FEEDING ACTIVITY, A STUDY OF CONTROL MEASURES, AND A SURVEY OF BLACK FLY PESTS (DIPTERA: SIMULIIDAE) OF HORSES IN VIRGINIA

by

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I. INTRODUCTION

The feeding activity of female black flies may cause considerable annoyance to saddle horses. Horses under attack become head-shy and difficult to manage, posing a potential hazard to riders. Stabling horses offers a means of protection against black fly feeding, but most horse owners cannot or will not stable their animals to prevent disturbance by noxious flies. Because stabling is an unsatisfactory control measure, I will evaluate various ear protectants to find easily applied materials of suitable effectiveness and duration on pastured animals to be of practical value to the owners.

Certain criteria are necessary to assess the effectiveness of control efforts directed at these pests, and to provide guidelines for treatment applications. Data gathered in control experiments will be used to determine if damage thresholds can be established based on the number of flies feeding in the ears, and the intensity of scabbing.

Survey techniques will be implemented to identify the extent of the black fly problem in Virginia, to estimate the numbers of horses exhibiting feeding damage, and to obtain information on the effectiveness of recommended control measures. Data on seasonal occurrance economic importance, and length of awareness of the problem are also of special interest.

Field studies of the pertinent species of black flies will provide data on blood meal volume and duration of feeding. The presence of diagnosed cases of equine infectious anemia, also known as swamp fever, in the area used in this study will make it desirable to investigate the detection of EIA antibodies in the blood meals of female black flies.

II. LITERATURE REVIEW

A. Simuliidae as Pests

Simuliids possess nefarious reputations as biters and vectors of human and animal diseases. *Onchocerca volvulus* (Leucart) is the most important human pathogen they are known to transmit. Blacklock (1926) established *Simulium damnosum* Theobald as the intermediate host for the nematode in West Africa and Uganda. *Simulium neavei* Roubaud was later found to be important in the etiology of the disease in Africa. Several species of black flies transmit these filaria in Central America and southern Mexico (Hoffman 1930, Strong et al. 1934). Simuliids and bovine onchocerciasis, caused by *O. gutterosa* Neumann, were investigated in England (Stewart 1937). Lee et al. (1963) reported simuliid-vectored equine onchocerciasis in Australia.

Several species of black flies have been shown to transmit avian blood parasites, *Leucocytozoon* spp. (O'Roke 1934, Skidmore 1932, Johnson et al. 1938, Shewell 1955, Anderson et al. 1962, and Tarshis 1972). This malarial disease has caused the deaths of large numbers of birds in North America (Wickware 1915, Nelson and Gashwiler 1941, and Laird and Bennett 1970).

Large swarms of the ubiquitous black flies are recorded as pests of livestock. Documented outbreaks include attacks on cattle around Poltava in the Ukraine (Dabizha 1970); deaths of cattle near Zurich, Switzerland (Eckert et al. 1969); and attacks of the golubatz fly in Romania (Ciurea and Dinlescu 1924). Zivkovitch (1970) cites three attacks in Yugoslavia: *Simulium colombaschense* Schoenberg in 1950 killed 800 head of livestock; *S. maculata* (Meigen) attacked poultry, 1938; and *Boophthora erythrocephalum* De Geer attacked livestock during 1969. During the past 200 years people in Kolumbacz, Hungary have lost several hundred head of livestock because of black fly feeding (Nicholson and Mickel 1950).

Cattle and horses in White Russia were attacked by *S. pusillum* Fries in 1956-57 (Dem'yanchenko 1960). Halgos and Ladislav (1971) recorded six species of simuliids attacking horses around Bratislava, Czechoslovakia. Rivosecchi (1970) captured two species of black flies feeding near the eyes of mules in the Castellano Valley of Italy. The first recorded simuliid-induced death of cattle in the Neotropical Region was reported from Paraguay (Coscaron 1968).

Animals in many areas of Canada have suffered from excessive black fly populations; particularly from *S. arcticum* Malloch (Cameron 1918). Millar and Rempel (1944) investigated the deaths of 132 farm animals, chiefly cattle and horses, in Sasketchewan valued at $20,000. Rempel and Aranson (1947) catalogued successive swarms of Simulium arcticum in the same region during May and June 1944-46, causing death to over 600 farm animals, most of which were cattle. Curtis (1954) estimated damages to cattle in British Columbia to be $24,000. Freeden (1969) cited losses to Alberta cattlemen attributed to attacks of S. arcticum on their herds. Specific causes were: disrupted feeding, breeding, and milk and beef production.

In the United States, nuisance reports of black flies accompanied the exploration and history of the Republic. Lugger (1896) cites the 1850 narrative of Louis Agassiz in "Lake Superior", which documents terrible black fly attacks on the members of the expedition and their resort to smudge fires for protection.

From 1861 until 1887, the southern buffalo gnat, Cnephia pecuarum (Riley), inflicted heavy losses on mules and other livestock. Livestock deaths were valued at $500,000 in west Tennessee. Thirty-two hundred head of livestock in Franklin Parish, Louisiana, were killed or had to be destroyed in 1882. Intense black fly attacks caused the deaths of numerous horses and mules in Memphis and Vicksburg, bringing streetcar traffic to a halt (Webster 1904).

The black fly situation in the Mississippi River system was studied comprehensively by Webster at the direction of C. V. Riley. Webster (1904) discovered that the outbreaks in the late 1800's coincided with periods of overflow from the river; a campaign of levee repair alleviated the problem. Bradley (1935) reported the deaths of over 1600 mules and horses in 1927, 1931, and 1934 in Mississippi and east Arkansas.

A broad spectrum of domesticated animals are affected by black flies. Studies include: cattle and horses, (Anderson and Defoliart 1961): nuisance to sheep, (Jones 1961); lowered egg production of laying hens, (Edgar 1953); turkey loses to disease, (Underhill 1944); and reduction of milk production of dairy cows, (Anderson and Voskiul 1963).

Determination of the specific cause of black fly-induced death is not clear, and may be due to a number of factors. Webster (1904) noted the term "Charbon" referring to the general effects of black fly bites. The symptoms were that of colic. Schmidt (1916) described symptoms of severely bitten animals as labored breathing, stumbling gait, rapid pulse, and painful swellings. Death occurred in one to two hours. He wrote that less severe cases were characterized by loss of appetite, abortion, marked depression, and blindness.

Cameron (1922) working in Canada, suggested that the direct cause of cattle deaths was suffocation. He based his views on the entrance of large numbers of black flies into the respiratory passages of the animals. Enderlein (1924) also felt death was due to inhaling flies in quantities sufficient to obstruct the bronchi. Cattle and horses were killed in one-half hour when the flies were exceedingly numerous. The rapid effect appeared to be due to asphixiation rather than toxins. Riley (1884) quoted Schonbauer, 1795, as witnessing the postmortem of a horse in which genital orifices, nasal passages, and bronchial tubes were filled with black flies.

Rempel and Aranson (1947) believed death was due to shock caused by an increased permeability of the capillaries, permitting a significant loss of fluid into tissue spaces and body cavities. Pogorelyi and Kovban (1967) attributed the deaths of cattle, dogs, and swine to anaphylaxis resulting from the bites of Simulium argyreatum Meigen. Deaths in acute cases occurred in four to six hours, taking three to four days in sub-acute cases. Chomczynski et al. (1971) and Minar and Kubec (1968) considered intoxication to be the causative factor in simuliid-related deaths. Often, the damage inflicted by tremendous swarms is such that, although death is not imminent, the animal has to be sacrificed (Eckert et al. 1969)

Simuliids occurring in Virginia and recorded as feeding on horses include: S. decorum Walker, S. jenningsi Malloch, S. luggeri Nicholson and Mickel, S. pictipes

Hagen, Simulium vittatum Zetterstedt, Prosimulium magnum (Dyar and Shannon), and Cnephia mutata (Malloch) (Stone 1964, Stone and Snoody 1969). A single reference is found in the records of the Cooperative Extention Service Virginia Polytechnic Institute and State University. Jones in 1961 stated:

> "these insects (identified as belonging to the S. jenningsi group by A. Stone) were found in large quantities and according to the owner, they affect livestock, especially a pony that is near the house."

McComb and Bickley (1959) studied pestiferous populations of black flies in Maryland counties along the Potomac River and found S. decorum, S. jenningsi, and S. vittatum to comprise the major proportion of specimens captured.

Simulium jenningsi and S. vittatum, determined by E. C. Turner, Jr., were collected from the ears of cattle in Montgomery County, June 6, 1958. Prosimulium sp. females were taken from cattle in Boutetourt County by an unknown collector. No data was recorded. These specimens were present in the Survey Entomologists' collection, Cooperative Extension Service.

B. Black Fly Control

Where black flies are vectors of human disease, control efforts have been primarily directed at the larval stage. However, this demands careful planning and execution. Requirements include large scale maps, knowledge of the terrain, and adequate assistance. Timing of larviciding activities is critical. Materials should be distributed when most of the larvae are maturing, but prior to pupation (Jamnback 1973).

Development of survey technoques are necessary to determine the extent of infestation and stage of development. Black fly larvae utilize caudal suckers to attach themselves to stream substrates. Knowledge of this behavior has been used to develop methods of population monitering (Wolfe and Peterson 1958, Curtis 1968, Johnson and Pengelley 1966, and Lewis and Bennett 1974). Kovban (1968) devised a method for using hydrometeorological conditions to predict the intensity and times of black fly outbreaks.

Muirhead-Thompson (1957) and Jamnback (1962) devised laboratory methods for studying the effects of black fly larvicides. They employed jars or containers for such evaluations. Travis and Wilton (1965) and Wilton and Travis (1965) reviewed these techniques and proposed diverting stream water through troughs to field test insecticides. This system allowed the use of flowing water, more conducive to black fly larvae than containers of standing water. The stream, however, was not contaminated by the procedure.

Kershaw et al. (1965) developed techniques utilizing particulate insecticide formulations. This method takes advantage of black fly larval feeding habits, which are different from those of other detritus feeders such as chironomids. Jamnback and Means (1966) investigated different formulations and the length of exposure of black fly larvae to insecticides as influencing factors. Freeden (1970) described a constant rate liquid dispenser for releasing larvicides into streams or rivers.

Aerial applications of simuliid larvicides were evaluated especially for use in forested or otherwise inaccessible areas (Anthony and Richey 1958, Brown 1952).

Muirhead-Thompson and Merryweather (1969) found that Simulium sp. eggs were unaffected by one hour exposures to DDT, fenthion, Abate®, methoxychlor, or chlorpyrifos at concentrations of 10 ppm. This was 100 times the field dose suitable for larval control. Recently discovered amine ovicides may prove effective against this stage (Wilton and Fay 1969, Wilton and Hopkins 1971). Limited testing by Hocking (1950) has shown the pupae to be more resistant to insecticides than larvae. Jamnback and West (1970) reported insecticide resistance to p, p'-DDT in larval Simuliidae.

The effects of larviciding practices on non-target organisms must be weighed against the gains experienced by control programs. Because of the persistance and lack of selectivity of many insecticides, non-chemical approaches to black fly control continue to receive serious consideration.

Three main groups of pathogenic parasites are commonly found in black flies: Microsporidia, (Beaudoin and Willis 1965, 1968; and Jamnback 1970); Nematoda, (Strickland 1913a, 1913b; and Anderson and Dicke 1960; and Phelps and Defoliart 1964); and Fungi, (Frost and Manier 1971). Davies (1959) noted aquatic parasitic mites of the genus Sperchon on black fly larvae. James (1968) studied bird predation on larvae and pupae of Simulium vittatum.

Elimination of breeding sites may play an important role in reducing black fly populations. In a few cases where breeding is restricted to short, turbulent parts of otherwise slow flowing rivers, removal of attachment sites such as trailing vegetation, large boulders, and sticks was effective (Jamnback 1973).

