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THE IMPACT OF RABON ORAL LARVICIDE AND DIMILIN ON THE INSECT FAUNA IN BOVINE MANURE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Charles William Cook, Jr.

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ABSTRACT

The use of oral insecticides for fly control is not a new approach, but recent research has developed safer, more effective chemicals for use on the market or for experimental studies. This study was designed to determine the impacts on the insect fauna in bovine manure of insecticide-fed cattle.

The insecticides, Rabon and Dimilin, were mixed with loose salt at recommended levels and fed free choice to pastured Angus cattle. Manure samples from the two treated herds were collected and their fauna compared to that of a control herd. Sixteen species were abundant enough to use as indicator species in the statistical analysis. Mortality of both pest and beneficial species were investigated and analyzed.

A field observation study was designed to further investigate the impacts of the two oral feed-throughs on the manure community. It was feared that a high mortality rate of beneficial insects may slow down or inhibit the decomposition of the manure back into the soil.

Both chemicals appeared very selective in their toxicity toward insects inhabiting the bovine manure community. Only one nontarget insect was positively shown to be significantly reduced by the treatments. Both formulations controlled face fly larvae in the breeding habitat, the manure pile.

Biodegradation rates of the fecal pats showed no significant differences between the untreated pats and the treated pats.

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CHAPTER I

INTRODUCTION

The fauna of bovine manure, composed almost entirely of insects, can be thought of as a distinct community type. It is distinct for two reasons; first, it is a community that is not oriented and dependent on the sun for its energy like most other plant and animal communities. The inhabitants living and competing in the community acquire all their vital nutrients from materials in the manure; therefore, each member must complete its development in the short time allotted by the original energy and water supply of the manure pile. This fact seems responsible for the keen competition which exists in cattle dropping communities. Secondly, the bovine manure insect community is distinct because it is an animal community that is successional in nature. In other words, as the manure pile ages, many flies, beetles, and wasps visit (or breed) and leave the cattle droppings in a certain order or succession. Mohr (1943) investigated cattle droppings as an ecological unit and listed in great detail the order of appearance of the insects living in this community.

Energy flow is a very important part of the bovine manure insect community. The energy is disbursed through different organisms of different trophic levels and is eventually removed from the systems when the insects leave the droppings. Within the cow-dung community we can recognize several different trophic levels. The original nutrients or

producers are thought of as the first trophic level. Utilizing these nutrients are the coprophagous (dung-eating) insects which are termed grazers or primary consumers. Eventually, many of these coprophagous insects are preyed upon while in their egg or larval stages by insect parasites and predators, termed secondary consumers. Also prevalent in this food web are various microflora and bacteria which act as decomposers. Eventually some of these microflora are consumed by other dung inhabitants.

When cattle are on pasture, their manure pats support a diversified complex of insects and mites. The most common orders found in these cattle droppings are Diptera, Coleoptera, and Hymenoptera. This insect complex associated with bovine manure includes both beneficial and pest species. Mortality of these pests, excluding climate factors, is concluded to be almost entirely due to entomophagous insects, as indicated by Poorbaugh et al. (1968). The parasitic wasps (Hymenoptera) are beneficial because they act as natural checks and reduce the number of certain fly populations. Blickle (1961) reported that in New Hampshire, parasitism by three species of wasps, Aphaereta pallipes (Braconidae) 13 percent, Xyalophora quinquelineata (Figitadae) 2.2 percent, and Eucoila sp. (Cynipidae) 1 percent, on the face fly, Musca autumnalis Degeer (Muscidae), totaled 16.2 percent for one season. Another group of entomophagous insects which significantly reduce coprophagous larvae in the manure are the predatory beetles of the families Histeridae, Hydrophilidae, and Staphylinidae. In several controlled experiments, Poorbaugh et al. (1968) demonstrated that the mortality of coprophagous species reared from droppings was directly correlated with the number of predators in each dropping.

Besides being efficient predators, adult Sphaeridium scarabaeiodes (Hydrophilidae) contribute to the aeration and desiccation of manure through meandering tunnels. Garry and Wingo (1971) reported that these tunnels allow larvae to descend to deeper portions of the pile earlier, because of the increased presence of oxygen. They also provide outlets for migrating fly larvae in what would ordinarily be a rather impenetrable crust and increase the probability of contact between fly larvae and hymenopterous parasites.

Another frequently occurring beneficial group of beetles are the Scarabaeidae or dung beetles. These dung beetles, are not predatory but are in direct competition for foodstuffs with coprophagous dipteran larvae. Hammer (1941) states that the Aphodius spp. (Scarabaeidae) feed on anything of nutritive value in the droppings and help deplete the organic nutrients and water in the pile, thus permitting the manure to eventually incorporate back into the soil. Acting together these beneficial insects help to reduce the population of two pest species, the horn fly, Haematobia irritans (Linnaenus) (Muscidae) and the face fly. It seems probable that without these beneficial insects in our state, cattle-fly related problems would be greatly increased.

Both the horn fly and face fly readily breed in droppings of pastured cattle in Tennessee. Sufficient control of the horn fly can usually be obtained with the correct use of backrubbers treated with recommended chemicals. But comparable control of the face fly, a relatively new pest in Tennessee, has not been achieved. The face fly was introduced from Europe and was discovered in Nova Scotia by Vockeroth (1953) in 1952. Since that time, it has expanded its range into the

United States, presently covering 44 states. During the warmer months face flies congregate around the animals' face and feed on mucous and other proteinaceous exudates. Decker (1961) reports that these exudates are possibly essential for the proper maturing of the female fly's eggs. Benson and Wingo (1963) found the face fly in Missouri in June, 1960. The spread of this species across Missouri was accompanied by the familiar complaints of cattlemen and the increased incidence of eye disorders in cattle. Steve and Lilly (1965) reported that the causative organism of pinkeye (infectious bovine keratitis), was a bacterium Moraxella bovis (Hauduroy) and was believed to be transmitted by the face fly.

In the early 1960's many investigations were carried out in attempts to control the face fly. Researchers tested various chemicals and application methods for face fly control, but were only partially successful (Bruce et al. 1960; Fales et al. 1961; Dobson and Huber, 1961; Jones and Medley, 1963). Presently, there are several chemicals which are toxic to the face fly and are registered for spraying and dusting cattle for face fly control. But these chemicals tend to wash off during rains and generally only persist at lethal levels for one week to ten days. This method is also expensive to the cattleman because of the necessary spraying equipment and the high frequency of applications.

Another approach to controlling flies is the method of adding insecticides to the manure. The concept of making manure toxic to fly larvae by feeding insecticides to cattle is not a new approach. Gallagher (1928) fed cows such compounds as tannic acid and linseed oil in an attempt to make their feces unsuitable for the development of horn fly

larvae. More recent research has proven several chemicals to be toxic to both horn fly and face fly larvae, when administered orally to cattle.

Miller (1970) cites the following criteria which are involved in a good feed additive larvicide. The compound must have the ability to pass through the digestive tract of an animal and appear in the feces in an amount sufficient to kill fly larvae. It must be palatable and have no detrimental effect on the animal to which it is fed. Neither the original compound nor any injurous metabolites must appear in the tissue, milk, or eggs at the level required for larvicidal action. In addition to the above criteria, the compound should be fairly selective and not destroy significant populations of beneficial insects.

Presently, only Rabon (Tetrachlorvinphos, Appex, Gardcide, Gardona, Rabond, Stirofos) (2 - chloro - 1 (2, 4, 5 trichlorophenyl) vinyl dimethyl phosphate), Ronnel (0, 0 - Dimethyl 0 - (2, 4, 5 trichlorophenyl phosphorothioate), coumaphos (0, 0 - Diethyl 0 - (3 - cholor - 4 - methyl - 2 - oxo - 2H - 1 - Benzapyran - 7 - yl) phosphorothioate, Methoprene (Altosid) Isopropyl (2E, 4E) - 11 - methoxy - 3, 7, 11 - trimethyl - 2, 4 - dodecadienoate and phenothiazine (thiodiphenylamine) are allowed by government regulations to be used as oral insecticides on cattle. These oral larvicides can be administered as capsules, mixed with salt and/or minerals, or mixed with rations as a feed additive. In Tennessee the last method seems preferable for dairy cattle, while the second method is well suited for pastured beef cattle.

