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Species composition and seasonal abundance of Aphodiine dung beetles (Coleoptera: Scarabaeidae) in dung from ivermectin-treated and nontreated cattle

Theresa A. Dellinger

To the Graduate Council:

I am submitting herewith a thesis written by Theresa A. Dellinger entitled "Species composition and seasonal abundance of Aphodiine dung beetles (Coleoptera: Scarabaeidae) in dung from ivermectin-treated and nontreated cattle." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Reid R. Gerhardt, Major Professor

We have read this thesis and recommend its acceptance:

Jerome F. Grant, Craig R. Reinemeyer

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Theresa A. Dellinger entitled "Species Composition and Seasonal Abundance of Aphodiine Dung Beetles (Coleoptera: Scarabaeidae) in Dung from Ivermectin-treated and Nontreated Cattle." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Dr. Reid R. Gerhardt, Major Professor

We have read this thesis and
recommend its acceptance:

Accepted for the Council:

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SPECIES COMPOSITION AND SEASONAL ABUNDANCE OF APHODIINE DUNG BEETLES (COLEOPTERA: SCARABAEIDAE) IN DUNG FROM IVERMECTIN-TREATED AND NONTREATED CATTLE

A Thesis

Presented for the

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Degree

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Theresa A. Dellinger

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ABSTRACT

Aphodiine beetles were collected from the dung of ivermectin-treated and nontreated pastured cattle during the spring and summer months of 1993 and 1994. Seven aphodiine species in the two genera *Aphodius* and *Ataenius* were found in both ivermectin-contaminated and noncontaminated dung in both years. In 1993, over 8,200 aphodiine beetles were collected, and approximately 3,000 aphodiine beetles were collected in 1994. The percentages of the total collected numbers for each species in 1993 and 1994, respectively, were: *Aphodius haemorrhoidalis* 83.16% and 64.90%; *Aphodius lividus* 3.88% and 2.70%; *Ataenius platensis* and *Ataenius spretulus* (combined together in 1993) 7.24%; *Ataenius platensis* 0.07% (1994); *Ataenius spretulus* 1.37% (1994); *Aphodius fimetarius* 1.27% and 5.93%; *Aphodius stercorosus* 2.67% and 5.67%; and *Aphodius erraticus* 1.78% and 19.37%.

About twice as many specimens were collected in ivermectin-contaminated dung (66.26%) as compared to noncontaminated dung (33.74%) in 1993, and the following year twice as many were again collected in ivermectin-contaminated dung (67.76%) as compared to noncontaminated dung (32.24%). Statistical analysis indicates that ivermectin itself did not affect the densities of individual species in either dung type. However, in 1993 the combined data of the three most abundant species (*Aphodius haemorrhoidalis*, *A. lividus*, and *Ataenius platensis* and *A. spretulus* added together) showed they were significantly more abundant in ivermectin-contaminated dung, but the combined data of the three most abundant species

collected in 1994 (*Aphodius haemorrhoidalis*, *A. fimetarius*, and *A. erraticus*) show no significant differences between densities in either dung type. Statistical analysis of the combined influence of treatment and the week in which samples were collected post-treatment are presented and discussed.

Seasonal distributions in 1993 and 1994 are presented for 6 of the 7 aphodiine species (except *Ataenius platensis*), based on the numbers of specimens collected in noncontaminated dung. In addition, the potential impact of ivermectin treatment on aphodiine beetles is discussed.

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CHAPTER I

INTRODUCTION

The dung pat of cattle has been well documented as a distinct ecological habitat with characteristic fauna (Mohr 1943, Valiela 1974, Stevenson & Dindal 1987). Numerous species of insects and other arthropods live in cattle dung throughout North America (Sanders & Dobson 1966, McDaniel & Balsbaugh 1968, Blume 1985, MacDonald & Combs 1985, Schreiber et al. 1987, Cervenka & Moon 1991). As a habitat, dung pats are subject to ecological succession with distinct waves of arthropod invaders colonizing the pat at specific intervals as the dung ages (Mohr 1943, Kessler & Balsbaugh 1972, Valiela 1974, Wingo et al. 1974).

Members of the orders Coleoptera, Diptera, and Hymenoptera comprise the vast majority of insect dung fauna. Hymenopteran dung fauna are usually parasitic on immature dipterans and visit dung pats to locate their hosts (Figg et al. 1983). While hymenopteran parasitoids are important regulators of coprophagous Diptera, they are unimportant contributors to the degradation of dung pats. However, coleopteran and dipteran dung pat fauna actively assist the degradation of dung pats and are thus instrumental in the subsequent recycling of the nutrients in the dung (Anderson et al. 1984).

Coleopteran dung fauna colonizing a newly-dropped cow pat soon form a network of tunnels throughout the pat, allowing air to circulate within the interior of

the pat. Coprophagous dipteran larvae are then able to penetrate the pat and continue to obtain air through their spiracles (Valiela 1974). In addition, the tunnels allow hymenopteran parasitoids access to dipteran larvae deep within the pat (Mohr 1943). Also, this network of tunnels undoubtedly eases the penetration of the dung pat by underlying vegetation such as grasses. A dung pat is eventually degraded and dispersed by the tunneling and feeding activities of dung pat fauna.

Most dung pat fauna are considered beneficial, or at least non-harmful, because they contribute to dung degradation. However, there are two economically important ectoparasites of cattle which breed in cattle dung in North America. Larvae of the horn fly, *Haematobia irritans* (L.), and the face fly, *Musca autumalis* De Geer, are both found in this habitat. As coprophagous larvae, both species are harmless and help degrade the dung pat by ingesting the dung and riddling the pat with tunnels. Adult *H. irritans*, however, are the most important pests of cattle in North America (Byford et al. 1992) with both sexes ingesting blood meals several times a day. Populations of *H. irritans* have been associated with subnormal weight gains in cattle (Haufe 1982). While adult *M. autumnalis* are not hematophagous, their presence around the head while they feed on nasal-lacrimal secretions is annoying to cattle. Adult *M. autumnalis* vector the causative agent of infectious bovine keratoconjunctivitis (pinkeye), *Moraxella bovis* (Hauduroy), but their primary harm is probably interference with feeding due to their annoying behavior. In 1987 it was estimated that *H. irritans* and *M. autumnalis* caused approximately $679 million and $59 million dollars, respectively, in yearly losses to the cattle industry (Drummond

1987). Despite the great economic importance of these ectoparasites, the majority of the numerous dipteran dung pat fauna is not considered pestiferous.

The coleopteran family Scarabaeidae includes three subfamilies often referred to as dung beetles. The subfamilies Scarabaeinae, Geotrupinae, and Aphodiinae all contain coprophagous (dung-feeding) and coprophilous (dung-attracted) members which aid in the degradation of mammalian dung. The extent to which dung beetles degrade dung depends on their attraction to the dung, and their feeding and nidification behavior (Stevenson & Dindal 1987). Larger species of dung beetles, particularly species of Scarabaeinae and Geotrupinae, may remove large portions of manure from the dung pat.

Dung beetles can be grouped according to their behavior in the dung pat. Paracoprids are dung beetle species which bury the portions under the pat while telecoprids are dung beetle species which roll the portions away from the pat before burying them. Either behavioral pattern can be effective in degrading the pat and incorporating it into the soil. Smaller species, such as the aphodiines, may behave as paracoprids, or they may, as endocoprid species, remain within the pat to feed and reproduce (Yoshida & Katakura 1992). While the behavior of endocoprids may not degrade the pat, their tunneling aerates the pat and contributes to the overall rate of pat disintegration.

As a habitat with a rich faunal population of arthropods and other invertebrates, cattle dung pats are often adversely affected by pesticide treatments applied to cattle to control parasites. Of particular importance are feed additives or

other substances which enter the digestive system and from there the dung pat. The insect growth regulators diflubenzuron and methoprene have been shown to eliminate both beneficial and pest species of insects which live in the dung pat habitat (Pickens & Miller 1975, Cook & Gerhardt 1977, Fincher 1991).

In 1981, Merck, Sharpe, and Dohme introduced the antiparasitic drug ivermectin for use in livestock. Ivermectin is a synthetic derivative of abamectin, a natural fermentation product of the actinomycete *Streptomyces avermitilis* Burg et al.. Abamectin has been widely marketed as a horticultural and agricultural pesticide, and as an antiparasitic drug for livestock in some countries. Together, ivermectin and abamectin, as well as other *S. avermitilis* fermentation products, are known as the avermectins.

Ivermectin is unique in that it offers strong protection against a number of endoparasitic and ectoparasitic arthropods, and against gastrointestinal nematodes of livestock as well. It is a systemic pesticide, spreading throughout the body after administration. Ivermectin may be administered to livestock in a variety of formulations, including topical applications, drenches, oral pastes, injections, and sustained-release boluses. Treated animals metabolize little ivermectin, and most of the drug is excreted in the feces (Campbell et al. 1983, Halley et al. 1989) where it continues to possess insecticidal properties. Regardless of the administration method, the concentration of ivermectin decreases daily after treatment until the drug is completely eliminated from the animal (Campbell et al. 1983).

Ivermectin, like other avermectins, is a 16-membered macrocyclic lactone which interacts with a glutamate-gated chloride channel found in invertebrates (Shoop & Mrozik 1994). Avermectins are believed to kill arthropods and nematodes by disrupting this channel and creating a chloride ion imbalance. The strong insecticidal activity of ivermectin appears to affect many species of dung pat fauna, and researchers began to question the adverse environmental impact of such a drug after its introduction to the market. Ivermectin excreted in dung acts as an effective larvicide of obligate coprophagous horn and face fly larvae, but it is effective against the immature stages of beneficial dung pat fauna as well. An intramuscular injection of 200 μg ivermectin per kg was sufficient to prevent the emergence of adult horn flies in cattle dung excreted up to 28 days after treatment; however, the numbers of emerging adult Sphaeroceridae and Sepsidae, two dipteran families typically found in cattle manure, were also severely reduced (Schmidt 1983). Several reviews have addressed the issue of ivermectin use and its impact on the dung pat fauna (Roncalli 1989, Strong & Wall 1990, Strong 1992, 1993).

Researchers have reported delays in the degradation of dung excreted by cattle treated with ivermectin. Artificial pats, made of dung from cattle fitted with experimental sustained-release boluses, failed to degrade within the time required for the degradation of artificial pats made of dung from nontreated cattle (Wall & Strong 1988). This delay in degradation was attributed to the insecticidal properties of ivermectin on the dung pat fauna as the artificial pats containing ivermectin were severely depleted of the dung fauna found in the noncontaminated pats. In another

study, artificial pats made from dung of cattle treated either by subcutaneous injection of 200 μg ivermectin per kg, or by topical treatment of 500 μg ivermectin per kg, showed no signs of degradation as measured as percentage of remaining organic matter until after 45 days of field exposure (Sommer et al. 1992).

Other studies have reported that ivermectin did not delay the degradation of dung pats. Artificial pats made of dung excreted by cattle treated with an intramuscular injection of 200 μg ivermectin per kg appeared to degrade at the same rate as artificial pats made of dung from nontreated cattle (Schmidt 1983). However, dung pat degradation was not quantitatively measured, but judged on appearance alone (Wall & Strong 1988). In Scotland, dung pats excreted by cattle treated with a pour-on formulation at 500 μg ivermectin per kg degraded at the same rate, as measured by diameter and thickness, as pats deposited by nontreated cattle (McKeand et al. 1988). However, it rained on 45 of the 65 days the study was conducted, and these results found during extremely wet conditions may not accurately reflect degradation rates seen in drier climates.

Cyclorrhaphan Diptera appear to be particularly sensitive to ivermectin. The dung-breeding muscid *Orthelia cornicina* (F.) was unable to complete its larval development in dung excreted by cattle for up to 32 days after treatment with an intramuscular injection of 200 μg ivermectin per kg (Wardhaugh & Rodriguez-Menendez 1988). Larvae of the muscid *Neomyia cornicina* (F.) did not survive in dung excreted by cattle given subcutaneous injections of 200 μg ivermectin per kg 1 day and 10 days after treatment (Lumaret et al. 1993). Cyclorrhaphan larvae were

killed in dung excreted 13 to 14 days after a topical treatment of 500 μg ivermectin per kg, and 28 to 29 days after subcutaneous injection of 200 μg ivermectin per kg, but nematoceran larvae present were unaffected by either treatment (Sommer et al. 1992).

Many studies concerning the effects of ivermectin on pestiferous dung-breeding flies have been conducted. Neonate larvae of Australian bush fly, *Musca vetustissima* Walker, did not survive in cattle dung excreted 3 to 25 days after subcutaneous injection with 200 μg ivermectin per kg, and mortality of larvae reared in dung excreted 35 days after treatment was still 93.6% (Wardhaugh & Mahon 1991). Larvae of *M. vetustissima* were unable to survive in dung excreted by sheep 1 to 6 days after drenching with 200 μg ivermectin per kg, but survival rates were normal in dung excreted 28 days after treatment (Wardhaugh & Mahon 1991). A subcutaneous injection of 200 μg ivermectin per kg eliminated larval *M. autumnalis* in dung excreted up to 9 days after injection; larvae survived in dung excreted between 10 to 15 days after injection, but produced malformed pupae with only a 10% adult emergence rate (Meyer et al. 1980).

Studies with avermectin, the parent compound of ivermectin, have shown a similar insecticidal effect on cyclorrhaphan Diptera. *M. vetustissima* was unable to complete its development to the adult stage when reared as larvae on dung from cattle treated with a subcutaneous injection of 200 μg avermectin per kg for up to 2 weeks after treatment. Eight weeks after treatment, however, mortality rates returned to the expected normal levels (Ridsdill-Smith 1988).

Larval exposure to sublethal levels of ivermectin may have latent effects on later development and reproduction. Experiments with the calliphorid *Calliphora vomitoria* L. have shown that a 300 μg dose of ivermectin topically applied to last-instar larvae suppressed adult emergence at significant levels, and a significant number of adult females which emerged successfully had oocytes which failed to develop to maturity (Strong 1989). A reduction in reproductive potential was also observed in the calliphorid *Lucilia sericata* (Meigen) when final-instar larvae were topically treated at a rate of 7.6 μg ivermectin per g; females dosed as larvae produced a higher number of sterile egg batches than females not treated as larvae (McGarry 1988).

Adult exposure to sublethal levels of ivermectin may also affect reproduction. Gravid *L. sericata* topically treated with ivermectin at a dosage of 0.05 μg per g produced a higher number of sterile egg batches than nontreated females (McGarry 1988). Adult female Australian sheep blowflies, *L. cuprina* (Wiedemann), fed dung from sheep drenched with ivermectin 18 hours earlier were unable to produce mature oocytes after 10 days of feeding (Cook 1991).

The effects of ivermectin on coprophagous and coprophilic Coleoptera have not been as well documented as the effects on Diptera, reflecting the earlier emphasis of research on the ability of ivermectin to control pestiferous dung-breeding flies. However, more studies on dung-associated Coleoptera have been conducted as concern about the environmental impact of ivermectin has risen. Ivermectin appears to be as equally severe on the immature stages of dung-breeding Coleoptera as it is on

immature dung-breeding Diptera. Larvae of the scarabaeine dung beetle *Copris hispanus* L. cannot survive in dung excreted by cattle injected with 200 μg ivermectin per kg until 16 days after treatment (Wardhaugh & Rodriguez-Menendez 1988). Larvae of aphodiine dung beetles (*Aphodius* spp.) were killed in significant numbers in dung from cattle treated 1 to 2 days earlier by topical application of 200 μg ivermectin per kg or by subcutaneous injection of 500 μg ivermectin per kg, but survival was normal in dung excreted 13 to 14 days after either treatment (Sommer et al. 1992). Larvae of the scarabaeine *Onthophagus gazella* F. were unable to develop in dung excreted by cattle up to 21 days after treatment by subcutaneous injections of 200 μg ivermectin per kg, but adult survival rates in ivermectin-contaminated dung were normal (Roncalli 1989).

