CHARACTERIZATION OF AGING BOVINE MANURE IN RELATION TO STABLE FLY (DIPTERA: MUSCIDAE) ADULT AND LARVAL PRESENCE

by

Merrilee Susan Haas

B. S., Kansas State University, 1980

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

Approved by:

A B Broce

Major Professor

DEDICATION

To my parents, whose encouragement to explore the world has channelled me into the sciences, and even inspired me to poetry, and to my two sons whom I hope will continue to ask "why?", I dedicate this thesis and this poem:

What begins as wonderment,
  Proceeds to persistant prying
    Into the reality of things.

And, having encompassed
  Yet deeper patterns
    Of the way things are,

And traced new paths
  Yet to be sought,
    Returns again to wonderment.

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INTRODUCTION AND LITERATURE REVIEW

The bite of the stable fly, Stomoxys calcitrans (Linnaeus), is an experience shared in common by humans and domestic animals in most habitable areas of the world. Brues (1913) reported the presence of the stable fly from Alaska through South America, throughout Europe, Africa and Asia, in Australia, and on numerous islands throughout the world. Subsequent studies have continued to document its cosmopolitan distribution. Muir (1914) differed with Brues' conjecture that the stable fly was a native of central Europe, and, on the basis of geographical distribution of the various species of Stomoxys and natural predator and parasite pressures in various regions, proposed the Indo-Ethiopian region as "cradle" of the species.

Despite worldwide distribution, ranging from tropical areas (Parr 1959) to subalpine zones at 9,000 feet (Eads 1979), the stable fly has maintained a homogeneity which enables the geographically separated strains to interbreed freely. Harris et al. (1972) found strains from Japan, Thailand, New Zealand, South Africa and the U.S.A. to be reproductively compatible. This would seem to indicate either a relatively recent development in its wide distribution, or an ongoing, continual interstrain sharing of genetic material, perhaps via human travel and transportation of livestock. A recent archaeological finding of stable fly puparia in northeast England (Seaward 1976) indicates that the

stable fly was associated with human habitation around 100 A.D., about the time of beginning Roman occupation of England (could the Roman Empire be responsible for the spread of the stable fly as well as government and culture?).

Linnaeus (1758) provided the first descriptive record of the stable fly, giving it the name Conops calcitrans. In 1762, Geoffroy created the generic name Stomoxys. During ensuing years, the stable fly was described under various synonyms; Empis calcitrans (Scopoli 1763), Stomoxis (Schaeffer 1766), Stomoxys calcitrans (Fabricus 1775), S. parasita (DeGeer 1776), S. inimica and S. dira (Robineau-Desvoidy 1830), S. cybira and S. occidentis (Walker 1849, 1852).

The first study of stable fly biology was Newstead's work on habitat, behavior, and life cycle in 1906. Previous to this, only a few sporadic observations had been recorded of stable fly larvae on manure (Bouche 1834; Howard 1900). Subsequent developmental studies by Mitzmain (1913), Bishop p (1913), Portchinsky (1910), Parr (1962) and others, have shown a range in life cycle timetables in response to various temperature, humidity, growth media and host regimes.

Parr (1962) reported life cycle completion (egg to adult) in 12 days at 80°F, 80% RH on a media mixture of dried cow dung, dried blood granules and sugar (Parr 1959), while Newstead's studies (1906) were conducted at 72°F (65°F night temperature) in moist sheep's dung, and resulted in a life cycle of 25-37 days.

Sutherland (1979) found that adults and pupae developed most

successfully at temperatures between 20-30°C, and eggs developed best at 30°C. Nieschulz (1933) reported an optimum temperature of 28°C for the stable fly, and in conjunction with Dutoit (Nieschulz and Dutoit 1933) found that European and South African strains reacted the same to temperature.

It was thought earlier that hibernation takes place in the larval and pupal stage in populations subjected to cooler climates (James 1948). Berry et al. (1978) found that nondiapausing larvae migrate inward in a manure mass and overwinter in a state of retarded development. Adults have been noted overwintering in barns and stables in Norway, reproducing at a slower rate during the winter (Sømme 1961).

Suitable larval media and adult blood meal hosts are both necessary to support a stable fly population, though the two need not be in the same immediate area. Ranging no farther than necessary for a meal (Todd 1964), stable flies have been found capable of travelling 3.22 km (Bailey et al. 1973), 9.6 km (Hodge 1913) or even 117 km (Rogers 1971).

Adults of both sex must find a bloodmeal source in order to complete reproductive development (Anderson 1978; Moobla and Cupp 1978). Mammalian hosts are preferred (Sutherland 1978), and any available exposed area of the body is a potential feeding site (Bishopp 1913). In piercing the host's skin with its proboscis, the fly traumatizes tissues by the boring action of its prestomal teeth and saliva is injected into the wound (Butler et al. 1977). Blood is then withdrawn by a sucking action. The painful bite terminates with a small drop of blood and a reddish spot left at

the feeding site (Bishop 1913, Newstead 1906). Depending on the fly's sex, age, and fill from previous meals, the process may take from two to twelve minutes (Mitzmain 1913).

A considerable variety of substrates have been found to be suitable for larval breeding, all having moisture and active microbial decomposition in common. While Newstead (1906) found larvae only in grass mowings and none in feces of domestic animals, Porchinsky (1910) noted larvae breeding in heaps of horse dung, though not in field deposits. Porchinsky (1910) speculated that the stable fly's preference for larger accumulations of manure may be due to the "voracious larva's" need for a larger food mass which is suitable for a longer time and free of natural enemies. Hafez and Gamal-Eddin (1959) noted that pure cow, horse and pig dung were not attractive to the stable fly for oviposition. Parr (1962) speculated that the texture of pure cattle dung prohibited larval development, and went on to state that, upon addition of straw or other vegetable matter, the cow and horse manures became suitable breeding media.

Decaying vegetable matter alone can serve as a very productive larval medium. Stable fly outbreaks noted by Iches (1909) and Bishopp (1913) were traced to prolific breeding in straw stacks left over from grain harvest. Hall et al. (1982) reported breeding in round hay bales in Missouri. Williams (1980), Guyer (1956), and Meyer and Petersen (1983) found stable fly larvae breeding in silage. Bay grass deposits (Simmonds 1944), pondweed deposits (Williams et al. 1980), deposits of dead mayfly bodies (Pickard 1968), sewage filters at a treatment plant

(Nettles 1934), fish scale waste from a sardine plant (Suenaga 1962), and decomposing trunks of oil palms (Urueta-Sandino 1972) have also been found to support stable fly breeding.

The stable fly's pest status results from its hematophagous feeding habit which causes direct physical discomfort in mechanical irritation of tissues, induces host immune reactons to the bite, and plays a key role in its realized and potential threat as vector of disease. Iches (1909) described a stable fly outbreak in Argentina that drove cattle frantic, resulting in considerable skin injury on some. Bishopp (1913) reported a similar outbreak in Texas with resultant loss of milk production, weight loss of cattle and of horse work teams, and general increased irritability of livestock. At times, this prevented teams from working in fields and caused horses to run away with wagons. Some horses became lame from stamping or prolonged standing in water. Further documentation of milk production loss was made in a 3-year study by Bruce and Decker (1958); loss was calculated at up to 0.7%/fly/cow. However, Cheng and Kesler (1961) found that fly control did not significantly affect milk production, and Miller and Pickens (1973) found that 650-1000 flies/cow did not affect milk production. Variable results in cattle weight gain were also obtained by Cutkomp and Harvey (1958), while the USDA in 1965 estimated $142 million loss in cattle production, a figure they increased to $735 million by 1975 (Anonymous 1976). Campbell et al. (1977) noted weight gain reduction in calves, and Cheng (1958) found that fly control increased weight gain. Human activities can also be disrupted.

