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*The Impact of Avermectin Usage on the Ecology of  
Dung Insect Communities and the Potential Implications  
for Foraging Birds*

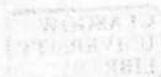
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Thesis submitted to the University of Glasgow for the Degree of  
Doctor of Philosophy

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December 2004

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*“...dealers in ordure, the scavengers of the meadows contaminated by the herd...notwithstanding their filthy trade, the dung beetles occupy a very respectable rank”*

Jean-Henri Fabre (1911)

## Summary

There is concern that the use of avermectin worming products in livestock and the subsequent presence of avermectin residues in dung could negatively affect the survival and development of dung-breeding insects in pastures. Such a reduction in natural populations of dung insects has potential implications for the vertebrate predators that forage in pastures for invertebrate prey.

This study compared the abundance, diversity and assemblage structure of adult dung insects (*Aphodius*, *Cercyon* and *Sphaeridium* beetles and yellow dung flies, *Scatophaga stercoraria*) between pastures grazed either by avermectin-treated or untreated cattle. Sampling was conducted using dung-baited pitfall traps in cattle-grazed pastures in Ayrshire, South West Scotland from April to July of 2002 and 2003. Twelve fields grazed by cattle that were not anthelmintically treated and fourteen grazed by cattle receiving either a doramectin or an ivermectin product were sampled. Six 'untreated' and six 'treated' fields were sampled in both years of the study while all other fields were sampled in only one of the years. Generalized Linear Models were used to investigate the significance of potentially influential factors for the abundance, diversity and assemblage structure of the dung insects under study. Those factors included avermectin treatment, seasonality, pasture management intensity, weather and various habitat variables. A multivariate ordination technique was used to explore differences in the species compositions of dung insect communities in study pastures.

In general, the factors found to be consistently significant for patterns of variation in dung insect abundance and diversity were year, seasonality and weather. There was no significant difference in the abundance of adult *Cercyon* beetles or yellow dung flies between pasture grazed by treated or untreated cattle. *Sphaeridium* beetles were trapped in numbers that were not sufficiently high to be modelled. Significantly more *Aphodius* dung beetles were trapped in fields grazed by treated cattle and evidence from additional fields trials suggested that this phenomenon could have been due to an avoidance of dung from avermectin-treated cattle. Wing length asymmetry was higher in yellow dung fly populations in pastures grazed by avermectin-treated cattle, suggesting that individual flies may have undergone developmental stress in dung from treated animals. However, higher asymmetry in

treated fields could not be solely attributed to avermectin exposure and other potential reasons for the difference in asymmetry are considered. Furthermore, there was no evidence that this possible sublethal effect impaired the overall density of yellow dung fly populations in pastures.

Variation in the size structure of *Aphodius* dung beetle assemblages was mainly due to seasonality and there was no effect of avermectin treatment. Therefore, the profitability of prey items for foraging birds is more likely to be a function of seasonal occurrence than due to any difference associated with avermectin treatment. Furthermore, basic observations of the foraging activity of birds in pastures did not show any major differences in the foraging activity of birds between pastures grazed by treated and untreated cattle. Hence, the availability of dung insects for foraging predators is more likely to fluctuate according to variation in season, weather and year than it is with avermectin treatment. However, the unsuitability of dung from avermectin-treated cattle for *Aphodius* dung beetles could potentially reduce beetle abundance in pastures when an alternative 'untreated' dung resource is not available.

A survey of the use of anthelmintics in livestock farms in South West Scotland was conducted to help guide the selection of study sites and to allow any results to be set in a wider context. This study was conducted in a region where dairy farming is predominant. The questionnaire survey indicated that aspects of livestock management and anthelmintic treatment on dairy farms increase the availability of avermectin-free dung in the landscape. Therefore, it cannot be disregarded that the observed minimal effects of avermectins on dung insects may have been mitigated by the presence of 'untreated' dung for insects to colonise. It is proposed that in areas where avermectin-free dung is limited, either because of farming type or geographical area, negative effects associated with unsuitability of dung from treated animals on populations could occur. Such effects could be mitigated by management practice. For example, only young animals should be treated and unnecessary treatment of immune adults should be avoided. Where possible, avermectin-treated cattle should be grazed in pasture adjacent to pasture that is grazed by untreated livestock. On grazed grassland being managed to benefit insectivorous species e.g. waders, a non-avermectin wormer or one of the less toxic avermectins could be used to worm livestock.

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## CHAPTER 1

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# GENERAL INTRODUCTION AND LITERATURE REVIEW

## 1. General Introduction and Literature Review

### 1.1 Interactions between Invertebrates and Farmland Birds

Farmland is widespread throughout Britain and thus it constitutes an important breeding habitat for many bird species. However, there has been a much-publicised decline in farmland bird populations and a contraction in the range of many species over the last twenty to thirty years (Krebs *et al.*, 1999; Chamberlain and Fuller, 2000). These declines have occurred in species of both arable (Siriwardena *et al.*, 2000) and grassland habitats (Stowe *et al.*, 1993, Henderson *et al.*, 2004). The intensification of agriculture has progressed over the last 50 years (Pain and Pienkowski, 1997), a process that has been attributed to the implementation of an ‘inefficient’ agricultural policy (Bowers and Cheshire, 1983). It is widely accepted that the farmland bird ‘crisis’ has ultimately happened as a consequence of agricultural intensification (e.g. Chamberlain *et al.*, 2000; Donald *et al.*, 2001b) although the specific causal factors are diverse, often interconnected, and require clarification.

The factors that could affect bird populations on pastoral land have recently been reviewed by Vickery *et al.*, (2001) who discuss pastoral intensification in terms of a switch in conserved grassland use from hay to silage, increased use of pesticides and inorganic fertilisers, and stocking pressure changes. These changes are discussed below in terms of their influence on the physical habitat structure and availability of invertebrate food for birds.

A move towards silage production from traditional haymaking and the mechanisation of cutting has proved detrimental for some bird species. The direct effects of silage cutting, nest loss and mortality, have been major factors in the demise of the corncrake *Crex crex* L. (Green, 1995). An indirect effect is that invertebrate abundance decreases following cutting, although this effect is thought to be a temporary one that is probably due to the disturbance incurred by cutting (Purvis and Curry, 1981; Good and Giller, 1991). Larger insects occurred more in silage fields (Purvis and Curry, 1981) however fields with long swards are not necessarily the

best foraging areas as many birds capture prey more successfully in shorter swards (e.g. Devereux *et al.*, 2004).

Pesticide use is an integral part of commercial agriculture methods. Herbicides control the broad-leaved weeds that are important host plants to invertebrates and the use of insecticides directly reduces the invertebrate food supply for birds (Campbell and Cooke, 1997). Not only are invertebrates essential for the survival of insectivorous species, but they are also important for granivorous species provisioning young during the breeding season (e.g. Green, 1978; Galbraith, 1989; Campbell and Cooke, 1997). Invertebrate protein is important for the development of young birds, for example skylark, *Alauda arvensis* L., chicks that were fed insect larvae had superior body condition than chicks not fed larvae (Donald *et al.*, 2001a).

The size, as well as abundance, of invertebrate prey may change with agricultural intensification. Blake *et al.* (1994) found body size in grassland carabids to decrease when management intensity increased. The profitability of insect prey is a function of both their size and abundance (Bryant, 1973; Kaspari and Joern, 1993) and many birds select prey on the basis of size. For example, the prey of swifts fell into the size range of 2-10mm (Lack and Owen, 1955) and pied wagtails discriminated against Sphaerocerid dung flies of 1-2mm in favour of larger Sphaerocerids and Scatophagids of 3-10mm in length (Davies, 1977). By examining the size and biomass structure of invertebrate assemblages, one can assess their relative value as prey for foraging birds.

An increase in stocking pressure occurred in the latter half of last century, particularly of sheep (Fuller and Gough, 1999). Such an increase has previously been associated with a decrease in the occurrence of pastoral bird species (Pain *et al.*, 1997). The implications of this for an individual species would depend on that species' particular habitat requirements, for example some birds prefer the shorter uniform sward that is characteristic of sheep-grazed fields to the longer heterogeneous sward of cattle-grazed fields. Jackdaws *Corvus monedula* L., rooks *C. frugilegus* L., carrion crows *C. corone* L., magpies *Pica pica* L. and starlings *Sturnus vulgaris* L. were observed more in fields grazed by sheep than in cattle-grazed fields (Tucker, 1992; Perkins *et al.*, 2000). For two of those species, carrion

crow and starling, the preference was attributed to shorter sward (Perkins *et al.*, 2000). Fieldfare *Turdus pilaris* L., golden plover *Pluvialis apricaria* L., and lapwing *Vanellus vanellus* L., were associated with cattle-grazed pasture (Lucker, 1992; Perkins *et al.*, 2000).

Livestock density and grazing regimes can influence the abundance and species composition of the grassland invertebrate community (e.g. Dennis *et al.*, 1997; Kruss and Tschamtkke, 2002). A high stocking density in a pasture provides a plentiful fresh supply of dung for the dung invertebrate community. A disadvantage of high stocking density in a pasture is that there is an increased chance of nest trampling of ground-nesting species, such as the lapwing (Beintema and Müskens, 1987; Baines, 1990). Insects that breed in dung are valuable prey for ground-feeding birds including lapwings and other waders (Galbraith, 1989; Beintema *et al.*, 1991), jackdaws (Laurence, 1954) and pied wagtails *Motacilla alba* L. (Davies, 1977). Swifts *Apus apus* L., house martins *Delichon urbica* L. and barn swallows *Hirundo rustica* L. prey upon dung-breeding flies (Lack and Owen, 1955; Bryant, 1973; Turner, 1982). Indeed, successful fledging of the barn swallow was positively associated with the number of cattle on farms (Ambrosini *et al.*, 2002). This relationship was probably linked to insect availability, because insect abundance decreased in areas where cattle had been removed (Møller, 2001). The research conducted for this thesis focuses on dung insects and considers the potential consequences of altered prey availability for farmland birds. However, other vertebrate predators e.g. bats would also be affected by changes in prey availability. For example, dung beetles are an important component in the diets of several bat species that forage over farmland (Wickramasinghe *et al.*, 2004).

As highlighted above, the dung-breeding invertebrate community is an important feeding resource for avian and mammalian predators on farmland. Hence, there is concern that any changes in livestock and/ or pasture management may ultimately exacerbate or contribute to the decline of some farmland bird and bat species. The following section describes the dung invertebrate community typical of Britain, and thereafter the effects of avermectin animal health products on dung invertebrates are reviewed.

## 1.2 Dung Invertebrate Communities

### 1.2.1 British dung insect communities

Herbivore dung supports many groups of invertebrates including beetles, flies, nematodes, mites and parasitoid wasps (Skidmore, 1991). These comprise different trophic groups, namely coprophages, carnivores and parasitoids (Koskela and Hanski, 1977). In north temperate regions, most coprophagous beetle species belong to the Scarabaeidae and Hydrophilidae families. The yellow dung flies (Scatophagidae), lesser dung flies (Sphaeroceridae) and soldier flies (Stratiomyidae) are among the many dipteran coprophilous species that utilise dung for some stage of development (Skidmore, 1991). Predatory rove beetles (Staphylinidae) and ground beetles (Carabidae) are common in dung where they feed on the adults, larvae and eggs of dung-breeding insects. Tiny Ptiliid and Cryptophagid beetles frequently occur in older dung where they feed on fungi and mould (Skidmore, 1991).

This research focuses on a selection of groups that are characteristic of livestock dung in temperate regions: *Aphodius* beetles (Scarabaeidae), *Cercyon* and *Sphaeridium* beetles (Hydrophilidae) and yellow dung flies (*Scatophaga* spp.). The remainder of this section, which places particular emphasis on these four genera, reviews the ecology of these dung insects.

The *Aphodius* have the main role in dung beetle communities in Britain (Hanski, 1986). Although there are approximately 40 species in Britain, the number of abundant *Aphodius* species in a typical British assemblage is approximately 10-13 (Hanski, 1986; Jessop, 1986). The *Aphodius* typically adopt a polygamous mating system where copulation takes place on the surface of dung or beside the dung (Landin, 1961). Thus, their reproductive strategy is simple in comparison to the strategies adopted by other Scarabaeidae that involve nest construction and parental co-operation (Cambefort and Hanski, 1991; Hanski, 1991b). *Aphodius* can exhibit interspecific differences in relation to oviposition site, number of eggs produced and larval development. For example, *A. ater* (Degger), *A. fimetarius* (L.), *A. rufus* (Moll) and *A. fossor* (L.) lay their eggs singly in drier parts of the dung, while *A. depressus* (Kug.) and *A. rufipes* (L.) lay clutches of eggs in the soil beneath dung (Hirschberger and Degro, 1996; Gittings and Giller, 1997). The larval development

of most species of *Aphodius* occurs within the dung, however the development of *A. prodromus* (Brahm) and *A. sphaecelatus* (Panzer) takes place in the soil where the larvae feed on plant roots and decaying organic matter (Gittings and Giller, 1997; Finn *et al.*, 1999). *Aphodius* typically have one generation per year (Hanski, 1991b) and most overwinter as adults although some species, for example *A. rufipes* and *A. rufus*, overwinter as third instar larvae (White, 1960; Gittings and Giller, 1997).

Seasonality is a major driving factor of patterns of abundance in the *Aphodius* community, and species can be categorised into ‘early summer’, ‘late summer’ and ‘spring/ autumn’ groups according to the timing of the adult flight period (Gittings and Giller, 1997). Early summer species include *A. ater*, *A. depressus*, *A. fossor* and *A. pusillus* (Herbst), and late summer species include *A. rufipes* and *A. rufus*. The species *A. prodromus* and *A. sphaecelatus* occur mainly in the spring and autumn (Hanski, 1980f; Gittings and Giller, 1997).

The small *Cercyon* (Hydrophilidae) beetles are common in dung although they are described as generalists (Koskela, 1979) because they breed both in dung and in other decaying matter (Hansen, 1987). While adult *Cercyon* are coprophagous or saprophagous, the larvae are carnivorous (Bøving and Henriksen, 1938 in Koskela and Hanski, 1977). There are 23 species of *Cercyon* in northern Europe and there may be approximately ten species in a typical British assemblage (Hanski, 1980f; Hansen, 1987). *Cercyon haemorrhoidalis* (F.) and *C. melanocephalus* (L.) are among the most abundant *Cercyon* species in Britain (Hanski, 1980c; Skidmore, 1991). Most *Cercyon* species that occur in Britain are multivoltine and are active as adults from spring until October, with *C. lugubris* (Olf.) and *C. atomarius* (F.) occurring mainly in spring and autumn (Hanski, 1980c; Hansen, 1987).

There are four species of *Sphaeridium* (Hydrophilidae) in Britain, *Sphaeridium bipustulatum* F., *S. lunatum* F., *S. marginatum* F. and *S. scarabaeoides* (L.) (Foster, 2005). *Sphaeridium* species have been described as specialist coprophages (Koskela, 1979) because the adults feed on dung and their larvae are predatory within the dung (Finn *et al.*, 1999). They have two generations per year with the summer generation resulting from larvae that emerge in spring (Hansen, 1987).

In temperate regions, the yellow dung fly *Scatophaga stercoraria* L. is a characteristic insect of cattle dung (e.g. Ward and Simmons, 1990; Strong and James, 1993). The reproductive behaviour of *S. stercoraria* has been described in detail by Parker (1970b). The sex ratio is skewed towards the males who compete strongly for mating access to the females that arrive at dung pats for oviposition (Otronen, 1995). The time taken for eggs to hatch is somewhat temperature-dependent with eggs hatching over approximately 6 days at 20°C and 12 days at 10°C (Gibbons, 1987). Elsewhere, hatching has been observed to occur in 1-2 days after which the hatched larvae move towards the centre of the pat to develop (Hirschberger and Degro, 1996). Pupation takes place either in the soil below the pat (Hirschberger and Degro, 1996) or in the dung itself, if suitable (Amano, 1989). Peaks in yellow dung-fly populations occur in the spring and the autumn with the latter peak persisting until the start of severe frosts (Laurence 1954; Parker, 1970a; Gibbons, 1987; Amano, 1989).

There are different stages of succession in dung invertebrate communities commencing with colonisation by flies, followed by beetles and their mites, then parasitoid wasps and finally soil invertebrates including earthworms (Mohr, 1943). Most *Aphodius* beetles are found in dung that is less than five days old (Hanski, 1980a) although a degree of succession is apparent within the genus. Early successional species of *Aphodius* include *A. depressus*, *A. prodromus*, *A. sphacelatus* and *A. rufipes*, mid-successional species include *A. ater* and *A. rufus* and late successors are *A. fimetarius* and *A. fossor* (Gittings and Giller, 1998). The late-successional *Aphodius* species colonised dung for up to 2-3 weeks after deposition (Hanski, 1980d). Of the Hydrophilidae, *Sphaeridium lunatum* and *S. scarabaeoides* are early colonisers of dung (Gittings and Giller, 1998). The species *Cercyon atomarius*, *C. haemorrhoidalis*, *C. lugubris* and *C. melanocephalus* could all be described as early successional species as they colonise dung up to 3-4 days old (Hanski, 1980e). Both *C. lateralis* (Marsh.) and *C. pygmaeus* (Ill.) colonise dung that is up to approximately 7-10 days old (Hanski, 1980e).

The above information on succession, adult and larval feeding strategies and seasonal occurrence is summarised below (Table 1.1).

Species	Succession in dung	Season (adults)	Adult feeding	Larval feeding	Site of larval development
<i>Aphodius ater</i>	Mid	ES	Co	Co	Dung
<i>A. depressus</i>	Early	ES	Co	Co	Dung
<i>A. fimetarius</i>	Late	ES - LS	Co	Co	Dung
<i>A. fossor</i>	Late	ES	Co	Co	Dung
<i>A. prodromus</i>	Early	S/ A	Co	Sa	In soil, on plant matter
<i>A. pusillus</i>	?	ES	Co	Co	Dung
<i>A. rufipes</i>	Early	LS	Co	Co	Dung
<i>A. rufus</i>	Mid	LS	Co	Co	Dung
<i>A. sphacelatus</i>	Early	S/ A	Co	Sa	In soil, on plant matter
<i>Cercyon atomarius</i>	Early	ES - LS	Co/ Sa	Carn	Dung/ decaying matter
<i>C. haemorrhoidalis</i>	Early	ES - LS	Co/ Sa	Carn	Dung/ decaying matter
<i>C. lateralis</i>	Mid	ES - LS	Co/ Sa	Carn	Dung/ decaying matter
<i>C. lugubris</i>	Early	ES - LS	Co/ Sa	Carn	Dung/ decaying matter
<i>C. melanocephalus</i>	Early	ES - LS	Co/ Sa	Carn	Dung/ decaying matter
<i>C. pygmaeus</i>	Mid	ES - LS	Co/ Sa	Carn	Dung/ decaying matter
<i>Sphaeridium lunatum</i>	Early	ES, LS, S/A	Co	Carn	Dung/ decaying matter
<i>S. scarabaeoides</i>	Early	ES, LS, S/A	Co	Carn	Dung/ decaying matter
<i>Scatophaga stercoraria</i>	Early	S/ A	Carn	Co	Dung

Table 1.1 – Summary of succession in dung colonisation (Early <5 dys, Mid 6-10 dys, Late >10 dys), seasonal occurrence (ES: early summer; LS: late summer; S/ A: Spring/ Autumn), adult and larval feeding strategies (Co=coprophagous ‘dung-feeding’; Sa=saprophagous ‘feeding on decomposing matter’ and Carn=carnivorous) and site of larval development for the dung insects studied in this research (Parker, 1970a; Hanski, 1980e; Gibbons, 1987; Hansen, 1987; Gittings and Giller, 1997, 1998; Finn *et al.*, 1999). Note that categories may not be strictly mutually exclusive.

### 1.2.2 Factors affecting the properties of dung and subsequent insect colonisation

Succession in insect colonisation of dung may be a consequence of optimal resource use for their ecological requirements because the physical properties of dung also change over time. As described by Mohr (1943), pats are either greenish-brown or uniformly brown and very moist on the first day after deposition. By day 3, they are brown with a dry crust, and within 30 days they are lighter brown with a thick crust or may even be dry throughout. Once a crust has formed over the pat, colonisation by insects is greatly reduced.

Assigning an age to dung is not always easy because its appearance and degradation rate can change according to a number of factors. The main contributory factors of dung decomposition, as reviewed by Marsh and Campling (1970), include rainfall, microbial decomposition, disintegration of pats due to foraging species and removal of dung by insects and earthworms. Once a pat has formed a hard crust, the eroding

effect of rain is lowered and decomposition is retarded (Weeda, 1967). The degradation time of dung has been shown to vary with season and geographical area. Cow pats deposited in late summer in a region of Denmark took 50-70 days to completely disappear (Holter, 1979a) while in a study in the west of Scotland, disappearance of cattle dung pats in May, June and July took 133, 109 and 79 days, respectively (Castle and MacDaid, 1972).

The suitability of dung for insect colonisation is largely dependent on the diet of the livestock animal, for example significantly more beetle larvae were collected from the dung of grass-fed animals than from animals fed hay or corn silage (Barth, 1993). Properties of dung can vary depending on the pasture vegetation type but mainly differ with the growth and maturity state of the grass ingested (Greenham, 1972). The moisture and consistency of dung may be affected by diet and Barth (1993) has stressed that dung moisture content is a critical factor for dung insects. Both *Aphodius fossor* and *A. sphaecelatus* prefer moister dung (Sowig and Wassmer, 1994; Vessby, 2001) and *A. ater* favours relatively dry dung (Gittings and Giller, 1998). The hydrophilids, *Sphaeridium*, burrow into dung that is too wet for some other species (Anderson *et al.*, 1984).

Another factor affecting dung 'quality' is the animal source of the dung, for example species composition, numbers and biomass of beetles can all differ between the dung of sheep, cattle and other mammals (Lunaret *et al.*, 1992; Hirschberger, 1999; Galante and Cartagena, 1999). Wassmer (1995) found that *A. fossor* and *A. rufipes* were most common in cattle dung while *A. prodromus*, *A. sphaecelatus* and *A. rufus* preferred sheep droppings. It is notable that the mean body size of beetle species in sheep droppings was significantly smaller than those in cow dung, therefore the preference could have been a function of the size and/ or shape of the dung resource. Moreover, Finn and Giller (2000) found a positive significant relationship between *Aphodius* species richness and pat size. The location of dung in a pasture may also govern how likely it is to be colonised by insects. For example, the yellow dung fly *Scatophaga stercoraria* opts to colonise dung pats that are shaded as opposed to pats in the 'open' (e.g. Laurence, 1954; Parker 1970a) and *Aphodius brevis* and *A. equestris* prefer shaded habitats (Hanski, 1980b).

### 1.2.3 Dung insect interactions and spatial dynamics

The main competitive pressures in north temperate dung insect assemblages are for food and space. Space could be a limiting factor for adults and larvae of dung beetles in small dung pats and for larvae in larger dung pats, for example mass occurrences of adult *Aphodius prodromus* can make the pat unsuitable for coprophagous larvae of other *Aphodius* species (Finn and Gittings, 2003). Species with slower larval development times are perhaps more at risk because, in rapidly decaying pats, larvae could lose their food and substratum before growth is complete (Holter, 1979b). High densities of *Scatophaga stercoraria* can result in intra-specific decreases in fecundity by way of increased larval mortality, reduced pupal volume and shortening of developmental period (Amano, 1983). There is evidence that certain species will assess the pat in terms of competition before breeding there. One study measured the egg-laying behaviour of *Aphodius ater* in relation to the density of *S. stercoraria* larvae in the dung (Hirschberger and Degro, 1996). They found that *A. ater* seemed to maintain normal laying behaviour at low larval densities (40 fly eggs per 50g dung), whereas oviposition was reduced at high larval densities (200 eggs per 50g dung).

Within individual pats, competition between species might be reduced via spatial distribution processes such as aggregation. Increased aggregation can lend itself to competitor coexistence (Atkinson and Shorrocks, 1981; Kneidel, 1985) by reducing interspecific competition (Shorrocks and Rosewell, 1987). A model describing these dynamics is the variance-covariance dynamics model (Hanski, 1991a). This model states that an increase in intraspecific aggregation across resource patches brings an increase in intraspecific competition relative to interspecific competition thus facilitating the coexistence of species. Hanski, (1980b) showed that the common species of *Aphodius* in pastures were more aggregated than the uncommon ones. When measuring aggregation in the field, it should be noted that dung beetle aggregation can be enhanced by variation in dung pat size (*pseudo-aggregation*), while *real aggregation* occurs when there is little variation in dung size and thus the effect is due to beetle density (Hutton and Giller, 2004). The proximate mechanisms (species' immediate responses to environmental factors) of aggregation are probably non-specific while an ultimate (evolutionary) cause might be searching for a mate

(Palestrini *et al.*, 1998). There is evidence that microclimatic differences between and within pats can enhance aggregation. For example, female yellow dung flies tend to oviposit on small hills on the dung surface rather than in depressions, which is possibly a mechanism to avoid drowning during rainfall (Ward *et al.*, 1999) hence aggregation occurs around optimal sites for oviposition.

The spatial distribution of *Aphodius* can be described by the core-satellite hypothesis (Hanski, 1980f). Species are described as ‘core’ if they have a wide regional distribution and are locally abundant, while ‘satellite’ species have the opposite qualities (Hanski, 1980f). Satellite species are much more likely to be affected by average distances between pastures (Hanski, 1991b), thus they are likely to be more susceptible to changes in the availability and management of pastoral land. Within a pasture, *Aphodius* beetles form patchy populations with lots of movement between pats although *A. pusillus* is sensitive to the spatial distribution of pastures and may exist as metapopulations (Roslin, 1999; Roslin and Koivunen 2001).

#### 1.2.4 Sampling dung insect communities

This section reviews the various methods with which dung insect communities can be sampled. Dung-baited pitfall traps are frequently used for sampling as they give a good indication of the level of activity of adult beetles and dung-flies (e.g. Doube and Giller, 1990; Ridsdill-Smith, 1993; Barbero *et al.*, 1999). Pitfall traps have been criticised because they cannot be used to provide absolute estimates of population density (e.g. Greenslade, 1964). Nevertheless, pitfall traps can give estimates of relative abundance, which are extremely useful when comparing sites. Furthermore, dung-baited pitfall traps can be used for experiments to assess the attraction of dung insects to a particular dung type (Wardhaugh and Mahon, 1991; Floate, 1998b). The traps can also reflect events at a wider landscape level, for example an increase in numbers caught in pitfall traps in one grassland field coincided with the removal of cattle from an adjacent pasture (Finn *et al.*, 1998). This may, of course, cause difficulty when trying to interpret data from pitfall traps because one might not be aware of all potentially influential events that have occurred in the wider landscape at the time of trapping. Error may also arise when using baited pitfalls if beetles remain in the bait and do not fall into the trap (Hanski, 1980d). However, few dung beetles

were observed to remain in the bait of pitfall traps used in this study (using the trap design described in Chapter 3) therefore this source of error is negligible (pers. obs.). Baited pitfalls could introduce bias because they may under-represent species that are late-colonisers of dung (Gittings and Giller, 1999), although this obviously depends on the length of time for which traps are exposed. Baited pitfalls are not suitable if one wishes to estimate the suitability of dung for colonisation because dung beetles cannot adequately assess the resource. For example, beetles may be trapped and it is inferred that they prefer this resource, whereas in a natural situation they may have approached the pat but then opted to colonise elsewhere (Dadour *et al.*, 1999).

Cores can be taken from dung to sample dipteran larvae and coleopteran adults and larvae (McCracken, 1990). As well as providing estimates of abundance within the dung, this method can provide information on the suitability of dung for insect colonisation and on the development of invertebrates in dung. A possible source of error is related to the aggregation that can occur within individual pats, for example the aggregation of beetle larvae in the ‘northern’ part of pats (Barth *et al.* 1994). To minimise the bias associated with intra-pat aggregation, dung cores should be taken from the same position on all pats or, alternatively, whole pats could be removed and sampled however the latter option can increase processing time. Furthermore, when sampling ‘natural’ dung pats in a pasture, one must age the dung as accurately as possible because the dung insect assemblage structure can change with time since deposition (McCracken, 1990). One could attempt to mark fresh pats when deposited and return to sample the insects in those droppings at a later date. However cattle trampling in a field that is highly stocked may make it difficult to obtain marked pats. Predation pressure by birds and mammals is another factor that should be considered (McCracken and Foster, 1993). Dung invertebrate abundance in dung could obviously be reduced if foragers have already disturbed the dung, although any disturbance to the pat would be visible.

Baited pitfall traps have a number of advantages over direct dung sampling. They do not suffer the disadvantage of being “unprotected” (Doube and Giller, 1990) as natural dung pats do. Therefore, one can use baited pitfalls and exclude the problem of predation pressure. Additionally, the number of dung-baited pitfall traps can be easily standardised, as can the size of the dung-bait, whereas it can be more difficult

to standardise the volume or weight of 'natural' dung sampled. However, direct sampling is ideal for studies of dung insect larvae. Furthermore, some species may not be as 'trappable' using baited pitfalls. For example, *Sphaeridium* are sampled more efficiently by direct sampling of dung pats than by pitfall trapping (Finn *et al.*, 1999).

Malaise traps are seldom used in dung insect studies but they have been used to sample beetles in pasture habitats in New Zealand (Harris and Burns, 2000). These traps are basically open-fronted tents, which have an opening in one of their top corners that leads to a trap (Southwood, 1966). Malaise traps must be positioned across 'flight paths' (Southwood, 1966) so they could be positioned anywhere in the field in order to intercept dung-breeding insects moving between pats. A disadvantage is likely to be disturbance of traps from cattle in actively grazed pastures. Emergence traps may be used to sample flies and beetles that are emerging from dung and therefore the development of insects in dung can be gauged (Vessby and Wiktelius, 2003). It may also be possible to conduct a count of eggs visible on the surface of pats to monitor the activity of dung-flies within a field and to assess their selection of oviposition sites. Ward *et al.*, (1999) made counts of yellow dung fly eggs on the surface of pats both in the laboratory and in the field.

When sampling dung insect communities, one should be aware of factors that might affect dung insect activity. The most obvious factor is weather as dung insects are less active during periods of heavy rainfall (Gibbons, 1987; Finn *et al.*, 1998) and are more active during periods of sunshine (Lobo *et al.*, 1998). Emergence of dung beetles might be affected by the aspect of a pasture, for example emergence occurred earlier on south-facing slopes than on north-facing slopes for *Aphodius fimetarius* and *A. fossor* (Vessby and Wiktelius, 2003). Soil type may also affect dung beetle abundance as Ryan *et al.*, (1978) found that more occurred in dung situated on peat soil than on mineral soil. The current structure of the dung insect community under study is also partially dependent on the past history of insect populations in the area and this should be borne in mind when comparing insect communities from different pastures or geographical areas. For example, Hanski (1980c) observed great variation in *Aphodius ater* abundance between pastures in the same local area, which may have been due to variation in historical distribution.

### 1.2.5 Analytical methods used to compare dung insect assemblage structure

This section provides the rationale for selecting the main statistical techniques that were used in the following chapters to analyse abundance, diversity and species assemblage data. The finer technical aspects of the analytical procedures are provided in the methodology sections of each chapter.

Generalised Linear Models (GLMs) were ideal for analyses of abundance, species richness and diversity data because they allow relationships (linear and curvilinear) between a dependent variable e.g. abundance of *Aphodius* beetles, and independent variables e.g. avermectin treatment, to be modelled (e.g. McCullagh and Nelder, 1989). Mixed models were used so that the variables could be included as either 'fixed' or 'random' effects. 'Field' i.e. the study field in which dung insects were sampled was included as a random factor so that the sampled fields were regarded as a random sample from the larger 'population' of fields. Therefore, the results could be interpreted with regards to pastures in general and not just the actual fields that were sampled. Mixed modelling allows different error distributions to be incorporated into the analyses, for example most of the invertebrate data were count data and therefore a Poisson distribution was used (e.g. Littell *et al.*, 1996). An important feature of GLMs is that they assume that observations are independent (McCullagh and Nelder, 1989). In this research, dung insect data were repeatedly collected from the same set of fields throughout the sampling season therefore those sampling occasions were regarded as repeated measures, rather than independent sampling occasions, to avoid pseudoreplication error.

Multivariate techniques analyse community data as a whole, with a view to summarising that data (Gauch, 1982), therefore they are ideally used for detecting relative changes in species composition within an assemblage. The multivariate ordination technique Detrended Correspondence Analysis (DCA) was used to analyse species assemblage data. Ordination analyses can be interpreted visually using graphical outputs to view the relative positions of samples along the axes. Samples are distributed spatially on the ordination axes with samples which are closer together being more similar in terms of their species compositions than those that are far apart. The gradient lengths of axes on the sample ordination plots

indicate the level of similarity between samples, as their lengths are expressions of the average standard deviations of species turnover (Gauch, 1982). Full turnover of species assemblages of samples occurs in approximately four standard deviations (Gauch, 1982). The DCA ordination analyses shown in this thesis were carried out using Community Analysis Package software (Pisces Conservation Ltd, 1999). It should be borne in mind that this software represents the gradient lengths of sample ordination axes as average standard deviations of species turnover multiplied by a factor of 100 (Pisces Conservation Ltd, 1999). Therefore (for sample ordination plots shown in this thesis), a gradient axis length between two samples of approximately 400 suggests that complete turnover of species assemblages has occurred between those samples. Positions of individual species on the ordination plots are calculated from the weighted averages of sample positions whereby the weights are related to that species' abundance in the samples (Lepš and Šmilauer, 2003). These points on the species plots are described as their 'optimum' position and the abundance of a particular species decreases symmetrically in all directions from that point (Lepš and Šmilauer, 2003). Thus, if a species' optimum position is close to the origin of both axes, then it is likely to have been present and/ or abundant in all or most of the samples. Furthermore, if a species' position is located in the upper right quadrant of the ordination plot then that species is more likely to have occurred or be more abundant in samples that lie towards the upper right quadrant position in the sample ordination plot.

To interpret the ordination of community data in a less arbitrary manner, the scores that are generated from the ordination can be placed in a mixed model analysis as a dependent variable and relationships with independent variables can be examined.

### **1.3 Avermectins and Livestock Health**

#### *1.3.1 History of the avermectins*

Commercial introduction of avermectins as animal health products began in the early 1980s (Campbell and Benz, 1984) and they are now used worldwide to control internal and external parasites in livestock. The first avermectin compound was discovered at a research institute in Japan where it was isolated via fermentation of

the soil actinomycete, *Streptomyces avermitilis* (Campbell *et al.*, 1983). Synthetic alteration of the original avermectin compound has given rise to several different compounds (Campbell, 1985) including abamectin, ivermectin, doramectin, and eprinomectin. The milbemycins e.g. moxidectin, are produced via the fermentation of *Streptomyces hygroscopicus* and *S. cyanogriseus* (Shoop *et al.*, 1995). Although there is a difference in the chemical structure between the avermectins and milbemycins (Shoop *et al.*, 1995), they are otherwise structurally similar hence compounds belonging to the two groups are collectively termed the ‘macrocyclic lactones’.

The proposed mode of action of avermectins was initially described as disruption of GABA ( $\gamma$ -amino butyric acid)-mediated processes resulting in somatic muscle paralysis of nematodes and arthropods (Campbell, 1985). This was supported further by the fact that ivermectin is not effective against either fluke or tapeworm, parasites that do not have the same GABA-mediated processes that ivermectin was believed to act upon (Campbell and Benz, 1984). However, the molecular target of avermectins is now debated and the role of GABA receptors in muscle paralysis has been described as being “open to question” (Feng *et al.*, 2002). It is proposed that avermectins act upon glutamate-gated chloride channels (e.g. Cully *et al.*, 1996), which causes pharyngeal paralysis and so disrupts feeding and hydrostatic pressure regulation within the parasite (Brownlee *et al.*, 1997). The mode of action of milbemycins is proposed to be the same as that of avermectins (Shoop *et al.*, 1995).

When an animal is dosed with an avermectin, the drug is absorbed systemically and residues of the parent drug and its metabolites are deposited in the liver and in fat, and can be detected in areas where target parasites are located e.g. gastrointestinal mucosal tissues and lungs (Campbell, 1985; Lanusse, 2003). The main route of excretion of ivermectin is in the faeces (Herd, 1995; Wratten and Forbes, 1996) although ivermectin is also excreted via the mammary glands (Toutain *et al.*, 1988). Each avermectin product has a designated withdrawal period to ensure that animal products for human consumption do not contain drug residues exceeding the recommended safety limits. Thus, a withdrawal period is the time that must elapse from the last anthelmintic treatment of an animal before the animal can be slaughtered or milked to provide meat and dairy products, respectively (Sainsbury,

1998). The pharmacokinetics of the avermectins are reviewed in detail by Steel (1993), Toutain *et al.*, (1997) and Cerkvenik Flajs and Grabnar (2002).

### 1.3.2 Use of avermectins in livestock farming

There are various methods of administering anthelmintic products to livestock. Cattle can be dosed via pour-on, subcutaneous injection or bolus, and sheep dosed with a drench or subcutaneous injection. Injections are given beneath the skin on an animal's neck so that the drug reaches the bloodstream via subcutaneous tissues. Pour-on formulations are applied along the midline on the back of the animal and the drug is absorbed through the skin, hence they cannot be used on sheep because lanolin in the wool interferes with drug absorption (Herd, 1988). Drenching is commonly used for sheep where a suspension of the anthelmintic drug is administered orally to the animal. A bolus is a device that is administered orally and remains in the gut of the animal, from where it emits the anthelmintic drug. Sustained-release boluses have continuous efficacy for a period of 90-140 days, depending on the product (Taylor, 2004). Pulse-release boluses are five or seven annular tablets of anthelmintic mounted on a metal core and, when the core erodes at intervals of approximately 21 days, a tablet is released (Taylor, 2004).

The excretion profile and concentration of avermectin residues in dung is influenced by the diet of the treated animal although the exact relationship is not clear. For example, absorption rates of ivermectin were higher in lambs fed hay and concentrate than in grass-fed lambs (Taylor *et al.*, 1992) indicating a higher faecal output of ivermectin in grass-fed lambs (Cook *et al.*, 1996). In contrast, faecal ivermectin concentrations were higher in grain-fed cattle than in grazed cattle (Cook *et al.*, 1996).

The concentration of avermectin residues in dung is also dependent upon the method of drug administration. Sommer and Steffansen (1993) compared ivermectin concentrations in dung from animals treated with either a pour-on or a subcutaneous injection. Concentrations were higher in the 'pour-on treated' dung but residues could be detected for a longer time in the 'injection treated' dung, thus implying that treatment with a pour-on causes excretion of ivermectin in higher quantities over a shorter period of time. Herd *et al.*, (1996) compared ivermectin concentrations in

plasma and faeces from cattle treated with a sustained-release bolus, pour-on or an injection. The highest faecal concentration of ivermectin occurred in the pour-on treated cattle two days after treatment. Faecal residues of bolus-treated animals did not peak as high as those treated with a pour-on but residues did persist in faeces from animals given a bolus for the duration of the study, which was seven weeks. In summary, excretion of avermectin residues is likely to have the highest peaks in animals dosed with a pour-on and residues are most persistent in dung from animals given a sustained-release bolus. The systemic uptake of avermectins is also dependent upon the behaviour of the treated animals, for example cattle that were allowed to perform normal licking behaviour following treatment with an ivermectin pour-on had higher concentrations of parent drug residues in dung (Laffont *et al.*, 2001).

The fate of avermectin residues in the environment is subject to various processes. Studies using radioactive labelling have shown that avermectins bind tightly to soil particles and laboratory tests showed that avermectin B<sub>1a</sub> (abamectin) did not leach into 'representative' agricultural soils (Bull, 1985; Halley *et al.*, 1989b). Ivermectin undergoes photodegradation rapidly when the residues are exposed to sunlight and a pat begins to degrade (Halley *et al.*, 1993). This explains why, in an outdoor environment in summer, ivermectin has a half-life of one to two weeks in soil/ faeces mixtures (Halley *et al.*, 1989a). However, photodegradation of avermectin residues will only begin as the pat begins to degrade (Halley *et al.*, 1993) or when the break-up of pats by foraging birds exposes the residues to sunlight (Wratten *et al.*, 1993). Levels of ivermectin and doramectin were measured in dung from cattle that had been treated at the recommended dose with the result that ivermectin and doramectin were detected in dung at levels considered lethal and sublethal to dung fauna for up to 180 days after deposition (Suarez *et al.*, 2003). The effects of avermectin exposure on dung invertebrates are discussed in the following section.

## **1.4 Direct and Indirect Effects of Avermectins on Dung Invertebrates**

### *1.4.1 Review of effects on dung insects*

Post-treatment persistence of avermectin residues in faeces was initially thought to be beneficial in the quest to control dung-breeding pest species. For example,

ivermectin has deleterious effects on larvae of the pest species: horn fly, stable fly, face fly, house fly and goat warble fly (Meyer *et al.*, 1980, 1981; Floate *et al.*, 2001; Giangaspero *et al.*, 2003; Miller *et al.*, 2003). Emergence of adult hornflies, *Haematobia irritans*, was prevented in dung from ivermectin-treated animals for up to 28 days post treatment (Schmidt, 1983), and was impaired in dung from ivermectin-injected animals for up to eight weeks after treatment (Fincher, 1992). Exposure to dung from cattle treated with doramectin or ivermectin suppressed adult emergence in *Musca domestica* (Marley *et al.*, 1993; Farkas *et al.*, 2003) and ivermectin delayed development and impaired the fertility of *Musca nevillei* (Kruger and Scholtz, 1995). Sheep blowflies *Lucilia cuprina* that fed on dung from ivermectin-drenched sheep showed reduced fecundity and delayed development (Mahon and Wardhaugh 1991), and males that fed on dung from ivermectin-treated sheep had fewer mating attempts than males fed on untreated dung (Cook 1993). Strong (1986a and b) found that ivermectin inhibited head eversion in blowfly, *Calliphora vomitoria* L., pupae thus resulting in their failure to develop to adult stage. It was further proposed that ivermectin also disrupts ovarian development in adult female *C. vomitoria* L. (Strong, 1989). However, regardless of these sublethal effects, Mahon and Wardhaugh (1991) concluded that ivermectin use at the recommended dosage was unlikely to cause any significant reduction in sheep blowfly numbers.

Concern was first shown in the late eighties (Wall and Strong, 1987), regarding the impact of avermectin residues on beneficial, rather than pestiferous, dung invertebrates. Since then, a large amount of research has been conducted on the effects of avermectins, as reviewed by Strong (1992, 1993). Species can be regarded as beneficial if they predate pest flies in dung and thus act as a form of biological control. For example, adult yellow dung flies are predators of numerous species of pest flies (Skidmore, 1999). Dung flies and beetles also have a valuable role in the decomposition of dung and therefore reduce the accumulation of dung in pastures (Anderson *et al.*, 1984).

Work by Sommer *et al.*, (1992) and McCracken and Foster (1993) found the larvae of Cyclorrhaphan diptera e.g. Sepsidae, Scathophagidae and Sphaeroceridae to be negatively affected by ivermectin. Conversely, other studies have found *Scatophaga*

*furcata* and *S. stercoraria* to be unaffected in dung from ivermectin-treated animals (Floate, 1998a). Larvae of the dung breeding muscid *Orthelia cornicina* F. (*Neomyia cornicina* F.) were killed in dung from cattle that had received an ivermectin injection at the recommended dose up to 32 days previously (Wardhaugh and Rodriguez-Menendez, 1988). Reproductive changes, including reduced percentage egg hatch, delayed first oviposition and inhibited larval development, were observed in *N. cornicina* after exposure to dung containing ivermectin (Lumaret *et al.*, 1993; Gover and Strong, 1995, 1997). Effects can also be sex-specific, for example exposing female *N. cornicina* to ivermectin had a greater impact on fecundity and alteration of male mating behaviour than did exposure of males to ivermectin (Gover and Strong, 1997). There is also evidence that negative effects of avermectins might cross trophic levels. The number of parasitoids, *Muscidifurax zaraptor*, emerging from host pupae was reduced from pupae that had been exposed to ivermectin during development (Floate and Fox, 1999).

It is unlikely that adult dung beetles are killed on entry into the pat (Strong and Wall, 1988) and deleterious effects are more likely to occur via sublethal effects and lethal effects on larvae. In Australia, adult beetles, *Onthophagus binodis* Thunberg, were not killed in dung from cattle treated with abamectin, however egg production and oviposition were reduced and larvae did not survive in dung for up to one week after treatment (Ridsdill-Smith, 1988; Houlding *et al.*, 1991). Survival of newly emerged *O. binodis* adults was reduced in dung from abamectin-treated animals for up to 6 days after treatment and in dung from doramectin-treated cattle for up to 9 days post-treatment (Dadour *et al.*, 2000). Following ivermectin exposure, no mortality was detected in the adult dung beetles *Bubas bubalus* Ol., but mortality was observed in newly emerged beetles of *Copris hispanus* L. and *Onitis belial* F. (Wardhaugh and Rodriguez-Menendez, 1988). A North American study found reduced emergence of adult beetles *Euoniticellus intermedius* (Reiche) and *Onthophagus gazella* F. from brood balls made from dung from ivermectin-injected cattle (Fincher, 1992). Development of *Euoniticellus intermedius* and *Onitis alexis* was delayed in dung collected from cattle 2-7 days after treatment with an ivermectin injection (Krüger and Scholtz, 1997). Similarly, larval development of *Aphodius* species was also delayed in dung from ivermectin-treated animals (Madsen *et al.*, 1990; Sommer *et al.*, 1992; Strong and Wall, 1994). Abundance of the hydrophilid beetles *Cercyon*

*quisquilius* and *C. pygmaeus* was reduced in dung from ivermectin-treated cattle, and this may have been a result of direct insecticidal effects on adults and predaceous larvae or indirect effects on the larvae via a prey shortage within dung (Floate, 1998a).

Research on ivermectin effects on earthworms has yielded various results depending on the study species used. Svendsen *et al.* (2002) found that ivermectin and its breakdown products had no deleterious effects on the survival and growth of *Lumbricus terrestris*. Similarly, Wratten *et al.* (1993) concluded that populations of earthworms are not affected on pastures grazed by ivermectin-treated cattle. However, deleterious effects were apparent in *Eisenia fetida* (Savigny) when survival and development were impaired in soil containing ivermectin at concentrations resembling those in faeces of treated animals (Gunn and Sadd, 1994). Earthworms colonised ivermectin-free dung from 40 days after deposition onwards while colonisation of dung from ivermectin-treated cattle occurred from 80 days onwards (Wall and Strong, 1987). This delay in earthworm colonisation could have been caused by a reduction in fly and beetle activity in treated dung, which would have slowed the breakdown of the soil-dung barrier that facilitates earthworm colonisation.

Some novel methods have been developed to investigate avermectin effects on dung beetle activity. For example, the reduction in sporangia of the dung fungus *Pilobolus* was measured because the sporangia are reduced through ingestion and disturbance by *Aphodius* beetles (Finnegan *et al.*, 1997). The reduction of sporangia was significantly lower in dung from an ivermectin-treated animal suggesting that either the abundance or activity of beetles was reduced. However, the results were confounded because the authors suggested that ivermectin may have had direct effects on the growth of *Pilobolus*. The potential impacts of avermectins on dung insect populations have also been investigated with the aid of population modelling techniques (e.g. Sherratt *et al.*, 1998; Wardhaugh *et al.*, 2001). Consequently, it was suggested that, in the absence of immigration, a single treatment of eprinomectin could reduce beetle activity in the next generation by 25-35% (Wardhaugh *et al.*, 2001).

Most ecotoxicological studies of avermectins to date have been carried out on ivermectin. Results from these studies cannot be extrapolated to include all avermectins since there is variable potency within the avermectin group. The avermectins were ranked in order of their adverse effects on dung insects as 'doramectin > ivermectin > eprinomectin >> moxidectin' (Floate *et al.*, 2002). This is supported by many studies on avermectin effects. For example, a doramectin injection had greater efficacy against gastrointestinal nematodes than either an ivermectin injection or pour-on thus suggesting that doramectin is more potent (Williams *et al.*, 1997). The dung beetle *Onthophagus taurus* was unaffected when fed on dung containing moxidectin, however there was high mortality of juveniles in the first two weeks after being fed with dung from eprinomectin-treated cattle (Wardhaugh *et al.*, 2001). A study of the larvicidal activity of avermectins against Muscids found doramectin to be the strongest larvicide followed equally by ivermectin and eprinomectin (Floate *et al.*, 2001).

#### 1.4.2 Effects on the suitability of dung for insect colonisation

The attractiveness of dung from ivermectin-treated livestock to members of the dung insect community has been widely discussed in the literature. The relative attractiveness of dung containing avermectin residues has wider implications, particularly if one supposes that avermectins have deleterious effects on dung insects. If dung with residues proved more attractive to insects then one might expect negative effects to be exacerbated while an avoidance of dung that contains residues might mitigate any harmful effects provided there is an alternative suitable dung resource within colonising distance. Several studies have suggested that the attraction of dung from treated animals is not affected, for example adult *Aphodius* beetles were attracted both to dung from ivermectin-treated and untreated cattle (Strong and Wall, 1994; Strong *et al.*, 1996). The same dung beetle species were present in dung from cattle given an ivermectin bolus and untreated cattle thus it was concluded that residues did not have a repellent effect (Barth *et al.*, 1993). However, that conclusion made no reference to the relative abundance of beetles in dung from treated or untreated animals. The dung fly *Neomyia cornicina* did not show a preference for dung from either untreated or ivermectin bolus-treated cattle (Gover and Strong, 1996). In a study where ivermectin was added to dung, there was no

attraction effect indicating that the ivermectin 'parent drug' does not itself increase or decrease attraction (Strong and Wall, 1988). However, there have been cases where dung beetles were more likely to show a preference for ivermectin-free dung. In Australia, dung containing ivermectin was less attractive to *Onthophagus taurus* than ivermectin-free dung (Dadour *et al.*, 1999) and in a Danish trial, *Aphodius*, *Cercyon* and *Sphaeridium* beetles were more attracted to dung from untreated animals (Holter *et al.*, 1993 a and b). There was a tendency for dung beetles to be more attracted to ivermectin-free dung in a Tanzanian study while mixed results were obtained from the Zimbabwean part of the study (Holter *et al.*, 1993 a and b). Earthworms *Eisenia fetida* chose not to enter or stay in soil containing high concentrations of ivermectin (Gunn and Sadd, 1994) indicating a degree of avoidance of ivermectin. Conversely, some studies have shown an apparent attraction of dung insects to dung containing avermectin residues. In a Canadian study, significantly more *Aphodius fimetarius* and *A. distinctus* were collected in dung from ivermectin-treated cattle, however this was not observed the following year (Floate, 1998b). Dung from avermectin-treated cattle attracted more dung beetles for at least 25 days post-injection than untreated dung (Wardhaugh and Mahon, 1991).

Avermectins could affect attraction by altering olfactory cues emitted from dung. Wardhaugh and Mahon (1991) stated that beetles are unable to discriminate, by smell or taste, against the presence of avermectin. Therefore, a difference in dung attraction has not been attributed to ivermectin *per se* (Holter *et al.*, 1993; Lumaret *et al.*, 1993), but to cattle diet (Floate, 1998b) or changes in gut flora or dung as a result of treatment (Wardhaugh and Mahon, 1991; Wratten and Forbes, 1996).

#### 1.4.3 Potential consequences of avermectin effects on dung decomposition

It has already been established that dung insects are a valuable resource for foraging predators (Section 1.1). Dung insects are also an important component of pasture ecosystems as they aid the biological control of pest species and parasites, for example the Scarabaeid dung beetles interfere with the development of free-living nematode larvae in dung (Grønbold *et al.*, 1996). Furthermore, dung-breeding insects have a beneficial role in the decomposition of dung in pastures (King, 1993).

Indeed, one potential problem associated with negative impacts on dung invertebrates is 'pasture fouling', a phenomenon defined as a build-up of dung on pastures due to retarded decomposition.

The decomposition of dung from ivermectin-treated livestock was investigated, and several studies (McKcand *et al.*, 1988; Jacobs *et al.*, 1988; Wratten *et al.*, 1993) concluded that ivermectin did not affect the rate of dung decomposition. However, those studies did not monitor dung insects in the pats. Jacobs *et al.* (1988) assessed degradation by looking for faecal remains in paddocks that had contained either control or ivermectin-treated animals some five months previously. Therefore, that study does not provide information on the relative degradation rates of dung from ivermectin-treated and untreated animals but simply tells us that degradation was not delayed beyond a five-month period. Retarded decomposition of dung from ivermectin-treated animals has been associated with a lack of dung insect activity (Wall and Strong, 1987; Strong *et al.*, 1996). The 98 day degradation time of dung from ivermectin-treated heifers was up to twice as long as from heifers that were not treated or that were treated with another anthelmintic (Madsen *et al.*, 1988). When Madsen *et al.* (1990) studied both the rate of dung degradation and effects on dung insects, they found that larval development of Cyclorrhaphan dipteran species in dung from animals treated with an ivermectin injection was inhibited for up to 30 days. They attributed retarded dung degradation to the adverse effects on the dung flies.

## **1.5 Thesis Aims and Structure**

As indicated above, there is evidence that exposure to avermectins can cause sublethal and lethal effects in dung insects and that they may alter the suitability of dung for colonisation. Most of that research has been carried out in a laboratory or in individual dung pats in an experimental field situation. Forbes (1993) proposed that certain aspects of pasture and livestock management might actually mitigate any deleterious effects on dung insects in a natural pasture situation. The aim of the research presented in this thesis is to assess whether temperate dung insect populations in pastures are resilient to any potential localised declines within dung, while considering the interactions with pasture management, weather and habitat.

In *Chapter 2*, data from a questionnaire survey of farmers in central and south-west Scotland are presented. The aim of the survey was to ascertain the most representative livestock systems and anthelmintic treatment strategies in those regions. This enabled the selection of study sites to ensure that results from this research could be set into a wider context. *Chapter 3* consisted of a series of small-scale field trials that investigated factors to be taken into account in the sampling methodology of dung insects, including attraction of insects to dung and days of trap exposure. The aim was to highlight factors influencing the sampling of dung insect assemblages in order to aid the interpretation of results from the wider study. In *Chapter 4*, results are presented from dung insect sampling in fields grazed by cattle that were not anthelmintically treated. By examining the abundance and assemblage structure of insects in untreated fields, a baseline was established with which to make comparisons of dung insect assemblages in fields grazed by avermectin-treated cattle. Comparisons between dung insect assemblages in fields grazed by untreated and treated cattle, in relation to environmental variables and pasture management, are presented in *Chapter 5*. The aim of *Chapter 6* was to assess whether avermectin residues in dung in a pasture situation affected the size and biomass structure of assemblages or caused sublethal effects on insects. This allowed an evaluation of whether the profitability of invertebrate prey for predators was impaired in fields grazed by avermectin-treated cattle. *Chapter 7* presents results from a series of bird observations made in treated and untreated fields. The aim was to make comparisons of the species richness and activity of foraging insectivorous birds in general and, specifically, of the barn swallow. Results from all chapters are discussed in *Chapter 8* with a view to drawing conclusions about what the results mean at a broader level and addressing potential questions regarding conservation management recommendations on avermectin usage.

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CHAPTER 2

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PATTERNS OF ANTHELMINTIC USE IN  
LIVESTOCK IN SOUTH-WEST AND CENTRAL  
SCOTLAND

## 2. Anthelmintic use in livestock in South-West and Central Scotland, with particular reference to the avermectins

### 2.1 Introduction

Anthelmintics are essential for controlling worm burden in livestock and their use prevents economic loss to farmers (Woolley, 1997) and the contravention of animal welfare standards. There is a multitude of anthelmintic products available in the United Kingdom with several manufacturers dominating the market. Anthelmintics are grouped into the following classes according to mode of action and chemical structure (e.g. Taylor, 2004):

Group 1-BZ	<i>Benzimidazoles ('white drenches')</i>
Group 2-LM	<i>Imidazothiazoles and tetrahydropyrimidines ('clear drenches')</i>
Group 3-AV	<i>Avermectins and milbemycins</i>

A fourth group of 'combination' anthelmintics has been recognised which combine a flukicide with a broad-spectrum wormer such as a Group 1-BZ anthelmintic (Taylor, 2004), thus Group 4 does not contain any novel anthelmintic compounds. Anthelmintics have one of two main modes of action. Some, including those in the 1-BZ group, act on biochemical target sites while 2-LM and 3-AV anthelmintics target the membrane ion-channels of the parasite (Martin *et al.*, 1997). The benzimidazole group includes albendazole, fenbendazole and thiabendazole. These drugs bind to beta-tubulin molecules within the parasite thus causing disruption of cellular function and starvation. All benzimidazoles are effective against roundworm, lungworm, gutworm and tapeworm and some e.g. albendazole also control fluke (Taylor, 2001). The imidazothiazoles e.g. levamisole and morantel tartrate act on the nicotinic acetylcholine receptor of nematodes causing spastic paralysis (Martin *et al.*, 1997). Levamisole is effective against gutworm, roundworm and lungworm while morantel controls gutworm and roundworm (Taylor, 2001). The avermectins and milbemycins control roundworm, gutworm and lungworm and ectoparasites (Taylor, 2001) by targeting glutamate-gated Cl<sup>-</sup> channels, and possibly GABA receptors (Feng *et al.*, 2002). The mode of action of avermectins is reviewed in more detail in Section 1.3.1.

Parasitic infestations occur in all livestock sectors (Cawthorne, 1984) and there are various recommended grazing regimes to minimise the spread of parasitic worms (Michel *et al.*, 1981). An evasive or 'dose and move' strategy is where cattle are turned out to pasture and then, in the middle of the grazing season when parasite burden increases, they are treated with an anthelmintic and moved to a clean pasture (Wratten and Forbes, 1996). Pasture is considered to be clean if it has been livestock-free for more than six months, if it has been ploughed and re-seeded, or if it has previously been used for hay or silage production. 'Strategic' worming involves the use of anthelmintics early in the grazing season to prevent worm burden reaching a high level in young cattle (Wratten and Forbes, 1996). The strategic method is prevalent on farms that do not have sufficient availability of clean pasture to adopt the evasive strategy. Alternatives to anthelmintic use are often sought particularly in light of growing anthelmintic resistance in certain parts of the world (Waller, 1997; Coles, 2002). However, organic farming or research into alternatives such as bioforage crops is likely to remain as a specialised niche and Waller (1997) proposed that today's grazing systems shall always require the use of anthelmintics.

The cattle grazing season is weather-dependent but dairy cattle are normally put out to grazing from late-April until October. They are housed during winter and fed on silage and other feedstuffs. Beef cattle can also be taken into housing during winter (Fuller, 1998), although it is not uncommon for them to be out-wintered. Cattle grazing systems can be broadly categorised into rotational or continuous ('permanent') grazing and their employment is dependent upon various factors including sward, topography of pasture and herd requirements (Mayne *et al.*, 1991). Usually, milking cows are grazed rotationally and younger cattle are in a continuous grazing system. There are few anthelmintic products that can be used on dairy cows providing milk for human consumption because the residues of some anthelmintics are excreted via the mammary glands into milk (L'outain *et al.*, 1988). Cattle can develop natural immunity with age, therefore only the younger animals in a herd tend to be wormed (Herd, 1988). Sheep are usually out-wintered and provided with supplementary feeding although profitability can improve by housing sheep in winter and during lambing (Speedy, 1980; Bryson, 1984).

Of the 75% of Scotland's land area that is used for agriculture, the predominant farm type varies across regions between arable, grassland and rough grazing (NFUS, 2004). Agriculture in the south-west of Scotland is dominated by dairy farming where Ayrshire and Dumfries & Galloway together hold 57% of Scotland's dairy cows while covering only 12% of Scotland's land area (Scottish Executive, 2002). The high rainfall and heavier soils in the west make it less suitable for arable cropping (Brockman and Wilkins, 2003) therefore grassland is the prevalent agricultural land-use there. In the upland parts of the Central region, sheep and beef cattle are typical farming enterprises.

While it is thought that avermectins are relatively widely used in British farming, there are no available data regarding their regional use. The aim of this survey was to ascertain which products and treatment strategies were most commonly used by livestock farmers in Central and South-West Scotland, with the purpose of setting a context within which to place the farms targeted for invertebrate sampling.

## **2.2 Method**

### *2.2.1 Survey method*

The questionnaire survey was carried out from November 2001 to April 2003. A number of questionnaire survey methods were attempted. Notices were posted at local cattle markets advertising the study and inviting farmers to participate in the survey, however there was a zero response rate with this method. Flyers were distributed via the Farming and Wildlife Advisory Group (FWAG) inviting farmers to contact the author. That method yielded nine responses however four out of the nine FWAG respondents had stopped using avermectins on their livestock because they were aware of the environmental concern associated with avermectins. Respondents from that method may have been unrepresentative of farmers in general therefore alternative survey methods were sought.

The majority of responses were obtained from the membership base of both the South West Scotland Grassland Society (SWSGS) and the Central Scotland Grassland Society (CSGS). These Societies exist to gain and transfer knowledge

about grass and forage crop production and their membership base represents a good cross-section of commercial farmers in those regions. The SWSGS Secretary provided the author with a list of Society members who would possibly be prepared to take part in the survey, and those members were contacted by telephone. Members of the CSGS were passed a short questionnaire at an Annual Meeting and the responses were gathered there. Each farm was surveyed only once. It was originally planned to carry out a follow-up survey to detect a shift in usage, however most farmers indicated that they would use the same treatment strategy for at least two years. Thus it would be more relevant to carry out a follow-up survey at a longer time interval, e.g. after five years.

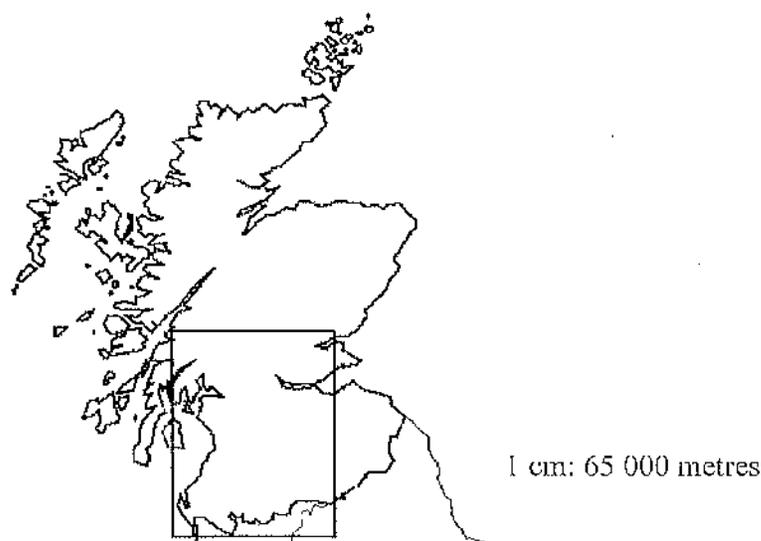


Figure 2.1 – Map of Scotland showing geographical area covered by questionnaire survey

### 2.2.2 Questionnaire design and data analysis

Farmers were asked the following questions via written questionnaire or telephone:

1. Which livestock type(s) do you farm?
2. Which worming product(s) do you use for each livestock type?
3. Method of dosing used for each product?
4. When and how often do you dose the livestock?
5. Are all livestock treated?

Questionnaire results are presented as percentage frequencies and are shown according to livestock type, anthelmintic class, dosing method, avermectin and treatment strategy. Chi-squared tests can be used to test for associations in nominal data but only when data meets the assumption of independence (Oppenheim, 1992). However, these data were not independent because often a response could be placed into more than one category. The revision of categories was attempted to gain independent data however this led to frequencies that were too low to apply a Chi-squared test.

## 2.3 Results

### 2.3.1 Distribution of respondents

Information on anthelmintic usage was obtained from 74 of 85 livestock farmers that were contacted therefore a response rate of 87% was achieved. Of those 74 responses, nine were from FWAG flyers, ten were from the CSGS meeting and 55 from telephone responses. The FWAG flyers were included in the data analysis because they did not make up a significant proportion of the responses hence they would not have biased results. Furthermore, they may have even reflected the proportion of farmers in the actual population that have altered their livestock treatment strategies because of concerns about avermectin use.

The surveyed farms were distributed across Ayrshire, Dumfries and Galloway, Clyde Valley, East Central, Lothian and Tayside regions (Figure 2.2). The uneven spread of responses over the surveyed area can be explained by gaps that occurred in urban areas and cities, and in areas that had few or no Grassland Society members. For example, the areas of South Ayrshire and Dumfries & Galloway that had few respondents overlapped with the Southern Uplands, a large tract of forest, moorland and acid grassland. The agricultural areas of the Southern Uplands are mainly extensive sheep farming with cattle farming on lower ground (SNH, 2001) and there were few members of the SWSGS that could be contacted in that area. Similarly, there were a relatively small number of respondents in the East Central and Tayside regions because there were fewer contacts in that area to administer questionnaires to.

