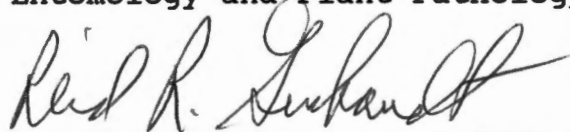


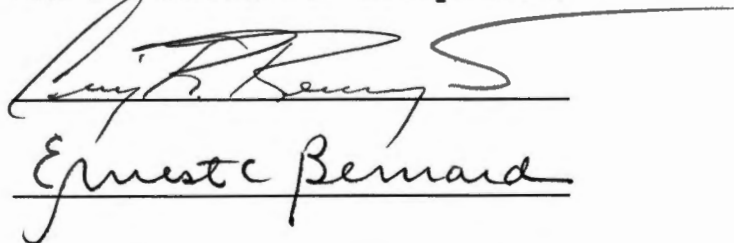
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SEASONAL DISTRIBUTION OF PARASITISM OF THE
FACE FLY (*Musca autumnalis* DE GEER) BY
Paraiotonchium autumnale (NICKLE) SLOBODYANYUK
IN EASTERN TENNESSEE

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Mark Caldwell Carder

December 1994

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ABSTRACT

The distribution and prevalence of the nematode *Paraiotonchium autumnale* in *Musca autumnalis* was studied at five sites in eastern Tennessee during 1993 and 1994. *Paraiotonchium autumnale* parasitized face flies at all sites in 1993 with a seasonal prevalence of 1.66%. Parasitized face flies were collected from four of the five study sites in 1994 and had a lower seasonal prevalence of 0.48%. Prevalence at the eastern Tennessee sites during 1993 and 1994 were the lowest reported in the United States, although Ontario had similar levels of parasitism in 1971 of less than 2%. *Paraiotonchium autumnale* appeared to have no effect on the numbers of face flies collected during 1993 or 1994. Experiments to examine the effects of simulated rainfall on larval mortality in *Musca autumnalis* were conducted by applying 0, 6, 12, and 24 mm of simulated rainfall to 24, 48, and 96 hr postoviposited larvae in artificial dung pats. Survival of 24 hr postoviposited larvae was significantly affected by simulated rainfall. Unexpected decreases in the numbers of face flies collected were not related to the application of insecticidal eartags. Numbers of face flies were correlated with rainfall of 8 mm or more prior to collections.

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CHAPTER I

INTRODUCTION

i - The Face Fly

The face fly, *Musca autumnalis* De Geer (Diptera: Muscidae), has been a pest of pastured cattle in North America since it was introduced into the maritime province of Nova Scotia in 1952 (Vockeroth 1953). The face fly has spread throughout North America with the exception of Alaska, Arizona, Florida, New Mexico, and Texas (USDA 1966, 1967, 1968, 1969, 1975; Poorbaugh and Smith 1968, Depner 1969, Pickens and Miller 1980).

The biology of *M. autumnalis* in Europe was described most extensively by Hammer (1942) and in North America was reviewed by Teskey (1960) and Wang (1964) under field and laboratory conditions, respectively. The life cycle of the face fly consists of egg, three larval instars, pupa and adult. Under pasture conditions, the life cycle of *M. autumnalis* is completed in 17-20 days (Hammer 1942, Wang 1964). Face flies are obligate dung-breeding flies, with gravid females ovipositing only on fresh bovine manure (Teskey 1960). Eggs hatch 18-24 hours after oviposition. Larval instars develop for 3 to 3.5 days and feed on and in the dung. The first instar lasts for 6.5-7.5 hours; the second 16.6-17.5 hours; and the final instar 55-57 hours.

Mature third instar larvae (pre-pupae) leave the dung and wander prior to pupation, which occurs outside the dung pats. The pupal period lasts 7.5-8 days.

West (1951) described *M. autumnalis* as a "facultative hematophagous" species, one which feeds upon blood opportunistically. Hammer (1942) considered it to be one of the muscoid flies in transition from general secretion feeding and obligate hematophagic feeding. Although blood offers the highest protein content for egg maturation, other foods are consumed more frequently, including dung liquids and oral and nasal secretions (Matthew 1960, Teskey 1960, Treece 1960, Turner and Hair 1967). The oral and nasal secretions of cattle offer *M. autumnalis* the most constant source of nutrition, thus the common name, face fly, is derived from its behavior of accumulating in and around the eyes and muzzles of cattle.

Face flies are an economic pest of both the beef and dairy industries. In 1968 the USDA (1968) estimated that approximately 80 million dollars annually was lost by the cattle industry due to face flies, rising to \$140 million dollars in 1977 (ARS 1977). The protective and evasive actions of cattle in response to feeding flies usually is considered to cause subclinical production losses, including reduced weight gain and decreased milk production (Teskey 1960, Decker 1961, Peterson and Boreherding 1962, Benson and Wingo 1963, Hansens 1963, Hansens and Valiela 1967, Ode and

Matthysse 1967). Reports of reduced weight gain due to face flies have been contradicted (Arends 1981).

The most economically significant damages caused by face flies are medical complications. Face fly feeding behavior may directly damage the cornea and conjunctiva of the eye and cause severe lesions due to rasping of the prestomal teeth (Brown and Adkins 1972, Shugart et al. 1979, Broce and Elzinga 1984). Face flies have also been shown to be vectors of some disease-causing organisms. Face flies are mechanical vectors of *Branhamella catarrhalis*, *Corynebacterium pyogenes*, infectious bovine rhinotracheitis virus (IBR), and *Moraxella bovis*, the causative organism of infectious bovine keratoconjunctivitis (IBK or pinkeye) (Steve and Lilly 1965, Cheng 1967, Wetzel 1974, Gerhardt et al. 1976, Gerhardt et al. 1982, Pickens and Miller 1980). IBK is the most frequent eye disorder of cattle (Pugh and Hughes 1975).

Face flies are the biological vectors of several nematodes, including *Parafilaria bovicola* and four species of *Thelazia*: *T. gulosa*, *T. lacrymalis*, *T. rhodesi*, and *T. skrjabini* (O'Hara and Kennedy 1991). In Europe, *P. bovicola* is the cause of hemorrhagic bovine filariasis, commonly known as green muscle disease (Bech-Nielsen et al. 1982). *Thelazia* spp. from *M. autumnalis* in North America were first reported by Chitwood and Stoffolano (1971). Species of *Thelazia* are parasites of the eyes of mammals and birds and

thus are commonly called eye worms.

Chemical devices are most often utilized to control face fly populations. Self-treating devices, such as dust bags and back rubbers, can effectively control face flies on cattle (Dobson and Huber 1961, Roberts 1963, Turner 1965, Hair and Adkins 1965). Feed additives and oral larvicides excreted in dung control face flies by killing the larval stages (Anthony et al. 1961, Wallace and Turner 1962, Miller and Pickens 1975, Gerhardt and Cook 1976, Miller et al. 1978). Insecticide-impregnated eartags applied to the ears of cattle can significantly reduce face flies on pastured cattle (Williams and Westby 1980, Williams and Westby 1982, Knapp and Herald 1980).

Acarina, Coleoptera, Diptera, and Hymenoptera that attack or affect adult or immature face flies apparently exert little or no natural control in the field (Pickens and Miller 1980). Hymenopteran parasitoids are considered unimportant mortality factors (Blickle 1961, Thomas and Wingo 1968, Turner et al. 1968) due to the inability of the parasites to emerge from the calcified puparia (Burton and Turner 1968). Predation on the immature stages appeared to be the highest mortality factor in the field (Valiela 1969, Burton and Turner 1970).

Abiotic factors such as wind speed, relative humidity, and rainfall may affect face fly activity (Engroff et al. 1972). Simulated rainfall has been demonstrated to reduce

larval survival of the Australian bush fly (*Musca vetustissima* Walker) by up to 83% (Hughes 1979).

ii- The Nematode

The face fly parasitic nematode, *Paraiotonchium autumnale* [Nematoda: Iotonchiidae], is a potential candidate for natural fly control. Members of this genus are all parasites of *Musca* (Slobodyanyuk 1975, Slobodyanyuk 1976, Siddiqi 1986, Remillet and Loumond 1991).

Paraiotonchium autumnale was discovered in 1965 by J.G. Stoffolano (Stoffolano and Nickle 1966) in a face fly colony at Cornell University and subsequently described as *Heterotylenchus autumnalis* by Nickle (1967). Slobodyanyuk (1975) transferred it to *Paraiotonchium*. Since the discovery of *P. autumnale* it has been reported from Canada and most areas of the United States, but with varying prevalences (Jones and Purdue 1967, Stoffolano 1968, Stoffolano and Nickle 1966, Thomas and Puttler 1970, Thomas et al. 1972, Wright 1972, Robinson and Combs 1976). The nematode was thought to have been introduced into North America from Europe with its host (Krafsur et al. 1983).

The life cycle of *P. autumnale* is similar to that described for *Heterotylenchus aberrans* (Christie 1950, Stoffolano and Nickle 1966, Nickle 1967, Treece and Miller 1968, Stoffolano 1970, Nappi 1973, Kaya and Moon 1980). The life cycle of *P. autumnale* is complex and, except for a

brief free-living stage, occurs entirely within the fly host. The nematode alternates between one gamogenetic generation and one parthenogenetic generation. Infected female face flies deposit gamogenetic J4 (fourth stage juveniles) male and female nematodes into fresh cow dung, where they molt once to maturity and mate. After copulation, the males die and the fertilized infective stage female penetrates a developing maggot. The nematode then enters the haemocoel. About five days after the fly emerges from its puparium, each nematode lays 15-24 eggs. These eggs develop into parthenogenetic females within two days, each of which lay 200-300 eggs. Juveniles hatching from these eggs are gamogenetic. Approximately 9-12 days after fly eclosion the juvenile nematodes molt to the J4 stage and migrate into the ovaries. The invasion of nematodes destroys the ovaries and eggs, sterilizing the female fly (Stoffolano and Nickle 1966, Stoffolano 1967, Treece and Miller 1968). Large numbers of worms in the ovaries distend the abdomen, causing the onset of oviposition behavior. In the absence of eggs in the ovaries, juvenile nematodes are deposited into the dung. This false oviposition is termed nemaposition.