There are still uncertainties about the feed additive larvicide approach for fly control. Two possible deleterious side effects are of immediate concern. First, the possibility of oral insecticides acting

indiscriminately and killing beneficial insects which act as natural checks on several pests species and secondly, the possibility of oral insecticides making the manure so toxic that few or no insects reside in the manure, thus inhibiting the biodegradation of fecal pats.

The former problem above is a historical one for entomologists ever since the development of powerful, nonselective chemical insecticides. The present trend is to combine the advantageous features of both chemical and biological control, which usually results in a more permanent pest suppression. The concern of the latter problem is that some oral larvicides could possibly make the cattle droppings lethal to all insect life, thus eliminating beneficial insects that are required to incorporate the manure back into the soil.

It is the purpose of this study to investigate how oral larvicides may change the population structure of some of the more important naturally occurring insect species found in the cow dung community. And secondly, to determine the impact, if any, of how these population changes may effect biodegradation rates of treated fecal pats. Two promising oral insecticides were fed to cattle, compared statistically against a control group, and field observations were recorded for all groups. The chemicals chosen for this study were (1) Rabon, described as a nonsystemic phosphate insecticide with a high level of activity to insects and a low hazard to warm blooded animals; and (2) Dimilin (TH6040) (N-(4-chlorophenyl)-N'-(2, 6-diflourobenzoyl) urea), described as an insect growth regulator (IGR) which causes eventual death by interfering with the formation of the insects' cuticle. It kills as a stomach poison.

CHAPTER II

LITERATURE REVIEW

I. CHEMICALS

For almost 50 years scientists have been experimenting with oral larvicides in an attempt to make cow manure unfavorable for the development of the larval stages of certain flies. The horn fly, a long time cattle pest of North America, aroused the first interests in the fauna of bovine manure. Attempts to control this pest were dropped when Gallagher (1928) fed cows such compounds as tannic acid, linseed oil, magnesium sulfate, and sodium chloride, and none were effective. The introduction of the face fly into North America in 1952 encouraged more research into the ecology of bovine manure. Since then, attention has shifted back and forth between chemical and biological control of the pests which breed in cattle droppings.

The history of chemical compounds tested as oral larvicides parallels the development of insecticides. Knipling (1938) reported that phenothiazine fed orally to cows at the rate of ca 100 mg/kg body weight caused the manure from these cows to be toxic to horn fly larvae. It was later reported by Bruce (1942) that rotenone and zinc oxide were also effective horn fly larvicides.

Eddy et al. (1954) found aldrin and dieldrin to be very effective larvicides against the horn fly, stable fly, and house fly when fed to

cattle at a level of 25 ppm. Lindane fed at 100 ppm was effective against horn fly larvae.

Sampson (1956) reported on certain insecticides, mainly the chlorinated hydrocarbons, which he found to be effective as a house fly larvicide. He found endrin, heptachlor, lindane, and parathion to be the most effective.

Harvey and Brethour (1960) found Polybor 3, a boron compound, to be larvicidal against the house fly when mixed directly with manure from a steer; however, there was no larvicidal activity in feces from steers fed up to 100 grams of Polybor 3 per day.

Bacillus thuringiensis Berliner was first fed to cattle in an experiment by Dunn (1960), and he reported an 89 percent reduction in house fly emergence. Gingrich (1965) fed three different commercial preparations of the pathogen to cattle in an attempt to control house, horn, and stable flies. He concluded that the pathogen could effectively control all three species of flies just mentioned. Ode and Matthysse (1964), however, reported essentially no control of face fly larvae when Bacillus thuringiensis Berliner was fed at levels of 30 mg/kg body weight to cattle. Miller et al. (1971) also found little control of house fly larvae in feces from dairy cows fed similar levels of the same pathogen.

Many of the organophosphorus insecticides and a few of the carbamates have been tested as feed through larvicides for cattle. Eddy and Roth (1961) screened 25 compounds, mainly organophosphates, for this purpose and showed that Bay 22408, coumaphos, Bay 21/200, Potasan, Dow ET-15, and ronnel were all lethal to house fly larvae when fed at levels of 5 mg/kg body weight. Anthony et al. (1961) also showed that coumaphos

and Bay 22408 when fed to cattle at a level of 1 mg/kg body weight inhibited emergence of both house and face fly larvae. Ronnel fed at a level of 5 mg/kg body weight was also lethal to larvae of both species.

Skaptason and Pitts (1962) showed that coumaphos at levels of 5, 10, and 50 ppm of the ration caused high larval mortality of the house fly. Treece (1962, 1964) presented more evidence on the effectiveness of coumaphos, Bay 22408, and ronnel for the control of face fly larvae. Jones and Medley (1963) showed coumaphos to be an effective larvicide against face flies when fed orally to cattle either in the grain ration or when sprayed on the pasture which cattle were grazing. Control of face fly larvae was obtained by Ode and Matthysse (1964) when dairy cows were fed zinc oxide, fenthion, bathrin, dimethrin, 3-4 dimethyl benzyl ester of chrysanthemumic acid, coumaphos, ronnel, and Bay 22408.

Drummond (1963) and Drummond et al. (1967) reported promising "feed-through" larvicides included Bay 37341, Bay 37342, chlorfenvinphos, famphur, Imidan, fenthion, R-3828, bromophos, and Rabon.

Knapp (1965) fed coumaphos as 2 percent of a salt mixture to pastured steers and reported 100 percent mortality of face fly larvae in the feces of the steers from a bioassay. However, control of adult face and horn flies was shown to be erratic. Similar results with face fly were obtained by Wallace and Turner (1964) after cattle were fed a salt mixture containing 5.5 percent ronnel.

Hoffman and Roberts (1963) reported control of the horn fly when cattle had access to a 5.5 percent ronnel--mineral salt block. Miller et al. (1970a) reported that coumaphos was found to have promise as a feed additive larvicide against the house fly. In another study, Miller (1970) showed that ronnel was ineffective against house fly

larvae, tended to be unpalatable to cattle, and was detected in the milk of cows fed this insecticide.

In three separate experiments it was found by Miller et al. (1970b) that Rabon (75 percent WP) fed at levels of 22, 37, and 48 ppm (actual Rabon) of an air-dry ration killed 94 percent or more of non-resistant house fly larvae seeded onto the feces. In a field trial conducted by Miller et al. (1970c) an encapsulated formulation of Rabon was fed to dairy cattle at a level of 64 ppm (actual Rabon) of the air-dry ration. This treatment caused a 94 percent reduction of house fly larvae in field manure compared with nontreated manure.

Miller and Gordon (1972a) demonstrated in a field trial that manure from cows fed 30, 45, 60, and 90 ppm of technical Rabon reduced larval populations by 81, 88, 93, and 98 percent, respectively, compared with manure from control cows. Miller and Gordon (1972b) also reported that in an insecticidal balance experiment 15 percent of the encapsulated Rabon which was fed orally to cattle reached the feces as compared with 3 percent that was found earlier with a 75 percent WP. Control of face flies in these experiments was excellent. Miller and Gordon (1973) found when dairy cows were fed Rabon at levels ranging between 36 to 252 ppm of an air-dry ration during an entire gestation, milk residues were generally nondetectable (<.005 ppm), but were occasionally as high as .014 ppm. Rabon was detected in only one tissue sample, and then at a level of only .02 ppm.

Rabon is an organic phosphate insecticide killing as a contact or stomach poision. In this experiment, Rabon was a specially prepared granulated material designed to provide optimum larvicidal activity in

the manure, when mixed with salt as an oral larvicide. When fed to cattle, Rabon Oral Larvicide passes through the cow's digestive system into the animals manure where it kills fly larvae on contact shortly after fly eggs hatch. (Technical Bulletin, Shell Oil Co., 1974). When fed to white rats, Rabon has an LD_{50} value of 4000 mg/kg. It has been shown to be relatively nonhazardous to fish and wildlife (Thompson, 1972).