Newson (1977) estimated that the Florida tourist industry may suffer as much as $1 million/day due to stable fly presence at beaches from mid-August to mid-September.

A possible host immune response to the stable fly bite was noted by Moorehouse (1972), and was demonstrated by work of Baker and Quinn (1978); an intradermal injection of an extract of the stable fly gave rise to an immediate tissue reaction in horses.

Flies were considered potential pathogen vectors during the 1800's. As early as 1871, Leidy, after observing the spread of disease in American Civil War hospitals, voiced suspicions that flies carried disease. His microscopic observation of fungi in the regurgitate of a fly that had been feeding in fungi added strength to this suspicion of the fly's role in disease transmission. Since then much research has confirmed that flies carry disease and increasingly clarified and detailed the fly's role as disease vector.

The stable fly, while being less of a mechanical vector than the house fly due to different food sources (Glaser 1923), is still a significant disease vector due to frequent and regurgitative feeding (Butler et al. 1977) and repeated sequential feedings (Venkatesh and Morrison 1980). Butler et al. (1977) suggest that the intermittant occurance of regurgitation may explain some difficulties encountered in establishing the stable fly's vectorship.

Curry (1902) found trypanosomes in the stable fly proboscis and gut. Mitzmain (1912) reported negative results when attempting to transmit Surra (Trypanosoma evansi) to horses,

monkeys and guinea pigs via the stable fly, but Nieschulz (1940) successfully transmitted the disease to guinea pigs in the lab. T. brucei (Nagana disease) is mechanically transmitted by the stable fly according to Taylor (1930). Duke (1913) failed to transmit T. gambiense (sleeping sickness) via the stable fly, but later Duke et al. (1934) were able to transmit T. rhodesiense, another form of the disease, from monkey to monkey in the lab.

Scott (1920) reported that the stable fly could mechanically transmit the viral disease equine infectious anemia (EIA or swamp fever), though tabanids were more active vectors (1924). Stein et al. (1942) could infect horses with EIA via the stable fly, but only after many bites, while Hawkins et al. (1973) was unable to obtain transmission of EIA via the stable fly.

Other pathogens found to be transmissible by the stable fly are hog cholera (Miller, et al. 1974; Morgan and Miller 1976), anthrax (Nieschulz 1928), yellow fever (Hoskins 1934) and fowlpox (Bos 1932). Parasites found to be transmissible are Dermatophilus congolensis (Richard and Pier 1966; Abu-Samra 1980), Habronema microstoma, a round worm (Bull 1919; Roubaud and Descazeaux 1922), Dermatobia hominis, the human bot fly (Pinto and Da Fonseca 1930; Zeledon 1957), and the nematode Stephanofilaria stilesi (Dadaev 1977). Woke and Konwinski (1972) demonstrated that the stable fly could potentially transmit circulating tumor cells of hamster reticulum cell sarcoma.

Mitzmain (1913), Aders (1916), and Glaser (1922) all noted the phenomenon of house flies and other flies feeding on the blood droplets left as a result of stable fly feeding. Mitzmain

(1916) found that the house fly could transmit Surra when feeding in such a way. The literature does not show any development of research on this stable fly-opportunistic blood feeder complex, perhaps a serious oversight in the study of disease vectorship, and yet another possible reason why studies on the role of the stable fly as a disease vector can be contradictory.

Control methods initially developed to combat stable fly attacks and discourage breeding included coverlets and/or petroleum based ointments for horses, screened environments, and sanitation which disrupted or eliminated breeding environments (Porchinsky 1910; Bishop 1913). With the introduction of DDT and other chlorinated hydrocarbon compounds during the 1940's, former methods of control received decreased emphasis in the face of the apparent great success of chemical control. By the 1960's, a pronounced pattern of insecticide resistance had emerged, including some resistance to more recently introduced chemicals such as the organic phosphates and carbamates, as well as the older chlorinated hydrocarbons. Biological magnification of persistant residues was also becoming an environmental concern.

Muma and Hixon (1949) and Quarterman et al. (1951) reported DDT effective against stable flies. House fly populations, however, had shown a DDT resistance as early as 1947 (McDuffie 1960). McDuffie (1960) noted that by 1960 stable fly resistance to DDT was confirmed in other countries and suspected in the U.S. Georghiou and Taylor (1976) reported that the stable fly had become generally resistant to DDT, cyclodienes and organophosphates; the house fly was noted by then to be

additionally resistant to carbamates and pyrethroids (seeming always to preceed the stable fly in developing resistance).

Research on alternate methods to control the stable fly has successfully produced chemosterilants (LaBrecque and Meifert 1975; Wright et al. 1971), bacterial larvacides (Gingrich 1965; Campbell and Wright 1976), physical attractants (Williams 1973; Meifert et al. 1978; Pickens et al. 1973), insect growth regulators (Harris et al. 1973; Wright 1974; Bowman et al. 1973; Campbell and Wright 1976), repellents (Matsumura et al. 1976; Schreck et al. 1978), and radiation sterilization (Williams et al. 1977; Patterson et al. 1980), and has placed renewed emphasis on the ongoing effort to find and use natural parasites (Legner et al. 1967) and predators (Peck 1970). A novel approach, such as host immunization against the stable fly (Schlein and Lewis 1976) shows promise for development of new strategies for the future. Legner and Olton (1968), Kennedy and Merritt (1980), and Patterson et al. (1980) have explored combinations of these control methods in IPM strategies, with improved results over chemical control alone.

By gaining greater detailed understanding of a pest and its environmental interactions, we are becoming more capable of disrupting selected aspects of these interactions with decreasing impact on the surrounding environment. This study was undertaken to further the development of such improved control systems through additional knowledge of the stable fly larva and its breeding environment.

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PAPER

CHARACTERIZATION OF AGING BOVINE MANURE IN RELATION TO STABLE FLY (DIPTERA: MUSCIDAE) ADULT AND LARVAL PRESENCE

ABSTRACT

Physiochemical and fly visitation patterns were followed in accumulations of bovine manure as it aged in a feedlot setting during two consecutive summers of study. Results of stable fly and adult population patterns found in this study demonstrated that the stable fly female presence around aging manure was due to an attractancy of the manure rather than random visitation from the general population of feedlot flies. Number and percentage of fully gravid female stable flies visiting manure increased with manure age. This number peaked, on the average, approximately 6 days before stable fly larval presence was detected (> 14 day old manure). Environmental factors (temperature, precipitation, solar radiation) influenced the expression of patterns of stable fly female visitation and the parameters reflecting manure aging processes. No correlations could be found between stable fly female presence and physicochemical parameters of the manure. Microbial populations showed patterns through time which were generally indicative of pysicochemical parameter trends.

Stable fly larval appearance in aged rather than fresh manure was considered to be a function of the female stable fly's attraction to the manure at that point in time, rather than the larvae's inability to survive in fresh manure. While competition with other fly species could have been a contributing factor in the evolution of the delayed appearance of the stable fly larvae

in this medium, the timing could as easily be a result of the female's response to particular attractants which appear late in this less commonly used breeding medium, but which could be critical timing stimuli to oviposition and successful larval development in other preferred media.

INTRODUCTION

As cosmopolitan as the housefly, Musca domestica L., the stable fly, Stomoxys calcitrans (L.) is also found to utilize a wide variety of breeding media. Spilled cattle feed and stored manure (Meyer and Petersen 1983), bay grass deposits (Simmonds 1944), and fish scale waste from a sardine plant (Suenaga 1962) are a few among the many substrates reported to support development of stable fly larvae. Hafez and Gamal-Eddin (1959) and Parr (1962) noted that stable flies show very little interest in ovipositing in pure manure, preferring that mixed with vegetable matter. Thomson (1937) reported stable fly avoidance of oviposition in fresh manure of several different types, but an interest in aged manure.