## 2.3.2 Livestock type

Relationships between prevalent livestock type and geographical region were apparent. Farms in Ayrshire and Dumfries & Galloway were predominantly dairy farms. In the Clyde Valley and East Central regions, surveyed farms were either dairy and beef enterprises or beef and sheep enterprises. Of the six respondents in Tayside, there were four beef farms, one dairy farm, and one beef and sheep farm. One of the farms in the Lothian region was dairy and the other was dairy and sheep.

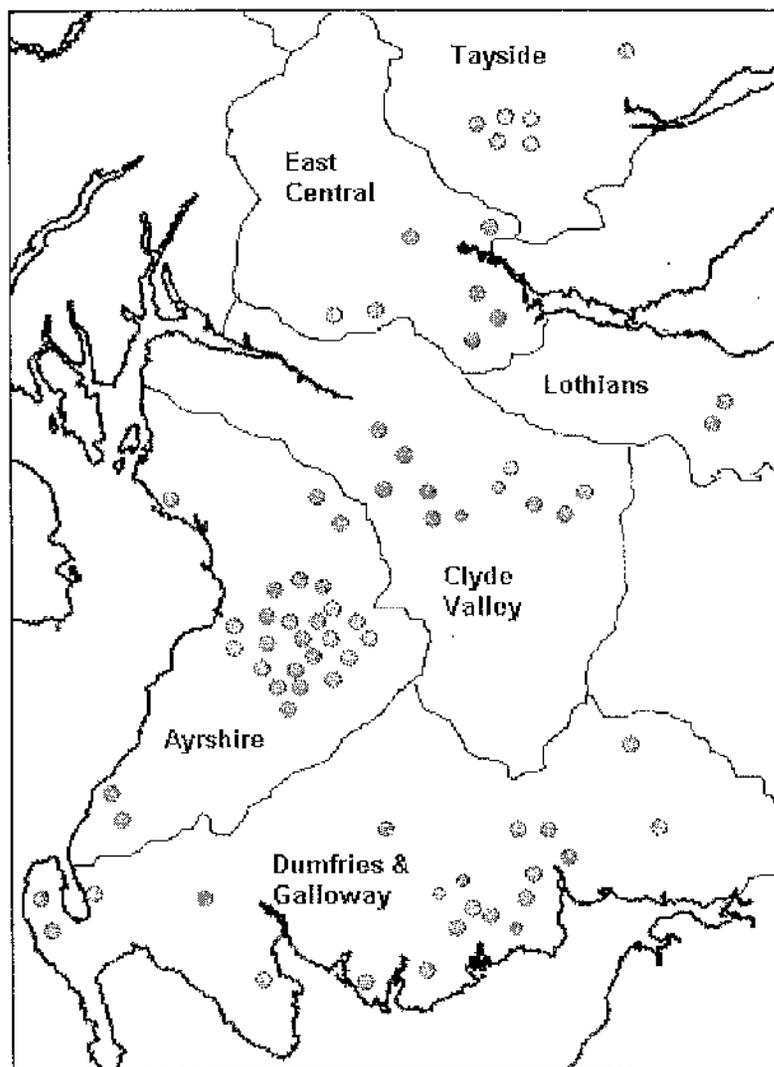


Figure 2.2 – Map showing distribution of questionnaire respondents from farms contacted via FWAG, CSGS and SWSGS (n=74)

Farms were placed into one of seven categories according to the types of livestock that they farmed (Figure 2.3). The categories were dairy only, beef only, sheep only,

beef/sheep, dairy/beef, sheep/dairy and sheep/beef/dairy. The majority of farms surveyed were 'dairy only' (45%). Similar proportions of dairy/beef, beef and beef/sheep enterprises occurred: 16%, 15% and 14%, respectively. Sheep were farmed exclusively on just 4% of all farms surveyed while only 1% farmed sheep, dairy and beef.

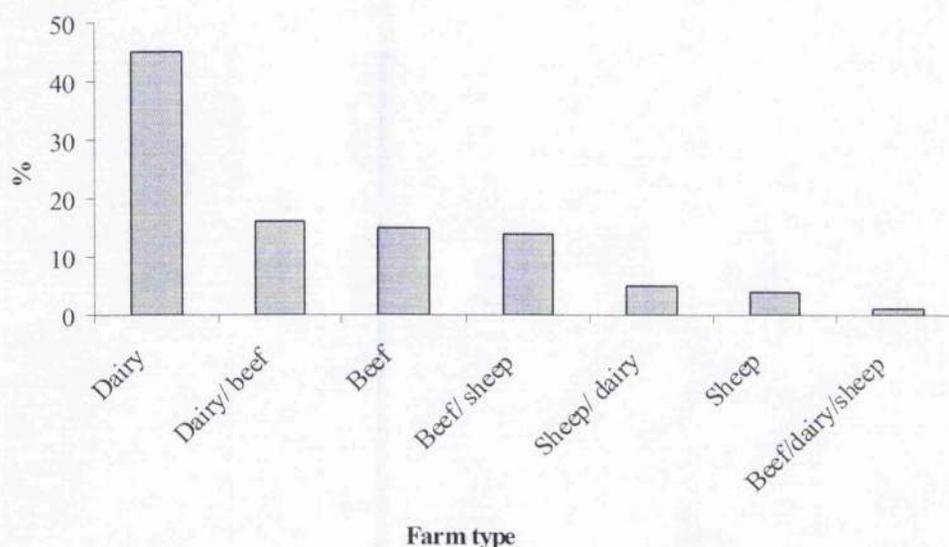


Figure 2.3 – Proportion of each livestock enterprise combination on surveyed farms in Central and South West Scotland (n=74)

### 2.3.3 Anthelmintic use

The trade name, manufacturer and active ingredient of each anthelmintic product that was recorded as being used on surveyed farms, are listed (Table 2.1). The table also lists the parasite groups against which the product is effective. The market share of anthelmintic products in each group is reflected somewhat by the number of products in each group given by surveyed farms, as shown in Table 2.1. For example, there are more Group 3-AV products on the animal health market than Group 2-LM products. Group 4 combination products were not provided in any of the questionnaire responses.

Product Name	Company	Active ingredient	Suitable for livestock	Controls parasites
<b>Group 1-BZ</b>				
Autoworm bolus	Schering-Plough	Oxfendazole	Cattle	R L T
Rycoben® drench	Young's	Albendazole	Cattle, sheep	R L T F
Mebadown drench	Janssen	Mebendazole	Sheep	R L T F
Panacur® bolus	Intervet	Fenbendazole	Cattle	R L T
<b>Group 2-LM</b>				
Paratect Flex™ bolus	Pfizer	Morantel tartrate	Cattle	R
<b>Group 3-AV</b>				
Cydectin® Drench	Fort Dodge	Moxidectin	Sheep	R L
Cydectin® Injection	Fort Dodge	Moxidectin	Cattle, sheep	R L E
Cydectin® Pour-on	Fort Dodge	Moxidectin	Cattle	R L E
Zernex® Drench	Fort Dodge	Moxidectin	Sheep	R L
Zernex® Injection	Fort Dodge	Moxidectin	Sheep	R L E
Zernex® Pour-on	Fort Dodge	Moxidectin	Cattle	R L E
Dectomax™ Injection	Pfizer	Doramectin	Cattle, sheep	R L E
Dectomax™ Pour-on	Pfizer	Doramectin	Cattle	R L E
Eprinex® Pour-on	Merial	Eprinomectin	Cattle	R L E
Ivomec® Bolus	Merial	Ivermectin	Cattle	R L E
Ivomec® Injection	Merial	Ivermectin	Cattle, sheep	R L F
Ivomec® Pour-on	Merial	Ivermectin	Cattle	R L E
Noromectin® injection	Norbrook	Ivermectin	Cattle, sheep	R L E
Noromectin® Pour-on	Norbrook	Ivermectin	Cattle	R L E
Oramec® Drench	Merial	Ivermectin	Sheep	R L
Panomec® Injection	Merial	Ivermectin	Cattle, sheep	R L F

Table 2.1 – Products used on surveyed farms. Information on products was collated from the Company websites and Taylor (2004). R – roundworm; L – lungworm; T – tapeworm; F – fluke; E – ectoparasites

Results are presented as ‘sheep’, ‘dairy’ and ‘beef’ categories, thus reducing the seven categories given in Figure 2.1 to the three aforementioned categories. For farms that kept more than one livestock type e.g. sheep, dairy cattle and beef cattle, each livestock type was considered as a separate unit (as per Gettinby *et al.*, 1987).

Therefore, a farm that kept a dairy and a beef herd was not considered as one farm but as one dairy unit and one beef unit. This was necessary because often there were different worming strategies for different livestock enterprises or ‘units’ on the same farm.

The use of each of the anthelmintic classes was summed for each livestock sector. For each livestock sector, independent usage of 1-BZ, 2-LM and 3-AV and the various combined uses of the groups are listed (Table 2.2). If a farmer used more than one product from the same class on any one unit then this was counted as one, for example if Ivomec® and Dectomax™ were used on a particular dairy herd then this was counted as one in the ‘3-AV only’ category.

The most common group was 3-AV, as sole use of avermectins occurred in 71.4% of sheep units, 46.9% of dairy units and 63.6% of beef units. The next common group in both dairy and beef sectors was the 1-BZ/ 3-AV combination that was used in 30.6% and 21.2% of the sectors, respectively. The least common strategies in all sectors involved the use of the 2-LM anthelmintics. It should be noted that although the use of a 2-LM product was recorded in the sheep sector (Table 2.2), a Group 2-LM product for sheep has not been listed in Table 2.1. This is because one farmer specified that a levamisole drench was used for his sheep but he could not recall the product name.

Anthelmintic class	Livestock sector					
	Sheep (n=14)	%	Dairy (n=49)	%	Beef (n=33)	%
1-BZ	1	7.15	5	10.2	4	12.1
2-LM	0	0	1	2.0	0	0
3-AV	10	71.4	23	46.9	21	63.6
1-BZ + 2-LM	0	0	0	0	0	0
1-BZ + 3-AV	2	14.3	15	30.6	7	21.2
2-LM + 3-AV	0	0	4	8.2	1	3.0
1-BZ + 2-LM + 3-AV	1	7.15	1	2.0	0	0

Table 2.2 – Numbers of anthelmintics used in each livestock sector according to ‘class’ and combinations of classes

Table 2.3 lists the methods of administering anthelmintics for all individual products used in each unit in each of the three sectors (see Section 1.3.2 for a description of dosing methods). Questionnaire responses were excluded if the farmer did not state

the dosing method of a product. Drenching (70%) and injection (30%) were used to administer anthelmintics to sheep. Pour-on was the most common method of treating dairy (48.1%) and beef cattle (47.6%). Use of a bolus was the next most popular method for dairy cattle (34.6%) followed by injection (17.3%). In beef cattle, injections (28.6%) were slightly more common than the bolus (23.8%).

Dosing method	Livestock sector					
	Sheep (n=20)	%	Dairy (n=81)	%	Beef (n=42)	%
Injection	6	30	14	17.3	12	28.6
Pour-on	0	0	39	48.1	20	47.6
Bolus	0	0	28	34.6	10	23.8
Drench	14	70	0	0	0	0

Table 2.3 – Dosing methods used to administer anthelmintic products in each livestock sector on questionnaire-surveyed farms

#### 2.3.4. Avermectin Use

The percentage use of avermectin products in each livestock sector was calculated according to active ingredient and method of dosing (Table 2.4). If more than one avermectin product was used on the same herd e.g. a doramectin pour-on and an ivermectin injection, then both of those products were included separately. The active ingredient was used for this analysis rather than the trade name because active ingredients can be marketed under different names.

Active ingredient and dosing method	Livestock sector					
	Sheep (n=14)	%	Dairy (n=52)	%	Beef (n=34)	%
Doramectin injection	5	35.7	2	3.8	6	17.6
Doramectin pour-on	0	0	20	38.5	9	26.5
Eprinomectin pour-on	0	0	6	11.5	2	5.9
Ivermectin drench	1	7.1	0	0	0	0
Ivermectin injection	3	21.4	8	15.4	4	11.8
Ivermectin pour-on	0	0	12	23.1	9	26.5
Ivermectin bolus	0	0	3	5.8	3	8.8
Moxidectin drench	5	35.7	0	0	0	0
Moxidectin injection	0	0	0	0	1	2.9
Moxidectin pour-on	0	0	1	1.9	0	0

Table 2.4 – Use of each avermectin and dosing method in each livestock sector on questionnaire-surveyed farms

For sheep, a doramectin injection and a moxidectin drench were equally the most common avermectin treatments. Dectomax™ was the only brand name given in the

survey regarding doramectin injections in sheep. The moxidectin drench was used under two brand names, which were Cydectin® and Zermex®. The manufacturer confirmed that these are effectively the same product marketed under different names (Fort Dodge Technical Department, pers. comm.). Doramectin pour-on was the most common treatment for dairy cattle (38.5%), attributed wholly to use of Dectomax™ pour-on. Ivermectin pour-on was the next common (23.1%) product, which was used as either Ivomec® or Noromectin® pour-on. In beef cattle, doramectin and ivermectin pour-on were equally popular (26.5%), again with use of the products Dectomax™, Ivomec® and Noromectin®. Moxidectin was the least used macrocyclic lactone in all livestock sectors.

Treatment strategies were grouped according to class and timing of treatment of which the latter was split into ‘at grass’ and ‘at housing’ (Table 2.5). The purpose of this was to gain an indication of whether avermectins were being used predominantly at grass or upon housing. This is particularly relevant because the use of avermectins only at housing would significantly reduce exposure of dung insects to avermectin residues. It should be noted that the same animals were treated at grass and at housing, i.e. the multiple dosing does not refer to two separate herds within the same livestock unit on a farm undergoing different treatment strategies.

Treatment Strategy	Livestock sector					
	Sheep (n=11)	%	Dairy (n=49)	%	Beef (n=29)	%
3-AV at grass	8	72.7	9	18.4	6	20.7
3-AV at housing	NA	NA	3	6.1	6	20.7
3-AV at grass and housing	NA	NA	14	28.5	8	27.6
1-BZ at grass	3	27.3	4	8.2	4	13.8
1-BZ grass/ 3-AV housing	NA	NA	15	30.6	4	13.8
1-BZ or 2-LM at grass/ 3-AV at housing	NA	NA	4	8.2	1	3.4

Table 2.5 – Treatment strategies in each sector according to anthelmintic class and timing of treatment. NA –not applicable

Of the surveyed sheep sectors, 72.7% used avermectins at grass and 27.3% used a benzimidazole at grass. For sheep, the ‘at housing’ strategies are not really applicable since sheep were only housed during lambing. Therefore, sheep were usually at grass when anthelmintically treated. On dairy farms, the most common

strategy was 'benzimidazole at grass and avermectin at housing'. The benzimidazole use in this strategy was attributed entirely to the use of the Autoworm bolus on young cattle in the grazing period, followed up by an avermectin treatment of either a doramectin or ivermectin product at housing. The next popular strategy in the dairy sector was 'ivermectin at grass and housing'. On beef farms, the most popular strategy was use of an avermectin at both grass and housing (27.6%) and the strategies of using avermectins only at housing and only at grass were equally popular (20.7%).

## 2.4 Discussion

### 2.4.1 Questionnaire design

Bias can be a source of error in all types of questionnaire survey although it can be minimised simply by recognising the limitations of a survey (Oppenheim, 1992). This survey may have been biased to give a larger representative proportion of dairy farmers for two main reasons. First of all, the predominant livestock type in a farming area can change with geographical region and the majority of responses were from Ayrshire, a region where dairy farming is prevalent. Secondly, the majority of the respondents were members of Grassland Societies, which exist to promote optimal grassland and forage crop production. Therefore, many Society members could be expected to be dairy farmers since the quality and production of grassland is paramount in good milk production. Having said that, the majority of contacted members of the Central Scotland Grassland Society were beef and sheep farmers although they made up a smaller proportion of the responses.

Non-response bias is common in postal questionnaire surveys. However it was reduced in this survey because the majority of respondents were contacted directly via telephone. Questions were kept simple to minimise any ambiguity and also to make the subsequent analyses of responses straightforward. Care was taken not to prompt farmers if they could not recall a product name, so that the survey remained impartial. This survey aimed to question farmers that were representative of commercial farmers, therefore when one realised that bias may have been introduced by obtaining responses from farmers via FWAG, that method of survey was not

pursued. Furthermore, one might have expected a degree of scepticism from some farmers if they believed that they were participating in a study to examine the environmental impact of worming products. However, few farmers queried exactly why the information on livestock worming strategies was sought, possibly because they were contacted via the Grassland Societies and the Scottish Agricultural College.

The response rate from this survey was 87 per cent which is high in comparison to similar surveys on livestock anthelmintic strategies (e.g. Wagner and Polley, 1997; Borgsteede *et al.* 1998; Stafford and Coles, 1999). The relatively low response rates in those surveys were probably due to their use of postal questionnaire methods. A direct telephone survey was used in this study to overcome problems associated with ambiguous questions and apathy in returning questionnaires and, consequently, response rate was high. Furthermore, individual farmers who were likely to respond to such a survey via a telephone call were specifically targeted, with help from the Grassland Society Secretary, therefore the survey succeeded in maximising responses.

A farmer's selection of an anthelmintic product can be guided by many factors including cost, veterinary advice, marketing, friend's advice etc, therefore it would have been interesting to ask farmers additional questions about what guided their choice of product. However, experience from the initial part of the survey suggested that asking such questions reduced the farmers' willingness to participate. Nevertheless, several farmers did stress that the cost-effectiveness of a product was most important for them and some had selected products on the basis that they were on special offer at the agricultural merchants. Thus, although the evidence is anecdotal, price could be considered to be an important factor guiding the choice of a particular anthelmintic product. However, it is suggested that price might only drive the choice of a product to a certain extent. For example, if a farmer wished to use a product from the avermectin group then it might be unlikely that he would then opt to use a levamisole product simply because it was cheaper. Instead, the cheapest product of the avermectins might be selected.

#### 2.4.2 Livestock type

Overall, dairy was the most common farm type and this could be partly attributed to the higher proportion of respondents from areas where dairy farming is common e.g. Ayrshire and Dumfries & Galloway. Beef and sheep farms were located mainly in upland areas e.g. Tayside and parts of the Clyde Valley. This can be explained by the grassland quality in the respective areas, as dairy cattle are often farmed in lowland areas where grass growth is high, and beef and sheep are more suited to extensive rough grazing on upland areas. The survey information on anthelmintic treatment strategies is discussed in turn for sheep, beef and dairy sectors.

##### *Sheep*

Avermectins (3-AV) were the most common anthelmintics used for sheep. Anthelmintics in the 2-LM group were the least used even though one of the compounds in that group (levamisole) is an effective anthelmintic, particularly for controlling benzimidazole-resistant worms (Andrews, 2000). The relative unpopularity of Group 2-LM products may have been because they control a narrow range of parasites in comparison to the other two groups. Fenbendazole (1-BZ) has broader efficacy against gastrointestinal nematodes than levamisole (2-LM), and some 1-BZ products have the added advantage of also controlling fluke (Williams and Broussard, 1995; Taylor, 2001). Anthelmintic products in the 3-AV group can be advantageous over 2-LM products because the former are also effective against ectoparasites (Taylor, 2001). Resistance to a particular group is unlikely to have affected the relative use of the anthelmintic groups as Bartley *et al.*, (2003) found that the same patterns of usage of the three groups occurred in Scottish sheep flocks regardless of whether they were 1-BZ resistant or not.

The finding that drenching was most common in sheep sectors concurs with Taylor *et al.*, (1992) who state that oral drenches are used “almost exclusively” in sheep. Pour-on is unsuitable for sheep as the lanolin in the wool interferes with absorption (Herd, 1988) and boluses are not commonly used for sheep.

Of the avermectin products used on surveyed sheep farms, a doramectin injection and a moxidectin drench were equally common. Dectomax™ was always used as the doramectin injection. That product is effective against roundworm, lungworm and ectoparasites including scab, and therefore it would appeal to farmers who wish to use a broad-spectrum anthelmintic with protection against sheep scab. On surveyed farms, the moxidectin drench was used as Zernex® or Cydectin®. Those products have an advantage over Dectomax™ injection in that they have a shorter withdrawal period for meat. In animals treated with a Dectomax™ injection, eight weeks must elapse before slaughter for human consumption whereas only two weeks must elapse after using the moxidectin drench (Taylor, 2001). In the analysis of anthelmintic use at grass and at housing, anthelmintics were not recorded as being used at housing presumably because sheep were at grass except for during lambing. The use of avermectins at grass was a more adopted strategy than use of benzimidazoles at grass, possibly because farmers opted to use products that were effective against sheep scab.

### *Beef*

Sole use of products from the avermectin group was the most prevalent strategy for beef cattle on the surveyed farms. Avermectins are desirable products for use by beef farmers because of their efficacy against ectoparasites, such as lice, mites and warble fly (*Hypoderma* spp.) infestations, of which the latter can compromise the quality and appearance of meat from infected cattle (Sainsbury, 1998). Other treatment strategies that were popular were the combined use of benzimidazole and avermectin, and use of benzimidazoles on their own. Group 2-LM products were not recorded as being used on any of the surveyed farms. Their lack of popularity may be because they are not as effective as the other anthelmintic groups in the control of the inhibited larvae of an important cattle parasite, the brown stomach worm *Ostertagia ostertagi* (Williams, 1991; Williams *et al.*, 1991).

The use of a pour-on may have been a popular dosing method because they are quick to administer and can be less labour-intensive than other methods. Administering anthelmintics to livestock can stress the animals, particularly if the dosing methods are intrusive, such as inserting a bolus. Even when held in a headlock, stressed

animals can become difficult to handle and this might increase the risk of injury to the farmer while also making the whole procedure more labour-intensive. Nevertheless, the use of a bolus could ultimately reduce labour costs (Strong *et al.*, 1996) because it needs to be administered only once as opposed to the three-dose strategy of most anthelmintic pour-ons and injections. A preference for a particular dosing method may govern the selection of a product. For example, most of the products in the least popular 2-LM category are administered to cattle via an oral drench (Taylor, 2004). Therefore, it is possible that farmers chose to use another product because it could be administered via their preferred method e.g. pour-on. Another factor that may determine the choice of an administration method is product cost.

Ivermectin pour-on (Ivomec® and Noromectin®) and doramectin pour-on (Dectomax™) were equally the most common avermectin products used on beef cattle. The most common strategy regarding anthelmintic group and timing of treatment was the use of an avermectin product at both grass and housing. The popularity of avermectins at both these times is due to their efficacy against endoparasites e.g. stomach worms and lungworm that cattle are exposed to at grazing, and ectoparasites such as lice and mites which pose a problem when cattle are housed together over winter.

### *Dairy*

In the dairy sector, the sole use of avermectin products was the most popular use of anthelmintic groups followed by combined use of a benzimidazole and an avermectin. The use of a Group 2-LM product was reported for one dairy herd, and the use of a Group 2-LM in conjunction with a Group 1-BZ and 3-AV product was reported for one other. As mentioned previously, 2-LM products may not be a popular choice by livestock farmers either because they control a relatively narrow range of endoparasites or because they are not available in the preferred formulation for dosing animals.

Pour-on was the most widely used administration method in dairy cattle, followed by a bolus and then injection. As mentioned previously, the pour-on was probably a

common choice for many cattle because it is easier, and safer for the farmer, to administer. Several of the dairy farmers said that the bolus was a popular choice for young stock that were sent to fields further away from the farm for grazing as it reduced costs and labour of repeated treatments throughout the grazing season. The most common strategy for young stock was the use of an Autoworm (1-BZ) bolus at grazing followed up by an avermectin product at housing. The popularity of the Autoworm bolus could not be attributed to a lower price since it had a similar market price to the Ivomec® bolus (Merial and Farmrite, pers. comm.). Although the Ivomec® bolus is now (2004) no longer on the market in Britain (Merial, pers. comm.), it was still available when the survey was carried out. Therefore the relatively high use of the Autoworm bolus was not due to a lack of alternative bolus products on the market. Its popularity may have been due to cost-effectiveness at the level of local suppliers. For example, if agricultural merchants had a special offer on Autoworm boluses at the time of the questionnaire survey, then many farmers may have opted to use that instead of e.g. the Ivomec® bolus. The most popular avermectin product used at housing was a doramectin pour-on (Dectomax™). The use of an avermectin at housing is valuable because they have good efficacy against adult and inhibited larvae of *O. ostertagi* (Forbes, 1993), and ectoparasites including lice and mites.

#### 2.4.3 Site selection

It was decided to concentrate the majority of invertebrate sampling on dairy farms because sampling was centred in Ayrshire, for logistical reasons, and the survey had ascertained that dairy farming was the prevalent farm type in that region. One beef farm was included in the study in order to increase the sample number of fields grazed by treated cattle. An advantage of sampling on dairy farms was related to the treatment strategies of the herds. The questionnaire survey highlighted that milking cows remained untreated while calves and young heifers were anthelmintically treated. This enabled invertebrate sampling to be carried out in fields containing treated young animals and untreated milking cows on the same farm, thereby minimising variation in dung insect fauna caused by inherent 'historical' differences in their distribution. Additionally, dung beetle assemblages can be expected to differ

naturally beyond a spatial scale of 100 km (Finn *et al.*, 1998). Therefore, an additional advantage of concentrating field sites within a limited geographical area (e.g. the furthest field sites were 17-18 km apart), was that variation caused by geographical location was minimised. Doramectin and ivermectin pour-on were the most popular choice of avermectin products on the questionnaire-surveyed dairy farms, therefore farms were selected for invertebrate survey according to their use of those products. The avermectin treatment strategies of livestock in fields that were surveyed for dung insects in the wider sampling study are provided (Table 5.1).

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## CHAPTER 3

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# FACTORS INFLUENCING THE ATTRACTIVENESS AND SAMPLING EFFICIENCY OF DUNG-BAITED PITFALL TRAPS FOR DUNG INSECTS

### 3. Factors influencing the attractiveness and sampling efficiency of dung-baited pitfall traps for dung insects

#### 3.1 Introduction

##### *Attractiveness of dung*

Insect colonisation of dung is dependent upon the 'findability' and the suitability of the dung resource (Gittings and Giller, 1998). Dung is detected by insects via olfactory cues (Landin, 1961), therefore finding dung is dependent on the strength and attraction of the emitted cues, and also on their reception. Once located, insects assess the dung resource and then colonise it if it is adequate. The dung insect species of Britain are typically *r*-strategists (e.g. Hanski, 1991b). They are mobile organisms with high fecundity; beneficial adaptations for the exploitation of an ephemeral, patchy resource such as dung.

There are many properties of dung that may influence its suitability for insects, but these are still poorly understood. Dung properties that are often measured in dung insect studies include moisture content, organic matter and fibre content, with moisture content deemed one of the most important (Barth, 1993; Gittings and Giller, 1998). When dung beetles are breeding, moisture content is critical because eggs could drown if dung is too wet. Early-colonising *Aphodius* species have adapted by laying eggs in the soil below the dung so that by the time the larvae hatch and colonise the dung, the dung has dried out sufficiently (Gittings and Giller, 1998). For species that are late colonisers, dung that is too wet is less likely to be a problem and they are more likely to be adversely affected by dung that dries out too much before larval development is complete. Indeed, the reproductive success of the late-colonising species *Aphodius fossor* was higher in relatively moist dung because the negative effects associated with dung desiccation were reduced (Vessby, 2001).

Avermectin treatment of livestock could potentially affect both the 'findability' and the suitability of dung. Previous studies on the attractiveness of dung from avermectin-treated cattle to dung insects have yielded mixed results (see section 1.4.2 for a review). Research in Australia and Canada has shown dung beetles to

prefer dung from avermectin-treated cattle (Wardhaugh and Mahon, 1991; Floate, 1998b). Conversely, some studies have found untreated dung to be more attractive than dung containing ivermectin residues e.g. dung from cattle treated with ivermectin attracted less *Aphodius* than dung from untreated cattle for up to 30 days after treatment (Holter *et al.*, 1993b). However, when ivermectin was added directly to dung, that dung attracted similar insect numbers to dung from untreated cattle (Holter *et al.*, 1993b). This suggests that any difference in attraction could be due to a change in dung quality caused by avermectin treatment (Wardhaugh and Mahon, 1991) rather than simply an avoidance of, or attraction to, the avermectin compound. It has been suggested that the control of internal parasites via avermectin treatment reduces diarrhoea in cattle and consequently dung is of lower moisture content (Barth, 1993; Wratten and Forbes, 1995). Such a change in moisture content could indirectly alter the attractiveness of dung from avermectin-treated animals to dung insect species.

In this study, a series of trials were carried out to investigate insect attraction to dung from untreated and avermectin-treated cattle. Two of the trials presented in this chapter investigated insect attraction to dung from treated and untreated cattle at two different spatial scales. In a further trial, the moisture content of dung was manipulated to assess the combined effect of moisture content and avermectin treatment on insect attraction.

### *Sampling efficiency*

As well as the quality of dung and its ‘findability’, having a sufficient quantity of the resource is also crucial for dung insect populations. Limitations to dung availability could impair reproductive success and magnify competitive interactions between dung insects. For example, a subsequent increase in larval density within individual dung pats would increase competition for space that is already a limiting factor (Finu and Gittings, 2003).

Grazing regime, for example permanent or rotational grazing, and livestock density can affect the supply of dung available within a pasture. Pastures that are permanently grazed by livestock provide a constant source of fresh dung whereas, in

rotationally grazed pastures, the supply of fresh dung is intermittent. Numbers of *Aphodius* beetles have been observed to increase in pitfall traps in a field when the cattle in the adjacent pasture had been removed (Finn *et al.*, 1998) indicating that mass emigration of beetles from a pasture could occur when cattle are removed for rotation. Furthermore, the density of livestock, and dung, in a pasture has implications for studies using dung-baited pitfall traps because the baited pitfalls are effectively competing with the natural dung pats in a pasture (Lobo *et al.*, 1998). For example, if the availability of fresh dung is low (e.g. through low livestock density or rotational grazing) then one might expect baited pitfall traps to be relatively more attractive to dung insects.

Given that the wider sampling study would compare dung insect assemblages across pastures with different levels of dung availability through the season, it was considered important to distinguish the level of influence that this could have on the number of dung insects attracted to baited pitfall traps. To this end, an experiment was carried out to test the 'dung density' hypothesis that dung insect abundance is lower in traps surrounded by a higher density of dung pats than in traps surrounded by no dung pats.

In addition, the successional pattern of dung colonisation by insects means that the dung insect assemblage is very much related to the age of dung (McCracken, 1990; Gittings and Giller, 1998; reviewed in section 1.2.1). Therefore, when dung-baited pitfall traps are used to sample dung insect communities, one must be aware that the species composition of insects in trap catches might differ with the duration of trap exposure. An experiment was therefore conducted to investigate whether the species composition of dung insects in traps changed significantly when traps were exposed for different periods of times, ranging from approximately one to three weeks.

Due to the time-consuming processing of invertebrate data, it can be beneficial to know the minimum number of traps required to sample the study taxa efficiently. Species-sample accumulation curves can be used to determine the optimal number of samples required to measure species richness in a particular habitat or geographical area (Gotelli and Colwell, 2001). Species-sample curves were constructed using

insect data collected from grazed pastures in Ayrshire in order to ascertain the optimum number of traps needed to sample dung insects in the wider field study.

### *Aim*

The aim of the work reported here was to determine ways in which to improve sampling efficiency and to gain knowledge of factors influencing the trapping behaviour of dung insects. An objective was to examine how the density and quality of dung might affect the abundance and species composition of dung insects in pastures. This information was considered necessary to aid the interpretation of data collected for subsequent chapters using dung-baited pitfall traps.

## **3.2 Method**

### *3.2.1 Data Collection*

Dung insects were sampled using dung-baited pitfall traps. The traps were 1 litre plastic containers of 11.5cm diameter sunk flush with the ground and containing approximately 3cm depth of 70% monopropylene glycol (MPG). Wire mesh was secured over the trap with a metal staple to minimise disturbance from animals and to serve as a support for the dung bait placed at the centre of the mesh. Dung baits were formed using a hemispherical mould of 6cm diameter and 3cm depth. The source of dung in each trial depended on the aim of the trial and this is detailed below for each individual trial. For all trials, dung was collected on one occasion from housed cattle rather than from cattle at pasture. Fresh dung deposited on pastures can be rapidly colonised by invertebrates therefore to use such dung as baits for pitfall traps may have confounded results. Once dung was collected, it was mixed thoroughly to homogenise it and then stored in a sealed container at 4°C.

The contents of each trap were sorted and *Aphodius* (Scarabaeidae), *Cercyon* and *Sphaeridium* (Hydrophilidae) adult beetles and yellow dung flies (Scatophagidae) were all identified to species level and counted. Identification was performed using standard keys (Jessop, 1986; Hansen, 1987; Skidmore, 1991) and with reference to collections at the Hunterian Museum and the National Museum of Scotland.

*Attractiveness of dung**Trial 1*

The aim of this trial was to determine whether dung insects exhibited a preference to, or an avoidance of, dung that contained avermectin residues. In 2003, dung was collected from housed cattle five days prior to avermectin treatment and then from the same cattle two days after treatment with a doramectin pour-on. The two-day time lapse was used because doramectin is excreted in faeces from treated cattle at that time (Goudie *et al.*, 1993) and the farmer wished to turn the cattle out to pasture after that time. Moisture levels were not measured in this trial although it was noted that the dung from treated and untreated cattle were visibly similar in terms of both consistency and wetness.

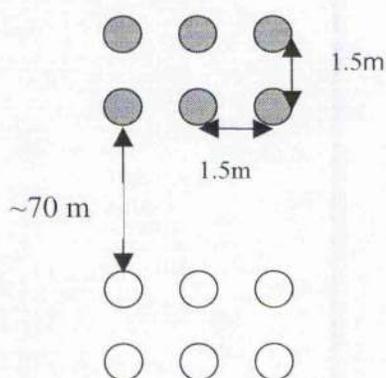


Figure 3.1 – Diagram showing the spatial location of pitfall traps baited with untreated dung (white) and treated dung (grey) for Trial 1

To prevent any disturbance of the traps, the trial was conducted in a field containing no livestock. Livestock grazed in surrounding fields and thus one could expect dung insects to immigrate into the study field to colonise traps. In the first part of the trial, traps were set in two grids of 3x2 (Figure 3.1). Within each grid, traps were set 1.5m apart and the two grids were established approximately 70m 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. Traps were set for five days and the contents were collected on 14 May 2003. Each grid of traps was then re-baited with the alternate dung type and the contents were collected on 19 May after a further five days exposure. In the second trapping period, the dung from treated and untreated

animals was swapped between grids to exclude any bias in the location of the grids. Trap collections from the two trap periods were pooled 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 (Figure 3.2). The aim of this trial was to determine whether dung insects made a choice between the two sources of dung at a scale of 1.5m. Traps were set for five days and collected on 9 June 2003 and then re-baited and collected on 14 June 2003. Trap collections from the two trap periods were pooled thus data from twelve untreated traps and twelve treated traps were analysed.

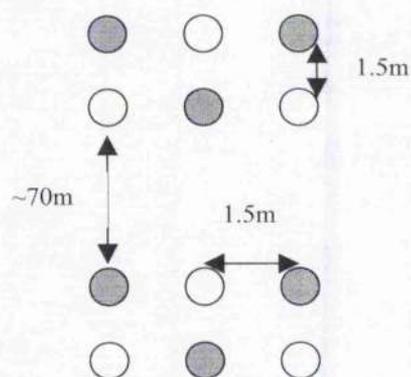


Figure 3.2 - Diagram showing the spatial location of pitfall traps baited with untreated dung (white) and treated dung (grey) for Trial 2

### *Trial 3*

The aim of this trial was to determine the combined influence of moisture content and avermectin residues on the attractiveness of dung to dung insects. In 2004, dung was collected from housed animals approximately five days before and two days after treatment with a doramectin pour-on. 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 which could still be supported on the mesh of the baited pitfall trap. Three samples (approximately 20g 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 on an area of ground not containing livestock but within 100m of grazed pasture. Traps were set in an 8x4 grid, with traps spaced approximately 5m apart, and each of the four baits were used in a repeated alternating sequence (Figure 3.3). Traps were collected on 16 May 2004 after six days exposure.

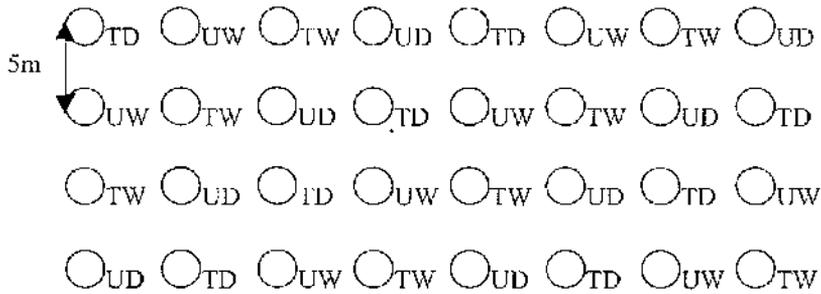


Figure 3.3 – Diagram of layout of traps baited with ‘treated dry’ (TD), ‘treated wet’ (TW), ‘untreated dry’ (UD) and ‘untreated wet’ (UW) dung for Trial 3

#### *Sampling efficiency*

##### *Trial 4*

An experiment was carried out to determine whether the presence of artificially formed dung pats within 1m of baited pitfall traps affected the abundance and composition of the catch of dung insects. In a field containing no livestock, two 4x2 grids of eight traps were set and all were baited with untreated dung. The two trap grids were approximately 70m apart and individual traps within a grid were spaced at 1.5m. At one grid, six ‘cow pats’ of approximately 20cm diameter were formed from the collected untreated dung and placed within 1m of the traps and at the other grid no ‘cow pats’ were placed around the baited pitfall traps (Figure 3.4). The aim was to simulate a field that was permanently stocked with cattle producing fresh dung and a field that was rotationally grazed with periods of no fresh dung, respectively. Traps were set for seven days exposure and collected on 17 May 2004.

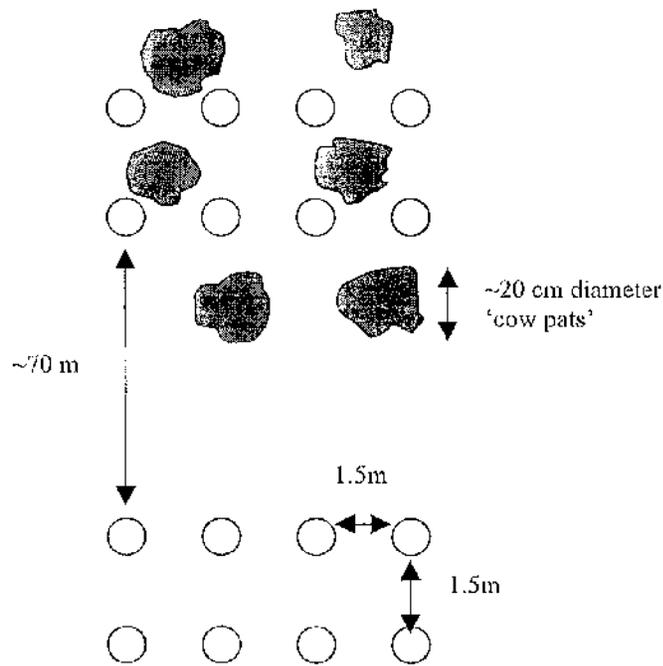


Figure 3.4 – Diagram of trap layout in Trial 4 with pitfall traps baited with untreated dung and surrounded either by simulated cow pats or no cow pats

#### Trial 5

A trial was conducted in June 2002 to investigate whether the number of days of trap exposure affected the assemblage structure of dung insects trapped. As dung insects have a successional pattern of dung colonisation, the species composition might be expected to alter with duration of trapping period. Eighteen pitfall traps were baited with dung, collected from untreated housed cattle, and set in a 6x3 grid in a field grazed by untreated dairy cows. Three random traps were lifted and the insect contents processed at 5, 8, 11, 14, 17 and 20 days after traps were set (Figure 3.5). The time range of 5-20 days was selected because it was anticipated that the majority of trapping periods in the wider sampling study would fall into this range.

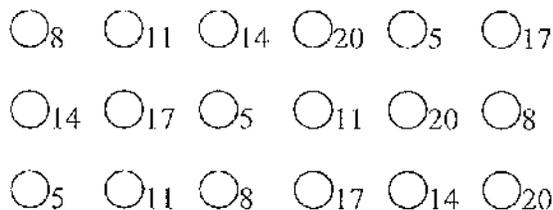


Figure 3.5 – Diagram of trap layout for Trial 5 showing the number of days for which traps were exposed, with three traps collected after each period of exposure of 5, 8, 11, 14, 17 and 20 days

### Trial 6

An experiment was carried out to ascertain the optimum number of dung-baited pitfall traps required to adequately sample the dung insect assemblage. In May and June 2002, eight traps were set out in five fields grazed by untreated cattle and collected after 10-14 days exposure. Traps were set out in two grids of four that were located in a central position at opposite ends of the field and, within each grid, traps were spaced 8m apart. Trap contents were identified to species level and the cumulative number of dung insect species collected was plotted against the number of traps. The minimum number of traps required to adequately sample a dung insect assemblage in a pasture was determined from those species-sample accumulation curves. The study fields used in the trial ranged in size from 2-6.8 ha to assess whether the optimum trap number was the same in pastures of different sizes.

#### 3.2.2 Data Analysis

Data analyses were carried out on adult *Aphodius*, *Cercyon* and *Sphaeridium* beetles and adult *Scatophaga stercoraria* flies. However *Sphaeridium* were excluded from abundance analyses because their numbers were consistently low. The *Aphodius* were further split into one of two guilds for data analysis according to larval feeding strategy (Table 1.1). ‘Guild 1’ comprised *Aphodius* individuals with coprophagous larvae and this included all species collected with the exception of *A. prodromus* and *A. sphaecelatus*. Species with saprophagous larvae, *A. prodromus* and *A. sphaecelatus*, were grouped in ‘Guild 2’ (Gittings and Giller, 1997). The guild distinction was made because although adults of both guilds feed in dung, only the adults belonging to guild 1 (with coprophagous larvae) could also be expected to select dung in terms of its potential suitability for larval development. A division into guilds was not extended to *Cercyon* beetles because all the *Cercyon* species recorded in this study can be found in both dung and decaying organic matter (Hansen, 1987).

The abundance, diversity and species composition of dung insects were examined for trials 1-4 and, in trial 5, the effect of number of days of trap exposure on species composition in traps was investigated using ordination. Abundance of *Aphodius* Guilds, *Cercyon* and *Scatophaga stercoraria* were compared between traps baited with treated and untreated dung using non-parametric statistics. In Trials 1 and 2,

data was collated across two trap periods 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 (Fowler and Cohen, 1990) and then analysed using a two-way Analysis of Variance to assess the significance of treatment, moisture level and the interaction between the two.

Dung insect diversity was calculated with the Shannon Index ( $H' = -\sum P_i \ln P_i$ ). The 'discriminant ability' of a diversity index describes its ability to detect differences between samples (Magurran, 1988). The Shannon index has relatively good discriminant ability in comparison to other diversity indices (Taylor, 1978) therefore it was considered suitable for this study which focussed on dung insect communities in a reasonably narrow geographical area i.e. south and east Ayrshire.

The species compositions of the dung insect assemblages were examined using an ordination method. Ordination methods can be used to summarise complex species/sample datasets so that similar samples, or species, are placed close together in 'ordination space' and dissimilar ones far apart (Gauch, 1982). Detrended Correspondence Analysis (DCA) is an ordination technique based upon reciprocal averaging but it has the advantage of correcting for the undesirable 'arch effect' associated with reciprocal averaging (Hill, 1979). The output from DCA is a number of axes of sample scores, and scores from the first two main axes of variation are frequently plotted to give a visual representation of the relative ordination positions of samples. The interpretation of the output is somewhat arbitrary, however axes scores can be correlated with environmental variables to investigate relationships. Each axis has an eigenvalue indicating the amount of variance that it accounts for, and the first axis accounts for the largest amount of variation (Gauch, 1982). The DECORANA option in the Community Analysis Package (Pisces Conservation Ltd, 1999) was used to ordinate species assemblage data. The analysis in that program is based on Hill (1979) with the correction as per Oksanen and Minchin (1997). Full turnover in the species composition of samples occurs in approximately four standard deviations (Gauch, 1982). It should be noted that one standard deviation is equal to 100 units on these axis scales of the ordination plots presented here (see Section 1.2.5). Transformation to proportional data was carried out prior to ordination to

examine variation in species composition while accounting for potential differences in overall abundance.

### 3.3 Results

#### *Attractiveness of dung*

##### *Trial 1*

The abundance of each dung insect species was compared for traps spaced 70m apart and baited with 'treated' and 'untreated' dung (Table 3.1). Abundance of *Aphodius* Guild 1 and 2, *Cercyon* and *S. stercoraria* were all significantly higher in traps baited with dung from untreated cattle (Figure 3.6). Mean dung insect diversity ( $\pm 1$  se) was  $1.06 \pm 0.04$  for traps baited with 'untreated' dung and  $1.42 \pm 0.08$  for traps baited with 'treated' dung (Mann-Whitney,  $n=24$ ,  $P=0.44$ ).

The ordination of dung insect assemblages gave eigenvalues of 0.449 for axis 1 and 0.133 for axis 2 (see Appendix II. for a list of scores). Traps baited with untreated dung were grouped close together low down on axis 1 and variation occurred mainly among traps baited with dung from avermectin-treated cattle (Figure 3.7). The spread of treated traps was probably due to the low occurrence of dung insect species in those traps which, in turn, made their ordination position more sensitive to the occurrence of a species even in low numbers. For example, the two treated traps situated to the far right of the graph had higher numbers of *C. lateralis* and *C. melanocephalus* relative to the other 'treated' traps (Figure 3.8).

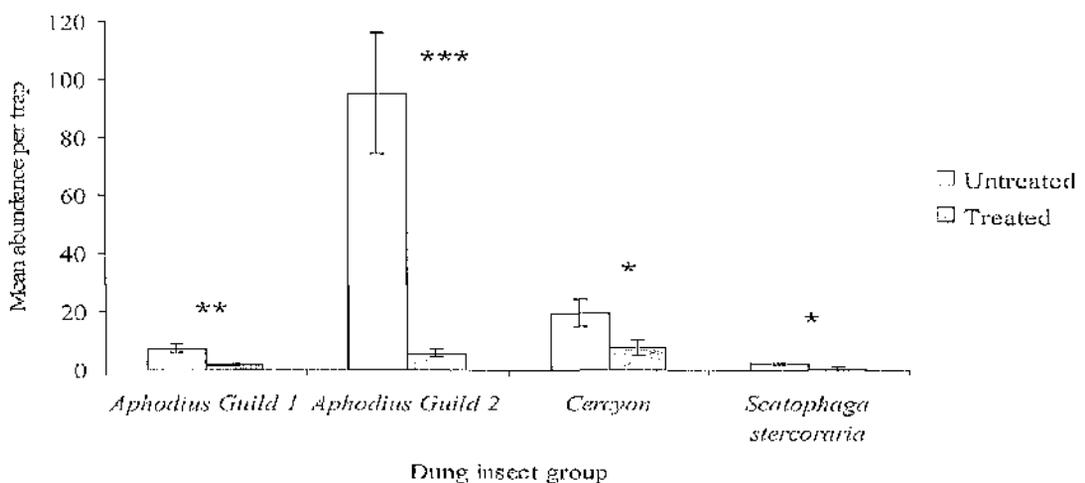


Figure 3.6 – Mean abundance ( $\pm 1$ se) of dung insects in 'treated' and 'untreated' baited traps separated by a distance of ~70m in Trial 1 (Mann-Whitney test \*  $P<0.05$ , \*\*  $P<0.001$ , \*\*\* $P<0.0001$ )

Species	Treated dung baits (n=12)	Untreated dung baits (n=12)
<i>Aphodius ater</i>	0	1
<i>A. depressus</i>	16	80
<i>A. pusillus</i>	1	8
<i>A. rufipes</i>	0	1
<b><i>Aphodius</i> Guild 1</b>	<b>17</b>	<b>90</b>
<i>A. prodromus</i>	68	1115
<i>A. sphacelatus</i>	2	26
<b><i>Aphodius</i> Guild 2</b>	<b>70</b>	<b>1141</b>
<i>Cercyon atomarius</i>	6	72
<i>C. haemorrhoidalis</i>	11	41
<i>C. lateralis</i>	23	27
<i>C. lugubris</i>	0	1
<i>C. melanocephalus</i>	51	86
<i>C. pygmaeus</i>	2	9
<b><i>Cercyon</i></b>	<b>93</b>	<b>236</b>
<i>Sphaeridium lunatum</i>	0	2
<i>S. scarabaeoides</i>	0	1
<b><i>Sphaeridium</i></b>	<b>0</b>	<b>3</b>
<b><i>Scatophaga stercoraria</i></b>	<b>10</b>	<b>25</b>

Table 3.1 – Numbers of individuals trapped in pitfall traps baited with dung from treated and untreated animals at ~70m spacing in Trial 1. The nomenclature for listed species is shown in Appendix I, n=number of traps

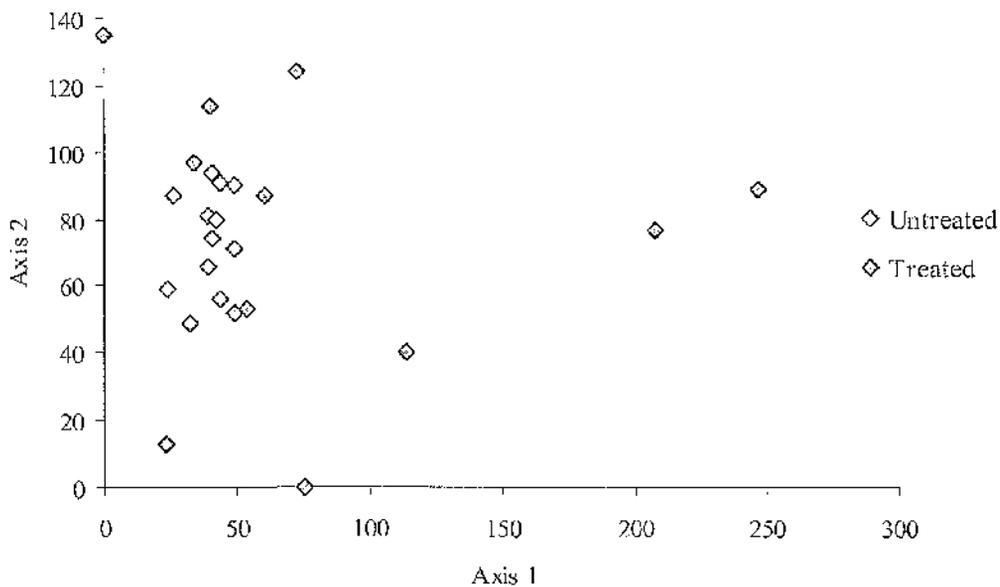


Figure 3.7 – Ordination of dung insect assemblages sampled in May 2003 with traps baited with either dung from avermectin-treated cattle or untreated cattle, and spaced at a distance of ~70m (Trial 1)

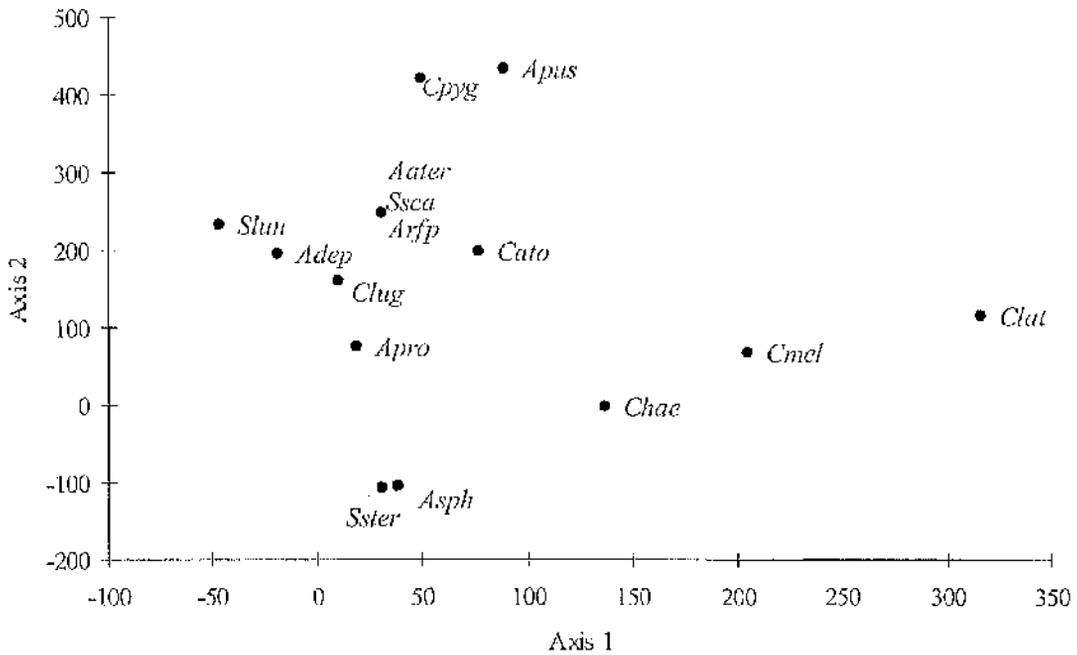


Figure 3.8 – Species scores from ordination of dung insect assemblages sampled in Trial 1 with traps baited with either dung from avermectin-treated cattle or untreated cattle. A list of species abbreviations is provided in Appendix I.

### Trial 2

The abundance of dung insects collected in traps spaced 1.5m apart and baited with dung from either treated or untreated cattle was summarised (Table 3.2). Significantly more individuals of *Aphodius* Guild 2, *Cercyon* and *S. stercoraria* were trapped in pitfalls baited with dung from untreated cattle than in those baited with dung from avermectin-treated cattle, and there was a non-significant trend for more Guild 1 *Aphodius* in ‘untreated’ traps (Figure 3.9). Mean dung insect diversity ( $\pm 1$  se) was  $1.37 \pm 0.08$  for traps baited with ‘untreated’ dung and  $1.36 \pm 0.06$  in traps baited with ‘treated’ dung (Mann-Whitney,  $n=24$ ,  $P=0.14$ ).

The ordination of dung insect assemblages showed that there was no distinct separation of dung insects in traps baited with either dung from treated or untreated cattle at 1.5m spacing (Figure 3.10). Eigenvalues for axis 1 and 2 were 0.298 and 0.052, respectively (see Appendix III. for a list of sample and species scores). With the exception of two traps baited with treated dung, the traps baited with untreated dung occupied a lower position on axis 2 and this was due to the relatively higher abundance of *Cercyon* in those untreated traps (Figure 3.11).

Species	Treated dung baits (n=12)	Untreated dung baits (n=12)
<i>Aphodius depressus</i>	16	27
<i>A. lapponum</i>	1	0
<i>A. rufipes</i>	0	7
<b><i>Aphodius</i> Guild 1</b>	<b>17</b>	<b>34</b>
<i>A. prodromus</i>	11	55
<i>A. sphaelatus</i>	0	3
<b><i>Aphodius</i> Guild 2</b>	<b>11</b>	<b>58</b>
<i>Cercyon atomarius</i>	54	154
<i>C. haemorrhoidalis</i>	25	82
<i>C. lateralis</i>	8	18
<i>C. lugubris</i>	1	15
<i>C. melanocephalus</i>	176	634
<i>C. pygmaeus</i>	4	14
<b><i>Cercyon</i></b>	<b>268</b>	<b>917</b>
<i>Sphaeridium lunatum</i>	0	1
<i>S. scarabaeoides</i>	1	9
<b><i>Sphaeridium</i></b>	<b>1</b>	<b>10</b>
<i>Scatophaga stercoraria</i>	24	78
<i>S. furcata</i>	0	2
<b><i>Scatophaga</i></b>	<b>24</b>	<b>80</b>

Table 3.2 – Numbers of individuals trapped in pitfall traps baited with dung from treated and untreated animals at 1.5m spacing in Trial 2, n=number of traps.

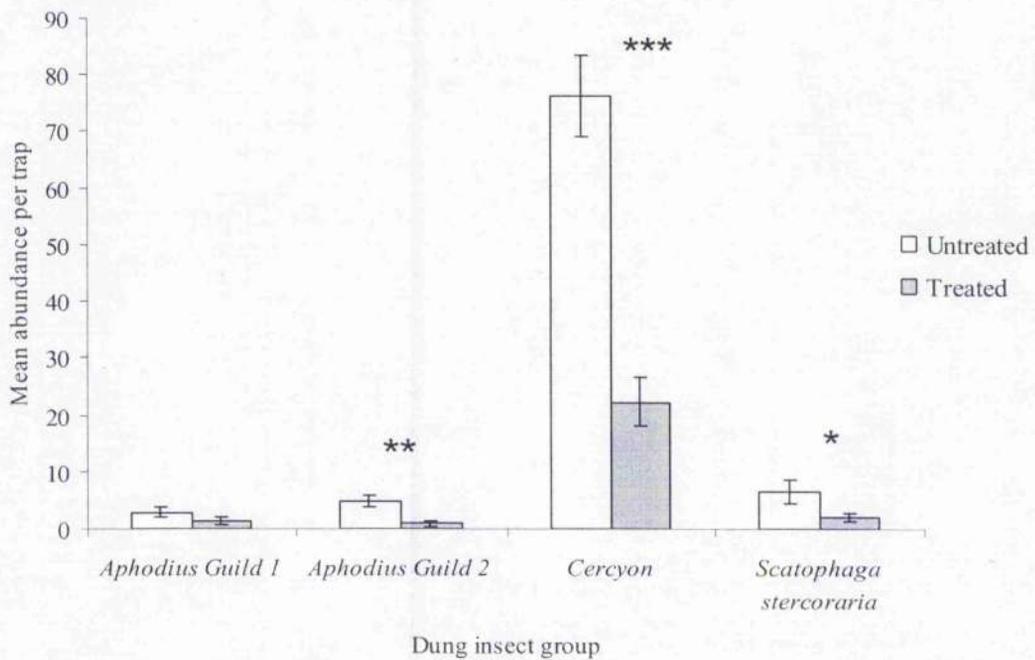


Figure 3.9 - Mean abundance ( $\pm 1$  se) of dung insects in traps baited with 'treated' or 'untreated' dung and spaced at 1.5m spacing in Trial 2 (Mann-Whitney test \*  $P < 0.05$ , \*\*  $P < 0.001$ , \*\*\*  $P < 0.0001$ )

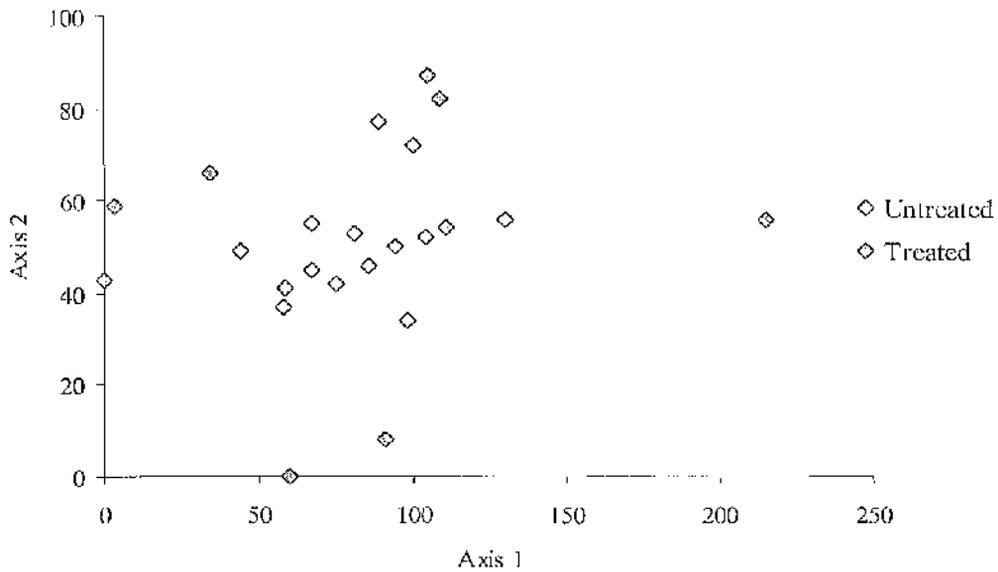


Figure 3.10 - Ordination of dung insect assemblages sampled in traps baited with either dung from avermectin-treated cattle or untreated cattle in Trial 2

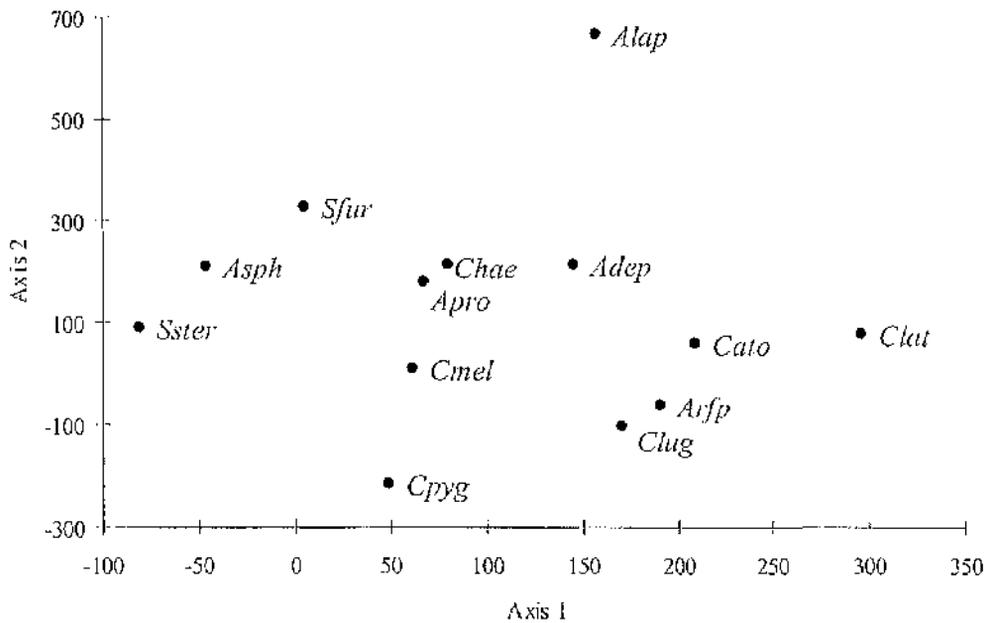


Figure 3.11 – Species scores from ordination of dung insect assemblages sampled in traps baited with either dung from avermectin-treated cattle or untreated cattle in Trial 2. A list of species abbreviations is provided in Appendix I.

## Trial 3

The abundance of dung insects in traps baited with dung of varying treatment and moisture level were compared (Table 3.3). For *Aphodius* species in Guild 1, significantly more individuals were attracted to traps baited with untreated dung than treated dung ( $F_{1, 28}=6.58$ ,  $P=0.02$ ) and the effect of moisture level alone was not significant ( $F_{1, 28}=0.68$ ,  $P=0.42$ ). The interaction between moisture and treatment was close to significance ( $F_{1, 28}=4.05$ ,  $P=0.054$ ). More beetles were attracted to untreated 'dry' dung than to treated 'dry' dung (Figure 3.12a). This difference was mainly driven by one species, *A. ater*, which was more attracted to traps baited with untreated dry dung than to all other dung types ( $H=8.67$ ,  $df=3$ ,  $P=0.03$ ). Guild 2 *Aphodius* species i.e. *A. prodromus* and *A. sphacelatus* (Figure 3.12b) were more attracted to untreated dung than to treated dung ( $F_{1, 28}=18.27$ ,  $P<0.001$ ), regardless of moisture level ( $F_{1, 28}=0.31$ ,  $P=0.58$ ). There was no difference in the number of *Cercyon* attracted to treated or untreated dung ( $F_{1, 28}<0.01$ ,  $P=0.98$ ) or to 'wet' or 'dry' dung ( $F_{1, 28}=0.26$ ,  $P=0.61$ ). However, numbers of *Cercyon* were extremely low therefore those results should be treated with caution (Figure 3.12c). More yellow dung-flies were attracted to dung baits from untreated cattle ( $F_{1, 28}=8.16$ ,  $P=0.008$ ). Moisture level ( $F_{1, 28}=1.45$ ,  $P=0.24$ ) did not significantly affect the number of flies attracted to baited pitfall traps (Figure 3.12d).

