Col., Scarabaeidae) in the Northern Pennines. *Entomologist's Monthly Magazine* **96**, 25–30.

Wratten, S.D. & Forbes, A.B. (1995) Environmental assessment of veterinary products with particular reference to the avermectins. *Pesticide Outlook* **6**, 20–24.