Ivermectin levels which may be lethal to immature stages may have no effect on adult survival rates, but may affect reproduction potentials instead. Larvae of the scarabaeine *Euoniticellus fulvus* (Goeze) were unable to develop in dung excreted by cattle 1 day after treatment by subcutaneous injection of 200 μg ivermectin per kg, but adult *E. fulvus* fed dung excreted 1 and 10 days after treatment survived. No significant difference was observed in the number of offspring produced by adults fed ivermectin-contaminated dung excreted 10 days after treatment and those fed noncontaminated dung, but a developmental delay was noticed in immature stages reared in the ivermectin-contaminated dung (Lumaret et al. 1993).

When ivermectin levels are not sufficient to kill adults, physiological aberrations may result from ivermectin exposure in addition to effects on

reproduction. Adult female *Copris hispanus* L. fed dung from cattle injected with ivermectin at a rate of 200 μg per kg 3 days earlier survived, but oviposited less frequently than females fed noncontaminated dung. Newly-emerged *C. hispanus* adults fed dung from cattle treated 16 days earlier showed delayed ovarian development and higher mortality rates compared to those fed dung from nontreated cattle. Adult beetles fed dung excreted 1 to 8 days after treatment exhibited abnormal gut contents, subnormal fat accumulation, and delayed ovarian development (Wardhaugh & Rodriguez-Menendez 1988).

As with dung-breeding Diptera, abamectin has effects similar to ivermectin on dung-breeding Coleoptera. Larvae of *O. gazella* are unable to survive in dung excreted by cattle up to 28 days after receiving a subcutaneous injection of 300 μg avermectin per kg, but adult survival rates in abamectin-contaminated dung were normal (Roncalli 1989). Newly-emerged adult *Onthophagus binodis* Thunberg fed dung excreted by cattle 3 to 5 days after treatment by subcutaneous injection of 200 μg abamectin per kg oviposited at significantly lower rates than adults fed noncontaminated dung (Houlding et al. 1991). Another study using *O. binodis* showed that larvae were killed in cattle dung excreted 1 week after treatment by subcutaneous injection of 200 μg abamectin per kg, but larval survival returned to normal levels in dung excreted 8 weeks after treatment (Ridsdill-Smith 1988).

Recently researchers have examined the potential of ivermectin or abamectin increasing the attractiveness of dung so that dung pat fauna are more attracted to avermectin-contaminated dung. An increase in the attractiveness of dung does not

appear to be a certain result of avermectin treatment. One study in Denmark showed that *Aphodius* spp., along with the hydrophilid species *Sphaeridium* and *Cercyon*, were more abundant in noncontaminated dung than in dung excreted by cattle 3, 10, 20, and 30 days after treatment by subcutaneous injection of 200 μg ivermectin per kg (Holter et al. 1993). However, the beetles were equally abundant in both contaminated and noncontaminated dung in two subsequent studies.

Pitfall traps baited with dung excreted by cattle 7, 10, and 17 days after subcutaneous injection with 200 μg ivermectin per kg attracted more beetles (4 scarabaeine spp. and 4 *Aphodius* spp.) than traps baited with dung from nontreated cattle (Lumaret et al. 1993). However, the authors were unable to detect with liquid chromatography the concentration of ivermectin in dung excreted 12 days or more after treatment. Also, no significant difference existed between the number of beetles attracted to traps baited with dung excreted 2, 4, 24, and 31 days after treatment and traps baited with noncontaminated dung. These researchers concluded that the presence of ivermectin itself was not responsible for the increased attractiveness observed in the dung excreted 7, 10, and 17 days after treatment.

In Australia, dung excreted by cattle within 3 days of treatment by subcutaneous injection of 200 μg abamectin per kg attracted significantly more *Onthophagus australis* Guerin-Ménéville adults than noncontaminated dung (Wardhaugh & Mahon 1991). Dung excreted 25 days after treatment was continued to be more attractive to adults, but no significant difference in numbers of beetles was noticed between dung excreted 35 days after treatment and noncontaminated dung.

However, *Onthophagus pexatus* Harold adults were more abundant in avermectin-contaminated dung only when traps were baited with dung excreted 25 days after treatment.

The same study found that *O. australis* was more numerous in dung excreted by sheep 1 day after drenching with 200 μg ivermectin per kg than noncontaminated dung, but no significant differences were observed in dung collected after the first day. The researchers speculated that the increased attractiveness of the cattle and sheep dung was not a direct result of the presence of avermectins, but rather that avermectin metabolites or an alteration in the intestinal flora of the cattle gut by the avermectins may be responsible instead.

The coleopteran subfamily Aphodiinae (family Scarabaeidae), also known as the aphodiine dung beetles, are common beneficial inhabitants of cattle manure. In the temperate northern areas of the world, aphodiine dung beetles have been reported as the dominant dung beetles in the numbers of species and individuals found (Balthasar 1964). A single cattle dung pat in Illinois contained more than 1,000 specimens of *Aphodius distinctus* Mueller (Mohr 1943). In Denmark, over 3,000 specimens of *Aphodius contaminatus* (Hbst.) were found in one cattle dung pat (Holter 1982).

Members of the coleopteran family Scarabaeidae possess a diverse range of physical characteristics. Like all scarabs, aphodiine dung beetles have five-segmented tarsi and lamellate antennae whose plates can be expanded or contracted into a club. Aphodiine beetles are small to moderate-sized, generally 2 to 10 mm in length.

Unlike the robust and stout-bodied Scarabaeinae and Geotrupinae, the Aphodiinae are usually elongate and somewhat cylindrical (Fig. 1). Their hind legs are located far back on the body, and are much closer to the tip of the abdomen than the middle legs. Members of the genus *Aphodius* are characterized by pronounced transverse carinae on the meso- and metatibiae, and to a lesser extent, deflexed heads (Dillon & Dillon (1972). Members of the genus *Ataenius* lack deflexed heads, and have only faint transverse carinae on the meso- and metatibiae if any carinae are present (White 1983).

The Aphodiinae is a large and diverse subfamily with varied ecologies. Despite the common name of dung beetles, some species feed primarily on decaying vegetation or plant roots. Immature *Ataenius spretulus* (Haldeman), a species often found in cattle dung, was reported as a turf pest in many states during the late 1970's (Davidson & Lyon 1987), and may be especially damaging to golf courses (Niemczyk & Dunbar 1976, Borror et al. 1989). Other aphodiine species are found in association with ants, termites, or the burrows of pocket gophers and gopher tortoises. Of the species which are found in dung, some species are found only in the dung of certain animals while others visit the dung of several animals. Some species are coprophagous while others are merely coprophilous. At least one species, *Aphodius rufipes* (L.), is kleptoparasitic on dung brood balls made by a scarabaeine dung beetle (Klemperer 1980).

Aphodius and *Ataenius* are the two largest genera in the Aphodiinae subfamily (Woodruff 1973). Approximately 210 species of *Aphodius* (Gordon 1983) and 63

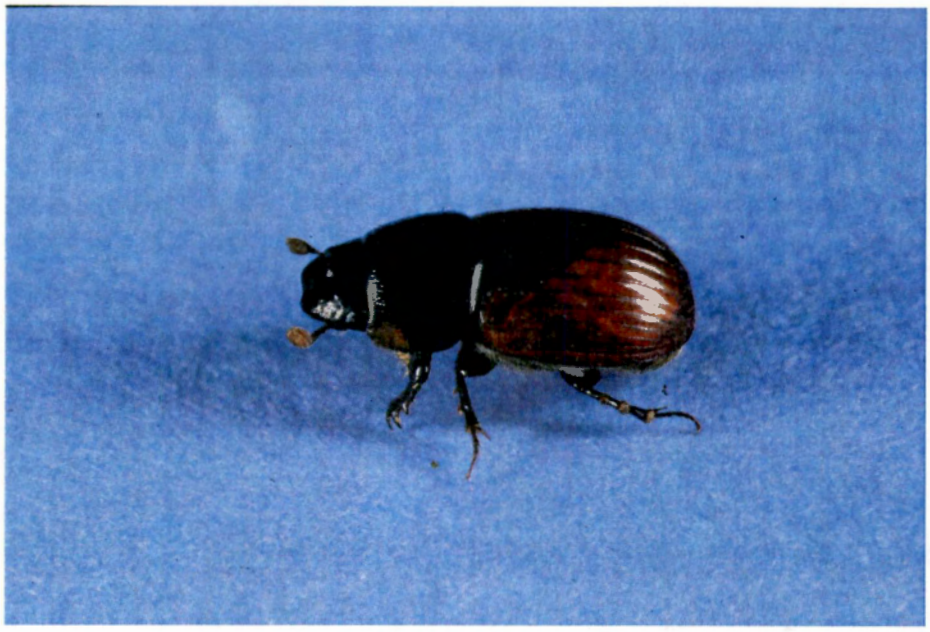

Fig. 1. *Aphodius fimetarius*, a common aphodiine species found in cattle dung.

species of *Ataenius* (Cartwright 1974) have been found in North America. Thirty-four species of *Aphodius* and 12 species of *Ataenius* have been found associated with cattle dung north of Mexico (Blume 1985). However, the classification of such a large subfamily has not yet been clearly established, and new species may yet be recognized, or recognized species may be moved from genus to genus within the subfamily. Several taxonomists have attempted to rectify this problem. Cartwright (1974) produced a taxonomic guide to the genera *Ataenius*, *Aphotaenius*, and *Pseudataenius* north of Mexico, and Gordon (1983) has studied the genus *Aphodius* in the United States and Canada.

Other contributions to the literature include Woodruff (1973), who surveyed the subfamily Aphodiinae in his study on the scarab beetles of Florida. On the west coast, Hatch (1972) catalogued *Aphodius* species in his work on Pacific Northwest Coleoptera. Several studies have been conducted on the ecologies of European members of the subfamily Aphodiinae (Landin 1961, 1968).

Many coprophagous aphodiine beetles are introduced species from Europe. It has been suggested that many of these beetles may have been transported in materials used as ship ballast, or in the dung found on ships carrying livestock or slaves (Woodruff 1973). Most of the introduced species are now found throughout North America.

Ivermectin treatment of cattle may pose a threat to populations of beneficial dung pat fauna. For this reason, the potential impact of ivermectin on adult aphodiine beetle populations in an eastern Tennessee cattle pasture was investigated in a two-

year study. Aphodiine dung beetles were chosen as the subject of this study because of the numerous species found in cattle manure and their frequently dense populations. The objectives of this research were to: 1) determine the species composition of adult aphodiine dung beetles collected in samples of cattle dung pats deposited by ivermectin-treated and nontreated cattle, 2) monitor the seasonal abundances of those species, and 3) evaluate the impact of ivermectin treatment on adult aphodiine populations.

CHAPTER II

SPECIES COMPOSITION AND SEASONAL ABUNDANCE OF APHODIINE DUNG BEETLES IN DUNG FROM IVERMECTIN-TREATED AND NONTREATED CATTLE

i. INTRODUCTION

Numerous species of insects live in cattle manure (Mohr 1943, Cervenka & Moon 1991), and their activity contributes to the degradation of the dung and the subsequent recycling of its nutrients (Anderson et al. 1984). While a few economically important pests of cattle, such as the horn fly, *Haematobia irritans* (L.), and the face fly, *Musca autumnalis* De Geer, breed in cattle dung, the vast majority of dung pat fauna are considered to be beneficial.

The coleopteran subfamily Aphodiinae, the aphodiine dung beetles, are common inhabitants of cattle manure (Cartwright 1974, Gordon 1983, Blume 1985). Numerous aphodiine species burrow into cattle dung to feed and reproduce. Thirty-four species of *Aphodius* and 12 species of *Ataenius*, the two largest aphodiine genera (Woodruff 1973), are associated with cattle manure in North America (Blume 1985).

Ivermectin treatment of cattle may pose a threat to populations of beneficial dung pat fauna, such as aphodiine dung beetles. The systemic antiparasitic drug ivermectin is effective against a number of arthropod parasites and gastrointestinal

nematodes in cattle. Regardless of the administration method used, treated animals metabolize little ivermectin, and most of it is excreted in the feces in concentrations decreasing daily after treatment until eliminated from the animal (Campbell et al. 1983). Ivermectin excreted in dung continues to possesses insecticidal properties, and researchers have questioned the adverse environmental impact of such a drug on dung pat fauna (Strong 1992). There is concern that ivermectin may have a deleterious effect on beneficial dung pat fauna, thus disrupting the successional nature of the dung community (Wall & Strong 1987, Wardhaugh & Mahon 1991).

The potential impact of ivermectin on adult aphodiine beetle populations in eastern Tennessee cattle pastures was investigated in 1993 and 1994. Research objectives were to: 1) determine the species composition of adult aphodiine dung beetles collected in samples of dung pats deposited by ivermectin-treated and nontreated cattle, 2) monitor the seasonal abundances of those species, and 3) evaluate the impact of ivermectin treatment on the aphodiine populations.

ii. Materials and Methods

Study Site and Treatment.

This study was conducted at Holston Farm, Knoxville Experiment Station, Knox Co., Tennessee, from April to September in 1993, and from April to August in 1994. Each year, two mixed-breed, predominantly Angus and Hereford, cow-calf herds were kept on adjacent pastures. Both herds were maintained in the same manner each year. In 1993, the treatment herd consisted of 23 cow-calf pairs, and the control herd consisted of 22 cow-calf pairs, while in 1994 the treatment herd

consisted of 22 cow-calf pairs, and the control herd consisted of 25 cow-calf pairs. The 1993 herds consisted of different animals than the 1994 herds. Adult cows ranged in age between 2 and 14 years. Dairy heifers were kept on surrounding fields during the duration of the experiment.

Each year, the treatment herd was first kept on a 14.18 ha pasture until low forage availability in mid-summer necessitated moving the cattle to a nearby 4.05 ha pasture. The control herd began the season on a 8.51 ha pasture until they were moved to a 4.86 ha pasture adjacent to the treatment herd. Both the treatment and control herds were moved to the smaller pastures at the same time. Forage on all four pastures consisted of red clover mixed with fescue or orchard grass. In 1994, the treatment herd was the subject of another study to examine pasture forage quality, and only 11 of the 22 cow-calf pairs were moved to the smaller pasture in mid-season. All of the cow-calf pairs in the control herd were moved to the second pasture.

Adult cows in the treatment herd were dosed with a pour-on ivermectin formulation (Ivomec® Pour-on for Cattle , MSD AGVET, New Jersey, USA) according to the label-recommended dosage of 500 μg per kg of body weight while the control herd received no treatment. Calves from either herds received no treatment. Treatments were made on 8 April, 20 May, 29 June, and 9 August 1993, and on 13 April, 25 May, and 18 July 1994.

Data Collection.

In 1993, dung pat samples were collected weekly for 5 weeks beginning about one week after treatment on 8 April and 20 May, beginning the day after treatment on 29 June, and beginning 2 days after treatment on 9 August 1993. In 1994, the sampling schedule was modified to include pretreatment samples. Pretreatment samples were randomly collected on the day of, but prior to, treatment of the cattle, except for 18 July, when pretreatment samples were collected 2 days before treatment. Post-treatment samples were collected weekly for 4 weeks beginning 5 to 8 days after treatment.

On each collection date, ten dung pats (2 to 5 days old) lacking thick surface crusts were randomly sampled in each pasture. Each sample, weighing between 250-300 g wet weight and measuring about 13 cm in diameter, was cut away from the edge of the pat so that the sample included both crust and interior of the pat. Samples were placed in individual 4.7 liter plastic pails with two large ventilation windows of fine-mesh fabric on the sides. About 1 cm of fine dry sand covered the bottom of each pail. Pails were covered with plastic lids, also having mesh-covered ventilation windows, and transported to an insect-rearing facility located at the Plant Sciences Farm, ca. 4.3 km from Holston Farm.