Distension of the abdomen induces behavioral changes causing infected face flies to become terminal dung feeders (Kaya and Moon 1979). Nematodes deposited 9-11 days after eclosion were capable of infecting face fly larvae (Jones

and Perdue 1967, Stoffolano 1967, Treece and Miller 1968) and could infect all three larval instars in the laboratory (Stoffolano 1970). Face flies exposed to *P. autumnale* in the laboratory have a skewed sex ratio in favor of males (Chirico 1990). Although male face flies infected with nematodes can transmit nematodes to fly larvae they are considered "dead end" hosts because there is no natural outlet to the environment (Nappi 1973).

The life cycles of *M. autumnalis* and *P. autumnale* are synchronized. Nematode reproduction ceases during diapause of the face fly (Stoffolano 1967). Diapause in face flies is characterized by hypertrophied fat bodies and arrested ovarian development (Stoffolano and Matthysse 1967). Lack of essential hormones or proteins were thought to limit egg production in the nematode (Stoffolano 1967). Only adult infective and parthenogenetic female nematodes have been found in diapausing face flies (Kaya and Moon 1978). Hypobiosis of the nematodes has been reported in diapausing face flies, thus *M. autumnalis* was deduced as the overwintering site of *P. autumnale* (Chirico 1994).

iii - Objective of this Study

Sterilization of the host, host specificity, and reinfestation of nematodes in the breeding media make *P. autumnale* an excellent candidate for biological control of *M. autumnalis*. The objectives of the study were to

determine: 1) the distribution of *P. autumnale* in eastern Tennessee and prevalences of *P. autumnale* parasitizing *M. autumnalis* at selected sites; 2) experimentally examine the effects of simulated rainfall on larval mortality of *M. autumnalis*; 3) to discuss observations on sampled face fly populations and possible relationships between decreased numbers of face flies and abiotic factors which could affect them.

CHAPTER II

SEASONAL DISTRIBUTION AND PARASITISM OF *MUSCA AUTUMNALIS* BY *PARAIOTONCHIUM AUTUMNALE* IN EASTERN TENNESSEE

i - Introduction

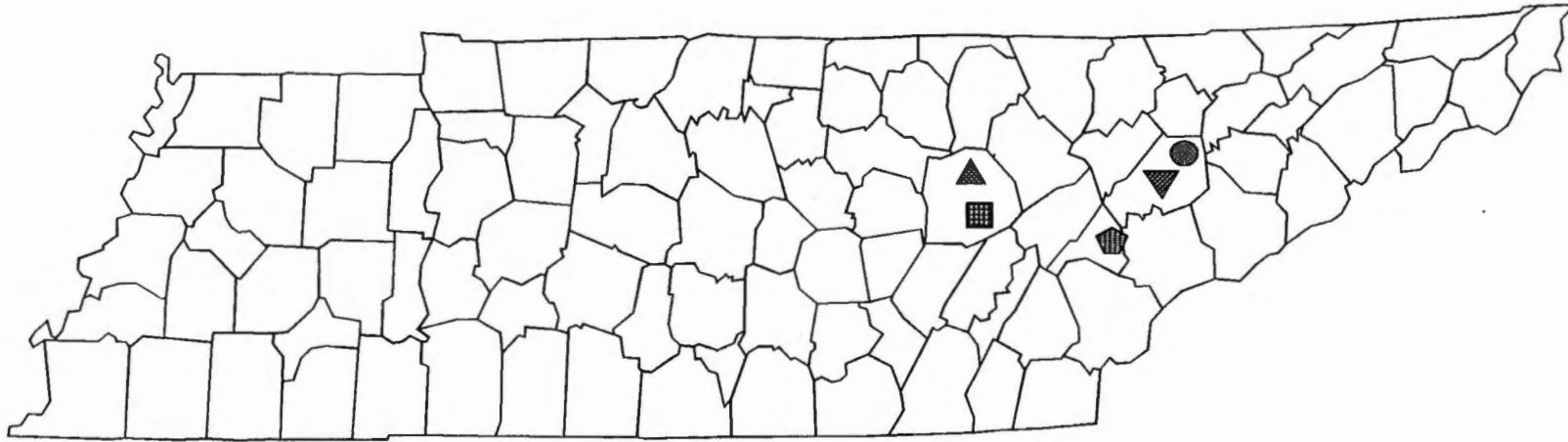
The face fly (*Musca autumnalis* De Geer [Diptera: Muscidae]) is an introduced pest of cattle in North America (Vockeroth 1953, Depner 1969). In Europe, where *Musca autumnalis* is endemic (Pickens and Miller 1980), it is considered a moderate pest of livestock (Hammer 1942). In North America, control of the face fly is warranted due to the monetary losses it may cause, through either disease organism transmission or avoidance behavior in cattle. Chemical control approaches have most often been employed against the face fly, but natural arthropod predators and parasites also have been investigated (Blickle 1961, Benson and Wingo 1963, Hair and Turner 1965, Houser and Wingo 1967, Thomas and Wingo 1968, Valiela 1969, Remmel 1971, Wylie 1973). Stoffolano and Nickle (1966) reported a nematode parasite, *Paraiotonchium autumnale* (Nickle) Slobodyanyuk [Nematoda: Iotonchiidae] (= *Heterotylenchus autumnalis* Nickle), which sterilized the female face fly. All members of the genus *Paraiotonchium* are parasites of *Musca* (Slobodyanyuk 1975, Slobodyanyuk 1976, Siddiqi 1986,

Remillet and Laumond 1991). *Paraiotonchium autumnale* is host-specific for *M. autumnalis* (Jones and Perdue 1967, Stoffolano 1967, Stoffolano and Streams 1971, Nappi and Stoffolano 1971, Nappi and Stoffolano 1972, Chirico 1994). *P. autumnale* is present in Europe and is thought to have been introduced into North America with the face fly (Krafsur et al. 1983). The distribution of *P. autumnale* probably is equivalent to that of *M. autumnalis*. The face fly has been reported in all states except Alaska, Arizona, Florida, Louisiana, New Mexico, and Texas (USDA 1969, 1975), while *P. autumnale* has been reported from California (Kaya and Moon 1978), Iowa (Krafsur et al. 1983), Mississippi (Robinson and Combs 1976), Missouri (Thomas and Puttler 1970; Thomas et al. 1972), Nebraska (Jones and Perdue 1967), and New York (Stoffolano and Nickle 1966, Stoffolano 1968). The objectives of this study were to determine the seasonal and temporal distribution of *P. autumnale* in face flies in eastern Tennessee pastures during 1993 and 1994, and to determine the impact of parasitism on face fly populations.

ii - Materials and Methods

Study Sites

Five study sites were selected in eastern Tennessee, two on the Cumberland Plateau and three in the Tennessee River Valley (Figure 2.1). The Cumberland Plateau sites (C.P.S.) were Grasslands Farm and Crab Orchard. The



- ▲ Grasslands Farm, Plateau Experiment Station, Cumberland Co.
- Crab Orchard, Cumberland Co.
- Holston Farm, Knox Station, Knox Co.
- ▼ Cherokee Farm, Knox Co.
- ◆ Smith Farm, Loudon Co.

Figure 2.1. Geographic location of cattle used to determine the seasonal and temporal distribution of *Paraiotonychium autumnale* in eastern Tennessee during 1993 and 1994.

Tennessee River Valley sites (T.R.S.) were Holston Farm, Cherokee Farm, and the Loudon County farm.

The westernmost site in the study, Grasslands Farm, Plateau Experiment Station, Crossville, Tennessee, is owned and operated by the University of Tennessee Agricultural Experiment Station. Cattle to be sampled were maintained on pastures of ca. 11 ha each with shaded resting sites and adequate water. During the 1993 and 1994 seasons, cattle in the experiment were moved among fields to maintain adequate forage. Experimental herds consisted of 12-15 mixed Angus and Hereford cow-calf pairs. The 1993 herd adults received two insecticidal eartags (Diazinon, 20% ai) each during the week prior to 22 June. The 1994 herd did not receive any treatment for face fly control.

The second Cumberland Plateau site was located in Crab Orchard, Cumberland County, Tennessee. The Crab Orchard site is owned and operated by Mr. R. L. Wheeler, a private land owner cooperating in this study. Cattle were kept on two pastures, each ca. 10.1 ha. The pastures were separated by a woodlot, but were connected by a lane ca. 75 m long. The herd each year consisted of 25 registered Polled Hereford cow-calf pairs. Adult cows received one insecticidal eartag (Diazinon, 20% ai) each during the weeks prior to 22 June, 1993 and 15 June, 1994.

Holston Farm, Knox County, Tennessee is owned and operated by the University of Tennessee Agricultural

Experiment Station. Two pastures, one 8.5 ha and the other 4.8 ha, were utilized on a rotational basis to maintain adequate forage. Herds were mixed breed Angus and Hereford, comprised of 22 cow-calf pairs in 1993 and 25 cow-calf pairs in 1994. The cattle received no insecticidal treatment in either year.

The University of Tennessee Cherokee Farm Facility, Knoxville, Tennessee, is managed by the University of Tennessee Veterinary Teaching Hospital. Cattle were rotated among four pastures of ca. 0.8 ha each during both seasons. Herds were of various breeds, but predominantly Angus, Hereford, and Holstein. Numbers of experimental animals varied throughout both seasons, but at least 10 animals were present at all times. No specific treatment for face flies was administered. Horses and goats were pastured adjacent to the cattle.

