To the Graduate Council:

I am submitting herewith a thesis written by Stephanie Camille French entitled "Effects of pat size, simulated rainfall, and temperature on the mortality of immature face flies (*Musca autumnalis* DeGeer); and lateral migration of infective trichostrongyle nematodes onto vegetation in eastern Tennessee." 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 dissertation
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

EFFECTS OF PAT SIZE, SIMULATED RAINFALL, AND TEMPERATURE ON THE MORTALITY OF IMMATURE FACE FLIES (*MUSCA AUTUMNALIS* DEGEER); AND LATERAL MIGRATION OF INFECTIVE TRICHOSTRONGYLE NEMATODES ONTO VEGETATION IN EASTERN TENNESSEE

A Thesis

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Degree

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Stephanie Camille French

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DEDICATION

I present this thesis as a dedication to my parents

Phil and Vo French

for all of their love, support, prayers,

and sacrifices over the years

so that I could have a quality education

with innumerable and priceless experiences.

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ABSTRACT

The effects of pat size, simulated rainfall, and temperature manipulated by different shading regimes on face fly (*Musca autumnalis* DeGeer) immatures were studied at the UT-KES Small Grains Unit in Knoxville, Tennessee, from May to August 1998. Pats of 600 grams in size and a density of 24 grams/larva had the highest face fly larval mortality (66.4 %) and the heaviest individual pupal weights (0.029 g). Smaller pats (below 200 grams), regardless of larva/gram density, caused lower mortality (trial #1: 40.8 % and trial #2: 67.2%) and had the lowest pupal weights. First instar face fly larvae suffered the greatest mortality after being exposed to 3 hours and 122.5 mm of simulated rain. Unexpectedly low face fly larval mortality (40.4 – 42.8 %) occurred in pats with high moisture loss (71 – 72 %) and high internal pat temperatures (44 – 47°C). The highest face fly larval mortality (60 – 70 %) was from pats with the lowest moisture loss (53 – 60 %) and the lowest internal pat temperatures (31 - 35°C).

An additional study was carried out to examine the lateral migration from cow pats of Trichostrongyle larvae at the UT-KES Small Grains Unit, Knoxville, Tennessee, from November 1998 to June 1999. *Cooperia* was the only trichostrongyle nematode recovered throughout the study. Four weeks after the November plot contamination, larvae of *Cooperia* spp. were recovered 5 cm away from the pat and were still present on the forage 6 months later.

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Chapter I

THE FACE FLY: *Musca autumnalis* (DeGeer)

Originating from European and Asiatic regions, the face fly, *Musca autumnalis* DeGeer (Muscidae: Diptera), was first reported in North America in 1952 in Nova Scotia, Canada. The first documented appearance in the United States was on Long Island, New York, in 1953 (Pickens and Miller 1980, Teskey 1969, Vockeroth 1953). Since its introduction, the face fly has spread throughout southern Canada and the area north of the 33rd parallel in the United States. This fly is typically found in the more temperate regions of this country, such as the eastern United States along the mountainous areas (Teskey 1969, Turner and Hair 1966), and is not to be found in Arizona, New Mexico, Texas, Louisiana, or Florida (Pickens and Miller 1980). In Tennessee, the face fly was first reported in 1974 (Burgess 1981).

The primary host of face flies is cattle, but occasionally one can find face flies on horses or swine (Bay et al. 1968, Teskey 1969). The typical life cycle under field conditions is as follows: eggs hatch within 24 hours of oviposition, larvae develop through three instars during the next three to five days, and proceed through the pupal stage which lasts from seven to ten days. The cycle is dependent on temperature and can be completed within 12 – 21 days (Pickens and Miller 1980, Teskey 1960, Teskey 1969, Burgess 1981).

Eggs are yellow-white, 3.1 mm long and 0.5 mm wide, and have a breathing tube or mast at one end. The first two larval instars are whitish-yellow, but as they develop, they become bright yellow. Pupae are dirty white in color, five to seven millimeters in length, and have a calcified texture to their cases (Cumming and Cooper 1998, Teskey 1969). Adults resemble house flies, but are slightly larger and darker, averaging seven to eight millimeters in length (Figure 1.1) (Cumming and Cooper 1998, Burgess 1981). Male face flies can be easily differentiated from females by looking at their abdomen. The majority of the male abdomen is dark orange-red with a dark band running down the middle of the tergites, whereas the female abdomen is mainly dark gray-black in color (Teskey 1960). Adult longevity is from 20 to 50 days and is dependent on air temperature (Pickens and Miller 1980).

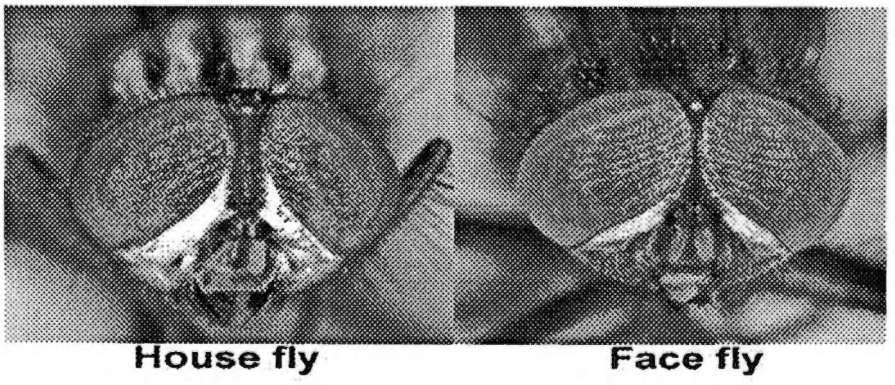

Figure 1.1. Comparison of a male house fly and face fly. Notice the face fly thorax is darker and slightly larger than the house fly.
Source: Cumming and Cooper 1998.

Mating occurs among flies 3 – 7 days old (Teskey 1969). Males will mate up to four times in their adult lifetime whereas females will mate only once, storing sperm and using it as needed (Pickens and Miller 1980). Typical of muscids, face fly females have polytrophic ovaries. The average number of mature eggs in a single batch is 20 to 26 eggs, and can be as high as 31 to 36 eggs (Killough and McClellan 1965, Teskey 1969). Different reports state that the initial ovarian cycle begins from the first to third day (Killough and McClellan 1965, Teskey 1969) or the fifth day (Pickens and Miller 1980, Teskey 1969) after emergence and cycles every two to three days afterward (Killough and McClellan 1965, Teskey 1969). During each cycle, all eggs mature at the same time and are usually laid at different times during that particular cycle. A female may lay two to eight batches of eggs in her adult lifetime (Killough and McClellan 1965) and has the potential to lay 141 to 230 eggs (Teskey 1969). A female will contain either full sized, mature eggs or none at all.

This fly species is probably a link between secretion lapping and blood-sucking Dipterans and is typically referred to as a facultative blood feeder (Turner and Hair 1967). It is thought that before becoming involved with pastured cattle their primary source of nourishment is nectar (Teskey 1960), which is still an essential food source for the flies during early spring before cattle are put out to pasture (Turner and Hair 1967). Host attractants appear to be odor, blood and perspiration (Teskey 1960). The face flies feed on the host's secretions such as fluids from the eyes, nose, mouth, and wounds, as well as nectar, sweat, and moist

manure (Cumming and Cooper 1998, Pickens and Miller 1980, Teskey 1969). Sugar is essential for adult survival, while a combination of sugar and protein is needed for egg production. The addition of milk as well as other dietary protein sources such as blood to the diet in laboratory colonies also increases egg production (Turner and Hair 1967).

Bovine feces is the choice substrate for breeding and larval development (Teskey 1969). The face fly is one of the first organisms to visit a cow pat usually arriving within five or ten minutes after the manure was voided. Female face flies will begin to wander over the surface of the pat, feeding on the fluids and locating suitable oviposition sites. Twenty minutes after arriving on the pat they will start to lay their eggs in small clusters of five to eight eggs. (Teskey 1960, Teskey 1969). As the pat ages and the top layer begins to crust, the females will wonder for longer periods of time, searching for openings to lay their eggs (Teskey 1969). In areas where fly numbers are quite large, it is possible to count several hundred eggs in a single cow pat (Teskey 1960). Pats older than two to three hours begin to lose their attractiveness for feeding and oviposition due to the dried up texture (Teskey 1969, Valiela 1969). Very wet cow pats are also unattractive to face flies (Teskey 1969). In areas of a pasture where there is high cattle activity, i.e. around water, salt, or feed stations, few eggs are oviposited in the fresh cow pats (Teskey 1969). It was assumed to be due to disturbance during oviposition by the activity of the cattle (Teskey 1969).

Female face flies seldom oviposit on cow pats under heavy shade (Pickens and Miller 1980).

Larvae emerge from their eggs through longitudinal slits along the sides (Teskey 1969). Most face fly eggs are positioned so that when the larvae emerge, they enter directly into the dung. However, some larvae emerge on the surface because the eggs were laid on their side and not inserted into the pat. These larvae may be able to enter the dung (Teskey 1969). It was reported that an average of 2 – 5% of the eggs never hatch, probably due to infertility or the larvae getting stuck on the surface of the cow pat and drying out (Teskey 1969).

First and second instar larvae stay near the surface of a fresh pat to obtain air, but as a crust forms over the pat, they develop holes to maintain the air supply. They are also known to utilize holes and tunnels created by burrowing dung beetles (Teskey 1969).

In three- to four-day old cow pats, larvae could be found at all levels of the pat (Teskey 1969). The first two larval stages tend to move in a random fashion whereas third instars will cluster together in one area of the pat (Teskey 1969). Emergence of third instars has been observed to take place at all daylight hours (Teskey 1969).

Face flies overwinter as adults in houses, public buildings, and sometimes under loose bark (Benson and Wingo 1963, Teskey 1969). Both overwintering male and female face flies are unmated (Benson and Wingo 1963). Overwintering face flies leave their hibernation sites around April, and rarely enter any type of

building during the active season (April through late October or early November) (Teskey 1969). Low temperatures and short days influence overwintering adults to diapause, at which time oocyte maturation ceases and low temperatures (i.e. 5°C) are tolerated for at least four months (Pickens and Miller 1980).

In the early season just after overwintering, there are peaks in the face fly population due to the production of a new generation by the overwintering face fly adults that had mated prior to leaving the hibernation site (Teskey 1969). Population numbers then slowly decline for the lack of available breeding sites. Once cattle are pastured, the first summer generation is soon produced and the population peaks throughout summer and early fall (Teskey 1969). Peaks in population are due to mass hatchings and usually occur in May, late June, and early July (Pickens and Miller 1980, Teskey 1960). Toward the end of the active season, fertile females are replaced by infertile females and numbers start to drop off. This event can be seen both in field and laboratory conditions (Teskey 1969).

During daylight hours, face flies can be found throughout the pasture (Teskey 1969). Their activity rises during the morning hours and peaks in the early afternoon (Engroff et al 1972, Teskey 1969). Because of drier afternoon conditions, the flies need to feed to replenish their bodily fluids (Teskey 1969). Face flies feed during the day and rest at night (Teskey 1960). Breaks from feeding or nighttime rests take place on fence posts, blades of grass, tree canopies, and other objects in the pasture (Teskey 1969, Burgess 1981). Face flies are known to have a rapid and extensive dispersal, covering from 0.5 to 11

kilometers over three to five days (Pickens and Miller 1980). Only four percent of the fly population can be found on cattle or cow pats (Pickens and Miller 1980) and about 90 % of that small group are females (Teskey 1969). Males are rarely found on cattle (Teskey 1960).

Several environmental factors have been analyzed for possible influences on face fly survival and activity. High wind speeds, high relative humidity, animal posture and location all are factors that can affect fly activity (Benson and Wingo 1963, Engroff et al 1972, Hansens and Valiela 1967, Teskey 1969). When windspeeds exceed 10 mph, face fly activity is reduced largely due to mechanical inhibition (Benson and Wingo 1963, Teskey 1969). At high relative humidity, face fly populations were low in numbers and when relative humidity was low, the fly populations were high (Benson and Wingo 1962, Teskey 1969). Partial cloud cover was observed to have little effect on face fly activity, whereas consistent cloud cover, indicative of an incoming storm, reduced activity (Teskey 1969).

Rain reduced or terminated feeding and activity of face fly populations (Engroff et al 1972, Teskey 1969, Pickens and Miller 1980). It was noted that when rain was light, a few flies stayed on the cattle and fed, but when rain became heavier, all activity stopped (Teskey 1969). The flies did not return to feed for several hours (up to 24 hours) after the rain, especially after a heavy rainfall or a long duration of rain (Teskey 1969).

Maximum activity was observed between 26.7 – 29.4°C, while there was minimal activity under 15.5 – 18.5°C (Hansens and Valiela 1967). Face flies were observed to feed on cattle when temperatures were between 12.8 – 30.6°C; maximum numbers occurred at 30.6°C (Teskey 1969). In one study, light intensity from the sun was found to be the most important environmental factor on face fly activity (Hansens and Valiela 1967).

Cattle in the sun, either grazing or standing, had fewer flies than cattle lying down in the shade or near woods (Benson and Wingo 1963, Hansens and Valiela 1967, Teskey 1969). Teskey (1969) stated that the only environmental factors that have observable influences on face fly activity are temperature, rain, light intensity, and wind speed. Among these, temperature is the most influential.

Although higher numbers of face flies have been observed on cattle lying down than those grazing or standing, large numbers of flies can be found on grazing individuals set apart from the rest of the herd (Schmidtmann and Berkebile 1985). Face fly activity has been known to decrease when cows begin to aggregate (Schmidtmann and Berkebile 1985, Hansens and Valiela 1967). Clustering can provide several hosts in a small location and those on the periphery tend to have the highest densities (Teskey 1969).

No relationship was found between face fly numbers and the head hair color of cattle (Steelman et al. 1993). Face flies appeared to be less attracted to white cows and more attracted to dark cows. Attraction increased when the coloration of the coat was a pattern of black spots on white (Engroff et al. 1972). No

preference of flies was noted for the age, breed, or type (beef or dairy) of cattle (Teskey 1969). If there were any differences between beef and dairy cattle then that was attributed to different management strategies, i.e. dairy operations tend to be cleaner situations and the cows are taken off pasture twice a day which limits exposure to face flies (Teskey 1969). Face flies take advantage of calf births and feed on the placenta and amniotic fluids (Teskey 1969).

Studies on the biology and ecology of the face fly began in 1958 when it became apparent that this introduced insect was a pest to livestock. One reason that the face fly is considered a major livestock pest is that it induces avoidance behavior in cattle that reduces grazing, and possibly impacts weight gain and milk production (Teskey 1969). Large numbers of face flies cause cattle to stop grazing and aggregate in order to seek protection (Burgess 1981).

In a study to determine the effects of face flies on calf weight gains, variable results were obtained and face fly feeding had no lasting effect on weight gain, (Gerhardt and Shrode 1990). Twelve to seventeen face flies per animal (Figure 1.2) were observed to cause irritation, but the irritation did not affect weight gain or the amount of feed consumed (Arends et al. 1982). Animals in this study had optimum access to feed and no reports of eye disease.

Face flies can also cause mechanical damage to the eyes of cattle (Shugart et al. 1979). One fly per eye per day causes mechanical eye injury, therefore this density has been proposed as the economic injury level for face flies (Unison Services 1998, Shugart et al. 1979). The sharp prestomal teeth of the face

Figure 1.2. Face flies. Face fly adults feeding around the eyes and nose of their bovine host. Source: Cumming and Cooper 1998.

fly's labellae scrape the hosts' eyes as the insect feeds, resulting in surface lesions and other irritations, and exposing the eye conjunctivae to the eyeworm nematode, Thelazia spp. (Figure 1.3) and disease (Johnson et al. 1991). Infectious bovine keratoconjunctivitis (IBK: bovine pinkeye), is caused by the disease organism Moraxella bovis and is highly contagious (Gerhardt et al. 1982, Steve and Lilly 1965).

The face fly can serve as a mechanical vector in the transmission of bovine pinkeye, spreading the disease through direct contact within a herd as they fly to feed from one individual bovine to another (Gerhardt et al. 1982). The face fly may possibly be a mechanical vector in the transmission of bovine herpesvirus-1 (BHV-1) (Johnson et al. 1991).

Cow pats can support a number of insect species and different types of microflora and microfauna. A study carried out in Kerrville, Texas listed 103 insect species representing 45 families and five orders (Termitidae, Orthoptera, Coleoptera, Diptera, and Hymenoptera) (Blume 1970). In Tennessee, one Coleopteran subfamily (*Aphodiine*) alone was represented by seven species in cattle droppings (Dellinger 1994). Mohr (1943) established that cattle droppings are ecological units and structured communities with flies as first invaders

Face flies are attracted to bovine feces primarily for nourishment, oviposition, and larval development (Bay et al 1968). Microflora within the fecal substrate, such as bacteria, are an essential food source for immature stages of the face fly

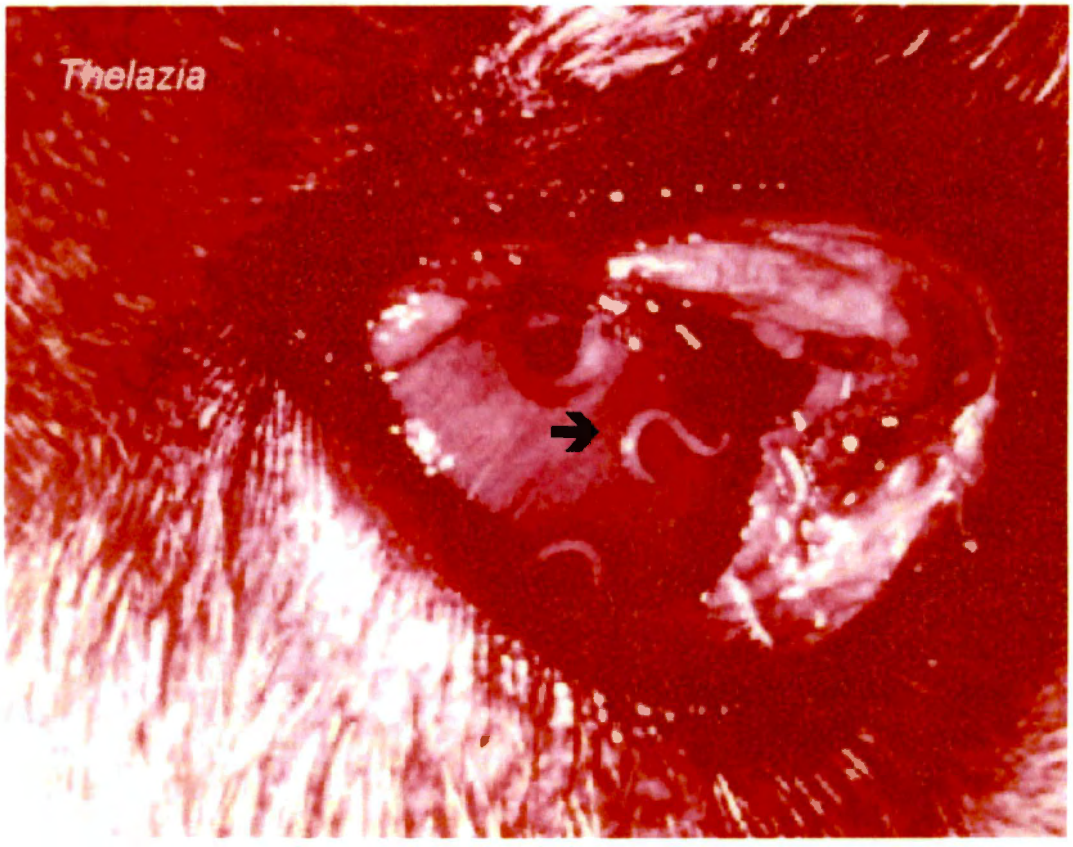

Figure 1.3. Thelazia spp. Nematode eyeworms in conjunctiva of a cow. Source: Corwin and Nahm 1998.

and possibly provide the larvae with accessory growth factors such as B vitamins and lactic acid (Hollis et al 1985). The moisture content of a cow pat is an important factor for face fly oviposition (Bay et al 1968). In field conditions, cow pats that were soft and had a moisture content of 80 – 85 % were attractive to face fly females up to two hours (Dougherty and Knapp 1993, Pickens and Miller 1980). Other potential breeding sites were tested for ovipositional preference, and it was found that sheep and deer feces were poor substrates due to the pellet form and low moisture content, and that the texture of horse manure was too coarse to allow for successful oviposition (Bay et al 1968).