Pails containing dung samples were later converted into emergence chambers (Fig. 2), similar to those described by Merritt & Poorbaugh (1975). Modifications included the ventilation windows and the funnel traps described below. These modifications allowed the most effective use of available space at the insectary, and

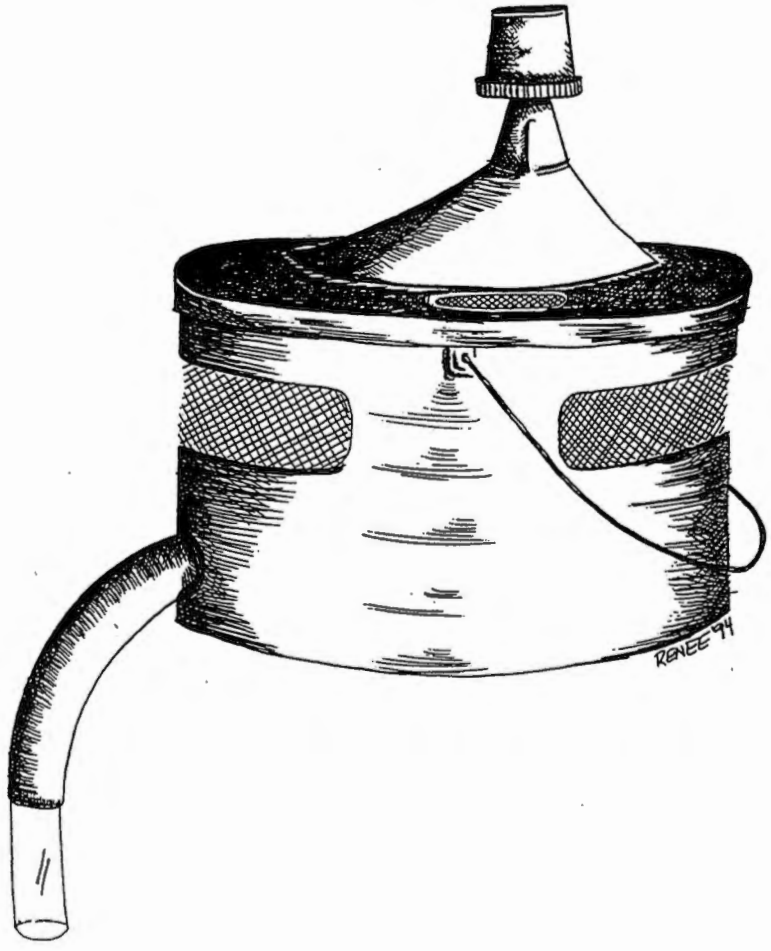

Fig. 2. Emergence chamber used to hold dung pat samples at the insect-rearing facility.

avoided problems of excessive fungal growth on the samples due to high moisture content.

While transporting samples in the field, a 3 cm diameter hole about 2 cm from the bottom of the pail was plugged with cotton batting. A 20 cm length of plastic tubing with a 1.9 cm inside diameter was inserted into the hole after the chambers were taken to the insect-rearing facility. A glass vial (23 X 85 mm) partially filled with about 20 ml of 70% EtOH was inserted into the free end of the tubing and secured with electrical tape. These alcohol traps collected any insects which entered the tubing.

The screened lids were left on the emergence chambers for 1 week to allow excess moisture to evaporate and were then replaced by funnel-trap lids. Funnel-trap lids consisted of another plastic lid with a 16 cm long and 11 cm wide opening covered by a 0.47 liter plastic funnel (1 pint; 16 cm long and 11.5 cm wide at base; 20 cm tall with a 1 cm diameter tip). A 3 cm hole was cut into the lid of a plastic hospital-use specimen cup (120 ml), and this lid was then fitted over the funnel tip and secured with silicone caulk. The specimen cup could then be screwed onto its lid upside down. Funnel-trap lids collected insects flying through the funnel and into the specimen cup.

Emergence chambers at the rearing facility were held on a screened porch protected from direct sunlight, but subject to ambient temperature and humidity. Chambers remained undisturbed for 5 weeks before the samples and sand were hand-searched for any aphodiine beetles not collected by the alcohol or funnel traps.

Alcohol and funnel traps were also removed, and numbers of any trapped aphodiine beetles were recorded.

Data on aphodiine species and numbers were log-transformed (log(n+1)) and analyzed with a repeated measures general linear model (GLM) (SAS Institute 1989) at $P < 0.1$. Treatment, densities in treatment periods of each year, and sampling intervals (meaning the week in which samples were collected prior to or after treatment) were considered main effects. Significantly different treatment means were analyzed by Sidak *t* Test (SAS Institute 1989). Differences were considered significant at $P < 0.1$. The combined influence of treatment and sampling date was also analyzed by least significant difference (LSD).

Because aphodiine beetles vary in seasonality and the number of generations produced each year, species were not compared to each other. Also, data from 1993 were not statistically compared to those of 1994. The data for the three most abundant species in each year were combined and statistically analyzed together to evaluate if treatment had an effect on a group of several aphodiine species.

iii. RESULTS AND DISCUSSION

Species Composition of Aphodiine Beetles.

1993. Four hundred dung pats were sampled in 1993, yielding more than 8,200 aphodiine beetles (Table 1). Seven aphodiine species in the two genera *Aphodius* and *Ataenius* were reared from both ivermectin-contaminated and noncontaminated dung. Almost twice as many aphodiine beetles were collected in samples from ivermectin-contaminated dung pats as compared to noncontaminated

Table 1. Numbers and percentages of aphodiine species collected in ivermectin-contaminated and noncontaminated dung in 1993.

Species	Author	Ivermectin-contaminated dung	Non-contaminated dung[2]	Total[2]
Aphodius haemorrhoidalis	(L.)	4782 (87.50%)	2077 (74.63%)	6859 (83.16%)
Aphodius lividus	Olivier	223 (4.08%)	97 (3.49%)	320 (3.88%)
Ataenius platensis	(Blanchard)	239 (4.37%)[1]	358 (12.86%)[1]	597 (7.24%)[1]
Ataenius spretulus	(Haldeman)	---	---	---
Aphodius fimetarius	(L.)	65 (1.19%)	40 (1.44%)	105 (1.27%)
Aphodius stercorosus	Melsheimer	91 (1.67%)	129 (4.64%)	220 (2.67%)
Aphodius erraticus	(L.)	65 (1.19%)	82 (2.95%)	147 (1.78%)
	Totals	5465	2783	8248

[1]*Ataenius platensis* and *Ataenius spretulus* treated as a group in 1993; numbers represent both species added together in 1993.
[2]Does not equal 100% due to rounding off.

dung pats. The most abundant species were *Aphodius haemorrhoidalis*, *Ataenius platensis* and *A. spretulus* (added together), and *Aphodius lividus*.

The two *Ataenius* species, *spretulus* and *platensis*, were not recognized as separate species until late 1993. The two species are morphologically similar (Woodruff 1973), and often difficult to distinguish from each other. For the purposes of this study, these species were combined and analyzed together in 1993.

1994. Three hundred dung pats were sampled in 1994, yielding around 3,000 aphodiine dung beetles (Table 2). Like 1993, twice as many aphodiine beetles were collected in samples from ivermectin-contaminated dung pats as compared to noncontaminated dung pats. The same seven aphodiine species found during 1993 were also found in 1994, again in both ivermectin-contaminated and noncontaminated dung. The three most abundant species were *Aphodius haemorrhoidalis*, *Aphodius erraticus*, and *Aphodius fimetarius*.

Seasonal Distribution of Aphodiine Species.

Eastern Tennessee experienced severe drought conditions during portions of the summer of 1993 (J. Logan, pers. comm.). The seasonal distributions of the aphodiine beetles collected that year may have been affected by the lower than normal amounts of rain, particularly during the month of June (Fig. 3). However, August and September in 1993 had higher than normal amounts of rain. In 1994, eastern Tennessee experienced higher than normal amounts of rain throughout most of the sampling season, which also may have affected aphodiine seasonal distributions.

Table 2. Numbers and percentages of aphodiine species collected in ivermectin-contaminated and noncontaminated dung in 1994.

Species	Author	Ivermectin-contaminated dung[1]	Non-contaminated dung	Total[1]
Aphodius haemorrhoidalis	(L.)	1430 (68.72%)	563 (56.87%)	1993 (64.90%)
Aphodius lividus	Olivier	42 (2.02%)	41 (4.14%)	83 (2.70%)
Ataenius platensis	(Blanchard)	1 (0.05%)	1 (0.10%)	2 (0.07%)
Ataenius spretulus	(Haldeman)	15 (0.72%)	27 (2.73%)	42 (1.37%)
Aphodius fimetarius	(L.)	138 (6.63%)	44 (4.44%)	182 (5.93%)
Aphodius stercorosus	Melsheimer	136 (6.54%)	38 (3.84%)	174 (5.67%)
Aphodius erraticus	(L.)	319 (15.33%)	276 (27.88%)	595 (19.37%)
	Totals	2081	990	3071

[1]Does not equal 100% due to rounding off.

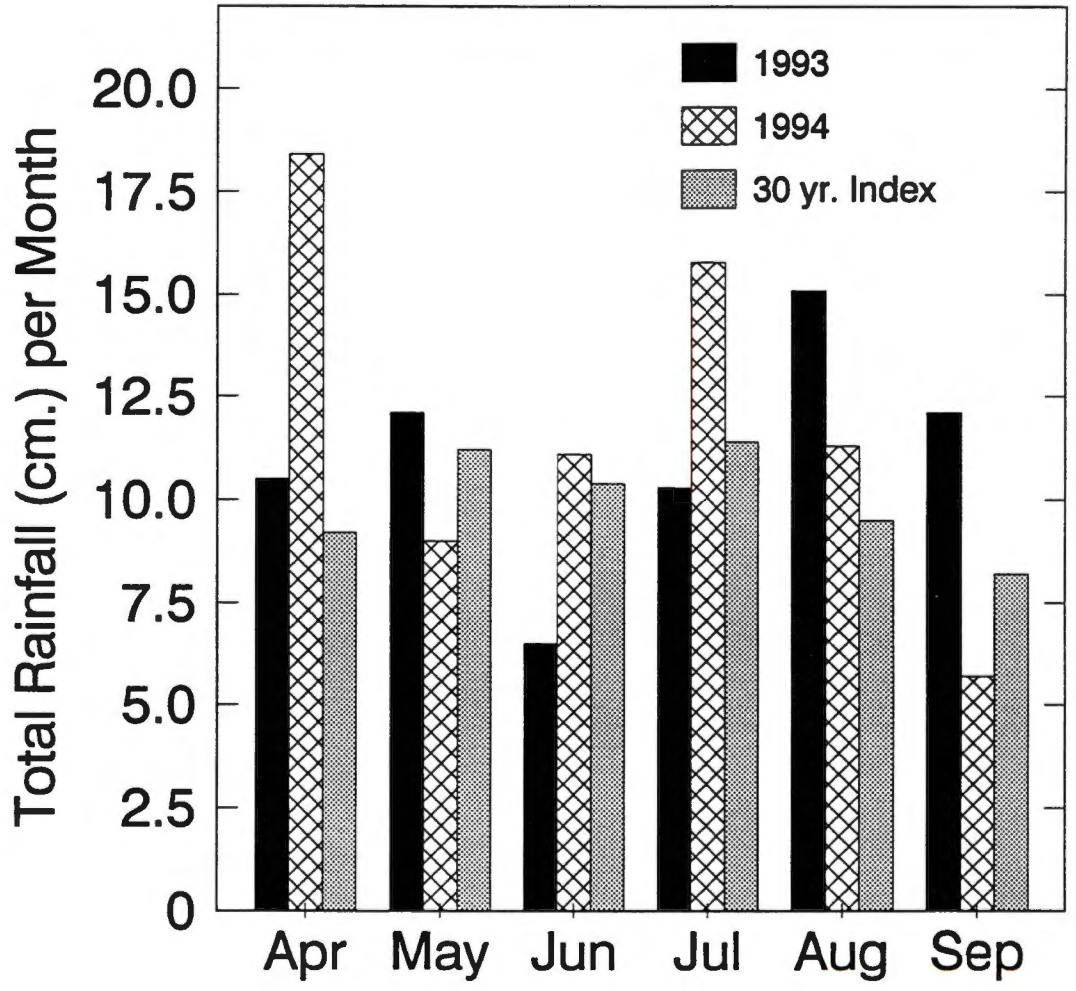

Fig. 3. Total rainfall (cm) per month in 1993 and 1994, recorded at Knoxville Experiment Station, Knox Co., TN. 30-year index represents average rainfall from 1960 to 1990, also recorded at Knoxville Experiment Station.

Discussions of seasonal distributions of each species are based on the population trends in noncontaminated dung only. However, comparisons are made between numbers of specimens collected in both ivermectin-contaminated and noncontaminated dung.

Aphodius haemorrhoidalis

Aphodius haemorrhoidalis, the most abundant species collected for both years, accounted for 83.16% (6,859 specimens) and 64.90% (1,993) of the total number of beetles collected in 1993 (Table 1) and 1994 (Table 2), respectively. More specimens of *A. haemorrhoidalis* were collected in ivermectin-contaminated than noncontaminated dung both years of the study.

An introduced species from Europe, *A. haemorrhoidalis* is well established throughout North America in cattle dung found in open pastures (Gordon 1983), occurring from California north to British Columbia and throughout the mid-western and eastern states (Blume 1985). Both larvae and adults are found in cattle manure (Ritcher 1966). *A. haemorrhoidalis* has been recorded as the predominant aphodiine species found in cattle dung in Indiana (Sanders & Dobson 1966), Minnesota (Cervenka & Moon 1991), western Nebraska (Schreiber et al. 1987), and east central South Dakota (Kessler & Balsbaugh 1972). It was one of the most abundant aphodiines in cattle dung in northeastern Mississippi (MacDonald & Combs 1985) and has been found in cattle dung in Texas (Blume 1970), Illinois (Mohr 1943), and Florida (Woodruff 1973).

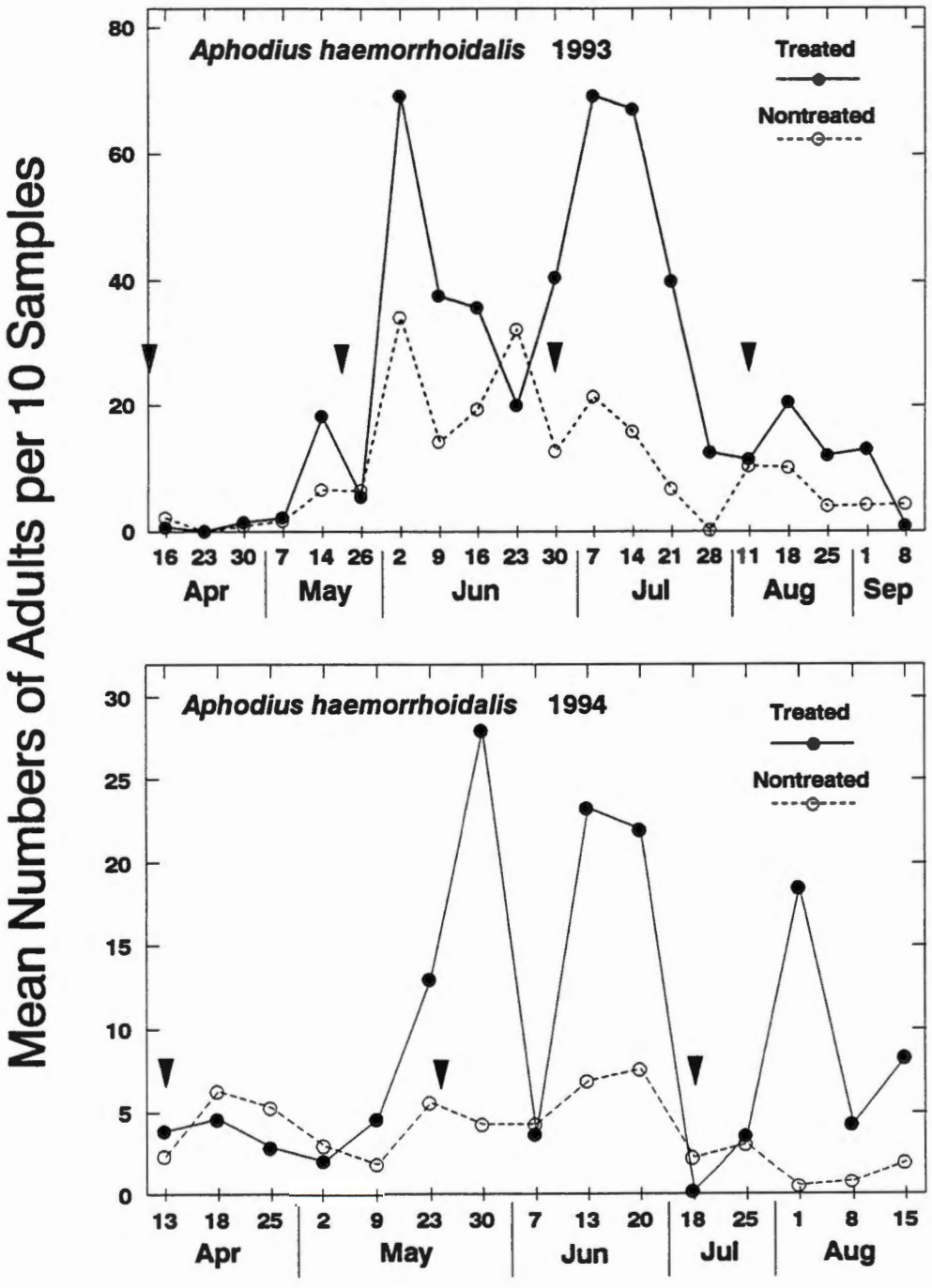

Fig. 4. Mean numbers (n = 10) and seasonal abundance of *Aphodius haemorrhoidalis* collected in dung from ivermectin-treated and nontreated cattle at Holston Farm, 1993 and 1994. Arrows indicate ivermectin treatment.