Properly constructed dams, which utilize vertical spillways, siphon dams, or sluce gates, eliminate breeding sites in the flooded stream above the dam. They also provide minimum attachment area on the dam itself. Dams with spillways constructed in a series of steps provide large breeding areas for long periods of the year and compound fly problems (Quellenec et al. 1968).

Webster (1904) brought about relief from attacks by the southern buffalo gnat using a program of levee repair along the Mississippi River. Snow et al. (1958) reported decreased annoyance from black flies after impoundments were constructed in the Tennessee River basin in conjunction with the Tennessee Valley Authority. Prior to these steps, black flies, particularly *Simulium vittatum*, disrupted farming operations by their attacks on work animals. Farmers used cloth hats, ear sleeves, leafy branches in the bridle, and grease or crankcase oil to protect their mules and horses.

Gross et al. (1972) reported on preliminary studies to evaluate utilization of the sterile male technique for black fly control. No judgement has yet been made on the efficacy of the method. This approach may be effective against species having a limited habitat range, such as *S. rugglesi* Nicholson and Mickel, a vector of *Leucocytozoon simondi* Mathis and Leger in ducks (Fallis and Bennett 1961).

Insect growth regulators have shown much promise in early trials. Cumming and McKague (1973) were able to obtain almost complete inhibition of adult emergence in several species of black flies by introducing R-20458, or methoprene, into rearing water.

Quellenec and Ovazza's (1969) summary of black fly control measures emphasized that reliance is placed entirely on insecticides, most of which are directed at the larvae. Until recently, DDT was used. Because of the danger of environmental pollution and insecticide resistance, other products are being sought. Biological control is being investigated as a supplementary measure, but extensive use depends on development of mass rearing programs for parasites. Genetic control is not presently practical because effective simuliid rearing techniques are not available.

C. Feeding and Attraction

With few exceptions, female simuliids obtain a blood meal from birds or mammals (Nicholson 1945). Davies and Peterson (1956) suggested that species with reduced mouthparts fed on nectar or not at all. The blood meal size has been determined for several species using weight measurements or radioactive P^{32} labeling techniques (Crosskey 1962, Anderson et al. 1962, and Bennett 1963).

Several species such as Simulium vittatum and S. jenningsi prefer feeding sites in the ears, along the underbelly, on the head and inner leg surfaces of horses, mules and turkeys (Underhill 1939, Jones and Richey 1956, Knowlton and Maddock 1944, and Stone and Jamnback 1955). Breev (1950) noted that black flies feeding on reindeer appeared to prefer the shaded parts of the animal. He felt visual stimuli were important in attraction.

Peschken and Thorsteinson (1965) observed S. venustum and S. vittatum to be more attracted to stationary cylinders or plaques than to those swinging or rotating in the wind. Fallis et al. (1967) experimenting with fan traps and silhouettes, found that few

S. venustum were taken without carbon dioxide. More flies were taken as the CO_2 output was increased from 50 ml/min. to 800 ml/min., although the increase was not linear. Davies (1951) working with colored cloth found *Simulium venustum* to be more attracted to dark blue and brown than to medium gray or white.

Olfactory stimuli have only rarely been noted as being attractive to simuliids. Lowther and Wood (1964) found the ornithophilic species, *S. euryadminiculum* Davies, was attracted to the ether extract of the tail of *Gavia immer* (Brunnich), the common loon. Fallis and Smith (1964) found *S. rugglesi* was attracted to ether extracts of the uropygial gland of ducks plus carbon dioxide. The flies exhibited lesser attraction to CO_2, and no attraction to purified ether extract. They postulated that carbon dioxide and the ether extract attracted the flies from a distance, and at close range orientation was visual. This was demonstrated by observing that the flies landed only on certain parts of a bird or raised objects on a silhouette. After landing, the texture of the feathers provided a tactile stimuli determining whether a fly would crawl in to feed or move off. Davis and James (1957) saw evidence of olfactory attraction. They collected *S. vittatum* in traps baited with putrid ground beef and tallow. Fallis (1964) reviewing attraction stimuli of simuliids stated that a combination of factors including temperature, light, color, odor, and appearance were important.

Wenk and Schlorer (1963) studied three species of black flies feeding on cattle and horses. Two species fed in the ears and one on the ventral surface of the abdomen. They stated the preferred feeding sites appeared to be related to the mating habits of the black fly species involved rather than skin texture of the host. Those species feeding in the hosts ears mated as the flies approached the ears. General body feeders mated some distance from the host.

The length of feeding times for the species studied vary considerably, but often range from two to five minutes (Fallis 1964, Davies 1957, and Hocking and Pickering 1954).

III. MATERIALS AND METHODS

A. Black Fly Survey

Horse owners, veterinarians, and county extension agents were identified as potential participants in a survey program. The practicing veterinarian was chosen as the focus for this study. Considerations regarding this decision were: an objective observer, uniform educational background with attention to animal pests, and frequent contact with horses in a defined region of the state.

Many owners treat a problem such as black fly damage with home remedies. However, veterinarians are consulted for innoculations and anti-parasite programs. This contact presented opportunities to receive reports of black fly feeding damage. It was assumed that in areas where the problem was severe, the owner would draw attention to the condition.

A questionnaire (Appendix A) was formulated presenting 21 responses. These were constructed to elicit the desired information in a concise form that would facilitate analysis of the returns. Multiple choice options were utilized where possible, though opportunities were provided for comment or elaboration. The questionnaire was accompanied by a cover letter, a line drawing of a black fly, and a brief description of the fly and its bite.

Copies were mailed in March 1973 to 337 Virginia veterinarians, whose addresses were supplied by the Cooperative Extension Service, Virginia Polytechnic Institute and State University. Timing of the distribution allowed the information to be available to the veterinarians prior to the 1973 fly season. A follow-up survey was scheduled to determine any changes in response in view of increased awareness of the fly.

The 1974 survey (Appendix B) was an abbreviated version of the initial form. It contained eight questions, a cover letter, and description of the problem. These were sent to respondants from the previous year.

A format was prepared for interviewing horse owners (Appendix C). It was used, with slight modification, to standardize telephone interviews with county extension agents.

B. Black Fly Control

Black fly control experiments were conducted on horses pastured along the New River in Pulaski County, Virginia. The area was chosen because of the large population of black flies, and a sufficient number of cooperators. The animals used were ponies and saddle horses over three years of age.

Formulations Tested: All vaseline formulations, with the exception of 2% stirofos (Shell Chemical Company, New York, New York), were prepared in the Virginia Polytechnic Institute Entomology laboratory using technical grade insecticide. Vaseline® petroleum jelly (Chesebrough-Ponds, Incorporated, New York, New York) was melted in an 85°C water bath. Sufficient insecticide was added to obtain the desired weight-percent dosage. Methoxychlor was dissolved in Risella® oil prior to incorporation into vaseline for the 10% dosage.

Many commercially available fly repellents for horses are oil formulations. Starbar Swish Fly Repellent® (Thuron Industries, Dallas, Texas) containing 0.10% pyrethrins, 0.20% technical piperonyl butoxide, 0.33% n-octyl bicycloheptene dicarboximide, 35% butoxypolypropylene glycol, and 64.37% hydrocarbons; and an experimental 1% stirofos oil formulation (Shell Chemical Company) were evaluated as representative of these preparations. A commercially prepared 3% stirofos dust formulation (Shell Chemical Company) was also used.

Vaseline®, often mentioned by horse owners as an ear protectant, served as the standard for comparison of smears. BPRL-7053-1 (Exxon Chemical Company, Baytown, Texas), a refined hydrocarbon, was used as the standard for oil wipes.

EXPERIMENT A

2% diazinon vaseline formulation
2% malathion vaseline formulation
3% stirofos dust formulation

1% stirofos oil wipe-on formulation
Vaseline®, standard

EXPERIMENT B

2% malathion vaseline formulation
2% stirofos vaseline formulation
Vaseline®, standard

EXPERIMENT C

1% stirofos oil wipe-on formulation
Starbar Swish Fly Repellent®
BPRL-7053-1 refined hydrocarbon, standard

EXPERIMENT D

5% malathion vaseline formulation
5% crotoxyphos vaseline formulation
10% methoxychlor vaseline formulation
Vaseline®, standard

Application: The horses were randomly assigned to treatment groups. Vaseline compounds were applied using cotton swabs. The inner surface of the horses ears was evenly coated with the formulation. By weighing applicators and containers, it was found that approximately 3 gm of the material or standard was dispensed to horses receiving vaseline base treatments.

Oils were poured on cheesecloth swabs prior to application. Initially, 6 ml were placed on the swab, then 2 ml were added before each treatment to standardize doses. One ear received the material to be evaluated, the other received the standard. The dust formulation was applied using a cheesecloth bag in the manner of a powder-puff.

Evaluation: A pretreatment count was made before application of the test materials. Ensuing counts were made at 24 hour intervals. The pretreatment averages for each treatment group were used for determining the amount of protection afforded. Fly counts during the postreatment period are expressed as a percent of numbers observed at the pretreatment count.

C. FEEDING AND ATTRACTION

Carbon Dioxide Baited Traps: Two modified canopy tabanid traps (Atkins et al. 1972) were placed 50 meters apart in a pasture at the Prices Forks Experimental Farm, Montgomery County, Virginia. Trap A was baited with seven pounds of dry ice to provide a carbon dioxide source. Trap B was unbaited and was designated a control. The traps were emptied morning and night. Experiments were started at 10:00 am and were terminated at 5:30 pm. On the second day trap B was baited, and trap A was designated the control. On the final day the position of the traps was switched. Specimens collected were identified in the laboratory.

Feeding Damage Study: An assessment of damage to the ear concurrent to fly counts were taken during control experiments and surveys. The following categories and abbreviations were used:

(N) None..........no scabs
(VL) Very Light....few scattered scabs
(L) Light.........sparse scabbing
(M) Medium........moderate scabbing

(MH) Medium Heavy...intense scabbing
(H) Heavy..........very intense scabbing

A standard t-test was used to detect differences between the mean number of flies in the left and right ears. Fly counts and damage assessments were analyzed using a test for the least significant difference (Sokal and Rohlf 1969). These tests allowed me to determine if any categories should be combined.

The percentage of surface area exhibiting scabbing was determined by projecting 35 mm color slides of ears onto 400 square per inch graph paper. The ear outline was traced and scab areas marked. The total squares in the ear and numbers of squares representing scabs were used to calculate a percent scabbing.

Determination of Blood Meal Volume: Engorged female *Simulium vittatum* were collected from the ears of horses pastured adjacent to the New River in Pulaski County, Virginia. Unengorged females were collected at the site using a canopy-type tabanid trap (Atkins et al. 1972) baited with dry ice. Collections were made during a four day period in August 1974.

All specimens were collected and frozen immediately in a cooler of dry ice for transport to the laboratory. They were weighed on arrival using a Mettler H-43 balance (Mettler Instrument Company, Princeton, New Jersey).

Due to the small size of both the engorged and unengorged flies, they were weighed in lots of 10, with the exception of one lot containing eight. The average weight was determined for each group. The blood meal volume (Vb) was calculated using the blood meal weight (BMw) and the specific gravity of horse blood (SGh) in the formula SGh x BMw = Vb. The specific gravity of horse blood is 1.053 at 20°C (Spector 1956).

Study of Feeding Duration: Female *Simulium vittatum* landing in the ears of a pastured Welsh gelding were allowed to find a suitable feeding location. Once settled, they were marked using a camels' hair brush and orange Super-Glo fluorescent tempra (The Morilla Company, Long Island, New York). Temperature and humidity were determined using a Psychron psychrometer (Model 556-3, Bendix Aviation Corporation, Baltimore, Maryland). A Gossen® exposure meter (Luna-Pro, Kling Photo Corporation, Woodside, New York) yielded light intensity data. The black flies were observed at one minute intervals until the abdomen became greatly distended. Checks were then made every thirty seconds to record when flies departed. A stop watch was used to measure elapsed time.

Blood Meal Analysis for EIA Antibodies: Engorged female *S. vittatum* were collected both from the ears of a chestnut gelding diagnosed as having Equine Infectious Anemia (EIA) and from an uninfected mare. The flies were immediately frozen and stored at -15°C prior to testing.