To determine larvicidal activity, effect on insect fauna, and biodegradation of fecal pats, Miller and Pickens (1973) fed the following treatments to dairy cows: (1) no insecticide, (2) 56 ppm coumaphos, (3) 56 ppm Rabon, and (4) 112 ppm ronnel. Larvicidal activity against Musca domestica L. was significantly greater in feces containing Rabon than in feces from cows fed either coumaphos or ronnel. Feces examined after six or nine weeks in a pasture showed no difference between the biodegradation of untreated and treated manure. In this test, Rabon was shown to significantly reduce the number of Scarabaeidae, Staphylinidae, Hydrophiladae, and Sarcophagidae.

Miller and Vebel (1974) showed that 1 ppm of Methoprene, an insect growth regulator (Altosid) completely inhibited development of face flies, and at 10 ppm markedly inhibited the development of house flies. Ro-20-3600, another insect growth regulator, at 10 ppm inhibited the development of face flies, but had no effect on house flies. R-20458 fed at 100 ppm had no effect on either species. Miller (1974) reported that TH6040 (Dimilin) at rates of 1 and .1 ppm in cow manure killed 100 percent and 71 percent house fly larvae, respectively. At .01 ppm 70 percent of the larvae pupated, but adults emerged from only 16 percent

of these pupae. Within the same paper he also demonstrated that .5 mg/kg body weight of TH6040 fed orally to dairy cows caused 99 percent mortality to face fly larvae.

Pickens and Miller (1975) evaluated growth-regulating chemicals against nontarget insect fauna in bovine manure. They found Methoprene was not active against nontarget insects, RO-20-3600 was active against Hydrophilidae and Scarabaeidae, and TH6040 was active against all the major nontarget insects except Scarabaeidae.

Ables et al. (1975) showed TH6040 (Dimilin) had no significant effect on the percent eclosion of house fly eggs, but after eclosion, 100 percent of the first instar (at all concentrations of Dimilin) were killed before developing further to the next instar. Late-instar larvae subjected to Dimilin pupated, but few pupae produced adult flies. He also reported no observable effects on emergence of the house fly parasitiod Muscidifurax raptor Girault and Sanders from host puparia which had been treated with Dimilin.

Dimilin, an insect growth regulator, is a relatively new compound which has not been registered with the E.P.A. for its use with cattle, thus it is still in the experimental stage. It is a representative of a new class of insecticide with an unprecedented mode of action, and has been proven successful against various types of insects.

Dimilin was shown to interfere with the egg hatch of the boll weevil, Anthonomus grandis Bohema when the adult was dipped in or exposed to a dry film of the chemical (Moore and Taft, 1975). Mulla and Darwazeh (1976) have proven Dimilin effective against the emergence of several genera of mosquitoes, using both a wettable powder and flowable

concentrate. The chemical also reduced the hatch of eggs of the stable fly, Stomoxys calcitrans (L.) and the house fly, Musca domestica L., with no effect on fecundity (of parent). The effect could be defined as ovicidal or larvicidal as fully developed larvae failed to emerge from eggs (Wright and Spates, 1976).

Mulder and Gijswijt (1973) described the mode of action of Dimilin. If treatment starts long enough before ecdysis (24 hours), the newly formed cuticle will consist only of epicuticular and exocuticular tissue (omitting endocuticle) which is not properly attached to the epidermis. Hence, the newly formed cuticle is very delicate and cannot resist the muscular traction and the increased turgor during molting. The actual cause of death is most probably, either rupture or the new cuticle or starvation, in case the cuticle remains intact. Third or fourth instar larvae that have been able to feed some time after ecdysis before they were treated, have thickened their endocuticles, which enable them to survive until pupation. Sowa and Marks (1975) further describe the mode of action of Dimilin by saying it inhibits the incorporation of glucose in the biosynthesis of chitin by immature insects. Post et al. (1975) found similar results and reported that the synthesis of cuticular glucose is affected by Dimilin, but the synthesis of cuticular protein is not affected.

When fed to white rats, Dimilin has an LD_{50} value of 4640 mg/kg and has been shown to be relatively nontoxic to fish and wildlife (Thompson, 1972).

II. ECOLOGY OF COW DUNG

Studies of the ecology of the bovine manure community are relatively scarce. Many authors have contributed lists of insects present in cow manure (Howard, 1901; Pratt, 1912; Mellor, 1919; Laurence, 1954; Sanders and Dobson, 1966; Poorbaugh et al., 1968). Other authors have reared certain inhabitants and given accounts of the biology of the more important species (Mellor, 1919; Hafez, 1939; Hammer, 1941; Mohr, 1943).

In the United States, the first real interests in the fauna of cattle droppings was stimulated by investigations of the horn fly. Howard (1901) and Pratt (1912) investigated the inhabitants of the bovine manure community in order to find a biological control for the horn fly. Graham-Smith (1916, 1919) investigated the inhabitants of several types of animal droppings in relation to parasites, predators, and micro-climates. Hafez (1939) listed 60 species of cattle dung inhabitants in Egypt and included a brief ecological synopsis on the major species present.

Hammer (1941) published the findings of a six-year ecological study of flies associated with pastured cattle and their excrement, in which he gave detailed accounts of the parasites and predators associated with dung inhabitants. He listed the Histeridae and Hydrophilidae as voracious carnivores both as adults and larvae. Adults devour large numbers of egg masses of certain flies while the larvae feed on larger fly larvae. Larvae of Sphaeridium sp. (Hydrophiliade) were reared exclusively on full grown larvae of Sepsidae (Diptera). Individuals devoured 32 to 36 larvae before they stopped feeding.

An increase in the interest in parasites which attack manure inhabitants was sparked by the introduction of the face fly into the United States in 1959. Turner et al. (1968) conducted a three-year survey and found four hymenopterous parasites and one staphylinid (Aphaereta pallipes, Eucoila sp., Spalangia nigra, Muscidifurax raptor, and Aleochara spp., respectively), which are known parasites of native hosts but will also attack the introduced host, Musca autumnalis.

Mohr (1943) studied the principles of animal succession using cattle dung and listed the order of appearance of insects within the cattle dung. He divided the inhabitants into well defined groups which attain their growth together and pupate at about the same time. He used Shelford's terms of microsere and microseral stages to describe these divisions. Mohr noted that the insects of the first 48-hour stage comprise most, if not all, of the species thought to be specific to fresh droppings of cows. Mohr also noted the physical changes of the dung which occur with aging and desiccation and how this effects which species are present.

Controlled laboratory studies have shown that larvae of the coprophilic Diptera have a remarkable ability to complete their growth from egg to pupa under a wide variety of physical conditions. Competition for food in cow dung is rarely, if ever, a mortality factor to larvae of the coprophagous species (Poorbaugh et al., 1968); however, the actual diet of coprophagous fly larvae is still in doubt. Baumberger (1919) proposed that the diet of coprophagous fly larvae consisted of various bacteria found in the microflora of cow droppings. Dowding (1967) concluded that the pharyngeal ridges occurring in larvae of some

of the cyclorrhapous Diptera act as a seine and filters out water and unwanted particles allowing the organism to concentrate a suspension of bacteria, protozoa, fungus spores or yeasts.

On the whole, the various cow dropping flies have a very rapid development, which is correlated with the fact that the medium in which they live offers suitable conditions for their growth and life for only a short time.

To give a more thorough understanding of the insect bovine community, brief biological sketches of some of the more important inhabitants are given.

Diptera

Muscidae

Musca autumnalis DeGeer. The female face fly is attracted only to fresh cattle droppings less than one hour old. As the dung aged, Teskey (1960) found that the flies were apparently unable to penetrate the thin crust with their ovipositor. He also noted that the eggs are laid singly and indiscriminately on the surface of the pile. Hammer (1941) found the ovipositing females to contain an average of 24.6 eggs in the ovaries. He also found the incubation period of the eggs to be 10-1/2 hours when laid in the morning and 22 to 23 hours when laid in the afternoon. Further studies of the life history of the face fly (Teskey, 1960) revealed the following. Under ideal field conditions the larval stages may be completed in two to three days (3 instars). The prepupae migrate several centimeters from the droppings and go about .6 cm under

the soil to pupate. The pupal stage is completed in 8 to 10 days, depending on temperature. Musca autumnalis (adult) is a facultative blood sucker, mainly of cattle, and feeds on nectar, secretions and dung liquids.