The Loudon County site is the property of Mr. Carl Smith and was the most southern site in the study. Herds were maintained on one field ca. 21.4 ha holding 19 cow-calf pairs in 1993 and 10 cow-calf pairs and 8 heifers in 1994. Animals were Brahman-Angus and Polled Hereford crosses. One insecticidal eartag (Diazinon, 40% ai) was applied to each adult the week prior to 7 April, 1993 and 7 April, 1994.

Face Fly Collections

Face flies were collected approximately every two weeks, May-September, 1993 and April-September, 1994. Flies

were collected by means of head sweeps, dung sweeps and dung pat collection. These collection techniques were similar to those used in other face fly studies (Stoffolano 1968, Thomas and Puttler 1970, Thomas et al. 1972, Wright 1972).

Face sweeps were made with an aerial insect net to collect face flies directly from the eyes and muzzles of cattle. Dairy feed was placed on the ground in the pasture and cows were allowed to feed. Sweeps were made around the nose and then directed upward across the eye as the animal fed. At least five cows at each location became conditioned to frequent sweeps of the net, allowing for a uniform 20 minute sampling period per site.

Adult face flies were collected from the surface of fresh manure at the same time as the face sweep. Face flies visiting fresh pats during the 20 minute time interval were collected with an aerial insect net. The sampled fresh pats were 0-20 minutes old.

Adult face flies collected from faces and pats were placed into separate meat/poultry bags and labeled by date and location. Bags were placed into a cooler containing crushed ice and transported to the Medical and Veterinary Entomology laboratory for dissection. Flies were held in the refrigerator 2-36 hours until dissection.

Six dung pats, 3-4 days old, were collected from each site on each sampling date. Pats selected had a dried outer surface or crust surrounding a moist inner area. Two pats

each were placed into a modified emergence trap and surrounded by sand. Each emergence trap was a wooden box, with a plywood bottom and hinged screen top, measuring 43 × 61 × 8.8 cm. Pats were labeled by date and location, transported to the Entomology insectary, and incubated at 27 °C, >50% relative humidity, with a 16 hr light/ 8 hr dark photoperiod (16:8 L:D). After 7 days, face fly pupae were collected by sifting the surrounding sand, counted, and placed into 0.3m³ screen/aluminum cages for emergence. Emergent adult flies were reared for 12 days under laboratory conditions, then dissected. Adults were fed an artificial diet of a 1:1 mix of powdered milk and granulated sugar, at 23 °C and 24-hr light regime. Pupae from which emergence did not occur were dissected and examined for parasites.

Dissections

Dissections were performed with the aid of a dissecting microscope at 8-10× power, with a black background stage. Face flies were dissected in Ringer's solution (NaCl, 8.0gm; NaHCO₃, 0.2gm; KCl, 0.2gm; CaCl₂, 0.2gm; Dextrose, 1.0gm; H₂O 1l). All adult, field-collected female face flies were dissected by opening the abdomen with forceps and removing the ovaries. The ovaries were examined for nematodes and age-graded according to Van Geem et al. (1983) (Table 2.1). Adult female face flies reared from emergence traps were only recorded as infected or non-infected, since all were

Table 2.1. Ovarian characteristics for physiological age grade stages for non-diapausing face flies (Van Geem et al. 1983).

Age Class	Gonadotrophic Cycle	Follicular Relics	Proximate Follicle
0	Nulliparous	Absent	undifferentiated
1	Nulliparous	Absent	no yolk
2	Nulliparous	Absent	yolk < 1/2 length
3	Nulliparous	Absent	1/2 < yolk < 3/4 length
4	Nulliparous	Absent	yolk ≥ 3/4 length
5	Nulliparous	Absent	Black mast present
6	Uniparous	YT	no yolk uncontracted
7	Uniparous	YT	yolk < 1/2 len; ET may be uncontracted
8	Uniparous	YT	1/2 < yolk < 3/4 len; ET partial contracted
9	Uniparous	YT	yolk ≥ 3/4 length
10	Uniparous	YT	Black mast present
11	Biparous+	DYB+YT	no yolk uncontracted
12	Biparous+	DYB+YT	yolk < 1/2 len; ET may be uncontracted
13	Biparous+	DYB+YT	1/2 < yolk < 3/4 len; ET partial contracted
14	Biparous+	DYB+YT	yolk ≥ 3/4 length
15	Biparous+	DYB+YT	Black mast present

Egg-tubes (EG).

Yellow tissue (YT) present in egg-tubes and oviducts.

Dense yellow body (DYB) and YT present in egg-tubes and oviducts.

the same age and nulliparous. Male face flies were dissected the same way, but only the presence or absence of infection was recorded.

iii - Results and Discussion

Seasonal Distribution in Tennessee

Of 10,004 face flies collected in 1993 and 1994, 111 (1.11%) were parasitized by *P. autumnale*. Prevalence of infected face flies was greater at all sites in 1993 than in 1994 (Table 2.2). The nematode was found at all sites in 1993, but was absent from the Loudon County site in 1994. The highest seasonal prevalence of 4.27% for 1993 was from the Grasslands site and the lowest prevalence of 0.49% was from the Loudon County site. The total 1993 sample population infected was 1.66%. The highest 1994 seasonal prevalence was 0.92% at the Grasslands site and the lowest was zero at the Loudon County site. The 1994 overall prevalence was 0.48%.

Face flies collected from the faces of cattle comprised 48.8% of the examined face flies, but only 36.7% of the infected flies came from that source. The seasonal prevalence in the face-collected flies in 1993 and 1994 were 1.2% and 0.48%, respectively, with an overall total prevalence of 0.82% for both seasons. The parasitism level of *M. autumnalis* by *P. autumnale* in eastern Tennessee during 1993 and 1994 was lower than those reported from

Table 2.2. Numbers of adult face flies examined for *Paraiotonychium autumnale* with numbers and percentage infected at five eastern Tennessee collection sites 1993 and 1994.

Site	Face Flies Collected 1993				Face Flies Collected 1994			
	No. Coll.	No. Exam.	No. Inf.	% Inf.	No. Coll.	No. Exam.	No. Inf.	% Inf.
Grass.	10	1100	47	4.27	12	1089	10	0.92
Crab.	10	1659	18	1.09	12	521	2	0.38
Loud.	10	409	2	0.49	12	244	0	0.00
Holst.	9	1793	19	1.06	12	2684	10	0.37
Chrkee.	9	290	2	0.69	12	215	1	0.47
TOTAL	48	5251	88	1.66	60	4753	23	0.48

Grass. = Grasslands Farm
 Crab. = Crab Orchard
 Loud. = Loudon County
 Holst. = Holston Farm
 Chrkee. = Cherokee Farm

No. Coll. = Number of face flies collected
 No. Exam. = Number of face flies examined
 No. Inf. = Number of face flies infected
 % Inf. = Percent of face flies infected

other states and Canada between 1965 and 1983. The prevalence of infection reported from New York was 23% (Stoffolano and Nickle 1966). New England states surveyed reported prevalences for Massachusetts (7.3%), Vermont (5.6%), New Hampshire (4.2%), Connecticut (7.3%), Maine (12.1%), and Rhode Island (11.2%) (Stoffolano 1968). Prevalences reported from Missouri were 40% (Thomas and Puttler 1970) and ca. 29% (Thomas et al. 1972). Prevalences from face flies collected in Ontario, Canada of 1.9%, from blood smeared boards, 4.5%, from dung pats, and 2.4%, from the overall season, were similar to those reported here from face and dung collections (Wright 1972).

Face flies collected from the dung of cattle comprised 13.1% of the examined face flies, but provided 57.8% of the infected flies. The modified behavior of terminal dung feeding by nematode infested face flies was expected (Kaya and Moon 1979). Collections from the surfaces of dung pats consistently yielded the highest numbers of infected face flies (Table 2.3). The 1993 and 1994 dung-pat-collected face flies had prevalences of 5.13% and 1.94% respectively, with a two season average of 4.80%. The prevalences of infection for face flies collected from dung pats were lower than the prevalences of 9.4% (1978), 7.4% (1979), and 19.3% (1980) reported for face flies collected from dung pats in Iowa (Krafsur et al. 1983). The Tennessee prevalences were also lower than the 4.9-52.7% prevalences reported in

Table 2.3. Numbers of face flies and percent parasitized by *Paraiotonchium autumnale* in face, dung pat, and reared collections for 1993 and 1994 at 5 sites in eastern Tennessee.

Site	Year	FACE			DUNG PAT			REARED		
		No. Col.	No. Inf.	(%) Inf.	No. Col.	No. Inf.	(%) Inf.	No. Col.	No. Inf.	(%) Inf.
Grass.	1993	720	13	(1.81)	207	34	(16.4)	173	0	(0.00)
	1994	655	1	(0.15)	163	8	(4.90)	271	1	(0.37)
Crab.	1993	982	6	(0.61)	181	11	(6.08)	496	1	(0.20)
	1994	368	0	(0.00)	50	2	(4.00)	103	0	(0.00)
Loud.	1993	208	1	(0.48)	72	0	(0.00)	129	1	(0.78)
	1994	153	0	(0.00)	51	0	(0.00)	40	0	(0.00)
Holst.	1993	677	11	(1.62)	159	5	(3.14)	957	3	(0.31)
	1994	923	6	(0.65)	380	3	(0.79)	1381	1	(0.07)
Chrkee.	1993	135	2	(1.48)	42	0	(0.00)	113	0	(0.00)
	1994	62	1	(1.61)	8	0	(0.00)	145	0	(0.00)
Total		4883	40	0.82	1313	63	4.80	3808	6	0.16

Grass. = Grasslands Farm
 Crab. = Crab Orchard
 Loud. = Loudon County

Holst. = Holston Farm
 Chrkee. = Cherokee Farm

No. Coll. = Number collected
 No. Inf. = Number infected
 % Inf. = Percent infected

California (Kaya and Moon 1979).