EIA immunodiffusion antigen and positive serum were obtained in a detection kit (Pitman-Moore, Incorporated, Washington Crossing, New Jersey). Negative serum (S^-) was obtained from laboratory stock. The microimmunodiffusion technique of Dommermuth et al. (1973) was used as follows:

1. A borate buffer solution, pH approximately 8.6, was prepared consisting of 2 gm NaOh, 3 gm H_3BO_3 and one liter of distilled water.

2. The borate buffer was used to prepare a 1% solution of Nobles' special agar.

3. A pipette was used to dispense three milliliters of the agar evenly over a 75 x 25 mm microscope slide.

4. After allowing the agar to dry, three rows of ten wells, 2.5 mm in diameter, were cut in the agar using a 12 gauge cannula. Well spacing was 5.5 mm between centers.

5. The macerated abdomens of five flies which had fed on an infected horse (F^+), and five flies from an uninfected horse (F^+), antigen (A), and sera (S^+, S^-), were placed in wells as shown in Fig. 1.

6. The slide was incubated at 25°C in a chamber containing water saturated air. The test was checked at 24 and 48 hours for the formation of precipitate lines.

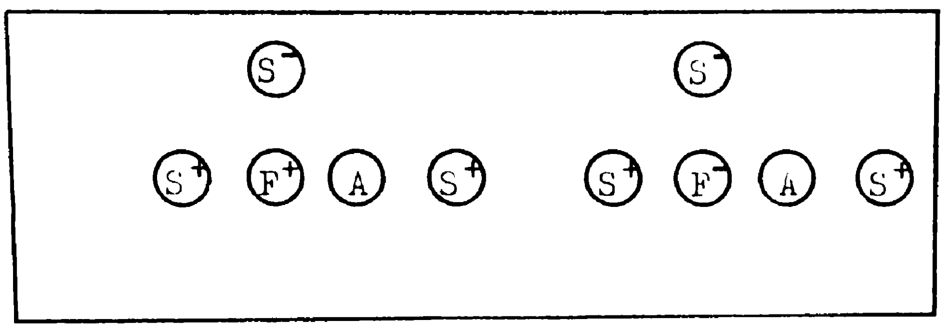


Figure 1. Arrangement of reagent wells for equine infectious anemia microimmunodiffusion test: S^- negative serum, S^+ positive serum, A antigen, F^+ macerated black fly abdomens with blood from diseased horse, F^- macerated black fly abdomens with blood from healthy horse.

IV. RESULTS AND DISCUSSION

A. Black Fly Survey

Twenty-nine replies were received from the initial mailing. Twenty-four veterinarians were selected from geographical areas not represented, and copies of the questionnaire remailed with an explanation of the value of their participation. Four replies from this group brought the grand total to 33, a 9.7% return for 1973.

Thirty-three questionnaires were distributed in March 1974. This follow-up survey served to verify previous replies. Thirteen were completed and returned for a 38% rate of reply.

Responses to the veterinarian's questionnaire are presented in Tables I and II. Data from the questionnaires and interviews were organized according to the physiographic regions of Virginia (Hoffman 1969). Black fly feeding estimates appear by county in Appendix D. Responses of the veterinarians were coded with a letter and number to facilitate tabulation and citation (Appendix E).

Thirty-eight horse owners were interviewed during the study. Most owned three to five horses, with a maximum of thirty-six. Awareness of the black fly problem among

Table I. A survey of estimated black fly infestation rates by Virginia veterinarians during 1973 and 1974. Part A: The Coastal Plain and the Piedmont Sections.

Vet Code	Observed* Fly	Observed* Scab	1973 Estimate of % Horses Affected	1974 Estimate of % Horses Affected
			Coastal Plain	
A-9	X	X	40-59	20-39
			Piedmont	
A-3	X	X	80-99	80-99
A-4	X	X	1-19	No reply
A-7	X	X	1-19	No reply
A-10	X	X	1-19	No reply
A-12	X	X	1-19	No reply
A-15	-	X	20-39	No reply
A-16	-	X	Few cases	Few cases
N-19	X	X	None	1-19
A-33	-	X	Few cases	No reply
A-35	-	X	No reply	Few cases
A-36	X	X	No reply	Few cases
A-37	X	X	No reply	50+

*/ X indicates a positive response

Table II. A survey of estimated black fly infestation rates by Virginia veterinarians during 1973 and 1974. Part B: The Blue Ridge, Ridge and Valley, and Appalachian Plateau Sections.

Vet Code	Observed* Fly	Observed* Scab	1973 Estimate of % Horses Affected	1974 Estimate of % Horses Affected
			Blue Ridge	
A-7	X	X	1-19	No reply
A-13	X	X	1-19	1-19
A-16	-	X	No estimate	No reply
			Ridge and Valley	
A-1	-	X	1-19	40-59
A-2	X	X	1-19	No reply
A-5	X	X	20-39	No reply
A-6	X	X	60-79	No reply
A-8	X	X	20-39	No reply
A-11	-	X	Many cases	No reply
A-14	X	X	1-19	20-39
N-20	-	X	None	1-19
N-24	-	-	None	1-19
			Appalachian Plateau	
A-5	X	X	20-39	No reply
A-34	X	X	No reply	40-59

*/ X indicates a positive reply

owners and veterinarians was generally two to six years, though one owner had been aware of the pest for 45 years and a veterinarian (A-3) 15 years.

Thirty owners were asked how much they would spend for protection of a horse against black flies. Responses ranged from $0.89 to $20 per animal per season, with an average of $4.05. In contrast, the average charges of 13 veterinarians was $14 for a call and treatment.

Treatments recommended by veterinarians were varied. Fly repellents and insecticides were considered to be of very little value. Various salves and ointments such as E. Q. 335, a 3% lindane wound dressing (USDA formulation), were rated as moderate to good. Horse owners consistently reported Vaseline® petroleum jelly as a satisfactory protectant.

Months of black fly occurrance based on owner and veterinarian's response are indicated in Fig. 2. Collection sites and dates for simuliids taken in the study appear in Appendices F and G. All species determinations were made by Dr. F. C. Thompson, United States Department of Agriculture, Systematic Entomology Laboratory.

Coastal Plain: A single response from the Coastal Plain (A-9) indicated the presence of black flies in Richmond, Essex, Middlesex, King & Queen, Gloucester, King William, and Caroline Counties. Twenty to thirty-nine percent of the horses observed were estimated to

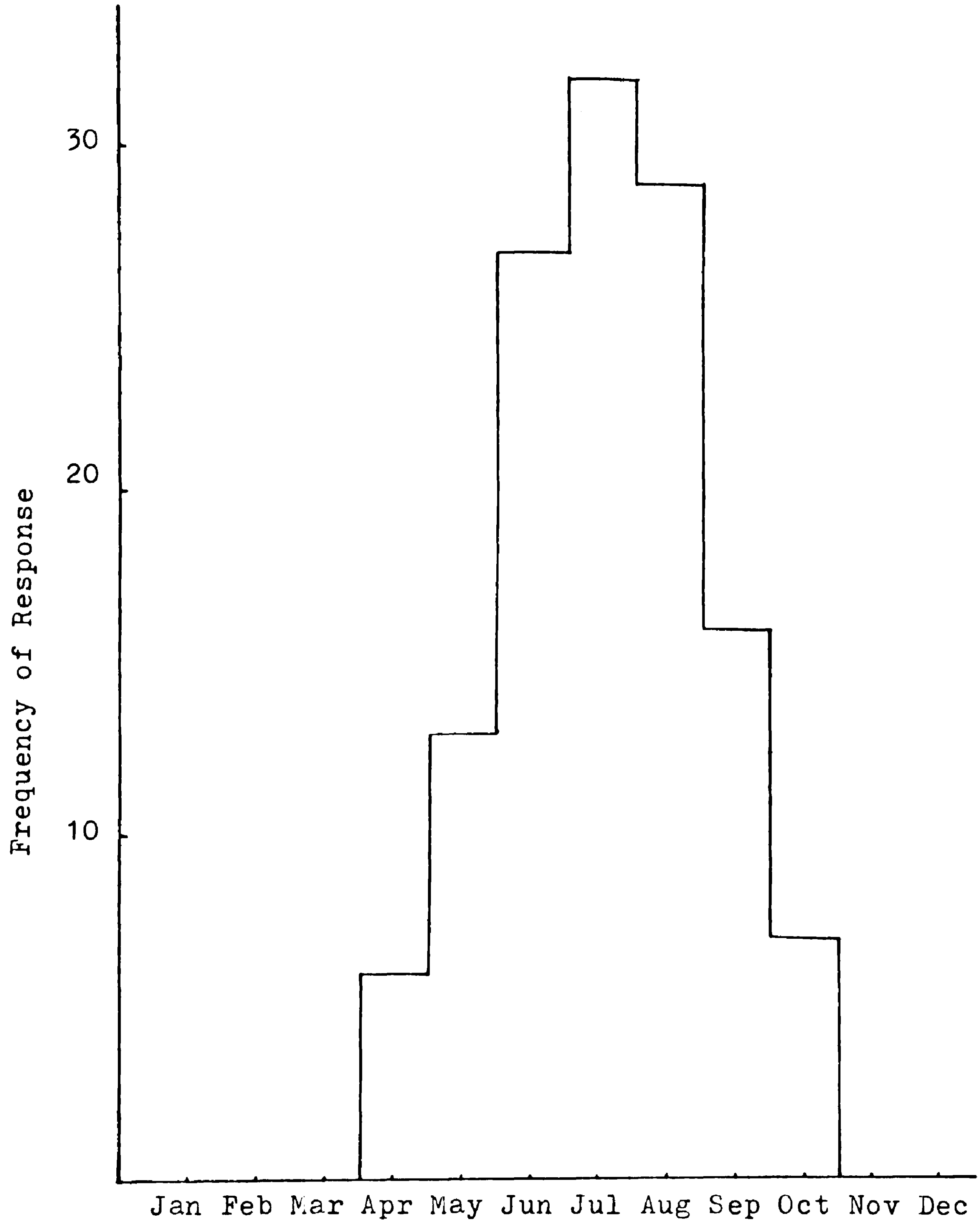


Figure 2. Seasonal occurrence of black flies based upon veterinarian's questionnaires and interviews with horse owners.

exhibit scabs resulting from black fly feeding. This was a decrease from the estimate of 40-59% reported in 1973. The veterinarian reported that both flies and scabbing were noted.

During a survey trip to the area in July 1973, one owner of ten horses in Isle of Wight County denied knowledge of the problem. Neither black flies nor feeding scabs were noted upon inspection of 18 horses at the Indian Point Stables, Nansamond County. No simuliids were observed in lightly scabbed ears of four pastured horses in Sussex County. Complaints of flies damaging the ears of a German shepherd dog were investigated, and stable flies, Stomoxys calcitrans Linneaus, were observed to be abundant on the side of the building to which the dog was secured. No simuliids were observed.

Extension agents for Middlesex, Essex, Lancaster, and King George Counties expressed no knowledge of the existence of the problem in telephone interviews. The agent for Caroline County had received inquiries concerning scabbing in the ears of horses, but had not had opportunity to observe the animals.

Piedmont Region: A low level of black fly infestation was indicated from responses from the Piedmont upland region. One of the seven veterinarians reporting from the area estimated that of the horses observed, 20-39% exhibited scabbing of the ears. The location of this respondant (A-15) (Table I) differed from the others in having a more easterly practice area, which comprised Prince Edward, Buckingham, and Cumberland Counties. The other respondants were located further west in the Campbell, Bedford, and Amherst areas. Reports from these counties indicated a 1-19% estimate of infestation. An interview with one horse owner from Bedford County revealed heavy damage to 22 horses; two other horse owners from Campbell County reported light to moderate damage to ten horses.

From May through July 1973, control experiments were performed on 20 horses at Deer Run Ranch Camp, Patrick County, Virginia. Black flies were numerous in May, and the horses, which were rented to campers, were extremely head-shy and difficult to handle as a result of the annoyance imposed by the feeding of these flies.