In 1993, *A. haemorrhoidalis* was present throughout the sampling season with the greatest number of specimens collected in June and July (Fig. 4). Three-hundred and forty-two specimens were collected on June 2, and 323 specimens were collected on June 23. In 1994, it displayed a similar seasonality, present from April through the end of the study in August. Similar seasonalities have been recorded for this species in Missouri (Wingo et al. 1974) and Mississippi (MacDonald & Combs 1985).

Aphodius lividus

Aphodius lividus accounted for 3.88% (320 specimens) and 2.70% (83 specimens) of the total number of beetles collected in 1993 (Table 1) and 1994 (Table 2), respectively. In 1993, a larger percentage of specimens was collected in ivermectin-contaminated dung, but in 1994 a larger percentage was collected in noncontaminated dung.

During 1993, few specimens were collected between April and late August (Fig. 5), with a small peak in numbers in late June and early July. Fewer specimens were collected in 1994 (Table 2), but this species was distributed more evenly throughout the sampling season. The greatest number of specimens was collected in mid-May when 14 specimens were taken in a pretreatment sample on May 23.

In Mississippi, where it was the most abundant aphodiine beetle in cattle dung, *A. lividus* was collected from late April through October and was most abundant in early July (MacDonald & Combs 1985). Another introduced species from Europe, *A. lividus* has a broad range across the southeastern, lower midwestern,

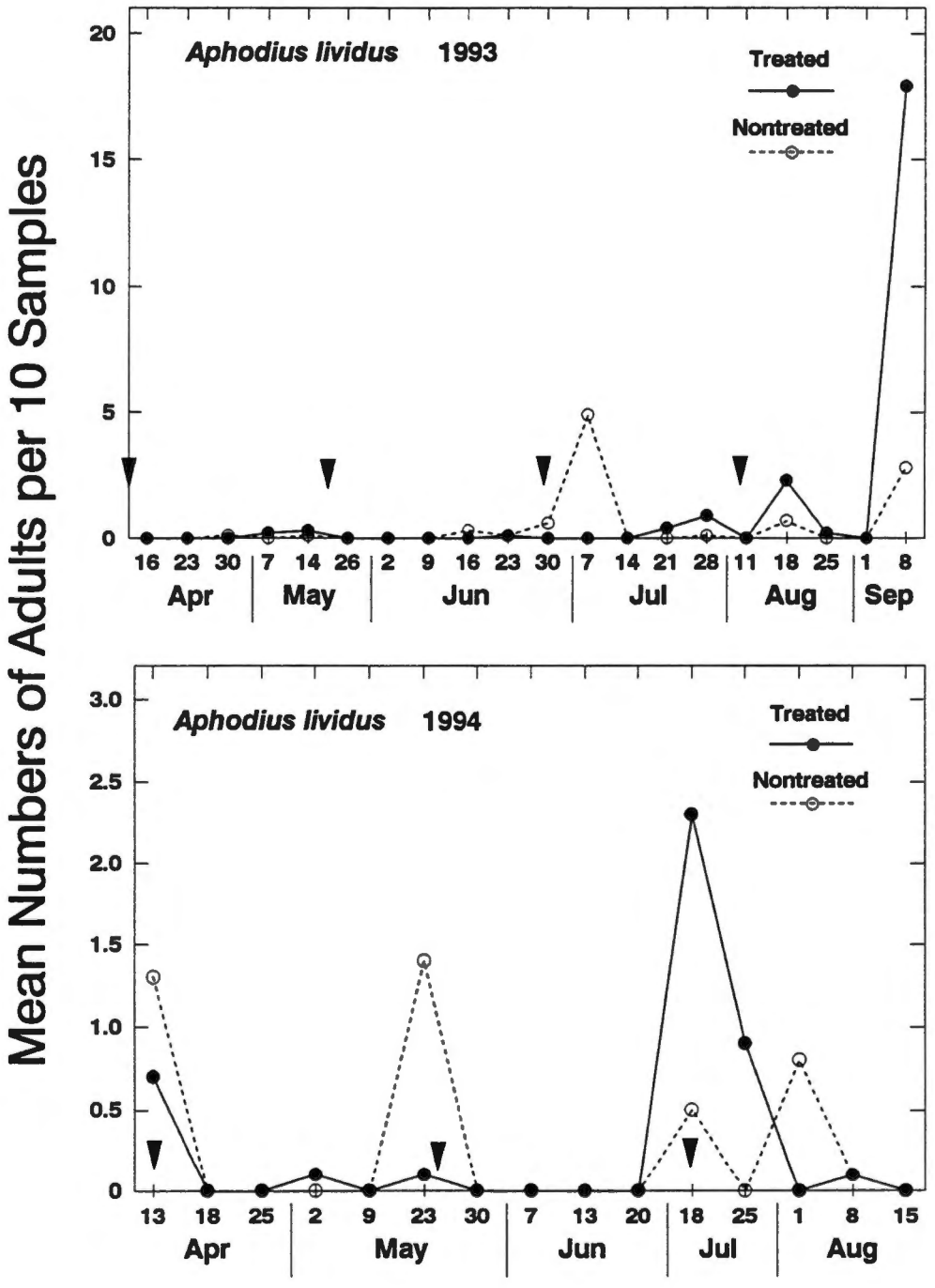

Fig. 5. Mean numbers (n = 10) and seasonal abundance of *Aphodius lividus* collected in dung from ivermectin-treated and nontreated cattle at Holston Farm, 1993 and 1994. Arrows indicate ivermectin treatment.

and southwestern portions of the United States (Woodruff 1973). This species was reported in cattle dung in Texas (Blume 1970) and Florida (Woodruff 1973). Both larvae and adults of *A. lividus* are found in cattle manure (Ritcher 1966).

Ataenius platensis* and *Ataenius spretulus

Data collected on *Ataenius platensis* and *A. spretulus* during 1993 are difficult to interpret as both *A. platensis* and *A. spretulus* may not have been present evenly over the entire season. Only one species may have been responsible for the peaks in collected specimens in early and midJune, late June and early July, and again in late July and early August (Fig. 6). The two species together accounted for 7.24% (597 specimens) of the total number of beetles collected in 1993, and, as a group, were the second most abundant aphodiine beetles recorded that year (Table 1).

In 1994, *A. platensis* and *A. spretulus* were tallied as separate species. *A. platensis* represented 0.07% (only 2 specimens) and *A. spretulus* represented 1.37% (42 specimens) of the total number of beetles collected (Table 2). Clearly, in 1994 the *Ataenius* species were not as abundant as they were in 1993. One possible explanation for their reduction may involve the drought conditions which occurred during June and July in 1993. While *A. spretulus* is often found in cattle manure, it is also frequently found in organic matter with no dung content. This species, commonly known as the black turfgrass beetle, has been reported as a pest of turfgrass and sod, where the grubs feed on grass roots and accumulated thatch (Wegner & Niemczyk 1981). It is possible that during the drought, when the ground

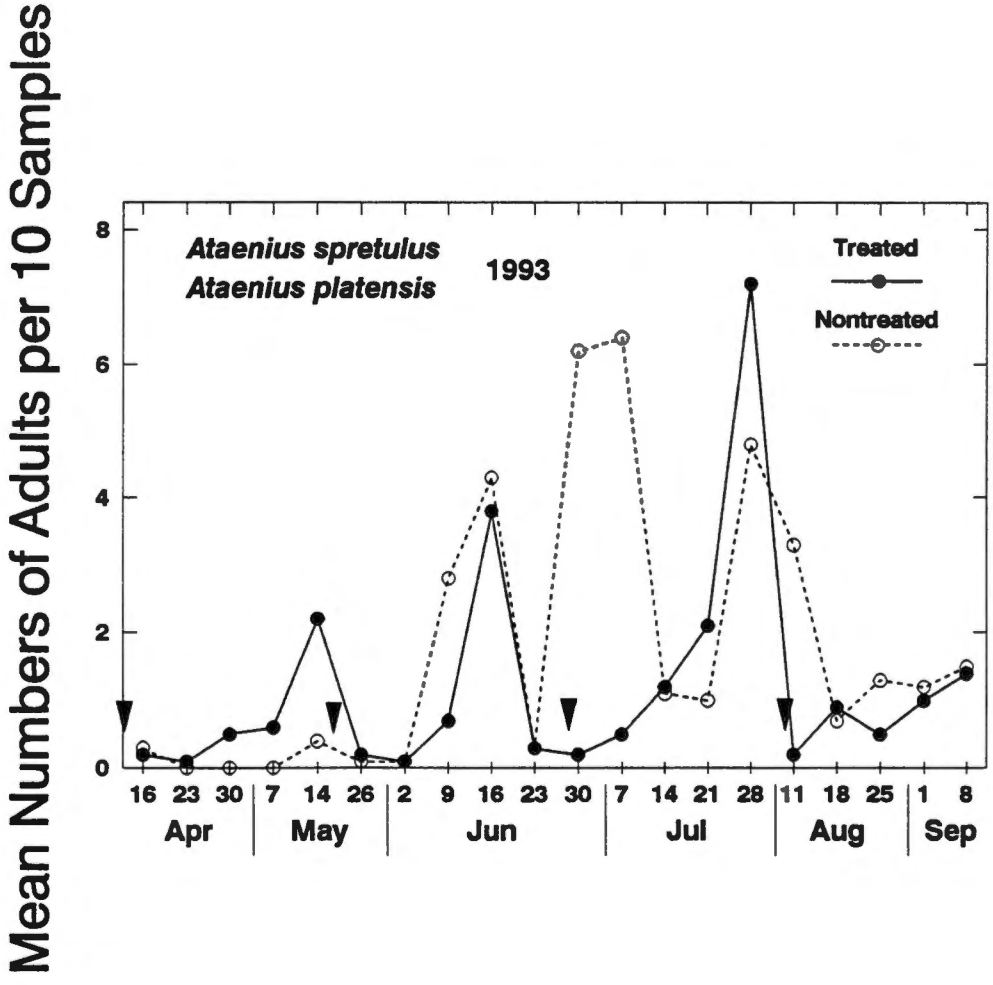

Fig. 6. Mean numbers (n = 10) and seasonal abundance of *Ataenius* species collected in dung from ivermectin-treated and nontreated cattle at Holston Farm, 1993. Arrows indicated ivermectin treatment.

and grasses were dry, *A. spretulus* was attracted to the moisture and organic matter found in cattle dung pats. The summer of 1994 was not as dry, and fewer numbers were collected in cattle manure, possibly due to the increased availability of alternate sources of organic matter other than cattle dung.

During 1994, a few *A. spretulus* specimens were collected in April, but this species was not collected again until late June (Fig. 7). A peak of 14 specimens was collected on August 15, and the numbers appeared to be increasing when the study ended in midAugust.

Only two specimens of *A. platensis* were collected in 1994, one in early June and the second in late July (Fig. 7). No seasonal distribution for this species could be established from these data.

In Ohio and Connecticut, *A. spretulus* is bivoltine (Niemczyk & Dunbar 1976). Adults were found in low numbers from April through June, with the first generation emerging in late June and early July. The second generation of adults emerged in late August and early September, after which the adult population declined to its previous low numbers. This species appears to be univoltine in cattle dung in Missouri, with low numbers of adults collected from early June to August, after which the population increases before declining again in early September (Wingo et al. 1974). In Mississippi, this species was collected in cattle dung from late April through October, and was most abundant in July (MacDonald & Combs 1985).

Ataenius spretulus was reported as the most abundant scarabaeid species collected in cattle dung in Mississippi (MacDonald & Combs 1985). It also has been

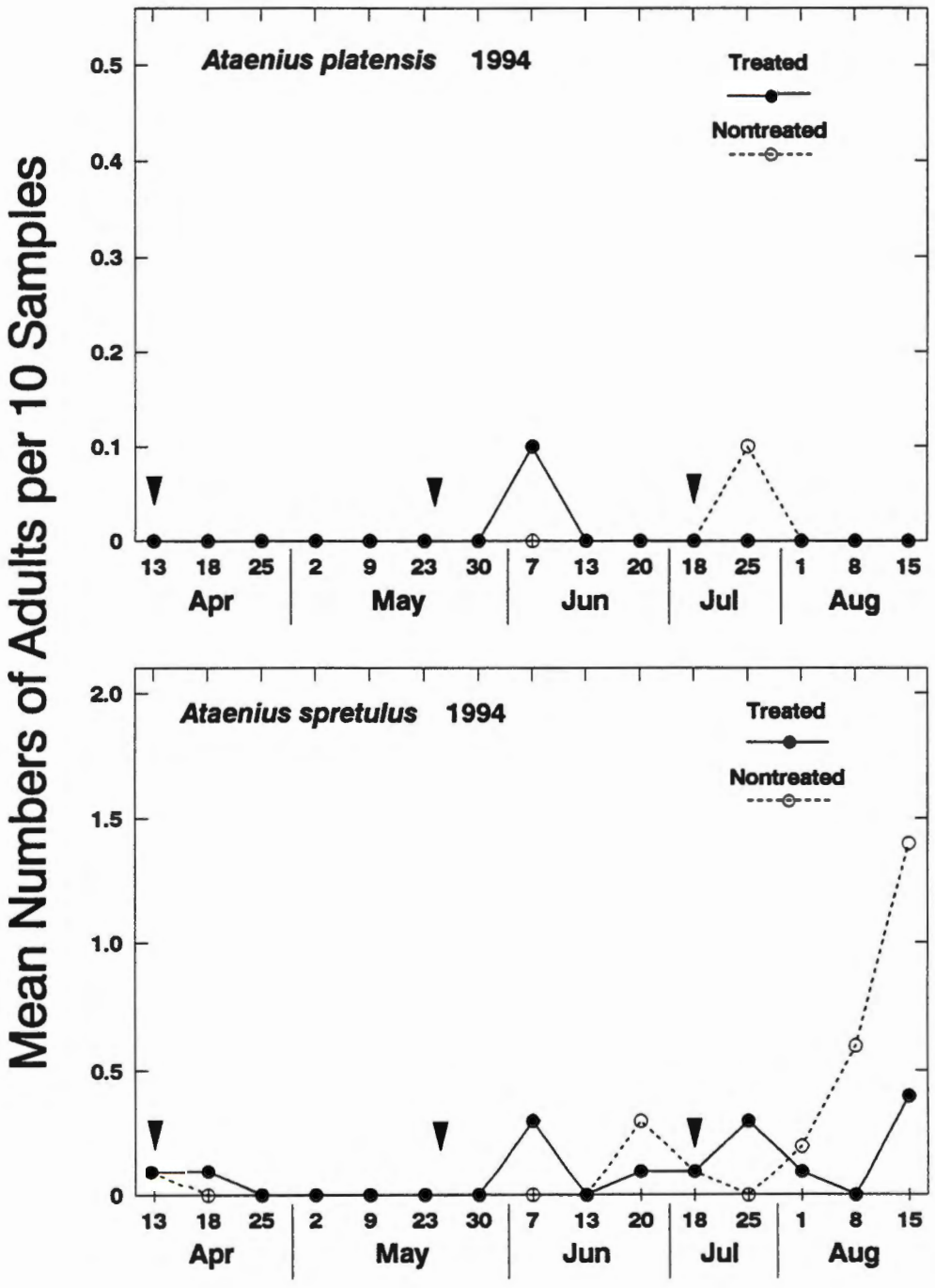

Fig. 7. Mean numbers (n = 10) and seasonal abundance of *Ataenius platensis* and *Ataenius spretulus* collected in dung from ivermectin-treated and nontreated cattle at Holston Farm, 1994. Arrows indicate ivermectin treatment.

reported in cattle dung in Minnesota (Cervenka & Moon 1991), Florida (Woodruff 1973), and east central South Dakota (Kessler & Balsbaugh 1972). Its geographical range includes most midwestern states and states east of the Mississippi (Woodruff 1973).