Flies reared from collected dung comprised 38.1% of the total face flies dissected, but only 5.5% of the infected flies. The face flies reared from collected dung pats in eastern Tennessee had prevalences of 0.25% in 1993 and 0.21% in 1994. The overall prevalence for the 3,808 reared face flies was 0.16%, lower than the 31.7% prevalence reported from Nebraska in 1967 (Jones and Perdue 1967).

Equal numbers of face flies collected between 1993 and 1994 at each site were expected to yield similar infection rates. Although approximately equal numbers of face flies were collected each year at Grasslands, there was a 4.5 fold decrease in parasitized face flies collected from 1993 to 1994. Face flies at each site in 1994 had lower infection prevalences than in 1993 (Table 2.2).

Face fly sex ratios may be altered in favor of males by *P. autumnale* (Chirico 1990). The distribution of infected male and female flies is presented in Table 2.4. Reared face flies which were infected with *P. autumnale* had a ratio of 2.5:1 favoring females. The face and dung sweeps did not adequately represent males in the sample populations. Collected field data from eastern Tennessee apparently does not support Chirico (1990). However, the numbers of infected flies in eastern Tennessee during 1993 and 1994 were low compared with the reported laboratory rates (Chirico 1990) and therefore should be considered

Table 2.4. Numbers of male and female face flies infected with *Paraiotonchium autumnale* collected by 3 methods from 5 sites in eastern Tennessee during 1993 and 1994.

Site	Year	Face			Dung Pat			Reared		
		♀	♂	Tot. Col.	♀	♂	Tot. Col.	♀	♂	Tot. Col.
Grass.	1993	13	0	(720)	34	0	(207)	0	0	(173)
	1994	1	0	(655)	8	0	(163)	1	0	(271)
Crab.	1993	6	0	(982)	11	0	(181)	0	1	(496)
	1994	0	0	(368)	2	0	(50)	0	0	(103)
Loud.	1993	1	0	(208)	0	0	(72)	1	0	(129)
	1994	0	0	(153)	0	0	(51)	0	0	(40)
Holst.	1993	10	1	(677)	5	0	(159)	2	1	(957)
	1994	6	0	(923)	3	0	(380)	1	0	(1381)
Chrkee.	1993	2	0	(135)	0	0	(42)	0	0	(113)
	1994	1	0	(62)	0	0	(8)	0	0	(145)
Total		40	1	4883	63	0	1313	5	2	3808

Grass. = Grasslands Farm
 Crab. = Crab Orchard
 Loud. = Loudon County
 Holst. = Holston Farm
 Chrkee.= Cherokee Farm

Tot.Col.= Total number collected

inconclusive in supporting a shift toward males.

Temporal Distribution

Numbers of face flies collected from face and pat sweeps and the percent of both parasitized were compared (Figures 2.2 through 2.7). The 1993 seasonal prevalence was 4.27% at Grasslands (Figure 2.2). The percent of parasitism exceeded 10% on 8 June 1993 and 30 August 1993 with 7 of the 10 sampling dates having parasitized face flies collected. The seasonal prevalence for 1994 was 0.92%, and never exceeded 5.0% during the season (Figure 2.3). Four of the 12 sampling dates had infected face flies collected with the highest parasitism for the season recorded on 10 August 1994.

Crab Orchard site had a 1.09% seasonal prevalence in 1993 and did not exceed 10% during the season (Figure 2.4). Eight of the 10 collecting dates had infected face flies collected. During 1994, parasitism remained at zero season long, except on 29 July; the seasonal infection prevalence was 0.38% (Figure 2.5). Infection prevalences were lower at Crab Orchard than Grasslands for both 1993 and 1994.

The seasonal infection prevalence at Holston Farm for 1993 was 1.06% (Figure 2.6). Parasitism exceeded 5% on 3 August 1993 and parasitized face flies were collected on 5 of the 10 dates. The 1994 seasonal prevalence was 0.37% (Figure 2.7). Infected face flies were collected on 4 of the 12 sampling dates. The seasonal prevalences at Crab

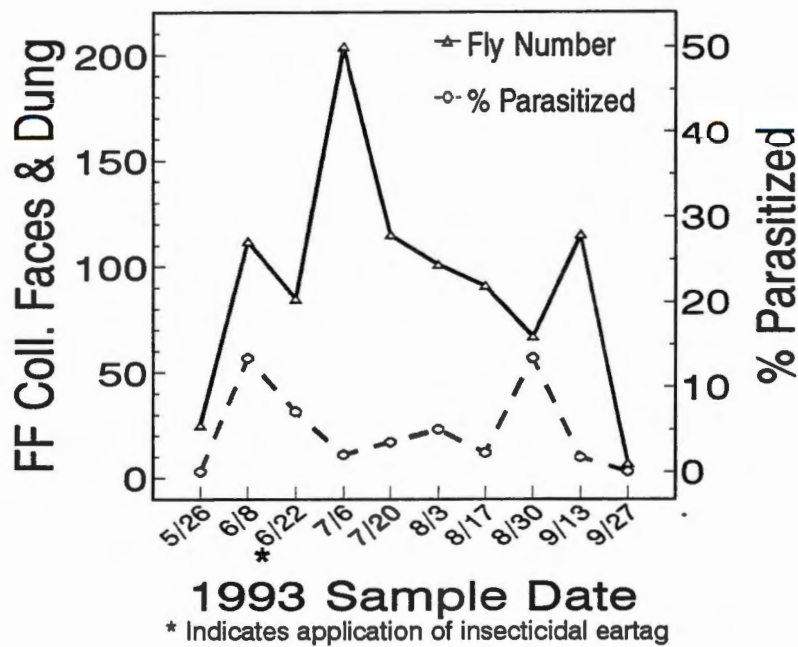


Figure 2.2. Composite numbers of face flies collected from face and pat sweeps and percent parasitism at the Grasslands site, Cumberland County, Tennessee during 1993.

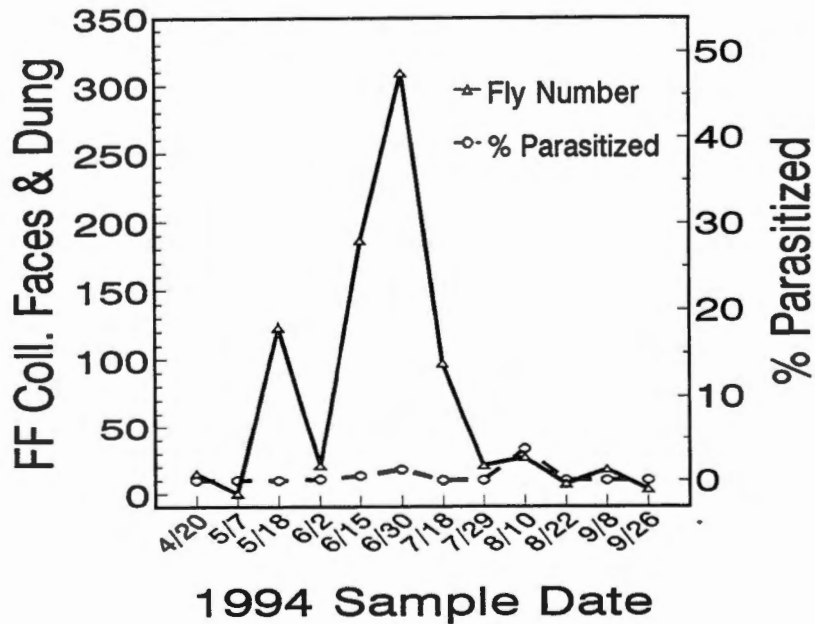


Figure 2.3. Composite numbers of face flies collected from face and pat sweeps and percent parasitism at the Grasslands site, Cumberland County, Tennessee during 1994.

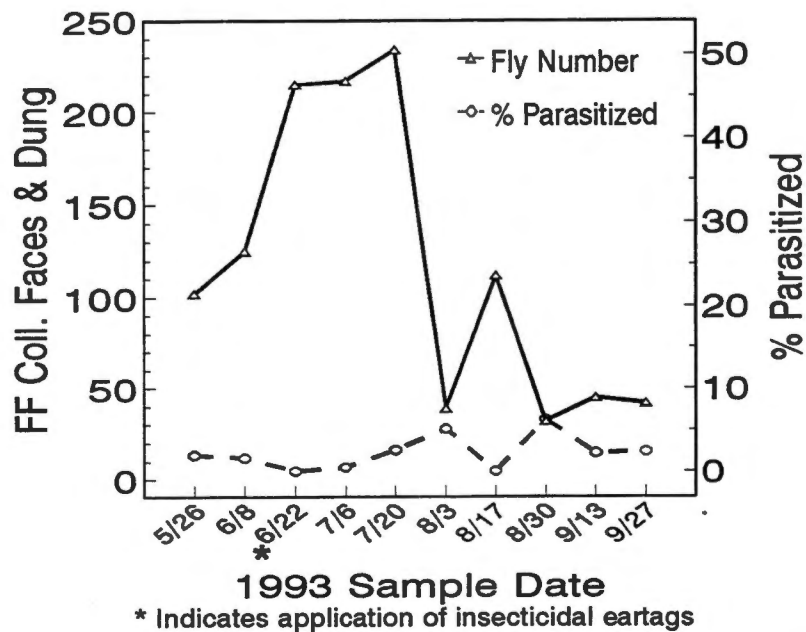


Figure 2.4. Composite numbers of face flies collected from face and pat sweeps and percent parasitism at the Crab Orchard site, Cumberland County, Tennessee during 1993.