	Treated 'dry' (n=8)	Treated 'wet' (n=8)	Untreated 'dry' (n=8)	Untreated 'wet' (n=8)
Dung moisture (mean $\pm$ 1se)	85.2 $\pm$ 0.3%	89.3 $\pm$ 0.2%	85.1 $\pm$ 0.2%	88.3 $\pm$ 0.2%
<i>Aphodius ater</i>	0	4	11	3
<i>A. depressus</i>	7	6	10	9
<i>A. fimetarius</i>	0	0	1	0
<i>A. fossor</i>	0	2	2	1
<i>A. pusillus</i>	1	0	0	1
<b><i>Aphodius</i> Guild 1</b>	<b>8</b>	<b>12</b>	<b>24</b>	<b>14</b>
<i>A. prodromus</i>	69	62	163	119
<i>A. sphacelatus</i>	10	14	53	50
<b><i>Aphodius</i> Guild 2</b>	<b>79</b>	<b>76</b>	<b>216</b>	<b>169</b>
<i>Cercyon atomarius</i>	3	3	2	6
<i>C. haemorrhoidalis</i>	0	1	1	2
<i>C. lateralis</i>	1	0	0	1
<i>C. melanocephalus</i>	1	0	0	0
<i>C. pygmaeus</i>	1	0	0	0
<b><i>Cercyon</i></b>	<b>6</b>	<b>4</b>	<b>3</b>	<b>9</b>
<b><i>Scatophaga stercoraria</i></b>	<b>1</b>	<b>5</b>	<b>10</b>	<b>13</b>

Table 3.3 - Numbers of individuals sampled in Trial 3 in traps baited with dung of different moisture levels collected from avermectin-treated and untreated cattle, n=number of traps

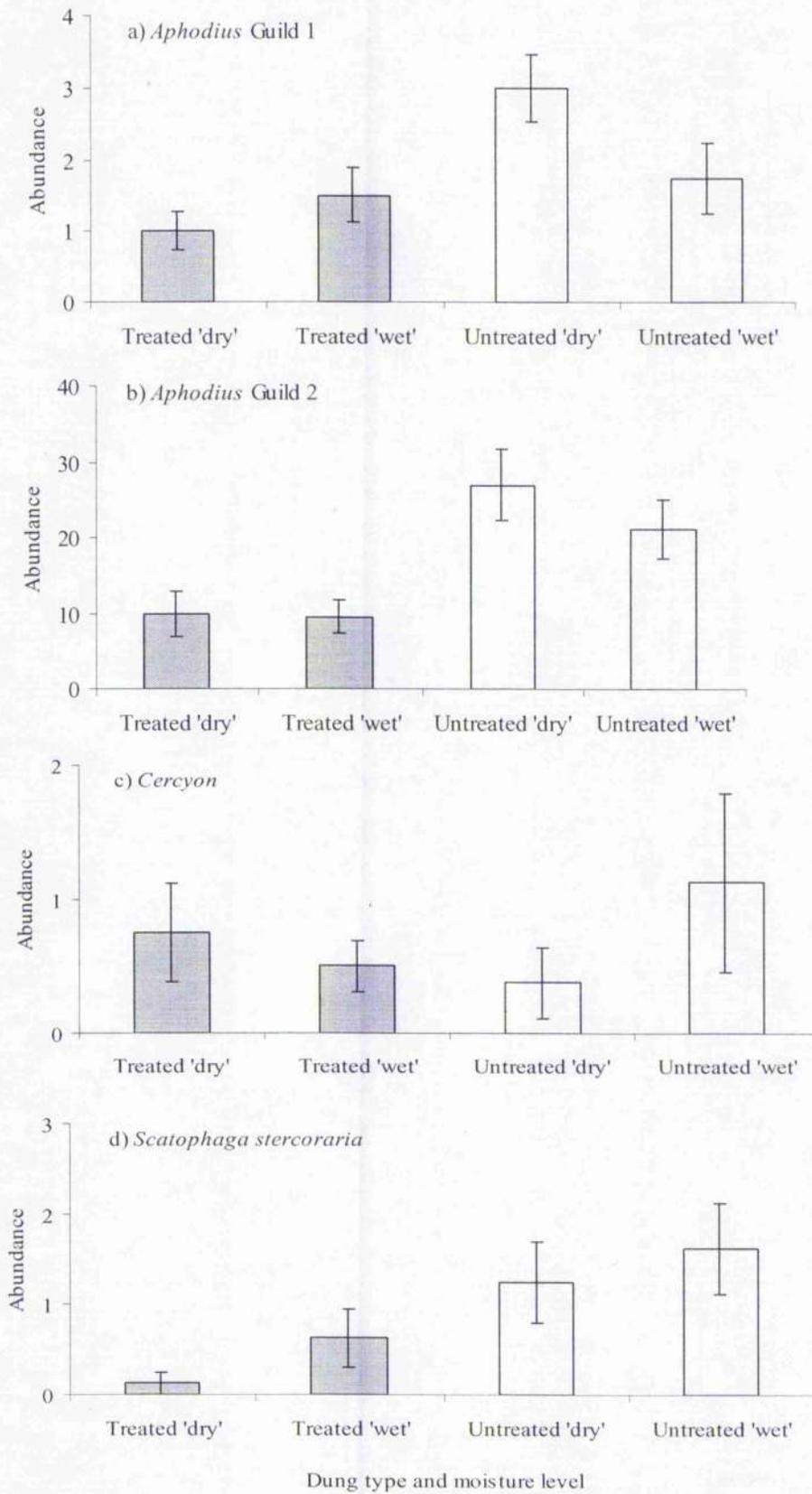


Figure 3.12 a-d – Mean abundance ( $\pm 1$  se) of dung insects in 'treated' and 'untreated' dung of different moisture levels in Trial 3

The ordination of dung insect assemblages in traps baited with dung of different 'treatment' and moisture level gave axes 1 and 2 eigenvalues of 0.266 and 0.14, respectively (Appendix IV. lists sample and species scores). Traps baited with untreated dung, of both moisture levels, were situated lower on axes 1 and 2 than traps baited with treated dung (Figure 3.13). The ordination positions of species suggests that untreated dung baits attracted relatively more *Scatophaga stercoraria*, *Aphodius ater*, *A. sphaecelatus*, *A. prodromus* and *A. fimetarius* (Figure 3.14). The latter species was only recorded on one occasion, in a trap baited with untreated dry dung. More variation occurred between traps with 'wet' and 'dry' treated dung than between traps baited with 'wet' and 'dry' untreated dung. This variation between treated traps of different moisture levels was partly due to the absence of *A. ater* in treated dry traps hence why those traps were situated further to the right of axis 1 than treated wet traps.

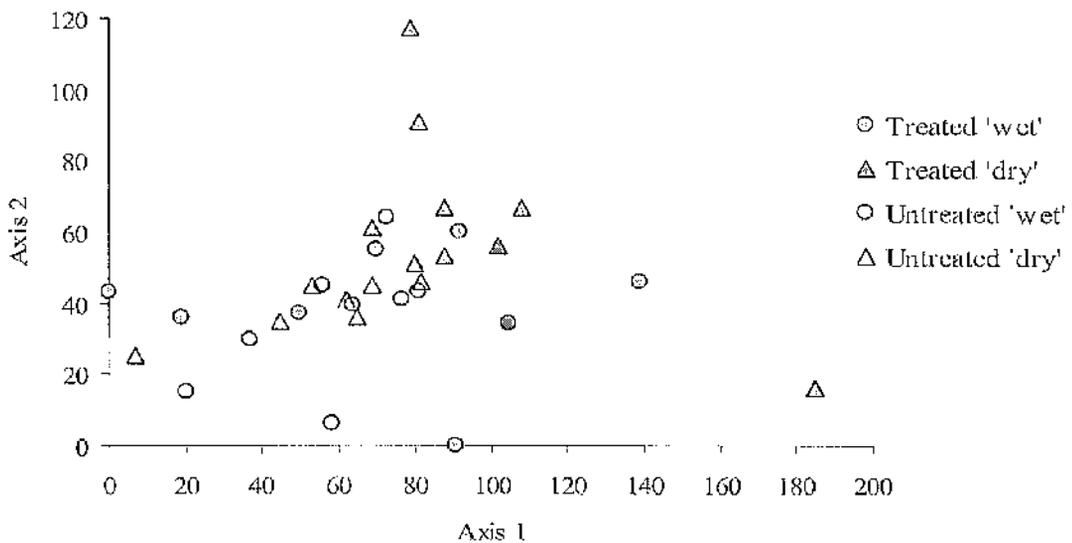


Figure 3.13 - Ordination of dung insect assemblages sampled in 'Trial 3' in traps baited with dung from either avermectin-treated cattle or untreated cattle at two different moisture levels

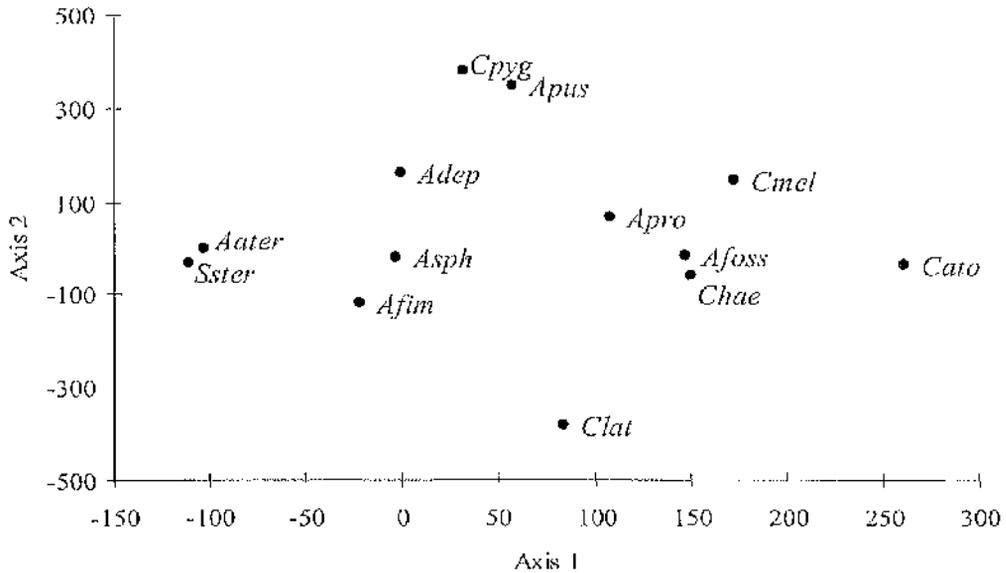


Figure 3.14 – Species scores from ordination of dung insect assemblages sampled in Trial 3 in traps baited with dung from either treated cattle or untreated cattle at two different moisture levels. Species abbreviations are listed in Appendix I.

### Sampling efficiency

#### Trial 4

The abundance of dung insects in baited pitfall traps surrounded by simulated ‘high’ and ‘low’ dung density is shown in Table 3.4. There was no significant difference in the abundance of *Aphodius* and *Cercyon* beetles or *S. stercoraria* flies between traps among high dung density or low dung density although there was a non-significant trend towards higher numbers of Guild 2 *Aphodius* in traps at low density (Figure 3.15). There was a significant difference in median diversity (Mann-Whitney,  $U_8$ ,  $n_1=89$ ,  $P=0.031$ ). Mean diversity was higher in traps among high dung density ( $0.88 \pm 0.11$ ) than in traps not surrounded by dung ( $0.55 \pm 0.04$ ). The lower diversity in traps at low dung density was attributed to the higher dominance of *Aphodius prodromus* in those traps.

Dung insect assemblages in traps in areas of high and low dung density were ordinated, and the ordination plot shows that traps at low dung density were grouped close together at the left of axis 1 (Figure 3.16). There was more spread among traps at high dung density because some traps were pulled towards the right of axis 1 because of relatively higher numbers of *Cercyon pygmaeus* and *Scatophaga*

*stercoraria* (Figure 3.17). However, eigenvalues of 0.118 for axis 1 and 0.008 for axis 2 (Appendix V.) were extremely low, indicating that the species composition of dung insects did not differ greatly between traps at low and high dung densities.

Species	Dung density	
	'Low' (n=8)	'High' (n=8)
<i>Aphodius ater</i>	30	14
<i>A. depressus</i>	336	317
<i>A. fimetarius</i>	1	1
<i>A. fossor</i>	2	3
<i>A. pusillus</i>	2	12
<i>A. rufipes</i>	1	1
<b><i>Aphodius</i> Guild 1</b>	<b>372</b>	<b>348</b>
<i>A. prodromus</i>	6593	3763
<i>A. sphacelatus</i>	144	105
<b><i>Aphodius</i> Guild 2</b>	<b>6737</b>	<b>3868</b>
<i>Cercyon atomarius</i>	200	168
<i>C. haemorrhoidalis</i>	12	9
<i>C. lateralis</i>	68	51
<i>C. lugubris</i>	2	0
<i>C. melanocephalus</i>	33	20
<i>C. pygmaeus</i>	1	8
<b><i>Cercyon</i></b>	<b>316</b>	<b>256</b>
<i>Sphaeridium lunatum</i>	1	1
<b><i>Scatophaga stercoraria</i></b>	<b>9</b>	<b>25</b>

Table 3.4 - Numbers of individuals sampled in Trial 4 in pitfall traps baited with dung and surrounded either by dung pats (high density) or no dung pats (low density), n=number of traps

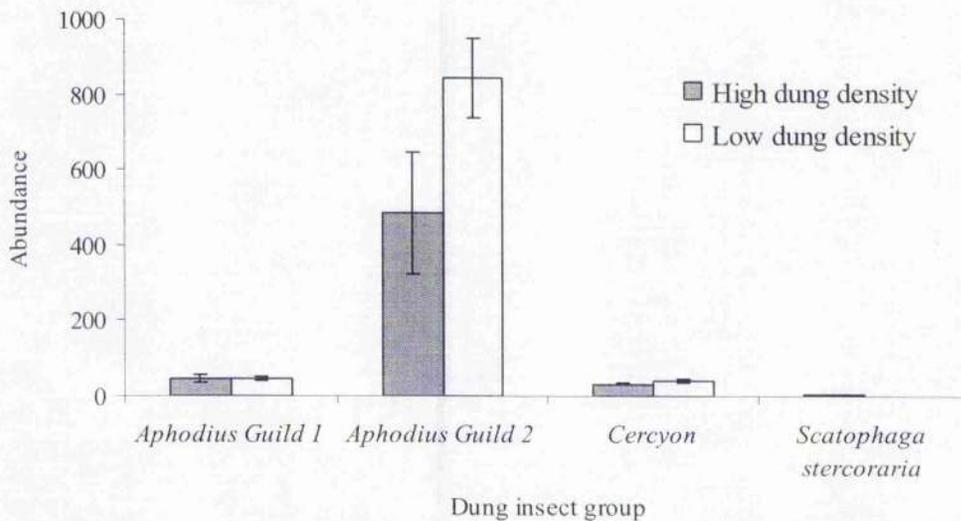


Figure 3.15 - Mean abundance ( $\pm 1$  se) of dung insects sampled in Trial 4 in dung-baited pitfall traps surrounded either by dung pats (high density) or no cow pats (low density)

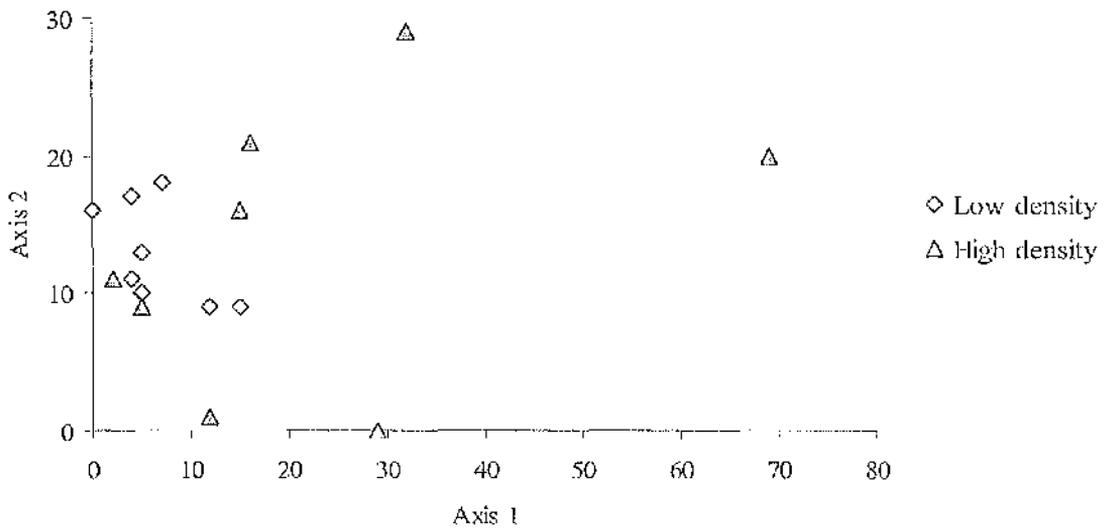


Figure 3.16 - Ordination of dung insect assemblages sampled in Trial 4 in dung-baited pitfall traps that were surrounded either by dung pats (high density) or no cow pats (low density)

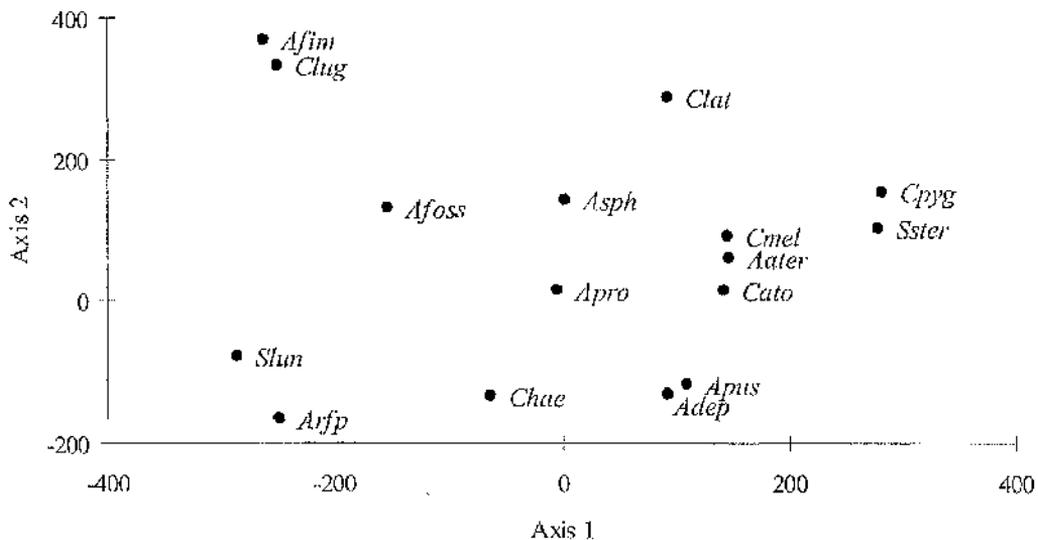


Figure 3.17 - Ordination of dung insect assemblages sampled in Trial 4 in dung-baited pitfall traps that were surrounded either by dung pats (high density) or no cow pats (low density). Species abbreviations are listed in Appendix I.

#### Trial 5

Four species of *Aphodius*, six *Cercyon* species and one Scatophagid were recorded in the trial investigating effects of duration of trap exposure (Table 3.5). Traps exposed for 5-17 days had 6-7 dung insect species, while traps exposed for 20 days had an average of 8 species (Figure 3.18). It is notable that all of the *Aphodius* species trapped are early colonisers of dung (Gittings and Giller, 1998) and that the species

*A. ater*, *A. fossor* and *A. fimetarius* that are regarded as mid and late successors (Finn *et al.*, 1998) were not recorded.

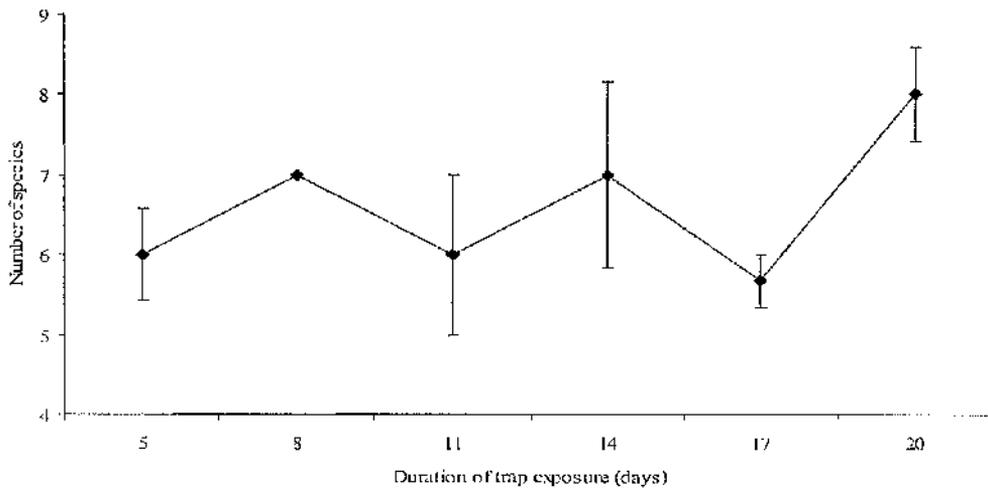


Figure 3.18 – Mean number ( $\pm 1$  se) of dung insect species in baited pitfall traps exposed for 5, 8, 11, 14, 17 and 20 days

The ordination of the dung insect data showed that there was no separation of traps according to the number of days that they were exposed (Figure 3.19, and Figure 3.20 for species scores). There was as much separation between the three traps collected on the same day as between those collected on separate days. However, eigenvalues were extremely low at 0.089 and 0.033 for axes 1 and 2, respectively, (see Appendix VI. for scores) and thus indicated that variation between traps was negligible.

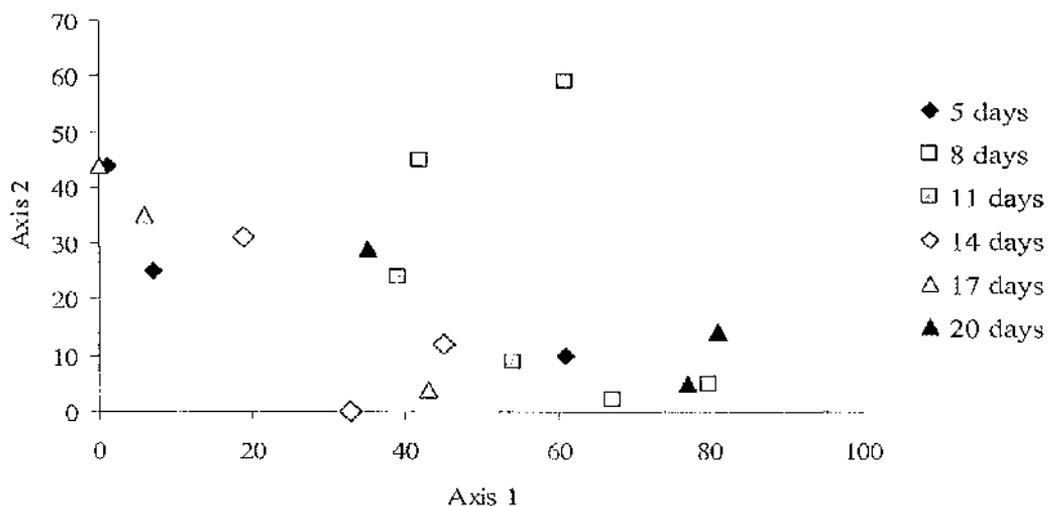


Figure 3.19 - Ordination of dung insect assemblages sampled in Trial 5 in dung-baited pitfall traps that were exposed for 5, 8, 11, 14, 17 and 20 days

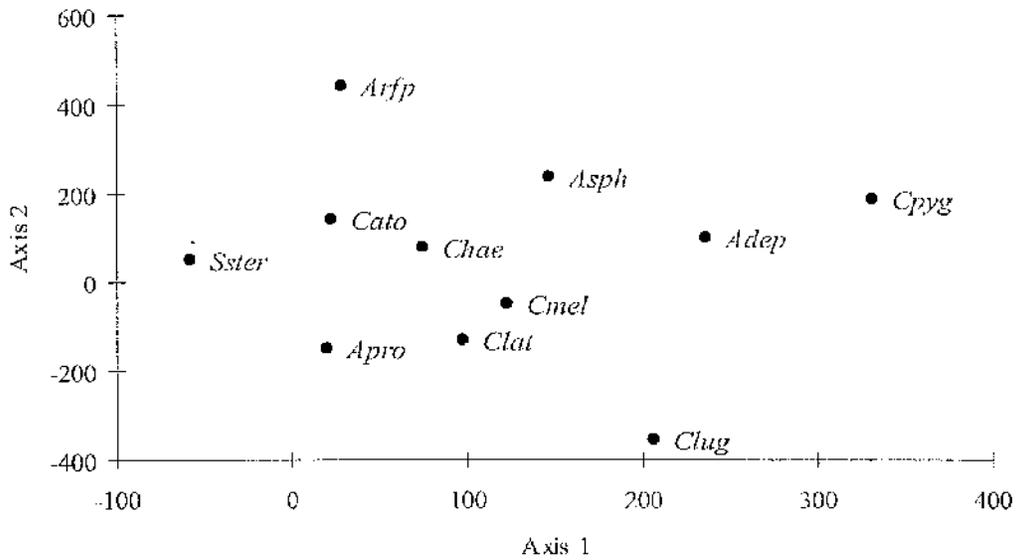


Figure 3.20 – Species scores from ordination of dung insect assemblages sampled in Trial 5 in dung-baited pitfall traps that were exposed for 5, 8, 11, 14, 17 and 20 days. A list of species abbreviations is provided in Appendix I.

Species recorded	Stage in succession of dung
<i>Aphodius depressus</i>	Early
<i>A. rufipes</i>	Early
<i>A. prodromus</i>	Early
<i>A. sphacelatus</i>	Early
<i>Cercyon atomarius</i>	Early
<i>C. haemorrhoidalis</i>	Early
<i>C. lateralis</i>	Mid
<i>C. lugubris</i>	Early
<i>C. melanocephalus</i>	Early
<i>C. pygmaeus</i>	Mid
<i>Scatophaga stercoraria</i>	Early

Table 3.5 – Species recorded in dung-baited pitfall traps exposed in grazed pasture for 5 to 20 days in Trial 5. ‘Early’: colonise dung up to 5 days old and ‘Mid’: 6-10 days old (collated from Hanski, 1980c; Gibbons, 1987; Gittings and Giller, 1998; reviewed in section 1.2.1)

### Trial 6

Eight baited pitfall traps had been set in each of five fields, and the cumulative number of dung insect species was estimated for the number of traps (Table 3.6). The species-sample accumulation curves suggested that 4-5 traps were the optimum number of traps to sample dung insect species in a typical grazed pasture, ranging in size from approximately 2-7 ha, in Ayrshire (Figure 3.21).

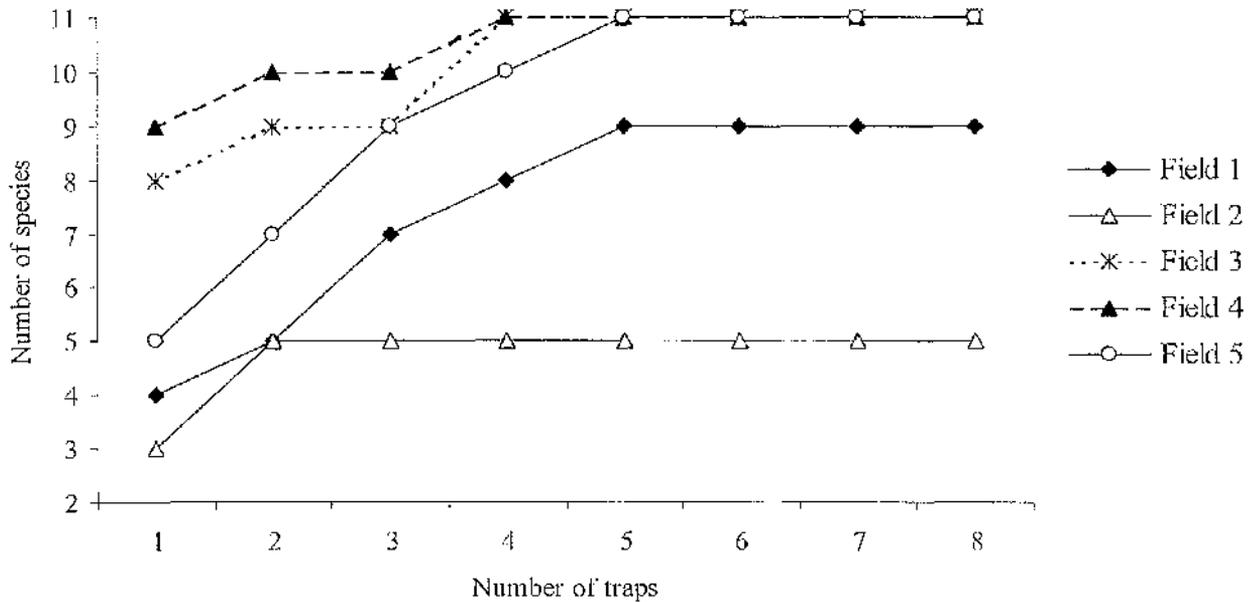


Figure 3.21 – Species-sample curves showing the cumulative number of dung insect species trapped in dung-baited pitfall traps in five grazed pastures in Trial 6

Field	Sampling time	Area (ha)	Number of dung insect species trapped	Number of traps to reach that
1	May 2003	2	9	5
2	May 2003	6.8	5	2
3	May 2002	4.8	11	4
4	June 2003	3.5	11	4
5	May 2003	4.1	11	5

Table 3.6 – List of fields used to construct species-sample curves for dung insects, the number of dung insect species in eight baited pitfall traps in each field and the cumulative number of traps in which maximum species richness was recorded

### 3.4 Discussion

#### *Attractiveness of dung*

The main conclusion from these results is that dung insects favoured dung from untreated animals. Similar findings have been observed previously for *Aphodius* and *Cereyon* with regards to ivermectin (Holter *et al.*, 1993 a and b). This study showed that, at a spatial scale of 70m, *Aphodius*, *Cereyon* and *Scatophaga stercoraria*, discriminated against dung from doramectin-treated animals. This has implications for the spatial dynamics of dung insects at the pasture level, for example where a field grazed by treated cattle is adjacent to one grazed by untreated cattle, the

majority of dung insects would be expected to potentially opt to colonise dung in the 'untreated' field.

When traps baited with dung from either treated or untreated cattle were spaced 1.5m apart, significantly more Guild 2 *Aphodius* and *Cercyon* beetles and *S. stercoraria* flies occurred in traps baited with untreated dung. Although not significant, there was also a tendency for more Guild 1 *Aphodius* beetles to occur in traps baited with untreated dung. Previous research has shown that insects can distinguish between dung from untreated and ivermectin-treated cattle at 3.5m (Holter *et al.*, 1993a) and this study suggests that insects can discriminate against dung from doramectin-treated cattle at 1.5m. Such findings are relevant when using dung-baited pitfall methods to sample dung insect communities. For example, if traps baited with untreated dung were set in a pasture where the natural dung deposited was from treated animals, then one might expect relatively more insects to be attracted to traps than if traps were set in a field grazed by untreated cattle. Hence, one might expect inflated trap catches in treated fields relative to untreated fields. The diversity or species composition of dung insects in traps, at both a 1.5m and a 70m scale, were not influenced by this attraction effect and there is no evidence that any one species was completely repelled by dung from doramectin-treated animals.

The experiment that examined the attraction of dung from treated and untreated dung at varying moisture levels (Trial 3) showed that more *Aphodius* Guild 1 were attracted to drier untreated dung than to drier treated dung. This difference was driven mainly by *Aphodius ater*, a species known to favour drier dung (Gittings and Giller, 1998). After colonising fresh dung, this species oviposits in the drier crust area of the pat (Gittings and Giller, 1997), and prefers to lay eggs in dung that is at least two days old (Hirschberger and Degro, 1996). This oviposition behaviour presumably reduces the possibility of eggs 'drowning'. There was an overwhelming preference by *A. prodromus* and *A. sphaelatus* for untreated dung regardless of moisture content. These two species oviposit in soil (Gittings and Giller, 1997), therefore they do not have the same constraints, in terms of moisture, as species that oviposit in dung. Yellow dung flies preferred untreated dung with either moisture level to dry dung from treated cattle thus suggesting that they were influenced more by the effect of doramectin treatment on dung quality than by moisture content. In

terms of oviposition, yellow dung-flies are more resilient to moisture changes in dung because the structure of their eggs allows the eggs to remain buried in the surface of dung with the respiratory features exposed (Hammer, 1941). Yellow dung-flies also make subtle choices about the oviposition site on the dung surface e.g. they avoid depressions that might become waterlogged with rain, possibly as a mechanism to avoid drowning (Ward *et al.*, 1999).

Species may exhibit seasonal differences in their attraction to dung according to their ecological requirements at that time. For example, *Aphodius fimetarius* showed no preference to dung from untreated or ivermectin-treated cattle when breeding in the spring but preferred dung from untreated cattle in the autumn when mainly feeding (Floate, 1998b). Unfortunately, one cannot assess the effect of season with this data because sampling was only conducted in the spring. However, the suggestion that *Aphodius* may select untreated dung as a feeding resource is supported here because beetles from both Guilds 1 and 2 would have been using dung for feeding at the time of these trials. It is not clear whether the greater attraction of Guild 1 beetles to untreated dung was entirely due to selection on the basis of feeding quality or also attributable to the selection of dung as a breeding resource.

In conclusion, dung insects were more attracted to ‘ivermectin-free’ dung than to dung from doramectin-treated cattle. Other studies have attributed such differences in attraction to changes in cattle diet (Barth, 1993; Floate, 1998b). Cattle diet can be excluded as a contributory factor here, as cattle were fed on the same silage diet during the collection of dung before and after treatment. It has also been suggested that attraction differences could be due to ivermectin therapy altering the moisture content of dung (Wratten and Forbes, 1995). However, this study has shown that the moisture content for some dung insect species e.g. *A. prodromus* and *Scatophaga stercoraria*, is not as important as the treatment effect.

Hence, these results indicate that doramectin or one of its metabolites could be making the dung less attractive to insects. Moreover, doramectin treatment may have indirectly diminished the quality of dung. For example, adult beetles feed on the energy-rich bacteria that are abundant in fresh dung (Hirschberger, 1999), and little research has been done to ascertain the effects on those bacteria and other dung

microflora. A significant reduction in those bacteria could make the dung less nutritious and less desirable to dung insects as a feeding resource.

### *Sampling efficiency*

The abundance, diversity and species composition of dung insects did not differ significantly with surrounding dung density, however there was a non-significant trend for more *Aphodius prodromus* individuals in traps surrounded by no dung. This suggests that a possible 'dilution effect' might have been apparent for that species, where fewer individuals occurred in traps surrounded by a higher density of dung.

There was no difference in the species composition of dung insects collected in traps on the same day or from traps collected after varying lengths of trap exposure from 5-20 days. The *Aphodius* species that were trapped are all early-successional species (e.g. Finn *et al.*, 1998). However, it was unlikely that the absence of late colonisers was due to dung exposure time since those species colonise dung up to 2-3 weeks old (Hanski, 1980d). Therefore, one would have expected the late colonising species to occur in traps exposed for 14, 17 and 20 days.

It is useful to know the number of traps that are required to give a representative sample of dung insect species in a pasture. Five was the optimum number of dung-baited pitfall traps to use for sampling spring and summer assemblages in a typical grazed pasture in Ayrshire. Four traps also gave a good representation of the dung insect species present and, for the wider field study, it was decided to process data from four traps rather than five in order to reduce processing time. It is interesting to note that Lobo *et al.*, (1998) proposed that using between two and five dung-baited pitfall traps was sufficient when sampling dung beetle assemblages in Mediterranean regions.

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## CHAPTER 4

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# INVESTIGATING FACTORS THAT INFLUENCE DUNG INSECT ASSEMBLAGES IN PASTURES GRAZED BY UNTREATED CATTLE

## 4. Investigating factors that influence dung insect assemblages in pastures grazed by untreated cattle

### 4.1 Introduction

A wide range of seasonal and environmental variables can influence insect population dynamics. Seasonality of insects can be related to temperature, day length and rainfall (Wolda, 1988) and, in north temperate dung insect communities, seasonal variation has been documented by many (e.g. White, 1960; Gibbons, 1987; Wassmer, 1994; see Section 1.21 for a review). While seasonal shifts in temperature and rainfall patterns might affect abundance, dung insects are also affected by weather at a finer scale. For example, fewer *Aphodius* beetles were trapped in baited pitfall traps during periods of heavy rainfall (Finn *et al.*, 1998). The reproductive success of *A. fossor* was reduced in dung exposed to sunshine since the subsequent acceleration in crust formation on the dung shortened the time available for oviposition (Vessby, 2001). Dung insect assemblages may additionally be affected by various habitat features of a pasture such as altitude, aspect, soil type and proximity to woodland (Ryan *et al.*, 1978; Wassmer, 1995; Romero-Alcaraz and Avila, 2000; Roslin, 2001; Vessby and Wiktelius, 2003).

The availability of a suitable dung resource is essential for the persistence of dung insect populations and several aspects of pasture management can affect that availability. In pastures where livestock are rotationally grazed, dung invertebrates often have to emigrate from the pasture to locate fresh dung (Finn *et al.*, 1998), whereas pastures that are grazed permanently throughout the grazing season provide a constant supply of fresh dung. If insects disperse from their pasture of emergence then they must be able to encounter a dung resource within a reasonable dispersal distance in order to maximise reproductive success. For example, a pasture surrounded by arable fields with little dung would provide more of a challenge to dung insects in terms of dispersal than if grazed pasture was adjacent. The stocking density of livestock in a field can directly affect dung availability. In highly stocked pastures a supply of dung is unlikely to be a limiting factor provided that the dung is of suitable quality.

The aim of this chapter was to determine the phenologies of *Aphodius* spp., *Cercyon* spp., *Sphaeridium* spp. and *Scatophaga stercoraria* in grazed pastures in the study area in South West Scotland, and to examine relationships between dung insect assemblages and habitat and management characteristics of pastures. All of the data presented in this chapter were collected from pastures grazed by cattle that had received no anthelmintic treatment. The purpose of analysing data from 'untreated' pastures was to establish a baseline to which insect data collected from pastures grazed by avermectin-treated cattle could be compared, in terms of avermectin treatment and other aspects of pasture management.

## 4.2 Method

### 4.2.1 Site Selection

The selection of study farms was guided by a questionnaire survey of farmers in Central and South West Scotland (see Chapter 2). Sampling was carried out on seven fields on four commercial dairy farms in 2002 and on eleven fields across seven dairy farms in 2003 (Table 4.1). The study fields contained cows that did not receive any anthelmintic treatment throughout the duration of the sampling period. It should be noted that Farm 3 is not listed because only 'treated' fields were sampled on that farm therefore those results are considered in Chapter 5. The relative locations of 'untreated' study fields are shown (Figure 4.1).

Farm	Field	Field size (ha)	Aspect	Boundary	Livestock	Grazing system	MIS	Year sampled
1	SC3	4.7	North	Hedge	Dairy cows	Rotation	10	Both
2	WMC3	4.8	South	Gappy hedge	Dairy cows	Rotation	16	Both
2	WMC4	4.8	South	Hedge	Dairy cows	Rotation	14	Both
4	BTBC1	3.5	South	Gappy hedge	Dairy cows	Rotation	12	2002
4	BTBC2	4.1	Flat	Gappy hedge	Dairy cows	Rotation	14	Both
4	BTBC3	6.8	South	Gappy hedge	Dairy cows	Rotation	12	2003
5	DC5	2	South	Fence	Dairy cows	Rotation	16	Both
5	DC6	3.3	South	Fence	Dairy cows	Rotation	17	Both
6	MTC	3.5	Flat	Gappy hedge	Dairy cows	Rotation	13	2003
6	BR	3.5	Flat	Gappy hedge	Dairy cows	Rotation	13	2003
7	GGC1	6.7	Flat	Gappy hedge	Dairy cows	Permanent	10	2003
8	LBC1	4.1	Flat	Hedge	Dairy cows	Permanent	14	2003

Table 4.1 – Description of study fields grazed by livestock not treated with anthelmintics (MIS: Management Intensity Score, see text below for description)

Scale 1cm: 1250m

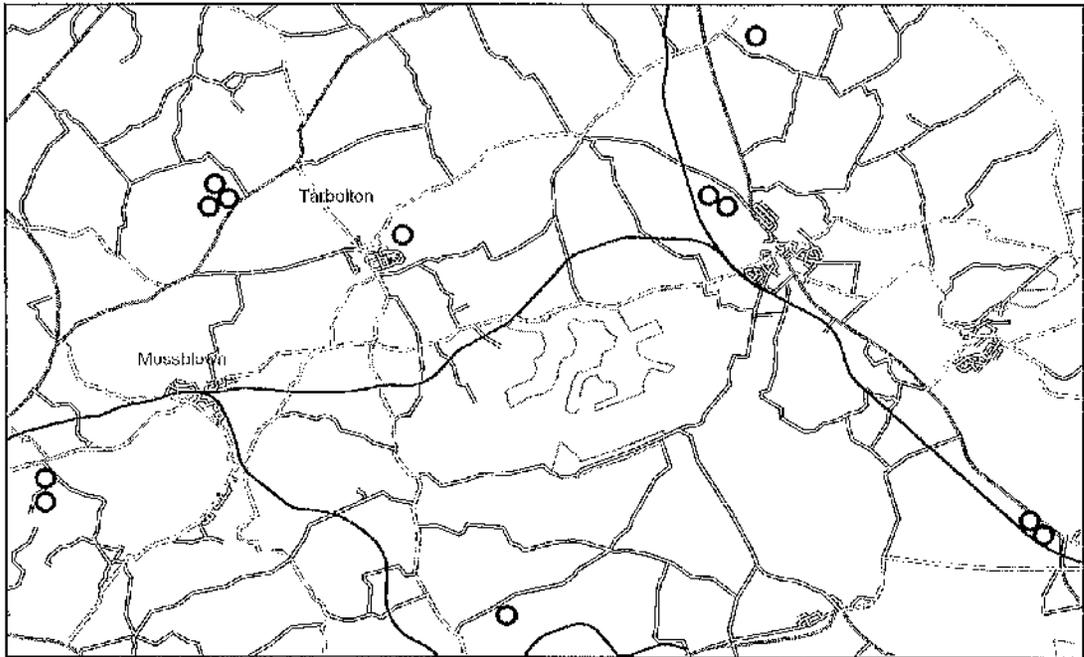


Figure 4.1 – Map showing relative location (O) of fields grazed by untreated cattle. Grid references of fields are provided in Appendix VII.

#### 4.2.2 Data Collection

##### *Dung invertebrate sampling*

Dung-baited pitfall traps were used to sample adult beetles (Scarabaeidae and Hydrophilidae) and adult dung flies (Scatophagidae) from April to July in 2002 and 2003. Sampling was carried out at that time of year because it is a time when cattle are grazing pastures, weather permitting, and when birds are foraging for invertebrates to provision young. Prior to setting the traps, dung for baits was collected from untreated housed cattle at each study farm (a full description of the trap design is provided in Section 3.3). Dung collected from each individual farm was mixed to homogenise it and stored in a sealed container at 4°C. In order to maintain good biosecurity practice, dung from different farms was kept in separate containers and care was taken to ensure that dung was used for baits only on farms from where it had been collected. Additionally, traps were set in October using dung that had been kept in cold storage since April. The dung attracted considerable numbers of *Aphodius* (unpublished data) and thus confirmed that the attractiveness of

dung kept in cold storage did not diminish over the course of the sampling period from April to July.

A pilot study suggested that four baited pitfalls adequately represented the dung insect species present in a pasture (Chapter 3, Trial 6) but eight traps were set as insurance against losing traps because of cattle trampling or disturbance. In each study pasture, eight baited pitfall traps were set in two grids of four with traps spaced approximately 8m apart within each grid. The reason for setting two separate grids in a pasture was to minimise loss of traps due to cattle trampling in one particular area of the field. One grid was set in a central position at each end of the field, i.e. away from field margins, to counteract possible edge effects. Traps were emptied and re-baited approximately every 7-10 days in 2002 and every 14 days in 2003. The trap period duration was extended in 2003 to enable a larger number of fields to be sampled. There were a maximum of nine and six collections per field over the sampling season in 2002 and 2003, respectively. The same number of traps were used in each pasture, regardless of field size, as has been done in other dung insect studies (e.g. Lobo *et al.*, 1998; Estrada and Coates-Estrada, 2002; Hutton and Giller, 2003). Field size was included as an independent variable in post hoc data analyses. The pitfall catch was not corrected to field area because the 'effective sampling area' of the dung-baited pitfall trap was unknown (Turchin and Odendaal, 1996).

Two traps from each grid were selected from each sampling date in each field and invertebrate material from those four traps was pooled and processed. Identification of dung insects was carried out using keys (Jessop, 1986; Hansen, 1987; Skidmore, 1991) and with reference to collections at the Hunterian Museum and the National Museum of Scotland. Individuals of the genera *Aphodius* (Scarabaeidae), *Cereyon* and *Sphaeridium* (Hydrophilidae) and *Scatophaga* (Scatophagidae) were identified to species level and counted. The same four traps in a field were processed for each trapping period unless those traps had been trampled in which case others were selected. When processing samples, the invertebrate composition in traps did not appear to differ greatly either within or between grids for a given sampling period (pers. obs.). To confirm this, Spearman rank correlations were performed on trap data from fifty trapping periods in 2002 (data not presented here). There was a high tendency for traps from the same trapping period in a field, either in the same grid or

in different grids, to be highly positively correlated (unpublished data) therefore one was confident that there was negligible inter-trap variance within each sampling period.

### *Environmental variables*

#### *Climate*

Climate data were obtained from Auchincruive weather station (NS 379 234), which was located at a maximum distance of ten miles from any one of the study fields. Total rainfall (mm) and sunshine hours and the mean maximum and minimum temperatures were calculated for each individual trapping period.

#### *Habitat variables – landscape*

In order to take into account the potential influence of avermectin use at a wider landscape scale, an 'Avermectin Index' was devised for each trapping period to estimate the proportion of land around the study field that was grazed by avermectin-treated livestock. This index allowed comparisons between untreated fields that were surrounded either by fields grazed by untreated cattle or by avermectin-treated livestock. To calculate the index, a circle of 0.5 km<sup>2</sup> was drawn to scale on a map with the study field as the centre-point. The percentage of land area grazed by avermectin-treated animals within that circle was then estimated to give the index value. The necessary information on avermectin use was obtained by contacting the farmers and landowners of fields within that 0.5 km<sup>2</sup> area.

Using the same method, the Pasture Index estimated the percentage area of grazed pasture in a 0.5 km<sup>2</sup> around the study field to reflect the potential availability of livestock dung in surrounding fields. The index was general in that it could only provide a snapshot of grazing land-use because information could not be obtained for the period between visits to the study area. For example, a cut silage field may have been grazed in the ten days between visits to a study site and this would not have been acknowledged.

For both indices, the 0.5 km<sup>2</sup> radius was chosen because it was relatively easy, logistically, to obtain land-use information at that scale. More importantly, that area i.e. a 400m radius was probably sufficient to cover the dispersal distances of most *Aphodius* travelling to and from adjacent fields. Roslin (2000) found that most *Aphodius* remained in the pasture in which they had emerged, and far fewer travelled distances up to 800m. While no comparable data was found for Scatophagid dung-flies, a mark-recapture study of the sheep blowfly *Lucilia sericata* estimated their dispersal distances to be between 100-200m (Smith and Wall, 1998). Therefore, one assumed that most Scatophagids were unlikely to disperse more than 400m from their pasture of emergence if there was a dung resource close by.

#### *Habitat variables - field*

The general characteristics of aspect, altitude, adjacency to woodland, field boundary and size of each field were recorded. Sward height was measured by the 'direct method' (Stewart *et al.*, 2001) using a ruler to measure the height at which about 80 per cent of the vegetation was growing, on approximately every second to third sampling occasion in 2002 and on every sampling occasion in 2003. Sward height was measured ten times in the area around the traps and mean sward height calculated.

In order to gauge the availability of fresh dung in a pasture, an index of dung deposition was devised for each trapping period:

$$\text{Dung Index (pats ha}^{-1}\text{)} = \frac{(\text{cows}) (\text{days}) (\text{rotation factor})}{\text{field area}}$$

where *cows* = number of cows in the study field

*days* = number of days that traps were exposed

*field area* = area of the study field in hectares

*rotation factor* = e.g. rotation factor was 1 when fields were permanently stocked; rotation factor was 0.5 if cows were rotated with one other field, and 0.33 if rotated with two other fields and so on

Soil pH, available phosphorous (P) and potassium (K), soil moisture and organic matter were determined for each study field. Six soil cores (10cm length x 6.5cm diameter) were taken from the area around the traps in all pastures and passed to a laboratory for analysis. 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 soil sampling meant that comparisons of soil characteristics between years were treated with caution, however comparisons could be made between fields for each year. Soil penetrability indicates how easy it is for soil to be probed by birds foraging for soil invertebrates (Green, 1988). The impenetrability of soil was measured using a penetrometer (ELE international) with an impenetrability index value range of 0-150 and a needle size of 0.65cm<sup>2</sup>. Ten measurements were taken around the trap grids on one sampling occasion in 2002 and on every sampling occasion in 2003 and mean soil impenetrability was calculated.

#### *Management intensity*

The intensity of pasture management can influence the abundance and species richness of dung insects (Hutton and Giller, 2003), therefore an index of management intensity was calculated for each study pasture. Blake (1996) derived a Management Intensity Score based on the intensity of various agricultural management practices. The scoring method was adapted by McCracken (pers. comm.), and a breakdown of that 'score system' is given in Appendix VIII. Management information was collected through interview of farmers who were asked about management practices in the fields e.g. sward type and age, soil disturbance, cutting regimes, grazing intensity, fertiliser input and herbicide use. Scores were assigned and totalled to give a potential overall Management Intensity Score (MIS) between 0-24, with 24 being the most intensively managed.

Ground beetles (Carabidae) and spiders (Araneae) were sampled in study fields because both of these groups have been recognised as indicators of management intensity differences (e.g. Gibson *et al.*, 1992; Blake, 1996; Pommeresche, 2002). Therefore, information on their assemblages can highlight the ecological similarity of study pastures in relation to management practice. To sample ground beetles and

spiders, five plastic cups of 75mm diameter were covered with wire mesh, part-filled with MPG and set in a line approximately 2m apart. Traps were set for three weeks in nine of the 'untreated' fields, i.e. a subset of the twelve study fields, in September 2003. The contents of all five traps were pooled and spiders and carabids were identified to species level and counted (by D. Beaumont and R. Morton, respectively).

#### 4.2.3 Data Analysis

##### *Environmental variables*

A 'habitat characteristic' score was derived by ordinating altitude, aspect, boundary, adjacency to woodland and 'pasture index' data (Table 4.2). Ordination can be used in such a way to simplify and reduce the number of parameters introduced into a model (Fox, 2004; Rushton *et al.*, 2004). Detrended Correspondence Analysis was the ordination technique used (see 3.2.2 for a description of this ordination method).

Variable	Level	Type
Altitude	m	Continuous
Aspect	3 levels: Flat, South, North	Categorical
Boundary	3 levels: Fence, Gappy hedge, Established hedge	Categorical
Pasture index	% (see text for estimation method)	Continuous
Woodland	2 levels: not adjacent to wood; adjacent to wood	Categorical

Table 4.2 – List of variables used to summarise habitat characteristics of pastures by ordination with DECORANA

Non-parametric statistics were used to assess differences in climatic, temporal, habitat and management characteristics between study fields between years. Data that was collected or estimated repeatedly over the sampling season e.g. sward height and dung density, were analysed using mixed models with repeated measures and a normal error distribution. Spider and ground beetle assemblage data were ordinated to compare their species compositions between the sub-set of the study pastures.

##### *Abundance, Species Richness and Diversity*

The species composition of dung insects in traps is unlikely to differ significantly in traps exposed for 7-14 days (Chapter 3, Trial 5 results). The abundance of *Cercyon*,

*Aphodius* and *Scatophaga stercoraria* were corrected to a period of 10 days to allow comparisons between trapping periods of different duration. Unless stated otherwise, abundance hereafter refers to values corrected to 10 days. Individuals of *Cereyon* and *Aphodius* species were pooled into their respective genera in order to gain sufficient numbers for analyses. The *Sphaeridium* beetles were trapped in numbers too low to analyse statistically. The *Aphodius* were divided into two guilds with Guild 1 comprising *Aphodius* species with coprophagous larvae that feed exclusively in dung and Guild 2 containing *Aphodius* species with saprophagous larvae that feed on plant roots and decaying vegetation (Gittings and Giller, 1997). Guild 1 included all *Aphodius* species collected with the exception of *A. prodromus* and *A. sphacelatus*, which were placed in Guild 2. Diversity of dung insects was calculated using the Shannon index (see Section 3.2.2 for details).

General Linear Modelling of abundance, species richness and diversity data was carried out using mixed models with repeated measures in the SAS® STAT program (SAS Institute, 2001). The GLIMMIX macro was used to analyse data for which a Poisson error and log link function were assumed e.g. abundance data, and *Proc Mixed* was used to analyse species richness and diversity data for which a normal error distribution was assumed.

In mixed models, variables are included as either fixed or random effects. Factors were listed as fixed when all of the levels of that factor were known or measured. Study farms and fields were classed as random effects as they were regarded as random samples from the larger population of fields and farms in that area. If fields were treated as fixed effects then one is limited to interpreting results in relation to only those specific fields. Sometimes the model could not estimate both farm and field as random factors in which case the model was re-run, including only field as a random factor. It was necessary for 'field' to be estimated as a random factor because otherwise the different trapping periods in any one field may have been regarded as individual fields. This would have inflated sample size and gave artificially small P-values, which obviously would have increased the risk of wrongly assuming that an independent variable was significant when it was not. Therefore, models were disregarded when the avermectin treatment variable was significant if the random factor 'field' could not be estimated. To overcome this, an alternative

dependent variable can be used in the model, for example a mean monthly abundance value instead of an abundance value per trapping period. The disadvantage of this is that the detail of the data may be lost, however it is sometimes necessary to ensure that the model is reliable.

Variable	Level	Type
Farm	7 levels	Categorical
Field	12 levels	Categorical
Year	2 levels: 2002, 2003	Categorical
Seasonality	Sampling date: days from 1 April (23-112 days)	Continuous; change
Days post-turnout	Days since cattle were put out to pasture (6-89 days)	Continuous; change
Sward height	(4-30 cm)	Continuous; change
Rainfall	mm per trap period (0.2-67.4 mm)	Continuous; change
Sun	hours per trap period (48-145 hours)	Continuous; change
Maximum temperature	Mean max. temp. per trap period (10.0-21.1°C)	Continuous; change
Minimum temperature	Mean min. temp. per trap period (3.4-13.8°C)	Continuous; change
Dung index	Density per ha per trapping period. See text for equation. (0-141 pats ha <sup>-1</sup> )	Continuous; change
Area of field	(2.0-6.8 ha)	Continuous; fixed
Soil pH	(pH 5.2-6.7)	Continuous; fixed
Available P	(11-82 mg l <sup>-1</sup> )	Continuous; fixed
Available K	(112-408 mg l <sup>-1</sup> )	Continuous; fixed
Soil Moisture	(23.1-47.26 %)	Continuous; fixed
Soil Impenetrability	(43-101 impenetrability index)	Continuous; change
Soil Organic content	% loss on ignition (8-13 %)	Continuous; fixed
Grazing System	2 levels: rotation or permanent grazing	Categorical; fixed
Management Intensity Score (MIS)	See Appendix VIII. for description (Score 10-17)	Continuous; fixed
Age of pasture (part of MIS)	4 levels: <5yrs, 5-10yrs, >10yrs, uncultivated	Categorical; fixed
Grazing Intensity (part of MIS)	4 levels: none, <0.8 LU ha <sup>-1</sup> , 0.8-1.14 LU ha <sup>-1</sup> , >1.14 LU ha <sup>-1</sup>	Categorical; fixed
Avermectin index	See text for description. (0-13.8 %)	Continuous; fixed
Habitat characteristic score	Ordination of aspect, altitude, boundary, adjacent woodland, pasture index	Continuous; fixed

Table 4.3 – List of environmental and management variables included in mixed model analyses of insect data. 'Fixed' variables did not change over the sampling season and 'change' variables altered with trapping period. The recorded ranges of continuous variables are given in parentheses.

'Sampling date' was listed as a repeated factor, because each field was sampled more than once over the sampling season, to exclude pseudoreplication error. The Autoregressive of order 1 or AR(1) covariance structure was thought to be most suitable for this repeated measures data because it assumes that measures further apart in time are less correlated than closer together measures (Littell *et al.*, 1996)

c.g. pitfall catches taken one week apart in May are likely to be more similar than catches from May and July.

To build a model for a particular dependent variable, the first step was to test all environmental and management variables (Table 4.3) independently. Those variables that were significant were then added to the model using a step-up procedure, whereby variables were retained in the model if they were significant and any variables that were no longer significant were removed. Significance was assessed at the  $P < 0.05$  level by Type 3 F-Tests and random effects were tested using Wald statistics. To consider curvilinear relationships, the quadratics of all continuous variables and interactions of interest were tested in the model and retained if significant. In SAS, F-tests are automatically adjusted for overdispersion (Littell *et al.*, 1996), which was necessary because the abundance data showed signs of overdispersion. The Akaike's Information Criterion (AIC) was used to support selection of the best covariance structure and model. The use of stepwise selection to build a model and the subsequent conclusion that the remaining variables in the model are 'important' has been criticised by Burnham and Anderson (2002). It should be noted that the discussion of important variables in this thesis is relative, i.e. it is assumed that the variables remaining in the final model are of greater importance to the dependent variable than those that were dropped through the process of model building.

#### *Dung beetle assemblage structure*

The total abundance of *Aphodius*, *Cercyon* and *Sphaeridium* in each individual trapping period from each untreated field in each year were ordinated after transformation to proportional data (see 3.2.2 for details of the ordination procedure). Axis 1 scores were used as the dependent variable in a mixed model with repeated measures because axis 1 accounts for relatively more variance than the other axes (Gauch, 1982). It was not necessary to use abundance data corrected to a 10-day period because, by using proportional data, the relative abundance of insects was considered. All of the independent variables (Table 4.3) were tested in the model.

### 4.3 Results

#### 4.3.1 Environmental variables

##### Climate

In individual trapping periods from April to July, recorded rainfall ranged from 0.2-67.4 mm with highest monthly rainfall occurring in June in 2002 and in May in 2003. Sunshine hours in individual trapping periods ranged from 48-145 hours with highest sunshine hours in 2002 occurring in May. In 2003, mean monthly sunshine was similar from April through to July with just a slight decrease in May. There was a negative correlation between rainfall and sunshine hours in both years (Figures 4.2a and b) thus rainfall was selected as the main variable to characterise both rainfall and sunshine hours in the models.

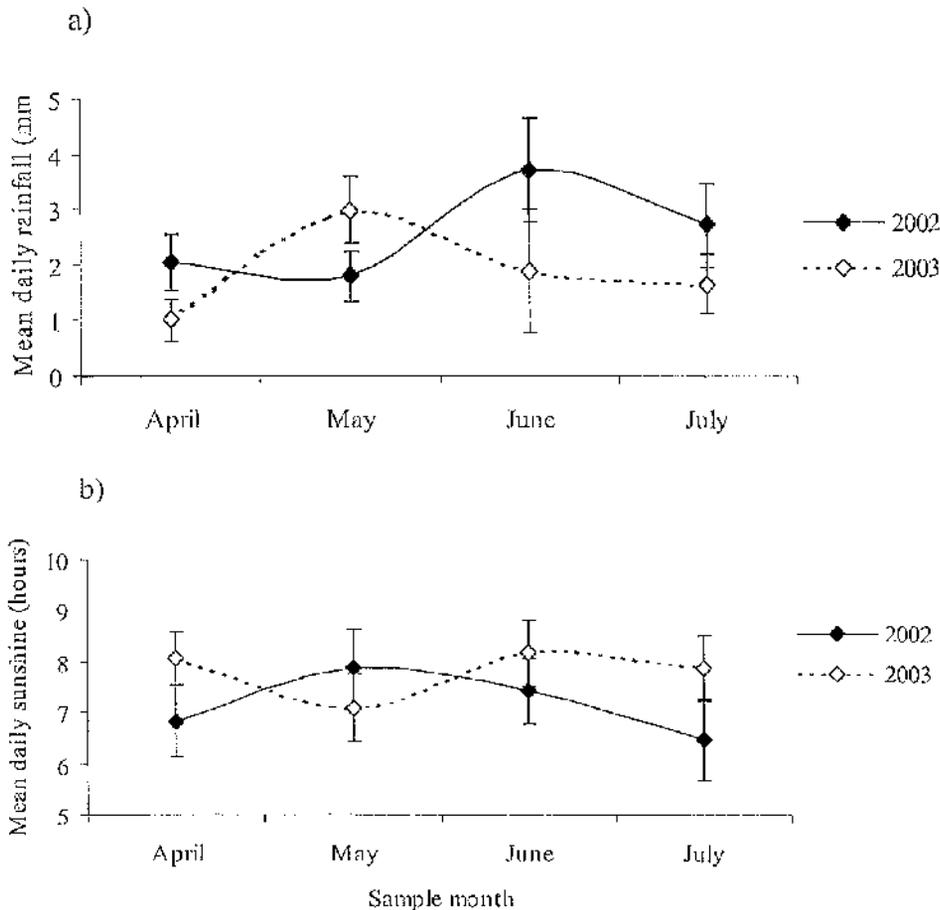


Figure 4.2 – Mean ( $\pm$  1 se) daily rainfall (a) and sunshine hours (b) for each sample month. Rainfall and sunshine were negatively correlated in 2002 ( $r_s=-0.47$ ,  $P<0.001$ ,  $df=120$ ) and in 2003 ( $r_s=-0.48$ ,  $P<0.001$ ,  $df=120$ )

The mean maximum and minimum daily temperatures were positively correlated with season (Figure 4.3a and b, respectively). There was little difference in mean minimum temperature between the sample years however mean maximum temperature was generally lower in 2002.

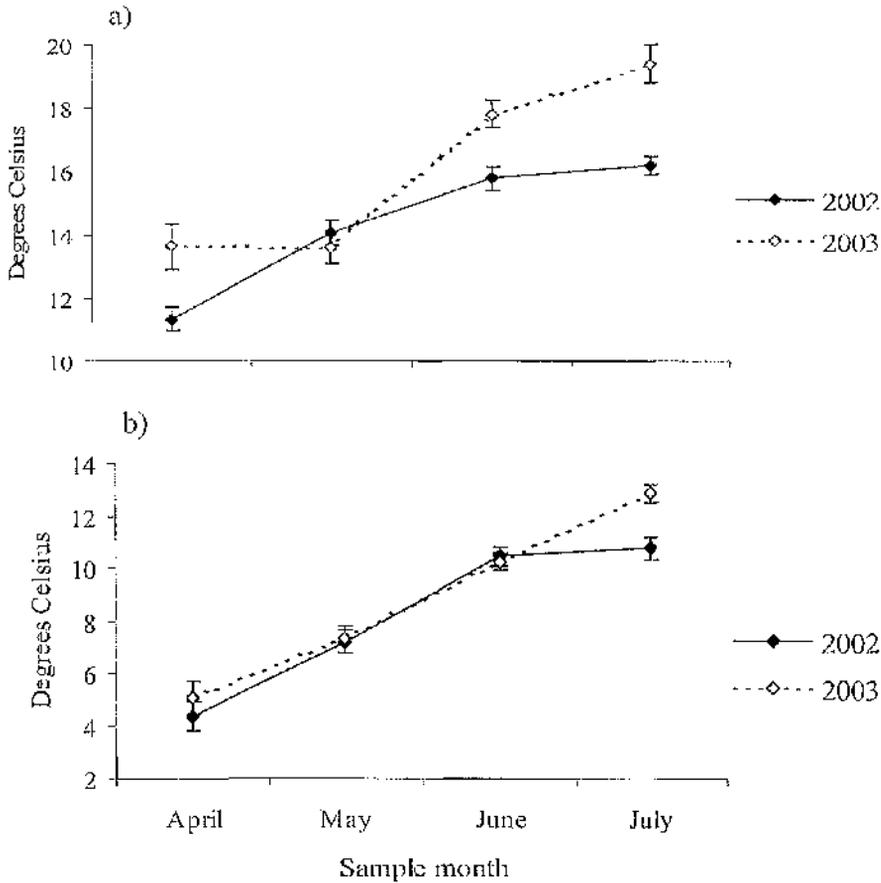


Figure 4.3 - Mean ( $\pm 1$  se) daily maximum temperature (a) and minimum temperature (b) for each sample month. There was a positive correlation between maximum temperature and days from 1 April in 2002 ( $r_s=0.66$ ,  $P<0.001$ ,  $df=120$ ) and 2003 ( $r_s=0.66$ ,  $P<0.001$ ,  $df=120$ ). Minimum temperature also increased with days since 1 April in 2002 ( $r_s=0.75$ ,  $P<0.001$ ,  $df=120$ ) and 2003 ( $r_s=0.83$ ,  $P<0.001$ ,  $df=120$ ).

#### *Temporal variables*

Two variables were used to assess changes in dung insect populations over time. 'Seasonality' was a measure of the number of days since 1st April and therefore gave a straightforward indication of change over the sampling season. It is worth noting here that for a pattern to be termed seasonal it must be shown to repeat year after

year (Wolda, 1988). Thus, 'seasonality' is used here in a general sense to mean the observed pattern of abundance across the season in the two study years.