Female face flies discriminated between cow pats from different dietary sources. Face flies preferred feces from animals that ate alfalfa-bromegrass over a bromegrass only diet, alfalfa being the key attractant in the diet (Dougherty and Knapp 1993, Treece 1966). In another report, feces from cows on a diet including alfalfa, with or without the addition of grain supplements, were most attractive to face fly females. Feces from animals on a grain or corn-silage were least attractive (Bay et al 1969, Pickens and Miller 1980). Changes in microflora and an increase in microbial fermentation as a result of dietary changes or compounds added to the ruminant's diet can adversely affect face fly larval stages (D'Amato 1980, Hollis et al 1985) and alter attractiveness of the feces to adult face flies (Treece 1966).

Face fly population densities are affected by amounts and properties of dung, larval competition, and natural predators (Dougherty and Knapp 1993). Most

field mortality appears to occur within the first day (Valiela 1969). Under field conditions, cow pats can be found in all sizes and densities. Food limitation can cause a reduction in pupal size and weight. This reduction in size is a first indication of competition and limited food resources (Valiela 1969). Valiela (1969) noted that when dung pat density was at or below 0.5 grams of dung per larvae, face fly pupal weight decreased. Dung supply affects the size of puparia, and emerging adults are stunted and die before completing expansion of wings (Moon 1980).

Higher muscids need a semi-aqueous medium on which to feed, and the churning of the dung substrate by face fly larvae could actually maintain pockets of dung fluid sufficient for feeding (Valiela 1969). Water content can be limiting when a pat dries out, preventing feeding by the face fly larvae and oviposition by adult females (Jones and Kunz 1996, Valiela 1969).

Studies on the relationship between moisture content and pupal weights had varying results. Treece (1966) found no correlation between pupal production and moisture content, but the author's values may be due to experimental error. They recommended that moisture should not be completely ruled out as having an effect on pupal production and weight. D'Amato (1980) reported a lack of significant relationship between larval mortality or pupal weight and fecal moisture content. However, in other studies, moisture content and pupal weights appeared to be correlated. With higher moisture content, the pupae were heavier, although percent adult emergence was not correlated to

moisture content (Bay et al. 1968). In a study in which fresh feces were compared to feces that was frozen and then reconstituted at 80 – 85 % moisture content, no significant differences were observed in pupal weight and percent adult emergence. However, no pupae were formed at 65 % and 95 % moisture content in the reconstituted pats (Bay et al. 1969). When face fly females were given the choice of fresh or reconstituted feces, they chose fresh feces for oviposition. Yet, when fresh feces were not available, the female flies oviposited on the reconstituted feces and larval development was found to be normal.

Cow pats and their inhabitants face the possibility of high moisture levels (above 90 %) due to heavy rain, flooding, or prolonged rain (Jones and Kunz 1996). The partially dried crust of a cow pat offers some protection during rain, yet when rain is heavy or persistant, the pat may become saturated, break apart, and spread out (Hughes 1979). As shown with the Australian bush fly, *Musca vetustissima* Walker, when a cow pat becomes waterlogged, fly larvae move to the surface or drown. Pupae within the soil either survive or drown, and eggs may get lost in the fluids (Hughes 1979).

Thomas et al. (1983) in Missouri reported that face fly prepupae migrating out of the cow pats were killed by midday temperatures which had exceeded 38 °C for most of the month. Face fly first instars seem to be the most sensitive to heat (Valiela 1969). Temperatures inside a cow pat are higher than surrounding daily air temperatures (Cook and Spain 1982, March and Bay 1983). Also in the field, there is a vertical temperature gradient within the cow pat in which the top

layer experiences extremes in temperatures, with reduced variation through the lower levels of the pat. Differences between the upper layer (0.5 cm) and lower layers of a cow pat are due to absorption of solar radiation at the surface of the cow pat (March and Bay 1983, Valiela 1969). Therefore, eggs and first instars are more likely to be affected by temperatures because they cannot move or move away from adverse temperatures as quickly as older instars (Valiela 1969).

Moisture and temperature affect the rate of development and pupal size of the buffalo fly, *Haematobia irritans exigua* deMeigere, (Cook and Spain 1982). Temperature also affects the rate of development and mortality of the horn fly (Palmer et al 1981). Horn fly, *Haematobia irritans irritans* (L.), immatures are unable to survive temperatures above 37°C and are less tolerant of high temperatures than the face fly. Thirty – eight percent of face fly larvae survived at 47 °C, and a maximum survivorship (68.8 %) was observed at 35 – 40°C (Palmer et al 1981, Valiela 1969).

Predation has been noted as the primary cause of mortality of early face fly instars (Thomas et al 1983). Predation by burrowing beetles caused substantial mortality (Valiela 1969) and parasites had little to no effect (Thomas et al 1983). Face flies are not a satisfactory host because parasites, typically Hymenopteran parasites, cannot emerge from their calcified puparium (Teskey 1969). Rapid growth of mold in a pat lowers the pH of the substrate which can cause face fly pupal death (Dougherty and Knapp 1993). Pupal mortality could

result from adverse influences by bacteria and pathogenic fungi (Burton and Turner 1970).

Dairy producers were the first to report problems with the face fly in 1958 (Ode and Matthysse 1964). When the face fly was acknowledged as being a pest to livestock, research on biology and control began in 1959 (Ode and Matthysse 1964). Hair and Adkins (1965) studied dust bags located in gateways and runways and cable backrubbers. Self-applicating devices gave an overall reduction of face fly population if they were set up over a large area (Turner 1965). Many insecticides and application methods were effective, but overall control was erratic. At first, poor control was thought to be due to low quality insecticides, but then it became clear that the dispersal and habits of the face fly were the cause of poor control (Pickens and Miller 1980). Recent control strategies combine various applications of insecticides in an integrated pest management approach. Applications currently in practice are: topical insecticides (permethrin, Dimilin – sprayed on manure); pyrethroid ear tags, organic phospate (O. P.) ear tags, spot-ons, and dust bags; subcutaneous injections of ivermectin; repellents; chemosterilants; and feed additives (Pickens and Miller 1980, Hutchinson 1998). Suggested integrated pest management strategies include cultural practices, such as keeping pastures clipped to eliminate resting and mating places, practicing good sanitation by spreading the manure in the field, and cleaning the manure from the pen as soon as possible (Burgess 1981, Hutchinson 1998). Stacking manure causes intense fermentation resulting in high

internal temperatures which will kill fly larvae and eggs. Prevention and destruction of fly habitat is recommended as an effective control (Peterson and Borcherding 1962). Control has been difficult because the face of a cow is a difficult area of the body to treat for pests, breeding places in pastures are nearly impossible to eliminate, and only a small portion of the population of face flies are on cattle at any one time (Burgess 1981, Pickens and Miller 1980). When the flies are not feeding on their host, they are off in the environment on objects in the field or blades of grass. Also, face fly populations redistribute themselves on a regular basis. If one farm experiences good control, new populations will move into the area. These habits make it difficult for a control program to estimate population size, to isolate specific problem farms, and to target the fly itself.

Other situations control are a decrease in pesticide availability, an increase in arthropod resistance to the available pesticides, an increase in cattle production costs, narrow profit margins, and a decline in livestock and poultry insect research in the past years. Thus, there is a need for a change in current arthropod pest management strategies. That change can come as the development and incorporation of an IPM program into U.S. livestock production. The present study was prompted by the need to research the mortality of face fly larvae under climatic conditions in eastern Tennessee.

The objectives of the face fly research described herein are to observe the effects of pat sizes, levels of rain, and temperature given by different shading

conditions on face fly larval mortality, under the climatic conditions of eastern Tennessee.

Chapter II

PAT SIZE

(i.) Introduction

Interest in the possibility that an intensive rotational grazing pasture management system could be employed as cultural control for pasture pests stimulated this investigation into the effects of pat sizes on immature face fly mortality. Even in areas where this particular management system is not in use, various sizes of cow pats occur in pasture conditions, and this size distribution may influence face fly populations.

It has been suggested that a combined influence of competition, dehydration, and dung quality could affect the size and the fecundity of the face fly under field conditions (Moon 1980). Under laboratory conditions, as dung supply per larvae decreased, the following results occurred: diminished fecundity at 3.4 gram/larvae, diminished size of adults at 1.0 gram/larvae, decreased survival to adulthood at 0.5 gram/larvae, and prolonged maturation at 0.4 gram/larvae (Moon 1980). The quantity of bovine feces required per larvae for maximal growth and development was determined to be 1.8 grams of feces/larvae where the heaviest pupal weight occurred at 27.7 mg. The highest percent of adult emergence (80.0%) occurred at 1.24 grams feces/larvae (Bay et al. 1970). Under field conditions, Moon (1980) found that at 3.8 gram of feces/larva, stunting occurred due to dehydration of dung and lower nutritive quality of the

dung. In laboratory conditions, a decrease in pupal weight occurred at a density below 1.25 grams/larvae, and a decrease in adult emergence at a density below 1.0 grams/larvae (Bay et al. 1970). When densities exceeded two larvae/gram, competition occurred (Moon 1980). Stunting basically indicated that the face flies were starving. Adult face fly females derived from larvae in a low supply of dung are stunted and must increase their feeding in order to restore their normal metabolic activity and be able to complete the first gonadotrophic cycle (Moon 1980). This could lead to an increase in feeding behavior resulting in an increase in annoyance and injury in cattle.

Cow pats in pasture conditions are found in various shapes and volumes due to the differences in amounts voided by cattle and by any pat disturbance caused by the trampling of cattle or burrowing of insects. A current pasture management system known as intensive rotational grazing is being investigated as a possible cultural control for pasture pests. The destruction of cow pats by a concentrated increase in cattle trampling as a result of high stocking rates in small sections of pasture is responsible for the control of pests (R. Moon and B. Stromberg, pers. comm.). The objective of this experiment was to observe the effects of different pat sizes on face fly larval mortality.

(ii.) Materials and Methods

Larvae: Throughout this study, a laboratory colony of adult face flies was

maintained in screened cages (30.5 x 30.5 x 30.5 cm) at 27 °C. They were fed a diet of one part fine granulated sugar and one part nonfat powdered milk. Water was supplied at all times (Bay et al. 1970, Wang 1964). In order to sustain the laboratory colony, the adult females were allowed to oviposit on manure-filled plastic dishes (100 mm dia. X 25 mm) placed in their cages once a week for one to two hours. The dishes were transferred to The University of Tennessee insectory on the Plant Science Unit located on Alcoa Highway, Knoxville, Tennessee where they were incubated at 27°C, 50% relative humidity, and a photoperiod of 16 hour light/8 hour dark. Pupae from the well-nourished face fly laboratory colony were weighed , and the average individual pupal weight was 0.0262 g (S. D. ± 0.0039).

For each experiment, the manure-filled plastic dishes with larvae were removed from the incubation room the day after the female flies were allowed to oviposit. Small portions of uninfested manure were weighted on petri dishes. First instar larvae were then removed from the incubation dishes with fine forceps and placed on the pre-weighed amounts of manure in each petri dish. The small portions of manure and larvae were then each added to more uninfested manure so that the combined weight made up the predetermined pat weights for all forty pats to be used in the experiment.

Manure: Freshly voided manure from mature dairy cows at The University of Tennessee-Knoxville Experiment Station (UT-KES) Dairy Unit was used for this

study. The cows were fed a diet mixture of corn silage, hay, and grain in bunkers, and were put out on rye-grass or fescue pasture twice daily. Manure was collected in a 75.7 L container, thoroughly mixed, placed into thirty plastic bags (ca. 2 kg manure/bag) for easier handling, and then frozen at −18 °C until needed. All experiments were conducted with manure that had been collected at one time. Sufficient numbers of bags of manure were set out one day prior to each experiment in order to allow the manure to thaw in time for preparation of the experiment.

Pans: Once the larvae were inserted into each pat, the pats were placed on about 2.5 cm of clean, white sand inside white plastic dishpans (14.0 cm deep, 20.3 x 30.5 cm) (Figure 2.1). Drainage holes had been drilled into the bottom of the pans and covered with small pieces of aluminum wire mesh to prevent any larvae from migrating out through the holes. Aluminum wire mesh lids were wired onto each pan to reduce larval mortality or disturbance of the pats and larvae by natural parasites or predators. Pans were numbered one through forty and were assembled with the designated pat size and larvae at the insectory. Pans were transported to the UT-KES Small Grain Unit.

Sand bed: A square (3.7 x 3.7 m) sand bed was constructed on an open grassy plot on the UT-KES Small Grain Unit, Knoxville, Tennessee (Figure 2.2). The

Figure 2.1. Cow pat in pan. 150 gram manure pat on sand inside a white plastic dishpan placed on the experimental sand bed.

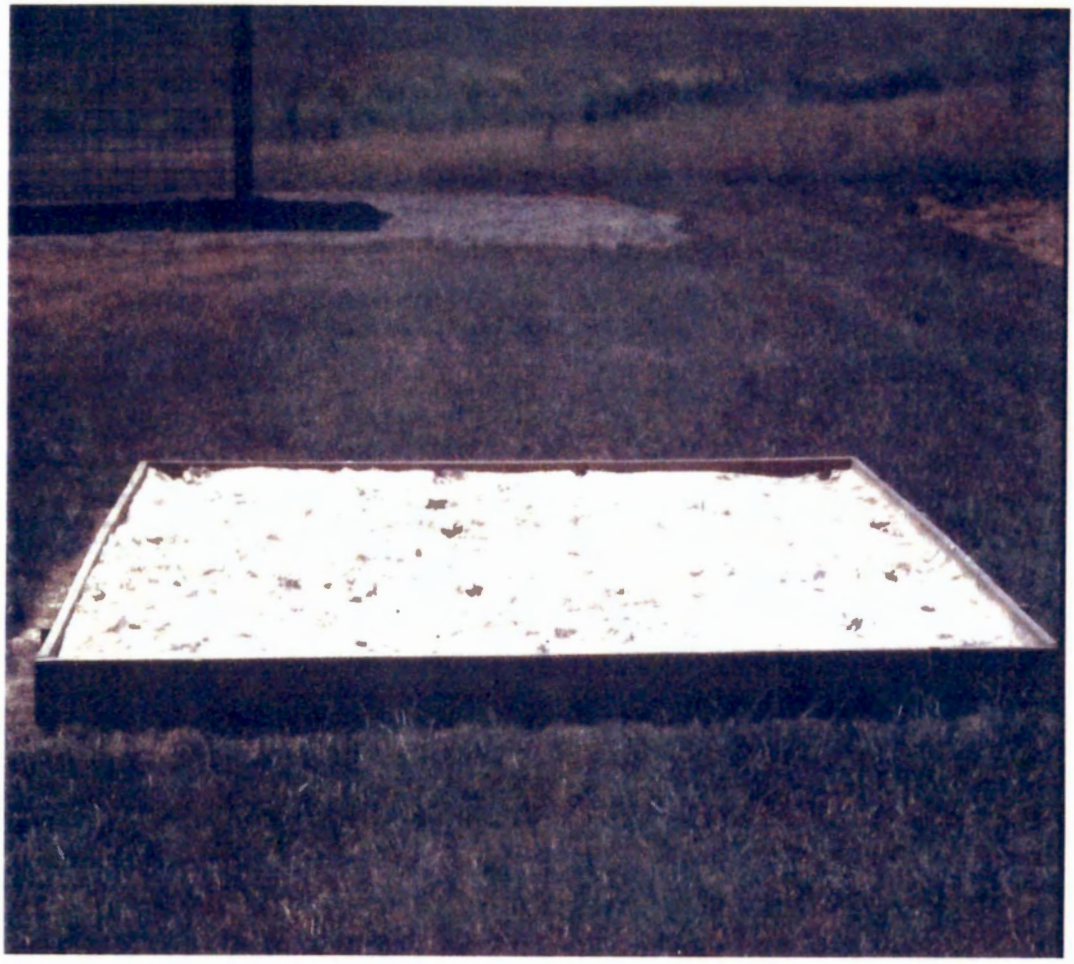

Figure 2.2. Experimental sand bed on an open grassy plot on the UT-KES Small Grain Unit, Knoxville, TN.

bed was constructed of four 3.7 m pressurized, wooden beams (5.1 x 15.2 cm) nailed together and supported to the ground at each corner by small 10.1 x 5.1 x 5.1 cm stakes. Fine Masonry sand (1250 kg) was deposited directly onto the grass inside the bed frame and smoothed out to a height of 15.2 cm flush to the height of the wooden frame. For all studies, with the exception of the shading experiment, all forty assembled pans were randomly placed in five rows of eight pans on the sand bed for each full week of experimentation. The purpose of the sand bed was to help absorb any excess moisture if rain occurred during each experiment.