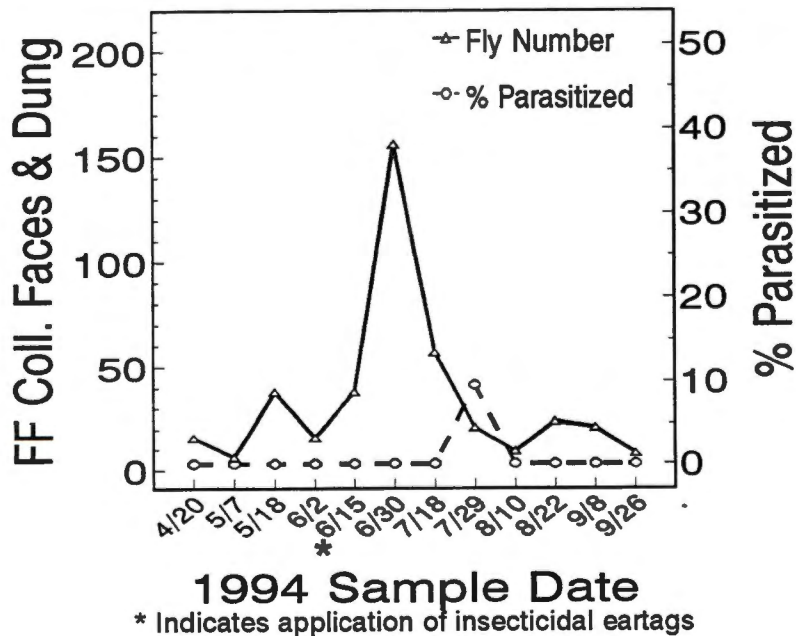


Figure 2.5. Composite numbers of face flies collected from face and pat sweeps and percent parasitism at the Crab Orchard site, Cumberland County, Tennessee during 1994.

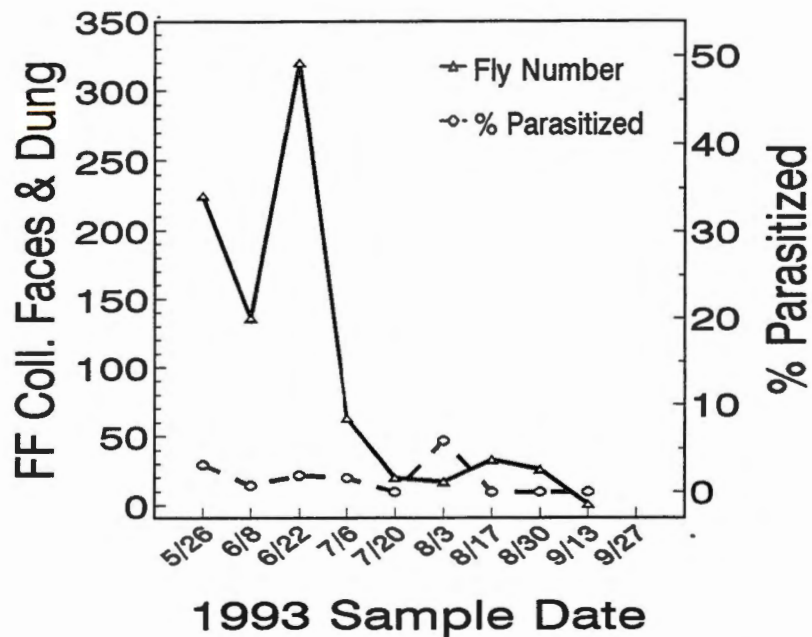


Figure 2.6. Composite numbers of face flies collected from face and pat sweeps and percent parasitism at the Holston Farm site, Knox County, Tennessee during 1993.

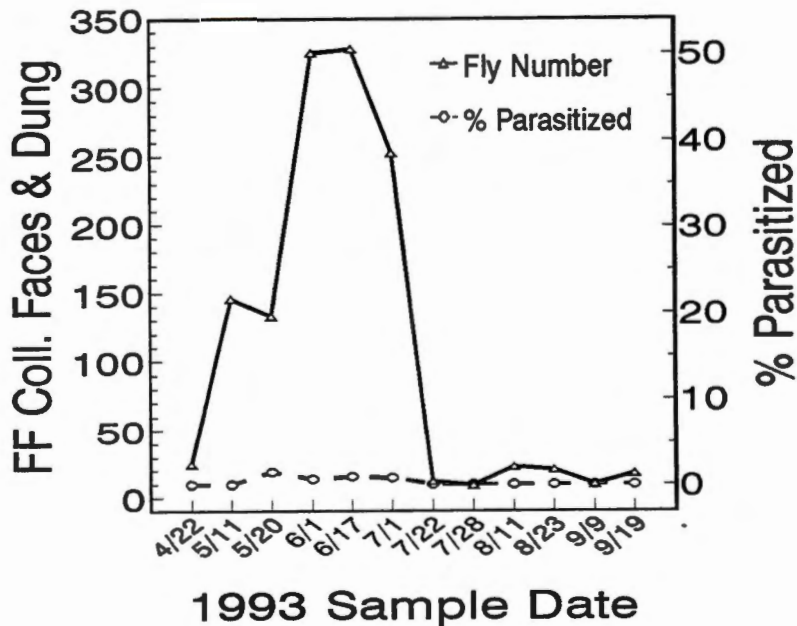


Figure 2.7. Composite numbers of face flies collected from face and pat sweeps and percent parasitism at the Holston Farm site, Knox County, Tennessee during 1994.

Orchard and Holston Farm were similar for both 1993 and 1994.

The Loudon County and Cherokee Farm sites had low face fly collections both years, thus infectivity data is not reported here.

Numbers of flies collected and their incidence of parasitism were similar in 1993 and 1994. Seasonal maxima of face flies at the Grasslands, Crab Orchard, and Holston Farm sites occurred in late June and early July for both years. The date of highest incidence of face fly parasitism varied among the sites, but occurred in late July and August of both 1993 and 1994. June appears to be the month of peak incidence of infected face flies elsewhere in North America (Wright 1972, Jones and Perdue 1967, Thomas et al. 1972, Kaya and Moon 1978, Krafur et al. 1983).

iv - Conclusions

Paraiotonchium autumnale occurs in eastern Tennessee. Seasonal prevalences of parasitism for 1993 and 1994 were 1.66% and 0.48%, respectively. The seasonal prevalences of infection during 1993 and 1994 in face flies collected from face sweeps, from pat sweeps, and reared from field-collected dung were lower in eastern Tennessee than reported elsewhere in the United States. Nematode parasitism of face flies were highest in August. *Paraiotonchium autumnale* was found more often in flies collected from the surface of

fresh cow manure. Face flies collected from the faces were more frequently parasitized than flies reared from field-collected pats. *Paraiotonchium autumnale* appeared to have no detectable effect on face fly populations during 1993 or 1994. Low observed prevalence may have been due to the young physiological age of the collected face flies, a problem discussed in Chapter 4.

CHAPTER III

SIMULATED RAINFALL AS AN ENVIRONMENTAL FACTOR AFFECTING LARVAL MORTALITY OF *MUSCA AUTUMNALIS* DE GEER

i-Introduction

Environmental factors, such as photoperiod, temperature, and humidity, affect the development of the face fly, *Musca autumnalis* De Geer (Wang 1964, Teskey 1969). Natural mortality of the face fly has been investigated in the field (Teskey 1969, Valiela 1969, Burton and Turner 1970, Thomas et al. 1983) and in the laboratory (Wang 1964, Killough & McCellan 1965). Biotic environmental factors such as predation and parasites adversely affect larval face fly populations (Blickle 1961, Drea 1966, Jones 1967, Wingo et al. 1967, Turner et al. 1968, Thomas and Wingo 1968).

Abiotic environmental factors that may decrease face fly activity include high relative humidity and wind speed (Engroff et al. 1972). Precipitation has not been examined as a factor affecting larval development or mortality. Rainfall increased mortality of Australian bush fly larvae, *Musca vetustissima* Walker (Hughes 1979). Reduced numbers of *M. autumnalis* collected on cattle at several locations in 1994 appeared to be associated with unusually high rainfall in July and August. The objective of this study was to determine the effects of simulated rainfall on larval

mortality.

ii - Materials and Methods

Manure

Fresh manure was collected from dairy cattle at the University of Tennessee Knoxville Experiment Station. The collection was made immediately after deposition to avoid contamination from other arthropods or dung-inhabiting fauna. Manure was sealed, transferred to the insectary, and used within two hr of collection.

Simulated manure pats were constructed from the collected dung with the aid of a 300-ml plastic flower pot liner. Each pat consisted of two full liners of dung dropped onto a piece of pressboard from a height of 0.5 m. The resultant pats, about 20 cm in diameter, closely resembled the shape of a natural pat. Pressboards were 1.3 × 25 × 28 cm as described by Thomas et al. (1983).

Larvae

Face flies used in these experiments were from the University of Tennessee's Medical and Veterinary Entomology stock colony. Plastic containers, 100 mm dia. × 25 mm, filled with fresh cow manure were placed into the fly cages and stock colony females were allowed to oviposit. The containers were maintained in the University of Tennessee's Insectary at 27° C and >50% relative humidity, with a 16 hour light/ 8 hour dark photoperiod (16:8 L:D). Three age groups of larvae were used in the experiments: 24, 48, or 96

hours old after oviposition. The ages approximated the three juvenile instar developmental times. First instar larvae were removed from the plastic containers 24 hr after oviposition. Other larvae in the experiments were maintained with standard procedures (Fales et al. 1961). Upon reaching the appropriate age, 25 larvae were removed with forceps and transferred into a 100 mm plastic petri plate with a small amount of fresh manure. A total of 25 petri plates containing larvae and manure was prepared for each experiment. The petri plates served as holding chambers until the larvae could be placed into the simulated dung pats. Larvae were held in the petri plates no longer than 60 minutes.

Rainfall Apparatus

Simulated rainfall was applied with a Ladybird® No. 25 part circle impact sprinkler head mounted inside a plexiglass box atop a steel stage (Figure 3.1). Directional stops on the sprinkler head allowed the area of spray to be set. Water pressure was 165.5 kilopascals (kPa) for the 3 experiments.