'Days post-turnout' was a measure of the number of days since cattle had been turned out to pasture for grazing. The purpose of the 'days post-turnout' variable was to give an indication of population characteristics before and after cattle were turned out to grazing. For example, if a trapping period occurred before a pasture was grazed, a lack of fresh dung for insects to colonise might have influenced the numbers of dung insects attracted to the baited pitfall traps. However, in all untreated fields, cattle had been out at pasture before trapping commenced hence seasonality and 'days post turnout' were highly correlated in 2002 ( $r_s=0.99$ ,  $df=55$ ,  $P<0.001$ ) and in 2003 ( $r_s=0.99$ ,  $df=60$ ,  $P<0.001$ ). As the two variables were confounded, seasonality was used as a measure of temporal change in dung insect populations.

#### *Habitat variables - landscape*

Fields containing untreated cattle were ordinated by habitat characteristics, which were aspect, altitude, boundary, pasture index and adjacency to woodland. Eigenvalues were 0.0169 for axis 1 and 0.0007 for axis 2 therefore variation in habitat characteristics between fields was less than two per cent (Appendix IX.). Such low eigenvalues would be expected because there was always a value for each habitat variable in the data matrix and only the level of the variable changed. However, ordination was not performed to determine the level of variation between pastures but to gauge how similar or dissimilar the study fields were in terms of habitat characteristics. Most of the habitat characteristics remained unchanged in any one field from one year to the next with pasture index being the variable most likely to change. Indeed, a change in pasture index explained the different ordination positions of fields that were sampled in both 2002 and 2003. The mean ( $\pm 1$  se) pasture index value for untreated fields in 2002 was  $60 \pm 7.1\%$  which was lower than the mean value in 2003 of  $71.8 \pm 3.9\%$ , but not significantly so (Mann-Whitney,  $n=18$ ,  $P=0.28$ ). Fields SC3, BTBC2, WMC3 and WMC4 all had a higher pasture index in 2003 thus explaining the lower position of 2003 fields on axis 2 relative to their 2002 counterparts (Figure 4.4). The fields DC5 and DC6 were the exception as

these had a slightly higher pasture index in 2002. MTC and BR occurred to the right of axis 1 because both fields were adjacent to woodland. SC3 and BTBC2 were also adjacent to woodland however BTBC2 was pulled towards the left of axis 1 because it had a higher altitude than the other 'woodland' fields (Figures 4.4 and 4.5).

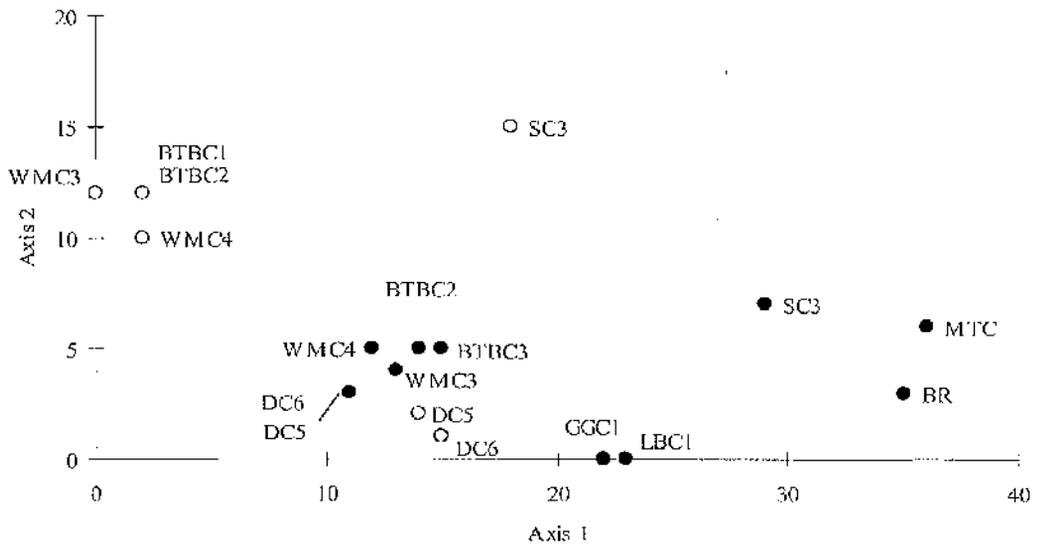


Figure 4.4 – Ordination of study pastures, grazed by untreated cattle, by habitat characteristics of aspect, altitude, boundary, pasture index and adjacency to woodland

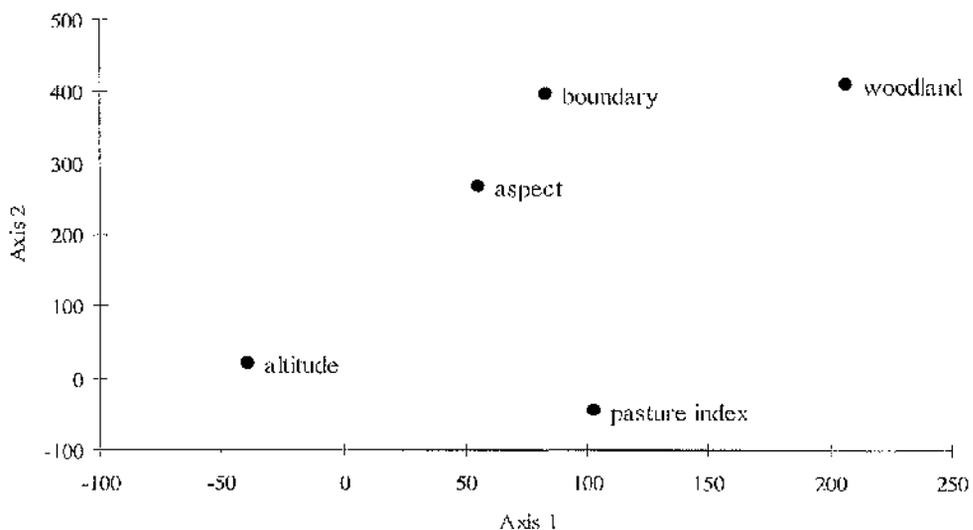


Figure 4.5 – Ordination of habitat characteristics in sampled pastures grazed by untreated cattle

The avermectin index is a measure of the proportion of pasture grazed by avermectin-treated cattle in a 0.5 km<sup>2</sup> area surrounding the 'untreated' study field. The index values were low for all fields grazed by untreated cattle with a mean ( $\pm$  1 se) value of  $3.9 \pm 2.1\%$  in 2002 and  $0.8 \pm 2.1\%$  in 2003. The avermectin index of

untreated pastures did not differ significantly between study years (Mann-Whitney,  $n=18$ ,  $P=0.21$ ).

#### *Habitat variables – field*

Over the two sample years, sward height in untreated fields ranged from 4-30 cm. Sward height was significantly higher in pastures sampled in 2002 than in 2003 ( $F_{1, 36.1}=20.08$ ,  $P<0.0001$ ) as mean sward height was  $16.6 \pm 0.7$  cm in 2002 and  $12.2 \pm 0.6$  cm in 2003.

The dung index estimated the number of dung pats deposited per hectare in each trapping period in a study field. To make inter-annual comparisons, the dung index was corrected to 10 days (as insect abundance was) to enable direct comparisons between trapping periods that occurred in 2002 and 2003. With the correction applied, the number of dung pats was significantly higher in fields sampled in 2003 ( $F_{1, 115}=32.65$ ,  $P<0.0001$ ). The mean number of pats deposited per 10-day period in fields was  $30.6 \pm 1.8$  pats  $ha^{-1}$  in 2002 and  $58.9 \pm 3.9$  pats  $ha^{-1}$  in 2003. In 2002, cattle in all sampled pastures were rotationally grazed with other fields. With the exception of two fields (LBC1 and GGC1) that were permanently grazed, all untreated fields sampled in 2003 were rotationally grazed.

#### *Management intensity of pastures*

Neither the use nor the management intensity of the six untreated fields that were sampled in both years changed from 2002 to 2003. The management intensity scores of untreated fields ranged from 10-17 in both years, and the mean intensity score was  $14.1 \pm 0.9$  in 2002 and  $13.6 \pm 0.7$  in 2003, therefore there was no significant difference in the management intensity between years (Mann-Whitney,  $n=18$ ,  $P=0.58$ ).

Carabid and spider communities were sampled to detect whether the composition of these 'management indicator' species differed between study pastures. Of the carabids, *Nebria brevicollis* was the most common species in all fields making up 93% of the catch. The remaining fifteen species that were recorded occurred in low numbers. Ordination of fields according to the ground beetle assemblages and species scores are shown in Figures 4.6 and 4.7, respectively. Seven fields were

situated in the middle of axis 1 with one field to the left of this, and one to the right. Field MTC contained five species, *Amara aenea*, *A. plebeja*, *Clivina fossor*, *Harpalus rufipes* and *Pterostichus strenuus*, that were not trapped in any other field and *Bembidion lampros*, *P. vernalis* and *Trechus quadristriatus* were only trapped in field BR. Axes 1 and 2 had eigenvalues of 0.146 and 0.012, respectively, indicating that variation in the carabid assemblages among the study fields was less than 15% (a list of the sample and species scores is given in Appendix X.). The lengths of the axes were small and indicated that the differences in the carabid assemblages between study fields were minor.

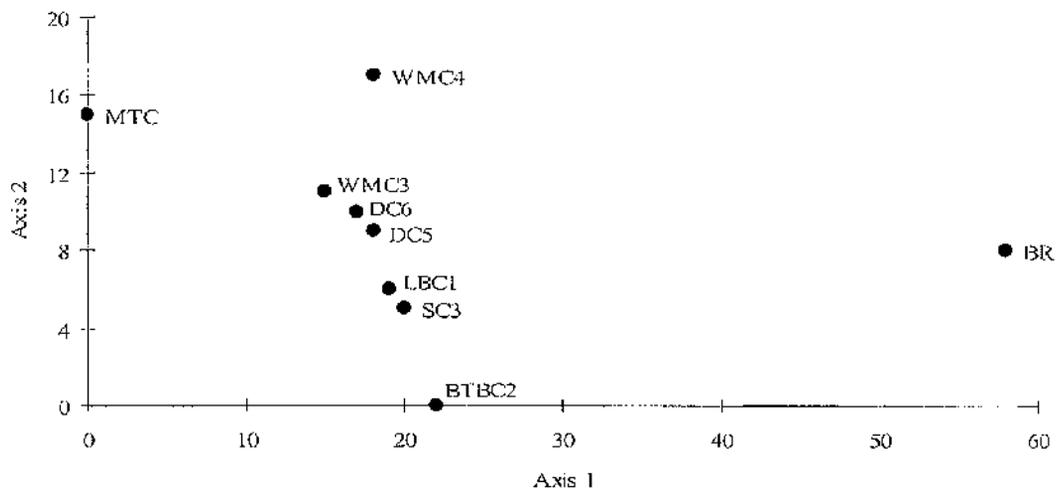


Figure 4.6 – Ordination of carabid assemblages sampled in September 2003 from nine fields grazed by untreated cattle

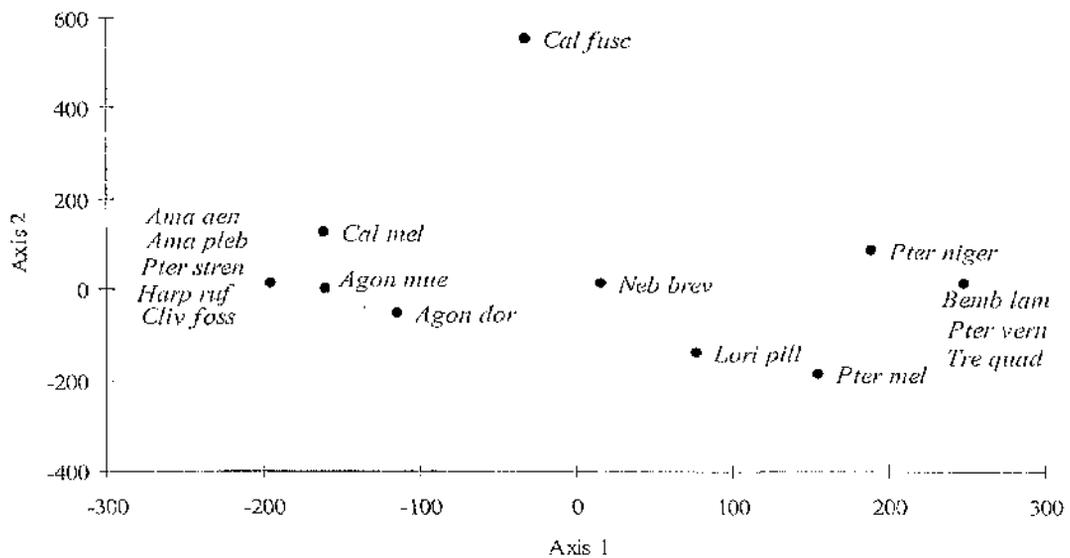


Figure 4.7 – Species scores from ordination of carabid assemblages sampled in September 2003 from nine fields grazed by untreated cattle. A list of species abbreviations is given in Appendix I.

The ordination of fields by spider assemblages and the species scores are shown in Figures 4.8 and 4.9, respectively. Eigenvalues were 0.105 for axis 1 and 0.029 for axis 2 (Appendix XI.) thus the variation in spider assemblages between fields was low. Fields were spread out along both axes although axes lengths were not large (Figure 4.8). Several species were recorded in all fields e.g. *Bathyphantes gracilis*, *Erigone dentipalpis* and *E. atra*. The low position of field DC6 on axis 2 was caused by the occurrence of *Allomengea scopigera* in that field. Field MTC was situated to the far left of axis 1, driven mainly by the absence of the otherwise ubiquitous species *Lepthyphantes tenuis*. Field BTBC2 was situated at the far right of axis 1 because it was the only field in which *L. pallidus* was recorded.

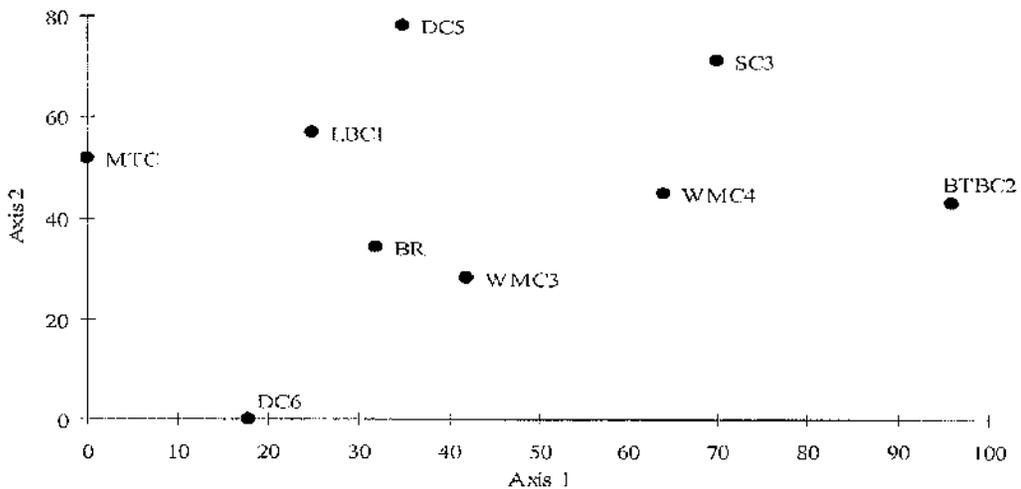


Figure 4.8 – Ordination of spider assemblages sampled in September 2003 from nine fields grazed by untreated cattle

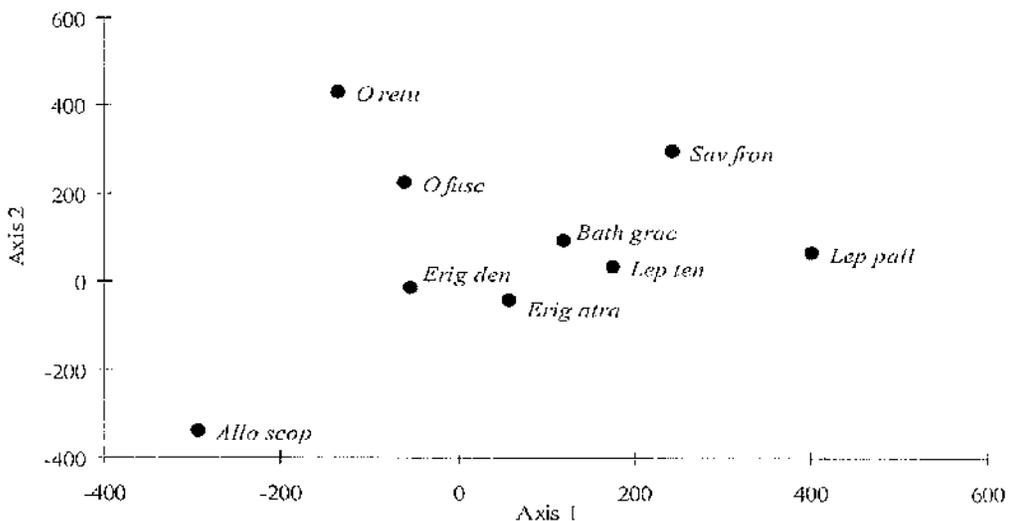


Figure 4.9 – Species scores from ordination of spider assemblages sampled from nine fields grazed by untreated cattle. Species abbreviations are given in Appendix I.

## 4.3.2 Abundance, species richness and diversity of dung insects

The numbers of dung insects trapped in pastures grazed by untreated cattle in 2002 and 2003 were summarised for all trapping periods (Table 4.4).

Species	2002 (n=57)	2003 (n=62)
<i>Aphodius ater</i>	36	15
<i>A. depressus</i>	1085	203
<i>A. fimetarius</i>	3	1
<i>A. fossor</i>	3	5
<i>A. pusillus</i>	0	3
<i>A. rufipes</i>	128	68
<i>A. rufus</i>	0	2
<b>Aphodius Guild 1</b>	<b>1255</b>	<b>297</b>
<i>A. prodromus</i>	373	412
<i>A. sphacelatus</i>	99	47
<b>Aphodius Guild 2</b>	<b>472</b>	<b>459</b>
<i>Cercyon atomarius</i>	783	1933
<i>C. haemorrhoidalis</i>	148	501
<i>C. lateralis</i>	233	823
<i>C. lugubris</i>	13	60
<i>C. melanocephalus</i>	1150	2193
<i>C. pygmaeus</i>	41	26
<b>Cercyon</b>	<b>2368</b>	<b>5536</b>
<i>Sphaeridium lunatum</i>	8	1
<i>S. scarabaeoides</i>	36	11
<b>Sphaeridium</b>	<b>44</b>	<b>12</b>
<i>Scatophaga furcata</i>	69	263
<i>S. inquinata</i>	63	4
<i>S. stercoraria</i>	11595	1740
<b>Scatophaga</b>	<b>11727</b>	<b>2007</b>

Table 4.4 - Abundance of each species of dung insects sampled in untreated fields from April to July in 2002 and 2003 (n = number of trapping periods). See Appendix I. for species nomenclature

The abundance model for *Aphodius* Guild 1 individuals is shown in Table 4.5. Significantly more Guild 1 *Aphodius* were trapped in 2002 (Figure 4.10a) and abundance had a mid-range low during periods with between 28-40 mm of rainfall. The abundance of the two most commonly trapped Guild 1 species, *A. depressus* and *A. rufipes*, are shown in Figures 4.10 b and c, respectively. Highest numbers of *A. depressus* were recorded in May although it was relatively common in all months of the sampling season. One can see from Figures 4.10a and b that *A. depressus* drives the overall seasonal abundance pattern of Guild 1 species. Very few or no

individuals of *A. rufipes* were trapped in April and May and their abundance increased in June through to July.

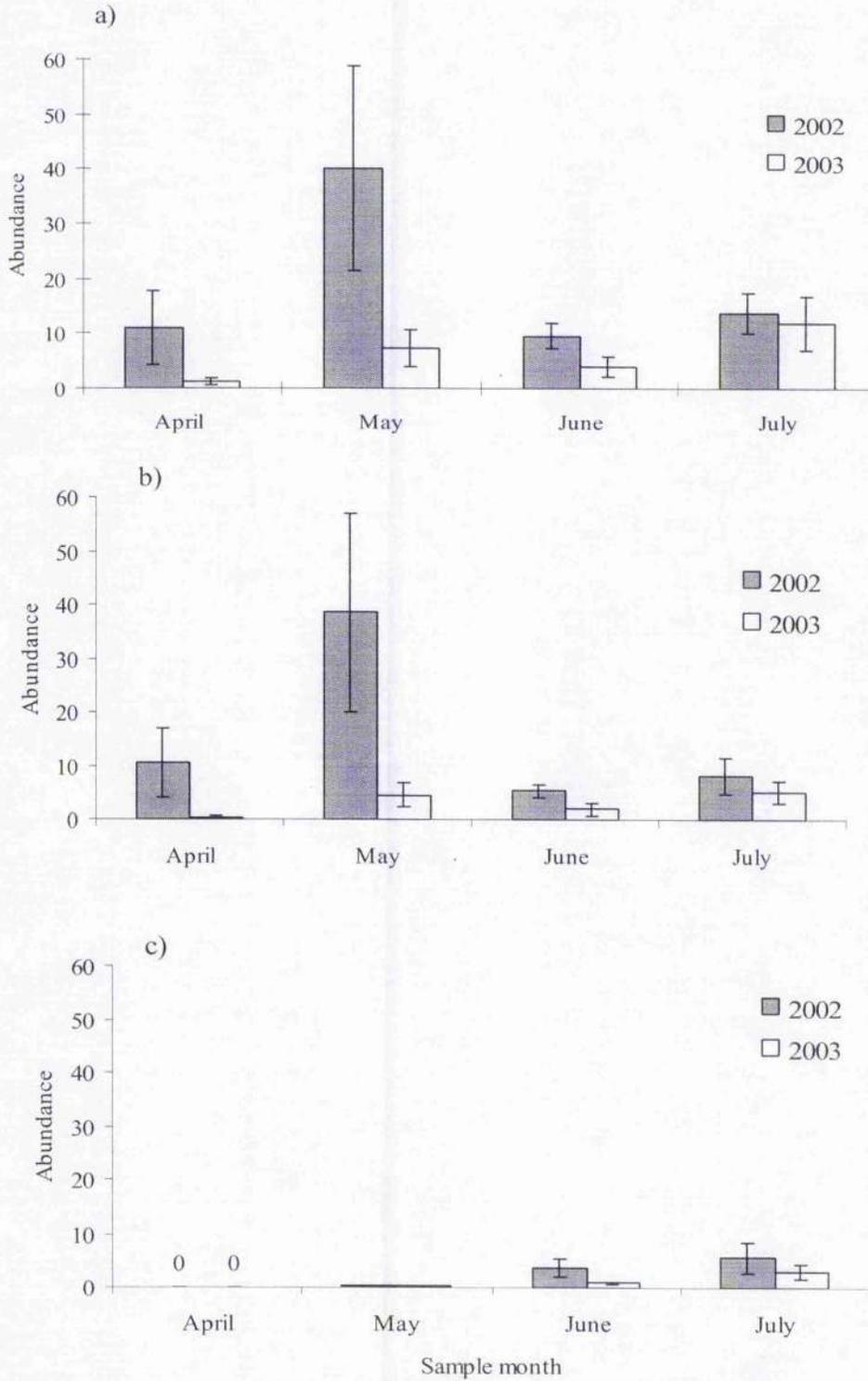


Figure 4.10 – Mean number ( $\pm 1$  se) of beetles trapped in fields grazed by untreated cattle in each sample month of 2002 and 2003 for a) all Guild 1 *Aphodius* beetles b) *A. depressus* c) *A. rufipes*

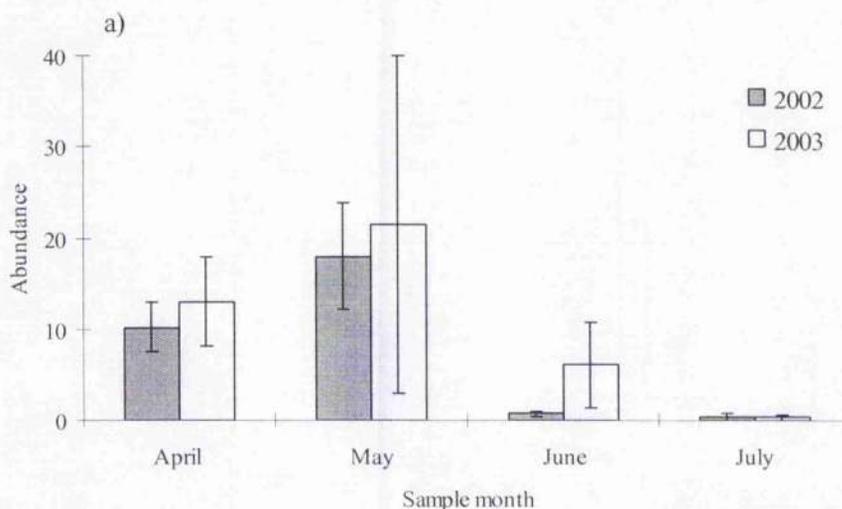
Variable	Estimate	se	Test statistics	P
<b><i>Aphodius</i> Guild 1</b>				
Field			Z=0.79	0.22
Farm			Z=1.35	0.09
Year	1.742	0.363	$F_{1,60.2}=23$	<0.0001
Rain	-0.164	0.021	$F_{1,99.2}=61.48$	<0.0001
Rain <sup>2</sup>	0.002	0.0004	$F_{1,104}=41.33$	<0.0001
Intercept	3.29	0.507		

Table 4.5 – Model of *Aphodius* Guild 1 abundance in fields grazed by untreated cattle, sampled from April to July in 2002 and 2003

The abundance of Guild 2 *Aphodius* i.e. *A. prodromus* and *A. sphacelatus* was negatively correlated with season (Figure 4.11a) and numbers were lowest in trapping periods with between 26-38 mm rainfall (Table 4.6). Abundance of both Guild 2 species, *A. prodromus* and *A. sphacelatus*, were highest in late-April and May and declined through June to July (Figures 4.11b and c, respectively).

Variable	Estimate	se	Test statistics	P
<b><i>Aphodius</i> Guild 2</b>				
Field			Z=0.73	0.234
Farm			Z=1.25	0.106
Seasonality	-0.083	0.008	$F_{1,55}=101.86$	<0.0001
Rain	-0.102	0.021	$F_{1,99.2}=23.31$	<0.0001
Rain <sup>2</sup>	0.001	0.0004	$F_{1,104}=9.42$	0.003
Intercept	7.606	0.685		

Table 4.6 – Model of *Aphodius* Guild 2 abundance in fields grazed by untreated cattle, sampled from April to July in 2002 and 2003



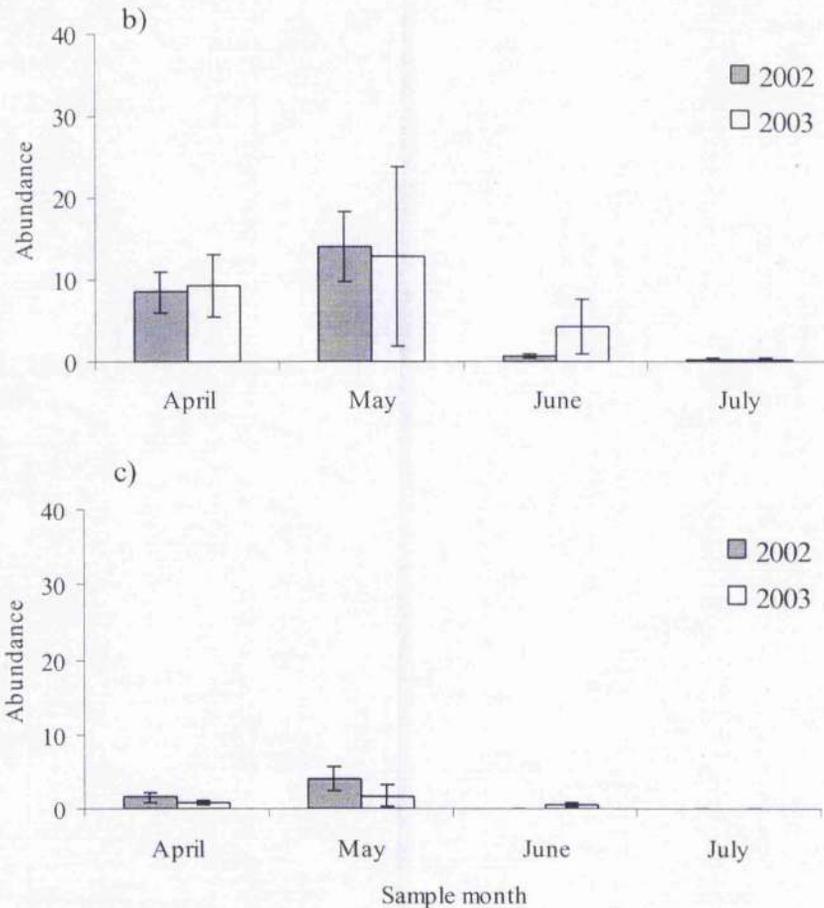


Figure 4.11 – Mean number ( $\pm 1$  se) of beetles trapped in fields grazed by untreated cattle in each sample month of 2002 and 2003 for a) all Guild 2 *Aphodius* beetles b) *A. prodromus* c) *A. sphacelatus*

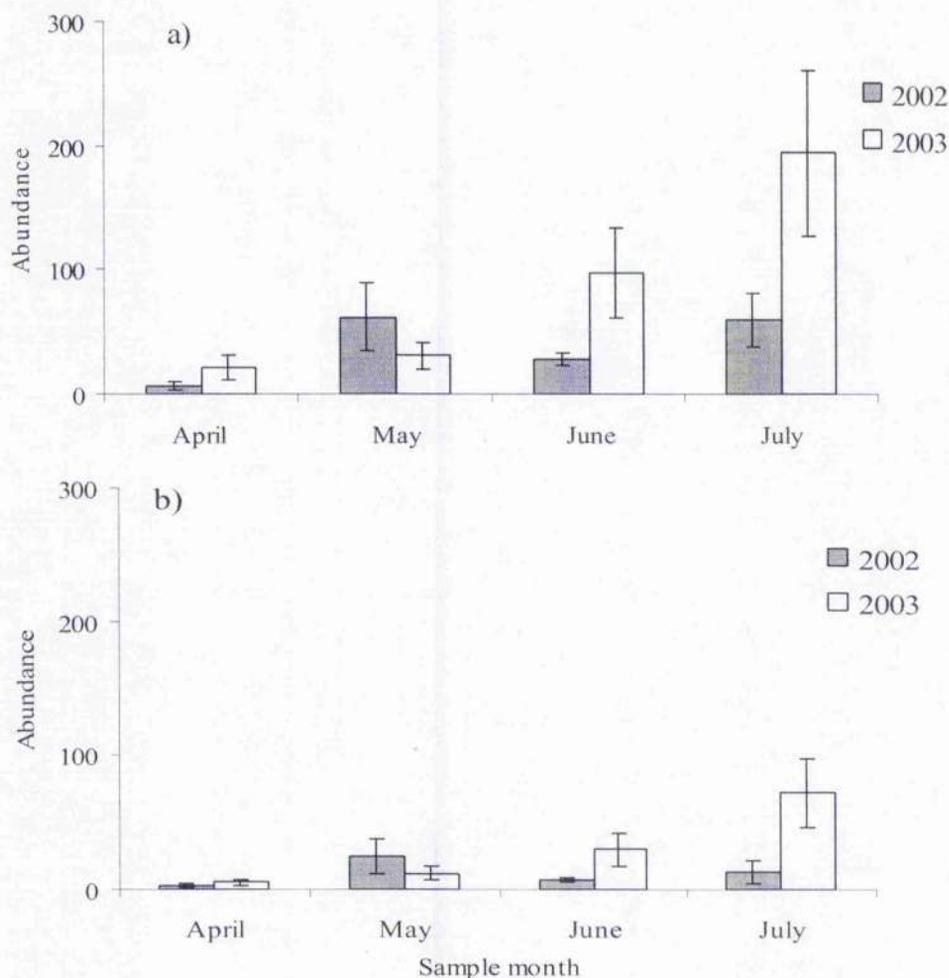
The *Cercyon* abundance model could not estimate 'farm' as a random factor (Table 4.7). The random factor 'field' was significant indicating that abundance differed significantly between fields. The graph of relative abundance of *Cercyon* per field shows that numbers of *Cercyon* were particularly high in two fields, MTC and BR, in relation to all other fields (Appendix XIX.). Models were constructed using data with and without those two fields to assess whether, in particular, inclusion of those two fields' data affected the significance of year since those two fields were only sampled in 2003. However, the factors included in the final models remained the same with and without the data from MTC and BR, therefore the model using the data from all fields is shown here.

Of the two sample years, *Cercyon* occurred in highest numbers in 2003 (Figure 4.12a). The effect of season on *Cercyon* abundance differed between the two years

(Table 4.7). There was no relationship between abundance and seasonality in 2002, however the number of *Cercyon* trapped increased from April to July in 2003. Figures 4.12b and c show the abundance of the two most abundant *Cercyon* species in each sample month. In 2002, seasonality of *C. atomarius* and *C. melanocephalus* is not discernible, but an increase in abundance across the season is apparent for both species in 2003.

Variable	Estimate	se	Test statistics	P
<b><i>Cercyon</i></b>				
Field			Z=1.67	0.047
Year	2.289	0.667	F <sub>1,47.8</sub> =11.78	0.001
Seasonality	0.0203	0.0051	F <sub>1,53.5</sub> =0.88	0.351
Year*Seasonality	-0.032	0.009	F <sub>1,47.8</sub> =11.98	0.001
Intercept	2.441	0.509		

Table 4.7 – Model of *Cercyon* abundance in fields grazed by untreated cattle, sampled from April to July in 2002 and 2003



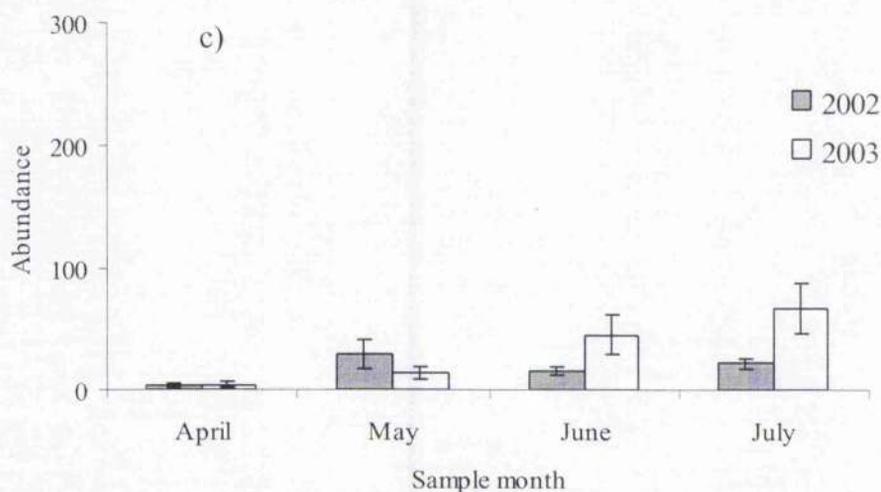


Figure 4.12 – Mean number ( $\pm 1$  se) of beetles trapped in fields grazed by untreated cattle in each sample month of 2002 and 2003 for a) all *Cercyon* beetles b) *C. atomarius* c) *C. melanocephalus*

The random factor 'farm' could not be estimated in the abundance model for the yellow dung-fly, *Scatophaga stercoraria*, (Table 4.8). Significantly more *S. stercoraria* were trapped in 2002, and there was a significant quadratic relationship with season (Figure 4.13). Abundance was highest in late April and early May and then declined so that abundance was lowest from 10<sup>th</sup> June until late June. From early July onwards, a slight increase in numbers was apparent. Seasonal patterns of abundance did not differ significantly between the two sample years ( $F_{1, 54.3}=0.02$ ,  $P=0.88$ ).

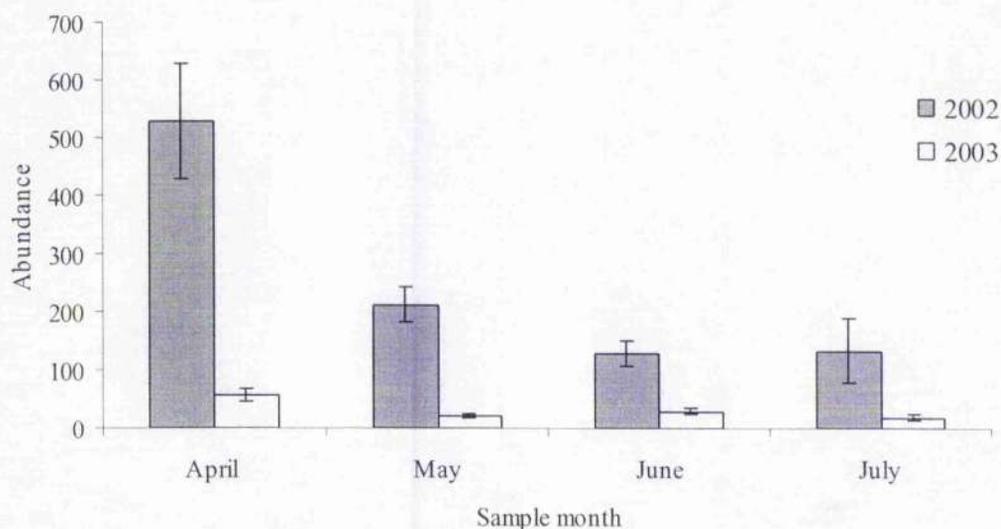


Figure 4.13 – Mean number ( $\pm 1$  se) of *Scatophaga stercoraria* trapped in fields grazed by untreated cattle in each sample month of 2002 and 2003

Variable	Estimate	se	Test statistics	P
<b>Yellow dung-fly</b>				
Field			Z=1.11	0.133
Year	1.891	0.185	F <sub>1,53.4</sub> =104.88	<0.0001
Seasonality	-0.117	0.015	F <sub>1,59.1</sub> =59.82	<0.0001
Seasonality <sup>2</sup>	0.0008	0.0001	F <sub>1,61.8</sub> =41.92	<0.0001
Intercept	7.302	0.459		

Table 4.8 – Model of *Scatophaga stercoraria* abundance in fields grazed by untreated cattle, sampled from April to July in 2002 and 2003

The number of *Aphodius* species trapped in pastures grazed by untreated cattle decreased throughout the sampling season (Table 4.9). The number of *Aphodius* species occurring in pastures was significantly higher in 2002 (Figure 4.14).

Variable	Estimate	se	Test statistics	P
<b><i>Aphodius</i> Species Richness</b>				
Field			Z=0.52	0.302
Farm			Z=1.34	0.09
Year	0.64	0.248	F <sub>1,33.4</sub> =6.67	0.014
Seasonality	-0.017	0.005	F <sub>1,54</sub> =14.27	0.0004
Intercept	3.164	0.51		

Table 4.9 – Model of *Aphodius* species richness in fields grazed by untreated cattle, sampled from April to July in 2002 and 2003

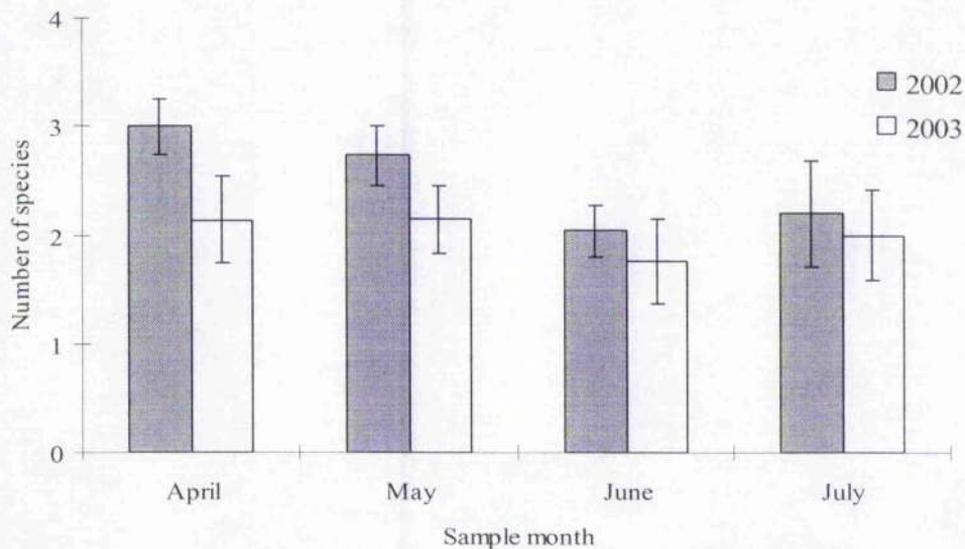


Figure 4.14 – Mean number ( $\pm 1$  se) of *Aphodius* species trapped in fields grazed by untreated cattle in each sample month of 2002 and 2003

The number of *Cercyon* species increased across the sampling season in both years (Figure 4.15). Species richness was lowest in trapping periods with between 28-50 mm rainfall (Table 4.10).

Variable	Estimate	se	Test statistics	P
<b><i>Cercyon</i> Species Richness</b>				
Field			Z<0.01	0.499
Farm			Z=1.27	0.102
Seasonality	0.025	0.005	F <sub>1,58</sub> =30.31	<0.0001
Rain	-0.109	0.028	F <sub>1,104</sub> =14.75	0.0002
Rain <sup>2</sup>	0.002	0.0005	F <sub>1,104</sub> =12.01	0.0008
Intercept	3.489	0.511		

Table 4.10 - Model of *Cercyon* species richness in fields grazed by untreated cattle, sampled from April to July in 2002 and 2003

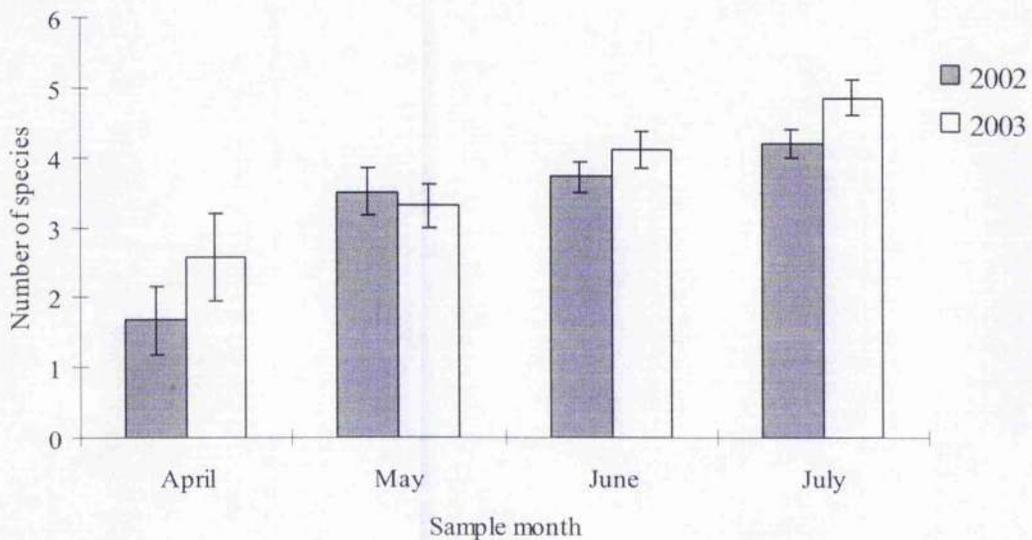


Figure 4.15 – Mean number ( $\pm 1$  se) of *Cercyon* species trapped in fields grazed by untreated cattle in each sample month of 2002 and 2003

The diversity of dung insects was significantly higher in 2003 (Figure 4.16). There were significant relationships between diversity and both seasonality and rainfall (Table 4.11). Diversity was lowest in late April and early May and then increased until early July before levelling off. Dung insect diversity was lowest in trapping periods with between 26-46 mm of rainfall.

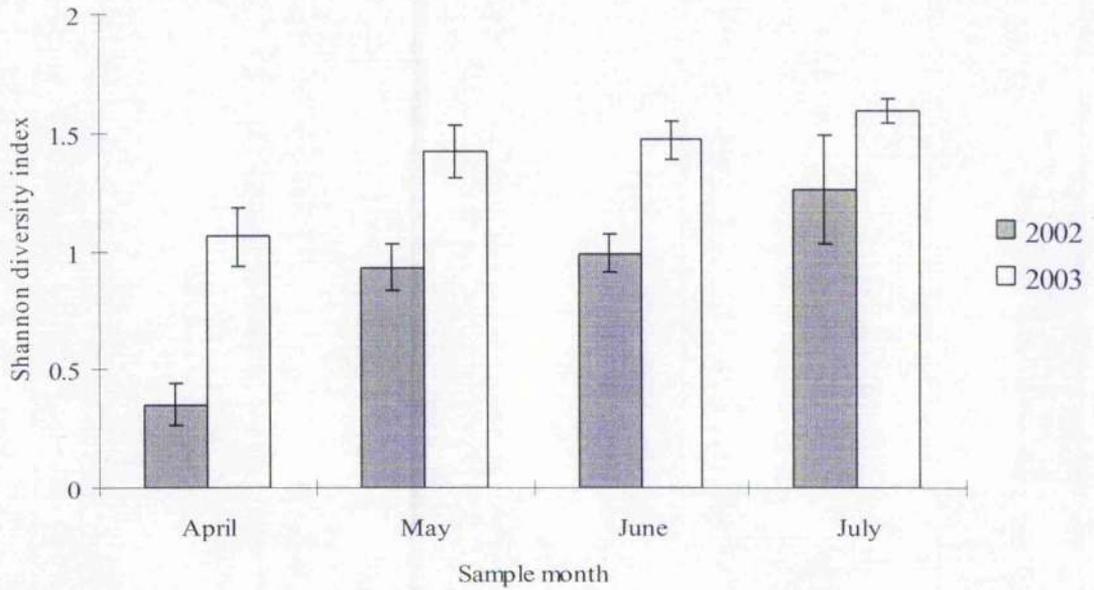


Figure 4.16 – Mean Shannon diversity index ( $\pm 1$  se) of dung insects trapped in fields grazed by untreated cattle in each sample month of 2002 and 2003

Variable	Estimate	se	Test statistics	P
<b>Diversity</b>				
Field			Z=0.66	0.255
Farm			Z=0.18	0.431
Year	-0.505	0.076	F <sub>1, 49.6</sub> =44.36	<0.0001
Seasonality	0.033	0.01	F <sub>1, 74.2</sub> =10.98	0.001
Seasonality <sup>2</sup>	-0.0002	0.00007	F <sub>1, 73.1</sub> =8.13	0.006
Rain	-0.026	0.01	F <sub>1, 99.2</sub> =7.2	0.009
Rain <sup>2</sup>	0.0003	0.00016	F <sub>1, 99.9</sub> =4.85	0.03
Intercept	0.596	0.33		

Table 4.11 - Model of dung insect diversity measured by the Shannon index in fields grazed by untreated cattle, sampled from April to July in 2002 and 2003

#### 4.3.3 Dung beetle assemblage structure

The species composition data of dung beetles in each trapping period were ordinated. The resulting axes 1 and 2 scores were 0.394 and 0.153, respectively, therefore explaining more than 50% of the variance in dung beetle assemblages (Appendix XII.). Axis 1 scores were placed in a mixed model with repeated measures and the final model indicated a significant relationship with season (Table 4.12).

The plot of the first two axes from the ordination of dung beetles (Figure 4.17) highlighted the seasonal effect, with trapping periods in April and May grouped to

the right of the ordination plot. This indicated that April and May periods had relatively more *A. prodromus*, *A. sphacelatus*, *A. ater*, *A. fimetarius*, *A. fossor*, *C. pygmaeus* and *Sphaeridium* species than June and July trapping periods (Figure 4.18). Numbers of *A. fimetarius* and *A. fossor* were extremely low but, when they were trapped, they occurred in May trapping periods.

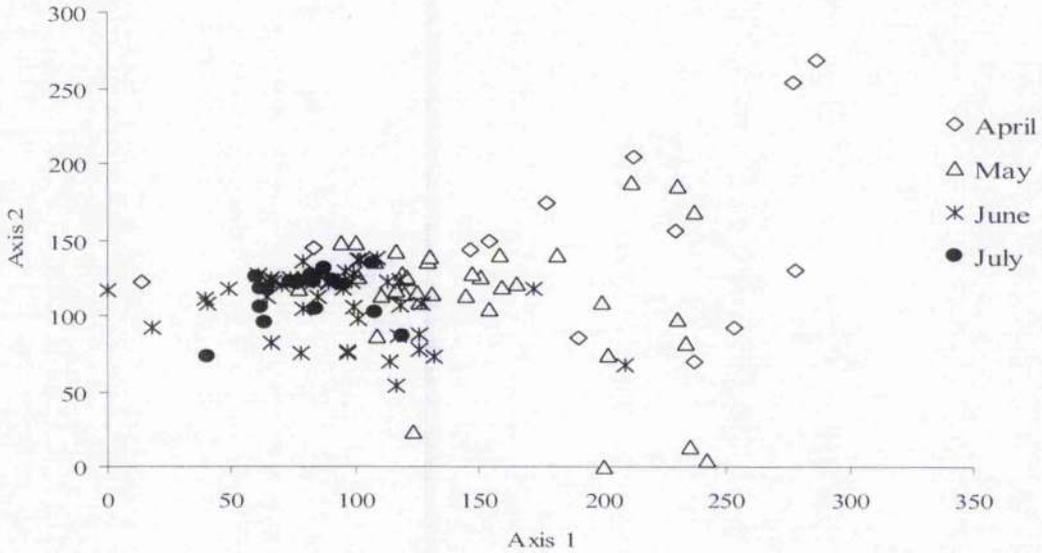


Figure 4.17 – Sample scores from ordination of dung beetle assemblages sampled from April to July in 2002 and 2003 in fields grazed by untreated cattle

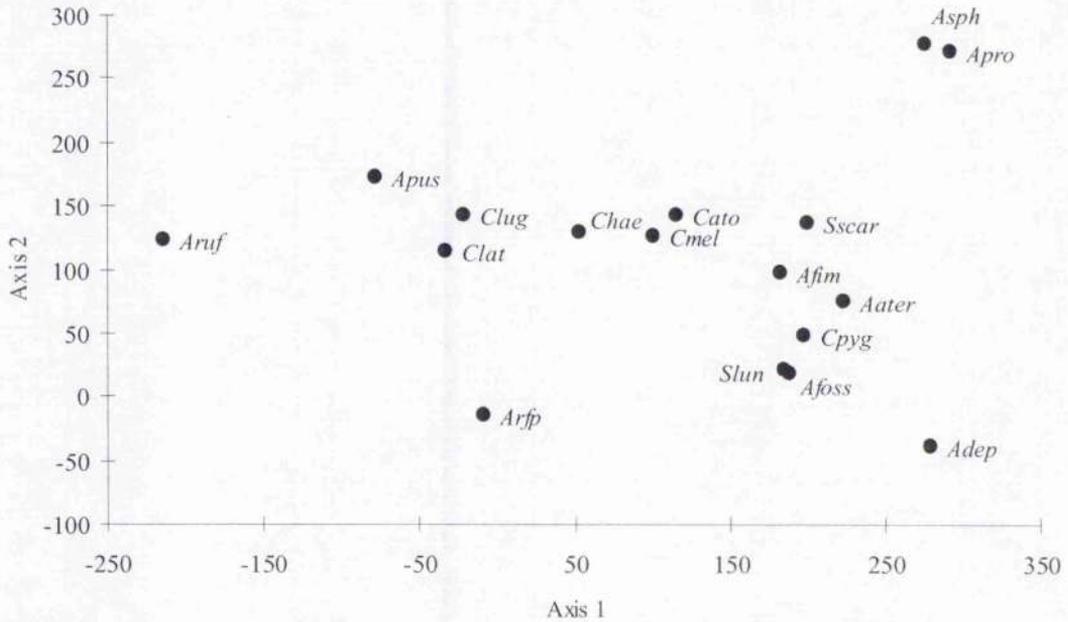


Figure 4.18 – Species scores from ordination of dung beetle assemblages sampled from April to July in 2002 and 2003 in fields grazed by untreated cattle. Species abbreviations are given in Appendix I.

Variable	Estimate	se	Test statistics	P
<b>Dung beetle assemblage structure</b>				
Field			Z=0.05	0.479
Farm			Z=0.65	0.257
Seasonality	-5.58	0.976	F <sub>1,67.3</sub> -32.66	<0.0001
Seasonality <sup>2</sup>	0.029	0.007	F <sub>1,67.3</sub> -17.52	<0.0001
Intercept	312.34	31.89		

Table 4.12 – Model of dung beetle assemblage scores from ordination of species abundance for each trapping period in untreated fields

#### 4.4 Discussion

Models were constructed using dung insect data collected in fields grazed by untreated cattle over the spring and summer of two consecutive years. Relationships between the abundance and diversity of dung insects and a range of variables relating to season, weather, pasture management and habitat were tested. The resultant models indicated that the factors that were most important for dung insect assemblages in general were inter-annual variation, weather and seasonality. These factors, which are themselves inter-related, are discussed below with reference to each of the dung insect groups studied. The untreated fields were found to be similar in terms of management intensity and this is also discussed below.

##### *Inter-annual variation*

The abundance of *Aphodius* Guild 1 and *Cercyon* beetles, and yellow dung-flies changed significantly between sample years. The significant inter-annual variation in the abundance of *Aphodius* Guild 1 beetles was driven mainly by the higher abundance of *A. depressus* in 2002. The next most common species in that guild, *A. rufipes*, was also trapped in higher numbers in 2002. It is not uncommon for *Aphodius* abundance to vary naturally between years (Finn *et al.*, 1999). However, dung availability and weather could also have potentially influenced the variation in beetle abundance between the two sample years.

In untreated fields, dung density was significantly lower in 2002 than in 2003. Therefore, it is possible that greater numbers of *Aphodius* may have been attracted to traps because of the lower availability of 'natural' dung pats in the pasture. This is supported by results from a previous trial (Chapter 3) which highlighted a non-

significant trend for higher numbers of *Aphodius* in traps when there is low availability of dung in the vicinity of those traps. Furthermore, the availability of other grazed pastures around the study fields was lower in 2002 than in 2003. Therefore, dung beetles may have been more active in study pastures in 2002 because there was less dung in surrounding pasture to colonise. Weather may have indirectly affected the availability of dung in study pastures and in the surrounding landscape. Periods of heavy rainfall in 2002 meant that some cattle were returned to housing thus causing lower density of dung in pastures and a lower proportion of grazed pasture around the study fields in that year.

Two Guild 1 species that occurred in low numbers in both years were *A. fimetarius* and *A. fossor*. They are late successional species that colonise dung up to 21 days old (Hanski, 1980d; Gittings and Giller, 1998), therefore there is a possibility that they were under-represented in traps exposed for 7-14 days. Alternatively, they may simply have been present in low numbers as studies in England and Ireland have shown that these two species occurred in relatively low numbers in comparison to other *Aphodius* species e.g. *A. ater*, *A. prodromus* and *A. rufipes* (Hanski, 1980b and c; Hutton and Giller, 2003).

Species richness of *Aphodius* was significantly higher within individual trapping periods in 2002, even though the overall number of species recorded over the whole sampling season was slightly higher in 2003. Similarly, in an Irish study, the species richness of *Aphodius* did not differ greatly between consecutive years (Finn *et al.*, 1999). Seven species of *Aphodius* were trapped in 2002 and nine in 2003, with the species *A. pusillus* and *A. rufus* recorded only in 2003. In comparison to other *Aphodius* species, *A. pusillus* is quite immobile and it exists as localised populations (Roslin, 1999; 2000). This might explain why it only occurred in 2003 as it was recorded in one field that was sampled only in that year. Similarly, *A. rufus* was only recorded in two fields that were sampled in 2003 and its presence in those two fields may have been due to their location immediately adjacent to woodland as *A. rufus* is known to prefer wooded areas (Wassmer, 1995).

Significantly more *Cercyon* beetles were trapped in 2003 and this was initially thought to have been due to their extremely high numbers in two fields (MTC and

BR) that were sampled in 2003 only. It is unclear why those two pastures, that were situated adjacent on the same farm, supported such high numbers of *Cercyon* although potential causes may have been some unmeasured factor of habitat, dung quality or historical distribution. However, inter-annual variation still occurred when data from those two fields were excluded from the model. Therefore, those two fields did not solely cause the significance of year in the model. Higher abundance in 2003 may have been a positive response to the higher summer temperatures that occurred in that year. Furthermore, the higher density of dung in fields in 2003 may have had a direct positive effect on the number of *Cercyon* that were trapped in pasture grazed by untreated cattle.

The significant inter-annual variation in the abundance of yellow dung-flies, *Scatophaga stercoraria*, in untreated fields, has been observed elsewhere for that species (Gibbons, 1987; Ward and Simmons, 1990). That variation may have occurred partly in response to differences in weather between the two years, as discussed below. Lower dung insect diversity in 2002 reflected the fact that *S. stercoraria* dominated the catch in that year.

#### *Weather*

The models indicated that the relationship between dung insects 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. Increased insect activity during low rainfall could be explained by the negative correlation between rainfall and sunshine, as beetle abundance is positively correlated with radiant energy (Lobo *et al.*, 1998). Moreover, insects may have been more active during periods with less rain because activity can be impaired by heavy rainfall (Gibbons, 1987; 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. Therefore, beetles may have been more active during short intermittent dry spells around dung baits whose colonisation 'life' would have been prolonged via rainy spells.

It should be borne in mind that the rainfall data used for the models was a total for each trapping period therefore it did not provide information on the duration of periods of rainfall or the intermittent dry spells. However, rainfall was generally higher in June and July of 2002 than 2003. Indeed, it was noted that fields sampled in 2002 became extremely waterlogged as a result of the high rainfall in the summer months (pers. obs). The *Aphodius* dung beetles that occurred in high numbers in late April and May of 2002 could have been expected to breed successfully in dung at that time. However, disintegration of pats and flooding via high rainfall could have impaired the subsequent development of larvae throughout the weeks when high rainfall occurred. Temperatures were also lower in 2002 than in 2003, thus the cooler wetter weather in 2002 could certainly have impaired *Aphodius* reproduction (Gittings and Giller, 1999). This could have caused the reduced population size in the following year that was observed in this study.

The higher numbers of yellow dung flies in 2002 may have been attributed to the cooler wetter weather in June and July as adult mortality can increase during periods of high temperatures (Ward and Simmons, 1990). It has also been proposed that adults acquiesce i.e. become inactive, during hot weather (Blanckenhorn *et al.*, 2001). Therefore, the generally high temperatures in the 2003 sampling season may have induced adult acquiescence thus causing lower numbers in baited pitfall traps.

### *Seasonality*

There was no distinct seasonal pattern for the *Aphodius* that were grouped together in Guild 1, presumably because the two most common species had different phenologies that partly 'balanced' each other. The most abundant species of that guild, *A. depressus*, occurred in all months of the study but peaked in abundance in May 2002. Its presence in all study months concurs with studies that state it is active from May through to August/ September (White, 1960; Finn *et al.*, 1998). The next most common species, *A. rufipes*, was typically abundant in the summer months of June and July (Holter, 1979b; Gittings and Giller, 1997).

Seasonality was an important factor for Guild 2 species, *A. prodromus* and *A. sphaecelatus*, which were trapped in higher numbers in April and May than in

summer. This seasonal pattern is typical of these species (Wassmer, 1994; Gittings and Giller, 1997). It was observed that *A. sphacelatus* occurred in much lower numbers than *A. prodromus* and this has also been noted elsewhere (White, 1960; Hanski, 1980f). Finn *et al.* (1998) proposed that for some *Aphodius*, inter-annual variation is not as great as seasonal variation and the *Aphodius* Guild 2 species supported that here. The species richness of *Aphodius* declined from April to July, reflecting the increase in *Aphodius* richness in early spring that was also observed by Hanski (1980f).

There was no seasonal pattern for *Cercyon* in 2002, however the abundance of *Cercyon* increased from April to July in 2003. The 2003 results agreed with observations made in Oxfordshire pastures where numbers of *Cercyon* increased from April to July (Hanski, 1980f). It is usual for *Cercyon* species not to exhibit distinct seasonal patterns, due to the overlapping generations of these multivoltine species (Hanski, 1980c). Species richness of *Cercyon* increased from April to July, as was observed elsewhere (Hanski, 1980c). In both years, *Cercyon melanocephalus* was the most abundant species and this is noted as being a common species in dung (Skidmore, 1991). Other studies in Britain found *C. haemorrhoidalis* to be the most abundant *Cercyon* species (Hanski, 1980c). In both years, six species of *Cercyon* were trapped which is considerably less than the eleven species recorded in pastures in Oxfordshire (Hanski, 1980f). Across the season, the number of species recorded was only just significantly higher in trapping periods in 2003.

The assemblage structure of *Aphodius*, *Cercyon* and *Sphaeridium* changed between spring (April and May) and summer (June and July) trapping periods. Spring trapping periods had relatively more *A. prodromus* and *A. sphacelatus* as one might expect from the seasonal distribution of these species. In this study, *Aphodius ater* was most common in May trapping periods, although this species can be active between April and July (Finn *et al.*, 1998). The hydrophilids, *Sphaeridium* spp. and *Cercyon pygmaeus*, occurred more in spring trapping periods than in summer trapping periods.

The *Aphodius* assemblage data generally followed the seasonal classification of *Aphodius* as proposed by Gittings and Giller (1997). The species that they class as

'spring/autumn', i.e. *A. prodromus* and *A. sphacelatus*, were most common in April and May and *A. rufipes* could definitely be classed as a 'late summer' species. The species, *A. ater*, was classed as an 'early summer' species by Gittings and Giller (1997) but was more typical of spring assemblages in this study as it was mainly trapped in May. The 'early summer' species, *A. pusillus*, was only trapped in late summer here, albeit in numbers far too low to place any confidence in its seasonal classification. The low occurrence of *Sphaeridium* in this study may have resulted from the sampling method used, as it has been suggested that baited pitfall traps are not as efficient for recording *Sphaeridium* as direct sampling of dung (Finn *et al.*, 1999). Two species, *S. lunatum* and *S. scarabaeoides*, were recorded. The third species found in Britain, *S. bipustulatum*, was not recorded and elsewhere it has been found to be the least common *Sphaeridium* species (Finn *et al.*, 1999).

The yellow dung-flies, *Scatophaga stercoraria*, followed the typical seasonal pattern of high numbers in spring followed by a decrease in summer months (Parker, 1970a; Gibbons, 1987). Yellow dung fly seasonality influenced the overall pattern of dung insect diversity, as diversity was lowest in April and May and then increased up until July when it levelled off.

#### *Management Intensity*

There were no major differences in the management intensity of untreated pastures, as determined by the Management Intensity Scoring process and by the relative similarity of both carabid and spider assemblages between untreated pastures. Most of the carabid species were trapped in low numbers and this was partly due to the time of sampling as relatively lower abundance and species richness of carabids is found when sampling in September in comparison to spring months (Thomas *et al.*, 2001; Meek *et al.*, 2002). The most common species in this study was *Nebria brevicollis* and it may have dominated the catch because it is highly active in the autumn (Thomas *et al.*, 2001). The low occurrence of some species may have been because they were transients in the grazed pastures, especially those species that prefer cultivated fields, e.g. *Agonum dorsale* and some *Pterostichus* species (Millán de la Peña *et al.*, 2003; Holland *et al.*, 2004). The response of carabid communities to changes in management intensity, for example inorganic fertiliser input (Blake,

1996; Döring and Kromp, 2003), makes them good indicators of pasture differences. This sampling did not detect any major differences in carabid assemblages that may have reflected differences in management intensity and habitat characteristics.

Management intensity and practice, for example cutting and grazing intensity, can alter the population size and species richness of spiders via disturbance and indirect changes to sward structure (Cherrett, 1964; Bell *et al.*, 2002; Pommeresche, 2002; Thorbek and Bilde, 2004). In this study, the two most common species were *Bathyphantes gracilis* and *Erigone dentipalpis* which are associated with improved pasture and low intensity grassland (Rushton *et al.*, 1989; Downie *et al.*, 2000). There were no major differences in spider assemblages between pastures grazed by untreated cattle. The low occurrence of Linyphiids and the absence of Lycosidae in untreated pastures may also have been due to autumn sampling as other studies have shown relatively low numbers of these families at that time of year (Meek *et al.*, 2002).

Hence, study pastures that were grazed by cattle that were not anthelmintically treated did not differ dramatically in terms of either management intensity, grazing regime or habitat structure. Of the variables that were measured and believed to be most influential for dung insects, the variation that was observed within the dung insect groups studied was mainly attributable to inter-annual fluctuations, to seasonality and to weather.