Stratiomyidae

Sargus cuprarius (Linnaeus). The adults of this striking purple fly are rarely seen in the field, but the larvae are abundant in cattle droppings. Mohr (1943) found a total of 448 stratiomyid larvae in one manure sample. The female oviposits in manure which has been exposed for two days. After the other muscoid flies have pupated, the hardy stratiomyid larvae are only about half grown and prefer to feed on dry dung several weeks before pupating (Poorbaugh et al. (1968). In many cases these larvae actually are responsible for the final disintegration of the pat. Sargus pupates in and under the dung, but not in the soil. Hammer (1941) reported the adult flies feed exclusively on nectar and appear at the droppings only when ovipositing.

Sarcophagidae

Ravinia sp. Robineau-Desvoidy. Female sarcophogids visit the dung during the first two to three hours after exposure (Hammer, 1941). They are larviparous and deposit first instar larvae on the surface of the dung (Mohr, 1943). Larvae develop within three to four days and pupate in the soil a few inches beneath the dung, apparently before any appreciable amount of predation can occur (Poorbaugh et al. (1968). Mohr (1943) noted that the larvae are very susceptible to the

hymenopterous parasite, Xyalophora quinquelineata (Say)(Figitidae). The new born larvae were attacked within minutes of larviposition by Xyalophora females who often used the tunnels created by Sphaeridium and Aphodius to search out their host. In the United States Ravinia larvae are readily parasitized by Aphaereta pallipes (Say), but the parasite is not successful in emerging from the puparia (Sanders and Dobson, 1966). Adult Ravinia feed mainly on the liquid of the dung and on nectar from flowers.

Coleoptera

Staphylinidae

The adult staphylinids that have been found in cow manure have been characterized by Hammer (1941) as specialized egg eaters. He observed smaller species biting off the top of fly eggs and thrusting their heads into the eggs where they chew the contents; and larger species engulfing both single eggs and entire egg masses. The staphylinids are also dependent on the tunnels created by Sphaeridium and Aphodius to find their food. Staphylinid larvae are also carnivorous, but feed mainly on fly larvae. Hammer (1941) reports that the larvae grow more slowly than their dipteran prey and are therefore disadvantaged.

Scarabaeidae

Aphodius spp. The ecological requirements for all stages of the life cycle of Aphodius can be satisfied within the cow manure (Hafez, 1939). Some Aphodius (i.e., A. granarius and A. sterocorosus) are

attracted immediately to dung while others seek dung exposed for one to three days (i.e., A. fimetarius, A. haemorrhoidalis, and A. ruricola) (Hafez, 1939; Mohr, 1943). Aphodius are frequent through early spring and occur again in large numbers in early fall. Hammer (1941) reports post-hibernating Aphodius appearing in April or May in such large numbers that they can harm the fly larvae present by eating everything of nutritive value. Dipteran larvae may perish of starvation or desiccation. He also notes their importance in tunneling through the manure, so that predators and parasites can come in contact with their pray or hosts.

Ataenius sp. Ataenius are very similar to Aphodius in their ecology and physical appearance. Both are coprophagous beetles, the main distinction being the presence of transverse ridges on the tibia of Ataenius.

Onthophagus pennsylvanicus (Harold). Onthophagus pennsylvanicus is a member of the group commonly called "dung-rollers." They remove the fresh dung from the pile and place it in burrows beneath the cake. The female deposits an egg in each small amount of manure, which is used for rearing the larvae (Sanders and Dobson, 1966).

Hydrophilidae

Sphaeridium scarabaeoides (Linnaeus). Sanders and Dobson (1966) report that Sphaeridium scarbaeoides is the first beetle to attack the manure. Within minutes after the dung is laid, the beetle arrives and dives into the pat. It tunnels through it in search of fly eggs, and larvae, and may emerge at a different location. This beetle is

responsible for early aeration of the pat. Hammer (1941) reported that the eggs of the beetle are spun just under the manure crust. The larvae emerges in one to two days and attacks newly hatched dipteran larvae. They are so voracious that they attack anything that moves in the piles, including other Sphaeridium larvae.

Cercyon sp. Cercyon sp. are coprophagous larvae which are much smaller than the above hydrophilid. Mohr (1943) reports that Cercyon breeds in older manure, and Sanders and Dobson (1966) found both adults and larvae to act as scavengers, feeding upon the manure.

Hister abbreviatus Fabricius. Sanders and Dobson (1966) reported that hister beetles were never present in large numbers, no more than four specimens taken per sample. Hafez (1939) notes that histers attack dipterous and Aphodius larvae in both the adult and larval stages. Hammer (1941) concluded that histers have an important effect on the abundance of flies. The adult female deposits one or two eggs in the surface of the ground beneath the dropping. Two or three days after deposition the first instar larvae hatch later than their dipteran prey, but have powerful mandibles and can successfully attack prey larger than itself. After the fly larvae are gone, the histers feed on Aphodius larvae (Hammer, 1941). Hafez (1936) observed that when the hister is disturbed, it simulates death and retracts its legs, head and antennae beneath the body.

CHAPTER III

MATERIALS AND METHODS

Three similar herds at Grassland Farm Plateau Experiment Station, Cumberland County, Tennessee (elevation, 2,000 feet), were used to determine the impact of two oral larvicides on the manure fauna of pastured cattle fed these insecticides. The two treated herds, Number 16 and Number 3, had 16 Angus cows and 18 Angus cows, respectively, ranging in age from yearling heifers to seven years. Each herd contained between 12 and 15 calves, all born between January 1 and April 1, 1976.

One herd (Number 10) remained untreated to compare with the herds which were fed oral larvicides. The relative position of the three experimental pastures can be seen from the map of Grasslands Farm (Figure 1). Table 1 shows the treatment, formulation, and feeding rate for each herd used in the experiment.

The feeding rate for both Rabon and Dimilin was 0.5 mg/kg body weight, a rate which had previously been shown to control face flies in feces. A 97.3 percent technical Rabon granule material was mixed with livestock grade salt to achieve the desired formulation. Likewise, a technical Dimilin dry powder (95 percent) was mixed with the salt to achieve the desired formulation.

All cattle in herds Number 16 and 3 had access to backrubbers (1 percent RaVap) located in the pasture and face dust bags (3 percent Rabon) on the salt box feeders. Due to the immigration pressure of