Calibration

The rainfall simulator was calibrated before each experiment. Sixteen of the pressboards were placed 6.2 m from the sprinkler head on the ground in a 4 × 4 pattern with the edges touching. Steel cans, 11 cm × 8.3 cm dia., were placed in the center of each board. Simulated rain was

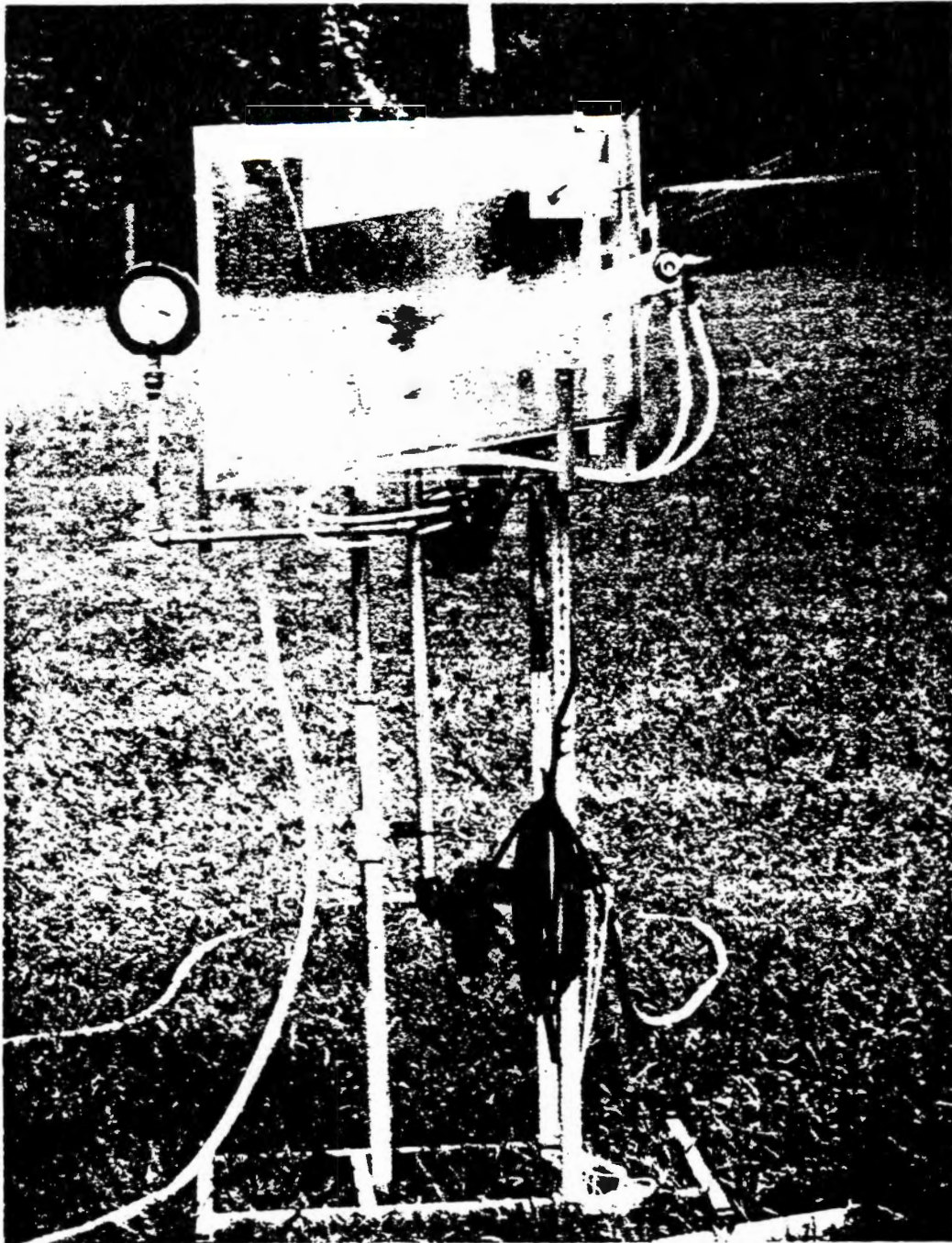


Figure 3.1 The rainfall simulator used in the experiments.

applied for 15 minutes at 165.5 kPa. The amount of water collected in the cans was measured and used to calculate the time necessary to administer the selected simulated rainfall amounts. According to an analysis of variance, there were no significant differences in the amount of water collected in any of the calibrations ($P < 0.05$).

Rainfall Simulation Experiment

Three experiments were conducted with larvae 24, 48, or 96 hr old after oviposition. Simulated rainfall at 0, 6, 12, and 24 mm was applied to artificially constructed dung pats containing *M. autumnalis* larvae. Each experiment utilized only one age of larvae for four different amounts of rain. Simulated dung pats were constructed on 20 boards; 15 were placed in the simulated rainfall area and 5 were not exposed to rainfall in a control area. The 25 previously selected larvae were put onto each of the dung pats and given 15 minutes to enter the pats and acclimate in the dung before rain was applied. Water was applied at 165.5 kPa for 15, 30, or 60 minutes. These times were calculated before the experiment to deliver 6, 12, and 24 mm, respectively. Five boards were randomly removed at each of the three calculated time intervals after initiation of rainfall. Each of the removed pats was labeled and placed with the control pats. At the conclusion of the simulated rainfall event each of the pats was placed into an emergence tray. Emergence trays were constructed of 30.5 × 10 × 43 cm

cardboard boxes lined with white plastic and filled with sand. Trays were kept in the insectary at 27° C, 16:8 L:D photoperiod, and >50% RH for one week. The sand was then sifted and *M. autumnalis* pupae were removed. The number of pupae collected was subtracted from 25 and the percent mortality calculated. Mortality was expressed in terms of mean percent dead. The data were analyzed with SAS Statistical GLM (General Linear Model) and Regression Analysis, with the GLM generated means separated by Duncan's Multiple Range Test (DMRT). The mean separation (DMRT) was performed using the arcsin square root transformation of the means.

iii - Results and Discussion

Across all ages, the amount of rainfall did not significantly ($P=0.44$) affect mortality. However, mortality was age-dependent and mortality at different larval ages was different ($P<.01$). Mortality of the first ($P=0.003$) and third instars ($P=0.0001$) generally increased with increasing rainfall, but second instar larvae ($P=0.07$) were not significantly affected by increasing rainfall (Table 3.1). The increased mortality of the larvae 96 hr old after oviposition may be due to wandering of pre-pupal instars. Mature larvae leave the dung to find pupation sites, and their absence may have artificially skewed the mortality calculation. The larvae 96 hr old after oviposition were

Table 3.1. Mean percent mortality of larval face flies for rainfall and age.

Rainfall (mm)	Age (Hrs)			Overall $\bar{x}$ Mortality by Rain
	24	48	96	
0	95.2	12.8	13.6	40.5ab
6	40.0	8.8	72.0	40.3b
12	63.2	8.8	88.8	53.6a
24	36.0	7.2	84.8	42.7b
Overall $\bar{x}$ Mortality by Age	58.6a	9.4b	64.8a	

Mortality is listed in terms of mean percent dead. The letters following means are from Duncan's Multiple Range Test of the arcsin transformation of the percent dead ($P=0.05$, DMRT).

Means within a column followed by the same letter are not significantly different.

sometimes observed migrating from treated and untreated pats.

The egg and the first instar face fly larvae consume more oxygen per gram of tissue per minute than do the other two instars (Guerra and Cochran 1970). The higher mortality in the larvae 24 hr old after oviposition may have been due to the developmental stage's increased oxygen requirement. Dung pats inundated by rainfall may have hindered gaseous exchange, suffocating the larvae. Bush fly larvae 1 day old were similarly affected by exposure to 8 mm of simulated rain, reducing survival 83% (Hughes 1979).

iv - Conclusions

Survival of first-instar *M. autumnalis* larvae was significantly reduced by simulated rainfall at 6, 12, and 24 mm. Mortality due to rainfall in the first instar larvae could be explained by dung pat environmental conditions which impede gaseous exchange.

CHAPTER IV

OBSERVATIONS ON THE EFFECTS OF RAINFALL AND INSECTICIDAL EARTAGS ON OVARIAN DEVELOPMENT AND POPULATIONS OF *MUSCA AUTUMNALIS* DE GEER IN EASTERN TENNESSEE

i-Introduction

Cattle dung has been recognized as a distinct ecological environment (Mohr 1943), and many studies have been conducted on its arthropod fauna (Sanders and Dobson 1966, Blume 1970, Wingo et al. 1974). The eggs and larvae of the face fly (*Musca autumnalis* De Geer) and the Australian bush fly (*Musca vetustissima* Walker) develop in cattle dung. Rainfall may reduce bush fly larval survival by up to 83% (Hughes 1979).

In eastern Tennessee, numbers of face flies collected declined dramatically in midsummer of both 1993 and 1994. Greater than normal rainfall in the second half of the summer of 1994 may have been partially responsible. The use of insecticidal eartags may also have resulted in reduction of fly numbers. The objective of this study was to correlate adult face fly numbers collected with rainfall and insecticidal eartag use.

ii - Materials and Methods

Collections

Face flies collected from cow faces and pats in the *Paraiotonchium autumnale* distribution research were used to estimate face fly populations during 1993 and 1994. Methods used to collect face flies and to age-grade the ovaries of field-collected female face flies are described in Chapter 2.

The Cumberland Plateau sites (C.P.S.) were Grasslands Experiment Station and Crab Orchard (Figures 4.1 and 4.2). The Tennessee River Valley sites (T.R.S.) were the Holston farm and the Loudon County farm (Figures 4.3 and 4.4).