Temperature probes: Four temperature probes (StowAway® XTI, Onset Computer Corp., Bourne, MA) were used to record the internal temperatures of the pats. Since only four probes were available, one probe was randomly assigned to a pat in each of the four replicates. The probe was inserted into the center of each pat and recorded temperature readings every 30 seconds for the one-week trial period.

Meteorological recordings: Daily precipitation was recorded on the UT-KES Small Grains Unit, Knoxville, Tennessee. Daily maximum and minimum temperatures were recorded at the UT-KES Plant Soil Science Unit, Knoxville, Tennessee.

Trial 1. Twenty-five larvae were added as described above to each of ten 75-gram pats, ten 150-gram pats, ten 300-gram pats, and ten 600-gram pats. The 75-gram pats were assigned to pans 1-10, the 150-gram pats to pans 11 – 20, the 300- gram pats to pans 21 – 30, and the 600-gram pats to pans 31 – 40.

Trial 2. A ratio of 3 gm/larva was used to vary the number of larvae in a given pat to observe any mortality due to effects of crowding or intra-competition. Therefore, 25 larvae were added to ten 75-gram pats, 50 larvae into ten 150-gram pats, 100 larvae into ten 300-gram pats, and 200 larvae into ten 600-gram pats.

In both trials, the forty assembled pans were exposed to natural conditions for seven days on the sand bed as described above. At the end of the week, the four probes were removed from the pats and all forty pans were taken off the sand bed and transported to the insectory. While at the insectory, the wire mesh tops were removed from each pan, the manure pats were re-weighed, and the results were recorded. The face fly pupae were sifted and collected out of the sand from each pan, put into a plastic specimen container designated for each group, and taken back to the laboratory to be counted, weighed as a group, placed back into the forty containers, and allowed to emerge. Once adults emerged, they were counted and released back into the laboratory colony. Mortality was determined by the difference between the initial larval numbers added to pats and adult numbers for each pat, and expressed as percent mortality. Percent mortality was compared among the four treatments. Moisture loss, determined as the

difference of the initial and end weights of each pat, was expressed as percent moisture loss and compared between each of the four treatments. The average individual pupal weights for each replicate were also determined. Percent mortality and percent moisture loss were statistically analyzed using ANOVA Least Significant Difference test (LSD, $P = 0.05$) and the average individual pupal weights for each replicate were analyzed using Duncan's Multiple range test ($P = 0.05$) (SAS 1987).

(iii.) Results and Discussion

All pats for both trials experienced a similar pattern of maximum and minimum internal temperatures as represented in Figure 2.3. Maximum temperatures, which ranged from 45 to 47°C, occurred from 1400 h to 1600 h. Minimum temperatures ranged from 17 to 19°C during 0400 h to 0600 h time period. Daily maximum and minimum air temperatures were recorded for trial #1 from June 19 through June 26, 1998 (Figure 2.4) and for trial #2 from June 29 through July 6, 1998 (Figure 2.5) at the UT-KES Plant Science Unit, Knoxville, Tennessee. Maximum air temperatures during the week of trial #1 reached 32.2 °C and minimum temperatures dropped to 17.2°C. During the week of trial #2, maximum air temperatures again reached 32.2°C, and the minimum temperature was recorded at 16.1 °C. Daily precipitation was recorded for both trials at the UT-KES Small Grain Unit, Knoxville, Tennessee.

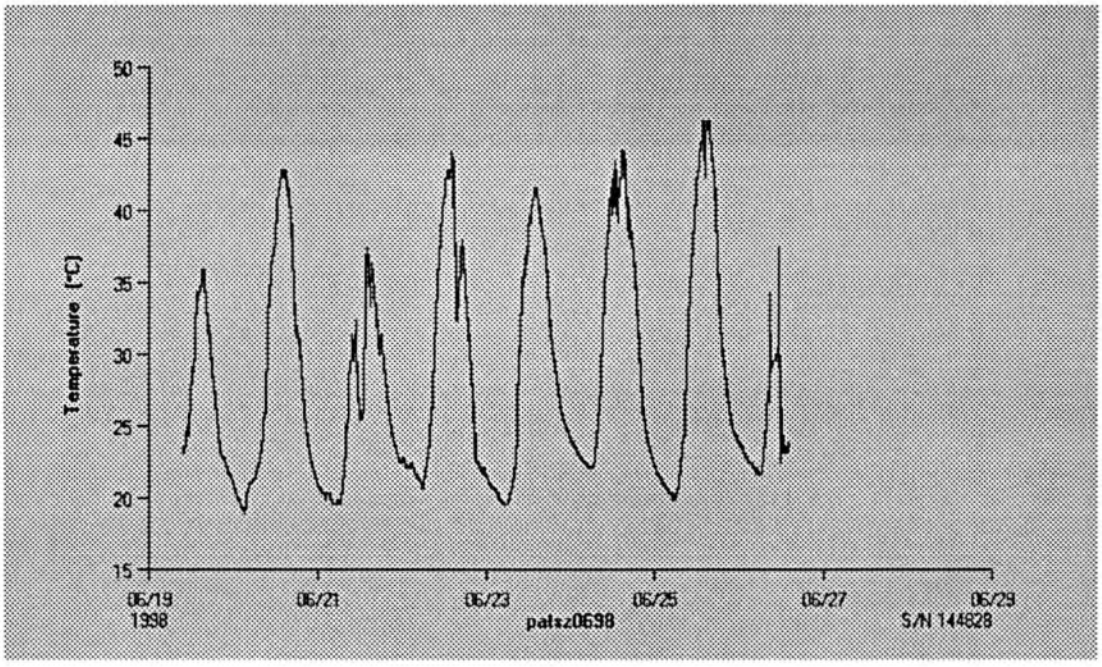

Figure 2.3. Internal pat temperature. A representative readout of the maximum and minimum internal temperatures recorded by the StowAway® XTI temperature probe for the pats in trial #1, 6/19/98 to 6/26/98, and trial #2, 6/29/98 to 7/6/98.

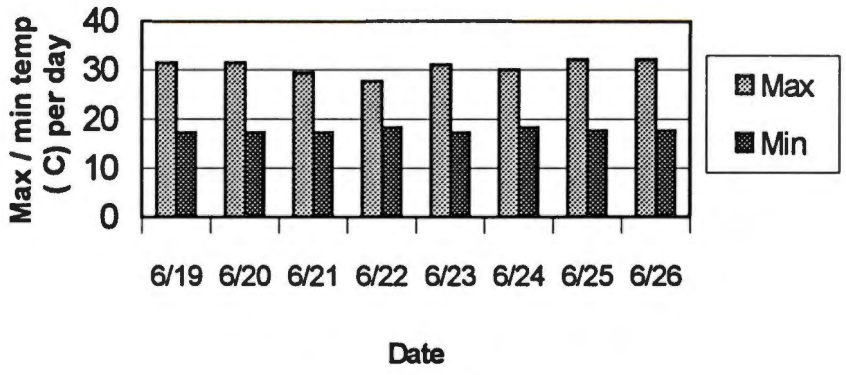

Figure 2.4. Daily maximum and minimum temperatures for trial #1, June 19, 1998 through June 26 1998.

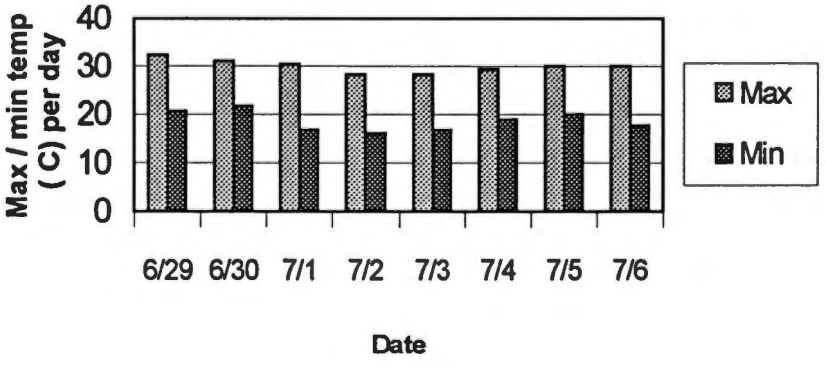

Figure 2.5. Daily maximum and minimum temperatures for trial #2, June 29, 1998 through July 6 1998.

For trial #1, precipitation occurred on three days, with the lowest level recorded as 1.52 mm and the highest as 22.35 mm. During trial #2, precipitation occurred only on two days, and those levels were 12.7 mm and 7.87 mm.

For trial #1, the 600-gram pats (24 g/larva) had the highest percent of mortality, 66.4 %, (Figure 2.6) which was significantly higher than the other three treatments. Percent mortality decreased as the pat sizes decreased, the 300-gram pats had 51.6 % mortality, the 150-grams pats had 45.6 % mortality, and the 75-gram pats had 40.8 %, but there were no significant differences among these pat sizes.

For all treatments in trial #2 (Figure 2.7), percent mortality was over 50 %, however there were no significant differences among the four pat sizes. Percent mortalities for the four pat sizes were determined as 60.95 % for the 600-gram pats, 55.30 % for the 300-gram pats, 64.2 % for the 150 gram pats, and 67.2 % for the 75-gram. Face fly survival has been observed to be affected by the volume (0.25, 0.5, and 1 L) of cow dung during the month of May but not during the month of July (R. Moon and B. Stromberg, pers. comm.). In this present study, trial #2 pats, which had 25 larvae/75 grams of feces, had much higher mortality than the 25/75 gram pats in trial #1 (the highest mortality among all treatments in both trials), as well as higher moisture loss and lower pupal weights.

Maximum air temperatures during trial #2 were lower than maximum temperatures during the week of trial #1, however, minimum temperatures were higher for trial #2. There were also fewer events of rainfall in trial #2, therefore,

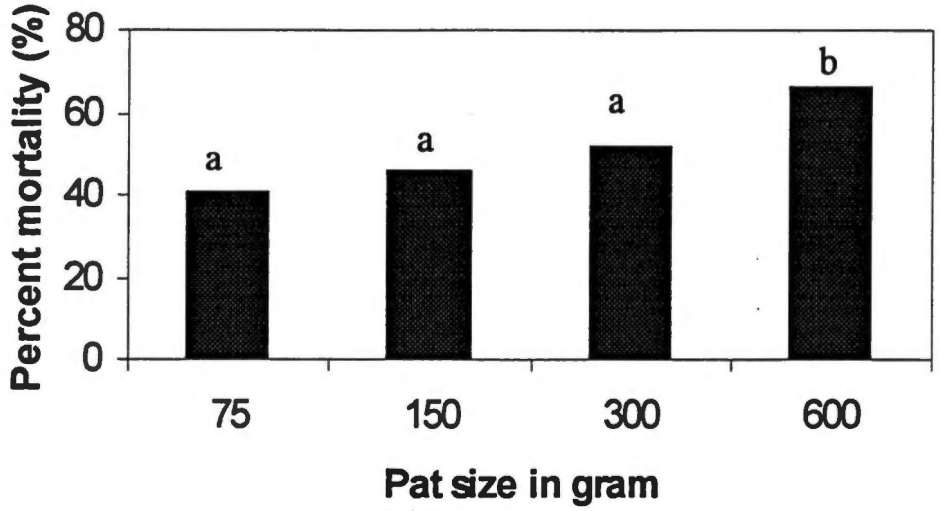

Figure 2.6. Percent mortality for Pat Size trial #1. All pats had a uniform number of larvae (25). Bars with the same letter are not significantly different according to LSD ($P = 0.05$)

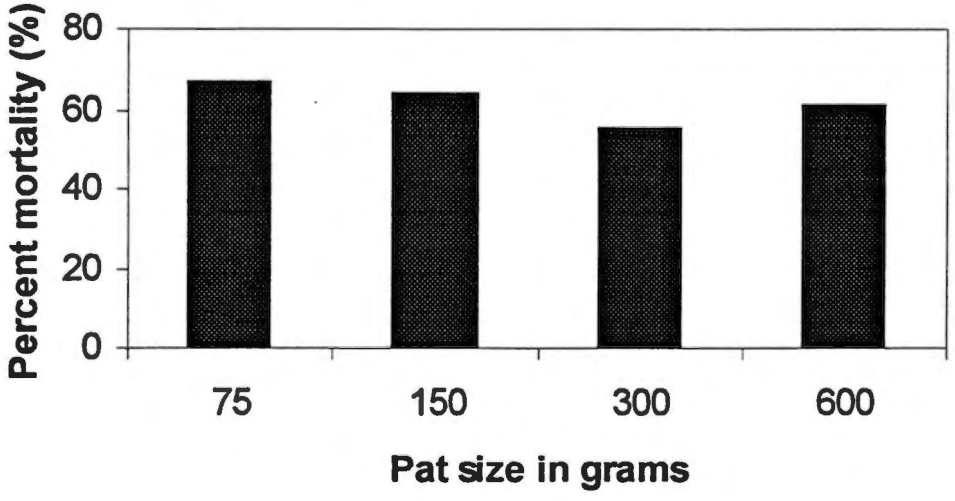

Figure 2.7. Percent mortality for Pat Size trial #2. All pats had a ratio of 3 gm/larvae.

conditions could have been much drier during trial #2 resulting in quicker desiccation of the smaller pats.

In trial #1 (Figure 2.8), pats originally weighing 75-, 150-, and 300-grams had the highest moisture loss (71.0 %, 71.7 %, 70.0 %, respectively), and were not significantly different. The pats originally weighing 600-grams had the lowest moisture loss (66.3 %) and were significantly different among themselves. Moisture loss could be low because of low surface-to-volume ratio in 600-gram pats preventing quick evaporation. Six day old pats show a decrease in water content by which time larvae are out of the pat. In this situation, pat moisture loss may not be an important mortality factor (Valiela 1969). A delay in drying could alter some cue for the larvae to migrate out of the pat and therefore prevent development into the pupal stages.

For trial #2 (Figure 2.9), the highest moisture loss was recorded again from the 150-gram pats as 73.9 %, and the lowest was from the treatment of 600-gram pats as 54.4 %. Percent moisture loss for the 75-gram pats and the 300-gram pats were 72.1% and 66.0 %, respectively. The 300-gram and 600-gram pats were significantly different from all other treatments. The 75-gram pats and the 150-gram pats were not significantly different from each other, but were significantly different from the 300-gram and 600-gram pats. Low pupal size is indicative of food supply shortage (Moon 1980). Pupal stunting as a result of low larval densities in to substrate is thought to occur due to the lack of available nourishment normally produced by the churning activity of higher densities of

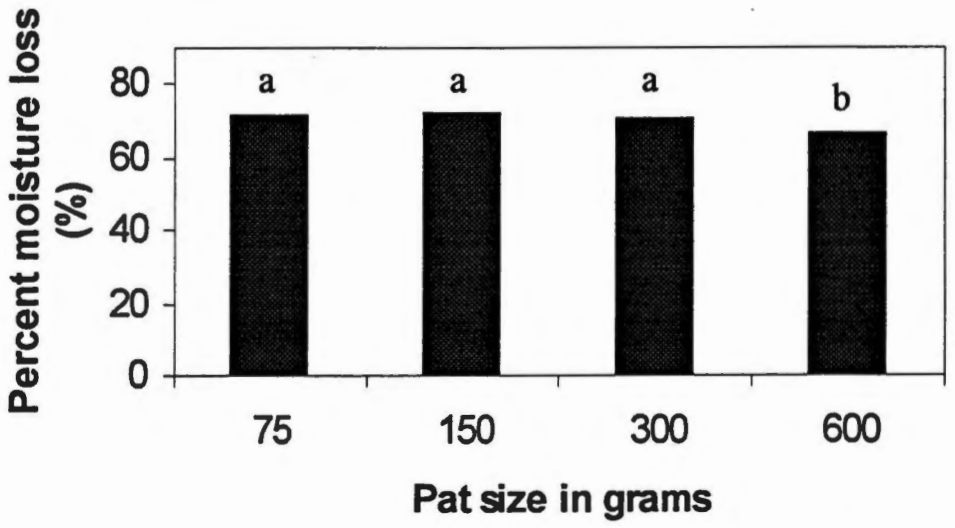

Figure 2.8. Percent moisture loss for Pat Size trial #1. All pats had a uniform number of larvae (25). Bars with the same letter are not significantly different according to LSD ($P = 0.05$).

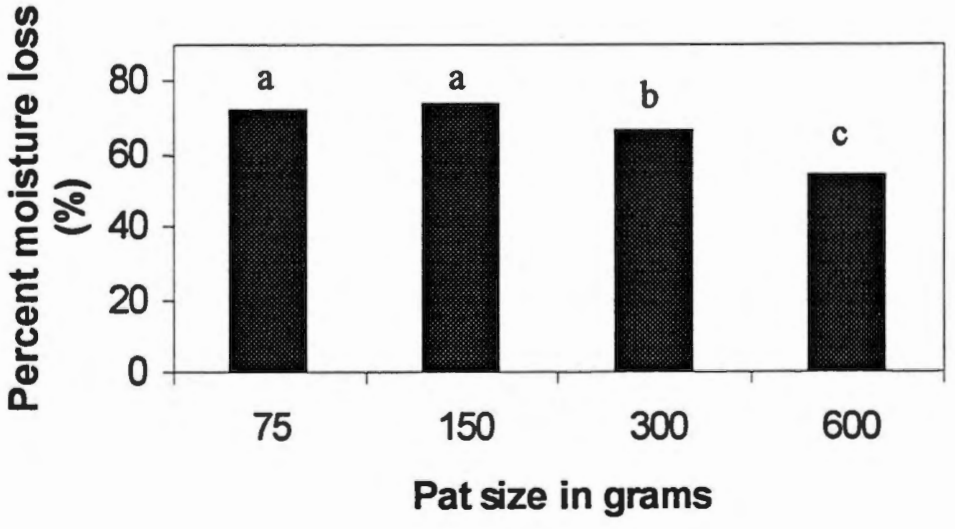

Figure 2.9. Percent moisture loss for Pat Size trial #2. All pats had a ratio of 3 gm/larva. Bars with the same letter are not significantly different according to LSD ($P = 0.05$).

face fly larvae, which keeps the substrate liquefied (Valiela 1969). At higher larval densities in substrate, competition could result in pupal stunting.