Previous field studies on stable fly larval habitats have included surveys of breeding sites (Campbell and McNeal 1979), a census of flies captured from various media (Guyer et al. 1956; Williams et al. 1980), and attempts to correlate environmental parameters with fly productivity in feedlot sites (Rasmussen and Campbell 1981).

In a preliminary study on characterization of the stable fly larval habitat conducted at Kansas State University (unpublished), stable fly larvae were not detected in aging bovine manure until approximately the 21st day. This distinct time window of larval appearance in this media was thought to

have potential as an indicator of a point in manure aging supportive of stable fly larval development and/or attractive to ovipositing adults. The present study was conducted to determine if there are physiochemical parameters which can be correlated with the timing of stable fly oviposition on aging manure and subsequent appearance of larvae.

Materials and Methods

Field studies were conducted at a research feedlot (Beef Research Unit) at Kansas State University, Manhattan, Kansas, during the summers of 1982 and 1983. Fresh manure was collected from feedlot calves fed a diet mixture of 86.8% sileage, 6.6% milo, and 6.6% of a protein and mineral supplement. Dairy manure was collected from high producing KSU dairy cows fed alfalfa hay and a mixture of 34% corn, 34% milo, 27% soybean meal and supplemented with vitamins and minerals (5%). Manure was collected as freshly or recently (5-15 minutes) dropped pats, mixed to provide homogeneity, and put in place at the experimental site on the same day collected.

Wooden frames, 55 cm x 55 cm were loaded with manure to a depth of 20 cm. Cone emergence traps topped with plastic collection cups were placed over each frame. During the 1982 study, eight replicates each of dairy and feedlot manure were set up. Four stations of each manure type were "covered" using plastic screen skirting at the base to exclude adult fly visitation to determine any effect of larval presence on the manure aging pattern. During the 1983 study, only dairy manure was used as it had previously (1982) been found to be more productive of stable fly larvae, and all traps were left open. Each station was assigned a randomized, equidistant position within a square plot.

The 1982 study followed changes on a set of manure plots for a period of 41 days, from July 7 to August 17. In 1983, to

determine if the 1982 results were dependent upon that particular time of summer, four consecutive plots of manure were set up at two week intervals for 27 days each from June 15 to August 22. Two "dummy" stations with cone traps but no manure were set out (1983) to ascertain if flies were attracted by the cone traps alone. Frames were recycled when the sequential sets were put out in 1983. Used frames were scraped clean, and old manure debris removed from each site before fresh manure was reloaded.

Physicochemical parameters of aging manure:

Each frame of manure was divided into a grid pattern and sampled randomly. Readings and samples were generally taken in the morning and analyzed that day or frozen for later analysis. Manure temperatures in 1982 were taken using a Campbell CR-5 Datalogger (Campbell Scientific, Inc., Logan, UT) with thermocouples, and manually in 1983 using a YSI Tele-Thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) with a spear-probe. pH measurements were taken on site at a depth of 6-8 cm. with a Model 5996-30 Horizon pH meter (Horizon Ecology Co., Chicago, IL), and a spear tip pH electrode (Cole Parmer Instrument Co., Chicago, IL).

A manure core, ca. 5 cm. diameter, was taken through the existing depth of the manure at the same site as temperature (1983) and pH readings. The sample was placed in a capped glass jar for transport to the lab. A capped, water-filled glass jar was placed in each sample site to minimize effects of the void

caused by sample withdrawal, i.e., increased surface area exposed to environmental influences. This was found to be less meaningful with dairy manure which retained a moist, semi-fluid consistancy longer than feedlot manure, and tended to fill in its own voids within a short time.

In the laboratory, samples were minutely sorted through to remove immature and adult fauna. The various core strata were mixed to provide a fully representative sample for subsequent analysis. Moisture content was determined gravimetrically, oven drying the samples at 90-100^{o} C for 24-48 h.

A Beckman Model 865 Infrared CO_2 gas analyzer was used to determine CO_2 production rates of manure samples in an attempt to quantify microbial activity. A 2-4 gm manure sample was placed in a 50 ml side-couple Erlenmeyer flask and diluted 1:1 with a Na_2HPO_4-NaH_2PO_4 buffer of similar pH. Osmolality was determined using a Wescor Vapor Pressure Osmometer (Wescor, Inc., Logan, UT). Manure samples for this analysis were centrifuged at 13,800 rpm for 30-45 min. The supernate was removed, placed in screw-capped glass vials and frozen for later analysis. Whole manure samples were frozen in glass vials for subsequent ammonia analysis using a Corning selective ion ammonia probe (Corning Medical and Scientific, Medfield, MA). and a Markson Model 95 digital pH/mv/temperature meter (Markson Science, Inc., DelMar, CA). A modification of the "Quick" method for ammonia measurement as described by Byrne and Power (1974) was used.

Larval presence:

Larvae sorted from the manure samples were washed with distilled water until free of manure residue. They were then placed in Super Skipper (Elzinga 1981) solution for 15-20 sec., then rinsed twice with, and stored in, Kahle's solution (Barbosa 1974) for at least 24 hrs. Larvae were subsequently dehydrated in an ethyl alcohol series from 30-100% and stored in 70% ethyl alcohol for identification and counting. Representative 1982 specimens were sent to the USDA/ARS Insect Identification and Beneficial Insect Introduction Institute, Beltsville, MD for identification. Identifications of specimens collected in 1983 were confirmed by personnel of the KSU Research Collection.

Adult fly visitation.

Flies captured in plastic collection cups were collected and cups replaced with fresh cups daily during 1982. Summer heat was found to desiccate a number of flies collected, making ovarian development determinations of some stable fly females difficult. Cups were collected twice daily in 1983 to minimize specimen desiccation, with minimal improvement. Flies were placed in a freezer temporarily to immobilize them, then transferred to petri dishes and identified and counted over ice. Flies were sorted into three categories: stable fly, house fly and "other" for counting purposes. General comment was made on what constituted "other". Stable flies were sexed and female stable flies dissected immediately to determine stage of ovarian development,

or placed in Ringer's solution for dissection the following day. Dissections were done using physiological saline (0.7% NaCl) in plastic tissue culture plates (VanGeem et al. 1983) containing a layer of blue-violet wax. The wax provided a medium for embedding specimen pins, and the wax color used enhanced the color identification of non-stained follicular relics via augmentation of the visual negative afterimage effect. Specifically, use of wax color (blue-violet) which was complimentary to relic color (yellow to yellow-orange), tended to heighten color perception of relic tissue, eliminating the need for staining. Developmental stages of ovaries were catagorized according to a variation of the method described by Scholl (1980). During 1983, mating status of flies was determined by checking for sperm in crushed spermathecae.

The overall feedlot fly population was monitored by placing two Alsynite-sticky traps (Williams 1973) of a cylindrical design (A. B. Broce, unpublished) in different areas of the feedlot during 1983. The northern trap farthest away from the cone traps, was placed near a feedmill, some distance away from cattle pens. The southern trap was placed next to a cattle pen, directly south of the first one and closer to the cone traps (Fig. 1). Sleeves of plastic covered with tack-trap were replaced and brought to the laboratory three times each week. Flies on a one inch strip through the middle of the sleeve were identified and counted as a representative sample of the entire sleeve. Up to 61 stable flies were then recovered from the whole surface of the sleeve, washed in kerosene to remove the adhesive, sexed, and the females

dissected to determine stage of ovarian development.