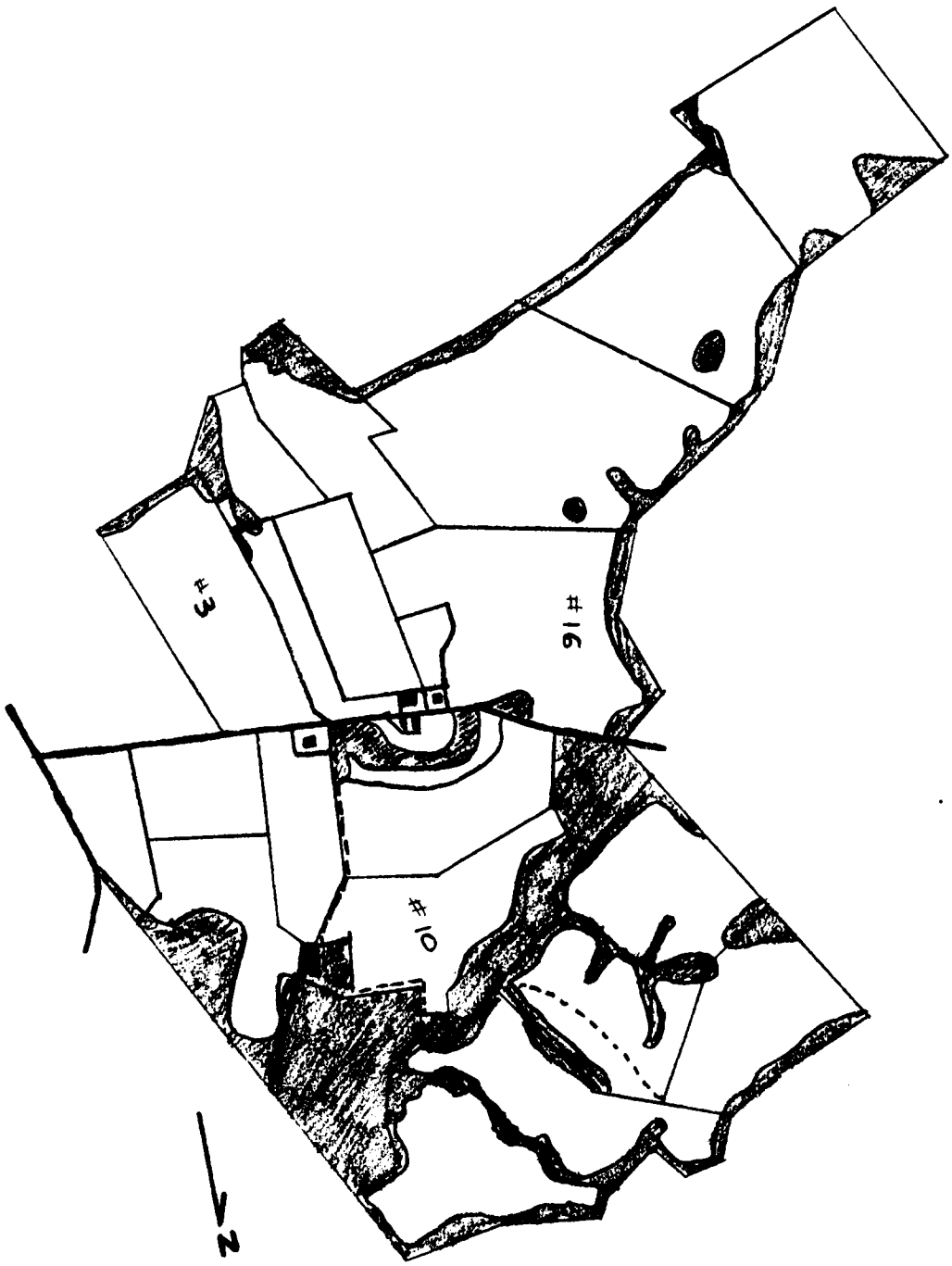

FIGURE 1. Diagrammatic drawing of Grassland Farm Plateau Experiment Station, showing relative positions of the three experimental herds.

TABLE 1. Treatment, Formulation, and Proposed Feeding Rate of A.I. per Day for Experimental Herds at Grassland Farm Experiment Station.

Herd No.	Treatment	Formulation (Percent)	Proposed Feeding Rate of A.I. per Day
10	Untreated		
16	Rabon	1.94 Rabon Oral Larvicide	.5 mg/kg body wt
3	Dimilin	.58 Dimilin	.5 mg/kg body wt

adult flies from the nearby pastures these control measures should not be significant in effecting the insects within the cow manure.

Ten manure samples from each pasture were gathered before any treatment on April 15 to ensure collecting methods and materials were working and to get some idea about which insects were already present. During the course of the experiment, manure samples were collected every two weeks from the three fields. Ten samples selected at random were collected from each treatment on each date. All samples collected were at least two days old and not older than five days. This was to provide ample time for many insects to visit the manure, and to prevent a significant mortality of the fragile, immobile eggs, caused by the handling of the manure. The pats also were lying in an area exposed to the sun, either all or part of the day. Manure lying in complete shade has a considerable different insect fauna (Poorbaugh et al., 1968).

Due to the small size of Burlese funnels (1 gallon), the entire manure sample size was standardized by volume, each sample ranging from 700 to 800 ml of manure. Thus, each sample consists of only part of a manure pat, roughly one-third to one-half. Also, due to the successional nature of the fauna of the manure community (Mohr, 1943), only those insects present in the manure at the time of collection are recovered. This is opposed to field emergence sampling where the manure is left in the field covered by a one-way screen and the natural succession is allowed to continue, with insects continuously arriving according to their successional patterns.

After the samples were collected, they were returned to Knoxville, Tennessee, where they were individually set into one-gallon Burlese

funnels for two weeks. This was found to be ample time for larval and pupal development of both flies and beetles. The contents of 73 percent of the samples collected dropped into small jars of alcohol where they were preserved until identifications could be made. The remaining insects were collected in crisper dishes containing sand so that inspection of pupal cases was possible. Specimens were sent to the Systematic Entomology Laboratory (ARS) and the Smithsonian Institution for identification.

Two separate field observation studies were designed to further investigate the impacts of the two chemicals on the manure. The first experiment was designed to check the degradation rate of both the treated and nontreated manure. Sixty individual pats were used, 20 from each treatment pasture. On June 10, 1976, 10 manure pats from each treatment were staked with tags to help find them in the future. All manure pats were obviously fresh, deposited that day. From that point on, until their incorporation back into the soil, all 30 pats were observed each week.

On July 1, 1976, the above experiment was replicated. Physical characteristics of the droppings were recorded as well as insect activity in and on the droppings.

The second field observation study involved the effects of both chemical treatments on one particular insect, Sphaeridium scarabaeoides (L.) a hydrophilid beetle which is an important predator of fly eggs and is present (adult) on the manure during the first few hours. It tunnels along the top of fresh manure while feeding voraciously on fly eggs (Figure 2). As the manure's upper crust began to dry, these tunnel

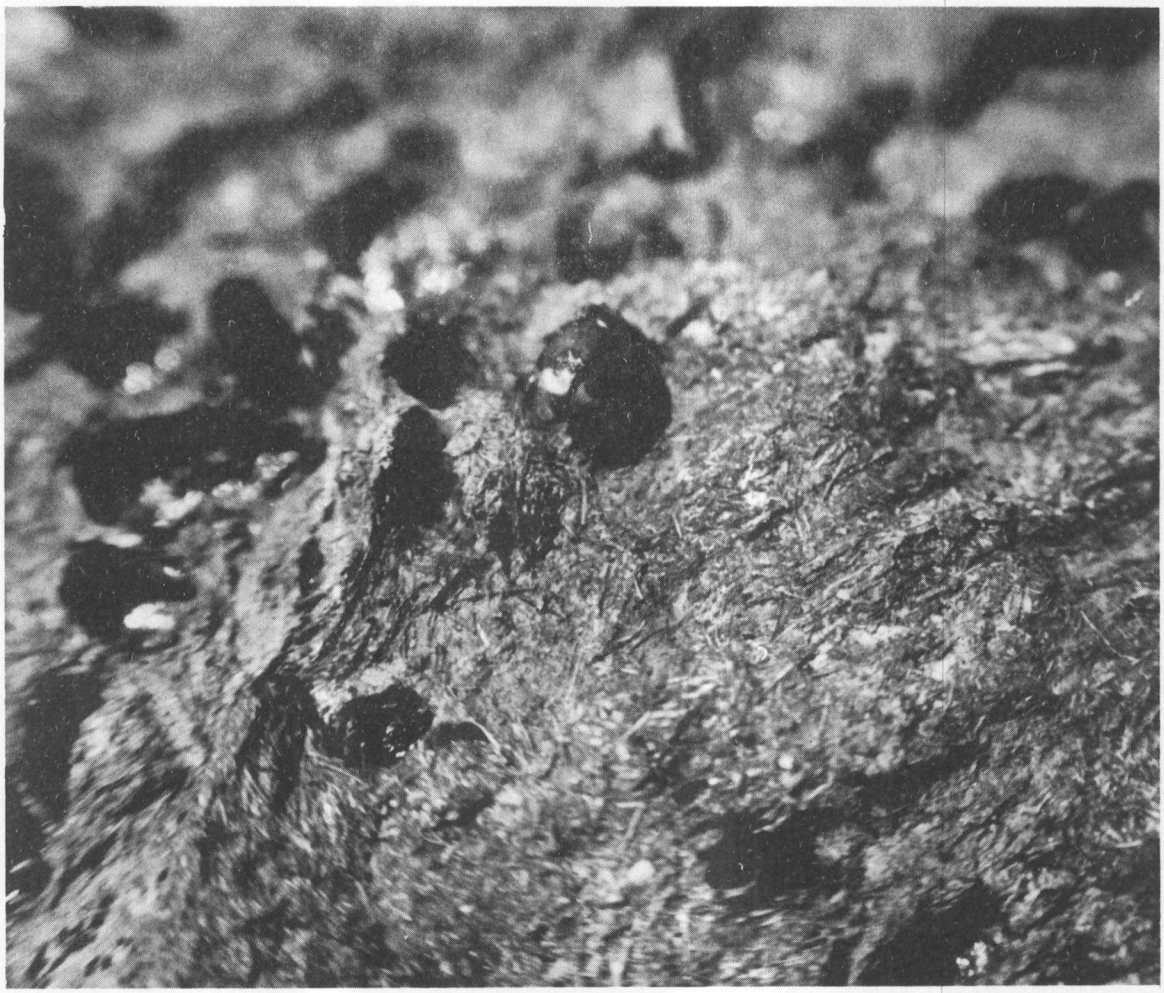

FIGURE 2. Sphaeridium scarabaeoides tunnelling through the bovine manure pat.