For trial #1, (Figure 2.10), there were significant differences among the average individual pupal weights of the four treatments. Average individual weights for the 75-, 150-, 300-, and 600-gram pats were 0.0258 g, 0.0270 g, 0.0293 g, and 0.0296 g, respectively. Pupae from the 75-gram pats were the lightest in weight and significantly different from the 300- and 600-gram treatments.

In trial #2 (Figure 2.11), where competition between larvae was stronger, the average individual pupal weights for the 300-gram pats (0.0234 g) and the 600-gram pats (0.0229 g) were significantly heavier than the other two treatment sizes (75 g: 0.0170 g, and 150g: 0.0203 g). The 75-gram pats in trial #2 was significantly different from all other treatments with the lightest pupae. This was an unexpected result because the four treatments in trial #2 were set up at the same ratio of 3 grams of feces/larva with the expectation that all results (i. e. percent larval mortality, pupal weight, and moisture loss) would be similar regardless of pat size. Although the ratio of g/larva was the same for the pats in trial #2, the smaller pat sizes seemed to quickly lose moisture due a greater surface–to–volume ratio. The quick drying of the 75-gram pats have limited proper larval nourishment resulting in pupal stunting or death. The opposite appears to have occurred in the larger pats, regardless of the ratio of grams of feces/larva, where the inner portion of the pat retained more moisture and may

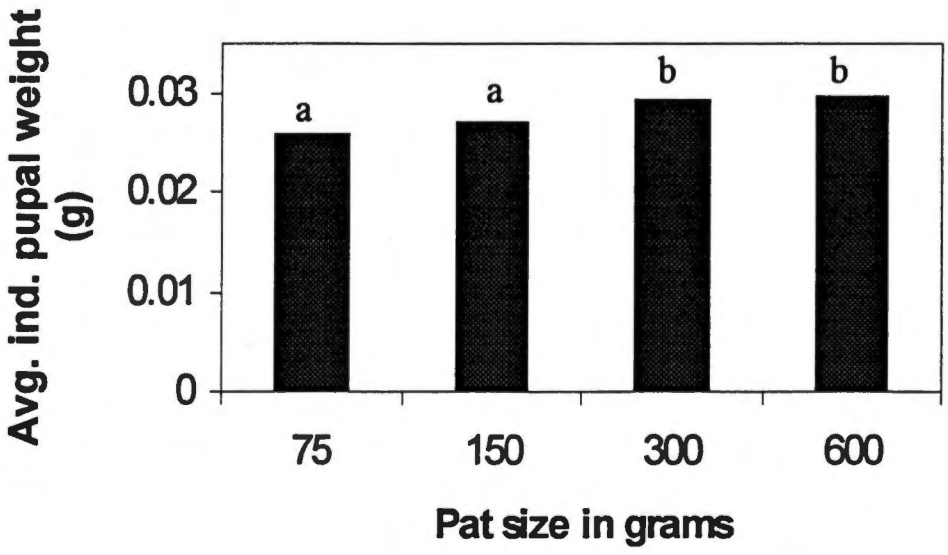

Figure 2.10. Average individual weight for Pat Size trial #1. All pats had an initial uniform number of larvae (25). Bars with the same letter are not significantly different according to Duncan's Multiple Range test ($P = 0.05$).

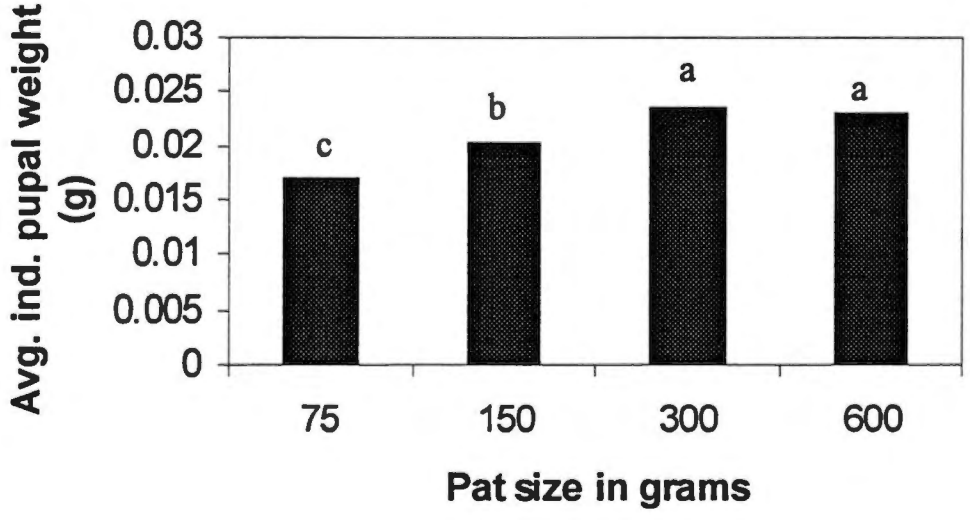

Figure 2.11. Average individual pupal weight for Pat Size trial #2. All pats had a an initial ratio of 3 g/larva. Bars with the same letter are not significantly different according to Duncan's Multiple Range test ($P = 0.05$).

have remained suitable as a food source, therefore producing heavier pupae.

A decrease in pupal weight was observed from densities below 1.25 grams of feces/larva (Bay et al. 1970) and our results also showed a decrease when densities of grams of feces/larva decreased whether competition was increased (Trial #2) or not. Therefore, our recovery of lighter pupae from small pats support the observations made by Valiela (1969) that face fly pupae decrease in size at high densities of larva/grams of feces.

Pupae recovered in trial #1 weighed as much as or a little above the average individual pupal weight of the pupae from the laboratory colony. Trial #2 pupae weighed less (0.009 g – 0.031 g) than those in the laboratory colony. Dung supply has been shown to affect the reproductive capacity of face fly females which survive to reproduce. As mentioned earlier (Chapter II), at 3.4 grams of feces/larva, adult face fly female fecundity decreased and their sizes diminished at 1.0 grams of feces/larva. It was also found that larger sized adult females, both lab-reared and field–collected , had greater numbers of ovarioles (Moon 1980).

Disturbance of the pat by face fly larval activity may also be important in survival by possibly allowing the pat to dry to the necessary state for normal face fly development. Low larva/gram density results in little disturbance of the pat (Moon 1980). Large amounts of dung with low numbers of larvae could also hinder the migration of larvae out of the pat and therefore result in significantly high mortality.

(iv.) Conclusion

A few studies have investigated the effects of densities, shapes, or volumes of cow pats on face fly development and survival (Bay et al. 1970, Hollis et al., 1985 Moon 1980, R. Moon and B. Stromberg, pers. comm., and Valiela 1969), but no studies exist climatic conditions in eastern Tennessee. Pats of 600-grams in size and a density of 24 grams of feces/larva had the highest mortality, the least moisture loss, and the heaviest individual pupal weights when compared to smaller pat sizes and higher larval densities in cow pats. Smaller pats (150 grams or less), regardless of larva/grams of feces densities, had lower mortality but had the lowest pupal weights.

These results, pats having low mortality and lighter pupae or high mortality and heavier pupae, appear to be contradictory. Perhaps the face flies that survived from the larger pats were close enough together to keep the substrate churned for nourishment but not too close to cause competition and stunting. The heavier pupae may have been positioned closer to the edge of the pats while in their larval stages and were able to leave the pats for pupation with little obstruction. The amount and/or length of time that pats dry out may be a cue in the development process, particularly the migration out of the pat to pupate, by face fly larvae. The smaller pats in this present study could have provided this necessity for development therefore resulting in a larger number of larvae leaving the pats to pupate as compared to the larger pats. The light weight of these pupae

may be a result of the small pats providing just enough substrate and nourishment for the larvae to survive, but not enough for them to be well nourished.

Lighter pupae from the smaller pat sizes may not be as fit as the heavier pupae from larger pats, therefore their future reproductive capacity may not be as high as that of their larger cohorts. Larger numbers of less fit face flies may not be as beneficial to a population's survival as a smaller number of larger, more fit adult fly individuals.

Further experimentation may produce more consistent results concerning pat-size effects on the immature stages of face flies. These results may support the hypothesis that the intensive rotational grazing pasture management system can function as a cultural control of pasture pests.

Chapter III

SIMULATED RAINFALL

(i.) Introduction

Face fly (*Musca autumnalis* DeGeer) development and activity have been shown through laboratory and field experimentation to be directly affected by both biotic and abiotic environmental factors. Natural predators can cause larval or adult mortality or prevent adult females from ovipositing in the pat (Teskey 1969; Pickens and Miller 1980). Abiotic factors such as temperature, wind speed, relative humidity, and light also affect larval development and survival of the face fly (Engroff et al 1972; Teskey 1969; Pickens and Miller 1980; Burton and Turner 1970; Moon 1980; Valiela 1969).

One abiotic factor that has received little attention in regard to its effects on face fly mortality, particularly the larval stages, is rainfall. Rainfall has been shown to reduce or terminate adult face fly activity depending on the duration and strength of the rainfall (Teskey 1969; Pickens and Miller 1980). The Australian bush fly, *Musca vetustissima* Walker, experienced increased egg and larval mortality as the amount and duration of simulated rain increased (expressed as percent survival, eggs: 5 minutes – 70 %, 20 minutes – 38 %, 80 minutes – 28 %; L1 larvae: 10 minutes – 83%, 40 minutes – 74%, and 80 minutes – 76%) (Hughes 1979). When manure pats were thoroughly wetted, a 30 % reduction in horn fly, *Haematobia irritans irritans* (L.), survival occurred (Jones and Kunz 1996). In a

study investigating the effect of immersion on the immature stages of the horn fly immersion in water for one hour caused an overall 37 % reduction of survival for all immature horn fly stages, with an increase in mortality as the immersion periods grew longer (6 hours: 53 %, 18 hours: 61 %, and 30 hours: 82 %) (Jones and Kunz 1996). This study reflects what could occur in a flooding situation, and results could be useful in predicting horn fly population fluctuations after such an event (Jones and Kunz 1996). Simulated rainfall levels at 0, 6, 12, and 24 mm did not significantly affect mortality of face fly larvae 24-, 48-, and 96-hour old (Mark Carder unpublished data 1994). The objective of this study was to observe the effects of high and duration of rainfall on the mortality of first instar face fly.

(ii). Materials and Methods

Larvae: Larvae for this experiment were obtained in the same fashion as described in Chapter II (ii). Twenty-five first instar larvae were added to each of forty 150-gram pats.

Manure: Manure was collected from the UT-KES Dairy Unit and prepared in the manner as described in Chapter II (ii). For this experiment, a uniform pat size of 150 grams was used for all forty pats.

Pressboards: After each pat was weighed out to 150 grams and contained 25

larvae, they were placed on a 1.3 x 22.9 x 18.4 cm pressboard. The pressboards with their pats were put into dishpans and transported to the UT-KES Small Grains Unit. The purpose of the pressboards was to provide a platform on the grass for the thirty pats and rain collecting cans to lay on while being rained upon in the experimental grid design.

Temperature probes: One temperature probe (StowAway® XTI, Onset Computer Corp., Bourne, MA) was assigned to one pat in each of the three treatments and the control. After the pats received the simulated rain, the tips of the four probes were inserted into the center of each of their designated pats to record the internal temperature at thirty minute intervals for the full week.

Meteorological recordings: Recordings were taken as described in Chapter II (ii.).

Simulated rainfall apparatus: Simulated rainfall was generated by an apparatus (Figure 3.1) with a Ladybird® No. 25 part circle impact sprinkler head. The sprinkler head was enclosed in a clear Plexiglas box mounted on a steel stand and water sprayed from side to side at an angular range of 150° out from an opening on one side of the box. The water pressure was at 40 psi allowing roughly 40 mm of simulated rain with large droplets to fall per hour in the experimental area. Three groups of ten pats (from pans 11–20, 21–30, 31–40) were chosen

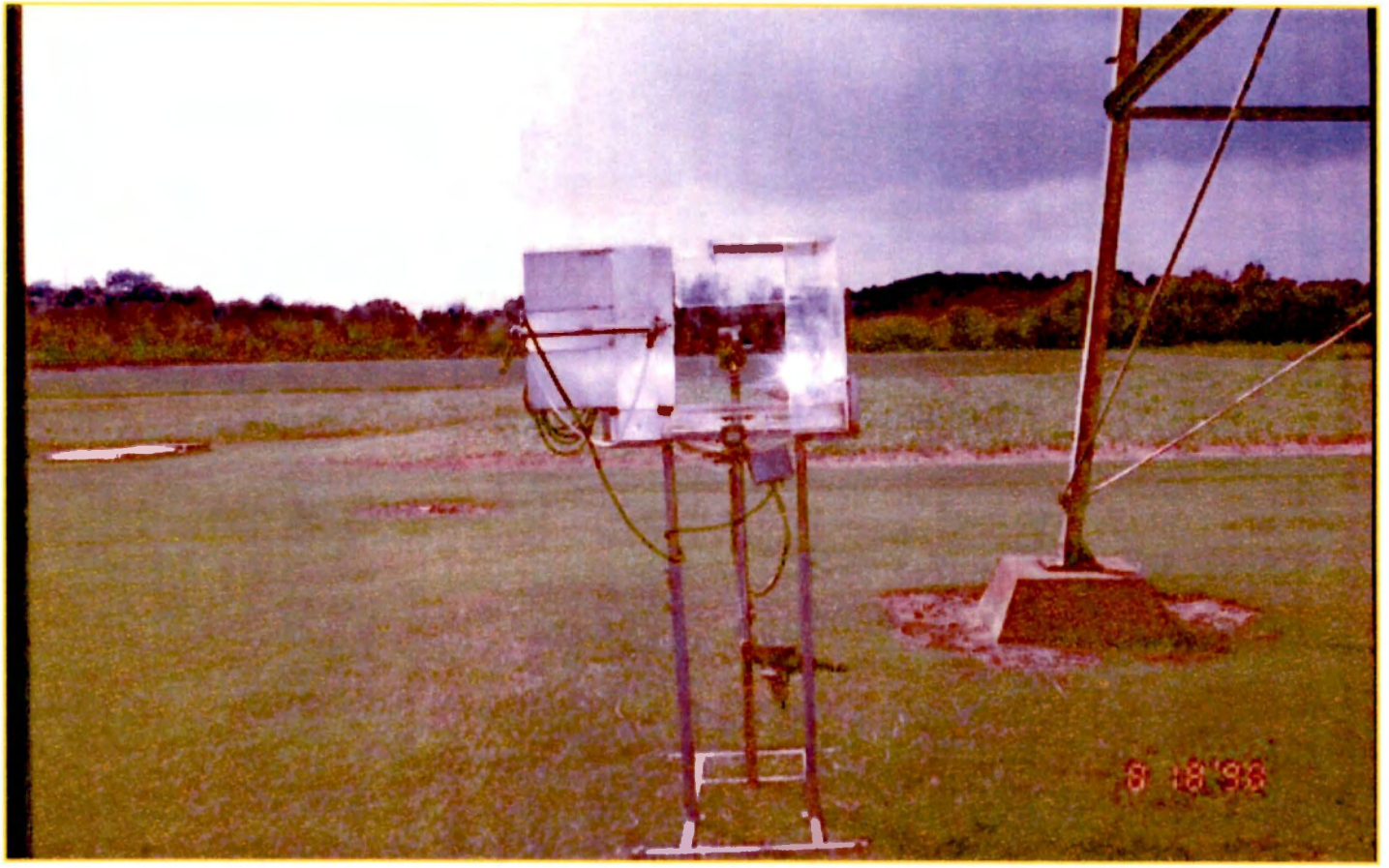

Figure 3.1. Simulated rainfall apparatus.

to receive simulated rain. The thirty pats (on pressboards) which had previously chosen to receive simulated rain, along with six more pressboards supporting six rain collecting cans (11 cm x 8.3 cm dia), were randomly arranged in a 6 x 6 grid design on a level grass plot. A fourth group of ten pats (from pans 1–10) served as the control group and were set several meters away from the plot to avoid any simulated rainfall.

All thirty pats received simulated rain for one full hour after which pats 11–20 were remove and placed into their respective pans. Water in the six rain cans was measured and then discarded. The cans were returned to their positions and the remaining twenty pats received simulated rain for a second hour. After the second hour of rain, pats 21 – 30 were taken off the grid and set into their designated pans. Water in the rain cans was measured and discarded and cans were returned to their position. Simulated rainfall on the remaining ten pats (31–40) was continued for a third hour. Again, the pats were removed and placed into pans numbered 31 – 40 and the water from the rain collecting cans was measured. Finally, after all the forty pats on pressboards were put into their respective dishpans, the dishpans were placed in a random fashion in five rows of eight pans on the sand bed. They were left out exposed to natural conditions for one full week.

At the end of the week, the temperature probes were removed and the pats were transported back to the insectory on the UT-KES Plant Science Unit. The pats were re-weighed and the face fly pupae were collected from each pan.

Pupae were taken back to the lab to be counted, weighed, and allowed to emerge as adults. Once adults emerged, they were counted and released back into the laboratory colony. Moisture loss (includes any material washed away during the simulated rainfall) was determined by the difference of the initial pat weights before exposure to rain and the final pat weights, and percent mortality, as determined by the difference in initial larval numbers and final adult numbers were analyzed using ANOVA Least Significant Difference test (LSD, $P = 0.05$). Average individual pupal weights from each treatment were analyzed by Duncan's Multiple Range test ($P = 0.05$) (SAS 1987).

(ii.) Results and Discussion

After the first hour of rain, 40.2 mm (≈1.5 in) of water was collected. The first two and all three hours of simulated rain produced cumulative totals of 79.2 mm (≈3.1 in) and 122.5 mm (≈4.8 in) of water, respectively. During the experiment while all forty pats were out on the sand bed, two events of natural rainfall occurred at trace amounts, and the weekly average maximum and minimum air temperatures were 28.1°C and 17.9°C, respectively.