Collections of flies from the ears of these horses were S. jenningsi and S. vittatum. Only S. jenningsi was obtained in two collections in July and August. The flies were numerous during late May but decreased significantly for the remainder of the summer. Simuliids were of no consequence during the 1974 season. Face flies were the most annoying horse pests encountered.

Added observations were obtained from Goochland County in the Piedmont uplands. At Hoof Print Hills Stables, Manakin, Virginia in July 1973, inspection of 29 stabled horses revealed very light to moderate scabbing. One simuliid was seen feeding in the ear of a pastured pony, but was not captured. An instructor there stated that black flies were a problem to the horses and a nuisance to the riders.

At Lithglow Stables, Goochland County in July 1973, four of the nine horses examined displayed moderate scabbing. Light scabbing was noted in the ears of the remaining animals. Three simuliids were seen feeding. According to the attendant, the flies presented no problem and control measures had not been implemented.

During the 1973 survey trip in the upland region of Albemarle County near Keswick, two thoroughbred farms were visited. Stable hands indicated the horses were turned out after dark during the summer, and fly problems were avoided. Six pastured quarter horse mares from a near-by herd were examined and showed light to moderate scabbing. Six Simulium jenningsi females were collected from the animals' ears.

During a 1974 survey trip to the northern section of the state, horse owners in Culpepper, Hanover, and Loudon Counties indicated knowledge of the pest. Two veterinarians (A-35, A-36) (Table I) reported observing the pest, but considered the incidence of the fly to be low.

The heaviest ear damage was reported in the returns originating from the Piedmont lowlands. An equine practicioner (A-3) reported 80-99% of the horses he examined displayed scabbing. The four counties forming the bulk of his practice, Loudon, Fairfax, Faquier, and Prince William, contained 17,778 horses. This observation was confirmed for Prince William County during an interview with a local veterinarian (A-37), who experienced the problem with his own horses. An equine veterinarian serving the same area reported no knowledge of the black fly as a pest in his initial response to the questionnaire. He indicated a 1-19% infestation rate for 1974. Two of five Faquier County horse owners had no knowledge of the problem, saying their horses remained stabled during the day. The remaining three were familiar with the problem. Veterinarian (N-19) did not observe the feeding in 1973, and saw only one case in 1974.

Blue Ridge: Reports from the northern Blue Ridge must be primarily based on the replies of practicioners from the Piedmont uplands whose responsibilities included this area. The response indicated a low level of infestation, 1-19%. The proprietor of a riding establishment in Sperryville, Virginia, acknowledged the problem, but experienced little trouble during the 1974 season. No scabs were observed on the five horses examined.

A low level of feeding damage was reported (A-13) from the southern section of the Blue Ridge. In a conversation with the manager of the Mt. Rogers Stables, Grayson County, Virginia, it was revealed that the flies were a formidible problem during the summer months. Stables had been constructed to provide protection for the horses during daylight hours. Eleven stabled horses examined in early fall exhibited light to moderate scabbing. One specimen of *Simulium jenningsi* was collected.

Ridge and Valley: Seven of the nine affirmative replies from this area came from the Middle Section of the Ridge and Valley Region of Virginia. Returns from this section indicated higher feeding estimates than elsewhere in the state, with the exception of northern Virginia.

Numerous field observations in Montgomery and Pulaski Counties substantiate the report (A-6) of a 60-79% damage rate. Collections of simuliids in these counties (Appendices F and G) yielded only two species: *S. vittatum* and *S. jenningsi*. Underhill (1944) in his collections indicated large numbers of *S. jenningsi* larvae were found in the streams of this region.

All fourteen horse owners interviewed from these two counties were familiar with the pest. The severity varied with the location of the animals. Those near the New River had moderate to heavy damage. In and around Blacksburg and Montgomery County, damage was light to moderate. Five horse owners in Giles County, and four in Roanoke County recognized the problem.

Horses examined in Shenandoah County (September, 1974) exhibited light to very light scabbing. The owner said the flies were no trouble that year, but had been a problem in the past.

A positive response was obtained from the one owner interviewed from Wythe County, though no specific information was given. Field observations of a pony in Smyth County (September 1973) revealed neither flies nor damage.

Return N-20 for 1973 reported no knowledge of the damage or flies. In the follow-up survey, he reported a 1-19% infestation rate. N-20 covered Rockingham County from which positive reports had been received.

Telephone interviews with extension agents in Warren and Clarke Counties (September 1974) indicated that no calls had been received concerning the problem.

Cumberland Mountains: One respondant (A-34) estimated that 40-59% of the horses were bothered by black flies. Black flies were seen flying about the ears of a pony and some Holstein cows at Big Stone Gap, Virginia, July 1974. None were collected. The owner interviewed had show horses, which were stabled except during workouts, so the flies posed no problems. The other reply (N-24) reported no observations in 1973, and a 1-19% feeding estimate for 1974.

B. Black Fly Control

The percent reductions and pretreatment counts of all control studies appear in Appendices H through K.

Experiment A: Two 2% vaseline formulations containing diazinon and malathion, and a 1% stirofos oil formulation were evaluated in the initial experiment of the series, from the 2nd to the 8th of July. Vaseline® was the standard of comparison for these trials.

The two percent malathion and diazinon in vaseline formulations performed comparably to the Vaseline® standard (Figs. 3a-3b). Each material provided adequate protection for two days. Thereafter, effectiveness decreased abruptly.

The 1% stirofos oil formulation protected the ears for a day, but was unsatisfactory beyond that time. Vaseline®, however, provided acceptable relief through the second day (Fig. 3c).

The 3% stirofos dust was the easiest material to apply. A powder-puff technique made it possible to cover the ear surface quickly. A shaker jar was tried, but I was able to dispense only small amounts of dust into the ear.

I observed that black flies swarmed around the ears and attempted to land immediately after treatment. Oil and vaseline caused the flies to drop off the ear. As each material disappeared, the flies were able to feed. Feeding began in the first spots to become free of treatment.

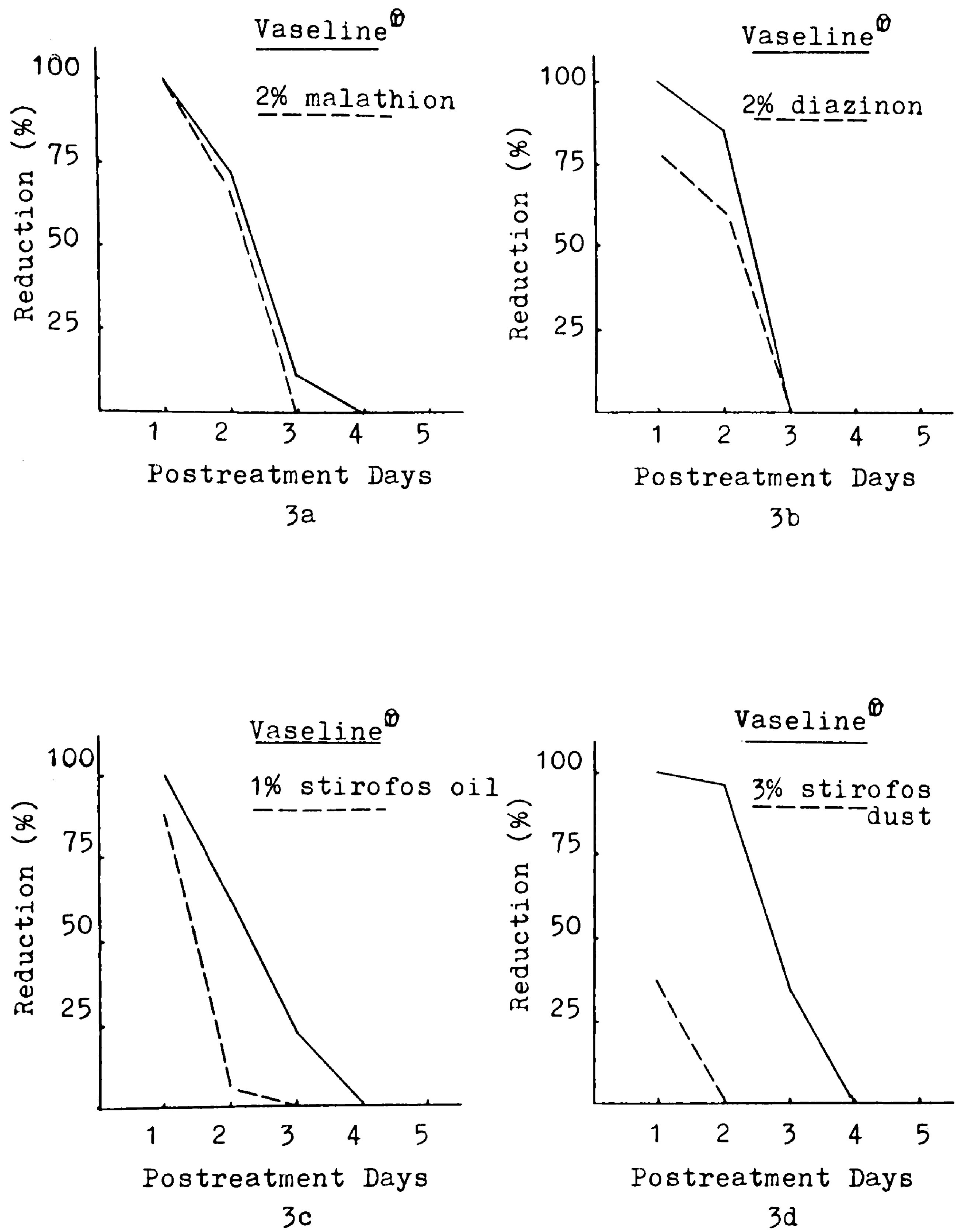
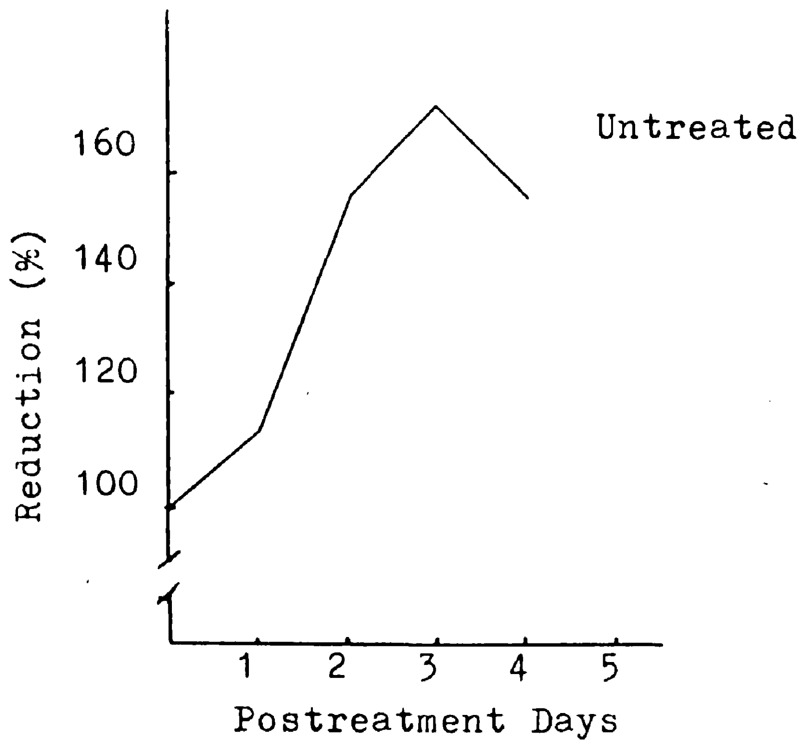


Figure 3. Experiment A Postreatment reduction of black flies based on pretreatment averages.



3e

Figure 3 (cont.) Experiment A Postreatment reduction of black flies based on pretreatment averages.