Microbial Analysis:

During 1982, manure samples were collected from each set twice per week, placed in sterile containers using aseptic technique, and taken to the KSU Department of Animal Science and Industry Food Microbiology Laboratory for microbial analysis. Viable cell counts for monitoring anaerobes, aerobes, lipolytic, saccharolytic and proteolytic bacteria were performed according to Lee (1982) using standard methodology (Richardson 1985).

Statistical Analysis:

Correlations, using the SAS correlation proceedure (p. 173, SAS User's Guide), were run on various parameters in an effort to find general indicators of non-causal linear relationships. Due to our non-standard application of this proceedure (time-series), p>R is not a reliable figure and an R-value must exceed 0.6 to be significant in indicating a linear relationship (D. Johnson, KSU Statistics Dept., personal communication).

Results and Discussion

Covered and Uncovered Traps:

Osmolality, pH, moisture, and CO_2 were selected as potential indicators of chemical and microbial changes occurring in the manure. Few differences in these four parameters were observed in covered vs. uncovered treatments of dairy and feedlot manure trials in 1982. t test comparisons of covered and uncovered treatments showed pH and osmolality in feedlot manure during week 4, and CO_2 in dairy manure during week 2 to be significantly different ($p>0.05$, Table 1). Osmolality and pH were found to have a strong inverse correlation ($R=-0.78$), thus, it is not surprising that both pH and osmolality values were significantly different in covered and uncovered treatments, though each was high in a different treatment. Moisture and pH are known to affect microbial CO_2 production (Alexander 1977). The lack of a corresponding significant difference in CO_2 production between both treatments during week 4 diminished the possibility that there is any overall significant difference between these treatments in feedlot manure. The one instance of significant difference in CO_2 production in dairy manure showed higher production in the covered treatment. While this result could be expected from this treatment in which lower larval numbers would provide less manure aeration, contributing to a more anaerobic environment and thus potentially more CO_2 production, again, the other parameters did not support this as a strong significant

difference.

Growing grass pushed up portions of the lower screening of the covered traps at day 12, allowing entry of adult flies, most notably evidenced by a large influx of house flies. A resultant increase in larvae was not seen, however, possibly due, in part, to the older age of the manure at that time. A small number of house fly and sarcophagid larvae were initially found in covered traps, probably a result of oviposition occurring during loading of the manure into the frames. Larval numbers in covered traps were much less than that in the uncovered traps (uncovered feedlot average = 24.5 X covered average, uncovered dairy average = 17.7 X covered average), and had dropped to near 0 by day 12, where they remained through day 41. These results suggested that larval densities seen during this study did not significantly alter the overall manure aging process as it was monitored by the physicochemical parameters used in this study. Subsequently, only uncovered traps were used in further studies.

Physicochemical Patterns and Environmental Influences:

Feedlot and dairy manures from the 1982 study showed similar overall physicochemical and adult fly patterns (Figs. 2, 3, 4, 5a, 5b, 6a, 6b). Differences in the timing and magnitude of each parameter seen in the two manure types were considered largely to be due to dissimilar diets of the cattle. According to D'Amato et al. (1980), two such contrasting feed regimens as the ones used for this study result in manures of different physical and

chemical composition, each supportive of somewhat different microbial populations (additional details of substrate-microbe correlations is discussed in the "Microbial Populations" section). Some of the differences could also be attributed to differences in age, sex, and breed of the two groups of cattle. The moisture content of feedlot manure appeared to be more responsive to changes in air temperature than did dairy manure, most likely due to the more fibrous, friable texture of feedlot manure allowing for more surface area to interact with atmospheric conditions, such as temperature and relative humidity (feedlot manure moisture showed an almost inverse relationship to air temperature, loosing moisture as air temperature rose, etc., Fig. 3a). Dairy manure moisture remained almost constant through significant changes in air temperature, probably a result of this manure's pasty texture.

Many factors, biotic and abiotic, influence the course and speed of manure decomposition; in a field setting, environmental conditions (temperature, precipitation, wind, and available moisture, etc.) can play a significant part. In the manure mass, decomposition, largely a function of microbial activity, produces chemical changes and generates heat from within. The 1983 study showed effects of both endogenous and exogenous factors on manure temperature. While manure temperature generally followed air temperature trends over the course of the summer (R=0.66, Figs. 7a & 7b), a range of stronger correlations was found among temperature patterns of the four manure sets (R=0.84 to 0.92). These figures helped demonstrate that the observed manure

temperature was the result of processes which produce a pattern somewhat, though not entirely, dependent upon environmental influences.

House fly-stable fly larval succession in bovine manure, and noted differences in the size of the larval osmoregulatory structures (that of the stable fly is larger, Stoffolano 1970), prompted an initial study of the impact of osmolality on the larval development and succession in the manure habitat. Both pH and osmolality were found to follow distinctive, reproducible patterns as manure aged (Figs. 2c, 3c & 7e). Decomposition processes occuring in the manure were reflected simultaneously, but inversely, in both parameters, and a correlation coefficient of R=-0.78 was found when run with both years' data. While following similar overall patterns, pH of 1982 dairy manure (Fig. 2c) was more gradually expressed than pH from a similar time of summer in 1983 (Fig. 7e). Average air temperatures were cooler during the 1982 experiment than the 1983's set 3 run (Figs. 2a & 7a). pH from set 1, 1983 run with cooler temperatures earlier in the summer, more closely resembled the 1982 pH pattern.

Environmental conditions have been found to affect fly behavior as well as manure decomposition. Valiela (1974) noted that cloud cover, when early in arthropod succession of a cow pat, can alter the pattern of species abundance by decreasing oviposition of certain fly species. In the present study, the number of flies and the timing of their appearance was found to vary from year to year. Adult fly data taken from corresponding periods of each summer showed similar overall patterns (Figs. 4,

5a, 5b, 6a, & 6b), with the 1983 pattern showing a greater number or percentage of flies appearing earlier. Relatively higher rainfall and lower temperatures or solar radiation (Fig. 8) during the summer of 1982 were probably responsible for this year's delayed fly pattern, causing either slower manure aging, indicated by the pH patterns mentioned above, and/or discouraging fly visitation during the earlier stages of manure aging.

Larval Populations:

Field studies of 1982 and 1983 confirmed the general observed timing of stable fly larval appearance first seen in preliminary studies. Initial detection of larvae ranged from day 14 to day 23, varying with the set (Figs. 2b, 3b, & 7c). A larval succession of several dipteran families was also noted (Fig. 9). Larviparous Sarcophagid larvae immediately colonized the fresh manure, quickly followed by house flies, then Stratiomyiids, and finally, stable flies. The magnitude of each family's colonization varied with the time of summer, but the order of appearance in manure remained constant, demonstrating fly population fluctuations throughout the summer as well as a succession at each set.

Adult Fly Populations:

Numbers of fully gravid stable flies (stage 5) showed no significant correlation through time with any one of the

physicochemical parameters monitored, i.e., pH, CO2, ammonia, moisture, or temperature. The data did, however, show a distinct pattern through time with regard to percent stable fly females, percent females in stage 0-2 (not yet capable of laying eggs), and percent females in stage 5.

When the number of stable fly females at stage 5 and larval numbers were offset by 1-9 days, the strongest correlation was found at 6 days offset (Table 4). This may indicate that, on the average, the time preceeding larval detection by 6 days is a period of highest manure attractancy to the gravid female.