Pats exposed to 3 hours of simulated rainfall had the highest mortality at 96.4 % (Figure 3.2) and was significantly different from the other three treatments. After the 3-hour simulated rain, these pats consisted of little more than fibrous material. If rain occurs soon after a cow pat has been dropped,

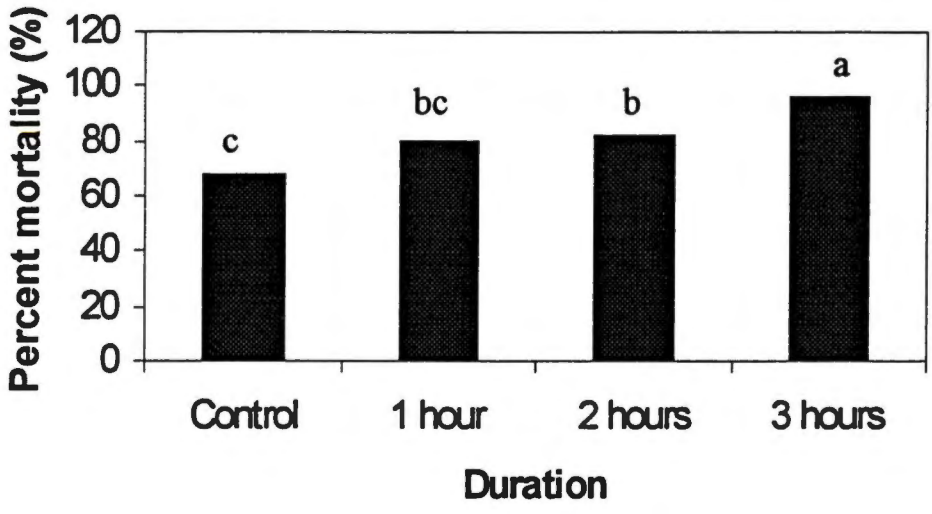

Figure 3.2. Percent face fly larval mortality for all treatments in the Simulated Rainfall experiment. Bars with the same letter are not significantly different according to LSD ($P = 0.05$).

the rainwater will mix with the pat material and can quickly wash the manure away (Hughes 1979, Jones and Kunz 1996). However, older pats can offer more protection due to the crust that forms on the surface (Jones and Kunz 1996). The control group had the lowest mortality (67.2 %) and was significantly different from the two- (82.0 %) and three- (96.4 %) hour treatments. Pats that received one hour of simulated rain had 79.6 % mortality which was much higher than results previously reported for bush fly first instars which suffered 24 % mortality after 80 minutes of simulated rain (32 mm) (Hughes 1979). Two possibilities for the lower percent mortality observed by Hughes 1979 could be (1) the lower simulated rainfall (32 mm), and (2) the lack of exposure to climatic conditions after the bush fly larvae received the simulated rain in contrast to conditions

in our experiments.

All treatments had high moisture loss (Figure 3.3). The highest loss occurred at 70.0 % from the 3 hour treatment and was significantly different from the other three treatments. The control, 1-hour, and 2-hour treatments, had 75.0 %, 75.15 %, and 75.18 % moisture loss, respectively, and were not significantly different at the $P = 0.05$ level.

Comparison of pupal weights (Figure 3.4) revealed no significance differences, although the average individual pupal weights slightly decreased as the duration of rain increased. The control had an average individual pupal weight of 0.0174 g while the 1-hour treatment average was 0.0169 g, the 2-hour treatment average was 0.01667g, and the 3-hour treatment average was 0.0144 g. Adults emerged from the pupae and pupae seemed to weigh less than the pupae from the laboratory colony and those in 150-gram pats in the Pat Size trial #1 and trial #2 experiments (Chapter II).

(iv.) Conclusion

In the horn fly immersion study, the period of ecdysis for both larvae and pupae occurred in the longer immersion periods and may have contributed to mortality (Jones and Kunz 1996). Ecdysis typically makes insects susceptible to their surrounding environment (Jones and Kunz 1996).

In our study, first-instar face flies suffered the greatest mortality after

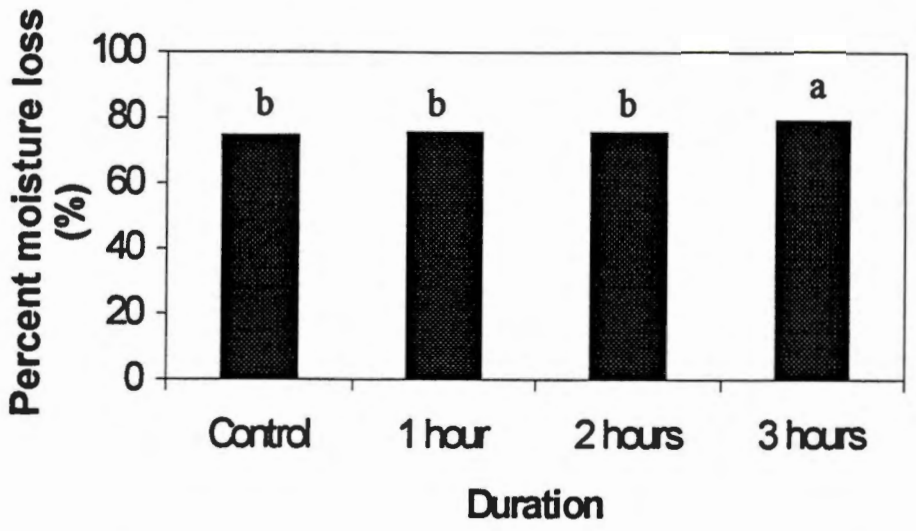

Figure 3.3. Percent moisture loss of the pats for all treatments in the Simulated Rainfall experiment. Bars with the same letter are not significantly different according to LSD ($P = 0.05$).

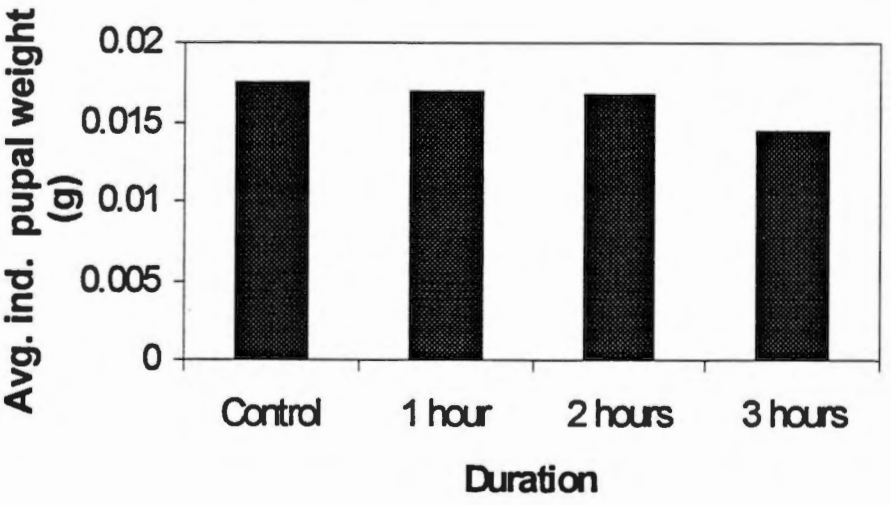

Figure 3.4. Average individual pupal weight for all treatments in the Simulated Rainfall experiment.

being exposed to 3 hours and 122.5 mm of simulated rain. Although moisture loss was high for all treatment groups, it was clear that the pats exposed to the longest simulated rain lost a large amount of fecal fluids before exposure for a week on the sand bed. Pupal weights were not significantly different, however, they were low.

Long periods of rain and heavy rainfall are not uncommon in eastern Tennessee. Further research using older larvae or pupae under the same experimental design could provide information useful in predicting face fly population fluctuations after rain events in eastern Tennessee.

Chapter IV

TEMPERATURE

(i.) Introduction

Since introduction from Europe in the early 1950's, face fly populations have been successful in their distribution across the North American continent. They can be found throughout Canada and in temperate regions of the United States above the 33rd parallel (Teskey 1969). Face fly populations are not found in the arid southwest and southern-most states such as Florida (Teskey 1969, Turner and Hair 1966). It is believed that temperature may be a key factor in determining the distribution of face fly populations in North America (Turner and Hair 1966). In Virginia, these authors found that temperatures above 29.4°C shortened the life of face fly adults, while cooler temperatures seemed to prolong life expectancies.

Maximum survival for face fly larvae has been recorded at 35 – 40°C and 38 % survival was observed at 47°C, while minimal survival was between 11 - 16°C (Palmer et al. 1981). Comparatively, the upper thermal limit for the buffalo fly (*H. irritans exiqua* de Meijere) is 30 – 37.5°C (Cook and Spain 1982) and for the horn fly, *H. irritans irritans* (L.), is 42°C (Palmer et al. 1981). It is well documented that the internal temperatures of a cow pat exceeds the daily maximum air temperatures for most of the day (March and Bay 1983). Even during the evening hours when little thermal stratification takes place in the pats

and heat is being radiated back into the atmosphere and down into the soil, the coolest strata, the pat-soil interface, is still warm (March and Bay 1983). Internal pat temperatures, recorded under sunny, hot conditions, were found to be 5°C warmer than surrounding air temperatures. Even on cloudy days, internal pat temperatures were similar to the maximum air temperatures (Palmer et al. 1981). Cow pats can absorb heat, resulting in a thermal gradient within the pat. The top of the pat has the highest temperatures while the lowest temperatures are at the bottom at the pat-soil interface (Hammer 1942, Valiela 1969). If temperatures within the pat become adverse, larvae probably move around until they find a more suitable spot (Valiela 1969).

Although some face fly populations have been observed to be more numerous on cattle under shading of trees than on cattle out in open pasture (Hansens and Valiela 1967, Parrish and Gerhardt 1976), face flies typically do not enter shaded areas such as barns, houses, or other shelters during the summer season (Pickens and Miller 1980). Female face flies have been observed to oviposit in cattle droppings in open pasture while avoiding those dropped in shaded areas (Teskey 1969). Fewer eggs were found in cattle droppings in tall, dense vegetation as compared to those in exposed pasture, suggesting partial shading from the tall vegetation had a negative effect on oviposition (Teskey 1969). In Virginia, an investigation of mortality in face fly field populations showed that shading from a 6-mil polyethylene plastic sheeting reduced heat

absorption in the manure and caused unfavorable conditions which prevented oviposition by wild face flies (Burton and Turner 1970).

The objective of this trial was to observe the effects of different temperature regimes in manure pats on face fly larval survival.

(ii). Materials and Methods

Larvae: Forty groups of twenty-five first instar face flies were obtained from the incubator and were each added to a pat in the same manner as described in Chapter II (ii). These pats were then placed on sand inside dishpans, again, in the same fashion as described in Chapter II (ii).

Manure: Manure was collected from the UT-KES Dairy Unit and prepared in the same manner as mentioned in Chapter II (ii). All forty pats were weighed out to 150 grams of cow manure.

Treatments: Three different shade covers, black plastic, clear plastic, and aluminum coated styrofoam house insulation (12.7 mm thick), plus an uncovered control (open treatment), were used in order to establish different temperature regimes. Wooden frames (1.2 x 1.5 m) were constructed from four wood pieces (5.1 x 5.1 cm) with four stakes (5.1 x 10.1 cm) attached at the corners. The two stakes in the front of the frame were cut 20.3 cm in height and the back two

stakes were cut 25.4 cm in height to allow for a slight slant to prevent water from collecting on the surface of the cover in the event of rain. The control group was not covered.

Temperature probes: One StowAway® XTI temperature probe was assigned to one pat in each of the four groups. The tip of the probe was inserted into the center of the pat and recorded the temperature at thirty-minute intervals for the full week duration of the experiment.

Meteorological recordings: Recordings were taken as described in Chapter II (ii.).

Once the forty pats were prepared and placed on dishpans, they were placed on the sand bed under their assigned cover. Pans 1 – 10 were under the reflective styrofoam cover, pans 11 – 20 were under the clear plastic cover, and pans 31 – 40 were under the black plastic cover. The control group were pans 21 – 30 and were fully exposed to the natural conditions (Figure 4.1)

Pats with face fly larvae were left out for a full week, after which all pans were taken off the sand bed, the temperature probes removed, and face fly pupae were collected from each pan. Pupae were counted and weighed and allowed to emerge as adults. Adults were counted and percent mortality was determined from these numbers. The pats were re-weighed and percent moisture loss per

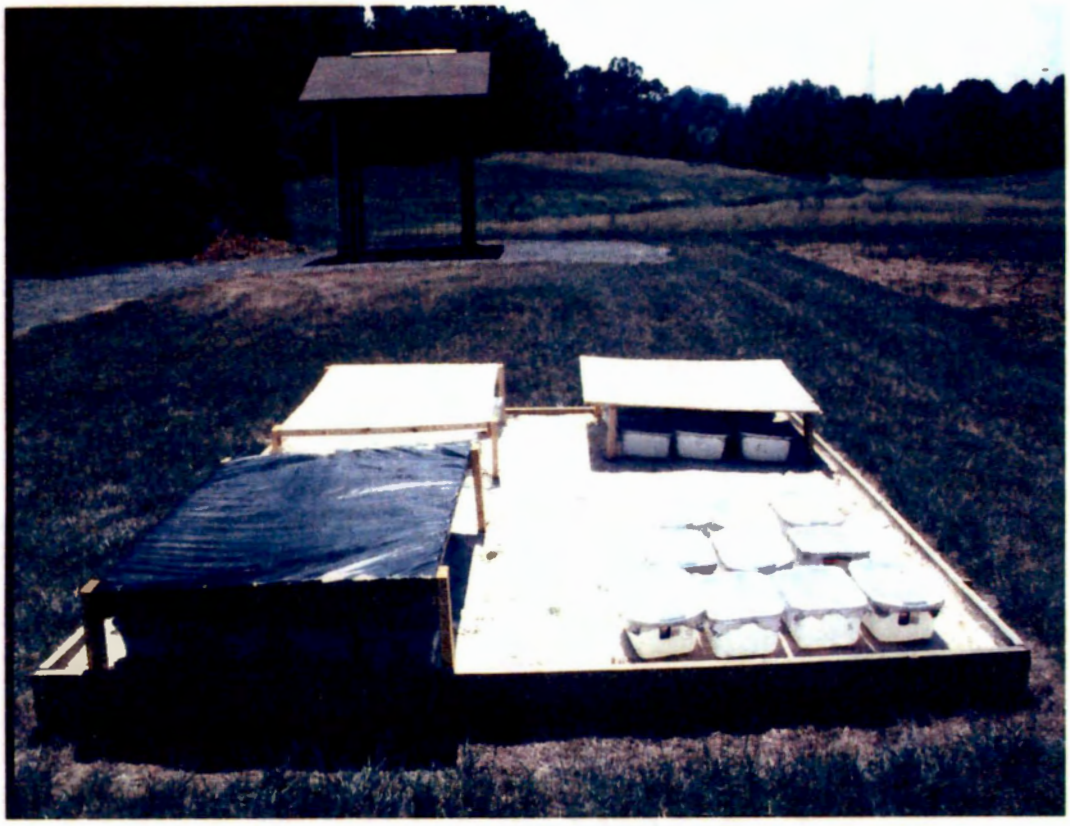

Figure 4.1. Setup of shading experiment. Top left: clear plastic; top right: reflective styrofoam cover; lower left: black plastic; and lower right: open (uncovered), control.

treatment was determined. Percent mortality per treatment and percent moisture loss per treatment were compared through ANOVA Least Significant Difference test (LSD, $P = 0.05$). Average individual pupal weights for each treatment were compared using Duncan's Multiple Range test ($P = 0.05$).

(iii.) Results and Discussion

During the week of this shading experiment (Figure 4.2), maximum air temperatures ranged from 28.3°C and 31.7°C, averaging 30.2°C, and the minimum temperatures were 16.1°C to 18.3°C, averaging 17.3°C. A precipitation event occurred (1.0 mm) on one day (the first day) during the week.

Recordings of the internal pat temperatures (Figures 4.3, 4.4, 4.5, and 4.6) revealed that the highest temperatures came from the pats under the open and clear plastic treatments, and the lowest temperatures came from the black plastic and reflective styrofoam treatments. These temperatures were measured at the center of the pat. The maximum internal pat temperatures ranged from 32.4 – 35.8°C in the reflective treatment, 44.2 – 47.1°C in the clear plastic treatment, 39.8 – 45.9°C in the open (control) treatment, and 31.8 – 35.2°C in the reflective styrofoam treatment. The minimum internal temperature recorded for each treatment was 20.1°C for the reflective styrofoam treatment, 19.4°C for the clear plastic treatment, 18.7°C for the open treatment, and 19.3°C for the black plastic treatment which was exposed to the intense sun during August, and the clear

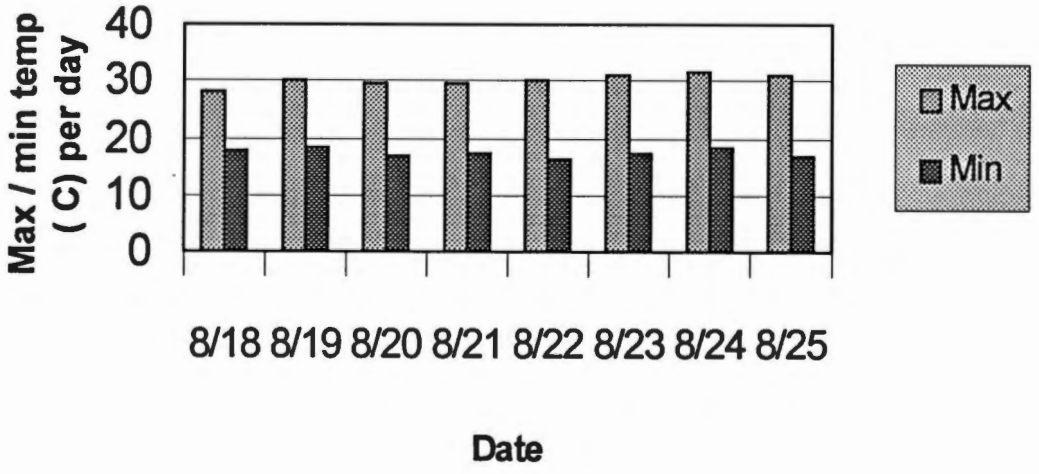

Figure 4.2. Daily maximum and minimum air temperatures recorded during the Shading experiment, August 18, 1998 through August 25 1998.