entrances remained intact and were counted at a later date to show the relative population of this insect's presence in each of the three treatments. Tunnel entrance scores were made by counting the number of holes in a 25 cm^2 square placed over the manure's crust. Three measurements (75 cm^2) were made for each manure pile placing the square over the middle and any two outer regions of the pat. The first 15 droppings found in each treatment pasture were counted.

Over 10,000 insects were collected from 330 manure samples. Some specimens were identified by the author but most were identified by the following: Dr. R. J. Gagne, Diptera (ARS); Dr. W. W. Wirth, Diptera (ARS); Dr. D. R. Smith, Hymenoptera (ARS); Dr. R. D. Gordon, Coleoptera (ARS); Dr. G. C. Steyskal, Diptera (ARS); Dr. E. W. Baker, Acarina (ARS); Dr. P. J. Spangler, Coleoptera (ARS); Dr. F. C. Thompson, Diptera (ARS); Dr. P. M. Marsh, Hymenoptera (ARS); and Dr. O. L. Cartwright, Coleoptera (U. S. National Museum).

The data were analyzed by a two-way analysis of variance on a 360IBM computer using the Statistical Analysis System (SAS 72) designed by Barr and Goodnight (1972). All data used in the analysis were transformed by the logarithmic transformation (Log x + 1) in order to stabilize the variance. Sixteen indicator species of insects were analyzed for both the effect of treatment and date on the occurrence of these insects in the manure.

CHAPTER IV

RESULTS AND DISCUSSION

Most cattle on pasture in Tennessee consume an average of 1 ounce of NaCl per day (McClaren, personal communication, 1976). Since the key to this experiment rested in the fact that the cattle would ingest a certain amount of salt per day (therefore insecticide was ingested), salt consumption was monitored in the treatment pastures. Results as shown in Table 2 proved consumption to be adequate and fairly stable. Neither Rabon Oral Larvicide or Dimilin proved to be unpalatable to cattle.

Although only 16 insect species were used as indicator species in this experiment, many other coprophilic fauna were encountered. Many were never identified because of the scope of this study, but several other species were identified and are included in the composite list with the indicator species in Table 3.

Sixteen species were found in sufficient numbers to be analyzed as indicators to help determine the impacts of the feed through chemicals. Of the 16 species, the occurrence of 4 was significantly effected, as shown in Table 4.

When Rabon Oral Larvicide and Dimilin were fed to cattle, face fly larvae were greatly reduced or completely eliminated in many manure samples. No face flies were found in 87 percent of both the Dimilin and Rabon Oral Larvicide treated manure samples (Table 5). Only 28 percent of the untreated manure samples were void of face fly larvae.

TABLE 2. Salt-Insecticide Consumption by Cattle in the Treatment Pastures.

	Ounces/Head/Day	
	Rabon	Dimilin
April 15 - May 3	1.03	1.16
May 3 - May 13	.75	.97
May 13 - May 27	1.0	.92
May 27 - June 10	.97	.95
June 10 - June 17	.74	.89
June 17 - June 23	1.07	.60
June 23 - July 1	1.1	1.3
July 1 - July 14	1.0	.82
July 14 - July 27	.84	.94
Mean	.95	.95

TABLE 3. A Composite List of Insects and Mites Found in Bovine Manure in Cumberland County, Tennessee, 1976.

Insect	Mite
Diptera	
Musca autumnalis DeGeer	(Muscidae)
Sargus cuprarius (L.)	(Stratiomyidae)
Phoenicia sp. Robineau-Desvoidy	(Calliphoridae)
Ravinia sp. Robineau-Desvoidy	(Sarcophagidae)
Bradysia sp. Winnertz	(Sciaridae)
Psychoda sp. Latreille	(Psychodidae)
Sepsis neocynipsea Melander and Spuler	(Sepsidae)
S. punctum (Fabricius)	(Sepsidae)
Leptocera sp. Olivier	(Sphaeroceridae)
Coleoptera	
Aphodius ruricola Melsheimer	(Scarabaeidae)
A. stercorosus Melsheimer	(Scarabaeidae)
A. haemorroidalis (Linnaeus)	(Scarabaeidae)
A. fimetarius (Linnaeus)	(Scarabaeidae)
A. granarius (Linnaeus)	(Scarabaeidae)
A. lividus (Olivier)	(Scarabaeidae)
Ataenius sp. Harold	(Scarabaeidae)
Onthophagus pennsylvanicus Harold	(Scarabaeidae)
Hister abbreviatus Fabricius	(Histeridae)
Cercyon sp. Leach	(Hydrophilidae)
Sphaeridium scarabaeoides Linnaeus	(Hydrophilidae)
Staphylinidae Leach	
Hymenoptera	
Aphaereta pallipes (Say)	(Braconidae)
Cermatogaster sp. Lund	(Formicidae)
Trichopria sp. Ashmead	(Diapriidae)
Acarina	
Parasitus sp. Latreille	(Parasitidae)
Macrocheles medarius Latreille	(Macrochelidae)

TABLE 4. Average Number of Insects per Manure Sample in Cumberland County, Tennessee, 1976.

	Treatment		
	Control	Rabon	Dimilin
Musca autumnalis	13.8	1.55**	2.56**
Sargus cuprarius	8.82	12.96	7.0
Phoenicia sp.	1.5	1.42	.45
Ravinia sp.	3.82	.89**	2.64
Aphodius spp. (six species)	5.21	7.68	6.83
Ataenius sp.	.4	4.74*	.45
Onthophagus pennsylvanicus	.27	.109	.25
Hister abbreviatus	.21	.3	.27
Cercyon sp.	2.67	3.3	3.78
Sphaeridium scarabaeoides	1.6	.16**	.25**
Staphylinidae	1.0	.70	1.74

*Significant at the 5 percent level ($p \leq .05$).

**Significant at the 1 percent level ($p \leq .01$).

TABLE 5. Frequency of Occurrence in Manure Samples for Indicator Species, Cumberland County, Tennessee, 1976.

	Percent		
	Control	Rabon	Dimilin
Musca autumnalis	72	13	13
Sargus cuprarius	47	50	43
Phoencia sp.	17	13	9
Ravinia sp.	47	9	41
Aphodius ruricola	9	18	19
A. stercorosus	36	34	22
A. haemorroidalis	20	22	23
A. fimetarius	5	6	5
A. granarius	5	18	9
A. lividus	28	36	32
Ataenius sp.	19	33	18
Onthophagus pennyslvanicus	17	5	10
Hister abbreviatus	20	14	17
Cercyon sp.	42	55	61
Sphaeridium scarabaeoides	34	10	14
Staphylinidae	25	34	28

Although these results show excellent control of face fly larvae in the manure, the control of adult face flies on the cattle in the field was erratic (unpublished data, Gerhardt). Immigration pressure of the adult face flies from nearby cattle pastures most probably account for the major difference.

The numbers of one nontarget species, Ravinia sp., were significantly reduced by Rabon but not by Dimilin. Numbers of Ataenius sp. were significantly increased by the Rabon treatment. The reason for this phenomenon cannot be explained by the author, but Table 6 shows the data involved. During the fall, large numbers of Ataenius sp. appeared in the Rabon treated dung, and not in the other treatments.