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## CHAPTER 5

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# DUNG INSECT ASSEMBLAGES IN PASTURES GRAZED BY AVERMECTIN-TREATED CATTLE

## 5. Dung insect assemblages in pastures grazed by avermectin-treated cattle

### 5.1 Introduction

Exposure to avermectin residues in dung can have lethal and sublethal effects on the many insect species that utilise dung for feeding and breeding (see review in Section 1.4). Much of the previous research that has investigated effects of avermectins on dung insects has been carried out in the laboratory or has made comparisons between experimental dung pats in a single field location (e.g. Lumaret *et al.*, 1993; Strong and Wall, 1994). However, avermectin effects at the pasture scale could differ from those at the individual pat scale if dung insect populations are resilient to any localised declines occurring within individual pats. Furthermore, in a real pasture situation, management factors and other processes may mitigate the effects of avermectins. For example, a 'dilution effect' might occur if a pasture containing treated cattle is surrounded by pastures that are grazed by untreated animals, and exposure to avermectins may be minimised if livestock treatments are asynchronous within a particular geographical area (Forbes, 1993). Also, avermectin effects may be 'overridden' by weather conditions. For example, cool wet weather during breeding periods can cause reproductive failure in *Aphodius* therefore exposure to avermectin residues at that time may not be as important if the insects are already adversely affected by weather (Gittings and Giller, 1999).

The aim of the research reported in this chapter was to compare the abundance, diversity and species composition of dung insects between pastures grazed either by avermectin-treated or untreated cattle on commercial farms, while considering overall differences in the grazing management of those pastures. Dung insect data were analysed in relation to habitat, management, climate and wider landscape variables. The research also aimed to highlight whether the potentially deleterious effects of avermectins were mitigated or exacerbated by any characteristic in a typical pasture situation.

## 5.2 Methods

### 5.2.1 Site Selection

Sampling was carried out on farms that were representative of livestock type and avermectin treatment strategy in the geographical region in which the research was conducted (see Chapter 2). Nine fields containing cattle treated with ivermectin or doramectin were sampled on four dairy farms and one beef and sheep farm in 2002. In 2003, eleven fields grazed by cattle treated with a doramectin pour-on were sampled on five dairy farms and one beef and sheep farm. Data were analysed and compared to data collected from fields grazed by cattle that had received no anthelmintic treatment of which there were seven fields in 2002 and eleven fields in 2003. Data collected from only those 'untreated' fields were analysed in relation to habitat, climatic and management factors and results were presented in Chapter 4.

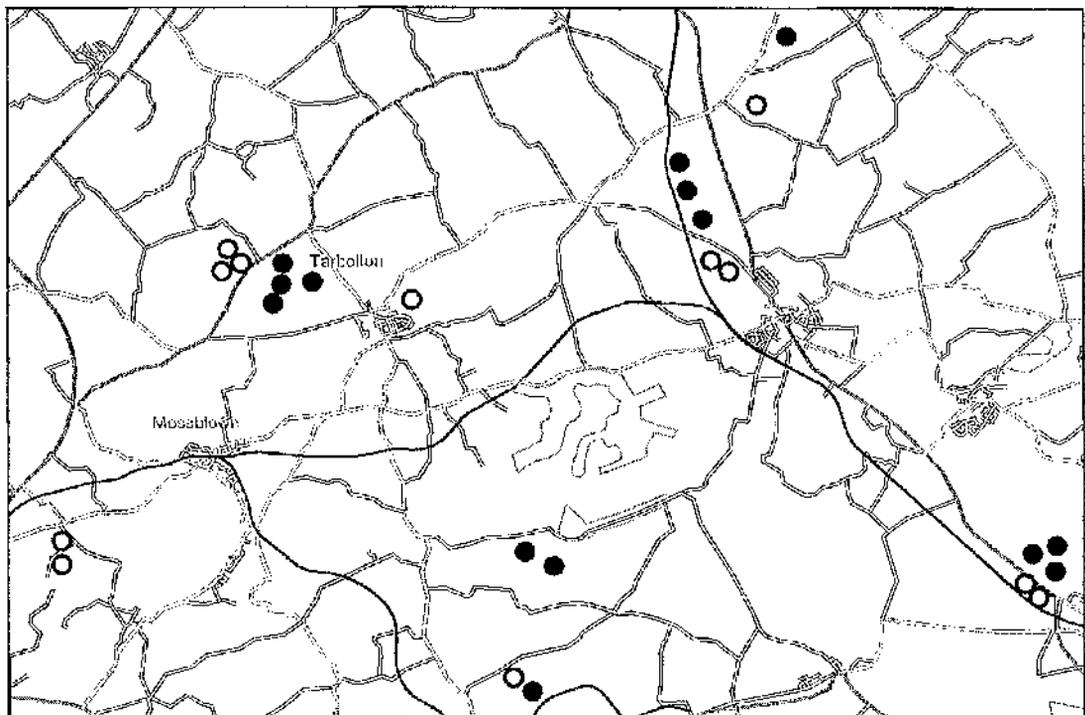


Figure 5.1 - Map showing relative location of fields grazed by untreated cattle ○ and avermectin-treated cattle ●. Grid references of fields are provided in Appendix VII. Scale 1cm: 1250m

The relative geographical locations of study fields are shown (Figure 5.1). The livestock types, treatment strategies and habitat characteristics of the total 26 fields sampled were summarised (Table 5.1). When possible, fields containing either avermectin-treated or untreated cattle were sampled on the same farm to minimise

variation due to the geographical location and the historical distribution of dung insects. However, only treated fields could be sampled on Farm 3 because all beef cattle were anthelmintically treated there, and on Farm 6 only untreated fields were sampled because livestock on that farm were not treated with avermectins at grazing.

### 5.2.2 Data Collection

#### *Dung invertebrate sampling*

Pitfall traps baited with dung from untreated cattle were used to sample dung invertebrates. Each pasture was sampled between 6-9 times throughout the sampling period from April to July in 2002 and 2003. Eight traps were set in each pasture and the trap contents from four of those traps were processed from each trapping period. Adult insects of the Scarabaeidae, Scatophagidae and the Hydrophilids *Cercyon* and *Sphaeridium* were identified to species level and counted. A full description of the sampling method and identification procedures is detailed in Section 4.2.2.

#### *Environmental variables*

This section summarises the environmental variables that were measured in pastures but for full details regarding the measurement and description of variables, refer to Section 4.2.2. Rainfall, sunshine and temperature data were collected from Auchincruive weather station and pasture characteristics including field boundary, altitude, soil impenetrability, sward height, aspect and adjacency to woodland were recorded for all fields. Soil was sampled on one occasion in each sample year to gain information on variables such as soil moisture, pH, phosphorous, potassium and organic content. The density of fresh dung deposited per trapping period in a pasture, and indices of avermectin use and pasture availability in the wider landscape around the pasture were estimated (a description of the indices is provided in Section 4.2.2). Field management information was collected through interview of farmers in order to assign a score to each pasture according to its management intensity (see Appendix VIII. for a breakdown of Management Intensity Scores).

Biotic indicators can be used to provide a comparison of management intensity and grassland characteristics between study sites, for example ground beetles and spiders are potential indicators of management intensity (e.g. Pommeresche, 2002; Døring

and Kromp, 2003). The purpose of sampling the carabid and spider communities was to obtain a one-off picture of how ecologically similar, or dissimilar, the study pastures were. Seventeen of the total 26 fields sampled for dung insects were selected for carabid and spider sampling on the basis of their ease of access and their current land-use in 2003. Grazed fields that were sampled in 2002 but which were converted to arable cropping in 2003 were excluded. In September 2003, non-baited pitfall traps were used to sample the carabid and spider assemblages in eight fields that contained doramectin-treated cattle and nine that contained untreated cattle (refer to Section 4.2.2 for details of the sampling method).

### *5.2.3 Data Analysis*

#### *Environmental variables*

Differences in climatic, temporal, habitat and management characteristics of pastures according to sample year and avermectin treatment were assessed using non-parametric statistics. Data that was collected or estimated repeatedly over the sampling season e.g. sward height and dung density, were analysed using mixed models with repeated measures and a normal error distribution (refer to Section 4.2.3 for details of the modelling procedure). A 'habitat characteristic' score was derived from an ordination of altitude, aspect, boundary, adjacency to woodland and 'pasture index' in order to reduce the number of habitat parameters that were included in analyses (the ordination method is described in Section 3.2.2). The spider and ground beetle assemblage data were ordinated to compare their species compositions in the sub-set of the study fields.

#### *Abundance, Species Richness and Diversity of Insects*

Generalised linear mixed models with repeated measures were constructed using data from fields grazed by untreated and avermectin-treated cattle in order to test for relationships between environmental variables and the abundance, species richness and diversity of dung insects. 'Field' and 'farm' were included as random factors and sampling date as a repeated factor, and all variables listed in Table 5.2 were tested in the mixed model analyses (for details of the modelling procedure see 4.3.3). All insect abundance data from trapping periods of different duration were corrected to 10 days to enable comparisons between trapping periods of different duration.

Farm	Field Code	Treated	Field area (ha)	Aspect	Boundary	Livestock	Grazing system	MIS	Avermectin treatment times	Year Sampled
1	ST1	Yes	8.3	Flat	Gappy hedge	Dairy heifers	Permanent	11	Doramectin: end Apr & end June	Both
1	ST2	Yes	4.6	Flat	Gappy hedge	Dairy heifers	Permanent	11	Doramectin: end Apr & end June	Both
1	ST3	Yes	6.3	North	Hedge	Dairy heifers	Permanent	11	Doramectin: end Apr & end June	2003
1	SC3	No	4.7	North	Hedge	Dairy cows	Rotation	10	N/A	Both
2	WMT1	Yes	2.3	Flat	Fence	Heifers/ bullocks	Permanent	7	Doramectin: beg May & end June	2002
2	WMT2	Yes	2.9	Flat	Fence	Heifers/ bullocks	Permanent	7	As for WMT1 in '02 and WMT3 in '03	Both
2	WMT3	Yes	2.3	Flat	Hedge	Dairy heifers	Permanent	11	Doramectin: 1 June and 30 July	2003
2	WMC3	No	4.8	South	Gappy hedge	Dairy cows	Rotation	16	N/A	Both
2	WMC4	No	4.8	South	Hedge	Dairy cows	Rotation	14	N/A	Both
3	CHT1	Yes	5.8	Flat	Fence	Bullocks/ sheep	Permanent	12	2002 Ivermectin: beg May & 20 Jun 2003 Doramectin: beg May & 10 July	Both
3	CHT2	Yes	4.2	Flat	Gappy hedge	Bullocks/ sheep	Permanent	12	2002 Ivermectin: beg May & 20 Jun 2003 Doramectin: beg May & 10 July	Both
4	BTBT3	Yes	7.5	South	Gappy hedge	Dairy heifers	Permanent	12	Ivermectin: 28 June	2002
4	BTBC1	No	3.5	South	Gappy hedge	Dairy cows	Rotation	12	N/A	2002
4	BTBC2	No	4.1	Flat	Gappy hedge	Dairy cows	Rotation	14	N/A	Both
4	BTBC3	No	6.8	South	Gappy hedge	Dairy cows	Rotation	12	N/A	2003
5	DT1	Yes	5.7	North	Gappy hedge	Heifers/ bullocks	Permanent	11	Doramectin: ~21 May & ~24 July	Both
5	DT2	Yes	5.6	North	Fence	Dairy heifers	Permanent	11	Doramectin: ~21 May & ~24 July	2002
5	DT3	Yes	3.3	East	Hedge	Dairy heifers	Permanent	11	Doramectin: ~21 May & ~24 July	2003
5	DC5	No	2	South	Fence	Dairy cows	Rotation	16	N/A	Both
5	DC6	No	3.3	South	Fence	Dairy cows	Rotation	17	N/A	Both
6	MTC	No	3.5	Flat	Gappy hedge	Dairy cows	Rotation	13	N/A	2003
6	BR	No	3.5	Flat	Gappy hedge	Dairy cows	Rotation	13	N/A	2003
7	GGT1	Yes	3.3	Flat	Gappy hedge	Dairy heifers	Permanent	12	Doramectin: beg May & beg Aug	2003
7	GGC1	No	6.7	Flat	Gappy hedge	Dairy cows	Permanent	10	N/A	2003
8	LBT1	Yes	5.5	Flat	Gappy hedge	Bullocks	Permanent	12	Doramectin: 13 May & 1 Aug	2003
8	LBC1	No	4.1	Flat	Hedge	Dairy cows	Permanent	14	N/A	2003

Table 5.1 – List of fields in which dung insects were sampled (MIS = Management Intensity Score)

*Abundance, Species Richness and Diversity of Insects (cont.)*

Analyses were performed on the abundance of yellow dung-flies (*Scatophaga stercoraria*), *Cercyon* individuals and *Aphodius* Guild 1 and Guild 2 individuals. Guild 1 included *Aphodius* species with coprophagous larvae that feed exclusively in dung and Guild 2 included *Aphodius* species with saprophagous larvae that feed on decaying vegetation (Gittings and Giller, 1997). Guild 1 included all *Aphodius* species collected except for *A. prodromus* and *A. sphaecelatus*, which were placed in Guild 2.

The 'peak abundance' of dung insects was defined as the maximum number of insects (*Aphodius*, *Cercyon*, *Sphaeridium* and Scatophagids) caught in any one trap in a trapping period in a study field. Peak abundance was used as an indicator of the relative value of a pasture as a foraging habitat for farmland birds, simply in terms of insect abundance. Differences in peak insect abundance between pastures were explored using a mixed model with a Poisson distribution, with field as a random factor, and all independent variables in Table 5.2 were tested in the model. The main breeding period of many farmland birds is from May to July therefore the temporal occurrence of the peak abundance is also important because, for birds provisioning young, there must be sufficient availability of invertebrates at the necessary time. A mixed model with a normal error distribution was used to determine whether avermectin treatment caused a shift in the timing of peak abundance.

*Dung beetle assemblage structure*

To explore changes in dung beetle assemblage structure over the sampling season and to detect effects of avermectin treatment over time, the abundance of individuals of *Aphodius*, *Cercyon* and *Sphaeridium* beetles in each trapping period in each field was ordinated. The ordination generates scores for the two principle axes, 1 and 2, and axis 1 scores account for more variation than axis 2 (Gauch, 1982). Therefore, axis 1 scores were placed in a mixed model with field and farm as random factors and sampling date as a repeated measure, and all other independent variables were tested in the model (Table 5.2).

Variable	Level	Type
Farm	8 levels	Categorical
Field	26 levels	Categorical
Year	2 levels: 2002, 2003	Categorical
Seasonality	Sampling date i.e. days from 1-April (23-112 days)	Continuous; change
Days post-turnout	Days since cattle were put out to pasture (-2 to 89 days)	Continuous; change
Avermectin treatment	2 levels: 1 – Untreated; 2 - Treated	Categorical; fixed
Days post-treatment	3 levels: 1-15 days, 16-40 days, 41+ days	Categorical; change
Sward height	(3-30 cm)	Continuous; change
Rainfall	mm per trap period (0.2-67.4 mm)	Continuous; change
Sun	hours per trap period (37-167 hours)	Continuous; change
Maximum temperature	Mean max. temperature per trap period (10.0-21.1°C)	Continuous; change
Minimum temperature	Mean min. temperature per trap period (3.4-13.8°C)	Continuous; change
Dung index	Density per hectare per trapping period. See 4.3 for equation. (0-197 pats ha <sup>-1</sup> )	Continuous; change
Area of field	(2.0-8.3 ha)	Continuous; fixed
Soil pH	(pH 5.0-6.7)	Continuous; fixed
Available P	(6.8-82 mg l <sup>-1</sup> )	Continuous; fixed
Available K	(112-408 mg l <sup>-1</sup> )	Continuous; fixed
Soil Moisture	(20.7-59.82%)	Continuous; fixed
Soil Impenetrability	(30-106 impenetrability index)	Continuous; change
Soil Organic content	% loss on ignition (6.3-24.0%)	Continuous; fixed
Grazing System	2 levels: rotation or permanent grazing	Categorical; fixed
Management Intensity Score (MIS)	See Appendix VIII. for description (Score 7-17)	Continuous; fixed
Age (part of MIS)	4 levels: <5yrs, 5-10yrs, >10yrs, uncultivated	Categorical; fixed
Grazing intensity (part of MIS)	4 levels: no livestock, <0.8 LUha <sup>-1</sup> , 0.8-1.14 LUha <sup>-1</sup> , >1.14 LUha <sup>-1</sup>	Categorical; fixed
Avermectin index	See 4.3 for description. (0-72%)	Continuous; fixed
Habitat characteristic score	Ordination of aspect, altitude, boundary, adjacent woodland, pasture index	Continuous; fixed

Table 5.2 – Environmental and management variables included in mixed models. 'Fixed' variables did not change over the sampling season and 'change' variables altered with trapping period. The recorded ranges of continuous variables are given in parentheses.

## 5.3 Results

### 5.3.1 Environmental Variables

#### *Climate*

As described in Section 4.3.1 (Figure 4.2), there was a negative correlation between rainfall and sunshine hours in both years of study thus rainfall was selected to characterise weather patterns in data analyses. In individual trapping periods from April to July, the recorded rainfall ranged from 0.2-67.4 mm and sunshine hours in individual trapping periods ranged from 37-167 hours. The mean minimum and maximum daily temperatures increased significantly from April to July in 2002 and 2003 (Section 4.3.1, Figure 4.3). Mean maximum temperature was lower in 2002 than in 2003.

#### *Temporal variables*

Relationships between the temporal variables of seasonality, number of days post-turnout and number of days post-ivermectin treatment were investigated for each year. The number of days post-turnout was used to assign a value to trapping periods that occurred in fields before cattle were turned out to grazing i.e. when there was no fresh cattle dung resource for dung invertebrates to colonise. However, because most of the invertebrate sampling in fields commenced after cattle were turned out to grazing, seasonality and days post-turnout were highly positively correlated for trapping periods in 2002 ( $r_s=0.98$ ,  $df=121$ ,  $P<0.001$ ) and 2003 ( $r_s=0.98$ ,  $df=118$ ,  $P<0.001$ ). Therefore, the seasonality variable was chosen for inclusion in the models because it is more directly comparable to other studies of dung insects in temperate regions in spring and summer than the 'days post-turnout' variable.

The variable 'days post-treatment' was used to detect potential changes in dung insect assemblages with time since treatment. For example, if ivermectin residues did have adverse effects on dung insect populations then one would be able to ascertain if there was any recovery from those effects. There was no correlation between seasonality and the number of 'days since ivermectin treatment' in either 2002 ( $r_s=-0.001$ ,  $df=121$ ,  $P=0.99$ ) or 2003 ( $r_s=0.07$ ,  $df=118$ ,  $P=0.45$ ). Additionally,

there was no correlation between the number of days post-turnout and the number of days after treatment in 2002 ( $r_s=0.13$ ,  $df=121$ ,  $P=0.14$ ) or in 2003 ( $r_s=0.18$ ,  $df=118$ ,  $P=0.052$ ).

#### *Habitat variables - landscape*

The study fields were ordinated by habitat characteristics, which were aspect, altitude, field boundary, pasture index and adjacency to woodland. Eigenvalues for axes 1 and 2 were low, 0.021 and 0.002 respectively (Appendix XIII.). One would expect such low eigenvalues in an ordination where the same set of characteristics is being compared between fields with only the level of each variable changing. As each habitat variable is regarded as being 'present' in the study field, the degree of dissimilarity between fields in ordination space is reduced. The value of the ordination of such data is in its ability to place fields with similar characteristics closer to each other and those that are dissimilar further apart.

There was no visible separation of fields by avermectin treatment, therefore there were no major differences in the habitat features of treated and untreated fields. Fields sampled in 2003 were positioned farther to the right of axis 1 than fields sampled in 2002 (Figure 5.2) and this was attributable to a change in 'pasture index' between years. In fields DC5 and DC6, the pasture index or 'proportion of grazed pasture in surrounding fields' decreased from 2002 to 2003 but in all other fields there was an increase in pasture index from 2002 to 2003. The mean ( $\pm 1$  se) pasture index was  $53.2 \pm 4.7$  % for fields sampled in 2002, which was significantly lower than the index value of  $72.2 \pm 2.0$  % for fields sampled in 2003 (Mann-Whitney,  $n=24$ ,  $P=0.006$ ). Two fields, with opposite patterns of change in pasture index between the two years, were labelled to highlight the shift in ordination position due to the change in pasture index (Figure 5.2). The ordination position of field SC3 moved to the right between the two years because of an increase in pasture index from 2002 to 2003 and the position of DC5 shifted to the left slightly due to a decrease in pasture index from 2002 to 2003 (Figure 5.2 and 5.3).

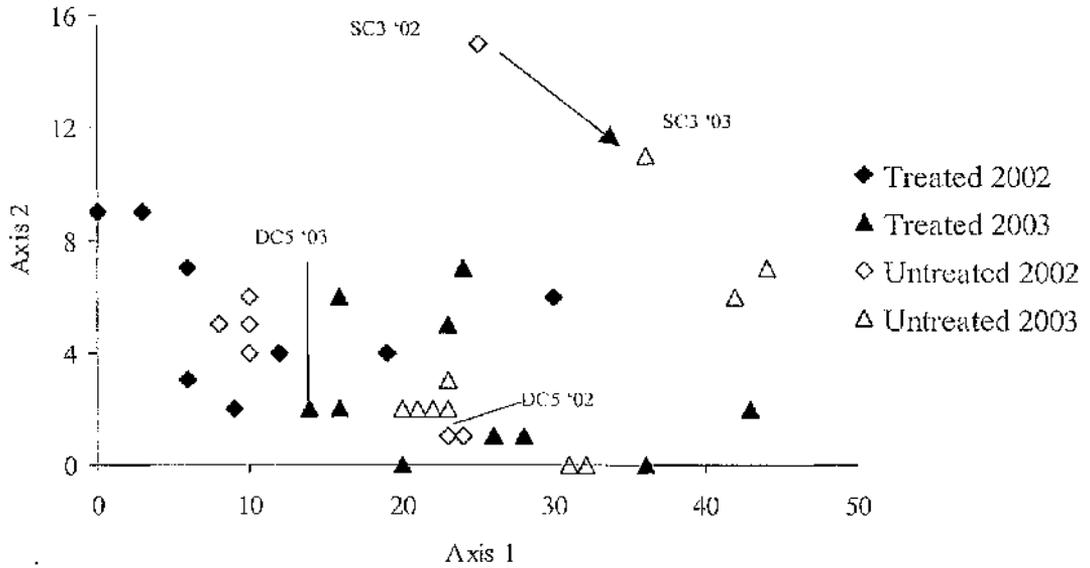


Figure 5.2 – Ordination of study pastures sampled in 2002 and 2003 by the habitat characteristics of aspect, altitude, boundary, pasture index and adjacency to woodland. Two fields (SC3 and DC5) are labelled to illustrate their shift in ordination position between 2002 and 2003, according to a change in pasture index.

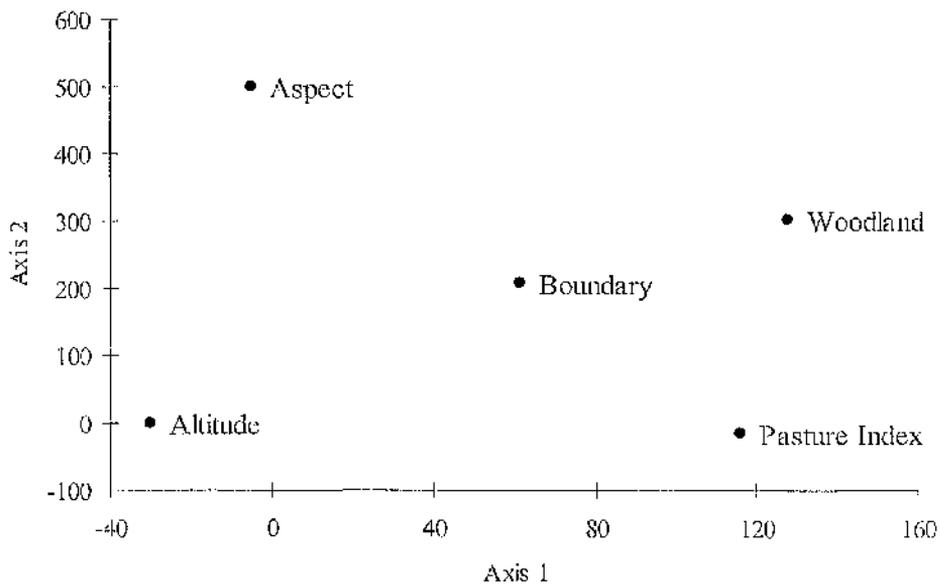


Figure 5.3 – Ordination of habitat characteristics in pastures sampled for dung insects from April to July in 2002 and 2003

The avermectin index was a measure of the proportion of pasture in a 0.5 km<sup>2</sup> circle surrounding the study area that was grazed by avermectin-treated cattle. The avermectin index was significantly higher for treated fields than for untreated fields

in 2002 (Mann-Whitney,  $n=16$ ,  $P<0.0001$ ) and in 2003 (Mann-Whitney,  $n=22$ ,  $P=0.0001$ ). Over the two years, the mean ( $\pm 1$  se) proportion of pasture grazed by treated cattle around 'treated' study fields was  $36.2 \pm 3.7$  %, and around 'untreated' study fields the proportion was just  $2 \pm 0.9$  %. The avermectin index of all fields did not differ significantly between years (Mann-Whitney,  $n=38$ ,  $P=0.75$ ) with a mean ( $\pm 1$  se) index of  $19.6 \pm 4$  % in 2002 and  $20.3 \pm 5.3$  % in 2003.

#### *Habitat variables – field*

There was no significant difference in the sward height between treated and untreated fields in either 2002 ( $F_{1, 14}<0.01$ ,  $P=0.99$ ) or 2003 ( $F_{1, 32}=0.68$ ,  $P=0.42$ ). Sward height was significantly higher in fields sampled in 2002 ( $F_{1, 83.5}=44.3$ ,  $P<0.0001$ ) with a mean ( $\pm 1$  se) sward height of  $16.4 \pm 0.4$  cm for fields sampled in 2002 and  $11.7 \pm 0.4$  cm for fields sampled in 2003.

The estimates of dung pat density were compared for individual trapping periods in pastures by year and by treatment. As with the insect abundance values, all dung density values were corrected to 10 days to ensure that the estimates of dung density per trapping period were directly comparable between 2002 and 2003. With the correction applied, trapping periods in 2003 had significantly higher dung densities than those in 2002 ( $F_{1, 217}=11.21$ ,  $P=0.001$ ). There was no significant difference in dung density between fields containing untreated or avermectin-treated cattle in 2002 ( $F_{1, 142}=0.2$ ,  $P=0.66$ ) or in 2003 ( $F_{1, 18.8}=1.6$ ,  $P=0.22$ ).

All of the study fields grazed by avermectin-treated cattle were permanently grazed. Conversely, the majority of pastures grazed by untreated cattle were rotationally grazed, with only two of the twelve sampled untreated pastures being grazed permanently.

#### *Management Intensity*

Neither the use nor the management intensity of fields sampled in both years changed from 2002 to 2003. Overall, the management intensity of study pastures sampled in 2002 did not differ from those sampled in 2003 (Mann-Whitney,  $n=38$ ,

$P=0.86$ ). Fields grazed by untreated cattle were managed more intensively than those containing treated cattle in 2002 (Mann-Whitney,  $n=16$ ,  $P=0.015$ ) and in 2003 (Mann-Whitney,  $n=22$ ,  $P=0.01$ ). The mean ( $\pm 1$  se) management intensity score was  $13.8 \pm 0.6$  for untreated fields and  $10.8 \pm 0.4$  for treated fields.

Carabid and spider assemblage data were ordinated to compare the species composition of these biotic indicators between fields. The ordination of fields by carabids and the species scores are shown in Figures 5.4 and 5.5, respectively. Eigenvalues were 0.198 for axis 1 and 0.054 for axis 2 (Appendix XIV.) indicating not only that the axes explained approximately 25% of the variation in carabid assemblages between all study fields but also that the variation in ground beetle assemblages across these fields was relatively low.

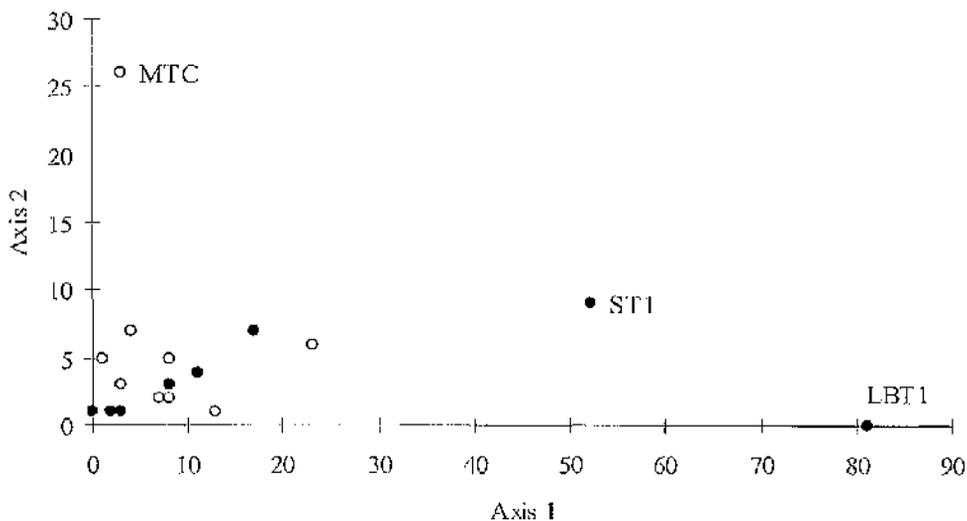


Figure 5.4 – Ordination of carabid assemblages sampled in September 2003 from 8 fields grazed by treated cattle (●) and 9 fields grazed by untreated cattle (○)

The most abundant carabid was *Nebria brevicollis* and the remaining eighteen species were recorded in low numbers. The field MTC is positioned high on axis 2 and this can be attributed to the presence of three species in that field only – *Pterostichus strenuus*, *Clivina fossor* and *Harpalus rufipes*. The fields ST1 and LBT1 are positioned to the right of the main cluster because *N. brevicollis* was less common in these two fields than in all other fields sampled. However, the length of each axis is short indicating that the difference between fields was negligible.

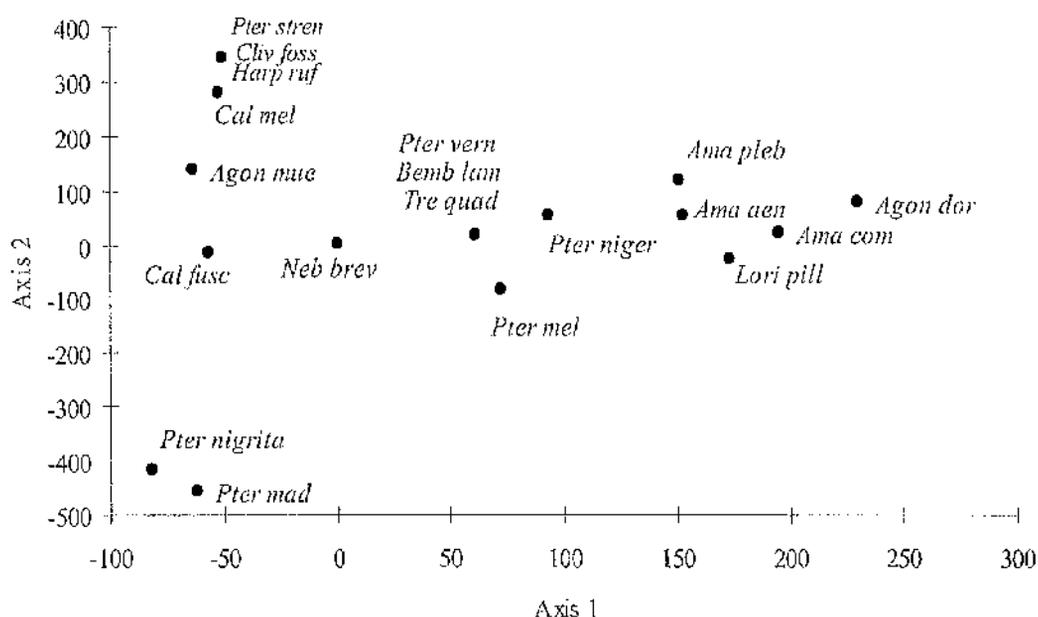


Figure 5.5 – Species scores from ordination of carabid assemblages sampled in September 2003 from 8 fields grazed by treated cattle and 9 fields grazed by untreated cattle. Species abbreviations are provided in Appendix I.

Variation in spider communities was very low between all study fields as indicated by the eigenvalues which were 0.094 and 0.06 for axes 1 and 2, respectively (Figure 5.6). The sample and species scores are listed in Appendix XV. The species *Bathyphantes gracilis*, *Erigone atra* and *E. dentipalpis* were recorded in all treated and untreated fields and *Oedothorax fuscus* and *Lepthyphantes tenuis* were recorded in the majority of fields (see Figure 5.7 for species scores). Six species were represented by only one individual per field, for example *O. retusus* in LBC1, *L. pallidus* in BTBC2, *Allomengea scopigera* in DC6, *Labulla thoracica* and *Dicymbium tibiale* in WMT2 and *Savignya frontata* in DT3 and SC3. The ordination suggested that there were no marked differences in the spider assemblages between sampled fields.

The ordination of carabid and spider assemblages independently mirrored the results for the Management Intensity Score process. The low variation between assemblages across the study fields suggested that the intensities of use were similar and no fields stood out as being distinctly different from the others.

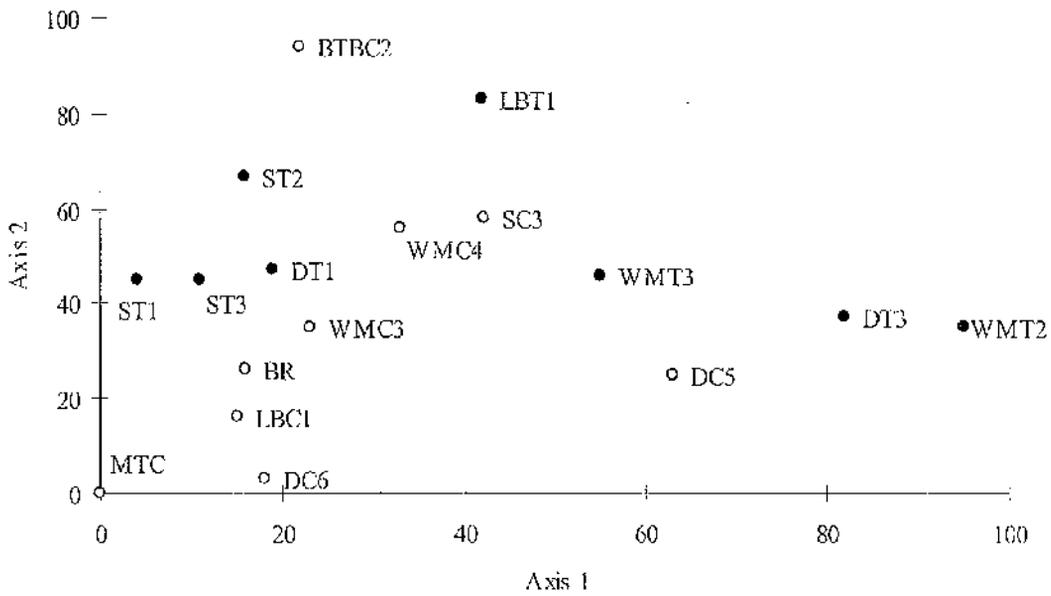


Figure 5.6 - Ordination of spider assemblages sampled in September 2003 from 8 fields grazed by avermectin-treated cattle (●) and 9 fields grazed by untreated cattle (○)

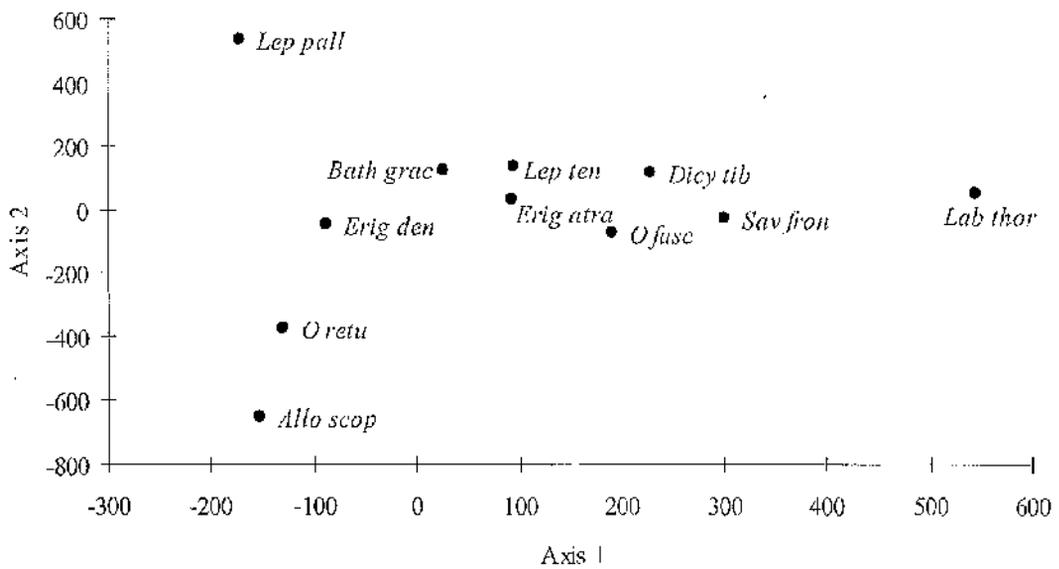


Figure 5.7 - Species scores from ordination of spider assemblages sampled in September 2003 from 8 fields grazed by treated cattle and 9 fields grazed by untreated cattle. Species abbreviations are given in Appendix I.

## 5.3.2 Abundance, Species richness and Diversity of Dung Insects

Numbers of dung insects trapped in pastures grazed by treated and untreated cattle in 2002 and 2003 were summarised for all trapping periods (Table 5.3).

Species	Fields with untreated cattle		Fields with treated cattle	
	2002 (n=57)	2003 (n=62)	2002 (n=66)	2003 (n=58)
<i>Aphodius ater</i>	36	15	198	14
<i>A. depressus</i>	1085	203	2189	599
<i>A. fimetarius</i>	3	1	4	0
<i>A. fossor</i>	3	5	51	0
<i>A. lapponum</i>	0	0	2	0
<i>A. pusillus</i>	0	3	0	0
<i>A. rufipes</i>	128	68	356	120
<i>A. rufus</i>	0	2	1	0
<b><i>Aphodius</i> Guild 1</b>	<b>1255</b>	<b>297</b>	<b>2801</b>	<b>733</b>
<i>A. prodromus</i>	373	412	3589	895
<i>A. sphacelatus</i>	99	47	1282	77
<b><i>Aphodius</i> Guild 2</b>	<b>472</b>	<b>459</b>	<b>4871</b>	<b>972</b>
<i>Cercyon atomarius</i>	783	1933	1004	1517
<i>C. haemorrhoidalis</i>	148	501	186	250
<i>C. lateralis</i>	233	823	654	641
<i>C. lugubris</i>	13	60	20	20
<i>C. melanocephalus</i>	1150	2193	2604	2236
<i>C. pygmaeus</i>	41	26	320	23
<b><i>Cercyon</i></b>	<b>2368</b>	<b>5536</b>	<b>4788</b>	<b>4687</b>
<i>Sphaeridium lunatum</i>	8	1	17	7
<i>S. scarabaeoides</i>	36	11	70	16
<b><i>Sphaeridium</i></b>	<b>44</b>	<b>12</b>	<b>87</b>	<b>23</b>
<i>Scatophaga furcata</i>	69	263	67	228
<i>S. inquinata</i>	63	4	3	7
<i>S. stercoraria</i>	11595	1740	11702	2427
<b><i>Scatophaga</i></b>	<b>11727</b>	<b>2007</b>	<b>11772</b>	<b>2662</b>

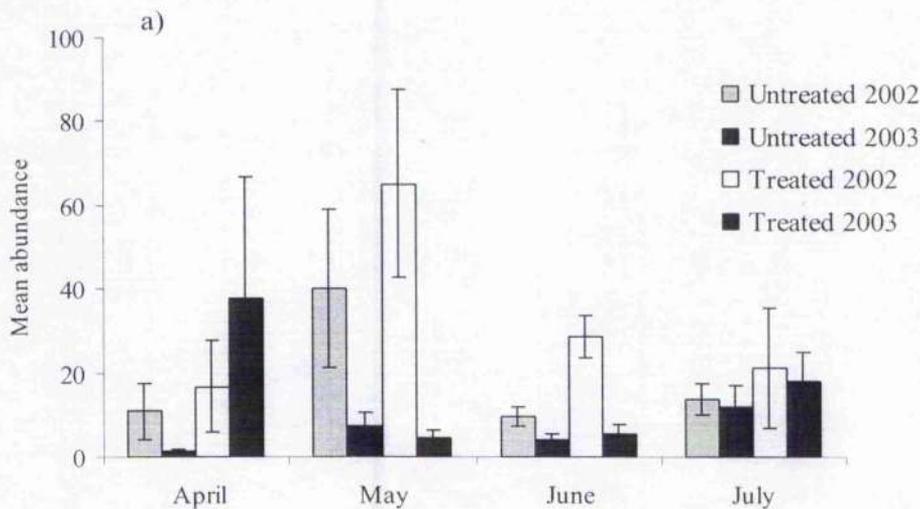
Table 5.3 - Abundance of each species in both sample years (abundance corrected to 10 days). Totals for genera and guilds are shown in bold text and n = number of trapping periods.

Table 5.4 shows the final model for abundance of *Aphodius* Guild 1 beetles. Numbers of these beetles were significantly higher in 2002 than in 2003 and in fields grazed by avermectin-treated cattle (Figure 5.8a). Highest numbers of Guild 1 individuals were recorded in late April and early May and then numbers declined from May onwards until the beginning of July when a slight increase occurred. The mean monthly abundance of the two most commonly trapped *Aphodius* Guild 1

species indicated how the seasonal abundance of those species drove the overall seasonal pattern of Guild 1 individuals (Figures 5.8b and c), as the seasonality of *A. depressus* is similar to that of the overall Guild. There was a greater tendency for *Aphodius depressus* to be trapped in April and May although the species was common in all months of the study, and a distinct seasonal pattern was apparent in *A. rufipes* which was caught more in June and July than in spring months. The 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. Hence, the model showed that year, avermectin treatment, rainfall and seasonality were all significant for the abundance of *Aphodius* Guild 1 individuals.

Variable	Estimate	se	Test statistics	P
<b><i>Aphodius</i> Guild 1</b>				
Field			Z=1.06	0.145
Farm			Z=1.06	0.145
Year	1.209	0.225	F <sub>1,122</sub> =28.99	<0.0001
Seasonality	-0.079	0.023	F <sub>1,142</sub> =11.42	0.0009
Seasonality <sup>2</sup>	0.0005	0.0002	F <sub>1,141</sub> =8.69	0.0037
Rain	-0.104	0.017	F <sub>1,220</sub> =36.96	<0.0001
Rain <sup>2</sup>	0.001	0.0003	F <sub>1,219</sub> =24.51	<0.0001
Avermectin treatment	-0.873	0.29	F <sub>1,16.1</sub> =9.12	0.008
Intercept	6.665	0.762		

Table 5.4 – Model 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



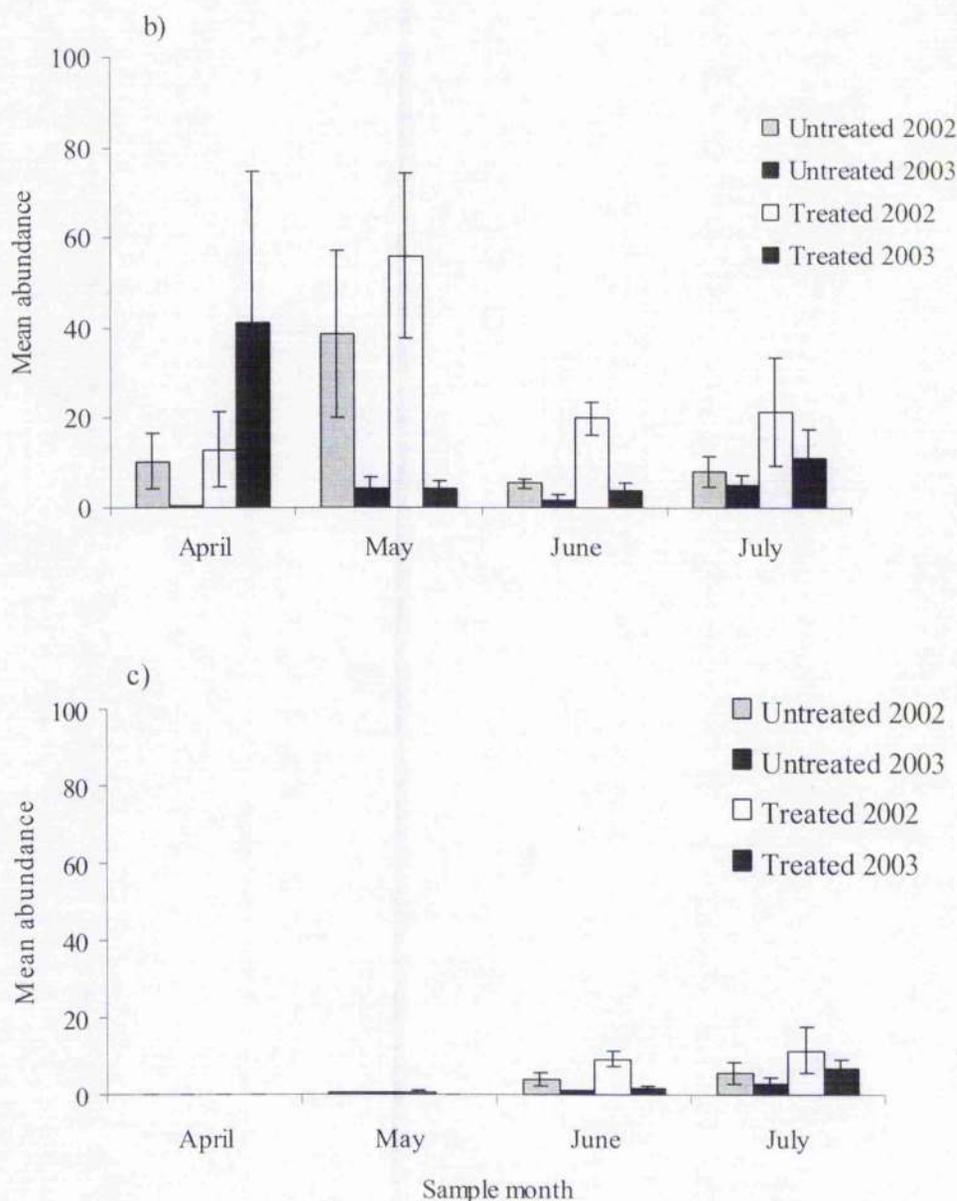


Figure 5.8 – Mean number ( $\pm 1$  se) of beetles trapped in treated and untreated fields in each month of 2002 and 2003 for a) all Guild 1 *Aphodius* beetles b) *A. depressus* and c) *A. rufipes*

The *Aphodius* Guild 2 abundance model is shown in Table 5.5. Individuals were trapped in higher numbers in 2002 and in both years their abundance decreased continuously as the sampling season progressed (Figure 5.9a). There was an interaction between year and avermectin treatment as significantly more *Aphodius* Guild 2 individuals were trapped in treated fields than untreated in 2002 ( $F_{1, 43.9}=7.27$ ,  $P=0.01$ ), but in 2003 there was no effect of treatment ( $F_{1, 22.9}=0.97$ ,

$P=0.34$ ). The Guild 2 species, *A. prodromus* and *A. sphacelatus*, followed a similar seasonal pattern of relatively high abundance in April and May with a decline in numbers in summer months (Figures 5.9 b and c, respectively).

In an initial model, the 'days post-treatment' variable was significant for *Aphodius* Guild 2 ( $F_{3, 69.6}=34.05$ ,  $P<0.0001$ ) with highest numbers occurring up to 15 days post-treatment. As cattle in treated fields were typically dosed with an avermectin at the beginning of May, this meant that the period up to 15 days after treatment coincided with the seasonal peak of Guild 2 *Aphodius*. To determine whether 'days post-treatment' was linked with seasonality, the effect of the former was tested on June and July data only as some cattle were given a repeat avermectin treatment during those months. For June and July data, 'days post-treatment' was not significant ( $F_{3, 19}=0.29$ ,  $P=0.84$ ) therefore the variable was not included in the model as it was confounded with seasonal patterns in abundance.

In summary, the final model showed that seasonality, year and avermectin treatment were significant for the abundance of *Aphodius prodromus* and *A. sphacelatus*, although the effect of avermectin treatment was year-dependent.

Variable	Estimate	se	Test statistics	P
<b><i>Aphodius</i> Guild 2</b>				
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 5.5 – Model 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

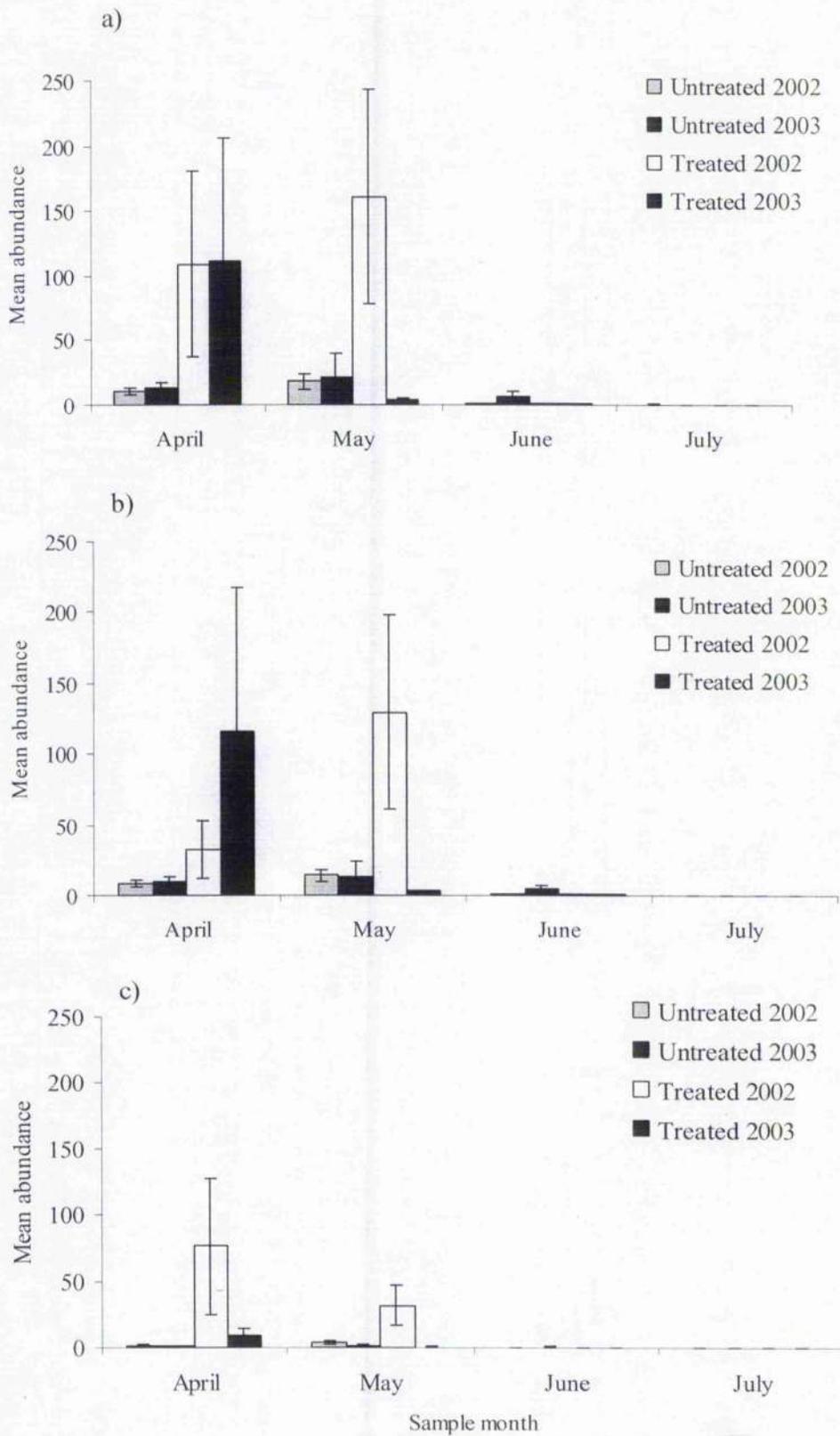


Figure 5.9 - Mean number ( $\pm 1$  se) of beetles trapped in treated and untreated fields in each month of 2002 and 2003 for a) all Guild 2 *Aphodius* beetles b) *A. prodromus* and c) *A. sphacelatus*

The model for *Cercyon* abundance could not estimate farm as a random factor, and 'field' was significant in the model indicating a difference in abundance between study fields (Table 5.6). As discussed in Section 4.3.2, this was probably due to the relatively high abundance of *Cercyon* in two of the untreated pastures, MTC and BR.

Numbers of *Cercyon* individuals were higher in 2003 than in 2002 (Figure 5.10a). There was no significant difference in the abundance of *Cercyon* between fields containing either untreated or avermectin-treated cattle ( $F_{1, 92.8}=3.63$ ,  $P=0.06$ ). A significant interaction occurred between year and seasonality because there was a quadratic relationship between abundance and season in 2002 ( $F_{1, 55.1}=14.67$ ,  $P=0.0003$ ) and a positive linear relationship in 2003 ( $F_{1, 60.5}=64.77$ ,  $P<0.0001$ ). In 2002, numbers of *Cercyon* were greatest at the beginning of May and then decreased and were lowest around mid- to late June before increasing thereafter. Lowest numbers of *Cercyon* were trapped in periods with between 35-50 mm rainfall.

The seasonal abundance of the two most common *Cercyon* species in treated and untreated fields are shown in Figures 5.10 b and c. For *C. atomarius*, the increase across sampling season in 2003 is clear, and in 2002, numbers were highest in May and July. *C. melanocephalus* showed the same seasonal pattern as *C. atomarius* therefore the overall seasonal pattern for all *Cercyon* is mirrored by these two species.

Variable	Estimate	se	Test statistics	P
<b><i>Cercyon</i></b>				
Field			Z=2.59	0.005
Year	2.726	0.578	$F_{1, 146}=22.27$	<0.0001
Seasonality	0.026	0.004	$F_{1, 135}=3.44$	0.066
Rain	-0.066	0.019	$F_{1, 222}=11.75$	0.0007
Rain <sup>2</sup>	0.001	0.0003	$F_{1, 220}=11.19$	0.001
Year*Seasonality	-0.04	0.008	$F_{1, 147}=25.96$	<0.0001
Intercept	2.991	0.506		

Table 5.6 – Model describing the variation in *Cercyon* abundance in fields containing avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003

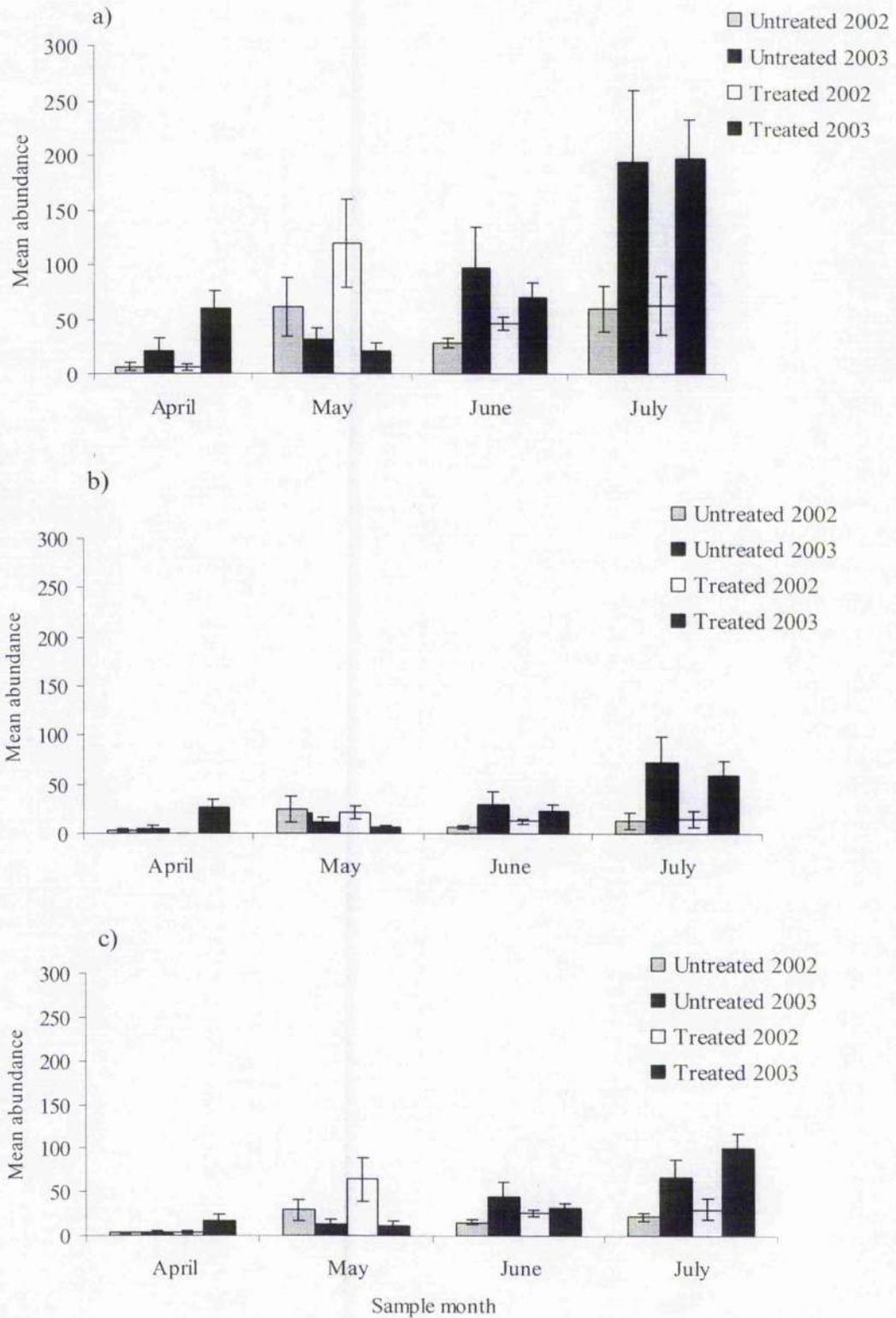


Figure 5.10 - Mean number ( $\pm 1$  se) of beetles trapped in treated and untreated fields in each month of 2002 and 2003 for a) all *Cercyon* beetles b) *C. atomarius* and c) *C. melanocephalus*

Abundance of yellow dung-flies, *Scatophaga stercoraria*, changed non-linearly through the season and this change differed between years (Table 5.7). In 2002, numbers were highest in April and then declined through May and June before beginning to increase slightly in July (Figure 5.11). In 2003, abundance was highest in April and then declined before levelling off in late June. There was no effect of avermectin treatment on the abundance of these flies ( $F_{1,18.9} < 0.01$ ,  $P = 0.97$ ).

Variable	Estimate	se	Test statistics	P
<b>Yellow dung-fly</b>				
Field			Z=1.14	0.127
Farm			Z=0.58	0.282
Year	1.217	0.189	$F_{1,125}=41.59$	<0.0001
Seasonality	-0.133	0.012	$F_{1,133}=117.98$	<0.0001
Seasonality <sup>2</sup>	0.0008	0.00009	$F_{1,135}=82.16$	<0.0001
Year*Seasonality <sup>2</sup>	0.0001	0.00004	$F_{1,120}=9.22$	0.0029
Intercept	8.289	0.394		

Table 5.7 – Model describing the variation in *Scatophaga stercoraria* abundance in fields containing avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003

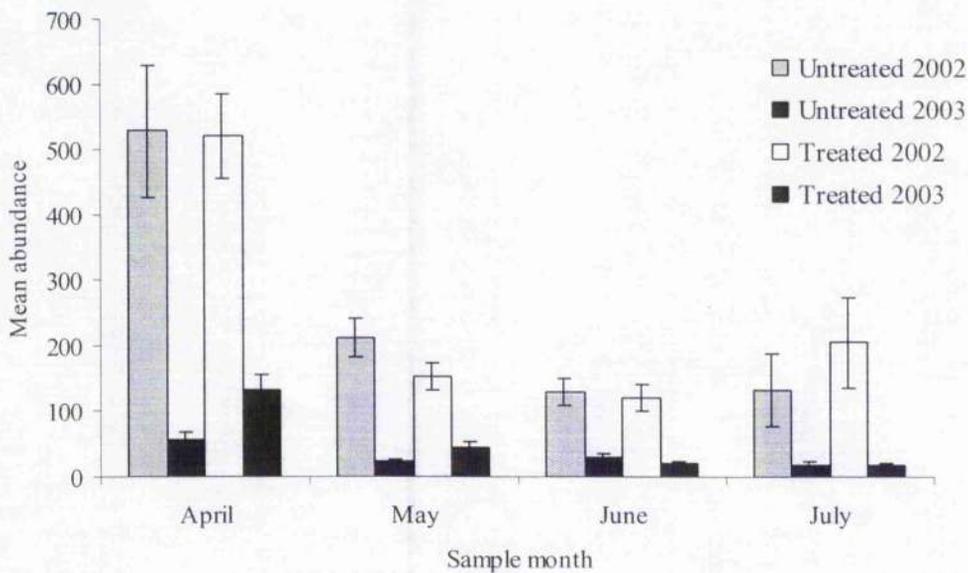


Figure 5.11 – Mean ( $\pm 1$  se) number of *Scatophaga stercoraria* flies trapped in treated and untreated fields in 2002 and 2003

Peak abundance was a measure of the maximum number of dung insects (Scarabaeidae, *Cercyon* and *Sphaeridium* and Scatophagidae) trapped in any one trap within a single trapping period in a field. Peak abundance was significantly higher in 2002 and declined with rainfall (Table 5.8). The random factor 'field' was significant indicating a difference in peak abundance between pastures. Peak abundance was higher in treated fields than in untreated fields but the difference was not statistically significant (Figure 5.12), and there was no interaction between avermectin treatment and year ( $F_{1,20.8}=0.02$ ,  $P=0.9$ ).

Variable	Estimate	se	Test statistics	P
Field			Z=1.76	0.039
Year	0.711	0.16	$F_{1,14.6}=19.65$	0.0005
Rain	-0.025	0.005	$F_{1,14.8}=24.72$	0.0002
Intercept	5.45	0.218		

Table 5.8 – Model of peak abundance of dung insects (*Aphodius*, *Cercyon*, *Sphaeridium* and *Scatophaga*) in treated and untreated fields sampled from April to July in 2002 and 2003

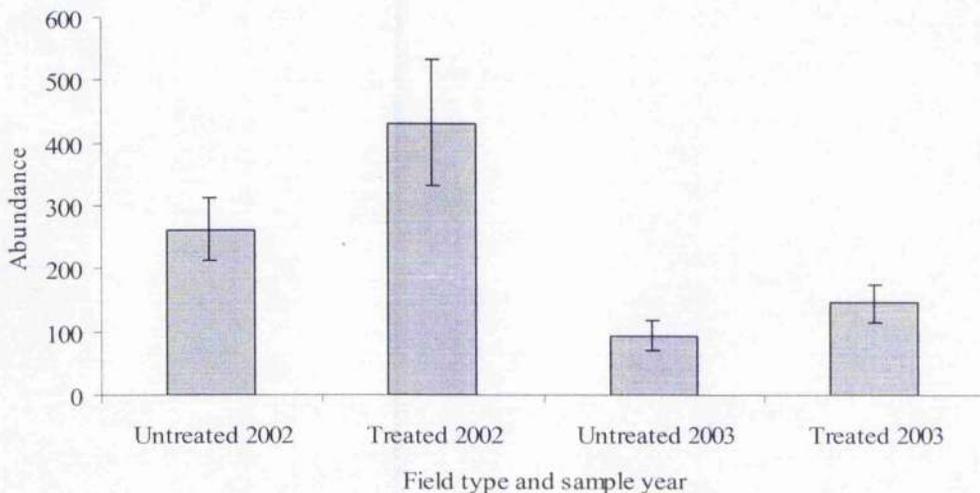


Figure 5.12 – Mean ( $\pm 1$  se) peak abundance of dung insects (*Aphodius*, *Cercyon*, *Sphaeridium* and *Scatophaga*) in treated and untreated fields in 2002 and 2003. Avermectin treatment did not affect peak abundance ( $F_{1,34}=2.82$ ,  $P=0.102$ )

The peaks in dung insect abundance happened mainly in late-April/ early-May and in July (Figure 5.13). There was a difference in timing of peak abundance between years ( $F_{1,15}=10.8$ ,  $P=0.005$ ) with most peaks occurring at the beginning of May in 2002, and in July in 2003. Avermectin treatment did not cause a shift in the timing of peak abundance in either 2002 ( $F_{1,14}=1.22$ ,  $P=0.29$ ) or 2003 ( $F_{1,19}=0.03$ ,  $P=0.86$ ).