Three treated and three untreated collection sites from 1993 and 1994 were observed for relationships (Figure 4.5 and 4.6) between decreasing numbers of face flies and application of eartags (Figure 4.5). Treated sites were Grasslands 1993, Crab Orchard 1993, and Crab Orchard 1994. Insecticidal eartags containing diazinon (20% ai) were applied during the week before 15 June 1993 and 1994 at the treated sites. Adult cattle at Grasslands 1993 received two eartags each and Crab Orchard 1993 and 1994 cattle received one eartag per adult. Untreated sites were Grasslands 1994, Holston 1993, and Holston 1994.

Rainfall

Rainfall data were obtained from Dr. Joanne Logan of the University of Tennessee's Department of Plant and Soil

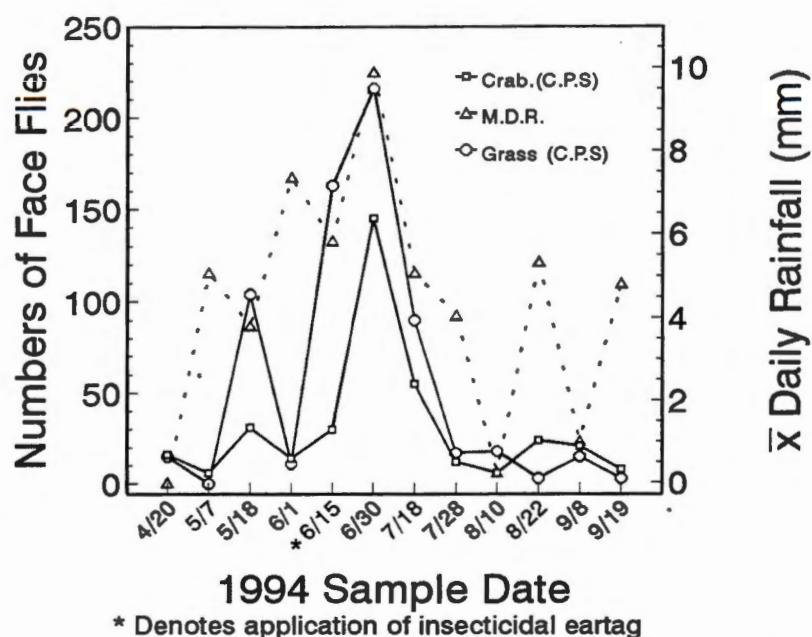


Figure 4.1

Mean daily rainfall and numbers of face flies collected from face and pat sweeps on the Cumberland Plateau sites in eastern Tennessee during 1994.

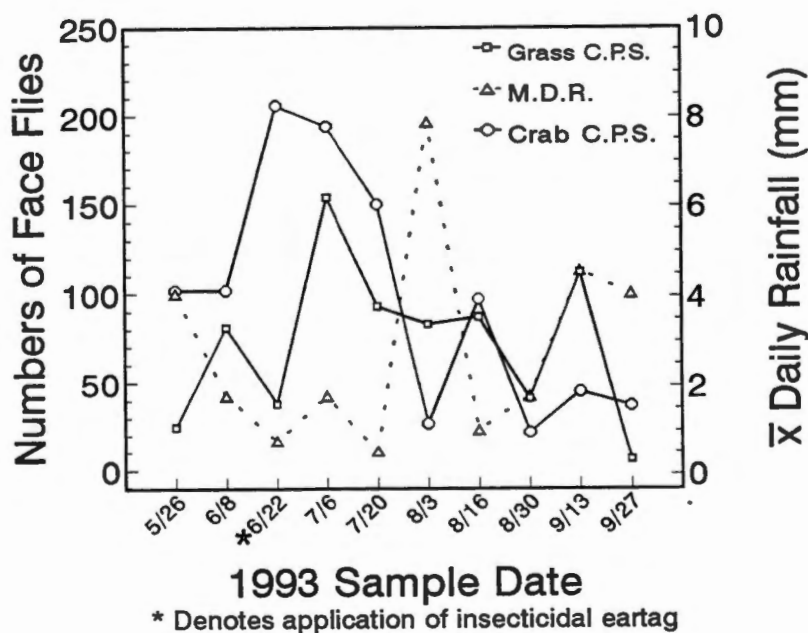


Figure 4.2

Mean daily rainfall and numbers of face flies collected from face and pat sweeps on the Cumberland Plateau sites in eastern Tennessee during 1993.

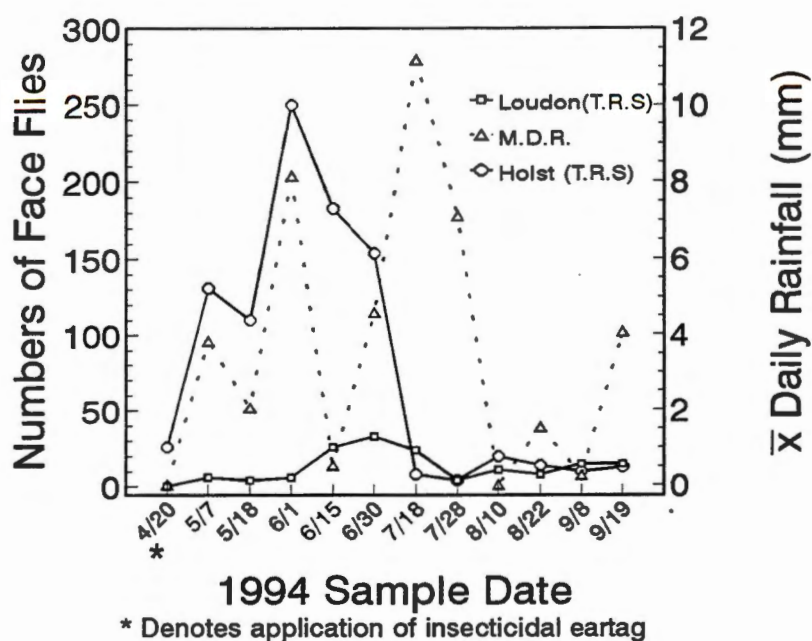


Figure 4.3

Mean daily rainfall and numbers of face flies collected from face and pat sweeps at the Tennessee River Valley sites in eastern Tennessee during 1994.

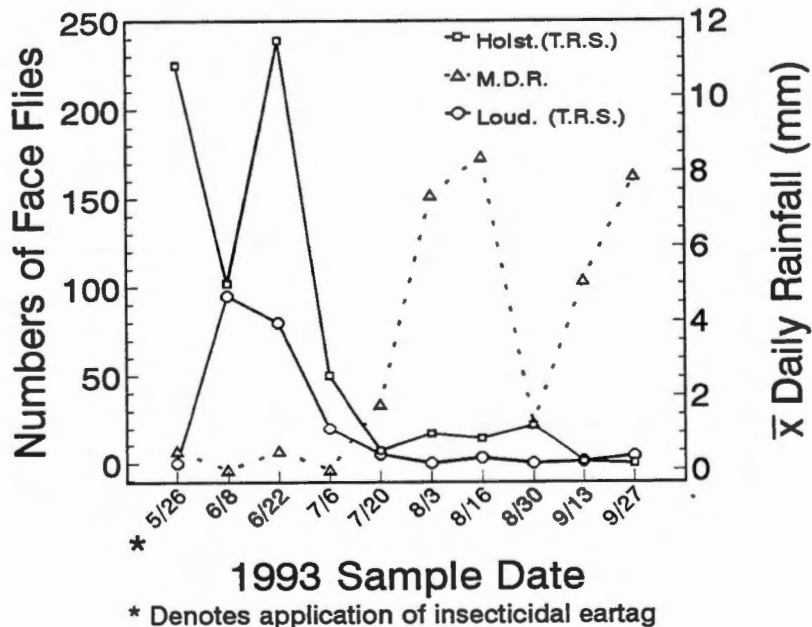


Figure 4.4

Mean daily rainfall and numbers of face flies collected from face and pat sweeps at the Tennessee River Valley sites in eastern Tennessee during 1993.

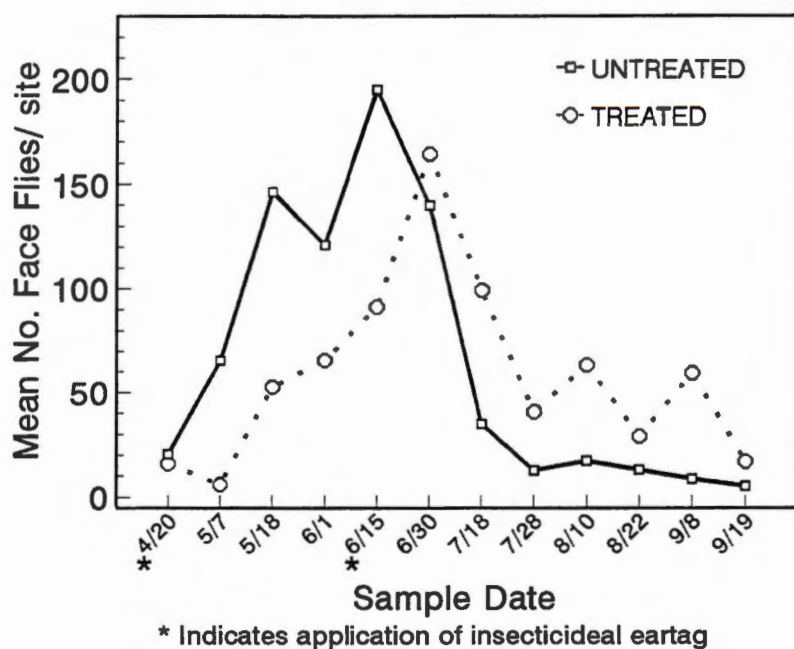


Figure 4.5

Mean number of face flies collected from face and pat sweeps from 3 treated and 3 untreated sites in eastern Tennessee during 1993, 1994.