Ataenius platensis has also been reported in cattle dung in Mississippi (MacDonald & Combs 1985) and Texas (Blume 1970). This species is found throughout the southeastern states (Woodruff 1973). *A. platensis* has also been observed in human and sheep dung, and may be responsible for root damage in some vegetables (Woodruff 1973).

Aphodius fimetarius

Aphodius fimetarius accounted for only 1.27% (105 specimens) and for 5.93% (182 specimens) of the total number of aphodiine specimens collected in 1993 (Table 1) and in 1994 (Table 2), respectively. *A. fimetarius* was the least abundant species collected in 1993, but it was the third most abundant species in 1994. The percentages of specimens collected in both types of dung were similar in 1993, but a larger percentage was collected in ivermectin-contaminated dung in 1994.

In 1993, *A. fimetarius* was most abundant in the early half of the sampling season (Fig. 8). The number of specimens peaked in June, with 18 specimens collected on June 16, but none were collected after July 21. More specimens were collected in 1994 (Table 2). *A. fimetarius* was most abundant in late May, with 17 specimens collected in pretreatment samples on May 30. The last specimens were collected on June 20.

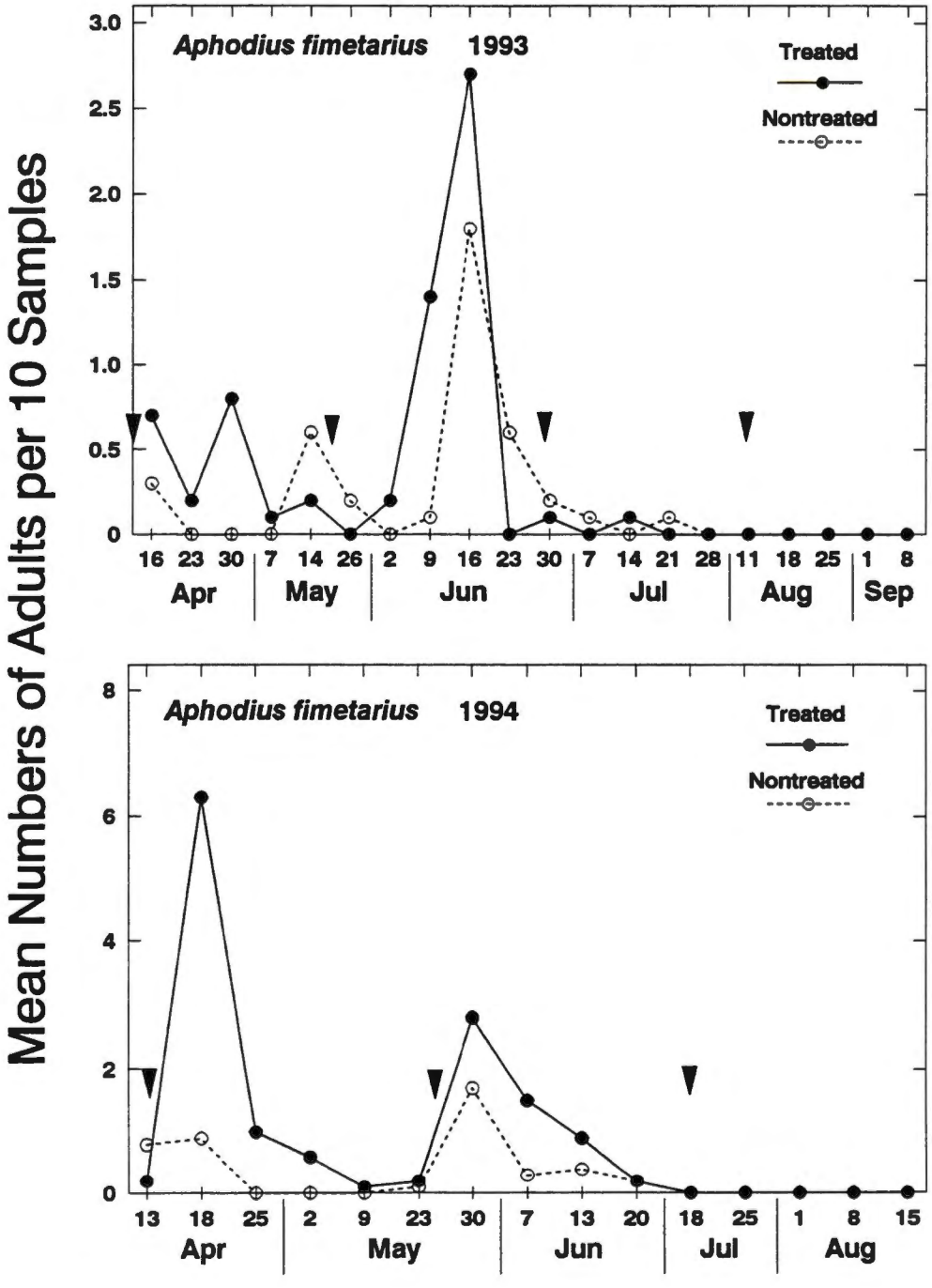

Fig. 8. Mean numbers (n = 10) and seasonal abundance of *Aphodius fimetarius* collected in dung from ivermectin-treated and nontreated cattle at Holston Farm, 1993. Arrows indicate ivermectin treatment.

In Mississippi, *A. fimetarius* has been reported in cattle dung from late April through June, peaking in late May (MacDonald & Combs 1985). No specimens were collected in July and August, but it reappeared in midSeptember and was collected through October. In Missouri, *A. fimetarius* was found in cattle dung during June and early July, after which it was not collected again until September, when the population began to increase (Wingo et al. 1974).

Populations of this species generally fall to extremely low levels during the hottest months of the year (Mohr 1943, Woodruff 1973). This species has been reported as a univoltine species in Illinois, with the adult population increasing in the fall with the annual emergence (Mohr 1943). If this study in Tennessee had continued into October, populations of *A. fimetarius* would have most likely increased in the cooler months.

Another imported species from Europe, *A. fimetarius* is widespread over much of temperate North America, except for the Rocky Mountain states and the southwest (Woodruff 1973). In addition to the previously mentioned states, this species has been reported in cattle dung in Texas, (Blume 1970), Indiana (Sanders & Dobson 1966), east central South Dakota (Kessler & Balsbaugh 1972), and western Nebraska (Schreiber et al. 1987).

Aphodius stercorosus

Aphodius stercorosus accounted for 2.67% (220 specimens) and 5.67% (174 specimens) of the total number of beetles collected in 1993 (Table 1) and 1994 (Table 2), respectively. A larger percentage of specimens was collected in noncontaminated

dung the first year, but in 1994 a larger percentage was collected in ivermectin-contaminated dung.

This species was collected throughout the season in 1993, generally in low numbers (Fig. 9). *A. stercorosus* was most abundant in late June, with 61 specimens collected on June 30, but the population declined to its previous low numbers the following week. In the following year, *A. stercorosus* was again present throughout the sampling season in low numbers. A peak of 19 specimens was collected in pretreatment samples on July 18.

This species has been reported in cattle dung from Florida (Woodruff 1973), northeast Mississippi (MacDonald & Combs 1985), and Minnesota (Cervenka & Moon 1991), east central South Dakota (misspelled "*A. stercorosa*", Kessler & Balsbaugh 1972), and Illinois (Mohr 1943). *A. stercorosus* has also been reported in human and sheep dung (Woodruff 1973).

Native to North America, *A. stercorosus* has a broad geographical range in the east, from southeastern Canada south to Florida, and west to Kansas and Texas, and is typically observed from May through October (Gordon 1983). In Mississippi, this species was collected from late April through October with a peak abundance in late May and early June (MacDonald & Combs 1985). Smaller peaks in abundance were observed in early July, late July, and again in late August.

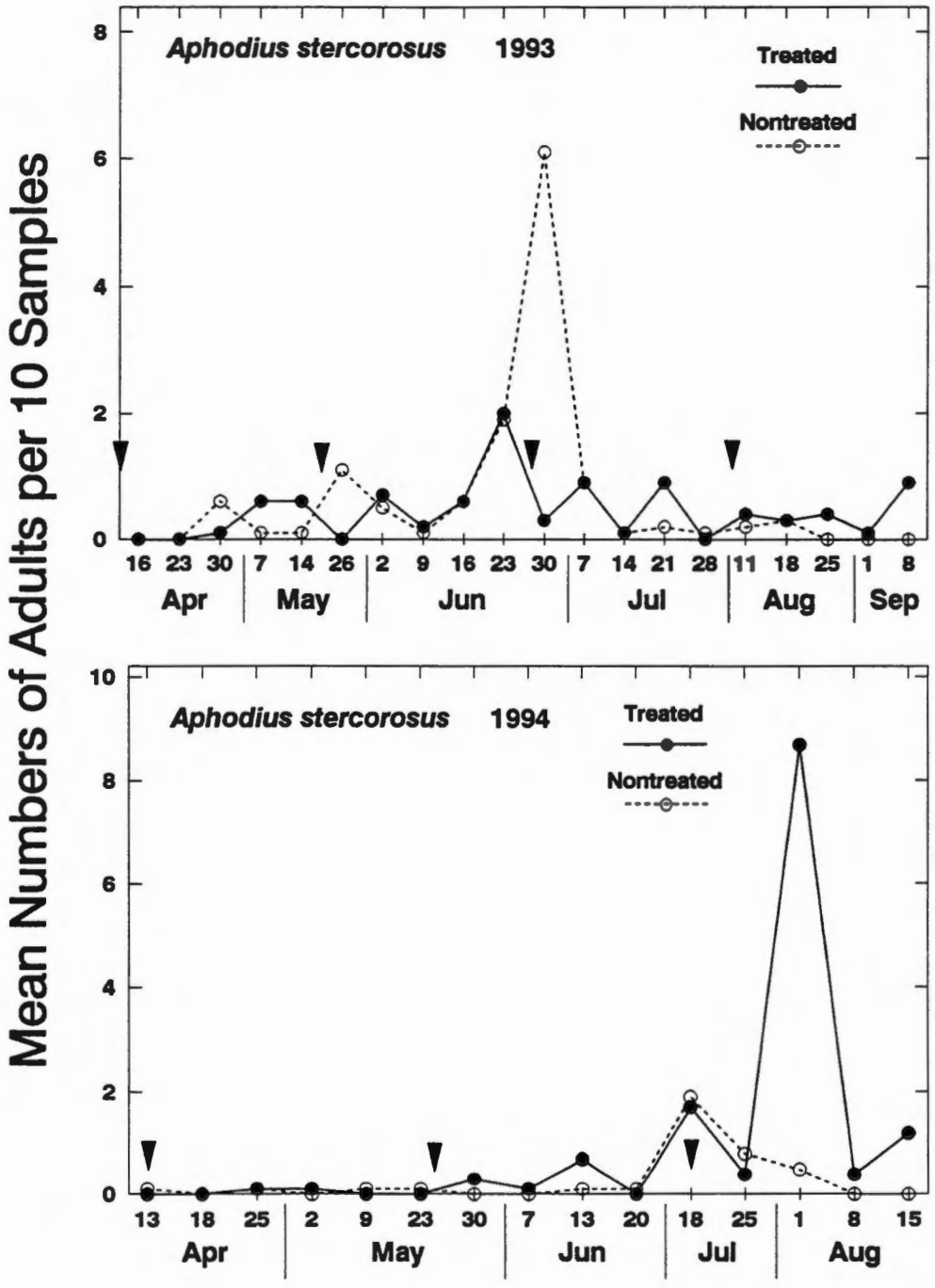

Fig. 9. Mean numbers (n = 10) and seasonal abundance of *Aphodius stercorosus* collected in dung from ivermectin-treated and nontreated cattle at Holston Farm, 1993 and 1994. Arrows indicate ivermectin treatment.

Aphodius erraticus

Aphodius erraticus accounted for 1.78% (147 specimens) and 19.37% (595 specimens) of the total number of beetles collected in 1993 (Table 1) and 1994 (Table 2), respectively. *A. erraticus* was the second most abundant species collected during 1994. A higher percentage of specimens was collected in noncontaminated dung during both years of the study.

In 1993, this species was most abundant early in the sampling season, with peaks in mid- to late April, and again in early June (Fig. 10). Totals of 23 and 25 specimens were collected on April 23 and 30, respectively. After late June, only three specimens were collected during the remainder of the study.

Aphodius erraticus was much more abundant the following year. Similar to 1993, it peaked in late April, and again, to a lesser extent, in late May. A peak of 161 specimens was collected on April 25. In both years of the study, only one specimen was collected after late June.

This species has been reported in cattle dung in Indiana (Sanders & Dobson 1966) and Minnesota (Cervenka & Moon 1991). It occurs in the mid- and eastern United States, and breeds in cattle dung (Ritcher 1966). Like most of the aphodiine species collected in this study, *A. erraticus* is an introduced species from Europe (Gordon 1983).