The overall percentage of female stable flies, and the percentage of female stable flies in stage 5 of ovarian development increased through time (Figs. 4, 5a & 5b), suggesting an increasing attractancy of the manure as it ages. This interpretation of the pattern was reinforced by t-tests on numbers of flies caught in cone traps with manure vs. those without manure ("dummy" traps, Table 2). For stable fly females, the difference between trap types initially was not significant (at the $p = 0.05$ level), but tended to become significant by the second week or thereafter. Differences in stable fly male numbers were never significant, indicating the manure held no particular attractancy for them regardless of its age. House fly numbers (not broken down according to sex) showed a consistent significant difference during week 1, reflecting this species' relatively earlier attraction to and colonization of the manure. The continuation of significantly higher house fly numbers at the manure traps into weeks 3 or 4, as seen in sets 3 and 4, could

reflect either increased use of manure by both sexes as a moisture source as summer heat increased, or its use as a default oviposition site as other suitable sites dried up after higher temperatures. The lack of data according to sex did not allow for clarification of this trend.

Successional patterns of percent of stable fly females and of females in different stages of ovarian development found visiting the manure repeated itself, set to set, with some variation, depending on the time of summer the manure was set out. The percentage of stable fly females was initially low (<50%), but rose to a consistent level of > 75% by day 1 - 19 (Fig. 4). Female populations monitored during mid-June took longer to reach this high percentage level than those toward the end of August. Percent of stages 0-2 peaked earlier and fell to 0 earlier as the summer progressed (Fig 5a). They were replaced earlier and by a higher percentage of stage 5 flies as the summer progressed (Fig. 5b). (Spermathecae from flies in stages 0-2 contained no sperm, while those from flies in stage 5 contained sperm with rare exception.) Variations in the timing of this pattern of female visitation were probably due to environmental effects on the rate of manure aging, and variations in the magnitude of the pattern expression probably due to the size of the feedlot stable fly population (Fig. 10) which diminished considerably by the end of the summer's experiments. Population trends of female stable flies during 1982 most closely resembled Set 3 data from 1983 which ran during a corresponding time of summer. This similarity in the two years' data showed

that the general environmental patterns which reoccur each summer apparently tend to produce comparable fly population trends.

Stable fly numbers from sticky traps showed no consistent pattern of correlation with stable fly numbers from cone traps (Table 3). Set 3 had the strongest correlation with sticky trap numbers, a trend which showed up weakly on the graph (Fig. 10). Percentage of stable fly females caught in sticky and cone traps showed no significant correlation (Table 3). This method of sampling failed to demonstrate a significant relationship between numbers of trapped flies from the feedlot population and those attracted to the manure and caught in the cone traps, suggesting that the pattern of fly visitation at manure is a function of the differential attractiveness of manure during the aging process, rather than random visitation from the feedlot population.

Microbial Populations:

The several assays run (Fig. 11) demonstrated patterns of microbial growth that could be responsible for, or contributing factors to, the physicochemical patterns found in aging manure. These patterns also reaffirmed feedlot manure's relatively faster rate of decomposition as compared to that of dairy manure.

Aerobic and anaerobic bacterial count data confirmed field and laboratory observations on the general physical characteristics of the two manures. Feedlot manure has a noticeably more fibrous texture, is less pasty, and becomes

earth-like more quickly than does dairy manure. This texture of feedlot manure would seem more conducive to aerobic population growth, and, in fact, this manure produced higher aerobic and lower anaerobic counts than did dairy manure. Dairy manure appeared to change less in texture through time in the portion below the crust, the lessened change reflected in the more random slope of these two parameters. The relatively greater speed of aerobic over anaerobic decomposition could be responsible for the speedier pattern of change seen in feedlot manure.

Proteolytic count patterns corresponded with pH changes due to production of alkaline compounds characteristic of this type of flora. Both parameters showed an initial fall and subsequent general rise. Ammonia levels also appear to reflect proteolytic patterns. Again, the feedlot pattern peaked earlier than dairy in both the proteolytic counts and ammonia levels.

Saccharolytic organisms showed a correspondance with CO_2 levels in both manures, resulting from the release of CO_2 by these organisms during fermentative degradation of carbohydrates. Feedlot manure saccharolytic populations and CO_2 levels generally exceeded those of dairy manure and peaked earlier, until approximately day 29. At that time dairy manure microbial and CO_2 levels began to generally exceed those of feedlot manure, accentuating dairy manure's relatively prolonged decomposition process.

Lipolytic counts reflected the same pattern of a continuous decrease through time as was seen in volatile fatty acid data from a 1981 field study, which was also run on dairy manure. A

decrease in these bacteria which break down fats into the shorter chain fatty acids would account for this decline in fatty acid levels.

Summary:

Results of stable fly and adult population patterns found in this study demonstrated that the stable fly female presence around aging manure was due to an attractancy of the manure rather than random visitation from the general population of feedlot flies. Number and percentage of fully gravid female stable flies visiting manure increased with manure age. This number peaked, on the average, approximately 6 days before stable fly larval presence was detected (> 14 day old manure). Environmental factors (temperature, precipitation, solar radiation) influenced the expression of patterns of stable fly female visitation and the parameters reflecting manure aging processes. No correlations could be found between stable fly female presence and physicochemical parameters of the manure. Microbial populations showed patterns through time which were generally indicative of physicochemical parameter trends.

Stable fly larval appearance in aged rather than fresh manure was considered to be a function of the female stable fly's attraction to the manure at that point in time, rather than the larvae's inability to survive in fresh manure. While competition with other fly species could have been a contributing factor in the evolution of the delayed appearance of the stable fly larvae

in this medium, the timing could as easily be a result of the female's response to particular attractants which appear late in this less commonly used breeding medium, but which could be critical timing stimuli to oviposition and successful larval development in other preferred media.

Table 1. t test analysis of pH, osmolality, moisture, and CO_2 production levels of covered vs. uncovered treatments[a] of feedlot and dairy manure plots, summer, 1982 (t values/degrees of freedom for unequal variances).

Week	pH	Osmolality	Moisture	CO2
		Feedlot Manure		
1	0.41/5.6	0.62/6.0	-0.76/5.2	-0.55/4.1
2	-1.01/3.8	0.95/5.8	0.01/5.4	-2.60/3.5
3	-1.41/3.4	1.48/3.8	-0.05/6.0	-1.45/3.2
4	-5.79/6.0**	2.91/4.9*	2.24/5.8	-0.27/3.9
		Dairy Manure		
1	0.11/5.0	0.13/4.0	0.70/3.3	1.95/6.0
2	-0.88/4.2	-1.53/4.0	1.62/4.4	4.58/5.7*
3	-1.94/4.7	-0.83/5.6	1.36/5.2	0.60/5.1
4	-1.65/5.9	-0.77/5.8	2.18/4.2	-0.61/5.0

* Significant at 0.05 level.
** Significant at 0.001 level.
[a] t value was calculated as covered minus uncovered values.

Table 2. t test analysis of numbers of female stable flies, male stable flies, house flies, and "others" caught in cone traps over manure versus no manure (dummy) plot treatments[a], summer, 1983 (t value/degrees of freedom for unequal variances).