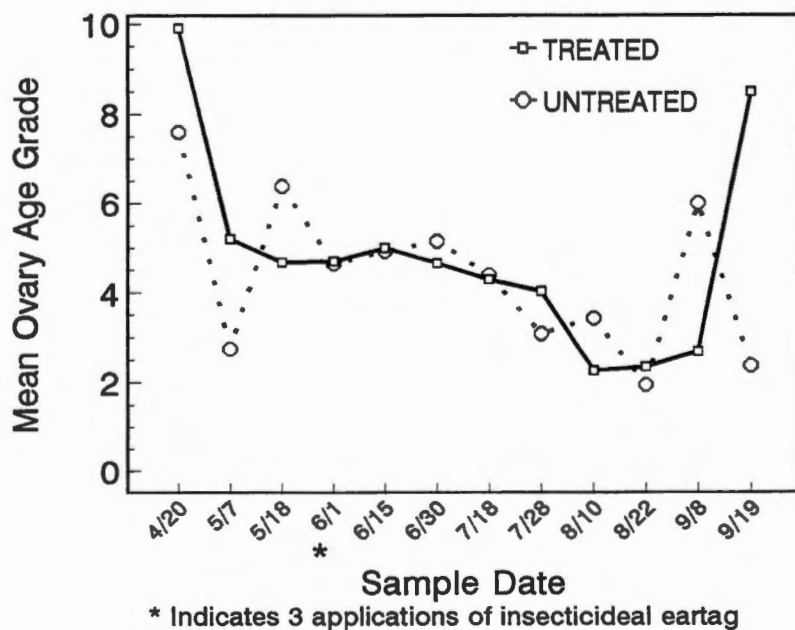


Figure 4.6

Mean ovarian age grade of face flies collected from face and pat sweeps from 3 treated and 3 untreated sites in eastern Tennessee during 1993, 1994.

Sciences. Monitoring stations were located at the Knoxville Plant Science Field Laboratory, Knox County, Tennessee, and the Plateau Experiment Station, Cumberland County, Tennessee. A square root transformation of the rainfall data was analyzed with the SAS General Linear Model procedure.

iii - Results and Discussion

Both the C.P.S. and the T.R.S. for 1993 and 1994 had increasing numbers of face flies from May until mid to late June, after which numbers declined (Figures 4.1-4.4). Face fly populations in eastern Tennessee typically increase throughout the face fly season and reach their highest numbers during July and August (Gerhardt et al. 1982). The numbers of face flies collected from C.P.S. and T.R.S. during 1993 and 1994 differed dramatically from the expected seasonal population dynamics of the face flies.

Mean numbers of face flies collected from untreated and treated cattle increased at approximately the same rate until 16 June and 30 June, respectively, after which numbers of face flies collected declined until the end of collecting in September. The increase in collected face flies approximately three weeks after eartag application suggested that eartag application was ineffective in controlling face fly populations in 1993 and 1994.

Effective eartag application has been demonstrated to

skew fly populations toward younger flies (Krafsur 1984). Adult face flies have a greater chance of contacting a lethal level of insecticide and leaving unexposed developing flies in the population. To determine if that phenomenon had occurred in eastern Tennessee, mean ovarian age-grade of flies from the three treated and three untreated sites was estimated (Figure 4.6). The ovarian age of face flies from treated and untreated cattle was similar.

Similar declines in fly numbers and mean ovarian age suggest that eartag application had no effect on face fly population dynamics. Declines in numbers of face flies collected during June and July of both 1993 and 1994 suggested that some other common factor had affected the populations at the C.P.S. and T.R.S.

Unusually high amounts of rainfall during June and July of 1994 may have been partially responsible for midsummer declines in numbers of face flies. Mean monthly rainfall amounts for June and July 1994 at C.P.S. and T.R.S. were higher than those of the same sites in 1993 (Figures 4.7 and 4.8). Statistical analysis of the 3 x 3 factorial showed significant differences only among month and year interaction. From the least square means (LSM) there were no significant differences in rainfall between May, 1993, and May, 1994 ($P>.5$); August, 1993, and August, 1994 ($P>.5$); or September, 1993, and September, 1994 ($P>.1$). The month of June, 1994, received significantly higher ($P<.02$)

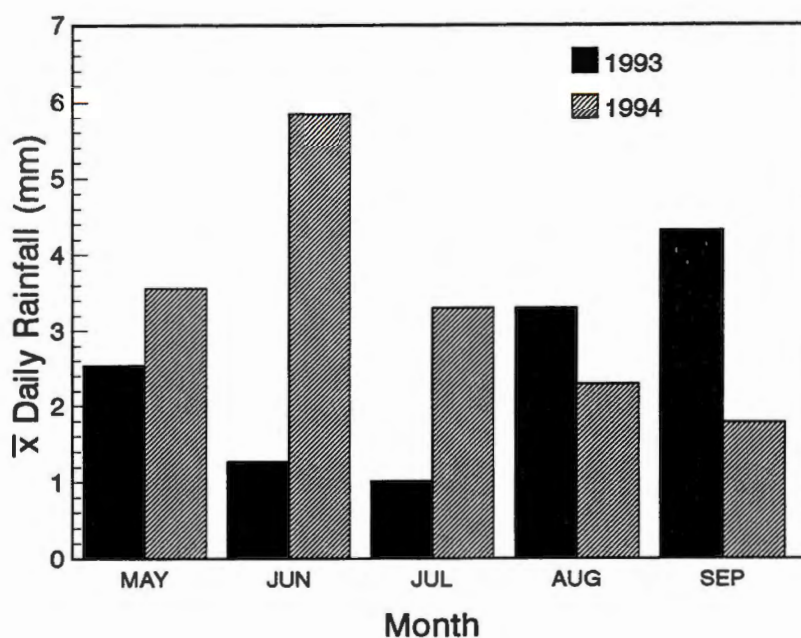


Figure 4.7 Mean daily rainfall May through September from the Cumberland Plateau sites, 1993 and 1994.

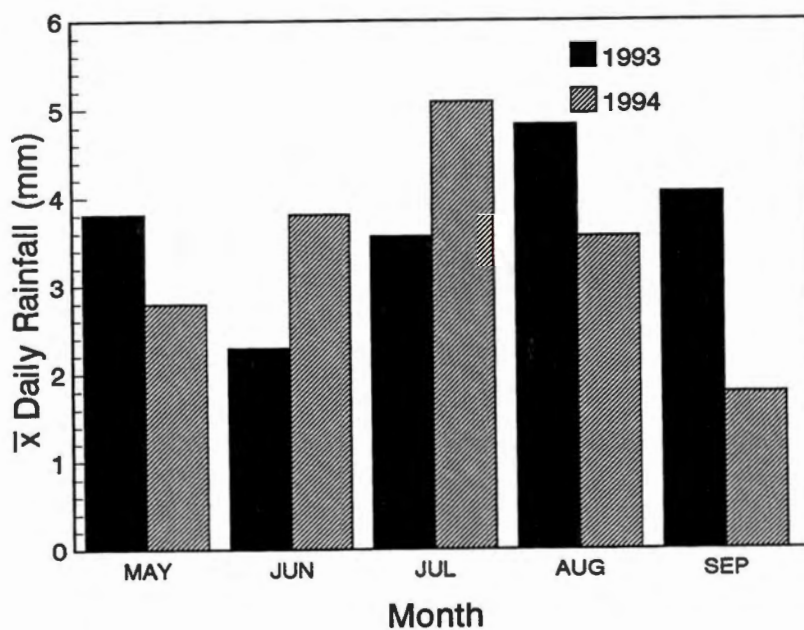


Figure 4.8 Mean daily rainfall May through September from the Tennessee River Valley sites, 1993 and 1994.

rainfall, LSM value of 4.76, than June, 1993, LSM value 1.75. July, 1994, received significantly higher ($P < .04$) rainfall, LSM value 4.16, than July 1993, LSM value 2.10.

Weeks with a mean daily average of rainfall greater than 8 mm coincided with subsequent declines in numbers of face flies (Figures 4.1 through 4.4). The C.P.S. in 1994 had one week, 8 June through 14 June, where mean daily rainfall was higher than 8 mm prior to face fly collecting (Figure 4.1). The numbers of face flies collected declined after the week of 30 June and throughout the 1994 season. Declines in face fly numbers collected at C.P.S. in 1993 did not appear to be related to the amount of rainfall and were therefore caused by unknown factors (Figure 4.2).

At T.R.S. in 1994 there were two different weeks where the mean daily rainfall was greater than 8 mm: 6 June and 18 July (Figure 4.3). Reduced numbers of face flies were collected after 1 June 1994 and throughout the end of the collecting season. Declines in face flies collected at T.R.S. in 1993 could not be associated with weeks receiving mean daily rainfall of 8 mm or greater, therefore declines were attributed to unknown factors.

iv - Conclusions

Unexpected decreases in face fly numbers during the 1993 and 1994 seasons could not be related to application of eartags, since declines in fly numbers and mean fly ovarian

age were similar for both the treated and untreated cattle sites. Rainfall may have been the limiting factor in face fly populations. Differences in rainfall amounts between June and July and subsequent decreases in numbers of face flies collected, at both C.P.S. and T.R.S. during 1994, appear to have been related. Also, weeks with mean daily rainfall of greater than 8 mm before face fly collections appeared to show a relationship between rainfall and decreased numbers of face flies collected.

The low numbers of parasitized face flies reported in 1994 from Chapter 2 also may have been related to the young ovarian ages of the face flies collected. The progressively younger ovarian ages of the face flies collected (Figure 4.6) suggests that the factors causing the decreases in face flies affected the populations before parthenogenetic nematodes could produce gamogenetic (infective stage) nematodes. The absence of older infected face flies in the population would have limited the numbers of reinfections to the dung pats resulting in the low 1994 prevalences.

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VITA

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