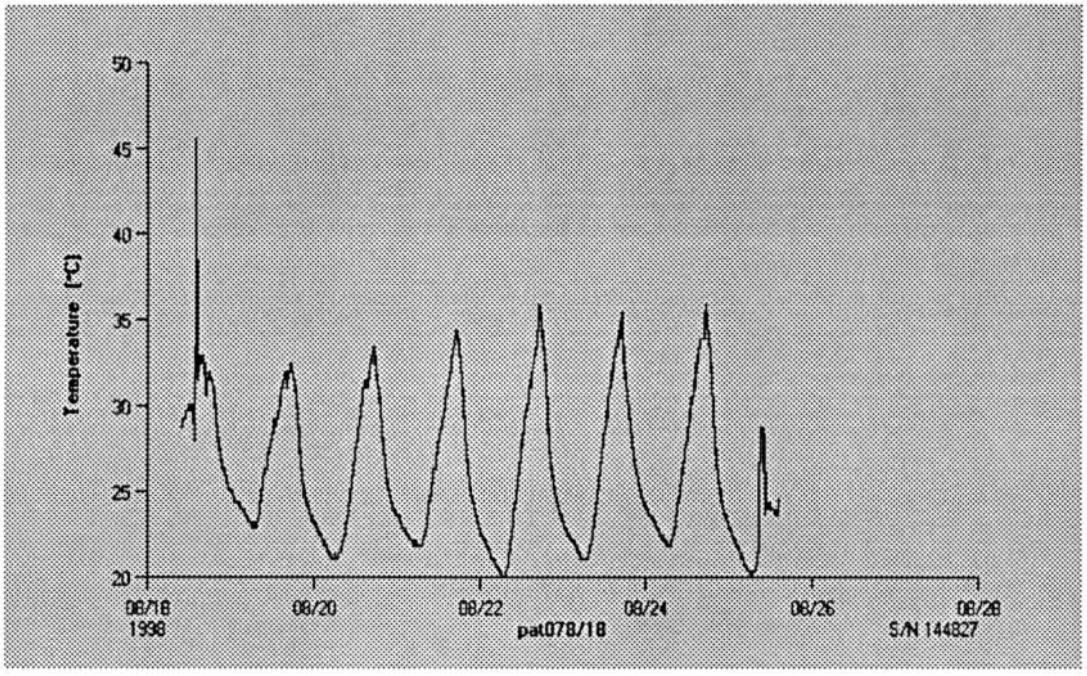

Figure 4.3. Temperature probe readout of the internal temperatures in a cow pat under the reflective styrofoam treatment from August 18 to August 25 1998. The initial peak seen on the graph is actually a recording of the surrounding air temperature before the probe was inserted into the pat.

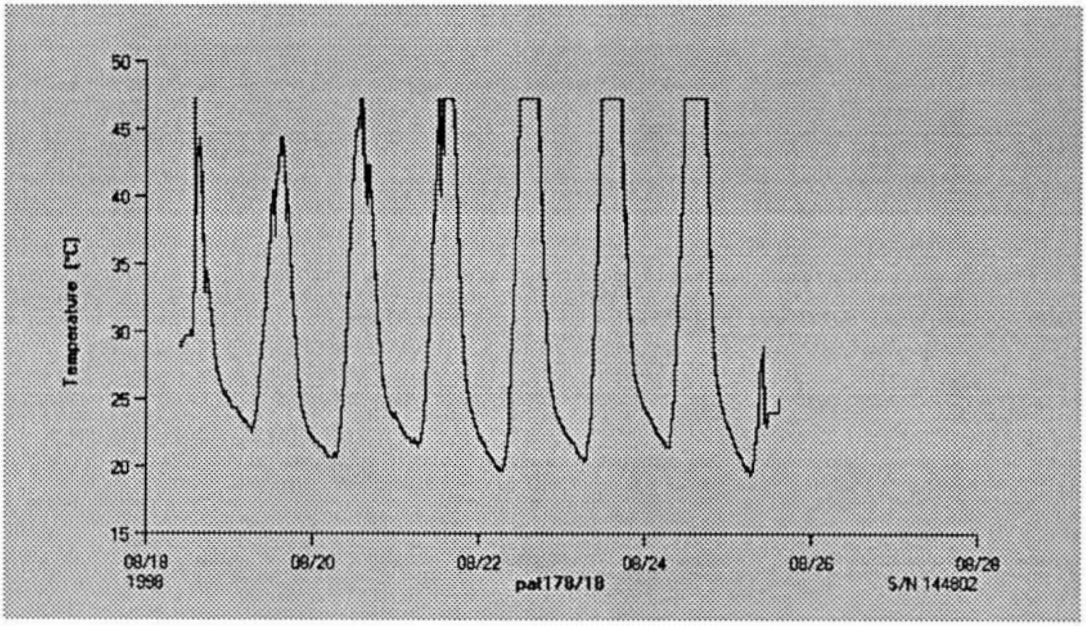

Figure 4.4. Temperature probe readout of the internal temperatures in a cow pat under the clear plastic treatment from August 18 to August 25 1998. The initial peak seen on the graph is actually a recording of the surrounding air temperature before the probe was inserted into the pat.

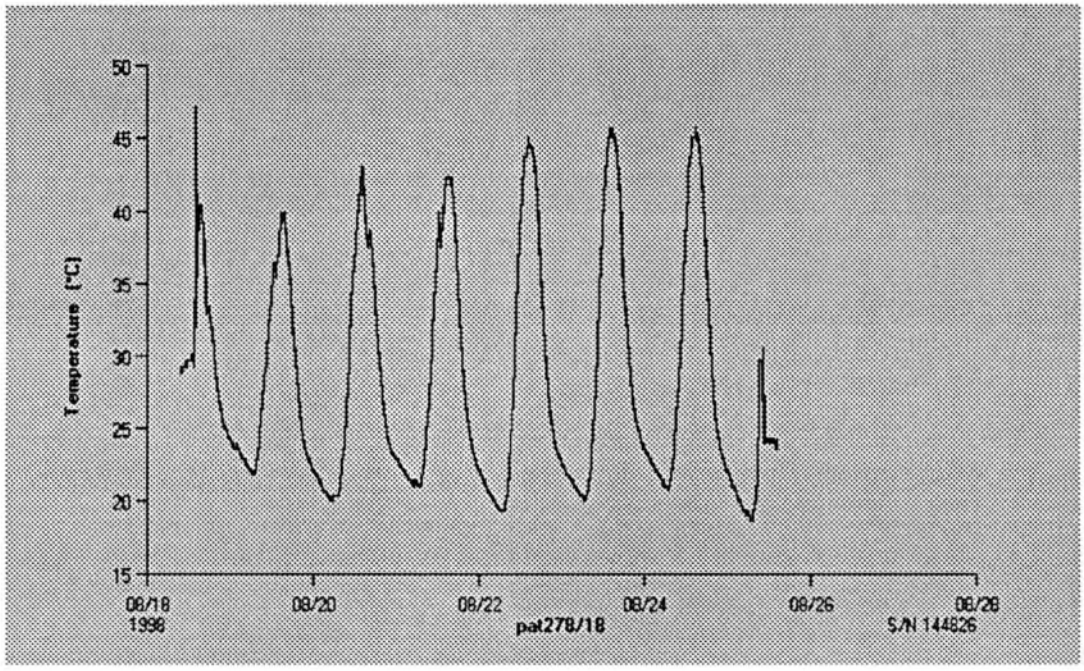

Figure 4.5. Temperature probe readout of the internal temperatures in a cow pat under the open (control) treatment from August 18 to August 25 1998. The initial peak seen on the graph is actually a recording of the surrounding air temperature before the probe was inserted into the pat.

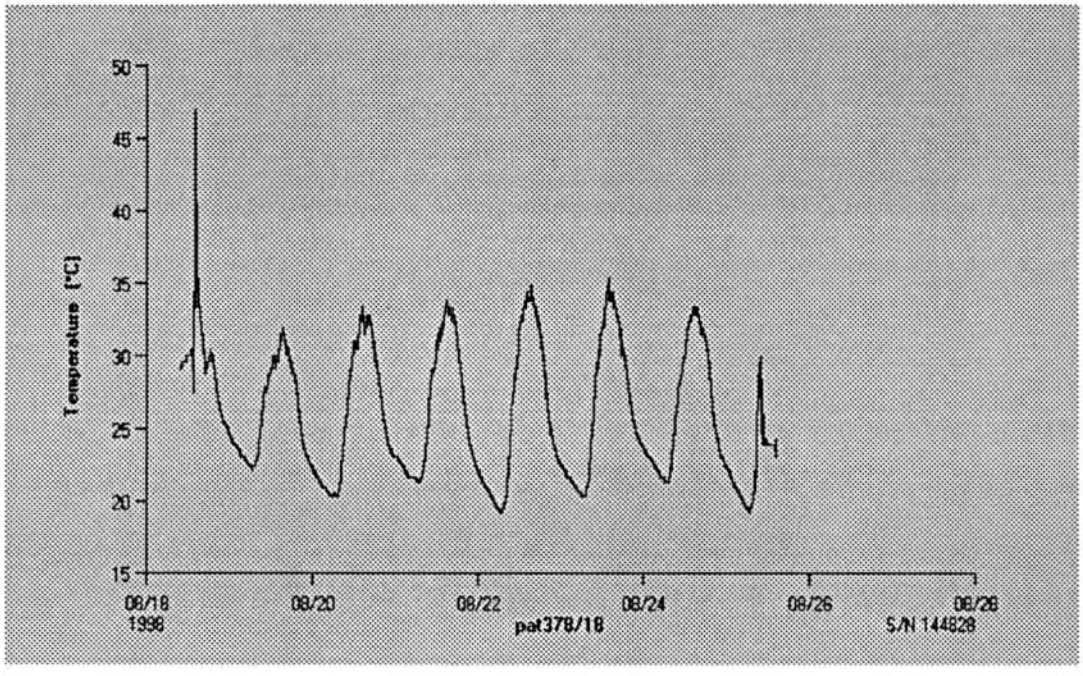

Figure 4.6. Temperature probe readout of the internal temperatures in a cow pat under the black plastic treatment from August 18 to August 25 1998. The initial peak seen on the graph is actually a recording of the surrounding air temperature before the probe was inserted into the pat.

treatment. The temperature probes did not record above 47°C, therefore, the four peaks which were flat at 47°C in Figure 4.4 could have represented higher temperatures if the recorder registered temperatures above 47°C. The daily maximum internal temperatures for all four treatments were considerably higher than the average daily air temperature maximum (30.0°C). Only two daily maximum temperatures, 31.8°C under the black plastic treatment and 32.4°C under the reflective styrofoam treatment, were close to the average daily air temperature maximum. The initial peak seen on the graphs is actually a recording of the surrounding air temperatures before the probes were inserted into the four pats.

There were significant differences among percent face fly mortality (Figure 4.7) in the four treatments. The reflective styrofoam treatment had the highest face fly mortality (70.0 %); the black treatment had 60.0 %; the open treatment had 42.8 %, and the clear treatment had 40.4 % mortality.

Higher mortality was originally expected to be from the black plastic treatment, while the lowest was expected to come from the reflective treatment. Quite the opposite occurred and the highest mortality came from the reflective styrofoam and the black plastic treatments. Black plastic caused lower temperatures in the pats beneath it. This treatment provided a shady situation as found under the reflective styrofoam treatment, but probably with a little higher temperature increasing moisture loss and allowing lower face fly mortality. Conditions under which we thought high mortality would occur, the open

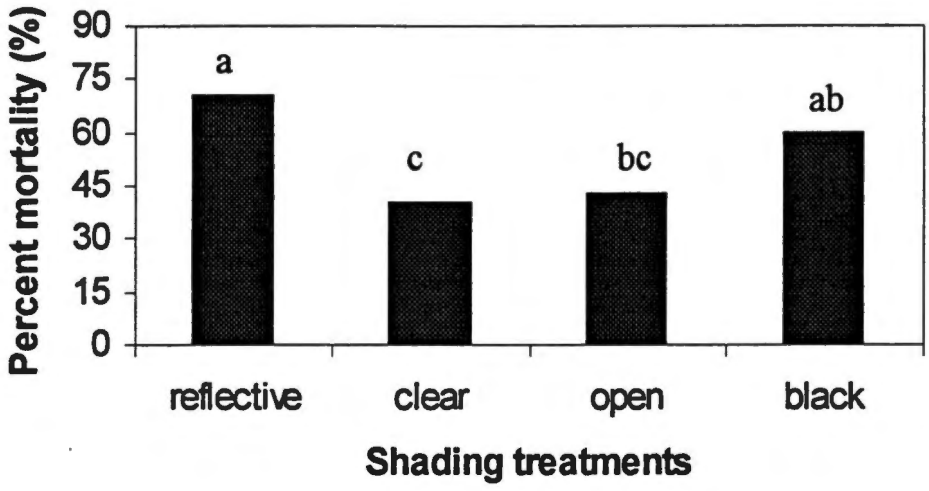

Figure 4.7. Percent face fly mortality for the Shading experiment. All pats had an initial population of 25 larvae/150 grams of manure. Bars with the same letter are not significantly different according to LSD ($P = 0.05$).

treatment, which was exposed to the intense sun during August, and the clear plastic treatment, which caused a greenhouse effect, actually were conditions under which the lowest face fly mortality occurred.

The lowest moisture loss (Figure 4.8) (53.2 %) occurred in the reflective treatment and it was significantly different from the other three treatments. The black plastic treatment (60.4 %) was also significantly different from all other treatments. The clear and open treatments were not significantly different having higher moisture loss and lower mortality than the other two treatments (clear treatment: 72.35 % and open treatment: 71.04 %). The reflective treatment had the lowest percent pat moisture loss when compared to all previous experiments.

The moisture loss was only 1.0 % lower than the 600-gram pats with the

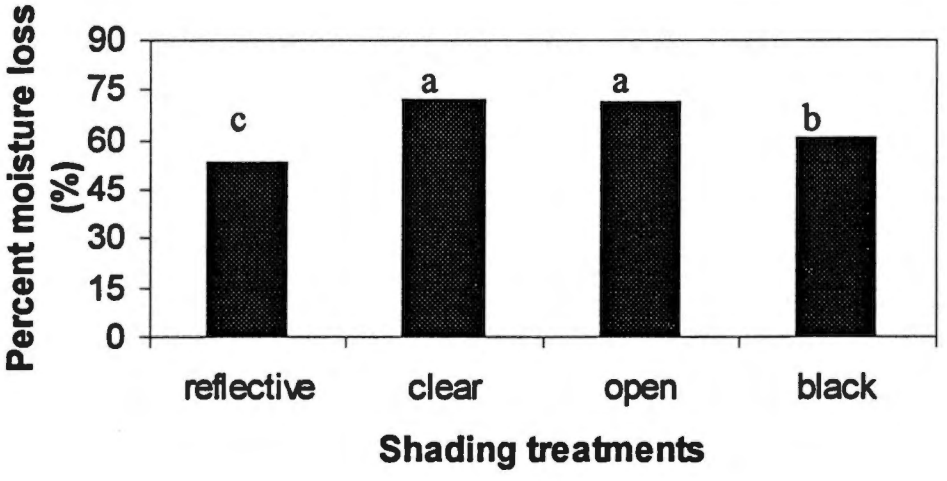

Figure 4.8. Percent pat moisture loss for the Shading experiment. All pats had an initial population of 25 larvae/150 grams of manure. Bars with the same letter are not significantly different according to LSD ($P = 0.05$).

1larva/3 grams of feces ratio in the Pat Size trial #2 experiment. The low level of moisture loss obtained under the black plastic and the reflective styrofoam treatments may reflect the reason why face fly females do not oviposit in shaded areas (Teskey 1969). Pats under these conditions may not be conducive to normal development and survival. There were no significant differences (Figure 4.9) among individual pupal weights of the four treatments.

Horn fly larvae exhibit thermotactic behavior and have been observed to move away from higher temperatures which were usually found in the upper strata of a cow pat (March and Bay 1983). Future research may reveal the same situation occurs with face fly larvae.

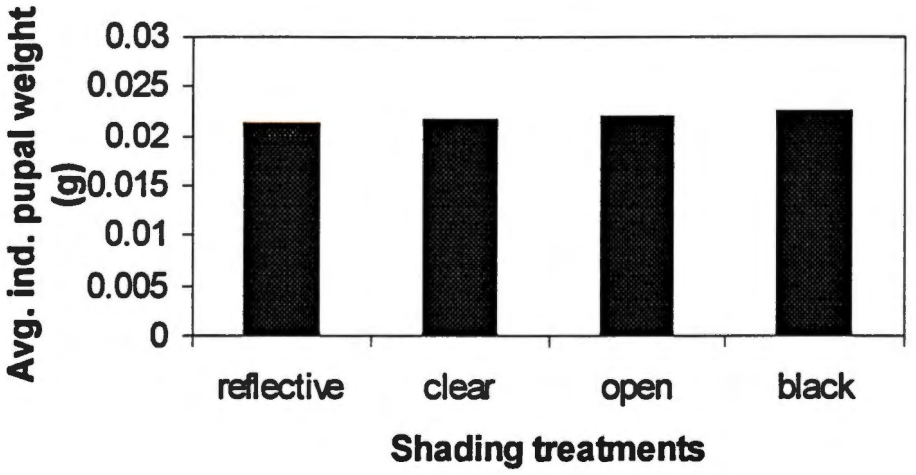

Figure 4.9. Average individual face fly pupal weights for the Shading experiment. All pats had an initial population of 25 larvae/150 grams of manure.

(iv.) Conclusion

The fact that face fly larvae can withstand higher temperatures than some other dipteran livestock pests, could be as result of a requirement for high internal pat temperatures for normal development and survival. In addition to higher internal pat temperatures, larvae may need pats to dry out, as was seen here in this shading experiment and in Chapter II in the Pat Size experiment, so development can occur.

If further experimentation should be carried out regarding the effects of temperature and shading on immature face fly mortality, more sensitive probes which could record a wider range of temperatures should be used at various levels

of the pat. A different material, possibly thicker plastic or some type of fabric, could be employed for the black cover treatment to increase absorption of solar radiation in order to produce higher temperatures under this type of cover than those which occurred in this present study. Also the combination of cover treatments with different pat sizes could be used in a factorial design to measure effects of pat temperatures and moisture loss on face fly survival.

The temperatures in this experiment may reflect why pats in shaded areas are avoided for oviposition by face fly females, as well as reveal that high temperatures here in eastern Tennessee may be necessary for normal face fly survival and development.

Chapter V

LATERAL MIGRATION OF INFECTIVE TRICHOSTRONGYLE LARVAE IN EASTERN TENNESSEE

(i.) Introduction

Gastrointestinal nematodes are considered to be the most important internal parasites of cattle (Herd 1980, Williams 1983). The group most responsible for parasite–related mortality and morbidity in ruminants is the superfamily Trichostrongylidae (Urquhart et al. 1996). Trichostrongylids feed on the mucosa within the abomasum or small intestine of their ruminant host (Figure 5.1) (Johnstone 1998, Urquhart et al. 1996, Williams 1983). Eggs are laid by adult female worms in the gastrointestinal tract and are passed out into the environment with the feces. These eggs hatch as first stage larvae (L1) and then molt into the second larval stage (L2). Both L1s and L2s are free-living and feed on bacteria within the feces and surrounding environment (Johnstone 1998, Lee and Atkinson 1974). As the larvae develop into the third stage (L3), they do not shed their second stage cuticle. Thus, the L3 infective larvae are ensheathed (Figure 5.2), all orifices are closed, and they are forced to utilize stored food as the sole source of energy.