Average fly counts in the ears of control animals increased during the experimental period. This indicated that the protective qualities of the materials were subjected to rigorous reinfestation pressure (Fig. 3e).

<u>Experiment B</u>: Two percent vaseline formulations of malathion and stirofos, and the Vaseline® standard were evaluated in this experiment, conducted from the 15th to the 19th of July 1974. The results from the Vaseline® and the malathion formulation were similar to those in Experiment A (Fig. 4a). However, the protection was adequate for only a day. The 2% stirofos provided much longer relief from black flies than any formulation previously examined. Fly numbers were still decreased by 55% on the fourth postreatment day (Fig. 4b). Other materials reach this level usually after two days of exposure. Petroleum gel with insecticide had lower viscosity than Vaseline® and the extended protection was due to this quality rather than any benefit derived from the insecticide.

Fly counts on control animals appear as a percent of pretreatment counts (Fig. 4c). Though fewer flies were present on day 2, a great increase was observed during the remaining days of the test.

<u>Experiment C</u>: Swish® (Fig. 5b) and the 1% stirofos oil formulation (Fig. 5a) performed well for one day. However, by the second day neither of them were protecting

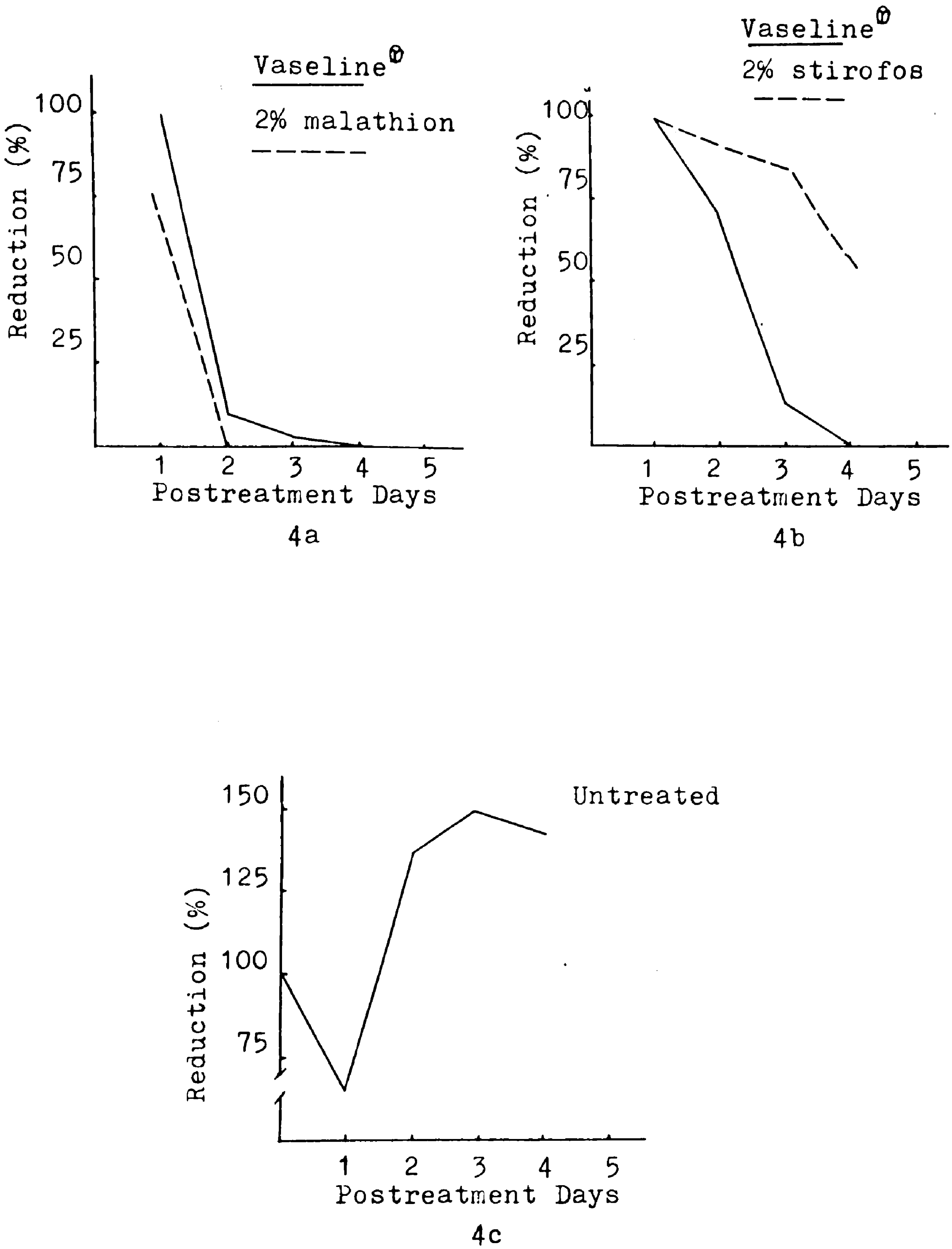
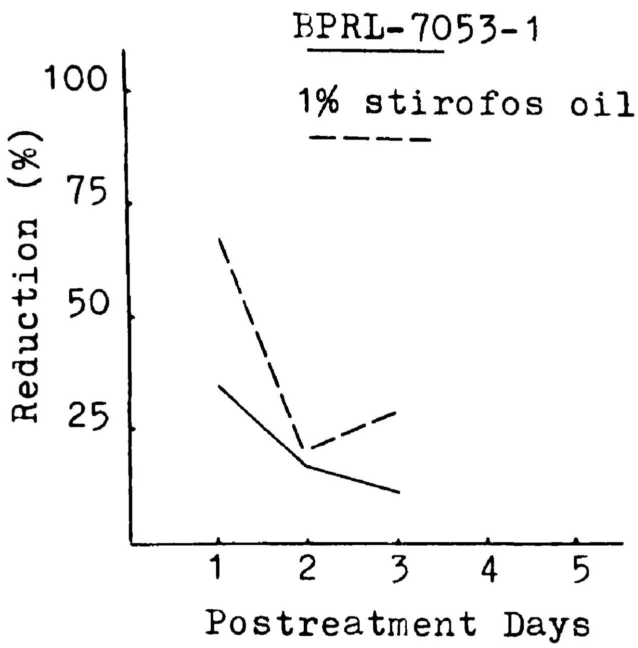
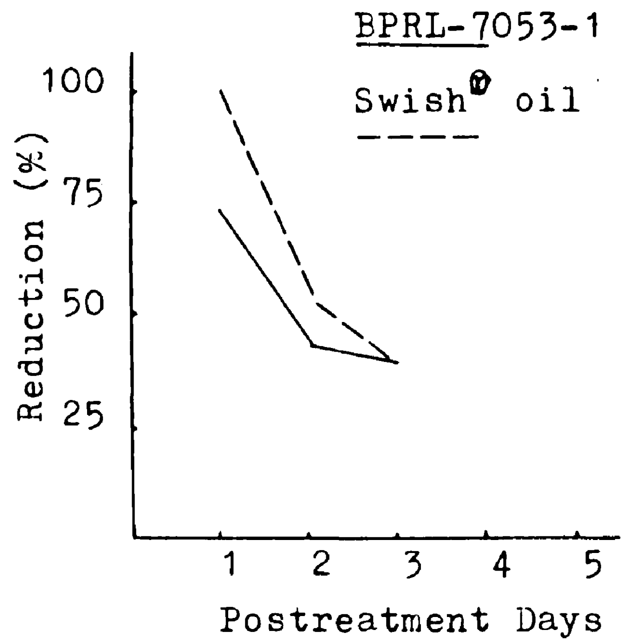


Figure 4. Experiment B Postreatment reduction of black flies based on pretreatment averages.



5a



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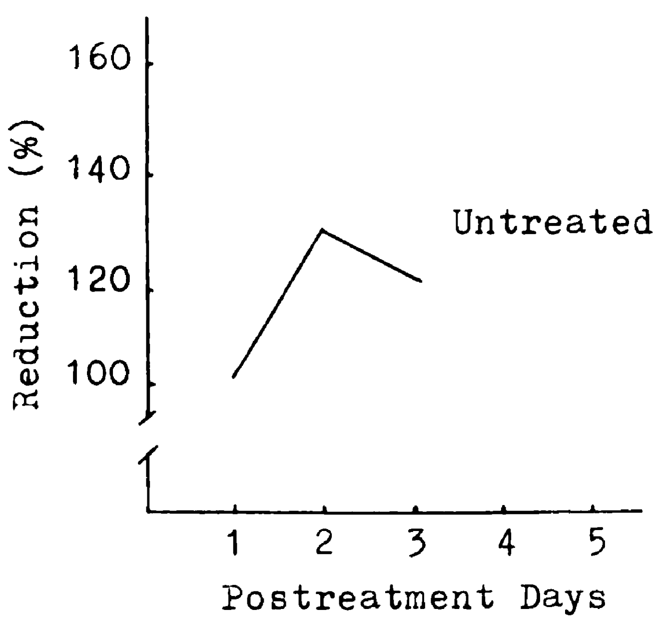

5c

Figure 5. Experiment C Postreatment reduction of black flies based on pretreatment averages.

the ears from black flies. The BPRL-7053-1 provided some protection, but was not effective beyond the first day. Fly counts on check animals (Fig. 5c) dropped on the first postreatment day and increased thereafter. This may have obscured the actual protective value of the oils, as a decrease in numbers of flies rather than protection may have been indicated.

All oils appeared to dry out the skin in the ears and in most cases there was an exfoliation of the skin in the ears. Oil formulations are more suitable than vaseline however, for protection of areas such as the ventral midline of the horses' abdomen. Applications must be made frequently. The 1% stirofos oil applied to the sides of animals brought about immediate and complete disappearance of horn flies, Haematobia irritans (Linnaeus), which were a pest at one location.

Experiment D: The previous experiments indicated insecticides at the 2% dosage did not enhance the protective action of Vaseline®. Malathion, crotoxyphos, and methoxychlor were selected for trial at increased dosages of 5%, 5%, and 10% respectively. Two trials were conducted from the 30th of July to the 3rd of August, and from the 7th to the 14th of August 1974. Performance of the three insecticide formulations closely paralled that of the Vaseline standard (Figs 6a-6c).