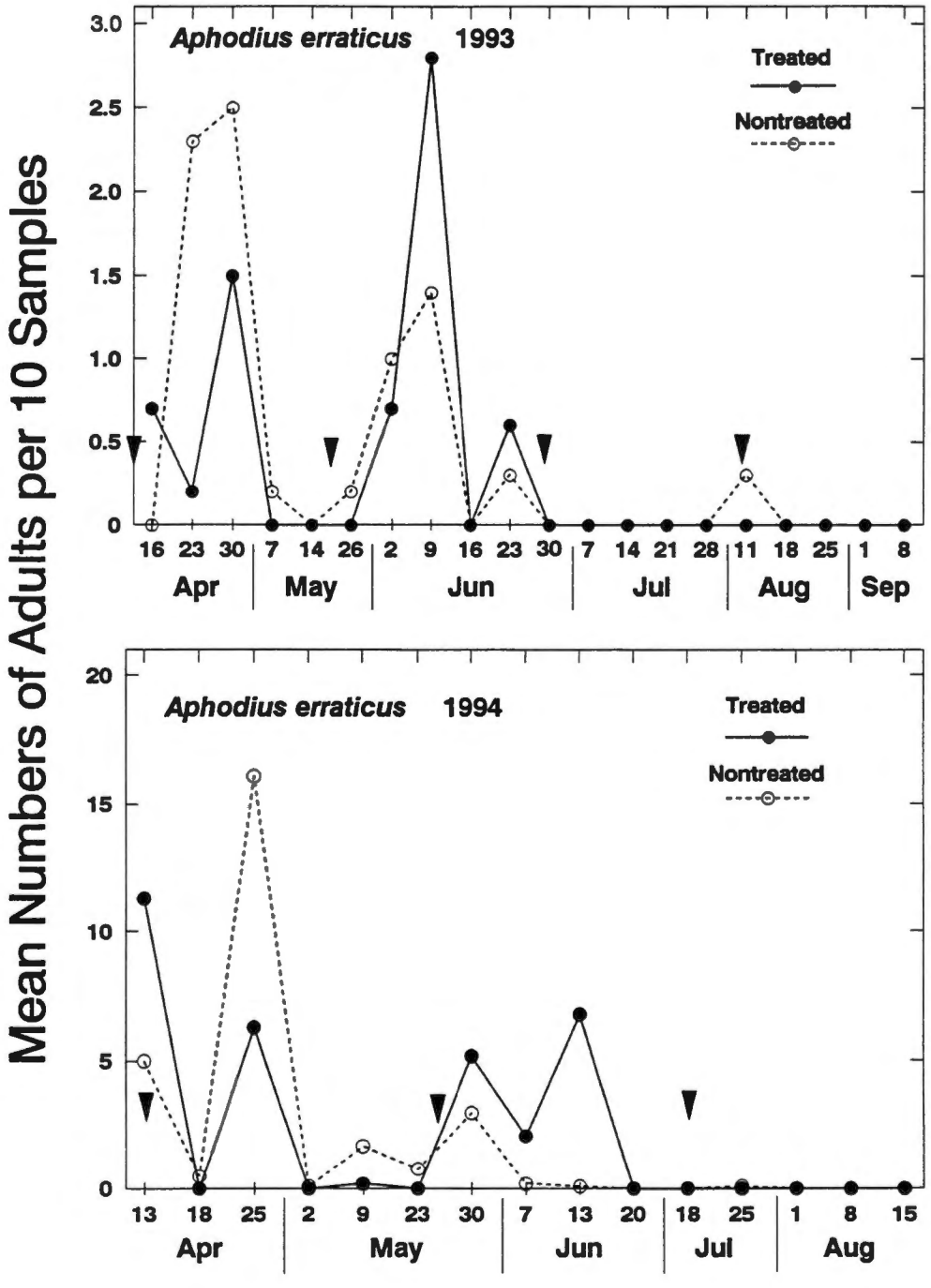

Fig. 10. Mean numbers (n = 10) and seasonal abundance of *Aphodius erraticus* collected in dung from ivermectin-treated and nontreated cattle at Holston Farm, 1993 and 1994. Arrows indicate ivermectin treatment.

Impact of Ivermectin on Aphodiine Species.

Significant differences were detected among densities of all seven aphodiine species in each of the four treatment periods in 1993, and in each of the three treatment periods in 1994. All seven species were more abundant at certain times than others throughout the sampling season in both years. These differences were expected because the seasonal abundance of a species fluctuates according to its seasonality and the number of generations it produces each year.

Aphodius haemorrhoidalis

1993. Ivermectin treatment did not affect the numbers of collected adult *A. haemorrhoidalis*. The total numbers of this species collected in ivermectin-contaminated and noncontaminated dung were not significantly different, even though twice as many specimens were collected in ivermectin-contaminated dung (Table 1). In addition, the week in which samples were collected after treatment did not affect the numbers of *A. haemorrhoidalis*. No significant differences in densities of this species were observed among the 5 weeks post-treatment of any treatment period in 1993.

Analysis by LSD shows that the influence of treatment and sampling date together affected the abundance of this species (Table 3). Densities of *A. haemorrhoidalis* were not significantly different between ivermectin-contaminated dung 1 and 5 weeks after treatment, or between densities collected in noncontaminated dung 1 and 5 weeks after treatment. However, significantly greater

Table 3. Influence of treatment and sampling interval on densities of *Aphodius haemorrhoidalis* in 1993.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	1	14.55 ± 26.52 bc
I	2	39.63 ± 52.86 d
I	3	29.58 ± 43.40 d
I	4	22.75 ± 40.05 d
I	5	13.08 ± 26.41 ab
N	1	7.93 ± 11.05 ab
N	2	16.43 ± 23.17 c
N	3	8.73 ± 17.65 a
N	4	8.00 ± 18.37 a
N	5	10.85 ± 34.96 a

[1]I = ivermectin treatment, N = no treatment.
[2]Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.306, $P < 0.1$, n = 40); values represent nontransformed data; statistical analysis was performed on log-transformed data.

numbers were collected in ivermectin-contaminated dung 2, 3, and 4 weeks after treatment.

1994. Ivermectin treatment did not affect the numbers of adult *A. haemorrhoidalis* during 1994. The total number of this species in ivermectin-contaminated dung was again twice as large as the number collected in noncontaminated dung (Table 2), but the totals were not significantly different. Neither did the week in which samples were collected prior to or after treatment have an affect on the numbers of collected specimens in 1994. *A. haemorrhoidalis* was equally abundant among the 5 weeks post-treatment of any treatment period.

Similar numbers of this species were collected in pretreatment samples from both ivermectin-contaminated and noncontaminated dung (Table 4). However, LSD analysis shows the densities of *A. haemorrhoidalis* changed after ivermectin-treatment. Abundance of this species increased in ivermectin-contaminated dung. Significantly more specimens were collected in ivermectin-contaminated than noncontaminated dung 2, 3, and 4 weeks after treatment.

Based on these results and the 1993 results, *A. haemorrhoidalis* appears to be more attracted to ivermectin-contaminated than noncontaminated dung. This increased attraction begins about 2 weeks after topical treatment of cattle, and lasts about 3 weeks. Possible implications of this trend and the role of ivermectin in the increased attractiveness of dung will be discussed in Chapter 3.

Table 4. Influence of treatment and sampling interval on densities of *Aphodius haemorrhoidalis* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	P	5.70 ± 11.53 ab
I	1	12.07 ± 32.13 bc
I	2	8.37 ± 13.97 cd
I	3	9.90 ± 18.61 c
I	4	11.63 ± 20.69 d
N	P	3.43 ± 5.22 ab
N	1	4.57 ± 5.89 ab
N	2	3.33 ± 4.33 a
N	3	3.57 ± 7.26 a
N	4	3.83 ± 6.14 a

[1]I = ivermectin treatment, N = no treatment.
[2]P = pretreatment sample; Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.303, $P < 0.1$, n = 30); values represent nontransformed data; statistical analysis was performed on log-transformed data.

Aphodius lividus

1993. The numbers of *A. lividus* collected at the study site were not affected by ivermectin treatment. Although twice as many specimens were collected in ivermectin-contaminated than noncontaminated dung (Table 1), the difference was not significant.

The week in which samples were collected post-treatment did have a significant effect on the densities of *A. lividus* (Table 5). Regardless of treatment, this species was more abundant during certain weeks than others within each 5 week post-treatment period. However, the Sidak *t* Test used to analyze these means failed to indicate significant differences among them at $P < 0.1$.

Analysis by LSD indicates there were significant differences in densities of *A. lividus* when the combined influence of treatment and sampling date was examined (Table 6). However, these differences did not appear to constitute a clear trend for this species. In fact, 7 of the 10 weeks were not significantly different from each other, regardless of treatment. Significantly more specimens were collected in noncontaminated than ivermectin-contaminated dung the second week after treatment, but a greater number was collected in ivermectin-contaminated than noncontaminated dung 5 weeks after treatment. Random fluctuations in the population or in sampling may have caused these differences rather than increased attraction in either dung type.

1994. Ivermectin treatment did not alter the numbers of *A. lividus* collected at the study site during 1994. This result was not surprising as almost the exact number of specimens was collected in both dung types (Table 2).

Table 5. Effect of sampling interval on densities of *Aphodius lividus* in 1993.

Week Samples Collected[1]	Mean ± SD[2]
1	0.11 ± 0.68 a
2	0.99 ± 1.44 a
3	0.04 ± 3.71 a
4	0.01 ± 0.22 a
5	2.79 ± 0.75 a

[1]Numbers represent weeks after treatment.
[2]Means followed by the same letter are not significantly different from each other (Sidak *t* Test, $P < 0.1$, n = 80); values represent nontransformed data; statistical analysis performed on log-transformed data.

Table 6. Influence of treatment and sampling interval on densities of *Aphodius lividus* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	1	0.00 ± 0.00 a
I	2	0.58 ± 3.32 a
I	3	0.05 ± 0.32 a
I	4	0.15 ± 0.48 a
I	5	4.80 ± 14.45 d
N	1	0.23 ± 0.92 a
N	2	1.40 ± 4.51 c
N	3	0.03 ± 0.16 a
N	4	0.08 ± 0.40 a
N	5	0.78 ± 2.59 b

[1]I = ivermectin treatment, N = no treatment.
[2]Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.122, $P < 0.1$, n = 40); values represent nontransformed data; statistical analysis was performed on log-transformed data.

Like 1993, the week in which samples were collected prior to and after treatment did affect the number of *A. lividus* collected in samples, regardless of treatment (Table 7). A significantly greater number of specimens was collected in pretreatment samples. This species was significantly less abundant in both ivermectin-contaminated and noncontaminated dung all 4 weeks after treatment and the average density decreased over that time period.

Since ivermectin treatment had no effect on the numbers of *A. lividus* sampled in either dung type, it is unclear why sampling date would have an affect. Perhaps the timing of pretreatment sampling coincided with natural declines in the population of this species, due to environmental conditions or the species' biology.

Analysis of data by LSD shows *A. lividus* was equally abundant in pretreatment samples from either dung type (Table 8). Densities of this species in pretreatment samples were significantly greater than those from either ivermectin-contaminated or noncontaminated dung any week following treatment (Table 7). Also, densities were not significantly different between either dung type all 4 weeks after treatment. The combined influence of treatment and sampling date did not affect the abundance of *A. lividus*.

Ataenius platensis* and *A. spretulus

1993. Ivermectin treatment did not affect the numbers of collected *A. platensis* and *A. spretulus* when the two species were analyzed together. A greater percentage of these two species was collected from noncontaminated dung (Table 1), but there was no significant difference between the totals collected in either dung

Table 7. Effect of sampling interval on densities of *Aphodius lividus* in 1994.

Week Samples Collected[1]	Mean ± SD[2]
P	1.05 ± 2.91 a
1	0.15 ± 0.76 b
2	0.13 ± 0.91 b
3	0.05 ± 0.22 b
4	0.00 ± 0.00 b

[1]P = pretreatment sample; Numbers represent weeks after treatment.
[2]Means followed by the same letter are not significantly different from each other (Sidak *t* Test, $P < 0.1$, n = 60); values represent nontransformed data; statistical analysis performed on log-transformed data.

Table 8. Influence of treatment and sampling interval on densities of *Aphodius lividus* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	P	1.03 ± 2.92 c
I	1	0.30 ± 1.06 ab
I	2	0.00 ± 0.00 a
I	3	0.07 ± 0.25 ab
I	4	0.00 ± 0.00 a
N	P	1.07 ± 2.96 c
N	1	0.00 ± 0.00 a
N	2	0.27 ± 1.28 ab
N	3	0.03 ± 0.18 ab
N	4	0.00 ± 0.00 a

[1]I = ivermectin treatment, N = no treatment.
[2]P = pretreatment sample; Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.112, $P < 0.1$, n = 30); values represent nontransformed data;statistical analysis was performed on log-transformed data.

type. Also, the week in which samples were collected after treatment did not alter the abundance of the two species during the first year of the study. Both species together were equally abundant among the 5 weeks post-treatment of each treatment period.

The influence of treatment and sampling date together may have affected the abundance of these species. Significantly more individuals were collected in noncontaminated dung the first and second weeks after treatment (Table 9). *A. platensis* and *A. spretulus* together were more abundant in ivermectin-contaminated dung the fourth and fifth weeks after treatment than the first 3 weeks in that dung type. These species may have been repelled by the higher levels of ivermectin present in the contaminated dung until 4 weeks after treatment, when the concentration of ivermectin most likely had dropped. Overall, fewer significant differences were observed among the densities in noncontaminated dung all 5 weeks after treatment than in ivermectin-contaminated dung.

As previously mentioned, the data collected on these two *Ataenius* species are difficult to interpret as the exact proportion of *A. platensis* to *A. spretulus* collected in 1993 is unknown. These results may reflect a predominance of one species over the other. If this was the case, then substantially different results might have been obtained had the second species been predominant. With this possibility in mind, caution must be taken in attributing the results observed in this study to either species alone.

Table 9. Influence of treatment and sampling interval on densities of *Ataenius platensis* and *Ataenius spretulus* in 1993.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	1	0.20 ± 0.52 a
I	2	0.40 ± 0.81 a
I	3	0.73 ± 0.88 b
I	4	1.88 ± 4.19 cd
I	5	2.78 ± 3.71 e
N	1	2.48 ± 4.80 d
N	2	1.80 ± 6.01 bc
N	3	1.30 ± 2.58 bcd
N	4	1.63 ± 4.32 bcd
N	5	1.75 ± 2.96 cd

[1]I = ivermectin treatment, N = no treatment.
[2]Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.166, $P < 0.1$, n = 40); values represent nontransformed data; statistical analysis was performed on log-transformed data.

1994. During 1994, *A. platensis* and *A. spretulus* were tallied separately so statistical analysis could be performed on each species. However, they were the two least abundant species collected that year (Table 2). Only two specimens of *A. platensis* were collected, so no analysis was performed on this species.

Although *A. spretulus* represented only 1.37% of the total aphodiine specimens collected in 1994 (Table 2), enough specimens were taken to analyze statistically. Like the previously discussed aphodiine species, no significant difference in the total number of *A. spretulus* was detected between ivermectin-contaminated and noncontaminated dung. Ivermectin treatment did not have an effect on the number of specimens collected. In addition, the week in which samples were collected prior to or after treatment had no effect on the population of *A. spretulus*. No significant differences were observed in densities of this species among the weeks of each treatment period.

This species was equally abundant in pretreatment samples of both ivermectin-contaminated and noncontaminated dung (Table 10). The density of this species in noncontaminated dung 4 weeks after treatment was significantly greater than densities from all other weeks in either dung type. As *A. spretulus* was equally abundant in all other weeks in both types of dung, the increased density observed in noncontaminated dung 4 weeks after treatment may have been a random occurrence. It would appear the combined influence of treatment and sampling date, as analyzed by LSD, does not greatly affect the abundance of *A. spretulus*.

Table 10. Influence of treatment and sampling interval on densities of *Ataenius spretulus* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	P	0.07 ± 0.25 ab
I	1	0.13 ± 0.43 ab
I	2	0.13 ± 0.35 ab
I	3	0.00 ± 0.00 a
I	4	0.17 ± 0.46 ab
N	P	0.07 ± 0.25 ab
N	1	0.00 ± 0.00 a
N	2	0.07 ± 0.37 ab
N	3	0.20 ± 0.81 ab
N	4	0.57 ± 1.10 c

[1]I = ivermectin treatment, N = no treatment.
[2]P = pretreatment sample; Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.072, $P < 0.1$, n = 30); values represent nontransformed data; statistical analysis was performed on log-transformed data.

Aphodius fimetarius

1993. The number of *A. fimetarius* collected in noncontaminated dung was slightly less than the number collected in ivermectin-contaminated dung (Table 1). Not surprisingly, ivermectin treatment had no affect on the collected number of adults. The week in which samples were collected after treatment did not affect the number of collected specimens, either. *A. fimetarius* was equally abundant among the 5 weeks post-treatment of any of the four treatment periods.

Data analysis by LSD indicates the combined influence of treatment and sampling date had some affect on the abundance of *A. fimetarius*, but not in a clearly defined trend (Table 11). No significant difference in the densities of this species was detected in either dung type in the first 2 weeks after treatment. *A. fimetarius* was more abundant in ivermectin-contaminated dung 3 and 5 weeks after treatment, but during the fourth week after treatment it was equally abundant in either dung type. The increased density seen in ivermectin-contaminated dung 3 and 5 weeks after treatment may have resulted from spurious significance at $P < 0.1$.

1994. During 1994, more individuals were collected in ivermectin-contaminated dung than in noncontaminated dung (Table 2), but the difference was not significant. As in 1993, ivermectin treatment had no affect on the collected numbers of *A. fimetarius*.

Unlike 1993, the week in which samples were collected prior to and after treatment did have an effect on the abundance of *A. fimetarius*, regardless of treatment (Table 12). Significant differences were observed in the densities of

Table 11. Influence of treatment and sampling interval on densities of *Aphodius fimetarius* in 1993.