Even though the population, Sphaeridium scarabaeoides (hydrophilid predator), was shown to be significantly reduced by both treatments, the reasons are unknown. S. scarabaeoides is a predator on various fly eggs and larvae, and since two species of flies were reduced significantly (Musca autumnalis and Ravinia sp.), an important food source may have been removed from the habitat. From the data in hand, it is not possible to tell whether the population of S. scarabaeoides was reduced because of lethal doses of insecticides or from the absence of an adequate food supply.

Because the adult Sphaeridium scarabaeoides is an early visitor to the dung (10 to 15 minutes) and leaves on the second or third day, it is possible that manure samples were collected after this species had left. Thus, further investigations were warranted about this important predator. This species is unique because the insects' activities in the pile in search of fly eggs and larvae leave a definite tunnel and tunnel entrance.

TABLE 6. Number *Ataenius sp.* per Manure Sample, Cumberland County, Tennessee, 1976.

	Rabon	Dimilin	Control
May 3	1.2	0	0
May 14	.1	.1	.1
May 21	.1	.2	0
June 3	.1	.1	.1
June 16	0	.1	.2
June 30	.7	0	.2
July 14	.1	0	0
July 28	.5	0	0
August 12	7.2	1.4	.6
August 26	31.4	1.5	1.9
September 9	10.8	1.6	1.3

These tunnel entrances were counted on 15 randomly selected dung piles within each treatment, to indicate relative abundance and activity of the species. The means that are shown in Table 7 are the number of tunnel entrances per 25 cm^2 on the surface of the pile, using three replications per pile.

The data in Table 7 were tested by the analysis of variance method and both treatments showed a significant reduction in number of tunnel entrances. Here again, from the information in hand it is not possible to determine that the decreased relative abundance of this species was caused from lethal dosages of insecticides or an absence of a good supply.

Seasonal occurrence and relative abundance figures were constructed from data collected from the untreated manure pats (Figures 3 and 4). These figures represent base-line data on the presence of some of the more important occurring inhabitants of the natural bovine manure community.

Due to the suspected seasonal nature of the occurrence of insects which inhabit the bovine manure, the data were analyzed to determine the effect of date. The analysis was performed with data from all treatment groups, corrected for the effect of treatment. These results are found in Table 8. The populations of three of the four dipteran species, Musca autumnalis, Phoenicia sp., and Ravinia sp. remained relatively stable throughout the 18-week fly season. The occurrence of Sargus cuprarius was most abundant during the middle of the season during the hot, dry months of July and August.

Many of the coleopteran species, Cercyon sp., Staphyliniade, Aphodius haemorroidalis, and Aphodius lividus, occurred in relatively

TABLE 7. Number of Tunnel Entrances/25 cm^2 Created by Sphaeridium scarabaeoides.

	Control	Rabon	Dimilin
Sphaeridium scarabaeoides	10.33	3.61*	3.93*

*Significant at 5 percent level ($p<.05$).

1 = Musca autumnalis
2 = Sargus cuprarius
3 = Ravinia sp.
4 = Cercyon sp.
5 = Sphaeridium scarabaeoides
6 = Staphylinidae

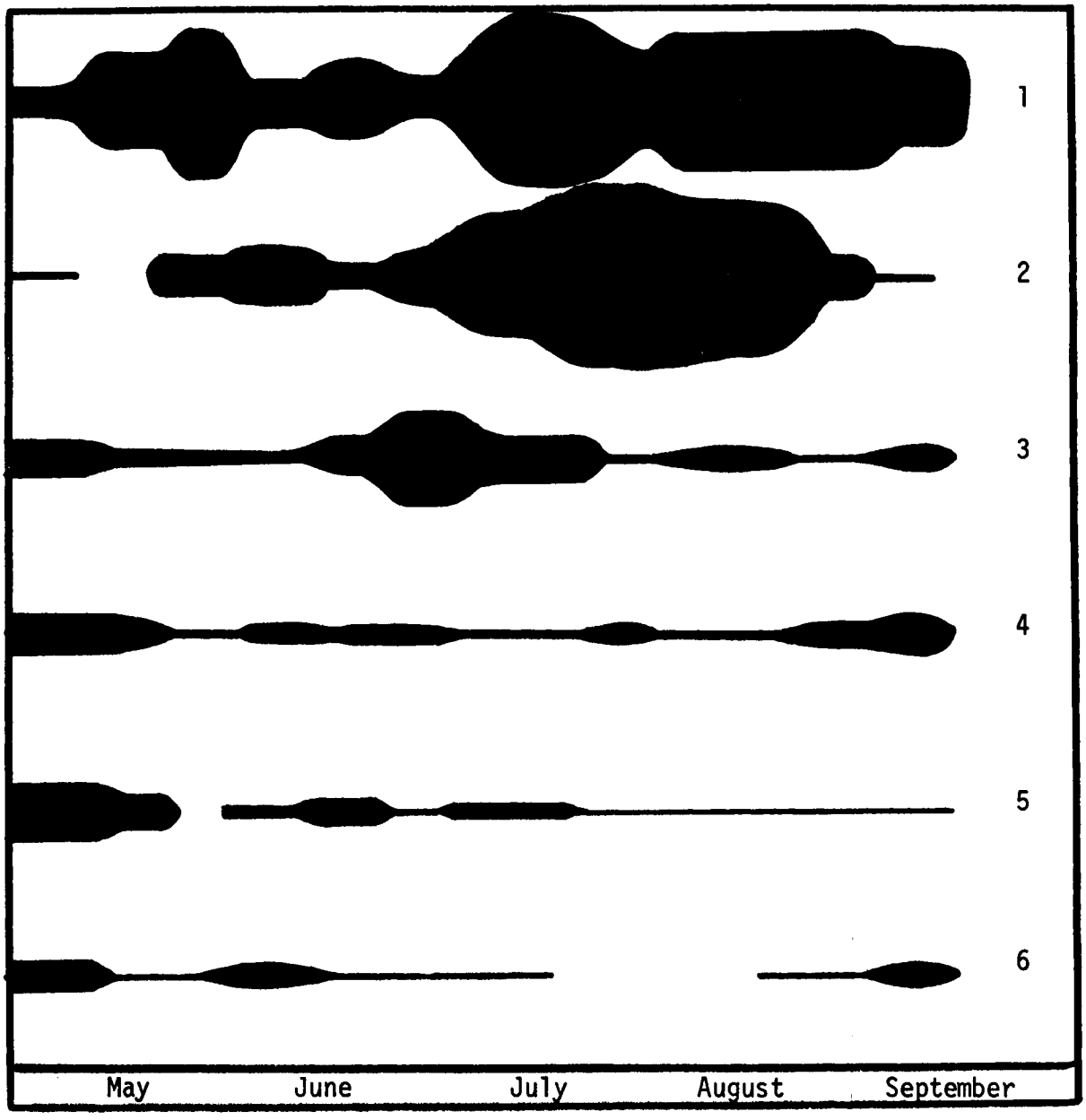

FIGURE 3. Seasonal occurrence and relative abundance of insects from the untreated manure in Cumberland County, Tennessee, 1976, with a scale of 1 cm = 1 insect.