Hatching and development of all free-living stages are dependent on climatic conditions, particularly temperature (Johnstone 1998, Urquhart et al. 1996, Williams 1983). Infective larvae migrate to the surrounding herbage and

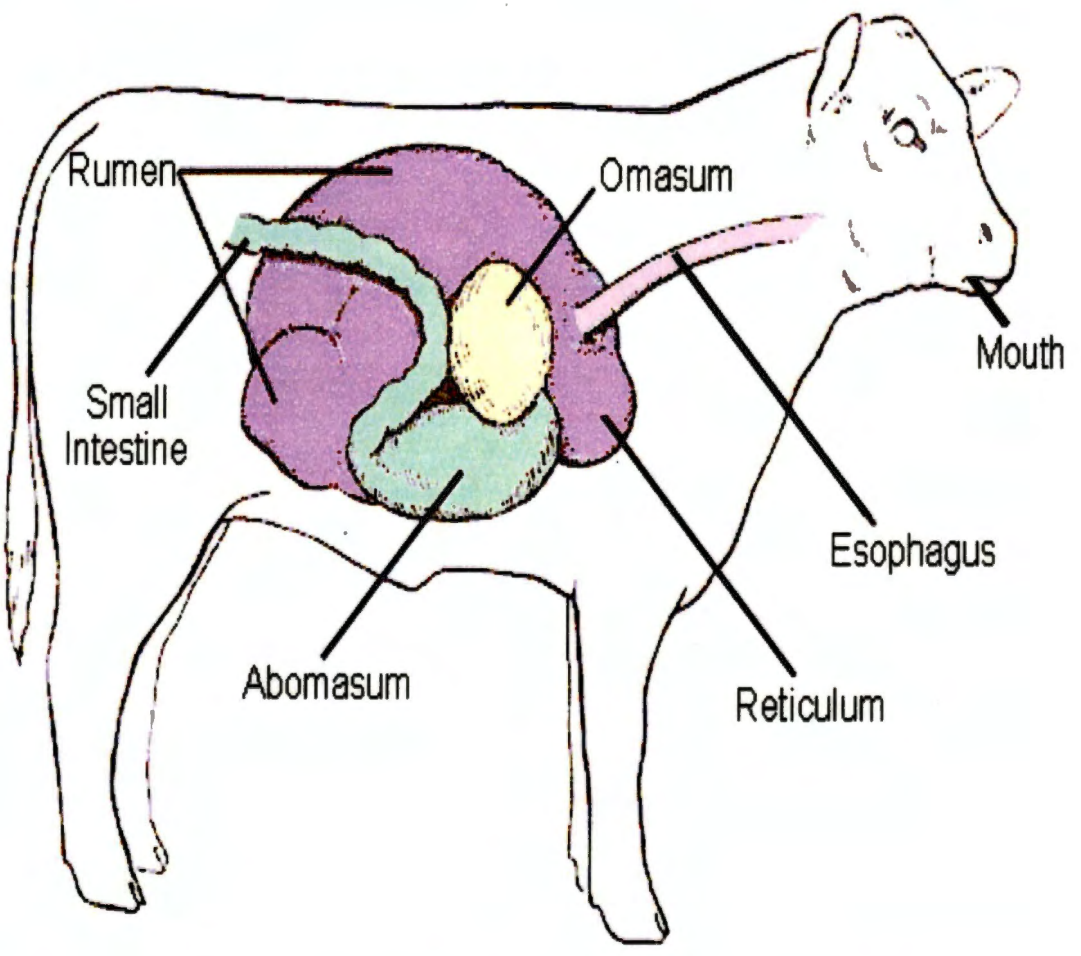

Figure. 5.1. Digestive tract of ruminants. Source: Anonymous 1997.

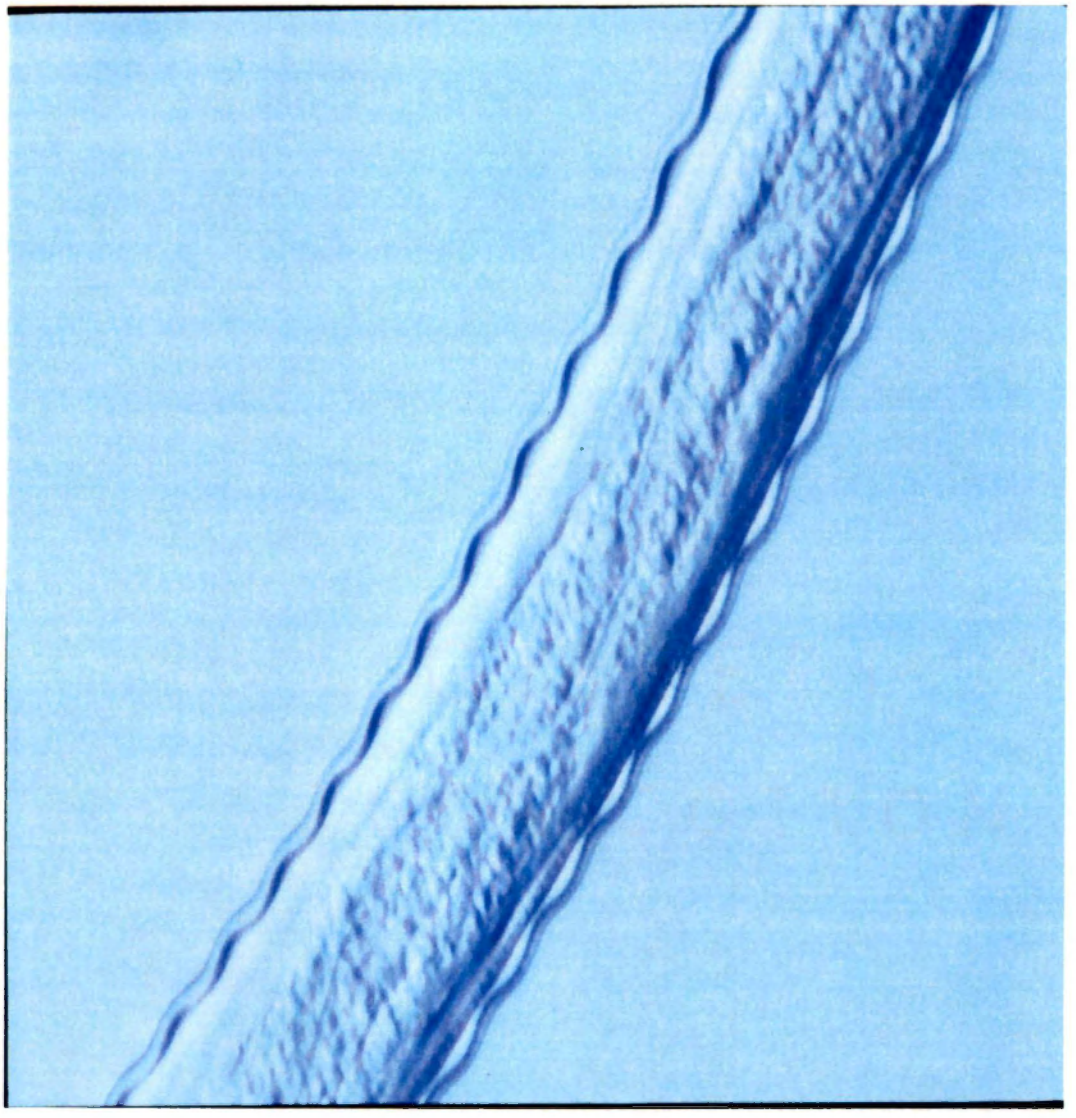

Figure 5.2. Ensheathed cuticle of an infective (L3) Trichostrongylid larva

are ingested as cattle graze on the contaminated forage. Infective L3's migrate into the gastrointestinal tract, exsheath from their L2 cuticle, and take up residence in the gastric glands of the abomasum. Cues from the alimentary tract that stimulate exsheathment are thought to be the low pH levels and the high concentrations of carbon dioxide, pepsin, bile and bile salts in the abomasum (Lee and Atkinson 1976). In these tissues, the parasitic L3's will develop into the fourth stage larvae and then gradually into the fifth larval and adult stages which mate and produce eggs (Johnstone 1998, Urquhart et al. 1996, Williams 1983). The interval from infection of the host to production of eggs by sexually mature females is called the prepatent period (PPP). The PPP varies among genera, but is typically two to three weeks (Johnstone 1998, Urquhart et al. 1996) for trichostrongylids.

A second pattern that can occur after ingestion is that infective L3's migrate to the gastric glands but enter hypobiosis (triggered by certain host and environmental conditions). Hypobiosis is a state of suspended animation in which metabolism slows and development is postponed for several weeks or months (Kaufmann 1996, Rogers and Petronijevic 1982, Urquhart et al. 1996). After some time, the infective larvae are stimulated to come out of hypobiosis synchronously and leave the gastric glands to complete the lifecycle on the mucosal surface (Reinemeyer 1990, Urquhart et al. 1996, and Williams 1983).

External environmental cues are probably the primary factors in influencing initiation and termination of hypobiosis. Adverse conditions, such as

the cold temperatures of autumn and winter, stimulate the larvae to enter hypobiosis upon ingestion in order to avoid reproducing while environmental conditions are still adverse. Emergence from hypobiosis occurs at times of the year when climatic conditions are favorable for development and survival of the free living trichostrongyle stages (Urquhart et al. 1996). Possible host stimuli for emergence are a decrease in the host's immune response due to parturition and lactation, number of adult worms, host resistance breakdown, or poor nutrition (Williams 1983).

Trichostrongylid infections of cattle usually involve mixed infections of several genera with a certain individual species causing major damage or being more pathogenic than the others (Williams 1983). All pastured animals are parasitized to some degree and parasitic infections are more a herd problem than an individual problem (Williams 1983). Cattle can develop immunity after their first grazing period, but yearlings in their second grazing season can become reinfected if their immunity wanes while they were housed for the winter. However, immunity can be reestablished in a short time when grazing resumes in spring (Herd 1980).

Two trichostrongylid genera found most commonly in the eastern Tennessee region are *Ostertagia* and *Cooperia*. *Ostertagia ostertagi* is found in the abomasum of its bovine host (Foreyt 1997, Kaufmann 1996, Urquhart et al. 1996). *Ostertagia* infections can cause profuse, watery diarrhea, weight loss, anorexia, and possibly death. *Cooperia* spp. (Figure 5.3) are worldwide in

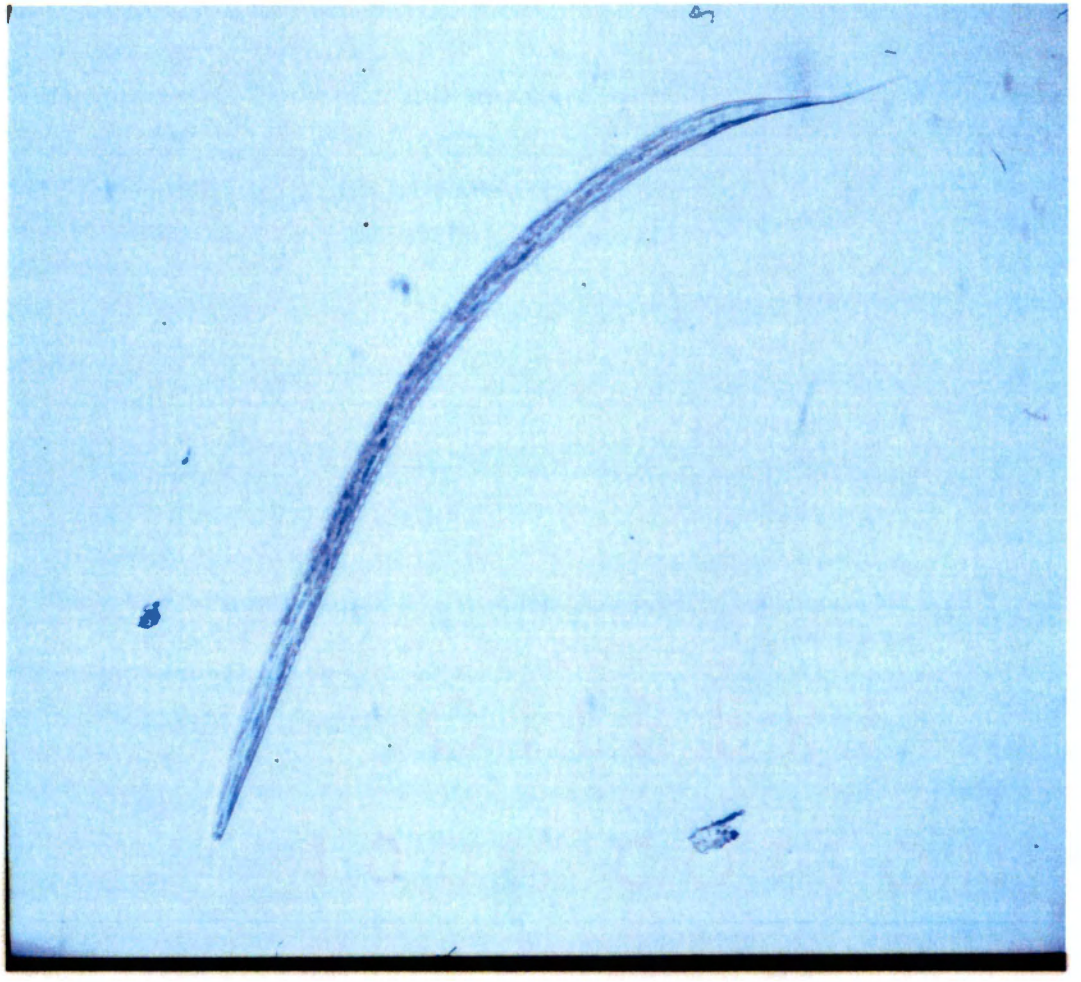

Figure 5.3. Trichostrongyle larva. Infective (L3) stage of Cooperia spp.

distribution like *Ostertagia* spp, but are located in the small intestine of cattle. Large numbers result in loss of appetite, reduced weight gain, and diarrhea in calves (Bowman 1995, Foreyt 1997, Kaufmann 1996, and Urquhart et al. 1996).

The infective stage (L3) is the bridge that connects the two different habitats (environment and host) of the nematode life cycle. The L2 cuticle acts as a protective barrier to environmental stresses that the nematode may encounter Rogers and Petronijevic 1982). The survival of the free living nematode is dependent on the sustainability of stored energy reserves and the nematode's ability to be infective (Williams 1983).

Factors which govern duration and fitness of the infective larvae are temperature, moisture, soil and herbage type, and pasture management (Williams 1983). Generally, development and migration of larvae onto the herbage surrounding the cow pat is favored when environmental conditions supply an abundant amount of moisture and moderate levels of heat, (Goldberg 1968). The major factor affecting larval developmental rate in cow pats is environmental temperature (Rossanigo and Gruner 1995). Optimal temperatures for development of trichostrongyle larvae are 5–32°C (Pandey 1972, Reinemeyer 1990) and 50–120 mm of monthly precipitation (Williams and Mayhew 1967) and for transmission 6–20°C (Williams and Mayhew 1967). Trichostrongyle development is delayed at temperatures of 4-8°C and lower (Williams and Mayhew 1967).

Cattle graze in a set pattern which results in a non-random distribution of feces (Stromberg 1997). Ruminants exhibit fecal avoidance behavior, and do not graze on forage surrounding feces at normal or low stocking densities (Goldberg 1970, Reinemeyer 1990, Stromberg 1997, and Williams and Bilkovich 1973). Dairy cattle, if given the option, will avoid forage within 10 to 20 cm of feces (Reinemeyer 1990). Cattle in low stocking situations do not graze forage as intensely and therefore do not ingest as many infective larvae as cattle in high stocking densities which are forced to graze all available forage (Williams 1983).

Therefore, migration of trichostrongyle larvae and dissemination of feces and larvae by various means (rain, dragging, trampling, insects and earthworms) are critical in making the trichostrongyle larvae available for infection of the host (Reinemeyer 1990, Rogers and Petronijovic 1982, Stromberg 1997, Williams and Biklovich 1973, and Williams 1983). Information on the migration and distribution of trichostrongyle larvae provides an idea of the potential level of infection and can aid in pasture management.

In Georgia, *O. circumcinta*, a sheep-specific species, was observed to migrate no farther than 12 cm from the feces, while *Cooperia punctata*, a parasite of cattle, migrated 15 to 20 cm away from the manure pats (Tarshis 1958). In Louisiana, 10 – 20 % of infective *Ostertagia ostertagi* larvae were recovered at 15 to 30 cm and 30 to 45 cm away from the cow pats (Williams and Bilkovich 1973). Optimal recovery of trichostrongyle larvae seems to be at a distance of 5 cm away from the fecal source (Stromberg 1997).

In Louisiana, the longest persistence for *Cooperia punctata* on the herbage from plots contaminated was 4 to 5 months (September to January), and then 3 to 4 months during the February to May (Williams and Mayhew 1967). *Cooperia punctata* survived 6.5 months (October through April) after plot contamination in Kansas (Williams and Mayhew 1967). *Ostertagia* spp. were found 12 months and 18 months after contamination in western Scotland (Bairdenand Armour 1985, and Saqur et al. 1982).

Optimal conditions in Louisiana, a southern temperate region, for trichostrongyle development occurred from October to November and again from March to May (Williams and Mayhew 1967), and high recovery of infective trichostrongyle larvae occurred from November through May (Williams and Bilkovich 1973). Differences between contamination months suggest climatic conditions that occur during deposition are important in the outcome of contamination (Barger et al. 1984).

In southern temperate regions, winters are usually mild enough to permit cattle to graze on pasture (Reinemeyer 1990). This extended grazing period provides an advantageous situation under favorable conditions for contamination and infection during those months (Reinemeyer 1990). By May, the number of larvae begins to decline (Reinemeyer 1990).

The objective of this experiment was to measure the lateral migrations of trichostrongyle larvae from contaminated bovine feces and onto the surrounding herbage under environmental conditions in eastern Tennessee.