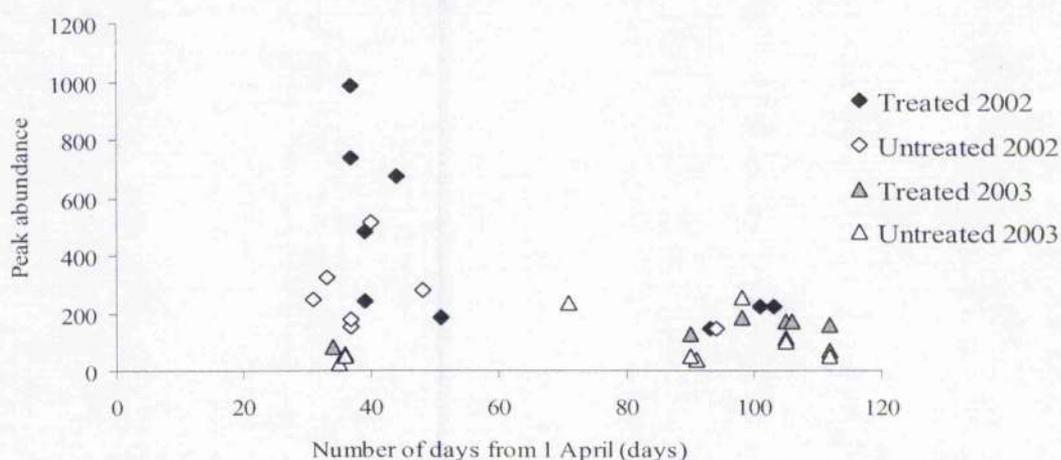


Figure 5.13 – Temporal occurrence of peak abundance of dung insects in treated and untreated fields sampled in 2002 and 2003

Species richness of *Aphodius* was significantly higher in 2002 and richness declined as the sampling season progressed (Table 5.9). The number of *Aphodius* species did not differ significantly between fields grazed by either untreated or avermectin-treated cattle ( $F_{1,65.3}=3.75$ ,  $P=0.06$ ), (Figure 5.14).

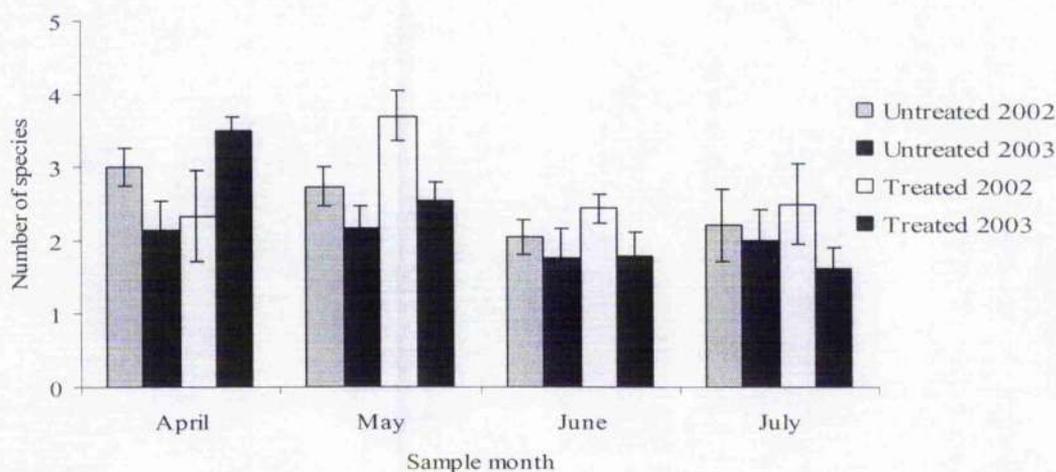


Figure 5.14 – Mean ( $\pm$  1 s.e.) species richness of *Aphodius* sampled in treated and untreated fields in 2002 and 2003

Variable	Estimate	se	Test statistics	P
<b><i>Aphodius</i> species richness</b>				
Field			Z=1.44	0.076
Farm			Z=1.58	0.057
Year	0.492	0.155	$F_{1,93.9}=10.14$	0.002
Seasonality	-0.022	0.003	$F_{1,122}=53.19$	<0.0001
Intercept	3.79	0.399		

Table 5.9 – Model describing the *Aphodius* species richness in fields containing avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003

The number of *Cercyon* species increased from April to July (Figure 5.15), and *Cercyon* species richness in pastures was not affected by avermectin treatment ( $F_{1, 85}=0.5$ ,  $P=0.48$ ). Species richness of *Cercyon* was lowest in periods with between 20-55 mm of rainfall (Table 5.10).

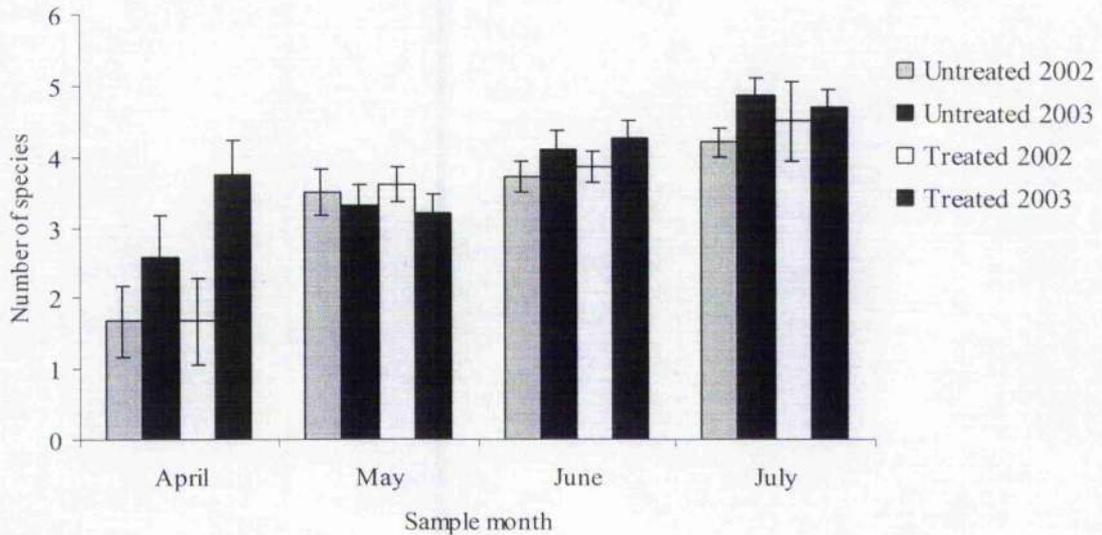


Figure 5.15 – Mean species richness ( $\pm 1$  se) of *Cercyon* for each sample month in fields grazed by treated or untreated cattle in 2002 and 2003

Variable	Estimate	se	Test statistics	P
<b><i>Cercyon</i> species richness</b>				
Field			Z<0.01	0.500
Farm			Z=1.20	0.115
Seasonality	0.024	0.003	$F_{1,128}=59.67$	<0.0001
Rain	-0.096	0.021	$F_{1,222}=21.76$	<0.0001
Rain <sup>2</sup>	0.001	0.0003	$F_{1,222}=18.28$	<0.0001
Intercept	3.375	0.358		

Table 5.10 – Model describing *Cercyon* species richness in fields containing avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003

The diversity of dung insects was highest in 2003 (Figure 5.16). Diversity was highest between mid-June and the first week of July and during periods of lowest rainfall (Table 5.11). Diversity was not significantly affected by avermectin treatment ( $F_{1, 13.2}=2.01$ ,  $P=0.18$ ). Hence, seasonality, year and rainfall were significant variables in the dung insect diversity model.

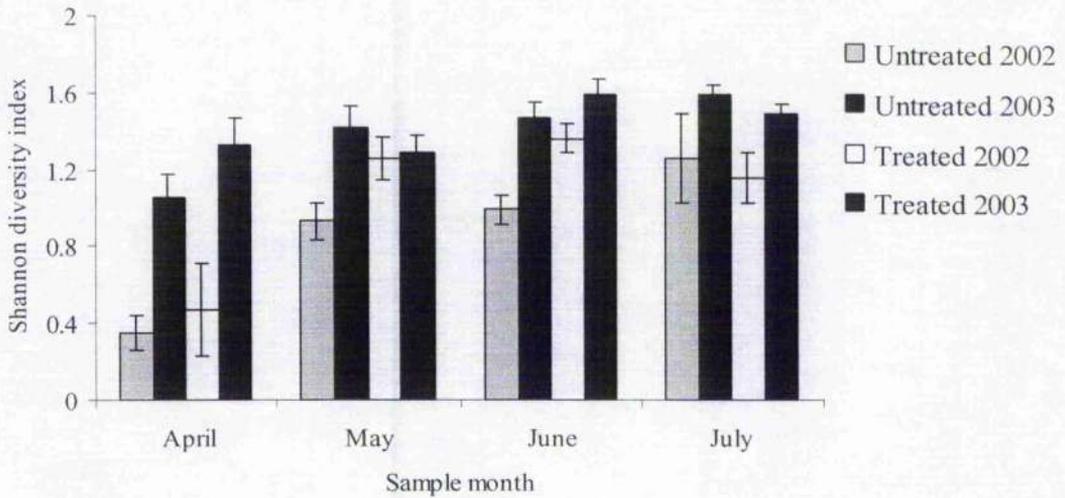


Figure 5.16 – Mean Shannon diversity index ( $\pm 1$  se) per month for dung insects collected from treated and untreated fields in 2002 and 2003

Variable	Estimate	se	Test statistics	P
<b>Diversity</b>				
Field			Z=0.65	0.259
Farm			Z=1.26	0.105
Year	-0.348	0.053	F <sub>1,108</sub> =42.81	<0.0001
Seasonality	0.033	0.007	F <sub>1,144</sub> =20.68	<0.0001
Seasonality <sup>2</sup>	-0.0002	0.00005	F <sub>1,144</sub> =16.59	<0.0001
Rain	-0.005	0.002	F <sub>1,190</sub> =9.01	0.003
Intercept	0.454	0.245		

Table 5.11 – Model describing dung insect diversity in fields containing avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003

### 5.3.3 Dung Beetle Assemblage Structure

Dung beetle assemblages were ordinated using data from each individual trapping period and axis 1 scores were put into a mixed model with repeated measures (for scores, see Appendix XVI). One can calculate the mean location, or centroid, of a group of samples in ordination space from the mean of scores along each axis. In order to make the graphical presentation of the individual trapping periods simpler, centroids were taken for axis 1 and 2 scores from treated and untreated trapping periods in 2002 and 2003. It should be noted that, although the centroids (Figure 5.17) do not appear orthogonal, independent data points were not correlated.

Trapping periods in 2002 had higher axis 1 scores which indicated that relatively more *Aphodius* individuals, with the exception of *A. rufipes*, *A. rufus* and *A. pusillus*,

occurred in the dung beetle assemblages in 2002 (Figure 5.18). There was a quadratic relationship with seasonality and a significant interaction between seasonality and year ( $F_{1, 79.9}=5.17$ ,  $P=0.026$ ). When explored further, the quadratic relationship with season occurred for both years. In 2002, axis scores generally decreased from the end-April onwards and then levelled off at the beginning of July. In 2003, a similar pattern occurred but axis scores levelled off in late June. There was no interaction between year and treatment ( $F_{1, 48.8}=0.01$ ,  $P=0.93$ ) and axis 1 scores were significantly higher in treated fields than in untreated fields (Table 5.12). The species scores from this ordination (Figure 5.18) indicated that the dung beetle assemblage in treated fields had relatively more of the species positioned higher on axis 1 (*Aphodius prodromus*, *A. sphacelatus* and *A. lapponum*) although it should be noted that only two individuals of the latter species were trapped in treated fields.

Variable	Estimate	se	Test statistics	P
<b>Dung beetle assemblage</b>				
Field			Z=0.08	0.468
Year	47.47	9.84	$F_{1, 72.1}=23.27$	<0.0001
Seasonality	-6.518	0.705	$F_{1, 135}=85.43$	<0.0001
Seasonality <sup>2</sup>	0.035	0.005	$F_{1, 132}=42.32$	<0.0001
Avermectin treatment	-19.23	5.204	$F_{1, 15.1}=13.65$	0.002
Year*Seasonality <sup>2</sup>	-0.004	0.002	$F_{1, 70.9}=7.29$	0.009
Intercept	360.86	24.296		

Table 5.12 - Model of dung beetle assemblage structure sampled from April to July of 2002 and 2003 in fields grazed by avermectin-treated and untreated cattle

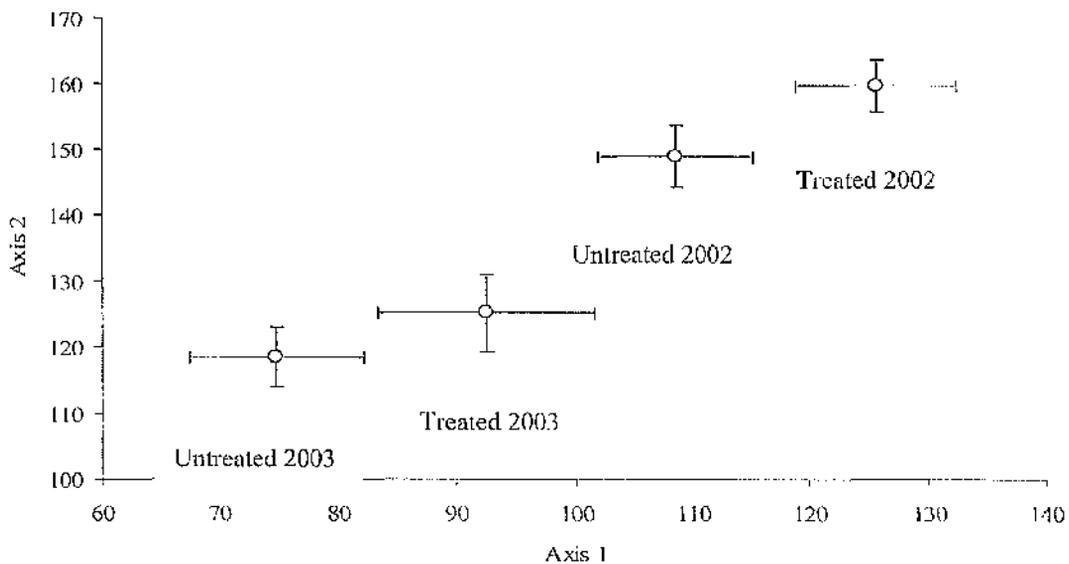


Figure 5.17 – Graph showing centroid (mean  $\pm$  1se) of axis 1 and 2 scores from ordination of dung beetles trapped in treated and untreated fields

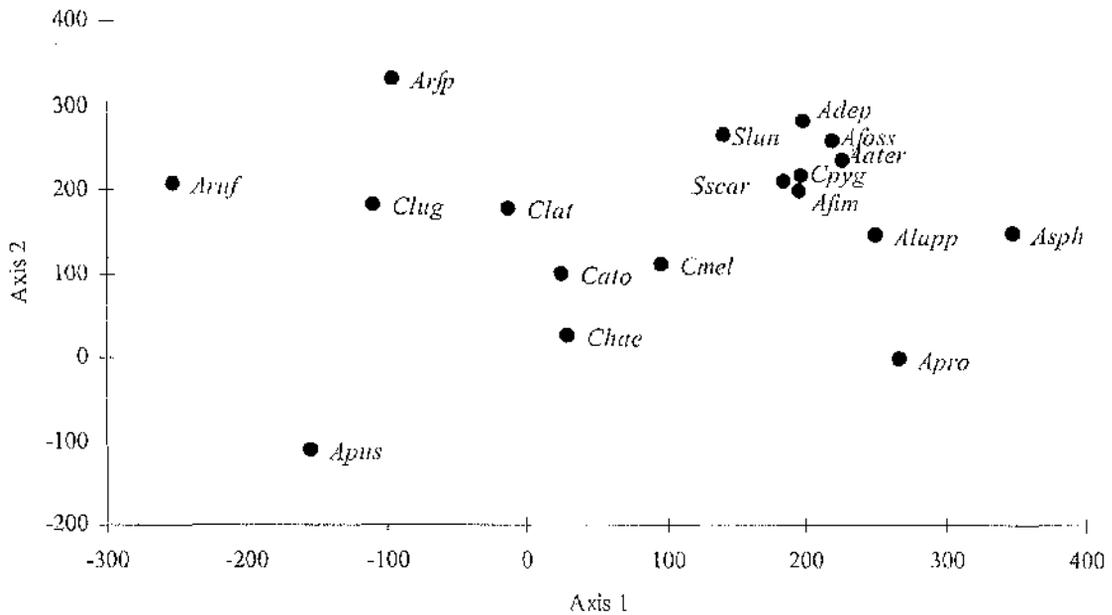


Figure 5.18 – Species scores from ordination of dung beetles trapped in individual trapping periods in treated and untreated fields from April to July in 2002 and 2003. List of abbreviations of species names is given in Appendix I.

#### 5.4 Discussion

These results showed that avermectin residues in dung, and/or the grazing management in pastures containing treated livestock, had a significant effect on the numbers of *Aphodius* dung beetles trapped. More *Aphodius* individuals were trapped in fields grazed by avermectin-treated cattle and that increase could not be attributed to pasture habitat characteristics such as aspect, field boundary, sward height or dung density, or wider landscape factors such as proximity to woodland or availability of surrounding grazed pasture. The assemblage structure of dung beetles (*Aphodius*, *Cercyon* and *Sphaeridium*) also differed between treated and untreated fields and that difference was due to the relatively higher numbers of *Aphodius* in treated fields.

As pitfall traps often reflect the activity of insects (Greenslade, 1964), the higher numbers in traps in fields grazed by treated cattle may indicate that *Aphodius* beetles were more active in those fields. Increased trapping of *Aphodius* in fields grazed by treated livestock could have been due to differences between pastures in terms of avermectin treatment, management intensity, grazing regime and/ or the proportion of surrounding pasture that was grazed by treated cattle. Each of these factors will

be discussed below in relation to how they may have caused higher numbers of *Aphodius* in pasture grazed by treated livestock.

The presence of avermectin residues in dung, in a pasture situation, could have affected *Aphodius* abundance via an “attraction/ repellency effect” as *Aphodius* beetles prefer to colonise dung from untreated cattle to dung from doramectin-treated cattle (see Chapter 3). All of the baits on the pitfall traps were formed from dung from untreated cattle, therefore inflated numbers of beetles may have occurred in traps in treated fields if beetles were avoiding the dung that occurred there naturally. It should be stressed that no other aspect of the sampling regime could explain the higher numbers of beetles in traps in treated fields since the same approach was taken in all treated and untreated fields.

Fields grazed by untreated cattle were managed more intensively than those grazed by treated cattle although the mean management intensity scores were not hugely different at 13.8 for untreated fields and 10.8 for treated fields. Indeed, Blake (1996) placed those scores in the same management intensity category, which indicates that the scores were not drastically different. Furthermore, spider and carabid assemblages did not show marked differences between treated and untreated fields suggesting that the pastures were quite similar ecologically in terms of management intensity. Therefore, it is unlikely that management intensity differences, to the degree observed in these study pastures, were a major contributory factor in the variation in *Aphodius* numbers. It should be noted however that abundance of *Aphodius* Guild 2 beetles was particularly high in two treated fields (Appendix XIX.) in April 2002. Those fields (WMT1 and WMT2) differed from the others in that they had not been ploughed for more than 70 years, and consequently the sward type was classed as semi-natural. However, it is difficult to ascertain whether that aspect of management caused the high numbers of *Aphodius* in those fields because the relatively high abundance was not sustained in either that trapping season in 2002 or in 2003.

Although dung density did not differ significantly in trapping periods between treated and untreated pasture, all treated fields were permanently grazed and the majority of untreated fields were grazed in rotation therefore the ‘pattern’ of dung

density differed between treated and untreated fields. The similarity in actual density in trapping periods in treated and untreated fields was due to the fact that the former are grazed permanently with a lower stocking rate while untreated fields are grazed rotationally at a higher stocking density. It is notable that 'groups' of grazed pastures on dairy farms usually contained the same livestock cohort, i.e. younger treated cattle were often grazed together in a cluster of adjacent fields and untreated milking cows were rotationally grazed through fields that were close together. This is because dairy farms are often managed so that young livestock are grazed farther afield while untreated milking cows are put to grazing in pasture close to farm buildings, for convenience when milking. There is evidence that dung beetles emigrated from a pasture when the cattle in that pasture were removed for rotation (Finn *et al.*, 1998). Therefore, the lower abundance of *Aphodius* in untreated fields in this study could have been a result of grazing regime whereby mass emigration of *Aphodius* occurred intermittently as cattle were rotated. However, one might expect that the periodic availability of fresh dung in pasture grazed by untreated cattle would only cause fluctuations in dung beetle numbers and would not seriously affect population density. This is because the 'on-off' supply of dung in one field would be mitigated by the availability of dung from untreated milking cows in another nearby pasture, provided that the cows were rotated through a group of adjacent fields.

In theory, a constant supply of fresh dung in treated fields, due to permanent grazing, could attract relatively more *Aphodius* beetles to those fields than to fields where cattle are grazed in rotation. However, most of the study fields were used for grazing the same type of stock i.e. treated or untreated, for a number of years. Therefore, if dung beetles were developing normally in dung in treated fields then the greater attraction to a continual supply of fresh dung would give the potential for greater emergence of insects in subsequent years. A greater number of insects due to increased emergence seems counterintuitive since previous research (reviewed in Section 1.4.1) suggests that emergence of dung insects would be impaired in dung from treated animals. Thus reduced emergence within individual pats should offset any increase in insect abundance in treated fields that results from a continual supply of fresh dung in those fields. Additionally, if more insects occurred in treated fields as a result of the continual availability of fresh dung there, then relatively fewer beetles could be expected in dung-baited pitfall traps in treated pasture as their

abundance would be 'diluted' by the colonisation of naturally-occurring dung in the field (as indicated by the dung density trial, Chapter 3). However, if the availability of a *suitable* dung resource was a limiting factor, then one might expect higher activity in fields due to increased search effort. Moreover, beetles in treated fields may not emigrate to adjacent fields because they are also likely to be grazed by avermectin treated cattle. Therefore, the dung in those fields would also be unattractive to the dung beetles. Higher abundance (activity) of *Aphodius* in treated fields was observed in this study which potentially indicates an avoidance of dung from avermectin-treated cattle. The implication of this is that dung beetles that are unable to locate dung that does not contain residues may have no option but to colonise dung from avermectin-treated cattle.

The remaining dung insect groups that were studied in this research did not exhibit significant differences in abundance between fields grazed either by untreated or avermectin-treated cattle. The abundance of *Cercyon* beetles did not differ significantly between treated and untreated pastures, and fluctuations in their numbers occurred mainly with year, seasonality and weather. Previous research on ivermectin effects found the abundance of only two species of *Cercyon*, *C. pygmaeus* and *C. quisquilius*, to be reduced in dung from ivermectin-treated cattle (Floate, 1998a). There was little variation in the number of yellow dung flies, *Scatophaga stercoraria*, trapped in treated and untreated fields and their pattern of abundance exhibited strong inter-annual and seasonal variation. It has previously been shown that yellow dung fly larvae are adversely affected by exposure to ivermectin (McCracken and Foster, 1993), however another study found them to be unaffected (Floate, 1998a). The peak abundance of dung insects trapped in fields did not differ with avermectin treatment. Peak abundance was significantly higher in 2002 indicating that availability of insect prey, and the value of a pasture as a foraging ground, can vary from year to year. The timing of the occurrence of peak abundance also differed between years. In 2002, peak abundance of insects occurred in May in all study fields and in 2003 peak abundance happened later (July). For bird species that are provisioning young in spring and early summer, insufficient availability of insects at that time may prove critical.

With the exception of the *Aphodius* beetles for which avermectin treatment was significant, the factors that were most likely to be significant in the dung insect abundance and diversity models were inter-annual variation, weather and seasonality (as was the case for untreated fields - Chapter 4). Significant inter-annual variation occurred for most of the insect groups that were studied among treated and untreated fields. Such inter-annual fluctuations are common in insect populations, although the variation may also have been partly due to the weather (as discussed in Section 4.4). Numbers of *Aphodius* beetles and yellow dung-flies were significantly higher in 2002 and peak abundance was also higher in that year, reflecting the higher abundance of those two groups. Dung beetle assemblage structure changed between the two study years, as there were relatively more *Aphodius* in catches in 2002 and more *Cercyon* in 2003.

There was no evidence of a shift or change in seasonal patterns of the dung insects in pastures that were grazed by treated cattle, in comparison to those grazed by untreated cattle. The *Aphodius* Guild 1 beetles were the exception, as a seasonal pattern was not significant in the abundance model from data collected in untreated fields (Chapter 4). However, when treated fields were also considered, a seasonal pattern was significant for *Aphodius* Guild 1. This was due to relatively higher numbers of *A. depressus* at the start of the sampling season, i.e. shortly after the first avermectin treatment of livestock, in treated fields. Again, this may have been a manifestation of the presence of avermectin residues in dung and grazing regime as discussed above.

In conclusion, inter-annual variation, seasonality and weather were important factors for dung insects sampled in fields grazed by treated cattle, as they were for dung insects in pastures grazed by untreated cattle. It is suggested that the higher activity of *Aphodius* beetles in treated fields could have resulted from increased search effort for a suitable dung resource, i.e. they avoided colonising dung from avermectin-treated cattle.

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## CHAPTER 6

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# AVERMECTIN EFFECTS ON THE SIZE STRUCTURE, BIOMASS AND ASYMMETRY OF DUNG INSECT POPULATIONS IN PASTURES

## 6. The effects of avermectins on the size structure, biomass and asymmetry of dung insect populations in pastures

### 6.1 Introduction

#### *Size structure*

Habitat quality may affect not only the abundance and diversity of its inhabitants, but also their size (Begon *et al.*, 1990). Sometimes, a change in some aspect of the habitat can subsequently alter the size structure of invertebrate assemblages. For example, successional changes in vegetation associated with the recovery of a polluted site caused a decrease in the average size of carabid species over time (Braun *et al.*, 2004). In grassland habitats, a shift towards smaller carabid species occurs as grassland management intensifies (Blake, 1994; Cole *et al.*, 2002). Hence, comparisons of the size structure of insect assemblages can potentially indicate differences in habitat characteristics.

In this chapter, the size structure of *Aphodius* assemblages is considered in relation to aspects of habitat, such as avermectin treatment, pasture management and other environmental variables. In particular, the hypothesis that smaller *Aphodius* species may be favoured in pastures grazed by avermectin-treated cattle is tested. One might expect such a change in size structure to occur in two ways. Firstly, one might expect relatively more smaller species to occur in 'treated' pastures as a result of a combined effect of the reduced attractiveness of dung from treated cattle and the dispersal ability of *Aphodius* beetles. The preference of *Aphodius* beetles for dung from untreated cattle over dung containing avermectin residues (see Chapter 3) means that beetles might disperse from a pasture containing only treated dung in an attempt to locate a dung resource that does not contain residues. Larger species of *Aphodius* have superior dispersal abilities (Roslin, 2000), therefore one might expect that larger species are more capable of emigration from a treated pasture than smaller species. This could potentially result in smaller species being more dominant components of the assemblage in pastures grazed by treated cattle. Secondly, in a pasture situation where *Aphodius* species have no option but to colonise dung from treated cattle (e.g. if no other dung resource can be located), then smaller species

might generally be favoured as a result of their larval feeding strategy and greater tendency to be saprophagous. The two species with dung-feeding coprophagous larvae that were most common in the study area were *A. depressus* and *A. rufipes* (see Chapter 4 and 5). These two species are larger in size than the two recorded saprophagous species that feed on decaying plant material, *A. prodromus* and *A. sphaelatus*. Species with coprophagous larvae might be expected to be more susceptible to any deleterious effects of residues because they have a higher degree of exposure to them during their development in dung. In addition, the species' phenologies means that any such difference is most likely to be apparent in May, since this is not only when cattle are typically given the first dose of avermectin but also when the 'coprophagous' *A. depressus* and the 'saprophagous' *A. prodromus* and *A. sphaelatus* all occur.

#### *Biomass and 'Weight Median Length'*

The productivity of a particular environment is sometimes expressed in terms of biomass (Begon *et al.*, 1990), thus the relative productivity of different habitats or sites can be assessed by such estimates. Biomass is a frequently measured parameter of dung insect communities (e.g. Galante and Cartagena, 1999; Finn and Giller, 2002) and it is known that dung beetle biomass can be affected by resource availability and pasture management. For example, dung beetle biomass increased when grazing regime changed from sheep to cattle grazing (Lumaret *et al.*, 1992), and *Aphodius* biomass was higher on organic farms than on intensive and rough grazing farms (Hutton and Giller, 2003).

When comparing habitats in terms of optimal prey availability for foraging birds then estimates of total biomass are not necessarily the most appropriate method because they do not provide information on the size of the insects that make up that biomass. The profitability of a prey item has been defined as a function of both the non-chitin mass of the prey and the handling time exerted by the predator (Kaspari and Joern, 1993). Thus, larger beetles are more profitable, provided they can be obtained and handled by the predators. The biomass of an adult beetle increases as approximately the cube of the length of the beetle (Jarošik, 1989) therefore longer beetles are of greater potential value than a similar total length of shorter beetles (Blake *et al.*,

1994). The Weight Median Length (WML) statistic was originally devised to measure the mid-point of the biomass distribution of ground beetles so that average body size of carabids could be compared across different habitat types (Blake *et al.*, 1994).

An objective of this chapter was to examine whether there was any relationship between *Aphodius* biomass and season or pasture management, particularly in terms of whether pastures were grazed by untreated cattle or by avermectin-treated cattle. Also, the WMLs of *Aphodius* assemblages were calculated for grazed pastures to explore whether *Aphodius* biomass distribution changed between pastures in spring and summer, in relation to avermectin treatment, pasture management and habitat variables.

### *Asymmetry*

The effects of environmental stress on a population, such as exposure to insecticide residues, can be assessed using measures of developmental stability (Clarke, 1995). For example, if a population of individuals is exposed to environmental stress during development, then developmental processes can be impaired thus causing bilateral traits to be more asymmetrical than would be expected by chance (Parsons, 1992). The ability of an individual to maintain developmental stability, when subjected to external environmental pressures, can be used to gauge its fitness (McKenzie, 1997).

Three types of asymmetry that can occur in bilateral characters of individuals are fluctuating asymmetry, directional asymmetry and antisymmetry (Møller and Swaddle, 1997). Fluctuating asymmetry is defined as minor random deviations from symmetry; directional symmetry is a handed-bias of one side of the character and antisymmetry is when either side of the character, left or right, is larger (Palmer and Strobeck, 1986; Møller and Swaddle, 1997). Of the three types of asymmetry, only fluctuating asymmetry is a suitable indicator of "perturbed development" (Leary and Allendorf, 1989).

Fluctuating asymmetry has previously been used to study the effects of avermectin exposure on populations of flies in the laboratory. That research has indicated that

exposure to avermectin can increase the asymmetry of some bilateral characters in flies. For example, asymmetry of wing characters was higher in bush flies *Musca vetustissima* that had bred in dung from abamectin-treated cattle than in those that had bred in dung containing no residues (Clarke and Ridsdill-Smith, 1990). In addition, asymmetry was found to be significantly higher in *Scatophaga stercoraria* L. reared in dung to which ivermectin had been added compared to those reared in dung with no avermectin residues (Strong and James, 1993).

A further aim of this chapter was therefore to investigate whether exposure to avermectin affected the asymmetry of natural populations of the yellow dung fly, *Scatophaga stercoraria*, in grazed pastures. Asymmetries of wing size and hind-tibiae length of individuals were compared between pastures grazed by cattle treated with an avermectin and pastures grazed by untreated cattle. Additionally, the biomass of individual yellow dung flies was compared between treated and untreated fields.

## 6.2 Methods

### 6.2.1 Size structure and biomass of *Aphodius* assemblages

#### *Data collection*

Beetles were sampled using pitfall traps baited with dung collected from cattle that had not been dosed with any anthelmintic product. Sampling was carried out from April to July in both 2002 and 2003 on a total of fourteen fields grazed by avermectin-treated and twelve grazed by untreated cattle. Six 'treated' and six 'untreated' fields were sampled in both years, therefore data was collected from a total of 38 pastures over the course of the two sampling seasons (see Section 5.3.3 and Table 5.1 in that section for details of the sampling procedure and a description of the fields). Each pasture was sampled between 6-9 times throughout the sampling period and trapping periods ranged in duration from 7-14 days. To allow comparisons between trapping periods of different duration, all abundance data were corrected to 10 days. Habitat, climate and environmental variables were collected for each trapping period or each study field, as necessary. Information on the

collection of those variables is provided in Section 4.2.2 and a list of the variables used in data analyses is given in Section 5.3.3 (Table 5.2).

#### *Data analysis – size structure*

For the size structure analyses, *Aphodius* beetle species were divided into one of five size classes according to average body length. The placement of a species into a category was carried out using the mean length provided in a Scarabaeidae key (Jessop, 1986) and personal observations regarding the typical length of a species obtained in Ayrshire. For example, the mean length of *A. prodromus* from the aforementioned Scarabaeidae key is 5.5 mm with a range of 4-7 mm. However, it was decided to assign a mean length of 7 mm to that species since one has noticed that *A. prodromus* individuals sampled in the study area were usually on the larger side of the published range. The mean lengths and size classes are listed for each recorded *Aphodius* species (Table 6.1).

Species	Mean length (mm)	Size class (mm)
<i>A. pusillus</i>	3.75	<4
<i>A. ater</i>	5	5-6
<i>A. lapponum</i>	5	5-6
<i>A. sphacelatus</i>	5	5-6
<i>A. rufus</i>	6	5-6
<i>A. fimetarius</i>	7	7-8
<i>A. prodromus</i>	7	7-8
<i>A. depressus</i>	9	9-10
<i>A. fossor</i>	11	11+
<i>A. rufipes</i>	11	11+

Table 6.1 – Mean body lengths (mm) assigned to each species according to published size range in Scarabaeidae key (Jessop, 1986) and personal observations of *Aphodius* beetles in Ayrshire

The abundance of *Aphodius* individuals in each size class was calculated for each individual trapping period in each field and ordinated using DECORANA (a description of that multivariate ordination technique is provided in Chapter 3). As the axis 1 scores from the ordination represent most variation in the ordinated assemblage (Gauch, 1982), those scores were used in a mixed model with repeated measures to investigate any relationships with environmental and management variables (see Section 5.3.3 and Table 5.2 for a description of those variables). A Poisson distribution was used in the analyses because the size structure data was

derived from count data, and field and farm were listed as random factors and sampling date as a repeated factor. For a full description of the modelling procedure, refer to Section 4.2.3.

#### *Data analysis – biomass*

If the mass-length relationship of a group of insects is known, then their dry mass can be more easily estimated from body length without the need for desiccation and weighing of individuals. A general mass-length relationship for insects was derived using individuals from approximately sixty insect families (Rogers *et al.*, 1976). However, it has been proposed that applying a general mass-length formula for any one specific taxonomic group may not give an accurate estimation of biomass (Lang *et al.*, 1997). Consequently, in this study, a mass-length relationship was calculated specifically for *Aphodius* beetles. To this end, the body lengths of 120 *Aphodius* individuals obtained from dung-baited pitfall traps in untreated pastures in May and June of 2002 and 2003 were measured from the anterior edge of the frons to the tip of the abdomen. Their dry masses were obtained by drying to constant weight in an oven at 50°C and then dry mass was regressed on length. A power function model was used to describe the relationship since this is regarded as the best predictor of biomass (Rogers *et al.*, 1977). The mass-length relationship for *Aphodius* was calculated to be:

$$\text{Mass} = 0.0248 (\text{Length})^{2.726}$$

This *Aphodius* mass-length relationship equation was used to estimate the typical biomass of an individual of each *Aphodius* species recorded during sampling, using the mean length for that species (Table 6.1). Then, the estimated mass for each species was simply multiplied by the number of individuals of that species trapped to calculate the total available biomass of each species in each trapping period. The total *Aphodius* biomass was calculated, by summing the biomass for each species for each individual trapping period in all fields, and included in a mixed model with repeated measures. As before, sampling date was included as a repeated factor and farm and field as random factors. In the initial model, treatment was highly significant however as neither of the random factors could be estimated, the

significance of treatment was unreliable (as described in Section 4.2.3). To overcome this, mean biomass per month was calculated for each field from all the trapping periods in a sample month and used as the dependent variable in the mixed model. Relationships with independent variables including avermectin treatment and pasture management and habitat variables were investigated.

A 'guild' approach was taken in previous chapters whereby *Aphodius* were placed into one of two guilds according to their larval feeding strategy. However, for the purpose of these results, both guilds were grouped together and the biomass availability of *Aphodius* was considered as a whole. The reason for doing so was that both species in Guild 2, *A. prodromus* and *A. sphaelatus*, were of similar size therefore their calculated biomass would merely have reflected their abundance (as in results presented in Chapter 5).

#### *Data analysis - Weight Median Length*

The Weight Median Length (WML) was calculated for *Aphodius* species in each study pasture from abundance data that was summed across the sampling season from April to July. Three fields were omitted from the analysis, because invertebrate sampling was not conducted across the full season for those fields, therefore data from 35 study fields were used. To calculate the WML of *Aphodius* for each pasture, the numbers of individuals of each *Aphodius* species were listed and species with the same mean body length were grouped together. The body mass of each species, or group of species with the same mean body length, was estimated using the mass-length equation described previously:  $\text{Mass} = 0.0248 (\text{Length}^{2.726})$ . For each field, the mass for each group of the same body length was multiplied by the number of individuals in that group to give a total biomass for each size class. The mass for each size class was totalled and expressed as a proportion of the total biomass for each pasture. An example of a WML calculation is shown in Appendix XVII. The cumulative percentage biomass for each class of body length was then plotted on probability paper. The Weight Median Length was taken as the length at the 50 per cent cumulative percentage biomass point i.e. the length at which half of the *Aphodius* biomass is made up of beetles shorter, and half is made up of beetles longer, than that length. Relationships between WML and avermectin treatment,

pasture management and habitat variables were investigated using non-parametric statistics.

### 6.2.2 Asymmetry and biomass of yellow dung-flies

#### *Data collection and analysis*

Yellow dung-flies (*Scatophaga stercoraria* L.) were collected from two fields grazed by doramectin-treated and two fields grazed by untreated cattle using dung-baited pitfall traps. The cattle in the treated fields had been dosed with a doramectin pour-on (Dectomax™) at the beginning of May when they were turned out to pasture. The fields were situated on two dairy farms with one 'treated' and one 'untreated' field on each farm. Traps were set 4-5 weeks after the dosing of cattle in 'treated' fields and collected ten days later in mid-June 2002 to ensure that most flies trapped in those fields would have developed from eggs oviposited in dung that contained doramectin residues. Doramectin residues can be excreted from an animal for up to 14 days after treatment (Toutain *et al.*, 1997) and the development time of *S. stercoraria* from egg to adult has been estimated at between 24-42 days (Gibbons, 1987; Strong and James, 1993). Flies were used from 2002 samples only because there were sufficient numbers trapped in that year to ensure that a sample of flies collected on the same day could be measured.

One possible problem with examining asymmetry in populations in pastures is that one cannot be certain that the flies trapped in a pasture have emerged from dung within that pasture. To overcome this, the four pastures from where flies were collected were selected on the basis that they were surrounded by the same grazing use i.e. treated study fields were adjacent to pasture that was also grazed by doramectin-treated cattle. Therefore, unless flies were dispersing large distances after emergence, which presumably they might not do if a dung resource was close by, the flies trapped were likely to have emerged either in the study pasture or in adjacent pasture containing the same 'type' of dung.

The left and right wing lengths and hind tibia lengths were measured on a total of 113 male flies, 54 of which were collected in treated pasture and 59 in untreated pasture. The asymmetry of individuals of only one sex was measured to exclude the

possibility that an effect may have been sex-specific. Males were measured because they were trapped in larger numbers than females therefore a sufficient sample size was obtained by measuring males. Wings and hind legs were dissected from each fly, placed under a coverslip, and measured under a microscope to the nearest 0.1 mm. Wing length measurements were made in a straight line from point A to point B (Figure 6.1), and hind tibia measurements were taken as the full length from where the tibia joined the femur to where it met the first tarsal segment.

The error of measuring a trait should be estimated to ensure that it does not exceed the actual measured asymmetry of that trait (Palmer and Strobeck, 1986). To estimate measurement error, the left and right wing and hind tibiae lengths of 30 flies were measured on three separate occasions on consecutive days, without reference to which individual was being measured at the time. Following Swaddle *et al.*, (1994), a mixed model ANOVA was used to test whether the variance of estimated asymmetry between individuals was greater than the variance due to measurement error. Variance was significantly greater between individuals than between repeated measurements for wing length asymmetry ( $F_{9, 36}=2.31, P<0.05$ ) but not for hind tibia length asymmetry ( $F_{9, 36}=0.86, P>0.05$ ). Therefore only wing length data was analysed and presented here, since one could not be certain that any differences in hind tibia length asymmetry were not due to measurement error.

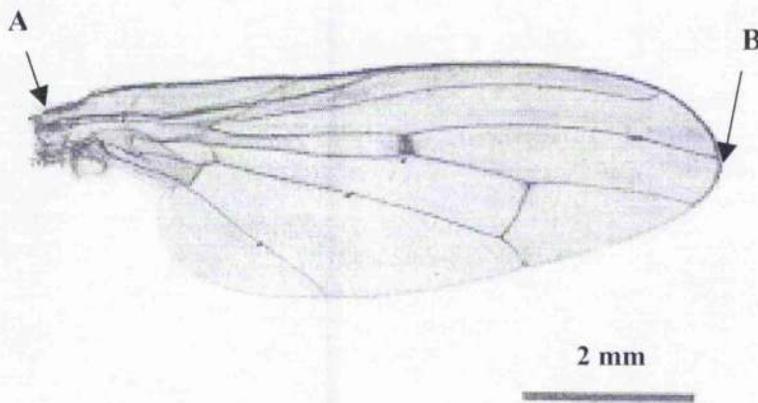


Figure 6.1 – Picture of a wing of *Scatophaga stercoraria* showing wing length measurements taken in a straight line from the proximal end of the costal vein (A) to the tip of the wing (B), photo adapted from Strong and James (1993)

The bilateral character that is measured among populations should meet the assumptions of fluctuating asymmetry, which are a normal distribution around a

mean of zero (e.g. Palmer and Strobeck, 1986). To do this, the signed asymmetry scores of wing length were calculated by subtracting the size of the right character from that of the left character. Normality of the signed asymmetry scores was assessed using the Kolmogorov-Smirnov test to check that antisymmetry did not occur in that trait in the study populations (e.g. Møller, 1996; Ahtiainen *et al.*, 2003). A 1-sample t-test was used to test that the mean signed asymmetry score did not differ significantly from zero to exclude the possibility that the data exhibited directional asymmetry (e.g. Møller, 1996; Sneddon and Swaddle, 1999). As the assumptions of fluctuating asymmetry were met, the absolute (unsigned) asymmetry scores for wing length were calculated by subtracting the smaller side of the bilateral character from the larger side. To examine fluctuating asymmetry between samples, non-parametric tests should be used on absolute asymmetry data (Palmer and Strobeck, 1986). Therefore, the wing length asymmetries of flies collected from fields grazed either by untreated or by doramectin-treated cattle were compared using a Mann-Whitney U-test.

As wing length and body mass are highly positively correlated ( $r^2=0.995$ ) in *S. stercoraria* (Borgia, 1982), wing length was used as an indicator of biomass in this study. The mean wing lengths of the flies used in the fluctuating asymmetry analysis, i.e. 54 flies from treated pasture and 59 flies from untreated pasture, were compared between fields grazed either by untreated or doramectin-treated cattle.

## 6.3 Results

### 6.3.1 *Aphodius* assemblages

#### *Size structure of Aphodius community*

The axis 1 scores from the ordination of size class data were used to determine whether there was a shift in size structure over the season or between treated and untreated fields (see Appendix XVIII. for ordination scores). The model could not estimate the random factor 'field'. However, avermectin treatment was not significant in the model thus there was no risk of wrongly concluding that there was a significant treatment effect (as discussed in Section 4.2.3). In the fields studied, size class distribution changed between years and with season (Table 6.2). There

was no effect of avermectin treatment on size class data ( $F_{1, 49.9}=0.01$ ,  $P=0.91$ ) and the effect of season on size distribution was the same for treated and untreated fields ( $F_{1, 153}=0.64$ ,  $P=0.43$ ). To show the ordination positions of trapping periods in each month and year, centroids were calculated as the mean of axes 1 and 2 scores and plotted (Figure 6.2). Axis 1 scores were lowest in April and May of both years and this was due to relatively more individuals in the 5-6 mm and 7-8 mm size classes e.g. *A. ater*, *A. prodromus* and *A. sphacelatus*. Trapping periods in June and July are positioned to the right of axis 1 due to a higher occurrence of larger species e.g. *A. rufipes*. The June 2003 centroid may also have been pulled to the right of axis 1 due to the occurrence of *A. pusillus* (<4 mm size class) in that month and year only (Figure 6.3).

Variable	Estimate	sc	Test statistics	P
Farm			$Z=0.27$	0.39
Year	0.178	0.058	$F_{1, 83.7}=9.47$	0.003
Seasonality	0.074	0.009	$F_{1, 163}=69.11$	<0.0001
Seasonality <sup>2</sup>	-0.0004	0.00006	$F_{1, 175}=37.72$	<0.0001
Intercept	1.487	0.318		

Table 6.2 – Model describing the variation in *Aphodius* size structure distribution in fields grazed by avermectin-treated and untreated cattle sampled from April to July in 2002 and 2003

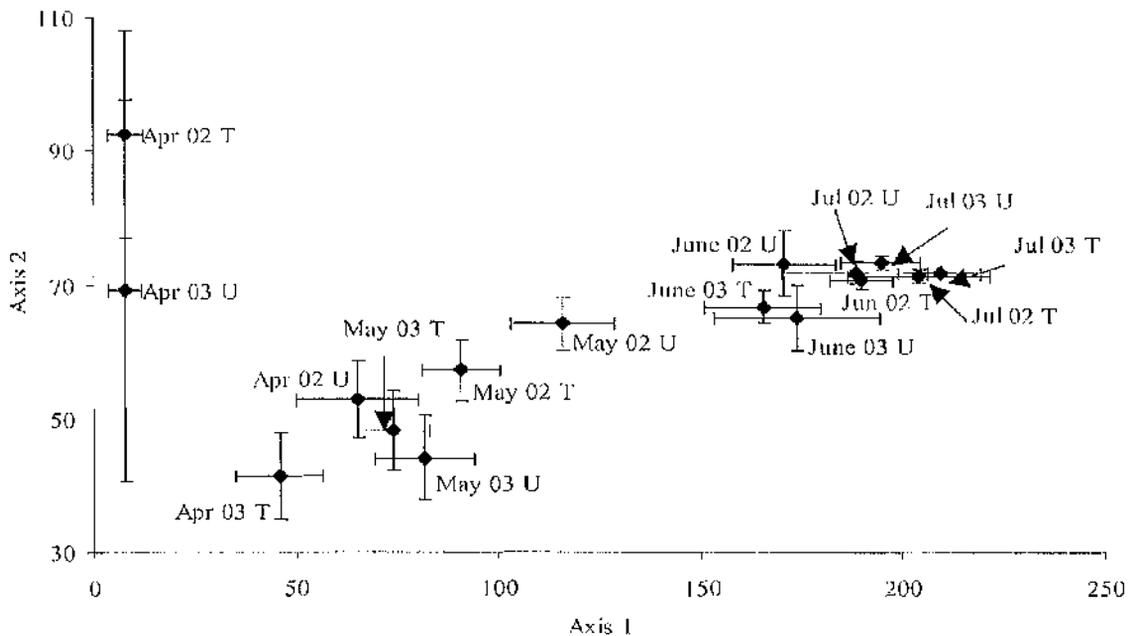


Figure 6.2 – Graph showing centroid (mean  $\pm$  1 se) of axis 1 and 2 scores from ordination of *Aphodius* abundance by size class, from individual trapping periods in treated (T) and untreated (U) fields sampled from April to July in 2002 and 2003

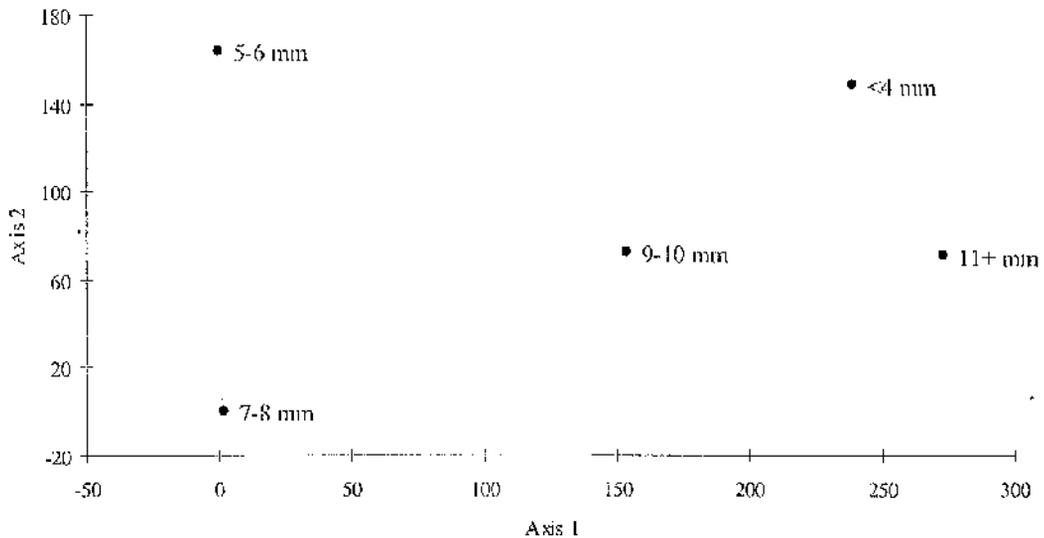


Figure 6.3 – Size class scores from ordination of *Aphodius* individuals by size class, sampled in treated and untreated fields from April to July in 2002 and 2003

Canonical Correspondence Analysis (CCA) is a ‘constrained ordination’ technique, which can be used to examine the variability of species assemblage data in relation to measured environmental variables (Lepš and Šmilauer, 2003). In such analyses, the data on the chosen environmental variables can be incorporated into the consideration of similarities between the species assemblages, thereby allowing the influence of these variables on the resulting ordination of the samples to be assessed directly. A partial CCA was performed to investigate the effects of avermectin treatment on the size class structure of *Aphodius* dung beetle assemblages. In a partial CCA, one can specify a particular variable to be examined, in this case avermectin treatment (ter Braak and Šmilauer, 2002). Two other variables (year and sample date) were included in the partial CCA as supplementary variables. Supplementary variables are considered posthoc and thus do not have a direct influence on the actual ordination. The analysis was conducted using the CANOCO 4.5 software package (ter Braak and Šmilauer, 2002). The resulting output graph showed that samples were classified into one of two distinct groups of samples, which corresponded to the categorical variable of treatment i.e. samples were divided into treated and untreated groups. There was no separation of samples according to differences in the size structure of the *Aphodius* assemblage. This observation occurred because the variation in size structure of *Aphodius* beetle assemblages between the samples was so low that there was insufficient power for it to be explained by the environmental variable under consideration (Palmer, 2005).

Therefore, variation in size structure of *Aphodius* beetle assemblages between treated and untreated pastures must have been extremely low. Consequently, the analysis is not considered further here.

### *Aphodius* biomass

Mean monthly biomass of *Aphodius* beetles was significantly higher in fields in 2002 (Figure 6.4). There was higher biomass of *Aphodius* in fields grazed by treated cattle although this was only just significant (Table 6.3). Sample month was not significant in 2003 ( $F_{3, 27.4}=2.27$ ,  $P=0.102$ ) however month was significant in 2002 ( $F_{3, 37}=15$ ,  $P=0.005$ ) when biomass peaked at the beginning of May (Table 6.3).

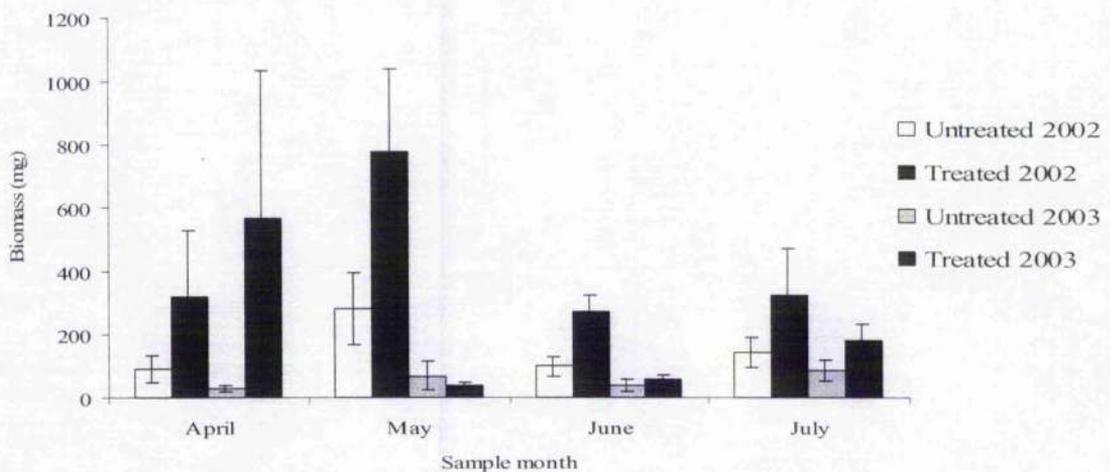


Figure 6.4 – Mean ( $\pm 1$  se) *Aphodius* biomass (mg) in pastures grazed by untreated and avermectin-treated cattle sample from April-July in 2002 and 2003

Variable	Estimate	se	Test statistics	P
Field			Z=0.74	0.231
Year	-83.11	137.56	$F_{1, 55.8}=6.12$	0.017
Month			$F_{3, 57.5}=1.64$	0.191
April	0	.	.	.
May	-236.44	119.01		
June	-241.45	116.89		
July	-189.04	116.56		
Avermectin treatment	174.05	79.97	$F_{1, 13.3}=4.74$	0.048
Year*Month			$F_{3, 83.5}=3.15$	0.029
Year*April	0	.	.	.
Year*May	533.99	178.29		
Year*June	226.23	177.51		
Year*July	233.74	187.06		
Intercept	212.49	104.46		

Table 6.3 - Model describing the variation in *Aphodius* biomass in each sample month in fields grazed by avermectin-treated and untreated cattle

### Weight Median Length

The 'Weight Median Length' (WML) of *Aphodius* species was compared to investigate differences in the *Aphodius* biomass distribution between study pastures. The mean ( $\pm 1$  se) WML was  $7.1 \pm 0.2$  mm in untreated fields and  $7.3 \pm 0.3$  mm in treated fields (Figure 6.5). The WML of *Aphodius* did not change with avermectin treatment, year or with grazing system i.e. permanent vs. rotational grazing (Mann-Whitney, all  $P > 0.52$ ), and there was no relationship between WML and the management intensity of pastures (Spearman Rank  $r_s = -0.05$ ,  $df = 33$ ,  $P = 0.78$ ).

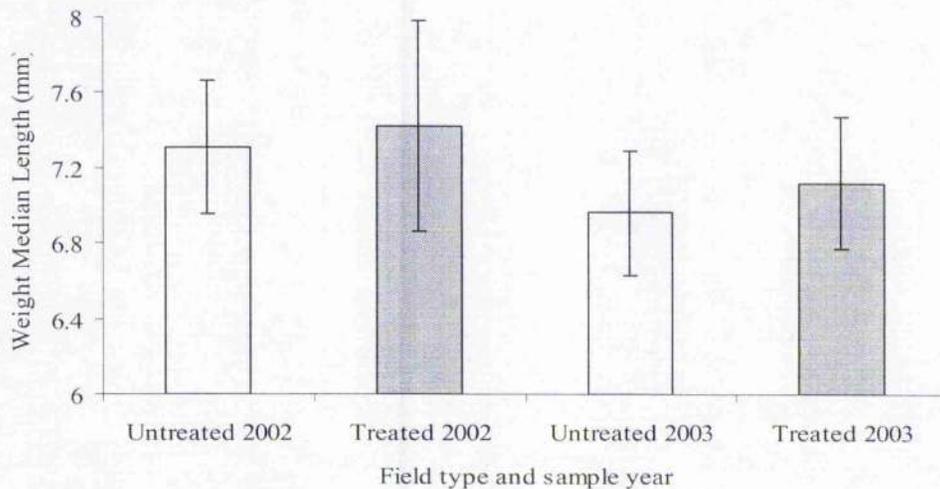


Figure 6.5 – Mean ( $\pm 1$  se) Weight Median Length of *Aphodius* biomass distribution in fields grazed by untreated and avermectin-treated cattle sampled from April to July in 2002 and 2003

#### 6.3.2 Asymmetry and biomass of yellow dung-flies

The signed asymmetry scores of wing lengths fitted a normal distribution ( $D = 0.047$ ,  $n = 113$ ,  $P > 0.15$ ) with a mean of zero ( $t = 0.27$ ,  $n = 113$ ,  $P = 0.79$ ) thus the assumptions of fluctuating asymmetry were met. The absolute asymmetry of wings was significantly higher in treated fields than in untreated fields (Figure 6.6).

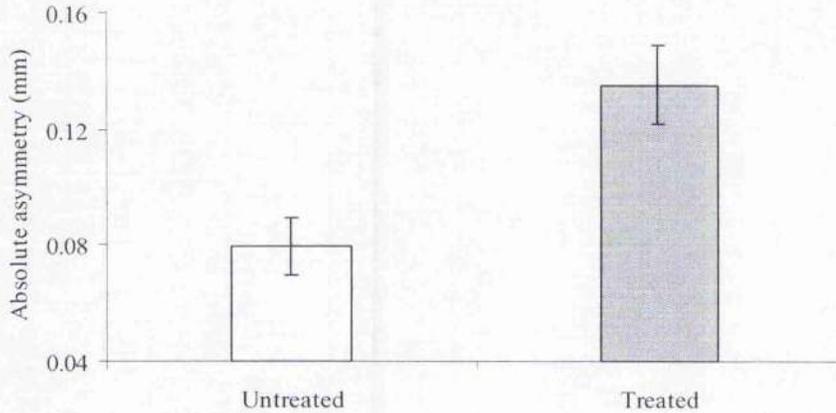


Figure 6.6 – Mean ( $\pm 1$  se) absolute asymmetry scores for wing lengths of *Scatophaga stercoraria* trapped in 2002 in fields grazed by untreated or doramectin-treated cattle. Asymmetry is significantly higher in fields with treated cattle (Mann-Whitney,  $n=113$ ,  $P=0.002$ )

The mean wing lengths of male flies measured for fluctuating asymmetry did not differ significantly between treated and untreated fields (Figure 6.7). This indicated that the body mass of flies exposed to doramectin during development was not significantly altered. However, there was a non-significant trend for individuals of higher biomass in treated fields.

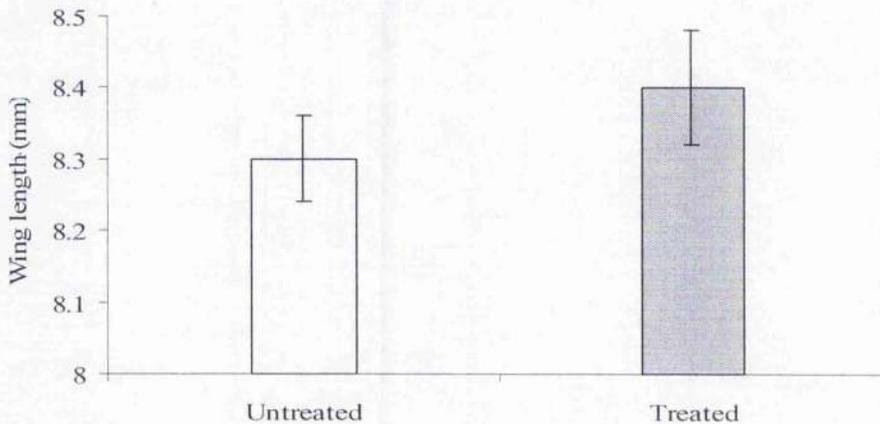


Figure 6.7 – Mean ( $\pm 1$  se) wing lengths of yellow dung-flies trapped in 2002 in pastures grazed by untreated cattle and pastures grazed by doramectin treated cattle ( $t=1.7$ ,  $df=224$ ,  $P=0.09$ )

## 6.4 Discussion

### *Size structure and biomass of Aphodius assemblages*

Analysis of size structure data showed that a shift towards smaller species did not occur in fields grazed by avermectin-treated cattle. Furthermore, the mid-point of the *Aphodius* biomass distribution (as measured by the Weight Median Length statistic) did not differ significantly between fields. Therefore, the average body size of *Aphodius* beetles in fields grazed by avermectin-treated cattle was not lower than in those grazed by untreated cattle. Biomass of *Aphodius* was significantly higher in treated fields but that was a function of their increased abundance in traps in those fields (Chapter 5) rather than greater availability of larger individuals in treated fields.

The size structure of *Aphodius* assemblages did change with season and with year. In April and May, there were relatively more individuals from the 5-8 mm size classes i.e. *A. ater*, *A. prodromus* and *A. sphacelatus* as those species are typically spring and early summer species (Hanski, 1980c; Gittings and Giller, 1997; Chapter 5). Relatively more individuals in the larger size classes, which mainly comprised the late summer species *A. rufipes*, were present in June and July. Seasonal patterns in *Aphodius* size structure did not differ between treated and untreated fields, thus indicating that the occurrence of any one particular size class was not delayed in treated fields.

A seasonal pattern was apparent for the biomass of *Aphodius* in 2002, with biomass highest in April and May and declining thereafter, as has been observed in other studies (Finn *et al.*, 1999). Biomass also tends to peak in late summer when high numbers of *A. rufipes* occur (Finn *et al.*, 1999) but that was not observed here. The lack of a seasonal pattern in 2003 may have been due to the lower numbers of *Aphodius* beetles in traps in that year.

If the profitability of a habitat for foraging predators is considered to be a function of the size of the insect prey there, then the profitability of pastures grazed by avermectin-treated cattle was not less than pastures grazed by untreated cattle. However, simply in terms of available biomass and abundance of *Aphodius* beetles,

treated fields would seem to be the most profitable feeding areas. However, as discussed in Chapter 5, higher numbers of beetles in treated fields may not be a simple expression of higher abundance but may instead be due to an attraction effect as a result of an avoidance of dung from avermectin-treated cattle. If the dung in treated fields is sub-optimal and is avoided for colonisation, then a subsequent reduction in the density of larval prey in dung could occur. This could ultimately diminish the availability of dung insect prey (larvae and adults) for vertebrate predators in fields grazed by treated cattle.

For predators of dung insects in pastures, optimal prey availability is most likely to change with season and also with year. Biomass of *Aphodius* dung beetles appears to be highest in early May, which would be beneficial for birds provisioning chicks at that time. Dung beetles of a larger size occur more in late summer therefore there could be good availability of profitable prey at that time of year. That is, of course, dependent upon those larger prey items being abundant at that time.

#### *Biomass and asymmetry of yellow dung-flies*

The mean individual biomass of male yellow dung-flies, as gauged by wing length, did not differ significantly between treated and untreated fields. Larger male yellow dung flies have higher mating success than relatively smaller flies (Borgia, 1982), however there was no evidence here that differences in biomass would impair the reproductive success of this species in treated fields. This also shows that the relative profitability of flies as individual prey items for foraging predators would not differ significantly between treated and untreated fields.

Asymmetry of wing lengths was significantly higher in flies trapped in fields grazed by doramectin-treated cattle (where the majority of trapped flies would have developed in dung containing doramectin residues). This potentially indicates that doramectin exposure during development has a sublethal effect on yellow dung flies in pastures grazed by treated cattle. One cannot rule out that the treated and untreated fields differed in some other unmeasured aspect that may have affected asymmetry. Ideally, a larger number of fields would have been included in this analysis to minimise such variation. However, there were only a limited number of

fields that could be sampled from where sufficient numbers of flies were collected some 5-6 weeks after cattle had been treated.

Increased asymmetry of a trait can compromise the survival of individuals in that population (Møller and Swaddle, 1997). Previous research has shown that the risk of being predated increases in asymmetric individuals (Swaddle, 1997), usually because more asymmetric prey are less able to evade predators than their symmetric conspecifics. For example, flies (*Musca domestica*) with higher wing asymmetry had an increased chance of being captured by foraging barn swallows (Møller, 1996). In these results, the relationship between predation pressure from birds and yellow dung fly asymmetry was unclear although predation by barn swallows could have reduced the level of asymmetry in the fly population. For example, if the foraging activity of swallows was lower in treated fields then selection pressure against asymmetric individuals would be reduced and would consequently result in relatively higher asymmetry in the yellow dung fly populations in those fields. This is feasible since treated fields are often situated farther from farm buildings i.e. nest sites for barn swallows, than untreated fields are. This could result in relatively higher foraging activity in untreated fields and thus increase the selection pressure against asymmetrical flies.