Treatment[1]	Sampling Date[2]	Mean ± SD[3]
I	1	0.20 ± 0.61 abc
I	2	0.10 ± 0.30 ab
I	3	0.58 ± 1.13 ef
I	4	0.70 ± 1.92 de
I	5	0.05 ± 0.22 f
N	1	0.13 ± 0.33 ab
N	2	0.03 ± 0.16 a
N	3	0.03 ± 0.16 a
N	4	0.48 ± 1.34 cde
N	5	0.30 ± 1.04 bc

[1]I = ivermectin treatment, N = no treatment.
[2]Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.80, $P < 0.1$, n = 40); values represent nontransformed data; statistical analysis was performed on log-transformed data.

Table 12. Effect of sampling interval on densities of *Aphodius fimetarius* in 1994.

Week Samples Collected[1]	Mean ± SD[2]
P	0.22 ± 0.67 b
1	1.95 ± 5.35 a
2	0.47 ± 1.47 ab
3	0.32 ± 0.81 ab
4	0.08 ± 0.28 b

[1]P = pretreatment sample; Numbers represent weeks after treatment.
[2]Means followed by the same letter are not significantly different from each other (Sidak *t* Test, $P < 0.1$, n = 60); values represent nontransformed data; statistical analysis performed on log-transformed data.

species among the weeks of each treatment period. *A. fimetarius* was most abundant in samples collected 1 week after treatment, but declined in abundance during the following weeks. The density of this species collected 1 week after treatment was significantly greater than the density in pretreatment samples and in samples collected 4 weeks after treatment. However, *A. fimetarius* was equally abundant in samples collected 2, 3, and 4 weeks after treatment. No significant difference was observed between those densities and that in pretreatment samples or samples collected 1 week after treatment.

Analysis by LSD shows the influence of treatment and sampling date together had some effect on the density of *A. fimetarius*. This species was most abundant in ivermectin-contaminated dung 1 week after treatment (Table 13). It was also more abundant in ivermectin-contaminated dung than noncontaminated dung 2 and 3 weeks after treatment. However, no significant difference was observed in densities between either dung type four weeks after treatment.

It would appear from these results *A. fimetarius* was more attracted to ivermectin-contaminated dung for up to 3 weeks after the topical treatment of cattle with ivermectin. During the fourth week after treatment either dung type was equally attractive. Implications of these findings and the role of ivermectin in the increased attractiveness of dung for this species and others will be discussed in Chapter 3.

Table 13. Influence of treatment and sampling interval on densities of *Aphodius fimetarius* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	P	1.13 ± 0.43 a
I	1	3.03 ± 6.75 d
I	2	0.83 ± 2.00 c
I	3	0.50 ± 1.07 bc
I	4	0.10 ± 0.31 a
N	P	0.30 ± 0.84 ab
N	1	0.87 ± 3.20 bc
N	2	0.10 ± 0.31 a
N	3	0.13 ± 0.35 a
N	4	0.07 ± 0.25 a

[1]I = ivermectin treatment, N = no treatment.
[2]P = pretreatment sample; Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.138, $P < 0.1$, n = 30); values represent nontransformed data; statistical analysis was performed on log-transformed data.

Aphodius stercorosus

1993. No significant difference was observed between numbers of *A. stercorosus* collected in either ivermectin-contaminated or noncontaminated dung, even though a larger number of specimens were collected in noncontaminated dung (Table 1). Also, the week in which samples were collected after treatment did not affect the numbers of collected specimens. *A. stercorosus* was equally abundant among all 5 weeks post-treatment in each treatment period in 1993.

Data analysis by LSD suggests the combined influence of treatment and sampling date had an effect on the abundance of this species, but the data are difficult to interpret (Table 14). A significantly larger number of *A. stercorosus* was collected in noncontaminated dung during week 1. This species was equally abundant in both dung types 2, 3, and 5 weeks after treatment. A significantly greater number of specimens was collected in ivermectin-contaminated dung 4 weeks after treatment. The increased densities of *A. stercorosus* in noncontaminated and ivermectin-contaminated dung 1 and 4 weeks after treatment, respectively, may have been a result of spurious significance at $P < 0.1$.

Increased densities of this species were observed in noncontaminated dung and ivermectin-contaminated dung 1 and 4 weeks after treatment, respectively. *A. stercorosus* was equally abundant in both dung types the other 3 weeks in each treatment period. Concentrations of ivermectin in dung decrease with time after treatment, therefore it is unlikely that ivermectin was solely responsible for the increased abundance seen in ivermectin-contaminated dung 4 weeks after treatment.

Table 14. Influence of treatment and sampling interval on densities of *Aphodius stercorosus* in 1993.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	1	0.15 ± 0.43 a
I	2	0.48 ± 1.11 bcd
I	3	0.20 ± 0.46 abc
I	4	0.55 ± 1.32 de
I	5	0.88 ± 2.10 ef
N	1	1.85 ± 7.08 f
N	2	0.43 ± 0.93 bcd
N	3	0.18 ± 0.68 a
N	4	0.23 ± 0.70 ab
N	5	0.53 ± 1.22 cde

[1]I = ivermectin treatment, N = no treatment.
[2]Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.118, $P < 0.1$, n = 40); values represent nontransformed data; statistical analysis was performed on log-transformed data.

1994. Again, ivermectin treatment did not affect the numbers of *A. stercorosus*. In 1994, three times as many specimens were collected in ivermectin-contaminated dung, but there was no significant difference between the totals in either dung type. The week in which samples were collected prior to and after treatment did not significantly alter the number of specimens, either. *A. stercorosus* was equally abundant among all 5 weeks of each treatment period in 1994.

The influence of treatment and sampling date together may have affected the densities of this species. No significant differences in densities of *A. stercorosus* were observed between pretreatment samples or samples collected during week 1 in either dung type (Table 15). During the second and third weeks after treatment, a greater number of specimens was collected in ivermectin-contaminated dung. *A. stercorosus* was most abundant in ivermectin-contaminated dung 2 weeks after treatment than in either dung type during any other week. Four weeks after treatment, densities fell to similar numbers in both types of dung. If ivermectin was responsible for the increased densities observed in ivermectin-contaminated dung during weeks 2 and 3, it is unknown why *A. stercorosus* was not more abundant in ivermectin-contaminated dung the week of treatment, when concentration of ivermectin would have been higher.

Aphodius erraticus

1993. Ivermectin treatment did not affect the numbers of *A. erraticus* specimens collected in either dung type. A larger number of specimens was collected in noncontaminated dung, but no significant difference was observed between the

Table 15. Influence of treatment and sampling interval on densities of *Aphodius stercorosus* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	P	0.57 ± 2.75 ab
I	1	0.20 ± 0.48 ab
I	2	2.97 ± 9.86 c
I	3	0.37 ± 1.00 b
I	4	0.40 ± 1.71 ab
N	P	0.70 ± 2.20 b
N	1	0.27 ± 1.11 ab
N	2	0.20 ± 0.61 ab
N	3	0.03 ± 0.18 a
N	4	0.07 ± 0.25 a

[1]I = ivermectin treatment, N = no treatment.
[2]P = pretreatment sample; Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.142, $P < 0.1$, n = 30); values represent nontransformed data; statistical analysis was performed on log-transformed data.

totals for this species collected in ivermectin-contaminated and noncontaminated dung.

The week in which samples were collected after treatment did have a significant effect on the densities of *A. erraticus* (Table 16). Regardless of treatment, this species was more abundant during certain weeks than others within each 5 week post-treatment period. However, the Sidak *t* Test used to analyze these means failed to detect the significant differences among them at $P < 0.1$.

Densities of this species, as analyzed by LSD, were similar in both types of dung within the same week after treatment for 8 of the 10 weeks (Table 17) . There were no significant differences between the densities of *A. erraticus* collected in ivermectin-contaminated and noncontaminated dung for the first, fourth, and fifth weeks after treatment. During the second week after treatment, significantly more individuals were collected in noncontaminated dung. However, the following week a significantly greater total was collected in ivermectin-contaminated dung.

The combined influence of treatment and sampling date appeared to have some affect on the abundances of *A. erraticus*, but not in a easily definable trend. While significant differences between the two types of dung were seen in weeks 3 and 4, the greater numbers were not found in the same dung type both weeks. These differences probably resulted from random fluctuations in the population rather than brief periods of increased attractiveness in the two dung types.

1994. Ivermectin treatment did not alter the adult population of this species during 1994. A larger number of specimens was collected in ivermectin-contaminated dung, but there was no significant difference between the totals collected in either

Table 16. Effect of sampling interval on densities of *Aphodius erraticus* in 1993.

Week Samples Collected[1]	Mean ± SD[2]
1	0.15 ± 0.68 a
2	0.55 ± 1.44 a
3	1.03 ± 3.71 a
4	0.03 ± 0.22 a
5	0.11 ± 0.75 a

[1]P = pretreatment sample; Numbers represent weeks after treatment.
[2]Means followed by the same letter are not significantly different from each other (Sidak *t* Test, $P < 0.1$, n = 80); values represent nontransformed data; statistical analysis performed on log-transformed data.

Table 17. Influence of treatment and sampling interval on densities of *Aphodius erraticus* in 1993.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	1	0.18 ± 0.84 abc
I	2	0.28 ± 0.78 bc
I	3	1.08 ± 3.19 d
I	4	0.00 ± 0.00 a
I	5	0.15 ± 0.95 ab
N	1	0.13 ± 0.46 abc
N	2	0.83 ± 1.85 d
N	3	0.98 ± 4.21 c
N	4	0.05 ± 0.32 a
N	5	0.08 ± 0.47 a

[1]I = ivermectin treatment, N = no treatment.
[2]Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.107, $P < 0.1$, n = 40); values represent nontransformed data; statistical analysis was performed on log-transformed data.

type. In addition, the week in which samples were collected prior to and after treatment had no effect on numbers of collected specimens. *A. erraticus* was equally abundant among all 5 weeks in each treatment period.

Analysis by LSD indicates the combined influence of treatment and sampling date did affect the population of *A. erraticus*. This species was more abundant in pretreatment samples from the dung of cattle which were later treated with ivermectin (Table 18). However, this species was equally abundant in either dung type sampled the following week. The density of *A. erraticus* increased in both dung types the second week after treatment, but no significant difference in density was observed between the two types of dung that week. *A. erraticus* was less abundant in noncontaminated than ivermectin-contaminated dung 3 weeks after treatment, but no significant difference in density existed between both dung types the following week.

Greater abundances of *A. erraticus* were not consistently found in one dung type. It is unlikely that ivermectin actually increased the attractiveness of contaminated dung 3 weeks after treatment, especially as the effect was not present the following week. Random fluctuations in the population may have been responsible for these trends.

Aphodius haemorrhoidalis*, *A. lividus*, and *Ataenius spretulus* and *A. platensis

1993. *A. haemorrhoidalis*, *A. lividus*, and the two *Ataenius* species together were the most abundant species collected in 1993 (Table 1), and were analyzed together as a group. Significant differences were observed among the densities of these species from each of the four treatment periods in 1993. This result was not

Table 18. Influence of treatment and sampling interval on densities of *Aphodius erraticus* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	P	3.77 ± 7.05 d
I	1	1.73 ± 4.41 bc
I	2	2.80 ± 6.57 cd
I	3	2.27 ± 6.64 bc
I	4	0.07 ± 0.37 a
N	P	1.93 ± 7.11 bc
N	1	1.20 ± 3.33 b
N	2	5.43 ± 14.22 d
N	3	0.07 ± 0.25 a
N	4	0.57 ± 1.52 ab

[1]I = ivermectin treatment, N = no treatment.
[2]P = pretreatment sample; Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.206, $P < 0.1$, n = 30); values represent nontransformed data; statistical analysis was performed on log-transformed data.

particularly surprising as each species showed significant differences when analyzed separately. As previously mentioned, these differences indicate that all species were more abundant at certain times throughout the sampling season. These differences were expected because the seasonal abundance of a species fluctuates according to its seasonality and the number of generations it produces each year.

Ivermectin treatment had a definite effect on these four species when they were analyzed together (Table 19). A significantly larger number of specimens was collected in ivermectin-contaminated dung. This result implies that these species, as a group, were more attracted to the ivermectin-contaminated dung than the noncontaminated dung. Implications of this finding will be discussed in Chapter 3.

The week in which samples were collected did not affect the numbers of specimens collected (Table 20). Together these species were equally abundant among all 5 weeks post-treatment in each treatment period. However, significant differences were observed among the 5 weeks regardless of treatment when untransformed data were analyzed. Significantly fewer specimens were collected during the first week than the second week after treatment. The species were equally abundant during the third, fourth, and fifth weeks after treatment. They decreased in number over weeks 3 and 4, but increased during week 5. Those densities were also similar to the densities collected the first and second weeks after treatment. The increase in abundance during week 2 and the gradual decline in the number of specimens collected in the following 2 weeks may reflect natural fluctuations in the populations of these four species.

Table 19. Effect of treatment on densities of *Aphodius haemorrhoidalis*, *Aphodius lividus*, and *Ataenius platensis* and *Ataenius spretulus* in 1993.

Treatment[1]	Mean ± SD[2]
I	26.23 ± 40.20 a
N	12.68 ± 23.55 b

[1]I = ivermectin treatment, N = no treatment.
[2]Means followed by the same letter are not significantly different from each other (Sidak *t* Test, $P < 0.1$, n = 200); values represent nontransformed data; statistical analysis performed on log-transformed data.

Table 20. Effect of sampling interval on the densities of *Aphodius haemorrhoidalis*, *Aphodius lividus*, and *Ataenius platensis* and *Ataenius spretulus* in 1993.

Week Samples Collected[1]	Log-transformed Data	Nontransformed Data
	Mean ± SD[2]	Mean ± SD[2]
1	1.74 ± 1.34 a	12.69 ± 21.03 b
2	2.29 ± 1.73 a	30.11 ± 42.21 a
3	1.95 ± 1.50 a	20.20 ± 34.87 ab
4	1.90 ± 1.37 a	17.24 ± 32.69 ab
5	2.03 ± 1.29 a	17.01 ± 31.90 ab

[1]Numbers represent weeks after treatment.
[2]Means followed by the same letter are not significantly different from each other (Sidak *t* Test, $P < 0.1$, n = 80); values represent nontransformed data; statistical analysis performed on log-transformed data.

Analysis by LSD indicates there were significant differences among the densities of this group as a result of the combined influence of treatment and sampling date (Table 21). During the first and second weeks after treatment, this group was equally abundant in both ivermectin-contaminated and noncontaminated dung within each week. Densities of the four species remained higher, however, in ivermectin-contaminated dung for the remaining 3 weeks in each treatment period in 1993. Significantly fewer specimens were collected in noncontaminated dung in the third, fourth, and fifth weeks after treatment.

It would appear that ivermectin-contaminated dung was more attractive to these species as a group. This period of increased attractiveness began the third week after treatment and lasted for at least 3 weeks.

Aphodius haemorrhoidalis*, *A. fimetarius*, and *A. lividus

1994. As in the previous year, and for the same reasons, significant differences were observed in the densities of the three most abundant species in each of the three treatment periods in 1994. For unknown reasons, ivermectin treatment did not affect the densities of these three species when analyzed together, unlike the previous year, when ivermectin altered the densities of the three most abundant species.

Again as in 1993, the week in which samples were collected in regard to treatment did not alter the number of specimens collected. These species occurred in equal abundance each of the 5 weeks in the treatment period. No significant differences were observed in untransformed data for this group.

Table 21. Influence of treatment and sampling interval on densities of *Aphodius haemorrhoidalis*, *Aphodius lividus*, and *Ataenius platensis* and *Ataenius spretulus* in 1993.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	1	14.75 ± 26.48 ab
I	2	40.60 ± 52.64 c
I	3	30.35 ± 43.43 c
I	4	24.78 ± 40.64 c
I	5	20.65 ± 28.97 c
N	1	10.63 ± 13.64 a
N	2	19.63 ± 24.80 bc
N	3	10.05 ± 19.12 a
N	4	9.70 ± 19.92 a
N	5	13.78 ± 34.57 b

[1]I = ivermectin treatment, N = no treatment.
[2]Numbers represent weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.298, $P < 0.1$, n = 40); values represent nontransformed data; statistical analysis was performed on log-transformed data.