(ii.) Materials and Methods

Collection and preparation of contaminated manure pats : Contaminated manure was collected at the beginning of each of the four experimental months (November, January, February, and March) from weaned, untreated steers kept on rye pasture at the UT-KES Blount Beef Unit, in Alcoa, Tennessee.

The manure was taken to the laboratory and thoroughly mixed. Ten 150-gram manure samples were prepared by weighing out each manure sample to 151 grams in pre-weighed plastic specimen cups (150 mL). Then 10 one-gram manure subsamples were taken from each of the ten larger samples and put into paper cups (89 mL.) which were used in the mixing of the manure sample and water (10 mL) in order to free all parasite products. This fecal-water suspension was then processed by a modified sucrose flotation and centrifugation technique (Cox and Todd 1962).

After centrifugation, the numbers of trichostrongyle eggs were counted to confirm contamination of the ten pat samples for the monthly treatments, and to estimate the number of trichostrongyle worms that could migrate out of each pat. Number of trichostrongyle eggs/kilogram of feces and were calculated from subsample counts.

After the manure samples had been confirmed to be contaminated with trichostrongyle eggs, the pats were transported to the UT-KES Small Grains Unit in Knoxville, Tennessee.

Experimental site and design : This experiment was carried out at the UT-KES Small Grains Unit, in Knoxville, Tennessee on a 6.7 m x 3.7 m plot of level land. Since the 1940's when mules were used on this experimental farm, this experimental plot and the area surrounding has never been used for grazing and therefore was determined to be free from contamination by domestic animal parasitic nematodes. The experimental plot was divided into four 1.2 x 3.1 m subplots made up of 10 60 x 60 cm sampling squares arranged in 2 x 5 rows.

One 150-gram pat was placed in the middle of each 60 x 60 cm sampling square on one day in the first week of the four treatments. Therefore, for the first month (November) of the experiment, only one subplot was contaminated. A second subplot was contaminated in January. The third subplot was contaminated in February, and the fourth subplot in March. A field sampling flag was inserted in each pat to mark its location for future identification.

After the pats were placed on the treatment plots, grass samples were collected at weekly intervals. One sampling square was used for each sampling interval. Clippings were taken at 5, 20 , and 30 cm away from the edge of the pat within the sampling square. Grass was cut (5 x 5 cm in area) around the point of distance from the edge of the pat and down to the soil surface. The weekly sampling intervals for the month of November were: 1, 2, 4, 6, 8, 10, 14, 18, 22, and 24 weeks after contamination. January, February, and March sampling intervals were 1, 2, 3, 4, 6, 8, 10, 14, 18, 22; 1, 2, 4, 6, 8, 10, 14, 18; and 1, 2, 3, 4, 6, 8, 10, and 14 weeks after contamination, respectively. February and March

only had eight weekly intervals because data from a ninth and tenth interval for both months would not be carried out in time to be included in this report.

Nematode recovery and analysis : Grass samples were taken to the laboratory for the recovery of any third stage trichostrongyle larvae. Each sample was first weighed and then nematodes were recovered in a modified rapid technique for the recovery of strongyloid nematodes from pasture samples as described in Young and Trajstman (1980). This technique, over a three to four day time period, basically washed the nematodes off the grass and funneled the nematodes into collection tubes for fixation and identification.

The dried herbage was reweighed five to seven days after the initial collection and recorded for the respective sample in order to calculated the number of trichostrongyle larvae per kilogram of herbage.

Once all samples had been examined for third stage trichostrongyle larvae, the number of L3 trichostrongyle larvae per kilogram of herbage was calculated using the following formula:

1000 / dry weight of herbage (g) x n = number of L3 larvae/kg of herbage, where (n) is the number of L3 larvae recovered for each sample.

The number of L3 larvae/kg of herbage for each treatment month as well as the number of L3 larvae recovered per weekly interval in each month and the number recovered at the three distances were compared and analyzed through ANOVA Repeated Measures Analysis at $P = 0.05$ level of significance (SAS

1987).

Daily air temperature was recorded at the UT-KES Plant Science Unit, in Knoxville, Tennessee. Daily precipitation was recorded at the UT-KES Small Grains Unit, in Knoxville, Tennessee.

Toward the end of the experiment, several herbage samples had to be processed at the same time. However, this situation did not affect the recovery of nematodes.

(iii.) Results and Discussion

Monthly mean maximum and minimum air temperatures and total monthly precipitation for the present study are presented in Table 5.1. Average maximum air temperatures for all seven months fell within the ranges (5-32°C) for optimal development and transmission of trichostrongyle larvae. Minimum air temperatures from November through March were at a level that could slow development of trichostrongyle larvae. All monthly precipitation totals with the exception of the months of April and June were in the range for optimal development (50-120 mm).

The initial egg counts for each 150-gram pat are presented in Table 5.2. Although the manure was homogenized, the initial number of eggs still varied among the ten samples for all four treatments. The average weight of herbage samples was not significantly different for all four treatment months (Table 5.3).

Table 5.1. Meteorological data recorded for the trichostrongyle lateral migration study from November 1998 through June 15, 1999.

	Temperature °C (Mean)		Precipitation (Avg.)
Month	Max	Min	(mm)
November	19.1	2.7	53
January	10.5	- 1.6	186
February	11.7	- 0.9	89
March	12.7	- 0.8	121
April	20.2	6.8	06
May	23.5	8.9	109
June*	30.7	18.4	14

* The readings for the month of June are only for June 1 through June 15, 1999 since June 15, 1999 was the last day of the experiment.

Table 5.2. Initial number of eggs per 150-gram pat per treatment and weekly sampling interval.

	Treatment (month)			
Weekly interval	November	January	February	March
1	13,350	6,900	10,800	750
2	7,050	4,000	43,950	4,800
3	----	9,000	----	3,900
4	13,200	21,000	7,200	900
6	21,450	19,000	8,250	600
8	3,600	50,000	9,150	2,100
10	9,000	26,000	13,650	2,100
14	16,950	1,000	7,050	8,400
18	8,250	26,000	2,250	----
22	2,400	39,000	----	----
24	3,300	----	----	----

Number of eggs for each 150-gram pat was derived from the number of eggs/1 g of manure multiplied by 150 (g).

(----) represents intervals when no sampling took place.

Table 5.3. Average weight (g) of herbage samples recovered from plots contaminated in November, January, February, and March.

Treatment Month	Average herbage weight (g)
November	3.75
January	3.25
February	3.06
March	3.23

Fifty-three percent of the weekly interval samplings recovered L3's after the November contamination (Figure 5.4). Only 10% of the weekly samples after the January treatment and 3% of the February treatments had L3's recovered. No L3's were recovered for the March treatment. Statistical analysis showed that the samples from the November treatment were significantly different from the other three treatments. January, February, and March treatments were not significantly different from one another.

The effect of the treatment month on the number of larvae proved to be significant (Figure 5.5). The November treatment had 457 L3/kg of herbage which was the highest number and significantly different from the other three treatments. The numbers dropped considerably to 43 L3/kg of herbage from the January treatment. The February treatment only had 17 L3/kg of herbage being recovered at the weekly intervals, and the March treatment resulted in no L3's

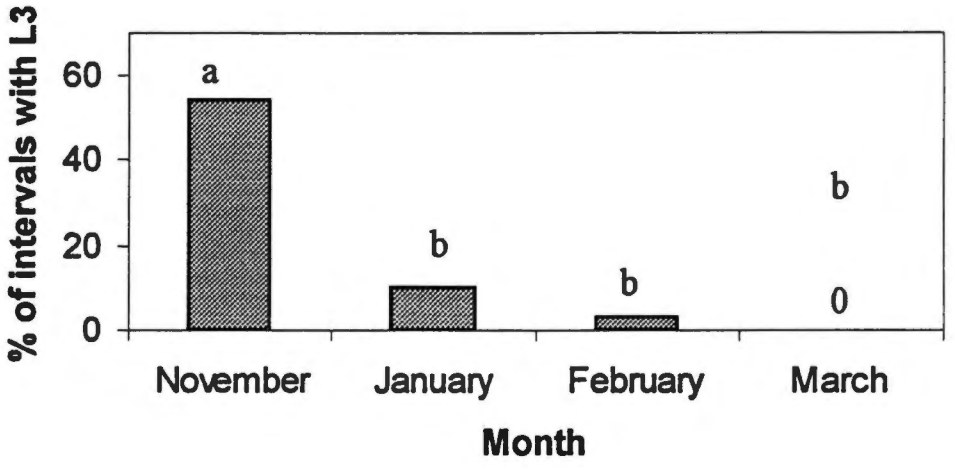

Figure 5.4. Percentage of intervals per monthly treatment in which infective trichostrongyle larvae (L3) were recovered. Bars with the same letter are not significantly different according to Repeated Measures Analysis ($P = 0.05$).

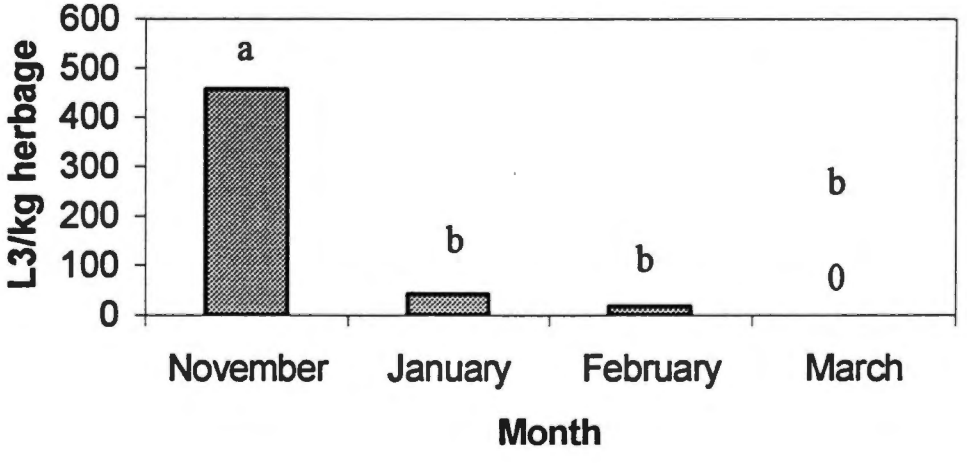

Figure 5.5. Number of infective trichostrongyle larvae (L3) recovered per kilogram of herbage. Bars with the same letter are not significantly different according to Repeated Measures Analysis ($P = 0.05$).

recovered.

The percentage of samples taken at the three distances (5, 20, and 30 cm) at which infective trichostrongyle larvae (L3) were recovered were not significantly different. However, these percentages decreased with an increase in distance (Figure 5.6). At 5 cm away from the pat, only 22 % of the samples clipped at that distance had L3's recovered. The percentage of samples taken at distances of 20 cm and 30 cm that had L3's recovered were 17 % and 11 %, respectively.

The effect of distance on the number of larvae/kg of herbage was not significant at the $P = 0.05$ level (Figure 5.7), but still shows a decrease in the number of larvae with an increase in distance per kilogram of herbage. Samples taken at 5 cm away from the pat had 176 larvae/kg of herbage, 20 cm had 149 larvae/kg of herbage, and 30 cm had 70 larvae/kg of herbage.

The effect of weekly intervals as significant for the percentage of positive samples for all four treatments (Table 5.4). However, it was not significant for the number of L3/kg of herbage (Table 5.5). Analysis of these intervals demonstrated that the larvae appeared in the herbage 4 weeks after contamination. This time period of 4 weeks could be due to the developmental time as influenced by temperature and precipitation. The highest numbers recovered were in the last height was not thought to be a factor in low numbers recovered because it was clipped down to the soil surface, therefore larvae at all levels of the grass blades should have been recovered. However, in the early spring months, the increase in

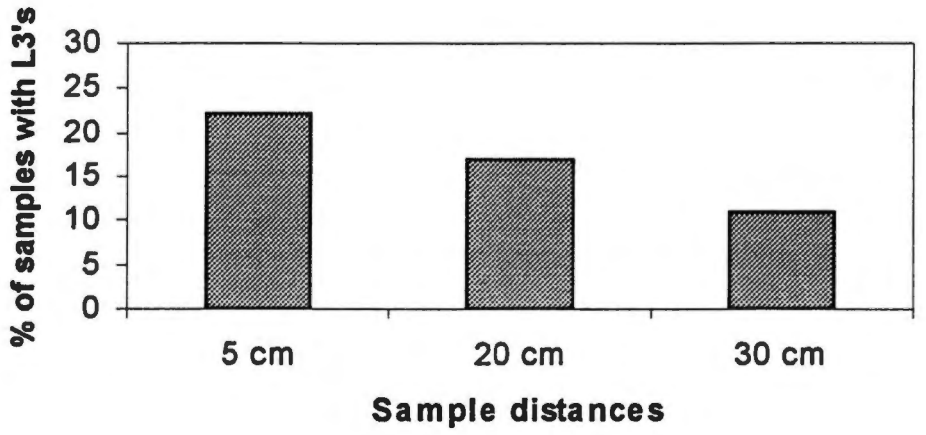

Figure 5.6. Percentage of samples from different distances for all four treatments in which infective trichostrongyle larvae (L3) were recovered.

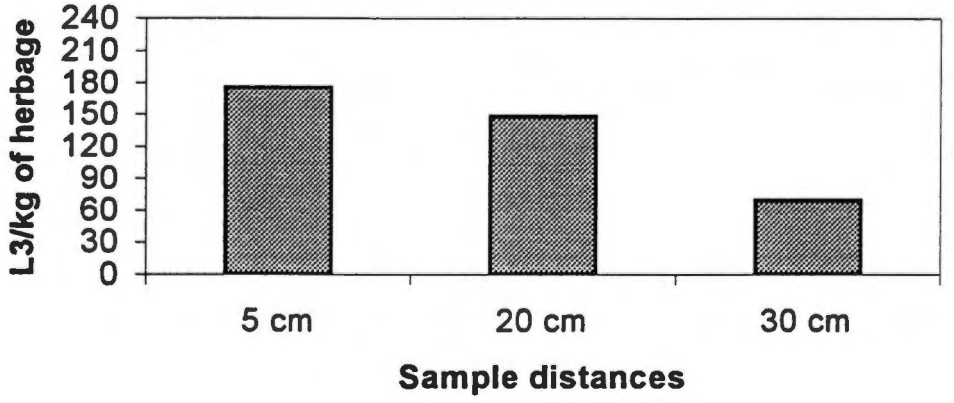

Figure 5.7. Number of infective trichostrongyle larvae (L3) per kilogram of herbage in samples taken at different distances from manure pats.

Table 5.4. Mean percentage of weekly intervals for all four treatments in which infective trichostrongyle larvae were recovered.

Weekly Interval	Percentage (%)
1	0
2	0
3	0
4	0
6	31
8	19
10	19
14	31
18	33
22	38
24	75

Table 5.5. Number of infective trichostrongyle larvae (L3) recovered per kilogram of herbage. Table represents intervals from all four treatments.

Weekly interval	L3/kg of herbage
1	0
2	0
3	0
4	0
6	287
8	91
10	107
14	341
18	213
22	317
24	550

two intervals of 22 and 24 weeks after contamination (5.5 and 6 months, respectively).

During this study, conditions seemed to be favorable throughout the seven months for development and transmission of the trichostrongyle L3's. Numbers in November were as expected. The January and February treatments should have had higher numbers similar to or higher than those recovered for the November treatment. This is because in a southern temperate region like eastern Tennessee, development and transmission of trichostrongyle larvae occurs in the winter and early spring months (Williams 1983, and Stromberg 1997). The majority of weekly samples for the January and February treatments occurred in the months of January, February, and March. In all collections, regardless of the month, distance, or time after contamination of the plot (weekly interval), free-living, plant-parasitic, or animal parasitic nematodes were collected.

Low numbers of nematodes recovered could be attributed to the sampling and recovery process. The herbage samples may not have been large enough and therefore missed larvae dispersed on the vegetation. Grass height probably was not a factor in the low L3 numbers recovered because grass was clipped down to the soil surface. Larvae at all levels of the grass blades should have been recovered.

Mwegoha and Jørgensen (1977) reported on two principles that recovery techniques depend on: (1) the specific gravity of the larvae which is important in flotation, sedimentation, and centrifugation, and; (2) the particle size of the

nematodes (relative to the sieve mesh size). The two principles, plus the fact that the nematode recovery process depends on nematode activity, pose a challenge in optimal recovery (Mwegoha and Jørgensen 1977). During the laboratory recovery process, it is quite possible that larvae were lost in the rinsing and funneling procedures.

We expected to recover infective larvae of both *Ostertagia* spp. and infective *Cooperia* spp. during this study, however all of the infective trichostrongyle larvae recovered were *Cooperia* spp, the most prevalent southern temperate trichostrongyle according to Reinemeyer (1990). In an Australian temperate region, similar to the southern temperate region in eastern Tennessee, more *Cooperia* than *Ostertagia* eggs were observed to be deposited by contaminated calves (Barger et al. 1984). *Cooperia* was most prevalent during all seasons in southeastern Queensland, Australia (Williams and Mayhew 1967). Our results do not necessarily mean that *Ostertagia* spp. are not to be found in eastern Tennessee. Perhaps their numbers are low and larger herbage samples than those used in this present study would be necessary to recover *Ostertagia* spp.

(iv.) Conclusion

There was little lateral migration of infective trichostrongyle larvae during this seven month study. What activity there was occurred mainly after in the November treatment. The numbers recovered during November reflected what is

usually expected of southern temperate regions where development and transmission increase in the winter and early spring months (Reinemeyer 1990, Stromberg 1997, and Williams 1983). Recovery of L3 larvae occurred beginning four weeks after contamination of the treatment plots. Meteorological conditions througout this study were conducive to development and transmission. The optimal distance from the pat for recovering infective larvae was at 5 cm away. Infective trichostrongyle larvae were recovered 5.5 to 6 months after contamination.

Recommendations for future research are: (1) take larger herbage samples that should compensate for the increased growth of herbage in the spring and recover larger numbers of larvae, (2) incorporate more equipment and staff in order to handle larger sampling sizes, (3) begin sampling earlier in the fall and carry all weekly intervals to 6 months or more, and, (4) use larger fecal samples to contaminate the plots. Other research could focus on distances farther than 30 cm away, different forage type or level of blade height, or carry sampling through the summer months. Also, identification of trichostrongyle larvae to species would be helpful information.

Rate of ingestion or transmission is influenced by grazing behavior and management procedures (Stromberg 1997). This type of information can give an idea about the contamination, distribution, and seasonal occurrence of infective trichostrongyle larvae in the field which can help guide pasture management decisions in eastern Tennessee.

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