Another potential explanation for greater asymmetry of flies in treated fields was competitive pressure because high intra- and interspecific competition may increase levels of asymmetry in populations (Rettig *et al.*, 1997). For example, larval crowding in the blowfly, *Lucilia cuprina*, increased asymmetry levels in that species (Clarke and McKenzie, 1992). Increased larval densities of the yellow dung flies may have occurred in dung in fields grazed by avermectin-treated cattle if a large number of adults were attracted to the fresh dung in those pastures, provided that the adults oviposited in that dung. This may seem unlikely since previous results indicated that yellow dung flies avoided dung from avermectin-treated cattle (Chapter 3). However, it has already been mentioned that the field was adjacent to other fields that were also grazed by treated cattle therefore perhaps adult flies had no option but to colonise treated dung pats. To this end, a lack of suitable dung in treated fields may have resulted in flies opting to colonise the 'best of a bad lot' of dung pats in those fields i.e. perhaps one or two pats in the fields were less

unattractive than others hence leading to overcrowding. However, the exact cause of increased asymmetry remains speculative.

Flies with higher levels of asymmetry often have lower reproductive success (Liggett *et al.*, 1993; Allen and Simmons, 1996; Møller, 1996), which could manifest itself as a reduced population size of at least one generation of yellow dung flies following exposure to avermectins during early development. However, a difference in abundance was not apparent when sampling yellow dung flies in treated and untreated pastures (Chapter 5 results) as one might expect if fecundity was significantly impaired. Hence, although increased levels of asymmetry can compromise survival (Møller and Swaddle, 1997), a decline in yellow dung fly populations in pastures was not evident in this research. Nevertheless, these results have highlighted that doramectin exposure during development, in a natural pasture situation, may cause sublethal effects in dung insects and this has implications for other more 'sensitive' species. For species that exist as metapopulations, a temporary loss of genetic diversity may occur via bottlenecks and such a loss of diversity may make populations less resilient to environmental stress (Brookes *et al.*, 1997; Keller and Waller, 2002). It has been suggested that the dung beetle *Aphodius pusillus* (recorded in only one location in this study) may exist as metapopulations and that the species is, at the very least, sensitive to changes in pastoral habitat connectivity (Roslin, 2000; 2001). Further investigation is required into the potential sublethal effects of avermectin exposure on species that may be more susceptible to environmental stress, because of their population dynamics and distribution.

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CHAPTER 7

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FORAGING ACTIVITY OF INSECTIVOROUS  
BIRDS IN PASTURES GRAZED BY AVERMECTIN-  
TREATED CATTLE

## 7. Foraging activity of insectivorous birds in pastures grazed by cattle treated with an avermectin

### 7.1 Introduction

#### *Foraging activity of insectivorous birds in pastures*

Invertebrates are an invaluable component in the diet of insectivorous birds and of granivorous birds that are provisioning young during the breeding season. The availability of this source of protein in the diets of chicks is necessary for good growth and body condition (Donald *et al.*, 2001a; Park *et al.*, 2001). The intensification of grassland management can alter the diversity and abundance of insects (Purvis and Curry, 1981; Kruess and Tschardt, 2002; Wickramasinghe *et al.*, 2004) which may in turn affect the profitability of a grassland habitat as a foraging area for predators. Indeed, reduced availability of insects has been implicated in the decline of farmland birds (Wilson *et al.*, 1999; Vickery *et al.*, 2001). Furthermore, the biomass value of invertebrate prey is dependent upon the size of the prey items as well as their abundance (see Section 6.1).

Insects that breed in dung are a good resource for foraging birds in terms of both adult and larval prey. For example, the dung flies Scatophagidae and Sphaeroceridae are preyed upon by swifts, house martins and wagtails (Bryant, 1973; Davies, 1977). Adult *Aphodius* dung beetles are eaten by waders such as lapwing, redshank and oystercatcher (Beintema *et al.*, 1991). Exposure to avermectin residues within dung can impair the oviposition and larval development of dung-breeding insects (Gover and Strong, 1997; Floate *et al.*, 2001; see Section 1.4). Therefore, in pastures where livestock have been treated with an avermectin product the amount of larval and adult prey available for foraging birds in pastures could ultimately be reduced. Indeed, McCracken (1993) has highlighted the potential effects of avermectin use on the vertebrate predators of dung insects.

The biomass distribution of a group of insects can be assessed using the Weight Median Length (WML) statistic (Blake *et al.*, 1994; and Section 6.1). By comparing the WML of a group of insects between habitats, one can assess whether that habitat

supports relatively more insects of larger body size than another habitat. Hence, the statistic can be used as a potential indicator of the profitability of a habitat for foraging predators.

The main aim of this thesis was to study the potential effects of avermectins on dung insect assemblages in pastures, rather than to assess directly the effects of avermectins on foraging birds. However, there was an opportunity to carry out a limited survey of insectivorous birds in pastures grazed by untreated and avermectin-treated cattle. Hence, these bird observations are not a comprehensive study of feeding activity in pastures but they do provide a general comparison of the activity of foraging birds in treated and untreated fields. One of the aims of this chapter was to ascertain whether there was a marked difference in the species richness and feeding activity of insectivorous birds between pastures grazed by avermectin-treated and untreated cattle. To allow consideration of potential factors causing any observed variation in bird feeding activity, the data were analysed in relation to treatment, dung insect abundance, *Aphodius* biomass distribution, pasture management and habitat characteristics.

#### *Swallow foraging activity*

The barn swallow, *Hirundo rustica* L., is a common species on British farmland in summertime. Swallows arrive in Britain in April and typically produce three broods of 4-5 eggs each summer (Dodds *et al.*, 1995). They are aerial foragers and feed on dung-breeding flies and insects, as well as other invertebrates (Turner, 1982). A reduction in the size of local swallow populations has been observed in many parts of Europe (Møller, 2001 and references therein). Such declines have been attributed to a loss of nest sites, but other changes resulting from agricultural intensification may also be important (Evans *et al.*, 2003). For example, declines may be linked to changes in dairy farm management. A study by Møller (2001), found that the size of local swallow populations on farms were smaller after active dairy farming had ceased. Since the nest sites of the swallows were not affected, the diminished breeding success may have been caused by reduced availability of insect prey on the farms that no longer kept dairy cattle.

An aim of this chapter was to investigate the foraging activity of the barn swallow in pastures grazed by avermectin-treated and untreated cattle. This species was selected for study because it is known to prey upon dung-breeding flies and is particularly associated with cattle-grazed pastures. Therefore, if avermectin residues in dung have a significant effect on populations of dung-breeding insects then one might expect the foraging behaviour of the barn swallow to change in response to altered prey availability.

## 7.2 Method

### 7.2.1 Species richness and feeding activity of insectivorous birds

#### *Data collection*

Bird observations were conducted on twelve fields on four commercial dairy farms in Ayrshire from late-May to July of 2003. The author selected study sites and conducted all data analyses, however all bird observations in 2003 were carried out by Becky Claws (Napier University MSc student). Table 7.1 lists the fields, details of avermectin treatment strategies, and the number of times that the observer visited each field.

On each visit, the field boundary was walked and any birds that were observed within the pasture were recorded along with their behaviour, e.g. feeding, collecting nest material. Birds flying over the pasture were noted but were not included in the analyses. When the fields could not be accessed, usually due to the presence of a bull, the observer stood at a fixed vantage point at the edge of the field from where observations were made.

The environmental and pasture management data collected for each field are summarised (Table 7.2). Sward height was measured by the 'direct method' (Stewart *et al.*, 2001) and soil impenetrability was measured using a penetrometer (as described in Section 4.2.2). Ten measurements of sward height and impenetrability were taken in the study field on each sample date and the mean values of each were used in the analyses. Both sward height and soil penetrability can be important

factors in the foraging success of ground-feeders (Green, 1988; Devereux *et al.*, 2004).

Farm	Field code	Avermectin treatment?	Dates of treatment	Number of times sampled
1	DT1	Doramectin pour-on	20 May & 7 July	5
1	DT3	Doramectin pour-on	20 May & 7 July	5
1	DC5	None	N/A	5
1	DC6	None	N/A	5
2	ST1	Doramectin pour-on	28 April & 23 June	5
2	ST2	Doramectin pour-on	28 April & 23 June	5
2	ST3	Doramectin pour-on	28 April & 23 June	6
2	SC3	None	N/A	4
3	LBT1	Doramectin pour-on	13 May & 1 August	5
3	LBC1	None	N/A	5
4	BTBC2	None	N/A	4
4	BTBC3	None	N/A	4

Table 7.1 – Avermectin treatment strategies of livestock in fields in which bird observations were made from late-May to July 2003

Rainfall and temperature data were collected at the weather station at Auchincruive (NS 379 234), which is situated within a ten-mile range of field sites. Each study field was assigned a Management Intensity Score (MIS) according to the intensity of a range of management factors in that field including livestock density, cutting regimes and fertiliser input (see Section 4.2.2 for a description of MIS). Avermectin treatment and ‘days post-treatment’ were included to detect whether there was a treatment effect and, if so, whether that effect diminished as time from treatment increased. The time of survey (am or pm), whether cows were present in the fields at the time of survey and the field boundary type were all recorded and included in the analyses as these factors could all have influenced bird activity.

Birds search for food over wide areas of agricultural land enabling them to exploit patchy or localised food supplies (Chamberlain *et al.*, 2000). Therefore, wider landscape features are likely to influence the foraging activity of birds in a particular pasture. Indices of ‘avermectin pasture’ and ‘grazing pasture’ were calculated for each field to account for the extent of avermectin use in surrounding pasture and the availability of surrounding grazed pasture, respectively. The former index estimated the amount of pasture grazed by avermectin-treated cattle within a 0.5 km<sup>2</sup> area around the study field and the latter estimated the proportion of grazed pasture within the same area (for detailed description of indices, see Section 4.2.2). The value of

the avermectin index is that it could highlight differences in the foraging activity of birds in pastures depending on whether the pasture is surrounded by fields grazed mainly by treated or by untreated cattle. With the grazing pasture index, one is able to examine whether there is a difference between fields surrounded by a high proportion of grazed pasture in comparison to those surrounded by other land-use types, e.g. urban areas, woodland.

Dung-baited pitfall traps were used to sample dung insects in the fields where bird observations were carried out and these data were collected as part of the wider sampling study (the trapping procedure is detailed in Section 4.2.2). The data used in this analysis were the maximum number of dung insects that occurred in four traps in a pasture, collected within two days of the bird survey. Traps were exposed for 14 days therefore the insect abundance data reflected what was trapped in the two weeks preceding the day that bird observations were made. The dung insects were adult *Aphodius*, *Sphaeridium* and *Cercyon* beetles and adult *Scatophaga* flies. Of those insects, the *Cercyon* species have the smallest body size with a size range of approximately 1-4mm (Skidmore, 1991). Therefore, the *Cercyon* may not be the most profitable group in terms of biomass but nevertheless, beetles of their size are predated by birds (e.g. wagtails: Davies, 1977). The Weight Median Length of *Aphodius* was calculated for each pasture using data that had been collected from the pastures from April to July of that year (see Section 6.2.1 for a full description of WML calculation). Fields that had the same Weight Median Length value were grouped together for data analyses.

#### *Data analysis*

Only insectivorous bird species and species that provision young with invertebrates were included in the analyses. Ordination of bird assemblages in each study pasture was performed using DECORANA to assess differences in species composition between pastures (see Section 3.2.2 for a description of the ordination method). The number of species observed in a pasture on each visit was included in a mixed model with repeated measures and a normal error distribution (for details of the modelling procedure, see Section 4.2.3.). The total numbers of birds that were observed actively foraging within or above the pasture on each visit were included in a mixed

model with repeated measures and a Poisson error distribution. In both models, field and farm were included as random factors and sampling date was included as a repeated factor to take into account that repeat visits were made to each field. Independent variables (Table 7.2) were tested in the model, including quadratics of continuous variables and interactions between variables.

Variable	Level	Type
Sampling date	Number of days from 1 April	Continuous; change
Field	12 levels	Categorical; fixed
Farm	4 levels	Categorical; fixed
Avermectin treatment	2 levels: 1-No; 2-Yes	Categorical; fixed
Days post-treatment	Days since cattle in field were treated with avermectin	Continuous; change
Field size	Area (2-8.3ha)	Continuous
Time of survey	2 levels: 1- am; 2- pm	Categorical; change
Cows present during survey?	2 levels: 1-No; 2-Yes	Categorical; change
Boundary of field	3 levels: 1-Fence; 2-Gappy hedge; 3-Hedge	Categorical; fixed
Sward height	(6-19cm)	Continuous; change
Soil impenetrability	(48-106 impenetrability index)	Continuous; change
Management Intensity Score	(Score 10-17)	Continuous
Rainfall	Rain on day of survey (0-3mm)	Continuous; change
Maximum temperature	Maximum temperature on day of survey (16-29.6°C)	Continuous; change
Minimum temperature	Minimum temperature on day of survey (7.7-15.5°C)	Continuous; change
Dung insects	Peak abundance (13-417 insects)	Continuous; change
<i>Aphodius</i> Weight Median Length (WML)	(5.9-8.4mm) see 6.2.1 for a description of WML	Continuous; fixed
Avermectin index	(0-53%)	Continuous
Pasture index	(62-81%)	Continuous

Table 7.2 – Variables measured for modelling species richness and foraging activity of insectivorous birds. ‘Change’ indicates whether a variable changes over time or whether it is ‘fixed’ over the whole sampling period. Recorded ranges of continuous variables are given in parentheses

For the analyses of foraging activity in relation to Weight Median Length, birds were initially divided into groups of ‘small’ and ‘large’ species because the optimal prey size of a bird would presumably reflect its body size and/ or gape size. However, the models could not estimate the random factors in the analyses for ‘small’ and ‘large’ birds. Therefore, the relationship between foraging activity and *Aphodius* Weight Median Length was considered for birds of all sizes together.

### 7.2.2 Foraging activity of Swallows

#### *Data collection*

Observations on the feeding behaviour of swallows were made, by the author, in seven fields on three commercial dairy farms in Ayrshire. The survey was carried out from late-May to July 2004 in four pastures grazed by untreated cattle and three pastures grazed by avermectin-treated cattle. In the ‘treated’ fields, cattle were dosed with a doramectin pour-on on 28 April and on 9 July. As only a small number of fields could be sampled, the seven fields were selected on the basis of their similarity of characteristics such as hedgerow type and proximity to swallow breeding colony in an attempt to minimise the inter-field variation that was not related to avermectin treatment of cattle. A total of 84 observation periods were conducted with each field surveyed between two and nineteen times with a mean of eleven visits to a field. The small number of visits to two of the fields was due to limited access to those fields. Observations were always carried out between 0700-0900 hrs for twenty-minute periods. Extremely rainy and windy days were avoided for surveys because swallows tend to avoid foraging in open pasture in preference for hedgerows in such weather (Evans *et al.*, 2003).

The maximum number of simultaneously foraging swallows and the duration of foraging bouts of individual swallows were recorded within a set area of the field (Evans *et al.*, 2003). The size of sample areas differed between fields because boundaries were defined using easily recognisable features and also because the natural topographies of fields sometimes obstructed views. Observations were made from a fixed vantage point that gave the best view of the sample area. Swallows were classed as foraging if they were flying low to the ground and changing flight direction rapidly. They were not recorded as foraging if they did not exhibit typical foraging behaviour e.g. if they were flying in one direction over a field. Also, birds were excluded if they were foraging exclusively along a hedgerow because the interest was in birds foraging over the pasture where the emergence of dung-breeding flies would occur.

### Data analysis

The maximum numbers of swallows observed foraging at any one time were included in a mixed model with a Poisson distribution. Sampling date was included as a repeated factor and field as a random factor. The independent variables (Table 7.3) tested in the model included factors that are known to influence swallow activity e.g. presence of trees in the field boundary, proximity to nest site and presence of cattle in fields (Ambrosini *et al.*, 2002; Evans *et al.*, 2003). 'Proximity to nest site' was ascertained by observing the location of the foraging swallows' nest site and then mapping the distance from that farm building to the sample area in the study field.

The mean time that swallows spent foraging in each twenty-minute period was calculated. The sample areas in fields ranged from 3000-8300m<sup>2</sup> therefore means were corrected to per hectare and then included in a mixed model as described above. Unfortunately, the initial model could not estimate the random factor 'field' therefore that model was unreliable (as highlighted in Section 4.2.3). To overcome this, the mean foraging time was calculated for each field over the whole sampling period from May to July. As the mean foraging time was calculated from all observations for each field, changes in time spent foraging activity could not be assessed with continuous variables such as sampling date. Thus, mean time spent foraging per hectare between treated and untreated pasture was analysed using non-parametric statistics.

Analyses were also performed on barn swallow data collected by Becky Clews in 2003, in order to allow comparisons with the 2004 swallow data collected by the author. It should be borne in mind that different observation methods were used in each year and that 2003 data refer to the number of swallows observed while 2004 data refer to the maximum number observed at any one time and the length of foraging bouts. Furthermore, there were no data on proximity to nest site in 2003. Dung insect data were collected in 2003 therefore the number of foraging swallows observed could be compared to yellow dung fly, *Scatophaga stercoraria*, abundance in pastures.

Variable	Level	Type
Sampling date	Number of days from 1 April	Continuous
Field	7 levels	Categorical; fixed
Farm	3 levels	Categorical; fixed
Avermectin treatment	2 levels: 1-No; 2-Yes	Categorical; fixed
Days post- treatment	Number of days since cattle in field were treated with an avermectin	Continuous; change
Cows present at survey?	2 levels: 1-No; 2-Yes	Categorical; change
Boundary of field	3 levels: 1-Fence; 2-Gappy hedge; 3-Hedge	Categorical; fixed
Rain	2 levels: 1 – no rain at time of survey 2 – raining at time of survey	Continuous
Maximum temperature	Max. temperature on day of survey (13.8-20.4°C)	Continuous; change
Minimum temperature	Min. temperature on day of survey (3.7-14.1°C)	Continuous; change
Trees in field or boundary?	2 levels: 1-No; 2-Yes	Categorical; fixed
Proximity to nest site?	(36-309m)	Continuous

Table 7.3 – List of variables tested in the models of swallow foraging activity. ‘Change’ indicates whether a variable changes over time or whether it is ‘fixed’ for the whole sampling period. Ranges of continuous variables are given in parentheses.

### 7.3 Results

#### 7.3.1 Species richness and species composition of insectivorous birds

A total of 22 insectivorous species were observed foraging in pastures in 2003 (Table 7.4). Only species that were present in more than 10 percent of observations were used in data analyses in order to exclude species that occurred infrequently (Table 7.4). The most frequently observed species was the barn swallow, which was observed in more than 90 per cent of surveyed pastures, and rooks, carrion crows and yellowhammers were all observed in almost 70 per cent of surveyed pastures. The ordination of pastures by bird species gave axis 1 and 2 eigenvalues of 0.326 and 0.195, respectively (Appendix XX.). There was no major separation according to whether fields were grazed by avermectin-treated or untreated cattle although untreated fields were situated slightly higher on axis 2 than treated fields (Figure 7.1). The lower ordination position of treated fields was due to the occurrence of skylark only in treated fields, and dunnoek was most often recorded in treated fields (Figure 7.2). Field BTBC1 was situated to the far right of axis 1 because the only species observed foraging there were pied wagtails and yellowhammers.

Feeding type	Common name	Species name	% occurrence in observations
Aerial insectivores	<b>Barn swallow</b>	<i>Hirundo rustica</i>	91.7
	<b>Spotted flycatcher</b>	<i>Muscicapa striata</i>	25
	<b>House martin</b>	<i>Delichon urbica</i>	16.7
	Sand martin	<i>Riparia riparia</i>	8.3
Insectivores	<b>Blackbird</b>	<i>Turdus merula</i>	58.3
	<b>Pied wagtail</b>	<i>Motacilla alba</i>	58.3
	<b>Dunnock</b>	<i>Prunella modularis</i>	58.3
	<b>Starling</b>	<i>Sturnus vulgaris</i>	50
	<b>Song thrush</b>	<i>Turdus philomelos</i>	25
	Meadow pipit	<i>Anthus pratensis</i>	8.3
	Curlew	<i>Numenius arquata</i>	8.3
Granivores (provisioning chicks with insects)	<b>Yellowhammer</b>	<i>Emberiza citrinella</i>	66.7
	<b>Tree sparrow</b>	<i>Passer montanus</i>	50
	<b>Skylark</b>	<i>Alda arvensis</i>	25
	Redpoll	<i>Carduelis flammea</i>	8.3
	Pheasant	<i>Phasianus colchicus</i>	8.3
	Greenfinch	<i>Carduelis chloris</i>	8.3
	Reed bunting	<i>Emberiza schoeniclus</i>	8.3
	Corn bunting	<i>Miliaria calandra</i>	8.3
Omnivores	<b>Rook</b>	<i>Corvus frugilegus</i>	66.7
	<b>Carriion crow</b>	<i>Corvus corone corone</i>	66.7
	Herring gull	<i>Larus argentatus</i>	8.3

Table 7.4 – List of ‘insectivorous’ species observed foraging in study fields sampled from May to July in 2003. Species included in data analyses are shown in bold type.

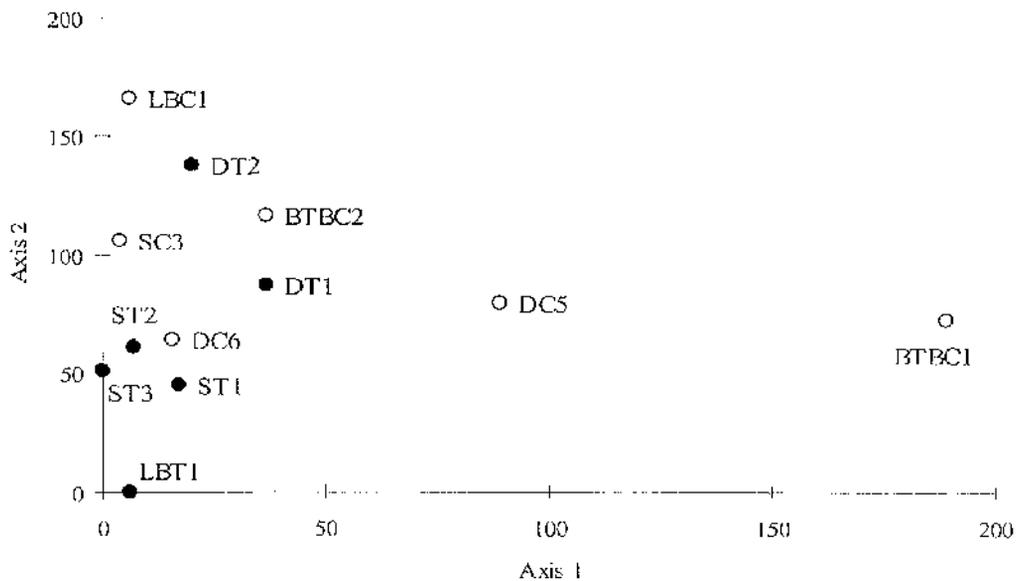


Figure 7.1 - Ordination of insectivorous bird assemblages in pastures grazed by treated cattle (●) or untreated cattle (○), sampled from May to July in 2003

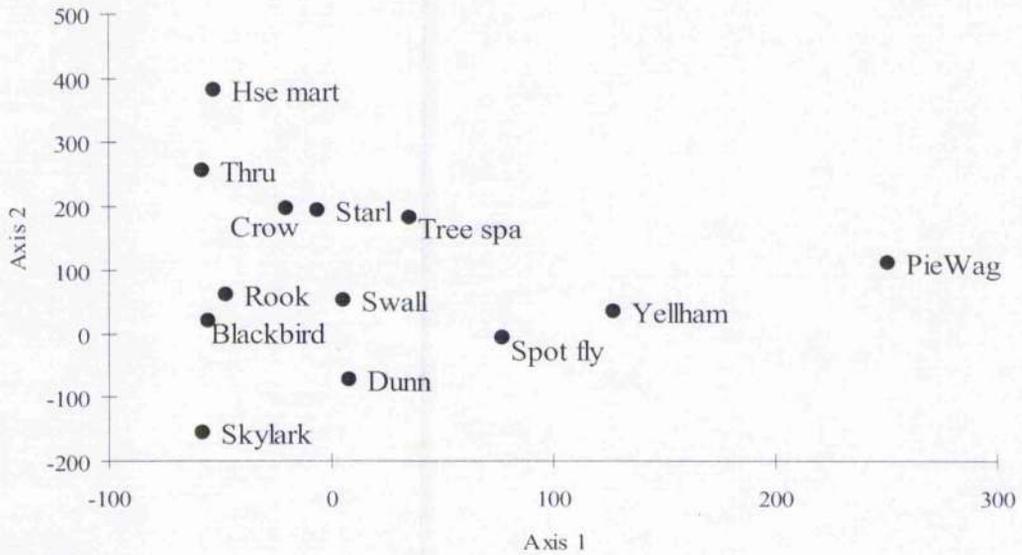


Figure 7.2 – Species scores from ordination of bird species observed in 2003 from 6 fields grazed by treated cattle and 6 fields grazed by untreated cattle. Species abbreviations are provided in Appendix XX.

The number of bird species observed foraging did not differ significantly between fields grazed by avermectin-treated or untreated cattle (Figure 7.3). Species richness of foraging birds was higher on days with lower rainfall, and soil was significantly more penetrable on days with higher rainfall (Table 7.5). Richness was positively correlated with the Weight Median Length of *Aphodius* beetles sampled in fields (Figure 7.4) and there was no evidence of a quadratic relationship with *Aphodius* Weight Median Length ( $F_{1, 6.68}=0.5$ ,  $P=0.501$ ).

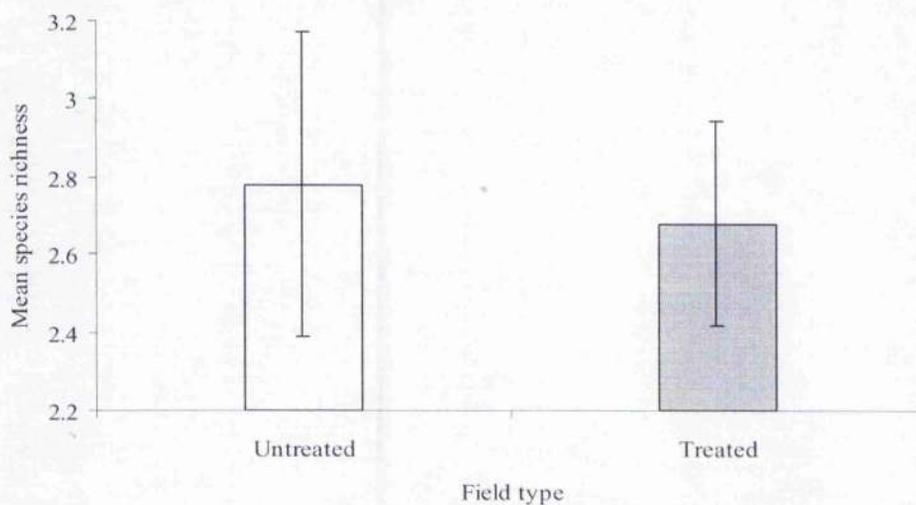


Figure 7.3 – Mean ( $\pm$  1se) species richness of insectivorous birds foraging in fields containing untreated cattle (visits  $n=27$ ) or avermectin-treated cattle (visits  $n=31$ ) from May to July 2003 ( $F_{1, 9.54}=0.03$ ,  $P=0.88$ )

Variable	Estimate	se	Test statistics	P
<b>Species richness of foraging birds</b>				
Field	0.981	0.541	Z=1.81	0.035
Impenetrability	0.019	0.017	F <sub>1,12.1</sub> =1.24	0.287
Rainfall	-5.107	1.082	F <sub>1,5.03</sub> =22.28	0.005
Impenetrability*Rainfall	0.074	0.016	F <sub>1,4.51</sub> =21.26	0.007
<i>Aphodius</i> Weight Median Length	1.217	0.374	F <sub>1,8.13</sub> =10.61	0.011
Intercept	-6.906	3.063		

Table 7.5 – Model for species richness of birds in fields containing untreated or avermectin-treated livestock from May-July 2003

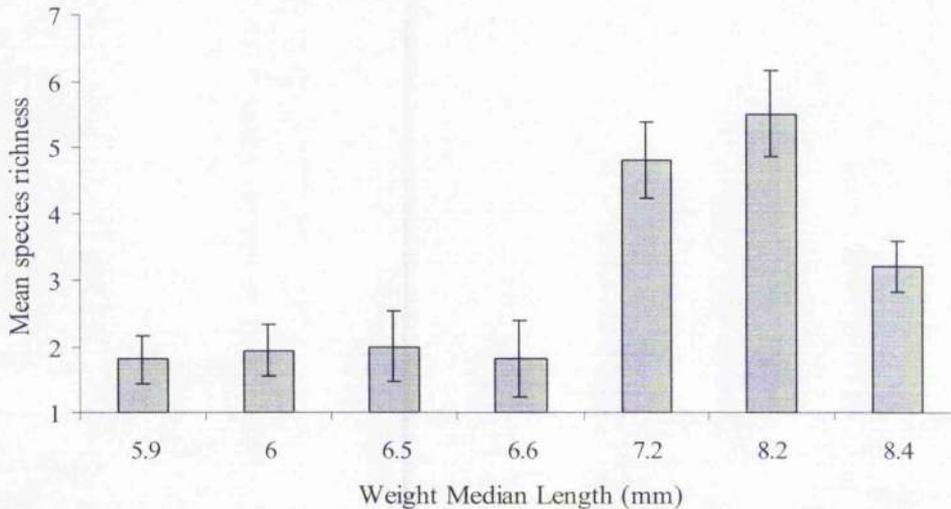


Figure 7.4 - Mean ( $\pm$  1se) species richness of insectivorous birds foraging in study fields (fields grouped according to *Aphodius* Weight Median Length)

### 7.3.2 Foraging activity of insectivores

Numbers of birds observed feeding did not differ significantly between pastures grazed by treated or untreated cattle (Figure 7.5). The abundance of foraging birds in a field was positively correlated to the number of dung insects trapped in the preceding two weeks in that field (Table 7.6). Dung insect abundance did not differ between the treated and untreated fields sampled ( $F_{1,9.19}=2.6$ ,  $P=0.14$ ).

Variable	Estimate	se	Test statistics	P
<b>Foraging activity of birds</b>				
Field			Z=0.56	0.287
Farm			Z=0.24	0.405
Dung insect abundance	0.006	0.002	F <sub>1,14.6</sub> =7.38	0.016
Intercept	1.886	0.471		

Table 7.6 – Model of number of insectivorous birds observed foraging in fields containing untreated or avermectin treated livestock from May-July 2003

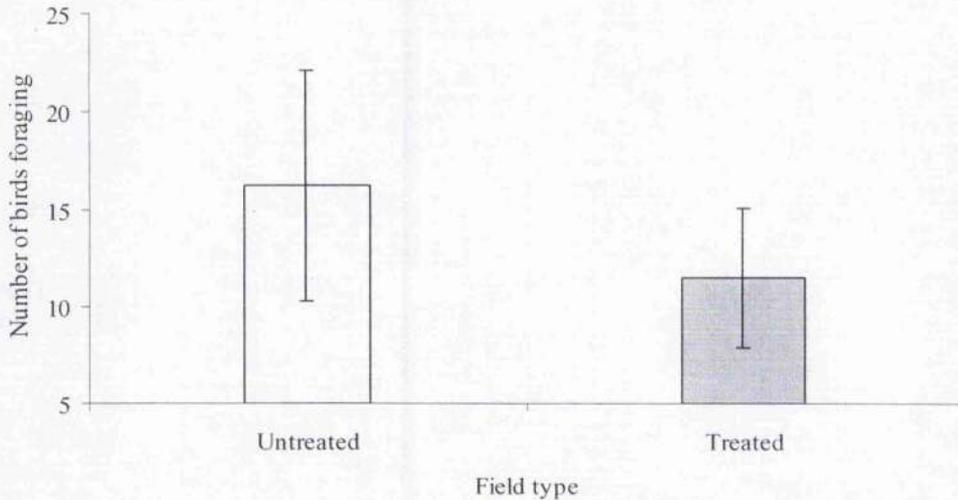


Figure 7.5 - Mean ( $\pm$  1se) number of insectivorous birds observed foraging in fields grazed by untreated cattle (visits n=27) or avermectin-treated cattle (visits n=31) from May to July 2003 ( $F_{1, 9.88}=0.03$ ,  $P=0.87$ )

The mid-point of the *Aphodius* biomass distribution (Weight Median Length) in study pastures ranged from 5.9-8.4 mm. Fields with the same Weight Median Length were grouped together for analyses thus giving seven WML categories. There was a non-significant trend for higher foraging activity in fields with higher Weight Median Length (Figure 7.6). The relationship shown in the graph seems curvilinear yet the quadratic relationship between foraging activity and Weight Median Length was not significant ( $F_{1, 3.79}=4.84$ ,  $P=0.1$ ).

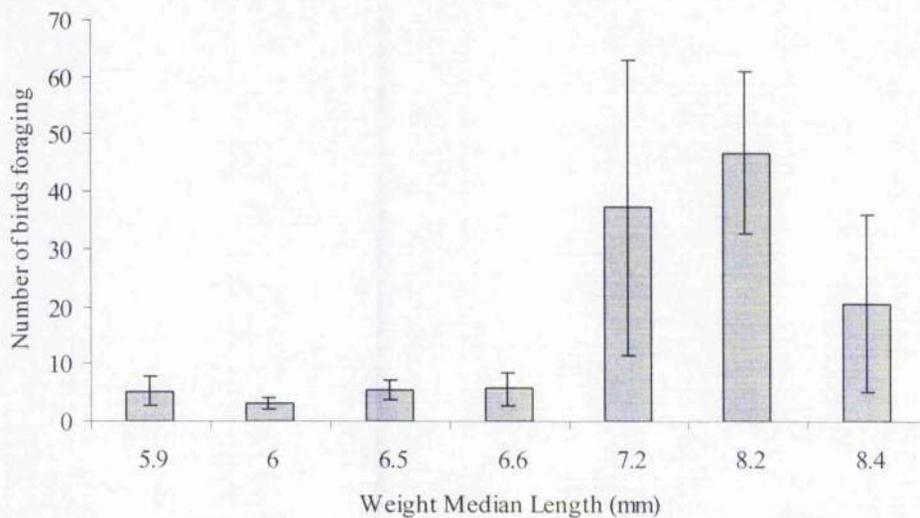


Figure 7.6 – Mean ( $\pm$  1 se) number of birds observed foraging in pastures with different Weight Median Lengths for *Aphodius* assemblages, calculated using *Aphodius* data collected from April to July of 2003 ( $F_{1, 2.42}=10.3$ ,  $P=0.07$ )

## 7.3.3 Swallow foraging activity

## 2003 data

The number of barn swallows observed foraging did not differ between fields grazed by either treated or untreated cattle (Figure 7.7). More swallows were observed feeding in pastures when abundance of yellow dung flies in the preceding two weeks was lower (Table 7.7). Yellow dung fly abundance did not differ significantly between treated and untreated fields ( $F_{1,6.85}=0.2$ ,  $P=0.67$ ).

Variable	Estimate	se	Test statistics	P
<b>Swallow foraging activity 2003</b>				
Field			Z=0.64	0.262
Farm			Z=0.69	0.246
Yellow dung fly abundance	-0.018	0.007	$F_{1,26.9}=7.3$	0.012
Intercept	1.238	0.542		

Table 7.7 – Model of foraging barn swallow abundance in six fields grazed by untreated cattle and six grazed by avermectin-treated cattle from May to July 2003

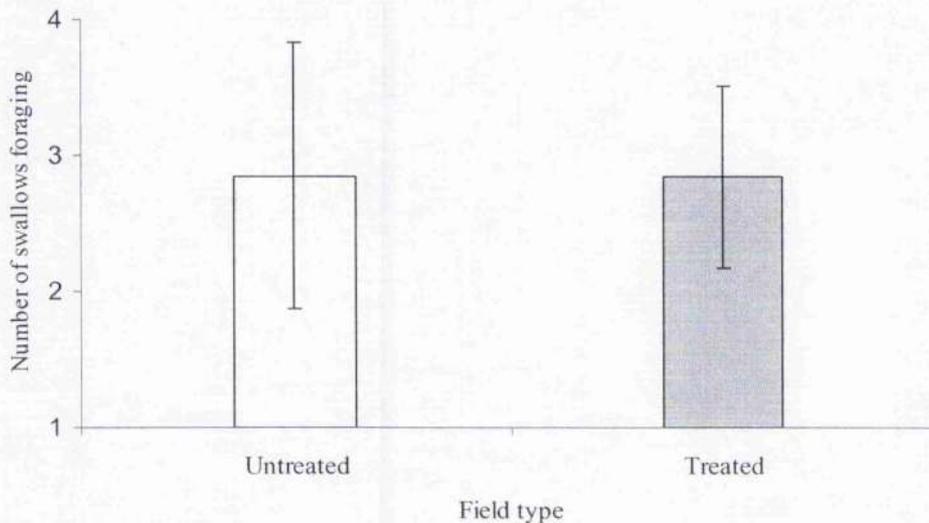


Figure 7.7 - Mean ( $\pm 1$  se) number of swallows observed foraging in fields grazed by treated cattle ( $n=6$ ) or untreated cattle ( $n=6$ ) in 2003 ( $F_{1,6.57}=0.18$ ,  $P=0.68$ )

## 2004 data

The maximum number of swallows observed foraging at any one time was significantly negatively related to sampling date and to proximity to nest site (Table

7.8). Highest numbers of swallows were observed at the start of sampling in late May. The distance range of the study fields to the nest sites was approximately 40-300m and maximum numbers of swallows were recorded in fields closest to the nest sites. Proximity to nest site did not differ significantly between treated and untreated fields (Mann-Whitney  $U_{3,4}=17$ ,  $P=0.11$ ) however the mean distance ( $\pm 1$  se) between nest site and sample field was higher for treated fields,  $224\pm 68$ m, than for untreated fields,  $73\pm 31$ m. Higher swallow numbers were observed foraging in untreated fields although that trend was not significant (Figure 7.8). The mean foraging time of swallows did not differ significantly between fields grazed either by avermectin-treated or untreated cattle although there was a trend for more time spent foraging in untreated fields (Figure 7.9).

Variable	Estimate	se	Test statistics	P
<b>Swallow foraging activity 2004</b>				
Field			Z=0.67	0.252
Sampling date	-0.011	0.0054	$F_{1,20.8}=4.55$	0.045
Proximity to nest site	-0.009	0.004	$F_{1,16.3}=4.95$	0.041
Intercept	2.146	0.594		

Table 7.8 – Model of maximum number of foraging swallows observed in treated and untreated fields from May to July 2004

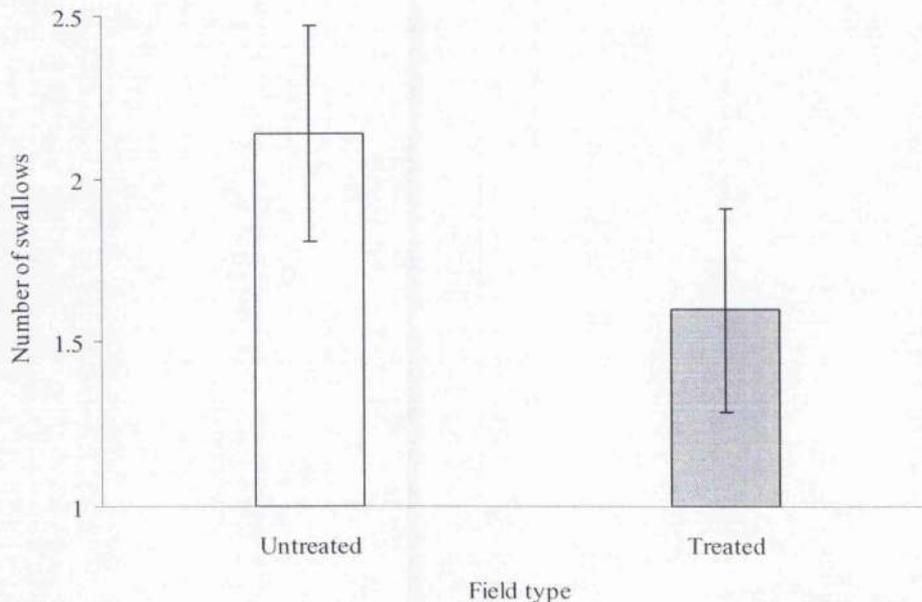


Figure 7.8 - Mean ( $\pm 1$  se) maximum number of swallows observed foraging simultaneously in a 20-minute period in fields containing avermectin-treated cattle (visits  $n=47$ ) or untreated cattle (visits  $n=37$ ) in 2004 ( $F_{1,4.61}=0.65$ ,  $P=0.46$ )

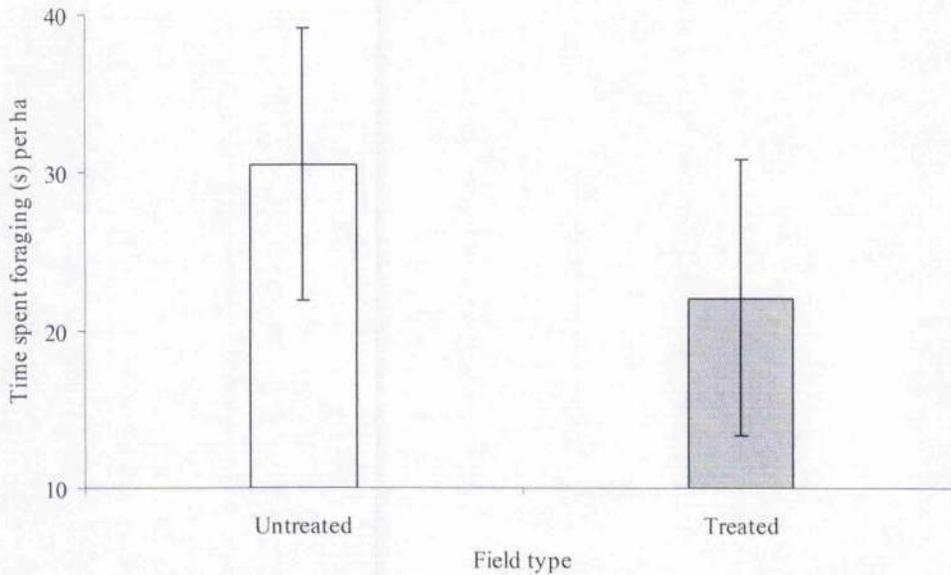


Figure 7.9 – Mean ( $\pm 1$  se) number of seconds spent foraging by swallows per hectare in a 20 minute observation period in fields containing untreated cattle (n=4) or avermectin-treated cattle (n=3) from late-May to July 2004 (Mann-Whitney,  $P=0.6$ )

#### 7.4 Discussion

##### *Foraging activity of insectivorous birds*

These results indicated that there was no major difference in the species assemblages of insectivorous birds between treated and untreated fields thus indicating that species were unlikely to avoid a pasture as a foraging ground simply because that field was grazed by treated cattle. Also, the species richness of foraging insectivorous birds did not differ between treated and untreated fields. Among the most common species in all fields were barn swallows, crows and rooks. As highlighted previously, barn swallows are associated with grazed pasture (Ambrosini *et al.*, 2002) and carrion crows and rooks are common in grazed pasture and improved grassland (Tucker, 1992; Barnett *et al.*, 2004).

The number of insectivorous birds observed feeding was positively correlated with dung insect abundance although neither bird nor insect abundance differed significantly between pastures grazed either by avermectin-treated or untreated cattle. It should be stressed that the positive relationship between foraging activity and dung insect abundance is not necessarily a direct one. For example, dung insects

may have been more active in response to some factor that may too have invoked a positive response in the abundance of other invertebrates. Hence, a greater activity of all grassland invertebrates could have sustained more foraging birds. Moreover, both the birds and dung insects may have been responding to some favourable aspect of the pasture habitat that was unmeasured in this study. It would be interesting to observe foraging activity in fields at a time when dung insect abundance was fluctuating significantly in order to ascertain whether the foraging activity of birds responded to that change.

The number of bird species observed foraging was higher in fields that supported *Aphodius* with a higher average biomass distribution i.e. a greater Weight Median Length. Additionally, there was a tendency for more birds to be observed foraging in those pastures too. For a prey item to be considered profitable by a predator, it must be of a size that can be handled, detectable and profitable i.e. the energy that it provides should be greater than that expended for search and capture (Zwarts and Blomert, 1992). The observation of more visits by birds to pastures with relatively more *Aphodius* individuals of higher biomass could be expected, as those fields would be more profitable feeding grounds than areas supporting insects of lower individual biomass. More work is needed to establish whether there is a relationship between the profitability of available insect biomass and the foraging activity of birds in pastures. Such work should aim to measure the biomass distribution of not only the dung beetles but also of other prey groups that are common in grassland e.g. ground beetles.

These results indicated that the number of insectivorous bird species in pastures was lowest on days with highest rainfall. While light rain is unlikely to affect the foraging activity of birds (Bibby *et al.*, 1992), one might expect them to take cover during periods of heavy rain and to resume foraging when rain ceases. That general avoidance of foraging during particularly rainy periods would have resulted in the observation here that fewer species were observed on rainy days.

*Foraging activity of Barn Swallows*

In 2003, there was no difference in the number of barn swallows observed foraging between treated and untreated fields. More swallows were observed foraging when the abundance of yellow dung flies trapped in the preceding fortnight was lower. There are at least two potential, contradictory, explanations for that finding. First of all, greater foraging activity by swallows may have been a response to reduced availability of prey. If the abundance of yellow dung flies in a pasture was low then other dipteran prey groups may also have been scarce and thus swallows would have had to forage for longer in order to find prey. Secondly, greater feeding activity by the barn swallows may have actually reduced the density of the yellow dung fly population via increased predation pressure. The relationship between foraging activity of barn swallows and the availability of yellow dung flies remains unclear.

The maximum number of swallows observed foraging in 2004 and the length of foraging bouts did not differ between treated and untreated pastures although there was a non-significant trend for more time spent foraging in untreated fields. This might have been a consequence of swallows having to spend more time searching for prey or for profitable prey (e.g. Lovvorn and Gillingham, 1996). However, there were no yellow dung fly, or other insect, abundance data in 2004 with which to compare swallow foraging observations.

Fields were selected on the basis of similarity, however fields grazed by untreated cattle were generally closer to farm buildings than fields grazed by treated cattle, which is not uncommon on dairy farms. Indeed, this may have caused the trend for higher foraging activity in untreated fields i.e. swallows would be more likely to forage in pasture closer to their colony than in more distant pasture in order to reduce time and energy expenditure during flight. Indeed, a negative relationship between swallow population density and distance from the colony has been observed elsewhere (Ambrosini *et al.*, 2002.). Proximity to nest site needs to be fully considered in any future studies that compare the foraging activity of swallows in pastures grazed by avermectin-treated and untreated cattle.

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## CHAPTER 8

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### DISCUSSION AND CONCLUSIONS

## 8. Discussion and Conclusions

### 8.1 Summary of main findings

The deleterious effects of avermectins on the survival and development of dung insects have been well-documented within 'individual' dung pats, however little is known about avermectin effects on natural populations of dung insects. The aim of this research was to examine the effects of avermectin usage in livestock on dung insect communities in pastures. Gaining knowledge of avermectin effects in pasture populations is not only important for dung insect conservation, but also for assessing the implications for birds and other vertebrate predators that forage in grazed pastures on farmland.

A pasture-scale study of dung insects (adult *Aphodius* and *Cercyon* beetles and adult yellow dung flies, *Scatophagidae*) was conducted on commercial farms, which were mainly dairy enterprises. Of the range of environmental factors that were tested in models of dung insect abundance and diversity, inter-annual variation, weather and seasonality were the factors most likely to be significant. Avermectin treatment was not found to affect the abundance of *Cercyon* beetles or yellow dung flies. Significantly more *Aphodius* beetles were trapped in fields grazed by avermectin-treated cattle. Results from trials investigating the relative attractiveness of dung from treated and untreated cattle indicated that the phenomenon of higher abundance in treated fields may have resulted from an attraction effect whereby *Aphodius* beetles were avoiding colonising the natural dung that occurred in fields grazed by avermectin-treated cattle. The size structure and biomass of adult *Aphodius* beetles and biomass of adult yellow dung flies were not affected by avermectin treatment. Variation in the size structure of *Aphodius* assemblages was related to seasonality and year. The foraging activity of insectivorous birds was not observed to differ between treated and untreated fields. The main findings from this research are summarised below and, in the following section, the observed effects of avermectins are discussed further:

- Year, seasonality and weather were significant factors for explaining aspects of variation in dung insect assemblages

- Abundance of adult *Cereyon* beetles and adult yellow dung flies, and dung insect diversity, were unaffected in pastures grazed by avermectin-treated cattle
- Dung from avermectin-treated cattle may be an unfavourable breeding resource for *Aphodius* dung beetles in pastures
- Avoidance of dung from avermectin-treated cattle could ultimately reduce the availability of larval and emerging adult insect prey for foraging predators
- Sublethal effects may occur in yellow dung fly (*Scatophaga stercoraria*) populations that have developed in naturally-occurring dung in pastures grazed by doramectin-treated cattle
- Size structure and biomass distribution of adult insects, and thereby profitability for foraging predators, is not impaired in fields grazed by avermectin-treated cattle
- Foraging activity of birds was not observed to differ between pasture grazed either by treated or untreated cattle

### 8.2 Avermectin effects on dung insects

In terms of the species that were present, there were no detectable differences in the dung insect species that occurred between fields grazed by avermectin-treated cattle and those grazed by untreated cattle. This suggests that exposure to avermectin residues in dung in a pasture situation has not compromised the existence of any one particular species that has a geographical distribution within the study area. However, sublethal effects may occur in dung insect species that have completed their development in dung from treated cattle. This research found that the wing length asymmetry of yellow dung flies was higher in fields in which flies would have been exposed to doramectin residues during development. It cannot be ruled out that the higher asymmetry in treated fields may have been due to a factor other than doramectin exposure. Flies that are asymmetric may be more easily predated by barn swallows because they are less able to evade predation because of their increased wing asymmetry (Møller, 1996). In this study, it is feasible that asymmetry may have been present at similar levels in yellow dung fly populations in untreated and treated fields but that higher swallow predation pressure in the former may have consequently reduced asymmetry levels. Nevertheless, an increase in asymmetry

could be an indication that individuals experienced stress during their development in dung from treated cattle. There was no evidence to suggest that this escalated to affect actual population densities of yellow dung flies in pastures since similar numbers of flies were trapped in treated and untreated fields. Indeed, the model of abundance for the yellow dung fly showed that year and seasonality were more important for explaining variation than avermectin treatment. However, species with more vulnerable life histories than the yellow dung fly, such as *Aphodius pusillus*, may not be so resilient to developmental stress and thus could be more susceptible in the long term.

Higher numbers of *Aphodius* beetles were trapped in treated fields, a finding that was potentially attributable to:

*i.) Relative attractiveness of dung*

A preference for ‘untreated’ dung would explain the higher numbers of beetles in traps in treated fields because the beetles in those fields may have avoided the natural dung excreted by treated cattle in favour of the ‘untreated’ dung baits on traps. Previous research regarding avermectin effects on insect attraction to dung has provided conflicting results (e.g. Wardhaugh and Mahon, 1991; Holter *et al.*, 1993a and b). However, a series of field trials (Chapter 3) showed that significantly more *Aphodius* beetles were attracted to dung from untreated cattle than to dung from cattle that had been treated with a doramectin pour-on two days previously. This supported the suggestion that higher numbers in treated fields were due to preferential attraction to untreated dung on pitfall traps. The mechanism by which avermectin therapy alters the attractiveness of dung is unclear.

*ii) Grazing regime*

The difference in grazing regime between treated and untreated fields may have caused more *Aphodius* to be trapped in fields grazed by treated cattle, since all ‘treated’ fields were permanently grazed and the majority of untreated fields were grazed on a rotational basis. Treated fields might have attracted and supported more dung insects because of the continual supply of fresh dung in those fields while the periodic supply of fresh dung in untreated fields may have limited the number of beetles attracted to those pastures.

Results from a small-scale 'dung density' trial (Chapter 3) highlighted that higher numbers in treated fields were probably due to an attraction effect rather than grazing regime. The trial compared the numbers of dung beetles that were trapped in dung-baited pitfalls when the traps were surrounded either by a high density or a low density of untreated dung, which resulted in a non-significant trend for more *Aphodius* beetles in traps at low dung density. Thus, a 'dilution effect' may have taken place whereby, at high dung density, more dung was available for insects to colonise and thus fewer beetles were attracted to baited pitfall traps. This discounts the grazing regime theory because even if relatively more beetles were attracted to treated fields than to untreated fields, then high numbers would not be expected in traps in treated fields as beetles would be 'diluted' across the available resource patches. However, if fresh dung deposited by cattle in treated fields was an undesirable resource for colonisation by beetles, then one might expect more beetles to be attracted to untreated baited traps in treated fields, as was observed in this study. It cannot be discounted that higher numbers of *Aphodius* in traps in treated fields were simply due to greater population densities in those fields. This seems unlikely though because previous laboratory research (reviewed in Section 1.4) has indicated that breeding success in individual dung pats would not be enhanced in dung from treated animals and certainly not to an extent that would result in greater population densities in treated fields. In addition, the measurement and analyses of pasture characteristics such as habitat features and management intensity suggested that the difference in *Aphodius* numbers between treated and untreated fields were not due to such characteristics.

As mentioned above, the factors that may cause dung insects to avoid dung from treated cattle remain unclear. Avermectin therapy lowers the moisture content of dung (Barth, 1993; Wratten and Forbes, 1995) and it has been suggested that this could alter its attractiveness. However, results from this study (Chapter 3) showed that moisture content did not seem to influence attraction to dung as much as whether the dung came from untreated or treated cattle. It is possible that avermectin treatment alters the bacterial content and consequently the nutritional quality and desirability of dung as a feeding resource. Additionally, the attractiveness of dung to beetles may be diminished as a result of reduced activity of pre-colonising species. Larvae of dung-breeding flies aerate the dung during their development and make it

more suitable for other insects to colonise (Suares *et al.*, 2003). Therefore, impaired activity of dipteran larvae in dung could have subsequently altered its suitability for beetle colonisation. The larval activity of dung-breeding flies may have decreased in dung from avermectin-treated cattle in the following ways. There may have been reduced fly activity in dung if flies were also avoiding colonising dung from treated cattle (as shown for yellow dung flies, Chapter 3). Also, it is known that avermectin residues in dung can be lethal for fly larvae (e.g. Floate *et al.*, 2001; reviewed in Section 1.4.1) and yellow dung fly larvae may undergo developmental stress in dung from treated cattle and therefore their activity might be impeded as a result.

If *Aphodius* dung beetles avoid colonising dung from avermectin-treated cattle in a pasture environment, then any potential negative effects on the development of beetles within dung pats could be minimised, provided that alternative ‘avermectin-free’ dung is attainable. It has previously been proposed that any ecological impact of avermectins would be limited by the dilution effects of untreated animals and by a lack of synchrony of treatments in any particular geographical area (Forbes, 1993). The survey of livestock farmers in south west Scotland did not seem to support that avermectin treatments were asynchronous, for example most beef farmers commonly administered a doramectin or ivermectin treatment to cattle at spring turnout. However, in some areas, there may be sufficient availability of pasture grazed by untreated cattle that are within reasonable insect dispersal distance of treated fields. This could potentially mitigate any effects associated with an unsuitable dung resource in treated fields.

It is interesting to note that *Cercyon* beetles and yellow dung flies (*Scatophaga stercoraria*) also displayed a preference for untreated dung (Chapter 3) and yet a significant difference in their abundance between fields grazed either by treated or untreated cattle did not occur. This could possibly be linked to differences in adult feeding strategies. Adult *Aphodius* beetles utilise dung as a feeding resource whereas *Cercyon* beetles also feed on decaying plant matter and such like, and yellow dung flies are predatory within dung. Why exactly a preference for untreated dung was apparent in *Cercyon* beetles and yellow dung flies in experimental trials and yet was not manifest at a pasture-level is unclear.

There was no suggestion that the seasonality of insects was altered in fields where cattle were treated with avermectins i.e. there was no delay in the time of their main occurrence. Furthermore, the seasonal occurrences of dung insect species in all sampled pastures were typical of those reported in other studies. It was proposed that the lower abundance of *Aphodius* dung beetles in all fields in 2003 could have been a consequence of the wet summer in the previous year, which could have impeded development in pats and soil due to flooding of pasture.

To conclude this section, reduced population sizes of dung insects were not apparent in pastures where cattle were treated with avermectin wormers. However, it seems that the ecology of *Aphodius* beetles, in terms of colonisation behaviour, may be affected in pastures grazed by treated cattle. Furthermore, there is evidence of sublethal effects in insects that have developed in dung in a pasture environment. Hence, while there is no immediate decline in the density of dung insect populations in pastures, one cannot exclude the possibility that the use of avermectins in grazing livestock may affect particularly vulnerable dung insect species or species in areas where exposure cannot be mitigated. The implications of these results for foraging predators, and how effects may be mitigated and/or exacerbated via livestock management are discussed in the sections below.

### *8.3 Implications for vertebrate predators of dung insects*

The body size structure of *Aphodius* dung beetle assemblages did not differ between treated and untreated fields thus indicating that no particular size class of *Aphodius* was more or less common in fields grazed by avermectin-treated cattle. As the profitability of invertebrate prey is a function of its body size (Section 6.1), it can be inferred that the value of individual prey items was not reduced in treated fields. This is further supported by the observation that the midpoints of the *Aphodius* biomass distribution (measured with the Weight Median Length statistic) were similar for treated and untreated fields. Additionally, there was no significant difference in the biomass of yellow dung flies between fields therefore the profitability of individual flies was unaffected in treated fields. The factors that were shown to influence size structure of *Aphodius* assemblages were year and seasonality, whereby more small beetles were present in April and May and larger

beetles occurred later in summer. This indicates that there may be a shift in the prey size of *Aphodius* beetles for foraging birds from small individuals in the spring and early summer to larger individuals later in summer.

Peak abundance of dung insects was a measure of the maximum number recorded in any one trapping period in a field over the course of the sampling season. The peak abundance differed significantly between sample years showing that the value of a pasture as a foraging ground can vary from year to year. Furthermore, the timing of peak abundance differed between the two sample years. In the first year, peak abundance tended to occur in May while in the second year it happened in July. Thus, for birds provisioning young in spring and early summer, the second year may have proved more of a challenge in terms of foraging for dung insect prey.

Although the profitability of treated pasture was not lower in terms of biomass or size of dung invertebrate prey, differences in dung insect behaviour may have consequences for foraging predators. If insects do avoid colonising dung from treated cattle then the availability of larval prey and emerging adults could ultimately be reduced in areas with a lack of avermectin-free dung. However, while insects are searching for a suitable dung resource to colonise, it is feasible that foraging birds may benefit in the short-term as they could locate insects more easily due to the higher activity of insects in treated fields.

It has already been acknowledged that the survey of bird observations was somewhat limited because the main focus of the research was to study dung invertebrates in pastures. Nevertheless, observations showed that there were no marked differences in the foraging activity of birds in fields grazed either by avermectin-treated cattle or untreated cattle. This indicates that any potential effects on the dung invertebrate community were not sufficient to directly affect bird foraging behaviour. There was a trend for higher foraging activity by barn swallows in untreated fields which was probably due to those fields being located closer to farm buildings and therefore nest sites. An interesting finding was that the number of birds observed foraging was higher in fields with relatively more *Aphodius* beetles of a higher average body size, highlighting that more birds may visit a particular habitat if they are able to locate larger, more profitable prey items there.

#### 8.4 Livestock management in relation to potential avermectin effects

As the insect sampling was carried out on dairy farms in a 'dairy-farmed area', the results are indicative of what might typically occur in geographical areas where dairy enterprises are commonplace. This section discusses the results in their original context of a predominantly dairy farming area, and how they might differ with regards to beef and sheep farming. Management recommendations are discussed in terms of mitigating any potential effects on dung insects with emphasis on the finding that dung from avermectin-treated animals may be an unfavourable resource for *Aphodius* beetles.

##### *Dairy*

The agricultural landscape in dairy regions is a mosaic of forage crops and pasture that is grazed either by untreated milking cows or treated young stock. The avermectins that are regarded as most ecotoxic (doramectin and ivermectin) are not administered to milking cows therefore 'untreated' pasture would always be present on dairy farms during the grazing season. Provided that pastures grazed by untreated cattle were within dispersal distance of beetles emerging in pastures grazed by treated cattle, then the beetles would be able to locate and preferentially colonise the dung in untreated fields. More work is required on the spatial scale at which dung beetles are able to discriminate between treated and untreated dung (as highlighted in Section 8.6) in order to gauge how close fields would need to be before insects would be able to preferentially colonise 'untreated' dung.

On dairy farms in south west Scotland, a popular worming strategy was to treat young cattle with a benzimidazole during the grazing period followed by an avermectin treatment upon housing. This would limit the exposure of dung insects to avermectin residues in dung in pastures. As there are no obvious detrimental effects of the benzimidazoles e.g. fenbendazole, on dung insect species (Strong *et al.*, 1996), effects on insects would be minimal even when young cattle were undergoing anthelmintic treatment.

If dung from cattle recently treated with an avermectin was unsuitable for colonisation then the presence of such dung on pasture could be avoided by keeping stock housed after treatment for the period of time that excretion levels in dung were highest. However, to delay the date of spring turnout may be undesirable because it would completely reduce the availability of dung in pastures for spring-breeding insects and consequently reduce the availability of prey for birds. Furthermore, delaying turnout would bring the associated costs to farmers of feeding housed cattle. Instead, it may be desirable to treat cattle at a certain prescribed time prior to normal turnout. For example, treating cattle five days before turnout would mean that the maximum excretion periods for doramectin and ivermectin would have passed and after that time, excretion levels decline (Toutain *et al.*, 1997). However, before this recommendation can be implemented, it should be confirmed that treating cattle five days prior to turnout would still afford them protection against parasitic infection at grazing. While cattle may be dosed with an avermectin up to three times throughout the grazing season (as recommended for a doramectin pour-on), it is not advised that cattle be returned to housing prior to the second and third treatments. Simply mitigating the first treatment of avermectin would be helpful, particularly as it would coincide with the peak in activity of many dung insects.

Ideally, the use of avermectins should be discouraged in sensitive areas, for example grassland that is particularly favoured by breeding waders. The agri-environment Rural Stewardship Scheme in Scotland advocates that wet grassland managed for waders should not be grazed for six weeks between April and mid-June (Scottish Executive, 2003). However, if stock exclusion is not possible because, for example the 'wader site' cannot be fenced off from adjoining pasture then it is recommended that grazing pressure should be kept low. Indeed, it is debatable whether total exclusion of livestock is the best management option since even a low level of grazing would provide a resource for dung-breeding insects that are predated by waders (Galbraith, 1989; Beintema *et al.*, 1991). Therefore, the best option may be to allow livestock to lightly graze wader-rich grassland, and to treat them with an alternative anthelmintic class to the avermectins, e.g. a benzimidazole. If a product from the avermectin class is desired, then moxidectin could be used because it controls the same range of parasites but is less toxic to non-target dung invertebrates

than doramectin and ivermectin (Strong and Wall, 1994; Taylor, 2001; Lumaret and Errouissi, 2002; Floate *et al.*, 2002).

### *Beef*

With regards to the cohort of livestock that are wormed, anthelmintic treatment of beef cattle is not as clearly defined as it is in dairy enterprises and certainly no clear pattern emerged after talking to beef farmers during the questionnaire survey. In beef herds, natural immunity to worms should be acquired in adults, which means that it is only really necessary to treat the young in the herd. Indeed, Forbes (1993) stated that adult beef cattle are rarely treated. However, the questionnaire survey indicated that treatment was often given to young only at grazing, the entire herd at grazing, or all of the cattle upon housing only. It is apparent that some farmers may take an 'on the safe side' approach and dose all animals before it is really necessary and this should be avoided. The benefit of treating only young stock is that, in grassland grazed by suckler herds, there would be a source of untreated dung for colonisation by dung insects. Only treating part of the herd would reduce the cost of purchasing anthelmintics and may also help to prevent anthelmintic resistance.

Avermectins were the most commonly used wormers in beef cattle in south west and Central Scotland, and doramectin and ivermectin were the most commonly used anthelmintics for cattle during summer grazing. When whole herds are treated, the use of avermectins in beef cattle should be restricted to housing and, if possible, an alternative anthelmintic class should be used during grazing. If the use of an avermectin is required then moxidectin could be used.

### *Sheep*

This research was most relevant to avermectin effects in cattle-grazed pastures. It is, however, possible to consider the findings in relation to sheep management. Particular emphasis has been placed on the recommendations that are made to prevent wormer resistance in sheep flocks, which has been documented in Britain (Yue *et al.*, 2003). These recommendations would also mitigate potential avermectin effects and therefore, such management would be beneficial economically and ecologically.