As in the previous trials, the amount of material

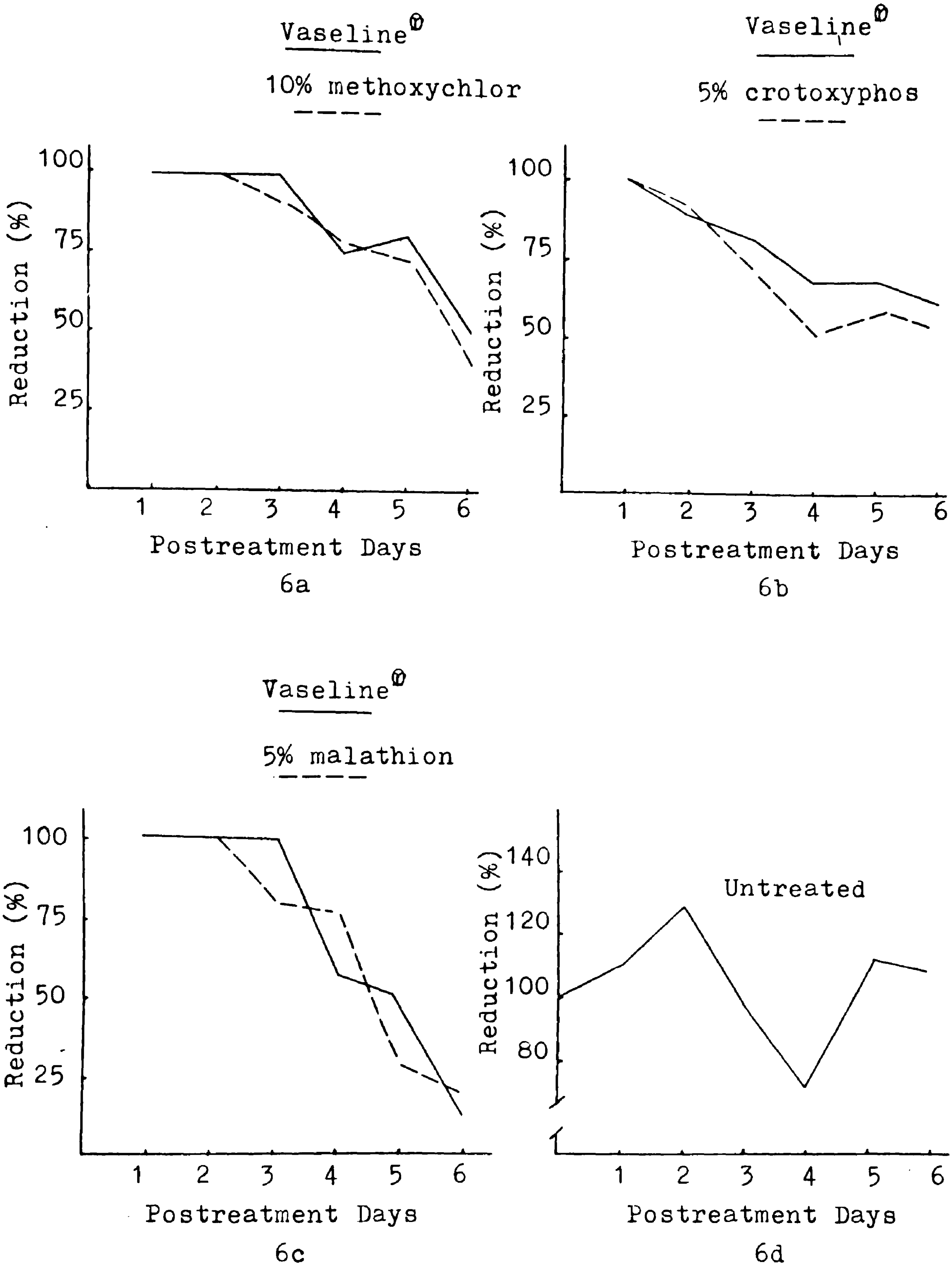

Figure 6. Experiment D Postreatment reduction of black flies based on pretreatment averages.

applied during the first week of experiment D was approximately 3 gm per ear. For the second week of experiment D, the materials were applied at the rate of 5 gm per ear.

Fly counts in ears treated with insecticide formulations or the Vaseline® standard were compared for the two weeks using the nested ANOVA test (Sokal and Rohlf 1969). The three categories compared were between postreatment days, between insecticide and Vaseline® standard, and between the 3 gm and 5 gm treatment rates. The F-values and significance levels appear in Table III.

There was a significant difference between fly populations in the ears during postreatment days. Flies began to feed in increasing numbers as the protective material disappeared. There was no significant difference in the reduction of black flies as a result of augmenting Vaseline® with the insecticides evaluated. Only a 50%-75% chance of significance was indicated by increasing the dosage applied from 3 gm to 5 gm per ear.

C. Feeding and Attraction

Carbon Dioxide Baited Traps: *Simulium jenningsi* and *S. vittatum* were the only species collected during three days of trapping. Flies were taken only when carbon dioxide was placed in a trap (Table IV). No animals were in the pasture, although cattle were present one-half kilometer away.

The pyramid configuration of the trap was not

Table III. An analysis of mean black fly counts from the ears of horses receiving vaseline base insecticide formulations and ears receiving the Vaseline® standard using 3 gm/ear and 5 gm/ear dosages.

Group	F-Value	Significance Level
Between Postreatment Days	2.252	0.025>F>0.05
Between Insecticide Formulations and Vaseline® Standard	0.1474 ns*	F<0.75
Between 3 gm/ear and 5 gm/ear dosages	1.06 ns*	0.25>F>0.5

Table IV. Catches of *Simulium jenningsi* and *S. vittatum* in baited and unbaited canopy traps during attraction experiments in Montgomery County, Virginia, 1974

Day	Trap	Species: *S. jenningsi*	Species: *S. vittatum*
		Baited	
1	A	104	11
2	B	187	12
3	B	58	7
		Unbaited	
1	B	0	0
2	A	0	0
3	A	0	0

especially suited for collecting simuliids. Many individuals were caught in the creases at the four corners of the trap and were difficult to retrieve. In all collections, several specimens had to be removed from the creases with an aspirator and were often damaged. A conical canopy would be more suitable for directing the flies up to the collecting container.

The largest concentration of flies was located in a bag hung under the canopy to hold dry ice. As the flies crawled up the canopy, many fell into the bag and were frozen. It is probable that many individuals missed the bag and fell to the ground. Only a portion of the flies collected had entered the killing jar at the top of the trap.

Weather conditions during the experiment were not closely monitered, though they were well within the activity parameters elucidated for S. jenningsi by Underhill (1940).

Feeding Damage Study: Analysis of black fly counts taken from the ears of 113 horses indicated no statistical difference in the mean number of flies in the right (7.1) and left (6.6) ears at the 99% confidence level. This information was considered important for control experiments and allowed me to treat each horse as a replicate.

Mean fly counts were calculated for five damage assessment categories where scabbing occurred (Table V).

Table V. Mean black fly counts associated with damage assessment categories.

Assessment Category	Number Ears Examined	Mean No.* Flies/Ear	Standard Error
	Using Five Categories		
Very Light (VL)	11	1.8a	0.87
Light (L)	30	4.5a	2.19
Medium (M)	56	12.7ab	5.16
Medium Heavy (MH)	26	20.3 c	4.86
Heavy (H)	50	22.7 c	8.0
	Using Three Categories		
VL+ L	41	3.8a	2.3
M	56	12.7 b	5.16
MH+ H	76	21.9 c	7.2

*/ Means indicated with the same subscript are not significantly different at the 95% confidence level.

Data from ears in the very light or light damage categories were combined, because no significant difference in the means was indicated at the 95% confidence level. More salient was the low level of ear scabbing in this group (Table VI). The same procedure was applied to the medium and medium heavy damage groups. The means were not shown to be significantly different, but the intensity of scabbing in both categories was sufficient to warrant prophylactic measures. Data from the medium group did not warrant further treatment and the category remained intact. The resulting categories of Light, Medium, and Heavy were a more tenable system than the previous five category arrangement.

The presence of eight or more flies per ear is proposed as a treatment threshold. Medium intensity scabbing was noted at and above this number.

Attempts at a treatment criterion based on the percentage of scabbing appear to be too subjective. From projected slides the percentage of scabbed area was estimated by the author and three others. I then determined the percentage of infested area by projecting the slides onto graph paper and drawing the outline of the ears and scabs. All estimates were consistently higher than the measured percentages of scabbed areas.

Determination of Blood Meal Volume: The weights of lots of 10 engorged and unengorged *Simulium vittatum*

Table VI. Surface area of scabbing associated with 35 mm color slides of damage assessment categories.

Assessment Categories	Number of Ears Examined	Average Area of Scabbing	Range
Light	6	4.3%	2.0%-7.0%
Medium	3	12.0%	7.6%-15.7%
Heavy	3	23.0%	18.5%-26.2%

appear in Table VII. There was a difference of 2.48 mg between the two groups, which was attributed to the weight of blood consumed by engorged flies.

The variance associated with mean blood meals was determined by analyzing data using the $\bar{d}$ test for comparison of paired observations (Sokal and Rohlf 1969). Mean fly weights from Table VII were written on pieces of paper. Engorged weights were placed in one box, unengorged weights in another. A weight was randomly drawn from each box, the difference calculated, and the papers returned to their respective boxes. These differences appear in Appendix L. This procedure was repeated to obtain 20 differences. Prior calculations based on variances of the weights of the two groups indicated the mean blood meal could be determined at the 95% confidence level with as few as seven differences.

The mean blood meal weight determined using these steps was 2.46 ± 0.33 mg. This mean is in close agreement with the previous value, but has the advantage of allowing calculation of the 95% confidence interval, 2.30- 2.62 mg.

The weight ratio of engorged to unengorged females was 2.55. Woodward and Chapman (1965) measuring blood meal volumes of female mosquitoes found that weight doubled after feeding. Foulk (1967) found the ratio for weights of engorged female Leptoconops kerteszi (Kieffer) to be approximately 2.5 times that of unengorged females.

Table VII. Weights of engorged and unengorged female *Simulium vittatum*.

Flies/Lot	Mean Weight (mg)
Engorged Females	
10	4.46
10	4.24
10	4.13
10	3.79
10	4.70
8	3.88
10	3.75
10	3.89
10	3.67
10	4.31
$\bar{X}= 4.08 \pm 0.35$	
Unengorged Females	
10	1.42
10	1.55
10	1.67
10	1.73
10	1.65
10	1.56
$\bar{X}= 1.59 \pm 0.11$	

Study of Feeding Duration: Feeding observations were made on July 22nd 1974 between 5:30 am and 10:30 am. Length of feeding periods appear in Table VIII. Average feeding duration for female *Simulium vittatum*, based on eight observations, was 10.9 minutes. One marked fly remained in the ear of a chestnut gelding for almost an hour. This fly appeared to be a female *S. jenningsi*.

During the first two hours of observation, the temperature rose from 53° to 57° F. Humidity was high as evidenced by fog.

The horses were lying on the ground at 5:30 am, and the ears displayed neither black flies nor fresh feeding wounds. The first simuliid appeared at a light intensity of approximately 30 foot-candles (f-c) at 6:25 am. As the light intensity increased, more flies appeared. At 840 f-c, seven flies were present; and 37 flies were feeding by 8:30. Light intensity was approximately 2700 f-c at that time.

Both *S. vittatum* and *S. jenningsi* were present; the latter in lesser numbers. Feeding of *S. vittatum* was confined to the ears, with infrequent exception. *S. jenningsi* fed in the ears, ventral midline of the abdomen, the prepuce, and inner sides of the legs. Once settled, both species were difficult to dislodge by prodding. Flies with distended abdomens left more readily.

Although a few engorged *S. vittatum* fell to the

Table VIII. Duration of feeding times for marked female *Simulium vittatum* on a pastured pony in Pulaski County, Virginia.

Observation	Time (minutes)
1	11.5
2	8.0
3	13.0
4	10.5
5	6.0
6	16.0
7	12.5
8	10.0

ground as they climbed out on the ear hairs, most flew away with no apparent difficulty. Marked flies exhibited no adverse effects due to the marking process, and all flew away upon repletion.

Blood Meal Analysis for EIA Antibodies: Precipitate lines formed after 48 hours are depicted in Fig. 7. Lines between the antiserum (S^+) and antigen (A) (1) demonstrated the kit materials were reacting satisfactorily. No precipitate formed between wells containing macerated fly abdomens (F^+, F^-) and negative serum (S^-). This indicated no reaction between the fly protein and the horse protein that could mask the antigen-antibody interaction.

A weaker precipitate line (2) was visible between the antigen well and well containing the abdomens of flies which had fed on the uninfected horse. Formation of this line indicated that EIA antibodies were present in the fly abdomen after the blood meal. The weak line was due to a low concentration of antibody. Use of more abdomens in a well should give a stronger response.

No precipitate formed between the antigen well and well containing abdomens of flies which had fed on the uninfected horse.

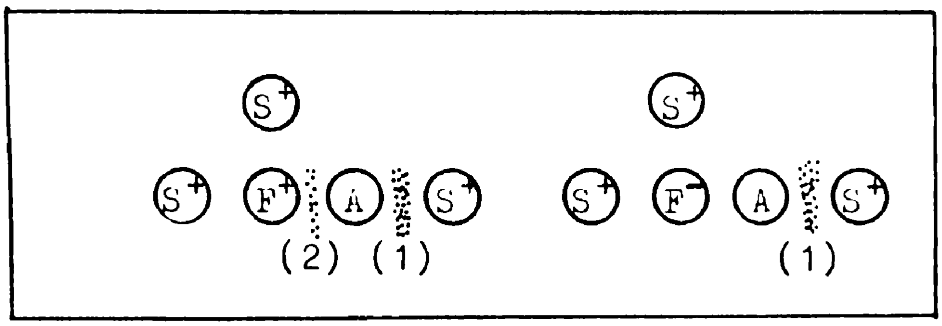

Figure 7. Precipitate lines formed after 48 hour incubation of EIA microimmunodiffusion test. (1) demonstrates reaction of antiserum and antigen; (2) demonstrates presence of EIA antibodies in abdomen of female S. *vittatum* engorged on a diseased horse.