The combined data for these species, as analyzed by LSD, suggest that the influence of treatment and sampling date did alter the densities of these species (Table 22). No significant differences were observed between densities of this group in pretreatment samples from either dung type. However, a significantly larger number of specimens was collected in ivermectin-contaminated dung each of the remaining weeks in the treatment period. As in 1993, it would seem that ivermectin-contaminated dung was more attractive to the three most abundant species. This period of increased attractiveness began immediately after treatment and continued for at least 4 weeks. Implications of these results, and those seen resulting from the combined analysis of the most abundant species in 1993, will be discussed in Chapter 3.

Table 22. Influence of treatment and sampling date on densities of *Aphodius haemorrhoidalis*, *Aphodius fimetarius*, and *Aphodius erraticus* in 1994.

Treatment[1]	Week Samples Collected[2]	Mean ± SD[3]
I	P	9.60 ± 12.55 c
I	1	16.83 ± 33.85 de
I	2	12.00 ± 14.27 e
I	3	12.67 ± 22.00 cd
I	4	11.80 ± 20.67 de
N	P	5.67 ± 8.79 bc
N	1	6.63 ± 6.88 c
N	2	8.87 ± 15.87 bc
N	3	3.77 ± 7.30 a
N	4	4.47 ± 6.17 ab

[1]I = ivermectin treatment, N = no treatment.
[2]P = pretreatment sample; Number of weeks after treatment.
[3]Means followed by the same letter are not significantly different from each other (LSD = 0.289, $P < 0.1$, n = 30); values represent nontransformed data; statistical analysis was performed on log-transformed data.

CHAPTER III

CONCLUSIONS

As a habitat with a rich faunal population of arthropods and other invertebrates, cattle dung pats are often adversely affected by pesticide treatments applied to cattle to control parasites. Some of these pesticide treatments have been shown to eliminate both beneficial and pest species of insects which live in the dung pat habitat (Cook & Gerhardt 1977, Fincher 1991). Researchers have recently evaluated the antiparasitic drug ivermectin to determine its effect on dung pat fauna. Treated animals metabolize little of the drug, and most is excreted in the feces (Campbell et al. 1983), where it continues to possess insecticidal properties. Ivermectin treatment of cattle may pose a threat to populations of beneficial dung pat fauna. For this reason, the potential impact of ivermectin on adult aphodiine dung beetles in eastern Tennessee cattle pastures was investigated in a two-year study. Aphodiine dung beetles, a coleopteran subfamily of scarabs, were chosen as the subject of this study because numerous species are often found in cattle manure and in frequently dense populations. Research objectives were to: 1) determine the species composition of adult aphodiine dung beetles collected in samples of cattle dung pats deposited by ivermectin-treated and nontreated cattle, 2) monitor the seasonal abundances of those species, and 3) evaluate the impact of ivermectin treatment on

aphodiine populations. Seasonal abundances were based on numbers collected from noncontaminated dung.

Seven aphodiine species in two genera, *Aphodius* and *Ataenius*, were found at the study site in both ivermectin-contaminated and noncontaminated dung both years. In 1993, more than 8,200 aphodiine beetles were collected, and almost twice as many (5,465 specimens) were taken from ivermectin-contaminated dung pats as compared to noncontaminated dung pats (2,783 specimens). The most abundant species were *Aphodius haemorrhoidalis*, *A. lividus*, and *Ataenius platensis* and *Ataenius spretulus* (the latter two species were combined and analyzed together). In 1994, around 3,000 aphodiine beetles were collected, and again twice as many (2,081 specimens) were collected in ivermectin-contaminated dung as compared to noncontaminated dung (990 specimens). The three most abundant species collected during the second year were *Aphodius haemorrhoidalis*, *A. erraticus*, and *A. fimetarius*.

Aphodius haemorrhoidalis was the most abundant species collected in both years. This species accounted for 81.92% (6,859 specimens) and 64.90% (1,993 specimens) of the total number of beetles collected in 1993 and 1994, respectively. *A. haemorrhoidalis* was present throughout the length of the experiment in both years, with the largest number of specimens collected in June and July. Ivermectin treatment alone and the week in which samples were collected in regard to treatment did not affect the densities of this species. However, analysis of the data by least significant difference (LSD) indicate, that for both years, the influence of treatment

and sampling interval may have affected the densities of this species. *A. haemorrhoidalis* appears to be more attracted to ivermectin-contaminated dung for a period which begins about 2 weeks after topical treatment of cattle and continues about 3 weeks.

Aphodius lividus accounted for 3.88% (320 specimens) and for 2.70% (83 specimens) of the total number of beetles collected in 1993 and 1994, respectively. This species was present from April through the end of the experiment in both years. During 1993, the largest number of specimens was collected in late June and early July, but the following year the peak number of specimens was collected in midMay. Ivermectin treatment had no effect on the densities of this species in either year. Significant differences were observed in the densities of this species according to the week in which samples were collected post-treatment in 1993 and 1994. Analysis of the data by LSD suggests the combined influence of treatment and sampling interval affected the densities of this species in 1993, but not in a clearly defined manner, and no effect was observed in 1994.

In 1993, data for *Ataenius platensis* and *Ataenius spretulus* were combined and analyzed. Together the two species accounted for 7.24% (597 specimens) of the total number of beetles collected that year, making them the second most abundant beetles. These species were collected from April through September, with the greatest numbers of specimens taken in midJune, late June and early July, and late July and early August. Ivermectin treatment and the week in which samples were collected post-treatment did not affect the densities of these beetles. The combined

influence of treatment and sampling interval may have altered the densities of these beetles, but not in a clearly defined manner. Data on these two species are difficult to interpret as the exact proportion of *A. platensis* to *A. spretulus* is not known, and caution must be taken in assigning these results to either species alone.

The following year the two species were tallied as separate species. *A. platensis* represented 0.07% (only 2 specimens) and *A. spretulus* represented 1.37% (42 specimens) of the total number of beetles collected. The *Ataenius* species were not as abundant in 1994 as they were in 1993, perhaps due to the drought conditions that occurred in the first year of the study. *A. spretulus* is often found in moist organic matter other than dung (Wegner & Niemczyk 1981), but during the drought, cattle dung may have been more readily available than other sources of moist organic matter, resulting in higher than normal densities in cattle dung.

During 1994, only a few specimens of *A. spretulus* were collected in April, and this species was not taken again until late June. The greatest number of specimens was collected in midAugust when the study ended. Ivermectin treatment and the week in which samples were collected prior to and after treatment had no effect on the densities of this species. It would appear that the combined influence of treatment and sampling interval, as analyzed by LSD, does not greatly affect the abundance of *A. spretulus*.

As only two specimens of *A. platensis* were collected in 1994, no seasonal distribution could be established for this species. No statistical analysis was performed as well.

Aphodius fimetarius accounted for only 1.27% (105 specimens) and for 5.93% (182 specimens) of the total number of aphodiine beetles collected in 1993 and 1994, respectively. *A. fimetarius* was the least abundant species collected in 1993, but the following year it was the third most abundant species. This species was most abundant from April through June in 1993, and was not collected again after mid-July. The following year it was also most abundant in the first half of the sampling season, with the highest number of specimens collected in late May. Specimens were not collected again after late June that year. Populations of this species generally decline to extremely low levels during the hottest months of the year (Mohr 1943), as observed in this study. Ivermectin treatment had no effect on the densities of this species in either year of the study. The week in which samples were collected post-treatment did not affect densities in 1993, but the following year this species was statistically more abundant in certain weeks than others during the treatment period. The influence of treatment and sampling interval combined did not appear to affect densities of *A. fimetarius* in the first year, but in 1994 it appeared to be more attracted to ivermectin-contaminated dung than noncontaminated dung.

Aphodius stercorosus accounted for 2.67% (220 specimens) and for 5.67% (174 specimens) of the total number of beetles collected in 1993 and 1994, respectively. This species was present throughout the sampling season both years in low numbers. In 1993, *A. stercorosus* was most abundant in late June, and in 1994 it was most abundant in mid-July. Ivermectin treatment and the week in which samples were collected post-treatment did not appear to alter the densities of *A. stercorosus* in

either year. The combined influence of treatment and sampling interval may have affected the densities of this species, but not in a clearly defined manner.

Aphodius erraticus accounted for 1.78% (147 specimens) and for 19.37% (595 specimens) of the total number of beetles collected in 1993 and 1994, respectively. In 1993, this species was most abundant in the early half of the sampling season, with the highest number of specimens collected in mid- to late April. The following year, more specimens were collected, but again it was most abundant in late April. Ivermectin treatment had no affect on the densities of this species, but the week in which samples were collected post-treatment did affect the densities of *A. erraticus* in 1993. In 1994, neither treatment or sampling interval had an affect on the densities of *A. erraticus*. The influence of treatment and sampling interval combined did have some affect on the densities of this species in both 1993 and 1994, but not in an easily definable manner. *A. erraticus* was the only species in 1994 which was significantly more abundant in one pretreatment sample than the other; more specimens were taken in dung of cattle which were later treated with ivermectin.

Statistical analysis was performed on the combined data for the three most abundant beetles in 1993, *Aphodius haemorrhoidalis*, *A. lividus*, and *Ataenius platensis* and *A. spretulus* (analyzed together), to determine if treatment and sampling interval had an affect on aphodiine dung beetles as a group. Ivermectin treatment had a definite effect on these beetles as a group. A significantly larger number of specimens was collected in ivermectin-contaminated dung. This result implies that

these species, as a group, were more attracted to ivermectin-contaminated dung than noncontaminated dung. However, ivermectin treatment did not have an effect on these species when they were analyzed separately. The week in which samples were collected did not affect the numbers of specimens collected.

Statistical analysis was also performed on the combined data for the three most abundant species in 1994, *Aphodius haemorrhoidalis*, *A. fimetarius*, and *A. lividus*. For unknown reasons, ivermectin treatment had no effect on these beetles as a group, although it did affect the most abundant species in 1993. Again as in 1993, the week in which samples were collected in regard to treatment did not affect the number of specimens collected.

Conclusions drawn from the statistical analyses of these data may be misleading. While roughly twice as many aphodiine beetles were collected in ivermectin-contaminated dung compared to noncontaminated dung, statistical analysis indicates that ivermectin treatment itself did not have a significant effect on individual species. However, analysis by LSD suggests that, for a few species, the combined influence of treatment and sampling date did affect species abundance. For example, significantly greater numbers of *A. haemorrhoidalis* were collected in ivermectin-contaminated dung in both years of the study. These larger numbers began to appear 2 weeks after the topical treatment of cattle and continued for about 3 weeks. In 1994, *A. fimetarius* was significantly more abundant in ivermectin-contaminated dung for up to 3 weeks after the treatment of cattle. These results imply that at least these

two species may be attracted to the dung of ivermectin-treated cattle for a varying period of time after treatment.

Limited information has been published on the possible attraction of scarab dung beetles to avermectin-contaminated dung, an area of potential environmental impact which has only recently come to the attention of researchers. An increase in the attractiveness of dung does not appear to be a certain result of avermectin treatment. One trial in Denmark showed that *Aphodius* spp. and other dung-inhabiting beetles were more abundant in noncontaminated dung than in dung excreted by cattle 3, 10, 20, and 30 days after treatment by subcutaneous injection of 200 μg ivermectin per kg (Holter et al. 1993). However, the beetles were equally abundant in both contaminated and noncontaminated dung in two subsequent trials.

In Australia, dung excreted by cattle within 3 days of treatment by subcutaneous injection of 200 μg abamectin per kg attracted significantly more *Onthophagus australis* Guerin-Méneville, a scarabaeine species, than noncontaminated dung, and dung excreted 25 days after treatment continued to be more attractive to this species (Wardhaugh & Mahon 1991). The researchers speculated that the increased attractiveness of the cattle dung was not a direct result of the presence of an avermectin, but rather that abamectin metabolites or an alteration in the intestinal flora of the cattle gut by abamectin may be responsible instead.

Pitfall traps baited with dung excreted by cattle 7, 10, and 17 days after subcutaneous injection with 200 μg ivermectin per kg attracted more beetles (4 *Aphodius* spp. and 4 scarabaeine spp.) than traps baited with dung from nontreated

cattle (Lumaret et al. 1993). However, the authors were unable to detect with liquid chromatography the concentration of ivermectin in dung excreted 12 days or more after treatment. Because no significant difference existed between the number of beetles attracted to traps baited with dung excreted 2, 4, 24, and 31 days after treatment and traps baited with noncontaminated dung, the researchers concluded that ivermectin itself was not responsible for the increased attractiveness observed in the dung excreted 7, 10, and 17 days after treatment.

The abundances of other aphodiine species collected in this study appeared to be affected by the influence of treatment and sampling date together, but not in a clearly defined manner. In 1994, *Aphodius erraticus* was equally abundant in both ivermectin-contaminated and noncontaminated dung 1 and 2 weeks after treatment. This species was more abundant in ivermectin-contaminated dung 3 weeks after treatment, but the following week it was again equally abundant in both dung types. In 1993, *A. stercorosus* was most abundant in noncontaminated dung 1 week after treatment, but was equally abundant in both dung types for the following 2 weeks. During week 4, it was more abundant in ivermectin-contaminated dung, but the following week it was again equally abundant in both dung types.

Similar fluctuations between dung types were seen in the densities of *A. lividus*, and the *Ataenius* species *platensis* and *spretulus* in 1993. Some species, such as *Aphodius fimetarius* in 1993 and *A. stercorosus* in 1994, were more abundant in ivermectin-contaminated dung only 1 or 2 weeks during treatment periods; during the remaining weeks in a treatment period, those species were equally abundant in both

dung types. Some of these fluctuations may be attributed to the natural rise and decline of the population of a species, due to environmental conditions or the biology of the species, or a spurious significance at $P < 0.1$, rather than an actual influence of treatment and sampling interval combined.

The possible increased attraction of ivermectin-contaminated dung may not be the only factor contributing to the larger number of aphodiine beetles collected in that dung type. One drawback to holding dung samples for 5 weeks before sorting them is that some specimens will be those which invaded the pat as adults, and others will be those which have developed and emerged from the pat sample. This limitation was unavoidable as the alternative would have been to float specimens out of the dung sample immediately after its collection. The flotation method would have avoided the probability of aphodiine reproduction within the sample, but specimens would have to have been identified to species from grubs, a more difficult task than identifying adult specimens to species. If grubs were not identified, one risked missing a species whose adults may have visited the pat, oviposited, and left before the sample was collected. This risk was avoided by holding the samples to allow the immature stages to complete their development before sorting them.

During the 5 weeks which samples were held, samples were protected from outside invaders which might have colonized the dung had it been left in the field. The only predators and parasitoids which could attack the aphodiine species inside the emergence chambers were those which were included in the pat sample. It is possible they may have been affected by the presence of ivermectin in the contaminated dung

samples, thus reducing their numbers and reducing potential attacks on aphodiine dung beetles.

Other dung pat fauna included in the sample, such as dipteran larvae, may also have been affected by the presence of ivermectin in contaminated samples. Adult aphodiine beetles in the emergence chambers may have mated and oviposited in dung pat samples, and the developing immatures, assuming they were not adversely affected by the presence of ivermectin, might have benefitted from the reduced competition for the resources of the dung sample. Reduced competition, and a possible reduction in the number of potential predators and parasitoids, may have resulted in the higher numbers of aphodiine beetles found in ivermectin-contaminated dung samples.

Regardless of whether ivermectin treatment had a significant impact on numbers of adult aphodiine beetles, it must be stressed that this study examined only the adult stage of these beetles. It should not be inferred from this study that ivermectin has no adverse impact on these species. Ivermectin cannot be deemed toxic or nontoxic for aphodiine dung beetles, or any other dung pat fauna, until studies have been conducted on the impact of the drug on all life stages.

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Vita

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