Week	Stable Fly Female	Stable Fly Male	House Fly	Other
		Set 1		
1	0.01/1.2	0.55/1.8	-3.99/3.8*	-7.47/3.0*
2	0.48/1.2	0.65/1.0	-1.00/3.0	-3.83/3.3*
3	-2.90/2.3	-0.06/1.4	-2.32/3.0	-2.49/3.0
4	-3.47/3.3*	-5.81/1.5	-2.61/3.0	-3.12/3.7*
		Set 2		
1	-1.72/2.7	-0.58/3.9	-13.94/3.0**	-4.95/3.0*
2	-9.46/3.0*	0.14/3.9	-2.61/3.0	-2.89/3.3
3	-2.44/3.2	0.00/1.3	-0.39/1.3	0.07/1.3
4	-3.94/3.0*	1.00/1.0	-0.58/3.0	-1.54/3.7
		Set 3		
1	-1.52/3.6	-2.34/3.6	-11.37/3.2**	-5.81/3.1*
2	-3.61/3.0*	0.48/1.2	-3.69/3.0*	-3.54/3.0*
3	-5.34/3.1*	0.45/1.5	-3.10/3.0*	-2.15/3.2
4	-2.92/3.8*	0.82/1.1	-1.00/3.0	-0.30/4.0
		Set 4		
1	-0.78/3.8	0.45/1.5	-4.34/3.0*	-3.95/3.0*
2	-1.01/3.5	1.00/1.0	-4.26/3.0*	-2.49/3.0
3	-3.46/3.9*	1.00/1.0	-2.45/3.0	-1.02/3.3
4	-3.66/4.0*	-1.26/3.0	-3.58/3.0*	-0.68/2.4

* Significant at 0.05 level.
** Significant at 0.001 level.
[a] t value was calculated as "dummy" minus manure plot values.

Table 3. Coefficients of correlations[a] between numbers of stable flies[b], or percentages of stable fly females[c], caught in Alsenyte sticky traps (two locations) and cone traps placed over dairy manure (Summer, 1983).

Cone Traps	Sticky Traps		
Set Number	North	South	N & S Average
	Total Stable Flies		
1	-0.09	0.73[a]	0.46
2	-0.48	0.12	-0.21
3	0.75[a]	0.50	0.63[a]
4	0.12	0.21	0.30
	Percent Stable Fly Females		
1	-0.58	0.03	-
2	-0.49	-0.59	-
3	0.15	0.25	-
4	-0.38	0.57	-

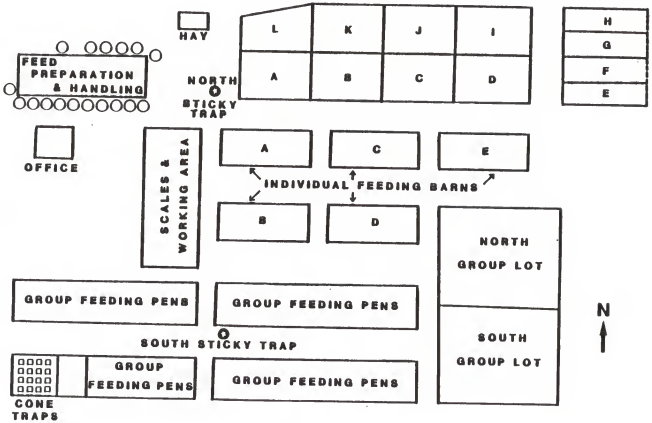
[a]Only coefficients > 0.6 are considered significant (see Materials and Methods for explanation).
[b]Stable Fly Accumulated Total (Accumulated for same periods of time as time duration between sticky trap collections.
[c]Stable Fly Female Percentage Accumulated Total (Accumulated for same periods of time as time duration between sticky trap collections).

Table 4. Coefficients of correlations[a] between numbers of female stable flies in stage 5 of ovarian development and number of larvae present, offset by 0 through 9 days.

Days Offset	Number[b]	Correlation Coefficient	Prob > R
0	34	-0.09	0.61
1	151	0.03	0.77
2	124	0.32	0.32
3	140	0.02	0.84
4	151	0.07	0.39
5	122	0.17	0.06
6	120	0.53	0.00**
7	180	0.24	0.00**
8	104	-0.04	0.70
9	88	-0.10	0.37

** Significant at 0.001 level.
[a]Only coefficients > 0.6 are considered significant (see materials and methods for explanation).
[b]Number of data points used in calculation.

Figure 1. Locations of sticky traps and cone traps at the Kansas State University Beef Research Unit, Manhattan, Kansas.



KSU Beef Research Unit

Figure 2. Changes in physicochemical parameters and fly larval presence of dairy manure, summer 1982 (with the exception of air temperature, points are averages of four replicates).

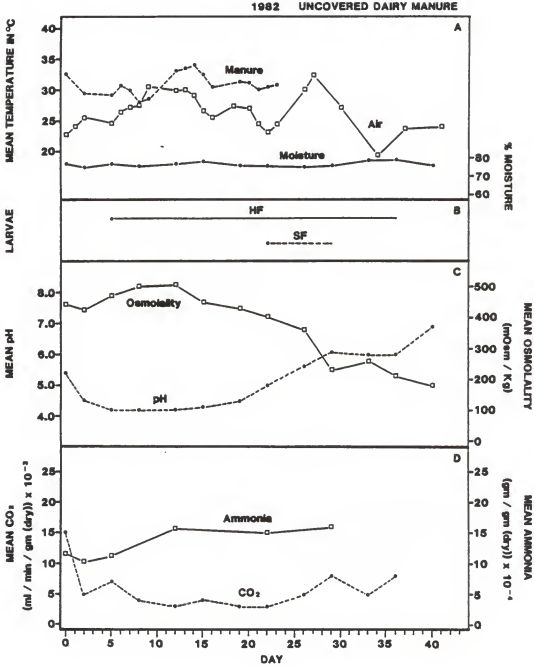

1982 UNCOVERED DAIRY MANURE
A
B
C
D
MEAN TEMPERATURE IN °C
Manure
Air
Moisture
% MOISTURE
LARVAE
HF
SF
MEAN pH
Osmolality
pH
MEAN OSMOLALITY (mOsm / Kg)
MEAN CO2 (ml / min / gm (dry)) x 10⁻³
Ammonia
CO2
MEAN AMMONIA (gm / gm (dry)) x 10⁻⁴
DAY

Figure 3. Changes in physiochemical parameters and fly larval presence of feedlot manure, summer 1982 (with the exception of air temperature, points are averages of four replicates).

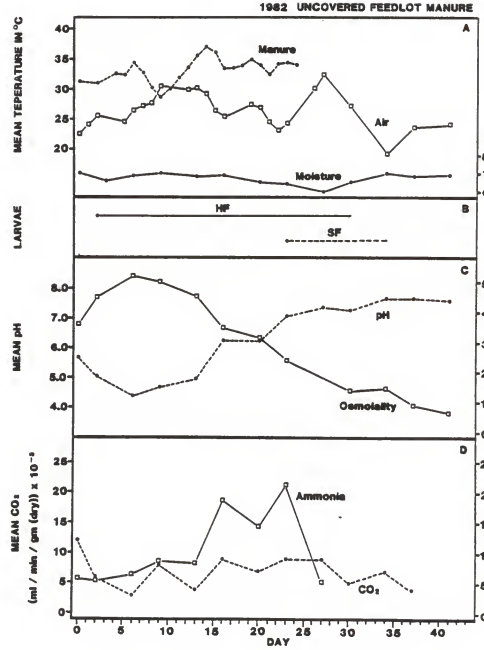

1982 UNCOVERED FEEDLOT MANURE
MEAN TEPERATURE IN °C
A
Manure
Air
Moisture
% MOISTURE
LARVAE
HF
SF
B
MEAN pH
C
pH
Osmolality
MEAN OSMOLALITY
(mOsm / Kg)
MEAN CO2
(ml / min / gm (dry)) x 10⁻³
D
Ammonia
CO2
MEAN AMMONIA
(gm / gm (dry)) x 10⁻⁴
DAY

Figure 4. Percent of female stable flies caught in cone traps during summers of 1982 (feedlot and dairy manures) and 1983 (dairy manure, sets 1-4). Open dots represent values for <4 flies.