V. CONCLUSIONS

A. Black Fly Survey

1. *Simulium vittatum* and *S.* (*Phosterodorus*) probably *jenningsi* were the only species of simuliids collected from horses during 1973 and 1974.

2. Questionnaire returns and survey trips indicated scattered instances of light to moderate feeding damage to Virginia horses caused by these flies. The most notable exceptions were in the northern and southwest regions of the State. Horses pastured near rivers in these areas were subjected to considerable annoyance as a result of large black fly populations.

3. Black flies were observed feeding on horses in January and March 1973. Responses indicated that the greatest problem period occurred during the summer months. Damage was often restricted to specific localities in an area. Owners and veterinarians indicated the intensity of black fly attacks may vary considerably from year to year.

4. Horse owners generally treat the condition themselves and seldom attempt to obtain professional assistance.

B. Black Fly Control

1. Vaseline® petroleum jelly provides adequate control against species such as *Simulium vittatum*, that feed in the ears of horses. Vaseline® also aids the healing of heavily scabbed ears by keeping the surface moist. Applied at the rate of 5 gm per ear, black flies were prevented from feeding for periods of four days in localities where severe damage was occurring.

2. Vaseline® supplemented with the insecticides tested did not provide increased effectiveness against the pests. Once the vaseline had disappeared from the ear, the flies returned and commenced feeding.

3. Oil and commercial fly wipes provided protection only for approximately 24 hours. In addition to being short-lived, they caused the skin in the ears to dry out. This did not enhance healing of the scabs. These formulations, however, were satisfactory for protecting the ventral midline of the abdomen from attack.

4. The dust formulation evaluated afforded no apparent protection.

5. Both the oil and vaseline appear to function as a physical barrier, which prevents the fly from remaining in the ear and feeding after it lands.

C. Feeding and Attraction

1. Experiments with canopy-type tabanid traps

demonstrated that carbon dioxide was an effective attractant for female _Simulium jenningsi_ and _S. vittatum_.

2. No preference was demonstrated by black flies between different ears on the same horse.

3. The presence of eight or more flies per ear produced sufficient scabbing to warrant prophylactic measures.

4. The blood meal weight taken by female _S. vittatum_ was determined to be 2.46 ± 0.33 mg, or approximately 2.6 ± 0.34 mm^3.

The average tome required for engorgement of female _S. vittatum_ was 10.9 minutes.

6. EIA antibodies may be detected in the blood meals of female _S. vittatum_, which have fed on an infected horse, by the use of Coggins test reagents and a micro-immunodiffusion technique. This may be a useful tool in studying potential vector relationships between the disease and various biting flies.

VI. SUMMARY

A survey of Virginia veterinarians and horse owners during 1973 and 1974 revealed black flies (Diptera: Simuliidae) to be a significant pest of horses in the northern counties of Fairfax, Faquier, Loudon, and Prince William and Pulaski, Montgomery, and Wythe counties in the southwest region of the state. The feeding activity of these flies caused annoyance to horses, making them head-shy and difficult to handle.

Simulium (*Phosterodoros*) prob. *jenningsi* Malloch and *S. vittatum* Zetterstedt were the only species encountered during field collecting. Questionnaire responses indicated that the greatest feeding damage occurred during the summer months. Black flies were often restricted to specific localities, primarily near rivers.

Simulium vittatum, an ear feeding species, was the predominant pest of horses in Pulaski County. Various measures were studied to find a suitable ear protectant. Vaseline® petroleum jelly, applied at the rate of 5 gm per ear, provided adequate protection for periods of 3-4 days. The materials also aided in the healing of heavily scabbed ears.

The petroleum jelly was supplemented with diazinon and malathion at 2% weight-dosages. Malathion, crotoxyphos, and methoxychlor were evaluated at 5%, 5%, and 10% weight levels respectively. The addition of these materials did not enhance the protective value of the vaseline base. Once the gel had disappeared from the ear, the flies returned and commenced feeding.

Oils and commercially prepared fly wipes provided protection for only a day. In addition to being short-lived, they caused the ears to dry out and did not aid in healing the scabs. These were satisfactory for protecting the ventral midline of the horses' abdomen from black fly attack. A 3% stirofos dust formulation afforded no protection. A commercial 2% stirofos formulation in petroleum jelly was superior to Vaseline®. The lower viscosity of the gel was the reason for its effectiveness rather than the insecticide.

Both the oil and Vaseline® appeared to function as a physical barrier, which prevented the flies from remaining in the ear and feeding. Black flies attempted to land in the ears immediately after treatment.

Experiments with canopy tabanid traps baited with dry ice demonstrated that carbon dioxide was an effective stimulus for attracting female *Simulium jenningsi* and *S. vittatum*. In host studies, no preference was demonstrated by black flies between different ears on the same horse. The presence of eight or more flies per ear was accompanied by sufficient scabbing to warrant prophylactic measures.

Mean time required for engorgement of female *Simulium vittatum* was 10.9 minutes. The blood meal taken by this species was 2.46 ± 0.33 mg, or 2.6 ± 0.34 mm^3.

Antibodies for equine infectious anemia were detected in the blood meals of this species, after they had fed on an infected horse, using Coggins test reagents an a microimmunodiffusion technique. This may be a useful tool in studying vector relationships between the disease and various biting flies.

VII. LITERATURE CITED

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VIII. APPENDICES

Appendix A. Black fly questionnaire packet distributed to Virginia veterinarians in March 1973.

Dear Sir:

Black fly damage to livestock, particularly horses, has become a serious problem in some areas of Virginia. Consequently, entomologists at VPI& SU have begun a study of the black flies and their damage associated with livestock.

The purpose of the attached questionnaire is to determine the geographical distribution, severity, and economic aspects of black fly damage as an initial step in the solution of this important problem. In addition, information on current control practices and their success is desired. To assist you is a picture and description of the fly and its damage for reference.

It is important that we have a high percentage of responses to the accompanying questionnaire. Won't you take 6 minutes to complete it and return it to:

Lee H. Townsend, Jr.
Department of Entomology
Virginia Polytechnic Institute
and State University
Blacksburg, Virginia 24061

Your interest and help will be sincerely appreciated.

Remember, it is also important that we receive information from uninfested areas so that we may define areas of positive infestation.

Sincerely,

Dr. Seymour L. Kalison
Professor of
Veterinary Science
VPI & SU

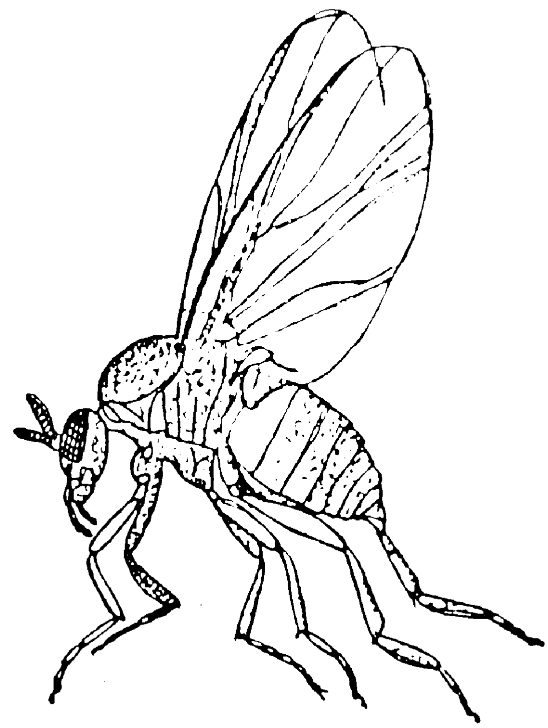
Dr. William A. Allen
Extension Specialist,
Insect Survey
Department of
Entomology
VPI & SU

Dr. E. C. Turner, Jr.
Professor of Entomology
VPI & SU

Mr. Lee H. Townsend, Jr.
Research Assistant
Department of
Entomology

ADULT

The black fly (Diptera: Simuliidae is a small, black, two-winged insect, about one-eighth inch in length. It has short, thick, horn-like antennae, and a robust hump-backed body, giving rise to the common name "buffalo gnat". The wings are broad and short with the veins near the front being much thicker than those behind.



BITE

The bite of the fly is painless at the time. The saliva is believed to be anesthetic, and to cause increased blood flow possibly by dilating the capillaries. Withdrawal of the beak is followed by bleeding which may be profuse. Bleeding may also occur beneath the skin. Many scabs are formed and are often obvious inside the ear of the animal. The bite usually becomes a source of irritation that may last for days or weeks.

Metcalf, C. L. and W. E. Sanderson. 1931. Black flies, mosquitoes, and punkies of the Adirondacks. N. Y. State Museum. CIVC. 5.

March 1973

BLACK FLY QUESTIONNAIRE

1) Name________________________________ Age_______
Address________________________________
____
Occupation________________________________

2) List the counties you serve.

____________________ ____________________
____________________ ____________________
____________________ ____________________

3) How long have you been serving your present area? (Check one)

___ 1-5 years ___6-10 years ___11-15 years ___over 15

READ THE ACCOMPANYING DESCRIPTION OF THE BLACK FLY AND ITS DAMAGE BEFORE CONTINUING

---

4) What observations concerning black flies have you made (Check one)

___ damage on the animal, but no black flies feeding.
___ black flies feeding and also some damage.
___ neither flies nor damage.

5) Damage or feeding was observed on: (Check all applicable)

__horses __turkeys __other animals.. Please list
__cattle __chickens ____________________
____

__ none of the above

6) Observations on feeding and/or irritation were:

__made upon direct request of the owner to treat damage.
__noted in general inspection while treating some other condition.
__noted in general inspection of healthy animal.

7) In cases of black flies feeding on horses, the horses were: (Check one)

__never stabled.
__occasionally stabled for short periods of time.
__stabled daily for regular periods of time.
__stabling habits not known.

8) Of the horses you observed last year, what percent did you notice showed signs of feeding by black flies? (Check one)

__zero __1-19% __20-39% __40-59% __60-79% __80-99% __100% __unable to estimate

9) First signs of infestation appeared in: (Check one)

__Jan __Feb __Mar __Apr __May __Jun __Jul __Aug __Sep __Oct __Nov __Dec

10) Peak damage occurred: (Check one)

__once yearly __twice yearly __three times yearly
__more than three times.

11) If several peak periods of damage occurred, check each month in which heavy feeding occurred.

__Jan __Feb __Mar __Apr __May __Jun __Jul __Aug __Sep __Oct __Nov __Dec

12) These periods of peak damage have been noted: (Check one and fill in the appropriate blank)

__yearly for the past ___years. ___ never noted
__only occasionally for the past___years.

13) Was black fly damage confined to the ears? (Check one)

__yes __if not, indicate other areas____________________
____

14) How would you rate black fly damage in your area? (Check cases and damage)

__many cases
- __heavy damage many scabs in ear
- __moderate damage few scabs in ear
- __slight damage scabs scarce

__few cases
- __heavy damage many scabs in ear
- __moderate damage few scabs in ear
- __slight damage scabs scarce

15) Have you collected and retained any black fly specimens? (Check one)

__yes __no

16) If provided with prepaid mailing tubes and vials of alcohol, would you be willing to send black fly specimens to VPI & SU? (Check one)

__yes __no

17) Do you have any case records concerning black fly problems which you would make available for study (Check one)

__yes __no

18) What success have you had in these treatments? (Check one)

__none __very little __moderate __good __excellent

19) What treatment did you recommend? (Please indicate)____________________________________

____

20) What, if any diseases have you noticed in animals you have treated having black fly damage? (Please indicate)

____

____

21) In order that we may accurately place an economic value on this insect pest, what is your average total charge for one animal? This information is extermely valuable and will be kept strictly <u>confidential</u>.