For sheep, it has been recommended to rotate anthelmintic classes annually and to treat lambs with a benzimidazole or levamisole (Abbott *et al.*, 2004). It has also been recommended to leave part of the flock untreated, which would mitigate any avermectin effects since there would be avermectin-free refugia in the pasture. However some farmers may have reservations about leaving part of a flock unwormed (Abbott *et al.*, 2004). This may be partly due to the fact that if sheep are being treated for scab, then all of the flock should be treated. However, use of a moxidectin product, which is administered in two doses at a ten-day interval, might limit negative effects on dung insects because it is less toxic than doramectin and ivermectin. If the whole flock is being treated for sheep scab with a doramectin injection (administered in one dose only) then it may be beneficial if sheep could be kept in a holding pen for 1-2 days after dosing and prior to turnout to grazing. Avermectins are excreted more rapidly from sheep than they are from cattle and maximum excretion happens within 24 hours after treatment (Steel, 1993). Therefore, to keep sheep from grass at the time of maximum excretion would reduce dung insects' exposure to high levels of avermectin residues in dung.

Sheep are often grazed together with beef cattle and mixed grazing can be beneficial in terms of efficient use of sward structure. Also, if sheep and beef were dosed with products from different classes e.g. cattle given a benzimidazole and sheep given an avermectin, then this would provide avermectin-free dung in pasture for insects to colonise. Dung insects may have differential preferences in terms of the livestock dung that they colonise, for example *Aphodius ater* prefers sheep dung (Hirschberger and Degro, 1996). Nevertheless, if the repellence of dung from treated animals was greater than the preference for dung from the livestock undergoing avermectin treatment, then beetles would still opt to colonise dung from untreated animals.

### 8.5 Conservation management recommendations

The following management recommendations have been proposed, on the basis that dung from avermectin-treated cattle may be an unsuitable resource for *Aphodius* dung beetles:

- Rotate the use of avermectins with other anthelmintic classes

- Treat stock only if necessary i.e. treat young susceptible stock but do not dose adults unless they require worming
- Treat cattle five days prior to turnout (provided that their protection against parasites at grazing is not compromised) in vulnerable areas e.g. water-rich grassland
- Alternatively, treat cattle with non-ivermectin wormers e.g. benzimidazoles, or a less toxic ivermectin (moxidectin) if they are grazing in vulnerable areas
- Retain sheep in a holding pen for 1-2 days after treatment with doramectin or ivermectin
- If possible, graze ivermectin-treated stock in pasture that is adjacent to fields that are grazed by untreated livestock

Furthermore, it has been proposed that any impact of ivermectins could be minimised if treatment coincided with cool, wet weather (Gittings and Giller, 1999). Results from this study suggested that the survival of *Aphodius* beetles was reduced in the year following a very wet summer. Thus, treating livestock with ivermectins when a wet summer is forecast could limit any potential effects on dung beetles.

### *8.6 Suggestions for further work*

Some key areas of research into ivermectin effects on dung insect populations in pastures need to be addressed:

#### *i.) Confirm why higher numbers were trapped in treated fields*

These results showed that activity of dung beetles was higher in treated fields but the exact cause of this remains unclear. It seems likely that the phenomenon was due to avoidance of dung from treated cattle (or lack of suitable dung) in pastures. However, grazing regime or a naturally higher abundance of dung insects in treated fields cannot be discounted at present. Dung insect sampling should be expanded to include cohort samples of naturally occurring dung in pastures. For example, if higher beetle numbers in pitfall traps in treated fields have a corresponding low occurrence of adult beetles in dung pats in the same fields then it could confirm that beetles were avoiding dung from treated animals in a pasture situation. Dung pat

samples were not taken in this study because of the disadvantages associated with that sampling method (reviewed in Section 1.2.4). However they may be crucial for understanding why this particular observation happened.

In connection with this, it would be interesting to assess whether adult beetles and flies were ovipositing in dung from treated cattle and whether those eggs and larvae were developing normally. This could be achieved using ‘bovine-proof’ emergence traps (Sheppard and Gibbons, 1980) to make comparisons of the development of insects in naturally-occurring dung in fields grazed by treated and untreated cattle.

*ii.) Effects in different livestock systems*

This work was conducted in a geographical area where dairy enterprises are prevalent. As discussed above, the typical worming strategies used on these farms may ultimately mitigate any negative effects. Therefore, it cannot be concluded that effects are minimal since effects on dung insect populations could be exacerbated in geographical regions where other livestock sectors do not provide avermectin-free refugia. Thus, further research into effects in those sectors would be desirable.

*iii.) Attractiveness of dung*

Further research is required to investigate the factors that determine the suitability of dung for insect colonisation. The means by which avermectin therapy influences the overall attractiveness of dung also need to be addressed, particularly in view of the conflicting evidence from many studies reported in the literature.

More work is needed to assess how far dung insects can travel in order to colonise dung from untreated animals in preference to ‘treated dung’. Results presented here (Chapter 3) have indicated that *Aphodius* beetles can discriminate at a spatial scale of approximately 70m, however more detailed investigation of this and of factors that may influence insects’ ability to make a choice between treated and untreated dung is required. It would also be interesting to determine whether there is any relationship between the period of time that residues in dung are most harmful to insects, in terms of results from laboratory bioassays, and the time after treatment for which the attractiveness of dung is altered.

*iv.) Sublethal effects on dung insect species*

A more thorough study of sublethal effects in dung insect populations in pastures grazed by avermectin-treated cattle is needed. In particular, the extent of asymmetry could be compared between species postulated to be more or less susceptible, for example species that are common and widespread e.g. *Aphodius depressus* in comparison to those that have more localised spatial distributions e.g. *A. pusillus*.

*8.7 Conclusions*

This research did not detect any major population effects on dung insects in relation to avermectin treatment, and factors such as inter-annual variation, seasonality and weather were more important. Thus, the availability of insect prey in pastures for predators is more likely to change with these environmental factors than as a result of livestock being treated with an avermectin. However, the research was carried out in an area where dairy farming was predominant which may have mitigated any effects. Avermectin and benzimidazoles are both commonly used for worming dairy cattle in the surveyed area, and the older cows in the herd remain untreated thus refugia of avermectin-free dung were present. Some species displayed avoidance behaviour to dung from avermectin-treated animals therefore exacerbation of any potential effects on insects in areas with a lack of untreated dung cannot be discounted. Such areas with a lack of refugia might be beef and sheep farming areas where avermectins are commonly used and where whole herds/ flocks are sometimes treated.

The continued use of avermectins in livestock farming is warranted for the effective control of parasites and for the prevention of anthelmintic resistance. However, management measures could be adopted to mitigate any potential harmful ecological effects of avermectin exposure and such measures could also forestall the development of anthelmintic resistance in livestock sectors.

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## APPENDICES

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Appendix I. – Species nomenclature and abbreviations used in ordination plots of species scores

**Family Scarabaeidae (Coleoptera)**

<i>Aphodius ater</i> (Degeer)	<i>Aater</i>
<i>Aphodius depressus</i> (Kugelann)	<i>Adep</i>
<i>Aphodius fimetarius</i> (Linnaeus)	<i>Afim</i>
<i>Aphodius fossor</i> (Linnaeus)	<i>Afoss</i>
<i>Aphodius lapponum</i> Gyllenhal	<i>Alap(p)</i>
<i>Aphodius prodromus</i> (Bralun)	<i>Apro</i>
<i>Aphodius pusillus</i> (Herbst)	<i>Apus</i>
<i>Aphodius rufipes</i> (Linnaeus)	<i>Arfp</i>
<i>Aphodius rufus</i> (Moll)	<i>Aruf</i>
<i>Aphodius sphaecelatus</i> (Panzer)	<i>Aspha</i>

**Family Hydrophilidae (Coleoptera)**

<i>Cercyon atomarius</i> (Fabricius)	<i>Cato</i>
<i>Cercyon haenorrhoidalis</i> (Fabricius)	<i>Chae</i>
<i>Cercyon lateralis</i> (Marsham)	<i>Clat</i>
<i>Cercyon lugubris</i> (Olivier)	<i>Clug</i>
<i>Cercyon melanocephalus</i> (Linnaeus)	<i>Cmel</i>
<i>Cercyon pygmaeus</i> (Illiger)	<i>Cpyg</i>
<i>Sphaeridium lunatum</i> Fabricius	<i>Slun</i>
<i>Sphaeridium scarabaeoides</i> (Linnaeus)	<i>Ssca(r)</i>

**Family Scatophagidae (Diptera)**

<i>Scatophaga furcata</i> (Say)	<i>Sfur</i>
<i>Scatophaga inquinata</i> (Meigen)	-
<i>Scatophaga stercoraria</i> (Linnaeus)	<i>Sster</i>

**Family Carabidae (Coleoptera)**

<i>Agonum dorsale</i> (Pontoppidan)	<i>Agon dor</i>
<i>Agonum muelleri</i> (Herbst)	<i>Agon mue</i>
<i>Amara aenea</i> (Degeer)	<i>Ama aen</i>
<i>Amara communis</i> (Panzer)	<i>Ama com</i>
<i>Amara plebeja</i> (Gyllenhal)	<i>Ama pleb</i>
<i>Bembidion lampros</i> (Herbst)	<i>Bemb lam</i>
<i>Calathus fuscipes</i> (Goeze)	<i>Cal fusc</i>
<i>Calathus melanocephalus</i> (Linnaeus)	<i>Cal mel</i>
<i>Clivina fossor</i> (Linnaeus)	<i>Cliv foss</i>
<i>Harpalus rufipes</i> (Degeer)	<i>Harp ruf</i>
<i>Loricera pilicornis</i> (Fabricius)	<i>Lori pill</i>
<i>Nebria brevicollis</i> (Fabricius)	<i>Neb brev</i>
<i>Pterostichus madidus</i> (Fabricius)	<i>Pter mad</i>
<i>Pterostichus melanarius</i> (Illiger)	<i>Pter mel</i>
<i>Pterostichus niger</i> (Schaller)	<i>Pter niger</i>
<i>Pterostichus nigrita</i> (Paykull)	<i>Pter nigrita</i>
<i>Pterostichus strenuus</i> (Panzer)	<i>Pter stren</i>
<i>Pterostichus vernalis</i> (Panzer)	<i>Pter vern</i>
<i>Trechus quadristriatus</i> (Schrank)	<i>Tre quad</i>

**Family Linyphiidae (Araneae)**

<i>Altonengea scopigera</i> (Grube)	<i>Allo scop</i>
<i>Bathypantes gracilis</i> (Blackwall)	<i>Bath grac</i>
<i>Dicymbium tibiale</i> (Blackwall)	<i>Dicy tib</i>
<i>Erigone atra</i> (Blackwall)	<i>Erig atra</i>
<i>Erigone dentipalpis</i> (Wider)	<i>Erig den</i>
<i>Labulla thoracica</i> (Wider)	<i>Lab thor</i>
<i>Lepthyphantes tenuis</i> (Blackwall)	<i>Lep ten</i>
<i>Lepthyphantes pallidus</i> (O.P.-Cambridge)	<i>Lep pall</i>
<i>Oedothorax fuscus</i> (Blackwall)	<i>O fuse</i>
<i>Oedothorax retusus</i> (Westring)	<i>O retu</i>
<i>Savignya frontata</i> (Blackwall)	<i>Sav fron</i>

Appendix II. - Axes 1 and 2 sample and species scores  
 from ordination of dung insect data in traps baited  
 with treated or untreated dung (Trial 1, Chapter 3)

Sample scores	Axis 1	Axis 2
Eigenvalues	0.4486	0.1327
Untreated 1	44	56
Untreated 2	39	66
Untreated 3	41	74
Untreated 4	24	59
Untreated 5	32	49
Untreated 6	49	71
Untreated 7	41	94
Untreated 8	39	81
Untreated 9	26	87
Untreated 10	44	91
Untreated 11	49	90
Untreated 12	42	80
Treated 1	0	135
Treated 2	75	0
Treated 3	23	13
Treated 4	54	53
Treated 5	49	52
Treated 6	61	87
Treated 7	34	97
Treated 8	73	124
Treated 9	208	77
Treated 10	247	89
Treated 11	114	40
Treated 12	40	114

Species scores	Axis 1	Axis 2
Eigenvalues	0.4486	0.1327
<i>Aphodius ater</i>	31	247
<i>A. depressus</i>	-19	196
<i>A. prodromus</i>	19	74
<i>A. rufipes</i>	31	247
<i>A. sphaecelatus</i>	38	-105
<i>A. pusillus</i>	89	432
<i>Cercyon atomarius</i>	77	198
<i>C. haemorrhoidalis</i>	137	-2
<i>C. lateralis</i>	316	116
<i>C. lugubris</i>	10	161
<i>C. melanocephalus</i>	205	67
<i>C. pygmaeus</i>	50	419
<i>Sphaeridium lunatum</i>	-47	232
<i>S. scarabaeoides</i>	31	247
<i>Scatophaga stercoraria</i>	31	-108

Appendix III. - Axis 1 and 2 sample and species scores  
 from ordination of dung insect data in traps baited  
 with treated and untreated dung (Trial 2, Chapter 3)

Sample scores	Axis 1	Axis 2
Eigenvalues	0.2982	0.0518
Untreated 1	59	41
Untreated 2	75	42
Untreated 3	67	55
Untreated 4	58	37
Untreated 5	81	53
Untreated 6	44	49
Untreated 7	98	34
Untreated 8	100	72
Untreated 9	130	56
Untreated 10	104	52
Untreated 11	81	53
Untreated 12	86	46
Treated 1	34	66
Treated 2	67	45
Treated 3	215	56
Treated 4	0	43
Treated 5	3	59
Treated 6	60	0
Treated 7	94	50
Treated 8	105	87
Treated 9	111	54
Treated 10	91	8
Treated 11	89	77
Treated 12	109	82

Species scores	Axis 1	Axis 2
Eigenvalues	0.2982	0.0518
<i>Aphodius depressus</i>	145	213
<i>A. lapponum</i>	157	667
<i>A. prodromus</i>	67	179
<i>A. rufipes</i>	191	-64
<i>A. sphacelatus</i>	-46	208
<i>Cercyon atomarius</i>	209	60
<i>C. haemorrhoidalis</i>	80	212
<i>C. lateralis</i>	296	77
<i>C. lugubris</i>	170	-105
<i>C. melanocephalus</i>	61	10
<i>C. pygmaeus</i>	49	-218
<i>Scatophaga stercoraria</i>	-81	87
<i>S. furcata</i>	5	328

Appendix IV. - Axes 1 and 2 sample and species scores  
 from ordination of dung insect data in traps baited  
 with treated or untreated dung of different moisture levels (Trial 3, Chapter 3)

Sample scores	Axis 1	Axis 2
Eigenvalues	0.266	0.1396
Treated Wet 1	139	46
Treated Wet 2	0	43
Treated Wet 3	91	0
Treated Wet 4	105	34
Treated Wet 5	70	55
Treated Wet 6	19	36
Treated Wet 7	50	37
Treated Wet 8	92	60
Treated Dry 1	81	91
Treated Dry 2	185	16
Treated Dry 3	108	67
Treated Dry 4	79	117
Treated Dry 5	69	61
Treated Dry 6	88	53
Treated Dry 7	88	67
Treated Dry 8	102	56
Untreated Wet 1	20	15
Untreated Wet 2	37	30
Untreated Wet 3	81	43
Untreated Wet 4	64	39
Untreated Wet 5	56	45
Untreated Wet 6	73	64
Untreated Wet 7	58	6
Untreated Wet 8	77	41
Untreated Dry 1	69	45
Untreated Dry 2	62	41
Untreated Dry 3	53	45
Untreated Dry 4	7	25
Untreated Dry 5	82	46
Untreated Dry 6	80	51
Untreated Dry 7	65	36
Untreated Dry 8	45	35

Species scores	Axis 1	Axis 2
Eigenvalues	0.266	0.1396
<i>Aphodius ater</i>	-103	0
<i>A. depressus</i>	0	163
<i>A. fimetarius</i>	-22	-117
<i>A. fossor</i>	147	-14
<i>A. prodromus</i>	108	67
<i>A. pusillus</i>	57	346
<i>A. sphaelatus</i>	-3	-21
<i>Cercyon atomarius</i>	261	-34
<i>C. lateralis</i>	84	-382
<i>C. haemorrhoidalis</i>	150	-59
<i>C. melanocephalus</i>	173	147
<i>C. pygmaeus</i>	32	379
<i>Scatophaga stercoraria</i>	-111	-30

Appendix V. - Axes 1 and 2 sample and species scores  
 from ordination of dung insect data in traps baited  
 with dung and surrounded by either 'high' or 'low' density of dung pats (Trial 4, Chapter 3)

Sample scores	Axis 1	Axis 2
Eigenvalues	0.118	0.00819
Low density 1	4	11
Low density 2	15	9
Low density 3	4	17
Low density 4	5	10
Low density 5	12	9
Low density 6	5	13
Low density 7	0	16
Low density 8	7	18
High density 1	32	29
High density 2	15	16
High density 3	69	20
High density 4	2	11
High density 5	16	21
High density 6	5	9
High density 7	12	1
High density 8	29	0

Species scores	Axis 1	Axis 2
Eigenvalues	0.118	0.008192
<i>Aphodius ater</i>	148	60
<i>A. depressus</i>	93	-133
<i>A. fimetarius</i>	-261	369
<i>A. fossor</i>	-152	132
<i>A. prodromus</i>	-4	14
<i>A. pusillus</i>	110	-117
<i>A. rufipes</i>	-248	-167
<i>A. sphacelatus</i>	2	142
<i>Cercyon atomarius</i>	142	16
<i>C. haemorrhoidalis</i>	-62	-136
<i>C. lateralis</i>	93	288
<i>C. lugubris</i>	-250	332
<i>C. melanocephalus</i>	145	92
<i>C. pygmaeus</i>	282	155
<i>Sphaeridium lunatum</i>	-286	-79
<i>Scatophaga stercoraria</i>	279	103

Appendix VI. - Axes 1 and 2 sample and species scores from ordination  
of dung insect data in dung-baited pitfall traps  
exposed for different numbers of days (Trial 5, Chapter 3)

Sample scores	Axis 1	Axis 2
Eigenvalues	0.0897	0.0334
5 days Trap 1	7	25
5 days Trap 2	61	10
5 days Trap 3	1	44
8 days Trap 1	80	5
8 days Trap 2	67	2
8 days Trap 3	42	45
11 days Trap 1	54	9
11 days Trap 2	61	59
11 days Trap 3	39	24
14 days Trap 1	33	0
14 days Trap 2	45	12
14 days Trap 3	19	31
17 days Trap 1	43	4
17 days Trap 2	6	35
17 days Trap 3	0	44
20 days Trap 1	81	14
20 days Trap 2	77	5
20 days Trap 3	35	29

Species scores	Axis 1	Axis 2
Eigenvalues	0.0897	0.0334
<i>Aphodius sphacelatus</i>	147	237
<i>A. prodromus</i>	21	-148
<i>A. rufipes</i>	29	443
<i>A. depressus</i>	236	99
<i>Cercyon atomarius</i>	23	141
<i>C. haemorrhoidalis</i>	75	77
<i>C. lateralis</i>	97	-132
<i>C. lugubris</i>	206	-353
<i>C. melanocephalus</i>	123	-49
<i>C. pygmaeus</i>	331	185
<i>Scatophaga stercoraria</i>	-58	52

Appendix VII. – Grid references of study sites

Farm	Field Code	Grid reference
1	ST1	NS 420 275
1	ST2	NS 416 274
1	ST3	NS 415 271
1	SC3	NS 434 275
2	WMT1	NS 481 293
2	WMT2	NS 482 294
2	WMT3	NS 486 288
2	WMC3	NS 483 285
2	WMC4	NS 486 284
3	CHT1	NS 458 231
3	CHT2	NS 460 231
4	BTBT3	NS 416 278
4	BTBC1	NS 409 284
4	BTBC2	NS 407 285
4	BTBC3	NS 407 282
5	DT1	NS 534 242
5	DT2	NS 532 238
5	DT3	NS 532 243
5	DC5	NS 539 225
5	DC6	NS 541 225
6	MTC	NS 379 232
6	BR	NS 378 231
7	GGT1	NS 461 210
7	GGC1	NS 458 213
8	LBT1	NS 497 318
8	LBC1	NS 490 309

Appendix VIII. - Management Intensity Scores for 'Treated' and 'Untreated' study fields, showing a breakdown of the scores and the scoring system for each category

Field	Sward Type	Soil disturbance	Age	Cutting	Grazing	Inorganic Input	Organic Input	Herbicide Input	MIS SCORE
BTBC1	3	0	1	0	3	3	2	0	12
BTBC2	3	0	1	0	3	3	2	2	14
BTBC3	3	0	1	0	3	3	2	0	12
BTRT3	3	0	1	0	3	3	2	0	12
CHT1	3	0	3	0	3	3	0	0	12
CHT2	3	0	1	0	3	3	2	0	12
DT1	3	0	1	1	3	3	0	0	11
DT2	3	0	1	1	3	3	0	0	11
DT3	3	0	1	1	3	3	0	0	11
DC5	3	2	3	1	2	3	2	0	16
DC6	3	2	3	2	2	3	2	0	17
WMT1	0	0	0	1	3	3	0	0	7
WMT2	0	0	0	1	3	3	0	0	7
WMT3	3	0	1	1	3	3	0	0	11
WMC3	3	2	3	1	2	3	2	0	16
WMC4	3	0	1	1	2	3	2	2	14
ST1	3	0	2	0	3	3	0	0	11
ST2	3	0	2	0	3	3	0	0	11
ST3	3	0	2	0	3	3	0	0	11
SC3	3	0	2	0	2	3	0	0	10
GGT1	3	0	1	2	3	3	0	0	12
GGC1	3	0	1	0	3	3	0	0	10
LBT1	1	0	1	2	3	3	0	2	12
LBC1	3	0	1	0	3	3	2	2	14
BR	3	0	1	1	2	3	3	0	13
MTC	3	0	1	1	2	3	3	0	13

Score	Sward Type	Soil disturbance	Age	Cutting	Grazing	Inorganic Input	Organic Input	Herbicide Input
0	Natural/ Semi-natural	None	Uncultivated	None	None	None	None	None
1	Sown/ improved sward now reverted	Harrowed only in last 3 years	In grass for >10 years	Topping only	<0.8LU*/ha	<50kg/ha NPK	This is subjective and	Fluaziflur only
2	Grass/ Clover mix	Ploughed once in last 3 years	In grass for 5-10 years	One cut	0.8-1.14 LU/ha	50-100kg/ha NPK	dependent upon number of	One herbicide product
3	Ryegrass	Ploughed >twice in last 3 years	In grass for <5 years	Two or more cuts of grass	>1.14 LU/ha	>100kg/ha NPK	slurry applications	Two or more herbicides used

\* LU - Livestock Units

Appendix IX. - Axes 1 and 2 sample and 'species' scores from ordination of pastures grazed by untreated cattle according to habitat characteristics

Sample scores	Axis 1	Axis 2
Eigenvalues	0.0169	0.00073
BTBC1 2002	2	12
BTBC2 2002	2	12
SC3 2002	18	15
WMC3 2002	0	12
WMC4 2002	2	10
DC5 2002	14	2
DC6 2002	15	1
BTBC2 2003	14	5
BTBC3 2003	15	5
SC3 2003	29	7
WMC3 2003	13	4
WMC4 2003	12	5
DC5 2003	11	3
DC6 2003	11	3
MTC 2003	36	6
BR 2003	35	3
LBC1 2003	23	0
GGC1 2003	22	0

Species scores	Axis 1	Axis 2
Eigenvalues	0.0169	0.00073
Altitude	-39	20
Aspect	55	266
Boundary	83	396
Woodland	207	410
Pasture index	103	-45

Appendix X. - Axes 1 and 2 sample and species scores from ordination of carabid assemblage data sampled in untreated pastures in September 2003

Sample scores	Axis 1	Axis 2
Eigenvalues	0.1462	0.012
DC5	18	9
DC6	17	10
WMC3	15	11
WMC4	18	17
SC3	20	5
LBC1	19	6
MTC	0	15
BR	58	8
BTBC2	22	0

Species scores	Axis 1	Axis 2
Eigenvalues	0.1462	0.0119
<i>Agonum dorsale</i>	-114	-52
<i>Agonum muelleri</i>	-160	0
<i>Amara aenea</i>	-195	14
<i>Amara plebeja</i>	-195	14
<i>Bembidion lampros</i>	248	14
<i>Calathus fuscipes</i>	-33	554
<i>C. melanocephalus</i>	-162	127
<i>Clivina fossor</i>	-195	14
<i>Harpalus rufipes</i>	-195	14
<i>Loricera pilicornis</i>	77	-138
<i>Nebria brevicollis</i>	16	12
<i>Pterostichus melanarius</i>	156	-186
<i>P. niger</i>	189	87
<i>P. strenuus</i>	-195	14
<i>P. vernalis</i>	248	14
<i>Trechus quadristriatus</i>	248	14

Appendix XI. - Axes 1 and 2 sample and species scores from ordination of spider assemblage data collected in untreated fields in September 2003

Sample scores	Axis 1	Axis 2
Eigenvalues	0.1052	0.02853
DC5	35	78
DC6	18	0
WMC3	42	28
WMC4	64	45
SC3	70	71
LBC1	25	57
MTC	0	52
BR	32	34
BTBC2	96	43

Species scores	Axis 1	Axis 2
Eigenvalues	0.1052	0.02853
<i>Bathyphantes gracilis</i>	121	94
<i>Lepthyphantes tenuis</i>	175	32
<i>Erigone dentipalpis</i>	-54	-16
<i>E. atra</i>	58	-43
<i>Oedothorax fuscus</i>	-59	223
<i>O. retusus</i>	-135	429
<i>L. pallidus</i>	403	65
<i>Savignya frontata</i>	245	293
<i>Allomengea scopigera</i>	-293	-342

Appendix XII. -Axes 1 and 2 sample and species scores from ordination of dung beetles collected in untreated pastures from April to July in 2002 and 2003

Sample scores	Axis 1	Axis 2
Eigenvalues	0.3936	0.1531
WMC3-1May02	212	205
WMC4-1May02	229	156
WMC3-7May02	230	185
WMC4-7May02	181	140
WMC3-16May02	151	126
WMC4-16May02	117	116
WMC3-24May02	202	74
WMC4-24May02	154	104
WMC4-31May02	126	109
WMC3-11June02	98	122
WMC4-11June02	127	108
WMC3-20June02	101	98
WMC4-20June02	99	106
WMC3-2July02	66	125
WMC4-2July02	70	120
SC3-9May02	237	168
SC3-17May02	147	128
SC3-28May02	77	118
SC3-5June02	117	86
SC3-14June02	79	105
SC3-24June02	66	82
SC3-3July02	97	75
SC3-12July02	40	73
DC5-3May02	146	144
DC6-3May02	253	92
DC5-10May02	159	119
DC6-10May02	199	109
DC5-23May02	165	121
DC6-23May02	145	113
DC5-30May02	101	126
DC6-30May02	120	115
DC5-7June02	118	124
DC6-7June02	118	107
DC5-16June02	113	123
DC6-16June02	95	118
DC5-25June02	114	70
DC6-25June02	67	123
DC5-4July02	108	102
DC6-4July02	119	87
BC1-1May02	278	130
BC2-1May02	237	70
BC1-9May02	230	98
BC2-9May02	234	82
BC2-18May02	242	5
BC1-27May02	235	14
BC2-27May02	123	24
BC1-5June02	209	67
BC2-5June02	172	118
BC1-16June02	116	54
BC2-16June02	78	75

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.3936	0.1531
BTBC1-24June02	126	88
BTBC2-24June02	132	73
BTBC1-3July02	97	76
BTBC2-3July02	126	77
BTBC1-12July02	84	105
BTBC2-12July02	63	96
DC5-7May03	83	145
DC5-21May03	129	136
DC5-6June03	109	87
DC5-18June03	85	112
DC5-2July03	0	117
DC5-16July03	60	126
DC6-7May03	14	123
DC6-21May03	94	148
DC6-6June03	100	148
DC6-18June03	61	127
DC6-2July03	86	127
DC6-16July03	74	123
BTBC2-6May03	287	268
BTBC2-20May03	158	140
BTBC2-4June03	131	115
BTBC2-17June03	40	108
BTBC2-1July03	89	121
BTBC2-15July03	87	132
BTBC3-6May03	277	254
BTBC3-20May03	119	128
BTBC3-4June03	108	136
BTBC3-17June03	49	118
BTBC3-1July03	18	92
BTBC3-16July03	62	106
WMC3-5May03	190	85
WMC3-30June03	99	128
WMC3-14July03	95	120
WMC4-5May03	154	149
WMC4-19May03	200	0
WMC4-3June03	116	117
WMC4-16June03	83	123
WMC4-30June03	65	123
WMC4-14July03	64	118
SC3-7May03	177	174
SC3-20May03	130	139
SC3-4June03	121	125
SC3-17June03	74	125
SC3-1July03	80	125
SC3-15July03	83	122
LBC1-13May03	121	126
LBC1-27May03	110	114
LBC1-10June03	96	129
LBC1-24June03	39	111
LBC1-8July03	62	118

Appendix XII. -Axes 1 and 2 sample and species scores from ordination of dung beetles collected in untreated pastures from April to July in 2002 and 2003

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.3936	0.1531
LBC1-22July03	82	126
GGC1-19May03	116	143
GGC1-3June03	125	116
GGC1-16June03	79	136
GGC1-2July03	102	137
GGC1-15July03	91	124
MTC-14May03	211	188
MTC-29May03	124	139
MTC-11June03	109	138
MTC-24June03	64	112
MTC-8July03	76	121
MTC-22July03	82	127
BR-14May03	198	48
BR-29May03	132	129
BR-11June03	101	137
BR-24June03	90	124
BR-8July03	78	123
BR-22July03	107	135

Species scores	Axis 1	Axis 2
Eigenvalues	0.3936	0.1531
<i>Aphodius ater</i>	224	75
<i>A. depressus</i>	279	-38
<i>A. fimetarius</i>	183	98
<i>A. fossor</i>	189	19
<i>A. prodromus</i>	292	272
<i>A. pusillus</i>	-78	172
<i>A. rufipes</i>	-9	-14
<i>A. rufus</i>	-214	123
<i>A. sphaelatus</i>	276	277
<i>Cercyon atomarius</i>	116	143
<i>C. haemorrhoidalis</i>	53	129
<i>C. lateralis</i>	-33	114
<i>C. lugubris</i>	-21	142
<i>C. melanocephalus</i>	100	126
<i>C. pygmaeus</i>	198	48
<i>Sphaeridium lunatum</i>	185	21
<i>S. scarabaeoides</i>	200	136

Appendix XIII. - Axes 1 and 2 sample and 'species' scores from ordination of habitat characteristics in pastures grazed by treated and untreated cattle

Sample scores	Axis 1	Axis 2
Eigenvalues	0.0209	0.002
BTBC1 2002	10	6
BTBC2 2002	10	4
BTBT3 2002	0	9
ST1 2002	12	4
ST2 2002	12	4
SC3 2002	25	15
WMT1 2002	6	3
WMT2 2002	9	2
WMC3 2002	8	5
WMC4 2002	10	5
DT1 2002	3	9
DT2 2002	6	7
DC5 2002	23	1
DC6 2002	24	1
CHT1 2002	30	6
CHT2 2002	19	4
BTBC2 2003	23	2
BTBC3 2003	23	3
ST1 2003	28	1
ST2 2003	26	1
ST3 2003	23	5
SC3 2003	36	11
WMT2 2003	20	0
WMT3 2003	14	2
WMC3 2003	22	2
WMC4 2003	21	2
DT1 2003	16	6
DT3 2003	24	7
DC5 2003	20	2
DC6 2003	20	2
CHT1 2003	43	2
CHT2 2003	36	0
MTC 2003	44	7
BR 2003	42	6
LB11 2003	26	1
LBC1 2003	32	0
GGT1 2003	16	2
GGC1 2003	31	0

Species scores	Axis 1	Axis 2
Eigenvalues	0.021	0.002
Altitude	-30	1
Aspect	-5	500
Boundary	61	210
Woodland	128	303
Pasture index	116	-15

Appendix XIV. - Axes 1 and 2 sample and species scores from ordination of carabid assemblage data collected in treated and untreated pastures in September 2003

Sample scores	Axis 1	Axis 2
Eigenvalues	0.1975	0.0539
DT1	0	1
DT3	3	1
DC5	8	5
DC6	3	3
WMT2	2	1
WMT3	17	7
WMC3	1	5
WMC4	4	7
ST1	52	9
ST2	11	4
ST3	8	3
SC3	7	2
LBT1	81	0
LBC1	8	2
MTC	3	26
BR	23	6
BTBC2	13	1

Species scores	Axis 1	Axis 2
Eigenvalues	0.1975	0.05389
<i>Agonum dorsale</i>	230	79
<i>Agonum muelleri</i>	-64	139
<i>Amara aenea</i>	153	56
<i>Amara communis</i>	195	24
<i>Amara plebeja</i>	151	118
<i>Bembidion lampros</i>	61	18
<i>Calathus fuscipes</i>	-57	-15
<i>C. melanocephalus</i>	-52	281
<i>Clivina fossor</i>	-51	342
<i>Harpalus rufipes</i>	-51	342
<i>Loricera pilicornis</i>	174	-25
<i>Nebria brevicollis</i>	0	4
<i>Pterostichus madidus</i>	-62	-454
<i>P. melanarius</i>	72	-84
<i>P. niger</i>	93	55
<i>P. nigrita</i>	-82	-414
<i>P. strenuus</i>	-51	342
<i>P. vernalis</i>	61	18
<i>Trechus quadristriatus</i>	61	18

Appendix XV. - Axes 1 and 2 sample and species scores from ordination of spider assemblage data collected in treated and untreated fields in September 2003

Sample scores	Axis 1	Axis 2
Eigenvalues	0.09351	0.0603
DT1	19	47
DT3	82	37
DC5	63	25
DC6	18	3
WMT2	95	35
WMT3	55	46
WMC3	23	35
WMC4	33	56
ST1	4	45
ST2	16	67
ST3	11	45
SC3	42	58
LBT1	42	83
LBC1	15	16
MTC	0	0
BR	16	26
BTBC2	22	94

Species scores	Axis 1	Axis 2
Eigenvalues	0.0935	0.0603
<i>Bathyphantes gracilis</i>	26	121
<i>Lepthyphantes tenuis</i>	95	136
<i>Erigone dentipalpis</i>	-88	-45
<i>E. atra</i>	92	32
<i>Oedoithorax fuscus</i>	190	-76
<i>O. retusus</i>	-130	-374
<i>L. pallidus</i>	-173	537
<i>Savignya frontata</i>	301	-29
<i>Allomengea scopigera</i>	-154	-652
<i>Labulla thoracica</i>	544	53
<i>Dicymbium tibiale</i>	229	115

Appendix XVI. - Axes 1 and 2 sample and species scores from ordination of dung beetles collected in treated and untreated fields from April to July in 2002 and 2003

Sample scores	Axis 1	Axis 2
Eigenvalues	0.5065	0.1612
WMT1-30April02	196	60
WMT2-30April02	276	99
WMC3-1May02	191	71
WMC4-1May02	205	109
WMT1-7May02	224	58
WMT2-7May02	225	59
WMC3-7May02	213	105
WMC4-7May02	144	123
WMT1-16May02	211	73
WMT2-16May02	202	72
WMC3-16May02	147	143
WMC4-16May02	94	128
WMT1-24May02	121	181
WMT2-24May02	153	156
WMC3-24May02	157	174
WMC4-24May02	125	127
WMT1-31May02	41	144
WMT2-31May02	67	186
WMC4-31May02	96	130
WMT1-11June02	48	146
WMT2-11June02	86	148
WMC3-11June02	76	117
WMC4-11June02	92	132
WMT1-20June02	18	205
WMT2-20June02	39	176
WMC3-20June02	65	153
WMC4-20June02	71	142
WMT1-2July02	67	135
WMT2-2July02	29	159
WMC3-2July02	55	120
WMC4-2July02	45	138
CHT1-16May02	230	46
CHT2-16May02	151	138
CHT1-24May02	176	169
CHT2-24May02	186	240
CHT1-31May02	88	199
CHT1-14June02	40	218
CHT2-14June02	33	243
CHT1-20June02	7	221
CHT2-20June02	97	155
CHT1-2July02	65	208
CHT2-2July02	67	208
CHT1-10July02	56	177
CHT2-10July02	120	204
ST1-2May02	308	122
ST2-2May02	284	136
ST1-9May02	191	183
ST2-9May02	214	188
SC3-9May02	211	103
ST1-17May02	198	140
ST2-17May02	192	145

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.5065	0.1612
SC3-17May02	125	114
ST1-28May02	144	215
ST2-28May02	159	189
SC3-28May02	56	140
ST1-5June02	102	212
ST2-5June02	143	216
SC3-5June02	69	175
ST1-14June02	122	213
ST2-14June02	66	171
SC3-14June02	56	144
ST1-24June02	59	140
SC3-24June02	12	184
ST1-3July02	76	186
SC3-3July02	58	167
ST1-12July02	39	171
SC3-12July02	0	192
DT1-4May02	321	97
DC5-3May02	147	148
DC6-3May02	204	157
DT1-14May02	129	138
DC5-10May02	114	129
DC6-10May02	154	142
DT1-21May02	115	156
DT2-21May02	131	122
DC5-23May02	139	125
DC6-23May02	112	125
DT1-30May02	117	142
DT2-30May02	138	182
DC5-30May02	71	115
DC6-30May02	86	120
DT1-9June02	136	229
DT2-9June02	157	215
DC5-7June02	95	122
DC6-7June02	77	137
DT1-18June02	121	177
DT2-18June02	102	120
DC5-16June02	90	115
DC6-16June02	71	122
DT1-26June02	94	116
DT2-26June02	94	113
DC5-25June02	81	184
DC6-25June02	69	127
DT1-4July02	51	123
DT2-4July02	76	132
DC5-4July02	91	147
DC6-4July02	99	164
BTBC1-1May02	241	146
BTBC2-1May02	179	178
BTBC1-9May02	190	156
BTBC2-9May02	186	172
BTBC1-18May02	171	238

Appendix XVI. - Axes 1 and 2 sample and species scores from ordination of dung beetles collected in treated and untreated fields from April to July in 2002 and 2003

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.5065	0.1612
BTBT3-18May02	188	102
BTBC1-27May02	171	228
BTBC2-27May02	66	240
BTBT3-27May02	149	168
BTBC1-5June02	150	178
BTBC2-5June02	117	126
BTBT3-5June02	154	192
BTBC1-16June02	70	200
BTBC2-16June02	52	186
BTBT3-16June02	63	237
BTBC1-24June02	86	159
BTBC2-24June02	97	163
BTBT3-24June02	85	167
BTBC1-3July02	55	182
BTBC2-3July02	96	173
BTBT3-3July02	91	179
BTBC1-12July02	48	144
BTBC2-12July02	39	163
BTBT3-12July02	37	162
DT1-7May03	83	119
DT1-21May03	127	101
DT1-6June03	86	103
DT1-18June03	51	115
DT1-2July03	75	114
DT1-16July03	73	105
DT3-7May03	51	132
DT3-21May03	138	94
DT3-6June03	115	99
DT3-18June03	59	117
DT3-2July03	69	128
DT3-16July03	61	118
DC5-7May03	68	118
DC5-21May03	95	113
DC5-6June03	69	133
DC5-18June03	62	119
DC5-2July03	13	160
DC5-16July03	48	115
DC6-7May03	8	155
DC6-21May03	68	110
DC6-6June03	82	45
DC6-18June03	48	66
DC6-2July03	74	118
DC6-16July03	53	122
BTBC2-6May03	259	0
BTBC2-20May03	101	105
BTBC2-4June03	91	136
BTBC2-17June03	13	160
BTBC2-1July03	66	115
BTBC2-15July03	46	111
BTBC3-6May03	253	11

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.5065	0.1612
BTBC3-20May03	97	108
BTBC3-4June03	84	95
BTBC3-17June03	34	146
BTBC3-1July03	1	163
BTBC3-16July03	43	149
WMT2-5May03	133	73
WMT2-19May03	117	123
WMT2-3June03	146	148
WMT2-16June03	79	147
WMT2-30June03	60	138
WMT2-14July03	86	119
WMT3-5May03	136	81
WMT3-19May03	31	151
WMT3-3June03	91	136
WMT3-16June03	48	124
WMT3-30June03	39	115
WMT3-14July03	63	117
WMC3-5May03	188	193
WMC3-30June03	59	109
WMC3-14July03	61	127
WMC4-5May03	123	119
WMC4-19May03	146	254
WMC4-3June03	91	111
WMC4-16June03	68	116
WMC4-30June03	49	124
WMC4-14July03	44	129
ST1-7May03	209	131
ST1-20May03	156	116
ST1-4June03	91	98
ST1-17June03	56	122
ST1-1July03	72	112
ST1-16July03	64	114
ST2-7May03	233	167
ST2-20May03	156	190
ST3-7May03	231	73
ST3-1July03	53	108
ST3-15July03	57	116
SC3-7May03	146	64
SC3-20May03	103	96
SC3-4June03	72	117
SC3-17June03	63	117
SC3-1July03	52	117
SC3-15July03	48	121
CHT1-13May03	198	121
CHT1-27May03	116	126
CHT1-10June03	74	148
CHT1-24June03	37	166
CHT1-8July03	39	143
CHT1-22July03	51	134
CHT2-13May03	179	154

Appendix XVI. - Axes 1 and 2 sample and species scores from ordination of dung beetles collected in treated and untreated fields from April to July in 2002 and 2003

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.5065	0.1612
CHT2-27May03	97	127
CHT2-10June03	101	119
CHT2-24June03	38	135
CHT2-8July03	53	150
CHT2-22July03	62	111
LBT1-13May03	150	66
LBT1-27May03	71	107
LBT1-10June03	56	127
LBT1-24June03	61	129
LBT1-8July03	54	119
LBT1-22July03	57	121
LBC1-13May03	106	115
LBC1-27May03	86	129
LBC1-10June03	67	115
LBC1-24June03	36	156
LBC1-8July03	48	138
LBC1-22July03	59	117
GGT1-19May03	199	280
GGT1-3June03	91	129
GGT1-16June03	53	124
GGT1-2July03	50	119
GGT1-15July03	78	140
GGC1-19May03	25	99
GGC1-3June03	78	123
GGC1-16June03	15	118
GGC1-7July03	39	103
GGC1-15July03	50	123
MTC-14May03	180	77
MTC-29May03	80	105
MTC-11June03	73	106
MTC-24June03	28	145
MTC-8July03	43	119
MTC-22July03	49	109
BR-14May03	197	215
BR-29May03	84	114
BR-11June03	67	101
BR-24June03	60	109
BR-8July03	44	117
BR-22July03	36	108

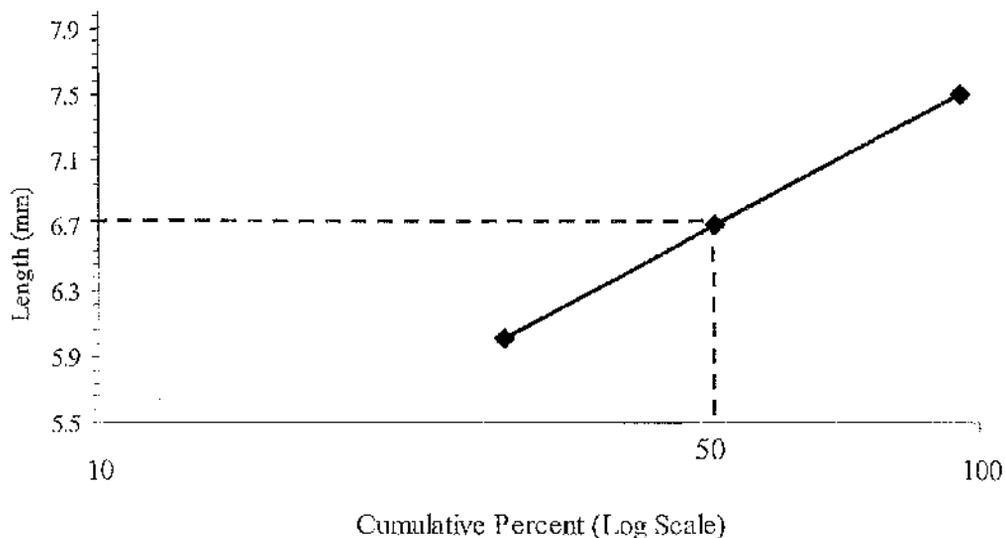
Species scores	Axis 1	Axis 2
Eigenvalues	0.5065	0.1612
<i>Aphodius ater</i>	227	235
<i>A. depressus</i>	199	280
<i>A. fimetarius</i>	184	209
<i>A. fossor</i>	219	258
<i>A. lapponum</i>	250	145
<i>A. prodromus</i>	267	-3
<i>A. pusillus</i>	-155	-111
<i>A. rufipes</i>	-96	331
<i>A. rufus</i>	-252	206
<i>A. sphacelatus</i>	348	148
<i>Cercyon atomarius</i>	25	99
<i>C. haemorrhoidalis</i>	29	25
<i>C. lateralis</i>	-13	177
<i>C. lugubris</i>	-110	182
<i>C. melanocephalus</i>	96	111
<i>C. pygmaeus</i>	197	215
<i>Sphaeridium lunatum</i>	141	265
<i>S. scarabaeoides</i>	196	198

Appendix XVII. - Example of Weight Median Length calculation

1. 'Weight' or mass was calculated using the mass-length relationship equation derived for *Aphodius*:  $\text{Mass} = 0.0248 (\text{Length})^{2.726}$ . Cumulative biomass was calculated for the total number of individuals in the sample (see table below).

Mean Length (mm)	Weight (mg)	Number of individuals, N	N x Weight (mg)	% Biomass	Cumulative % Biomass
5	1.994	27	53.835	1.955	1.955
6	3.277	225	737.427	26.777	28.731
7.5	6.021	303	1824.47	66.248	94.979
10	13.190	4	52.761	1.916	96.895
11	17.103	5	85.516	3.105	100
Total biomass			2754.01		

2. Using values in the above table, mean length was plotted against cumulative % biomass.
3. From the graph, the length (mm) was taken at the 50% cumulative biomass point. One can see from the above table that the 50% point would be between 6 and 7.5 mm. The WML was calculated as **6.7 mm** from the graph below.



Appendix XVIII. - Axes 1 and 2 sample and 'species' scores from ordination of  
*Aphodius* assemblage data according to size class

Sample scores	Axis 1	Axis 2	Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.6058	0.3033	Eigenvalues	0.6058	0.3033
WMT1-30April02	2	32	ST2-28May02	128	88
WMT2-30April02	1	94	SC3-28May02	183	47
WMC3-1May02	11	45	ST1-5June02	175	75
WMC4-1May02	53	42	ST2-5June02	151	73
WMT1-7May02	14	28	SC3-5June02	160	74
WMT2-7May02	8	37	ST1-14June02	164	75
WMC3-7May02	28	66	ST2-14June02	173	65
WMC4-7May02	52	64	SC3-14June02	198	64
WMT1-16May02	27	18	ST1-24June02	187	77
WMT2-16May02	28	16	SC3-24June02	240	73
WMC3-16May02	77	118	ST1-3July02	208	72
WMC4-16May02	116	95	SC3-3July02	202	72
WMT1-24May02	123	66	ST1-12July02	220	66
WMT2-24May02	105	50	SC3-12July02	255	77
WMC3-24May02	119	69	DT1-4May02	0	109
WMC4-24May02	107	60	DC5-3May02	52	79
WMT2-31May02	154	72	DC6-3May02	93	43
WMC4-31May02	154	72	DT1-14May02	78	43
WMT1-11June02	223	71	DC5-10May02	90	64
WMT2-11June02	154	72	DC6-10May02	88	50
WMC3-11June02	154	72	DT1-21May02	129	60
WMC4-11June02	145	65	DT2-21May02	55	44
WMT1-20June02	255	71	DC5-23May02	68	60
WMT2-20June02	246	71	DC6-23May02	104	48
WMC3-20June02	243	71	DT1-30May02	137	64
WMC4-20June02	233	71	DT2-30May02	140	65
WMT1-2July02	243	71	DC5-30May02	273	71
WMT2-2July02	228	59	DC6-30May02	154	72
WMC4-2July02	273	71	DT1-9June02	167	72
CHT1-16May02	23	19	DT2-9June02	150	70
CHT2-16May02	84	58	DC5-7June02	0	164
CHT1-24May02	105	55	DC6-7June02	154	72
CHT2-24May02	139	74	DT1-18June02	147	77
CHT1-31May02	154	72	DT2-18June02	104	48
CHT1-14June02	215	71	DC5-16June02	104	48
CHT2-14June02	226	77	DC6-16June02	154	72
CHT1-20June02	251	71	DC5-25June02	194	72
CHT2-20June02	174	72	DT1-4July02	273	71
CHT1-2July02	200	74	DT2-4July02	154	72
CHT2-2July02	194	73	DC5-4July02	174	72
CHT1-10July02	203	74	DC6-4July02	154	72
CHT2-10July02	164	72	BTBC1-1May02	67	54
ST1-2May02	14	114	BTBC2-1May02	116	53
ST2-2May02	22	113	BTBC1-9May02	86	52
ST1-9May02	86	83	BTBC2-9May02	103	50
ST2-9May02	91	80	BTBC2-18May02	147	70
SC3-9May02	47	48	BTBT3-18May02	53	35
ST1-17May02	53	94	BTBC1-27May02	147	68
ST2-17May02	87	56	BTBC2-27May02	213	72
SC3-17May02	76	31	BTBT3-27May02	118	70
ST1-28May02	151	81	BTBC1-5June02	131	61

Appendix XVIII. - Axes 1 and 2 sample and 'species' scores from ordination of  
*Aphodius* assemblage data according to size class

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.6058	0.3033
BTBC2-5June02	93	43
BTBT3-5June02	126	62
BTBC1-16June02	200	72
BTBC2-16June02	184	67
BTBT3-16June02	204	71
BTBC1-24June02	176	72
BTBC2-24June02	174	72
BTBT3-24June02	181	73
BTBC1-3July02	213	72
BTBC2-3July02	128	88
BTBT3-3July02	188	72
BTBC1-12July02	162	66
BTBC2-12July02	196	72
BTBT3-12July02	210	72
DT1-7May03	41	37
DT1-21May03	23	57
DT1-6June03	53	24
DT1-18June03	273	71
DT3-7May03	72	59
DT3-21May03	40	18
DT3-6June03	33	14
DT3-18June03	154	72
DT3-2July03	154	72
DT3-16July03	213	72
DC5-7May03	2	10
DC5-21May03	63	62
DC5-6June03	154	72
DC5-18June03	213	72
DC6-7May03	0	164
DC6-21May03	2	0
DC6-6June03	2	0
DC6-16July03	154	72
BTBC2-6May03	2	0
BTBC2-20May03	63	29
BTBC2-4June03	96	42
BTBC2-17June03	273	71
BTBC2-1July03	154	72
BTBC3-6May03	7	7
BTBC3-20May03	78	36
BTBC3-4June03	2	0
BTBC3-17June03	154	72
BTBC3-1July03	273	71
BTBC3-16July03	213	72
WMT2-5May03	9	23
WMT2-19May03	63	29
WMT2-3June03	88	51
WMT2-16June03	156	66
WMT2-30June03	170	68
WMT2-14July03	171	72
WMT3-5May03	14	31

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.6058	0.3033
WMT3-19May03	53	24
WMT3-3June03	126	66
WMT3-30June03	78	36
WMT3-14July03	194	72
WMC3-5May03	0	164
WMC3-30June03	178	72
WMC3-14July03	194	72
WMC4-5May03	31	113
WMC4-19May03	154	72
WMC4-3June03	104	48
WMC4-16June03	273	71
WMC4-30June03	146	54
WMC4-14July03	213	72
ST1-7May03	65	51
ST1-20May03	78	36
ST1-4June03	54	49
ST1-17June03	169	66
ST1-1July03	233	71
ST1-16July03	256	71
ST2-7May03	82	65
ST2-20May03	127	69
ST3-7May03	38	24
ST3-1July03	263	71
ST3-15July03	253	71
SC3-7May03	12	26
SC3-20May03	73	24
SC3-4June03	116	45
SC3-1July03	253	71
SC3-15July03	223	71
CHT1-13May03	57	53
CHT1-27May03	99	46
CHT1-10June03	136	60
CHT1-24June03	190	72
CHT1-8July03	223	73
CHT1-22July03	209	72
CHT2-13May03	86	68
CHT2-27May03	104	48
CHT2-10June03	51	53
CHT2-8July03	224	71
CHT2-22July03	191	72
LBT1-13May03	17	23
LBT1-10June03	154	72
LBT1-24June03	154	72
LBC1-13May03	63	62
LBC1-27May03	149	85
LBC1-10June03	78	36
LBC1-24June03	154	72
LBC1-8July03	178	72
LBC1-22July03	154	72
GGT1-19May03	154	72

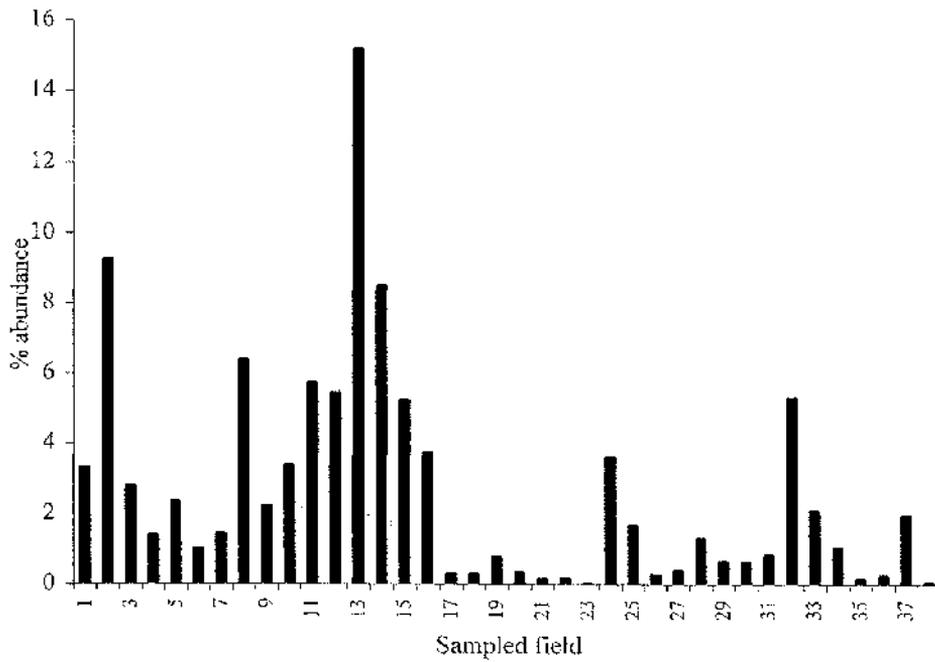
Appendix XVIII. - Axes 1 and 2 sample and 'species' scores from ordination of  
*Aphodius* assemblage data according to size class

Sample scores (cont.)	Axis 1	Axis 2
Eigenvalues	0.6058	0.3033
GGT1-3June03	77	118
GGT1-16June03	154	72
GGT1-2July03	154	72
GGT1-15July03	155	72
GGC1-3June03	154	72
GGC1-15July03	182	72
MTC-14May03	25	27
MTC-29May03	79	53
MTC-11June03	47	27
MTC-24June03	183	85
MTC-8July03	192	81
MTC-22July03	273	71
BR-29May03	91	64
BR-11June03	25	34
BR-24June03	205	93
BR-8July03	162	70
BR-22July03	198	81

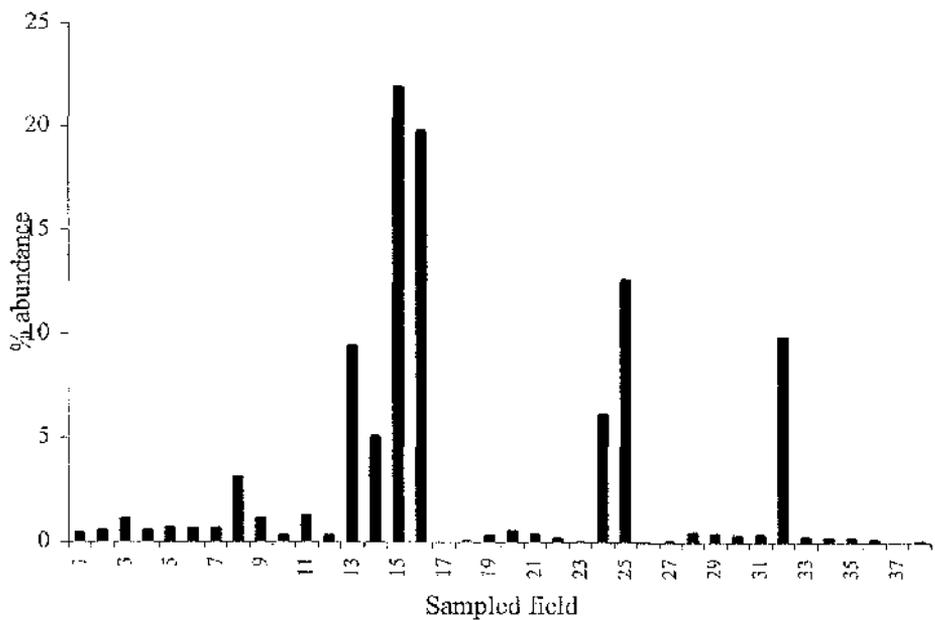
Species scores	Axis 1	Axis 2
Eigenvalues	0.6058	0.3033
<4 mm	239	148
5-6 mm	0	164
7-8 mm	2	0
9-10 mm	154	72
11+ mm	273	71

Appendix XIX. - Proportional abundance of dung insect groups trapped  
(*Aphodius*, *Cercyon* beetles, and yellow dung fly) in each study field

*Aphodius* Guild 1



*Aphodius* Guild 2

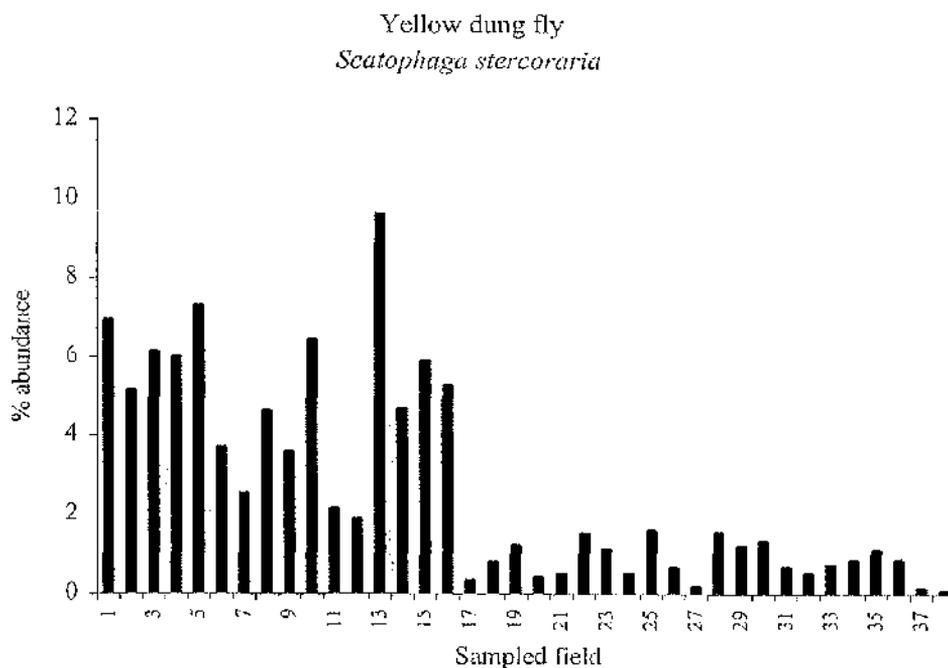
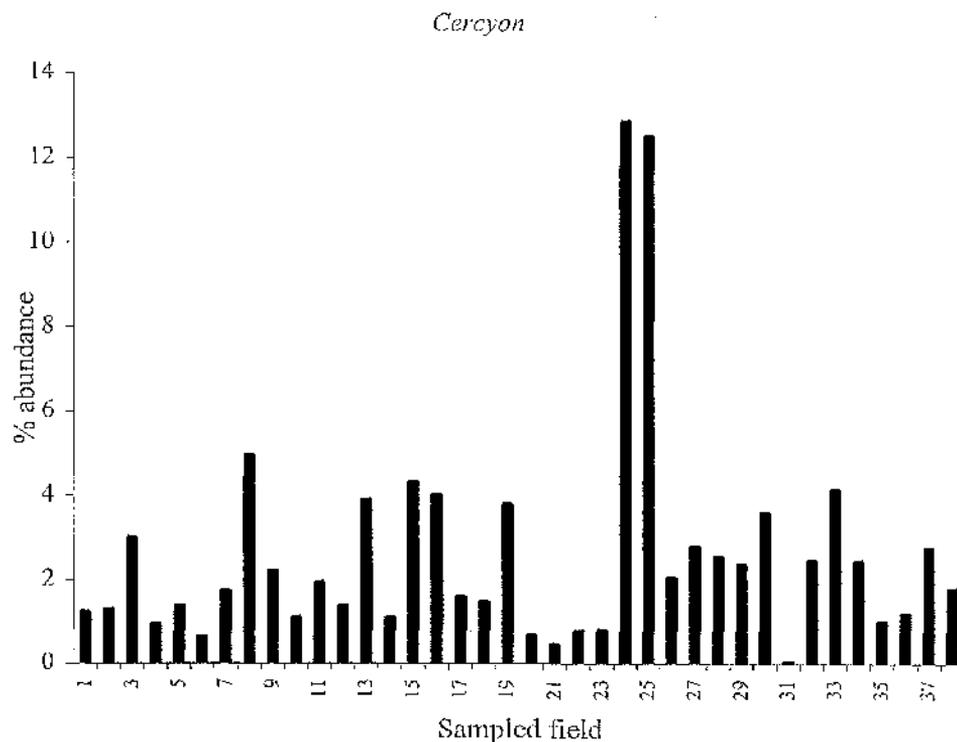


Key to field numbers\*

- 1-7        Untreated 2002
- 8-16      Treated 2002
- 17-27     Untreated 2003
- 28-38     Treated 2003

\*Full code is provided overleaf

Appendix XIX. - Proportional abundance of dung insect groups trapped (*Aphodius*, *Cercyon* beetles, and yellow dung fly) in each study field



Key to field numbers\*

- 1-7      Untreated 2002
- 8-16     Treated 2002
- 17-27    Untreated 2003
- 28-38    Treated 2003

\*Full code is provided overleaf

Appendix XIX. - Proportional abundance of dung insect groups trapped (*Aphodius*, *Cercyon* beetles, and yellow dung fly) in each study field

Field Number	Field Code	Sample year	Treated/ Untreated
1	BTBC1	2002	Untreated
2	BTBC2	2002	Untreated
3	DC5	2002	Untreated
4	DC6	2002	Untreated
5	SC3	2002	Untreated
6	WMC3	2002	Untreated
7	WMC4	2002	Untreated
8	DT1	2002	Treated
9	DT2	2002	Treated
10	BTBT3	2002	Treated
11	CH T1	2002	Treated
12	CH T2	2002	Treated
13	ST1	2002	Treated
14	ST2	2002	Treated
15	WMT1	2002	Treated
16	WMT2	2002	Treated
17	WMC3	2003	Untreated
18	WMC4	2003	Untreated
19	SC3	2003	Untreated
20	BTBC2	2003	Untreated
21	BTBC3	2003	Untreated
22	DC5	2003	Untreated
24	MTC	2003	Untreated
25	BR	2003	Untreated
26	GGC1	2003	Untreated
27	LBC1	2003	Untreated
28	WMT2	2003	Treated
29	WMT3	2003	Treated
30	ST1	2003	Treated
31	ST2	2003	Treated
32	ST3	2003	Treated
33	CHT1	2003	Treated
34	CHT2	2003	Treated
35	DT1	2003	Treated
36	DT3	2003	Treated
37	GGT1	2003	Treated
38	LBT1	2003	Treated

Appendix XX. - Axes 1 and 2 sample and species scores from ordination  
of bird species data from observations made in treated and untreated  
pastures from May to July 2003

Sample score:	Axis 1	Axis 2
Eigenvalues	0.3256	0.1945
BTBC1	189	72
BTBC2	37	116
ST1	17	45
ST2	7	61
ST3	0	51
SC3	4	106
LBC1	6	166
LBT1	6	0
DT1	37	87
DT2	20	138
DC5	89	79
DC6	16	64

Species scores	Axis 1	Axis 2
Eigenvalues	0.326	0.195
Pied wagtail ( <b>PieWag</b> )	251	110
Yellowhammer ( <b>Yellham</b> )	127	34
Carrion crow ( <b>Crow</b> )	-20	197
Barn swallow ( <b>Swall</b> )	6	52
Tree sparrow ( <b>Tree spa</b> )	36	183
Dunnock ( <b>Dunn</b> )	8	-71
Blackbird ( <b>Blackbird</b> )	-55	20
Spotted flycatcher ( <b>Spot fly</b> )	77	-6
Starling ( <b>Starl</b> )	-6	195
Song thrush ( <b>Thru</b> )	-58	257
House martin ( <b>Hse mart</b> )	-52	381
Rook ( <b>Rook</b> )	-47	62
Skylark ( <b>Skylark</b> )	-58	-156

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