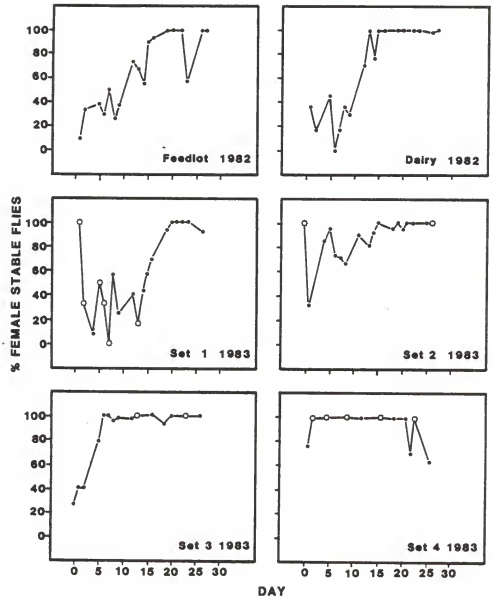

% FEMALE STABLE FLIES
100
80
60
40
20
0
Feedlot 1982
Dairy 1982
Set 1 1983
Set 2 1983
Set 3 1983
Set 4 1983
0 5 10 15 20 25 30
DAY

Figure 5a. Percentage of female stable flies in stages 0-2 (nulliparous) of ovarian development during 1982 and 1983 summer studies. (Open dots represent values for <4 flies.)

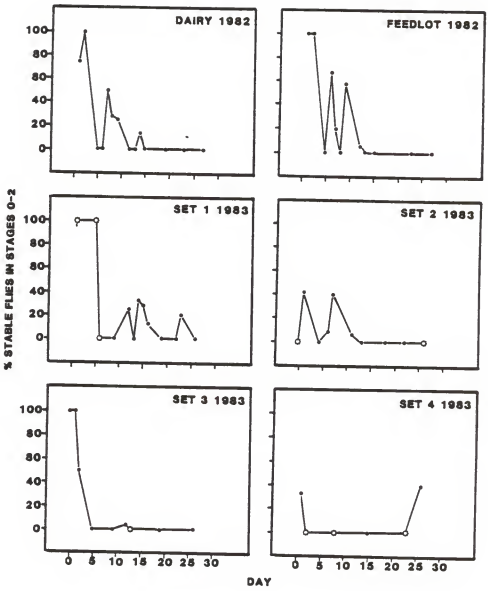
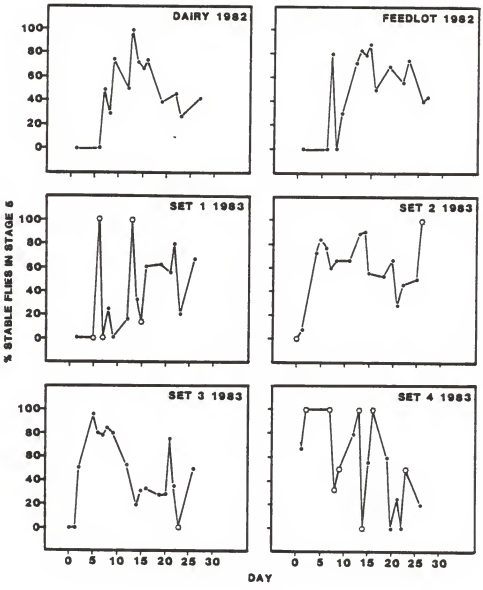
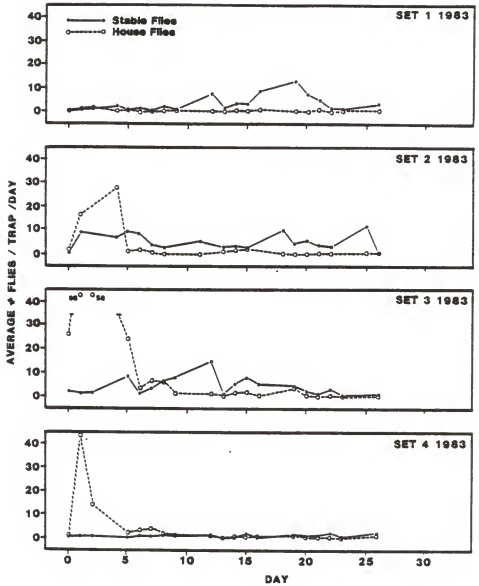

DAIRY 1982
FEEDLOT 1982
SET 1 1983
SET 2 1983
SET 3 1983
SET 4 1983
% STABLE FLIES IN STAGES 0-2
100
80
60
40
20
0
0 5 10 15 20 25 30
DAY

Figure 5b. Percentage of female stable flies in stage 5 of ovarian development (gravid) during 1982 and 1983 summer studies. (Open dots represent values for <4 flies.)


DAIRY 1982
FEEDLOT 1982
SET 1 1983
SET 2 1983
SET 3 1983
SET 4 1983
% STABLE FLIES IN STAGE 6
0
20
40
60
80
100
0 5 10 15 20 25 30
DAY

Figures 6a & b. Average number of stable and house flies caught daily in cone traps during summers of 1982 and 1983.


Stable Flies
House Flies
SET 1 1983
SET 2 1983
SET 3 1983
SET 4 1983
AVERAGE # FLIES / TRAP /DAY
DAY
0
5
10
15
20
25
30
40

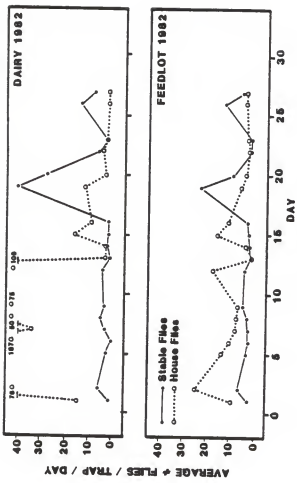

DAIRY 1982
FEEDLOT 1982
Stable Flies
House Flies
AVERAGE # FLIES / TRAP / DAY
DAY
0
5
10
15
20
25
30
40

Figure 7. Changes in physicochemical parameters and fly larval presence of dairy manure, summer of 1983 (points of all parameters except air temperature represent averages of four replicates).

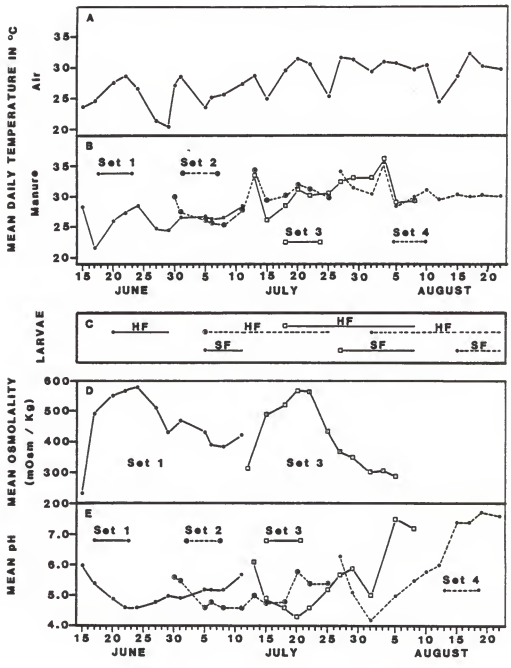

1983
A
B
C
D
E
MEAN DAILY TEMPERATURE IN °C
Air
Manure
Set 1
Set 2
Set 3
Set 4
LARVAE
HF
SF
MEAN OSMOLALITY (mOsm / Kg)
MEAN pH
JUNE
JULY
AUGUST

Figure 8. Precipitation and solar radiation during summers of 1982 and 1983. Duration of 1982 dairy manure set and 1983 Set 3 are included for comparison of environmental parameters.