1 = Aphodius ruricola
2 = Aphodius stercorosus
3 = Aphodius haemorroidalis
4 = Aphodius fimetarius
5 = Ahpodius granarius
6 = Ahpodius lividus
7 = Ataenius sp.
8 = Hister abbreviatus

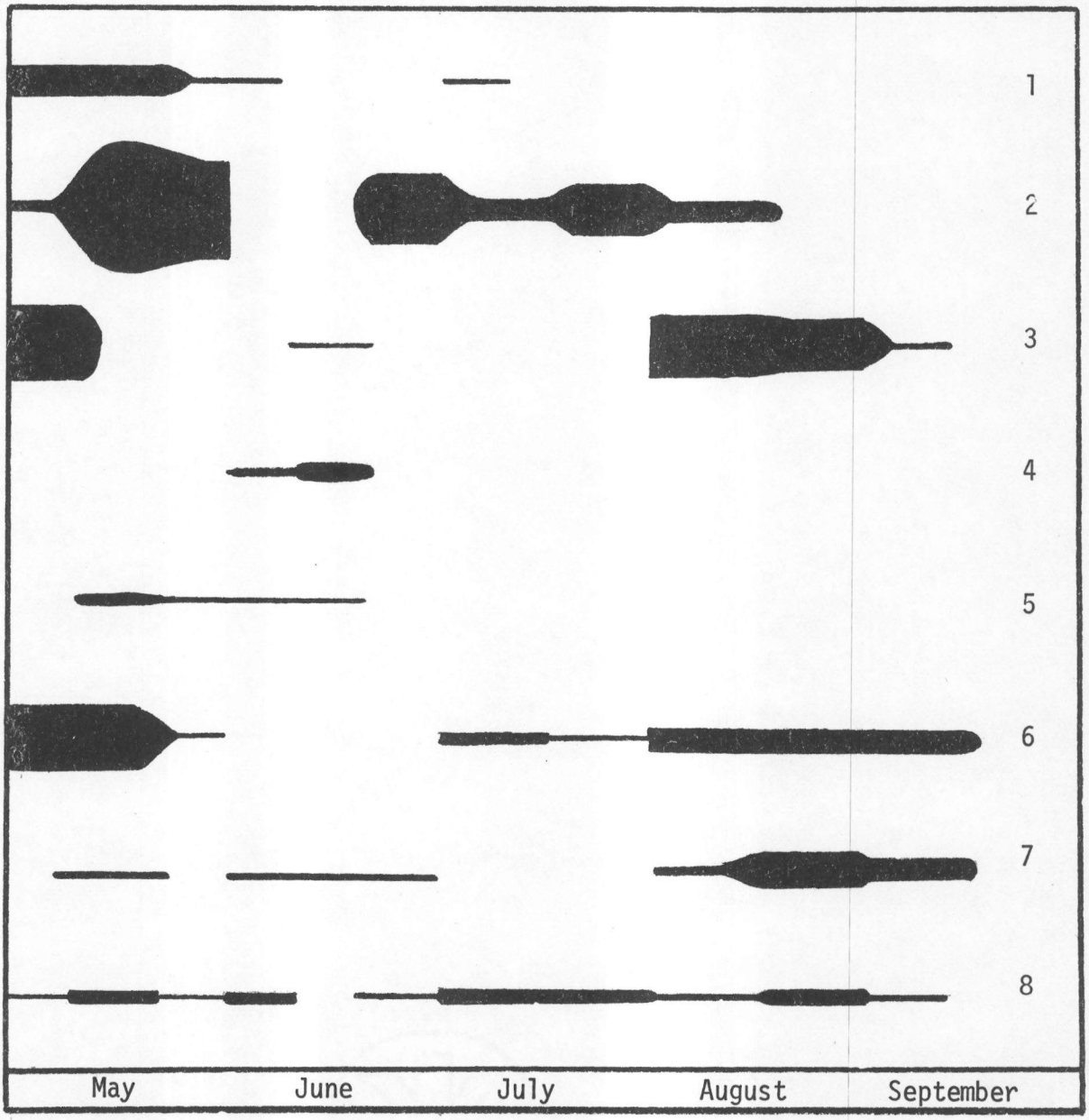

FIGURE 4. Seasonal occurrence and relative abundance of insects from the untreated manure in Cumberland County, Tennessee, 1976, with a scale of 2.5 cm = 1 insect.

TABLE 8. Analysis of Variance Testing the Effects of Date on Insect Populations.

Insect	Variance
Musca autumnalis	ns
Sargus cuprarius	**
Phoenicia sp.	ns
Ravinia sp.	ns
Aphodius spp. (six species)	*
Ataenius sp.	**
Onthophagus pennyslvanicus	ns
Hister abbreviatus	*
Cercyon sp.	**
Sphaeridium scarabaeoides	ns
Staphylinidae	*

*Shows significance at 5 percent level ($p < .05$).

**Shows significance at 1 percent level ($p < .01$).

high numbers during the early and late stages of the fly season. Aphodius ruricola, Aphodius stercorosus, and Sphaeridium scarabaeoides were most abundant during the early fly season and tapered off significantly during the remaining weeks. Ataenius sp. were present throughout most of the fly season but their numbers increased during August and September.

A biodegradation study of cattle droppings was conducted to see if the insects which were killed in treated pats had a considerable effect on the decomposition rate of bovine manure. Sixty manure pats were observed weekly until they were incorporated into the soil. Pats were inspected for moisture content, friability, insect life, and other physical characteristics to see how biodegradation was progressing. Results are presented in Table 9.

The results indicated in Table 9 show that neither insecticide interfered with the natural decomposition rate of cattle droppings in the pasture. Treated pats decomposed slightly slower than the normal pats, but no deleterious effects were observed.

As indicated by Tables 4 and 5, pages 31 and 32, respectively, the face fly seems to be the dominant species samples in the bovine manure community. It appears in 72 percent of all pats collected in the control group, averaging 13.88 larvae per sample. Because the face fly is an introduced pest, the natural control agents which keep the pest in check in Europe (its native home) are mostly not present in North America. For example, in Denmark, Hammer (1941) reports six species of predaceous maggots in droppings where the face fly breeds, compared to one in the United States (Sanders and Dobson, 1966; Poorbaugh, 1968).

TABLE 9. Manure Biodegradation Rates for Manure Pats Deposited June 10, and July 1, 1976, Cumberland County, Tennessee.

	Accumulative Number of Pats Incorporated into Soil		
Treatment (June 10)	34 days	44 days	54 days
Control (10 pats)	4	9	10
Rabon (10 pats)	1	7	10
Dimilin (10 pats)	0	7	10
Treatment (July 1)	40 days	51 days	56 days
Control (10 pats)	1	10	10
Rabon (10 pats)	0	8	10
Dimilin (10 pats)	0	7	10

Because of the new relationship between host and parasite, natural control is much less efficient here than in the hosts native country. Wingo (1970) studied the newly established relationships of the face fly and Aphaereta pallipes and observed the following. The parasite frequently killed the first instar host larvae by the act of oviposition and in other larval hosts, the larvae of the parasite died before reaching the pupal stage. And even when the parasite reached maturity it usually failed to emerge from the hard calcified pupal case. These and many other factors contribute to the success of the establishment of the face fly into North America.

Overall, the insect fauna of cattle droppings in Europe is reportedly much richer than in North America (Hammer, 1941), but this is to be expected since there are more native species of bovine in Eurasia than in North America. Correspondly, most of the major fauna of the dropping community which occur in North America have been introduced from Europe to the east coast and spread westward (Poorbaugh et al., 1968).

CHAPTER V

SUMMARY AND CONCLUSIONS

Both Rabon Oral Larvicide and Dimilin proved to be palatable to pastured cattle (Angus) in this experiment. Intake of the salt-chemical formulations were only .05 ounce per day lower when compared to the average consumption rate of untreated salt in Tennessee. Face fly larvae breeding in the manure were significantly reduced by Rabon Oral Larvicide and Dimilin treatments. Only 13 percent of these treated pats (both Rabon and Dimilin) contained face fly larvae, whereas 72 percent of the untreated pats were infested with face fly larvae. Although these results show excellent control of face fly larvae in the manure, adult face fly control on cattle was erratic. The difference resulting from immigration of adult flies from nearby pastures.

A seasonal occurrence and relative abundance chart was constructed to give base line data concerning the presence of some of the inhabitants within the bovine manure community. Statistical analysis showed that the date (season) was in fact a controlling influence on whether or not eleven species of insects were present in the manure at the particular sampling time.

Statistical analysis of the impacts of the two oral larvicides proved that neither acted indiscriminately by killing large numbers of beneficial insects. No reduction in the histers or staphylinids were exhibited, and no conclusive evidence showed the treatments reduced the

efficient hydrophilid predators. Fourteen of the fifteen beneficial species analyzed were not significantly reduced by either treatment. Because of this selectivity, no disruption of the interactions of the manure community were found. These beneficial insects, so important to the recycling of nutrient materials back into the system, were allowed to continue their role of helping incorporate the manure back into the soil.

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VITA

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