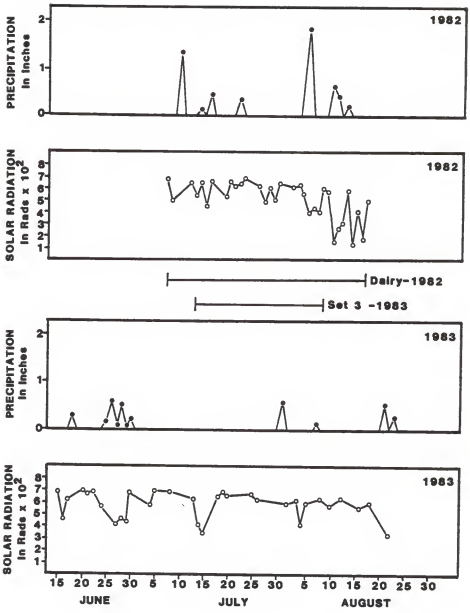

PRECIPITATION
In Inches
1982
SOLAR RADIATION
In Rads x 10²
1982
Dairy-1982
Set 3 -1983
PRECIPITATION
In Inches
1983
SOLAR RADIATION
In Rads x 10²
1983
15 20 25 30 5 10 15 20 25 30 5 10 15 20 25 30
JUNE
JULY
AUGUST

Figure 9. Populations of Diptera larvae in dairy manure, summer 1983. Set 1 placed in field June 15, with subsequent sets started at 2 week intervals. (Sarc. =Sarcophagid spp., Strat.=Stratiomyiid spp., HF =house fly, SF=stable fly.)

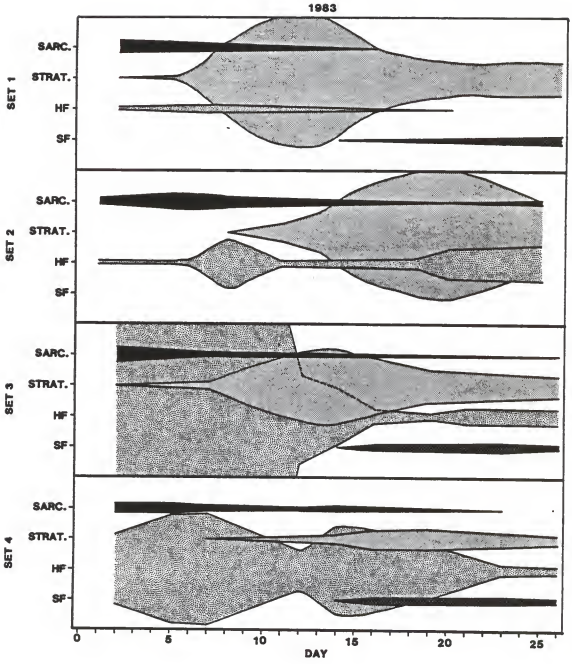

1983
SET 1
SET 2
SET 3
SET 4
SARC.
STRAT.
HF
SF
0
5
10
15
20
25
DAY

Figure 10. Number of flies caught in sticky and cone traps during 1983 summer study. Sticky traps were collected 3x/wk. For comparison, cone trap fly numbers were totaled over the same number of days that sticky traps were in field collecting flies.

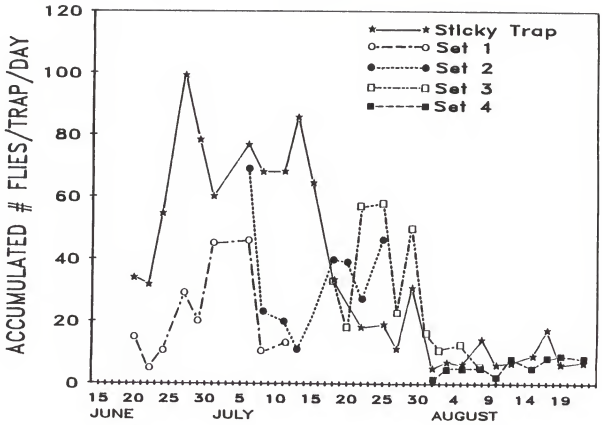
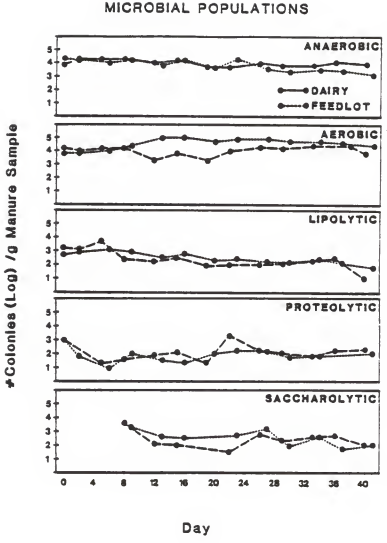

ACCUMULATED # FLIES/TRAP/DAY
120
100
80
60
40
20
0
Sticky Trap
Set 1
Set 2
Set 3
Set 4
15 20 25 30 5 10 15 20 25 30 4 9 14 19
JUNE
JULY
AUGUST

Figure 11. Microbial populations of feedlot and dairy manures during summer of 1982.


MICROBIAL POPULATIONS
ANAEROBIC
DAIRY
FEEDLOT
AEROBIC
LIPOLYTIC
PROTEOLYTIC
SACCHAROLYTIC
#Colonies (Log) /g Manure Sample
Day
0
4
8
12
16
20
24
28
32
36
40

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ACKNOWLEDGMENTS

I would like to sincerely thank Dr. A. B. Broce for his guidance and patience during my prolonged program of research and study. Thanks also go to the other members of my committee, Drs. D. Y. C. Fung and G. L. Greene for their insights and helpful suggestions offered in the preparation of this thesis.

To all those people who were such a help with the lab and field work; Glenn Brownlee, Brad Shores, Geneva Diamond, Mary Bagladi, Laura Smallwood, Chris Albrecht, and Kent Hampton, and to the IIBII Lab in Beltsville, MD, and Larry Corpus of KSU for their assistance with identification of insects, my gratitude.

This research wasa supported by USDA-ARS Cooperative Agreement No. 58-519B-0894.

CHARACTERIZATION OF AGING BOVINE MANURE IN RELATION TO STABLE FLY (DIPTERA: MUSCIDAE) ADULT AND LARVAL PRESENCE

by

Merrilee Susan Haas

B. S., Kansas State University, 1980

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

ABSTRACT

Physiochemical and fly visitation patterns were followed in accumulations of bovine manure as it aged in a feedlot setting during two consecutive summers of study. Results of stable fly and adult population patterns found in this study demonstrated that the stable fly female presence around aging manure was due to an attractancy of the manure rather than random visitation from the general population of feedlot flies. Number and percentage of fully gravid female stable flies visiting manure increased with manure age. This number peaked, on the average, approximately 6 days before stable fly larval presence was detected (> 14 day old manure). Environmental factors (temperature, precipitation, solar radiation) influenced the expression of patterns of stable fly female visitation and the parameters reflecting manure aging processes. No correlations could be found between stable fly female presence and physicochemical parameters of the manure. Microbial populations showed patterns through time which were generally indicative of pysicochemical parameter trends.

Stable fly larval appearance in aged rather than fresh manure was considered to be a function of the female stable fly's attraction to the manure at that point in time, rather than the larvae's inability to survive in fresh manure. While competition with other fly species could have been a contributing factor in the evolution of the delayed appearance of the stable fly larvae

in this medium, the timing could as easily be a result of the female's response to particular attractants which appear late in this less commonly used breeding medium, but which could be critical timing stimuli to oviposition and successful larval development in other preferred media.