$______

Appendix B. Black fly questionnaire packet distributed to Virginia veterinarians in March 1974.

Dear Sir:

Investigations concerning black fly damage to horses are continuing at VPI & SU. Information obtained from the questionnaires returned by Virginia veterinarians in 1973 has contributed significantly to the project. To supplement this data, an abbreviated follow-up questionnaire is enclosed. We would appreciate you completing thie form primarily in view of observations from March 1973 to March 1974. To assist you is a picture and description of the fly and its damage for reference. Please return the questionnaire to:

Lee H. Townsend, Jr.
Department of Entomology
Virginia Polytechnic Institute
and State University
Blacksburg, Virginia 24061

Your interest and cooperation is sincerely appreciated. Information from uninfested areas is important in order to define areas of positive infestation.

March 1974

BLACK FLY QUESTIONNAIRE

1) Name________________________
Address____________________
____

2) List the counties you serve. ________ ________
________ ________ ________ ________ ________

3) Estimate the number of horses you encounter in your practice_______.

READ THE ACCOMPANYING DESCRIPTION BEFORE CONTINUING

4) Of the horses you observed last year what percent did you notice showed signs of feeding by black flies? (Check one)

___zero
___1-19%
__20-39%
__40-59%
__60-79%
__80-99%
__100%
__unable to estimate

5) Check months during which the black flies were a problem. (Check all applicable)

__Jan
__Feb
__Mar
__Apr
__May
__Jun
__Jul
__Aug
__Sep
__Oct
__Nov
__Dec

6) How would you rate black fly damage in your area? (Check cases and damage)

__many cases
- __heavy damage many scabs in ear
- __moderate damage few scabs in ear
- __slight damage scabs scarce

__few cases
- __heavy damage many scabs in ear
- __moderate damage few scabs in ear
- __slight damage scabs scarce

7) Indicate treatment prevention techniques you recommended and their relative success (good, fair, poor).

8) Approximately how many calls did you receive regarding this problem this year?__________

9) Comments (If any)

THANK YOU FOR YOUR COOPERATION

Appendix C. Black fly questionnaire used to interview Virginia horse owners.

March 1974

HORSE OWNERS
QUESTIONNAIRE

Name________________
County______________

1) How many horses do you own or board that are kept outside most or all of the time?________

2) Have you noticed ear damage of flies like that shown on the slides? ___yes ___no

3) How long have you noticed this problem?_______years

4) During which months do you notice the damage or flies? (Please list)

5) How would you rate the damage to your horses? (Please check)

___ OVER 50% OF HORSES
__heavy
__moderate
__light

___ LESS THAN 50% OF HORSES
__heavy
__moderate
__light

6) List remedies you have tried and their success as to good, fair, poor.

7) Estimate your expenditures per horse for treating this condition including vet fee, salves, etc. $_________

8) How much do you feel it is worth to you to prevent these flies from feeding on your horse?
$_________ per horse

Appendix D. Black fly feeding estimates of Virginia horses by county, as estimated by veterinarians responding to 1973 and 1974 questionnaires.

County	No. Horses	Estimated % Horses Affected	Vet. Code Affirm.	Vet. Code Negative
Alleghany	709	20-39	A-8	
Albemarle	3115	0		N-26
Amherst	1106	1-19	A-10	
Appomattox	410	0		N-19
Augusta	2878	1-19	A-1	
		Many	A-11	
		None		N-23
Bath	495	1-19	A-14	
Bedford	907	1-19	A-4	
		1-19	A-10	
		1-19	A-12	
Bland	482	60-79	A-6	
		20-39	A-5	
		None		N-18
Botetort	1349	1-19	A-4	
		1-19	A-12	
		20-39	A-8	
		None		N-17
Buchanan	979	20-39	A-5	
Buckingham	791	20-39	A-15	
		None		N-19
Campbell	1706	1-19	A-10	
		1-19	A-4	
		1-19	A-12	
		None		N-19
Caroline	410	40-59	A-9	

Appeddix D. (cont.) Black fly feeding estimates of Virginia horses by county as estimated by veterinarians responding to 1973 and 1974 questionnaires.

County	No. Horses	Estimated % Horses Affected	Vet. Code Affirm.	Vet. Code Negative
Carroll	709	1-19	A-13	
Charolette	762	None		N-19
Clarke	1530	None		N-27
Craig	343	20-39	A-8	
Culpepper	1217	None		N-21
Cumberland	569	20-39	A-15	
Essex	179	40-59	A-9	
Fairfax	4949	80-99	A-3	
		Positive	A-36	
		None		N-25
		None		N-28
Faquier	6348	80-99	A-3	
		Positive	A-16	
		None		N-27
Floyd	1228	1-19	A-13	
Frederick	1647	None		N-22
Giles	833	60-79	A-6	
Gloucester	490	20-39	A-9	
Grayson	1779	1-19	A-13	
		None		N-18
Greene	399	None		N-21
Highland	306	1-19	A-1	
		1-19	A-14	
King & Queen	195	40-59	A-9	
King William	199	40-59	A-9	
Lee	1905	40-59	A-34	

Appendix D. (cont.) Black fly feeding estimates of Virginia horses by county as estimated by veterinarians responding to 1973 and 1974 questionnaires.

County	No. Horses	Estimated % Horses Affected	Vet. Code Affirm.	Vet. Code Negative
Loudon	4147	1-19	A-7	
		None		N-25
		80-99	A-3	
		Positive	A-16	
		Positive	A-35	
		Positive	A-36	
Louisa	1164	None		N-21
Madison	675	None		N-21
Mathews	203	20-39	A-9	
Middlesex	174	40-59	A-9	
Montgomery	1838	60-79	A-6	
Orange	926	None		N-21
Page	801	1-19	A-2	
Prince Edward	510	20-39	A-15	
		None		N-19
Prince William	2334	80-99	A-3	
		None		N-28
		Positive	A-37	
Pulaski	969	60-79	A-6	
Richmond	200	40-59	A-9	
Rockbridge	1424	1-19	A-1	
		None		N-23
Rockingham	2745	1-19	A-1	
		None		N-20
		None		N-23
		Many cases	A-11	

Appendix D. (cont.) Black fly feeding estimates of Virginia horses by county as estimated by veterinarians responding to 1973 and 1974 questionnaires,

County	No. Horses	Estimated % Horses Affected	Vet. Code Affirm.	Vet. Code Negative
Russell	3014	20-39	A-5	
Smyth	2158	20-39	A-5	
Spotsylvania	1097	None		N-21
Tazewell	2772	20-39	A-5	
Warren	909	1-19	A-2	
Washington	2716	None		N-24
Wise	924	40-59	A-34	
Wythe	1444	60-79	A-6	

Appendix E. Identification of returns cited from 1973-74 surveys of Virginia veterinarians.

Code	Name	Location
A-1	D. B. Cromer	Churchville
A-2	E. A. Martin	Luray
A-3	R. S. Mouser	Clifton
A-4	S. C. Gardner	Bedford
A-5	T. R. Barnett, Jr.	Tazewell
A-6	D. C. Stanley	Dublin
A-7	N. A. Laing	Purcellville
A-8	J. H. Burr	Covington
A-9	Dillman-Wilkins	Tappahannock
A-10	Forest Hill Hosp.	Lynchburg
A-11	G. F. Foley	Harrisonburg
A-12	D. L. Gardner	Bedford
A-13	H. C. Epperly	Hillsville
A-14	P. M. Givens	Hot Springs
A-15	J. B. Gates, Jr.	Farmville
A-16	R. L. Booth	Middleburg
N-17	J. Q. Adams	Buchanan
N-18	J. Tate	Wytheville
N-19	E. C. Tutwiler	Appomattox
N-20	W. P. Schwobel	Harrisonburg
N-21	R. F. Estes	Orange
N-22	R. L. Fish	Winchester
N-23	S. J. Simon	Verona
A-24	W. D. Fletcher	Bristol
N-25	H. Howard	Leesburg
N-26	J. J. Huckle	Charlottesville
N-27	E. J. Myer	Boyce
N-28	R. H. Grothaus	Springfield
N-29	W. B. Allison, Jr.	Mt. Sidney
N-30	J. I. Gray	Richmond
A-31	B. H. Watson	Petersburg
A-32	E. A. Schoen	Virginia Beach
A-33	W. R. Gaines	Warrenton
A-34	B. S. Riggs	Big Stone Gap
A-35	Rokus	Middleburg
A-36	Howard	Leesburg
A-37	Prince William Hospital	Prince William County

Appendix F. Collection dates and county records for female black flies taken during survey work. *Simulium vittatum* Zetterstedt

County	Collection Date	Number of specimens
Montgomery	May 7, 1973	4
	May 25, 1973	10
	June 12, 1973	7
	July 9, 1973	8
	August 11, 1973	3
	August 13, 1973	4
	August 20, 1973	2
	August 31, 1973	1
	March 28, 1974	7
	June 19, 1974	12
Patrick	May 25, 1973	1
	June 22, 1973	4
Pulaski	May 26, 1970	11
	May 3, 1972	1
	June 12, 1972	8
	July 10, 1973	24
	August 29, 1973	19
	August 31, 1973	9
	January 29, 1974	8
	February 14, 1974	7
	June 12, 1974	24
	July 16, 1974	16
	July 18, 1974	4

Appendix G. Collection dates and county records for female black flies taken during survey work. Simulium jenningsi Malloch

County	Collection Date	Number of specimens
Albemarle	July 19, 1973	6
Grayson	September 7, 1973	1
Montgomery	May 25, 1973	2
	July 3, 1973	3
	July 9, 1973	1
	July 24, 1973	5
	August 9, 1973	1
	August 11, 1973	1
	August 13, 1973	6
	August 20, 1973	1
	August 27, 1973	11
	August 31, 1973	31
	June 19, 1974	49
Patrick	May 25, 1973	1
	June 22, 1973	1
	July 27, 1973	11
	August 24, 1973	10
Pulaski	July 10, 1973	10
	August 29, 1973	1
	September 11, 1973	8
	July 16, 1974	1
	July 18, 1974	4

Appendix H. Pretreatment averages and postreatment percent reductions, black fly control Experiment A.

Material	Pretreatment Average*	+1	+2	+3	+4
		Percent Reduction			
2% malathion	12.3	100	67	0	0
Vaseline®		100	72	8	0
3% stirofos dust	17.6	37	0	0	0
Vaseline®		100	97	35	0
1% stirofos wipe	21.3	88	5	0	0
Vaseline®		100	72	21	0
2% diazinon	14.3	79	62	0	0
Vaseline®		100	83	0	0
Untreated	10.0	+18	+70	+90	+70

*/Based on 4 horses.

Appendix I. Pretreatment averages and postrearment percent reductions, black fly control Experiment B.

Material	Pretreatment Average*	+1	+2	+3	+4
		Postreatment Days / Percent Reduction			
2% stirofos	12.3	100	92	86	55
Vaseline®		100	70	13	0
2% malathion	5.4	78	0	0	0
Vaseline®		100	8	4	0
Untreated	12.5	-38	+40	+50	+43

*/ Based on 5 horses.

Appendix J . Pretreatment averages and postreatment percent reductions, black fly control Experiment C .

Material	Pretreatment Average*	Postreatment Days +1	+2	+3
		Percent Reduction		
1% stirofos oil	8.2	67	18	30
BPRL-7053-1		35	20	12
Swish® wipe-on	5.1	100	55	38
BPRL-7053-1		74	45	40
Untreated	4.1	0	+34	+24

*/ Based on 6 horses.

Appendix K. Pretreatment averages and postreatment percent reductions, black fly control Experiment D.

Material	Pretreatment Average*	+1	+2	+3	+4	+5	+6
		Percent Reduction					
10% methoxychlor Vaseline®	17.9	100	100	92	77	73	41
		100	100	100	75	80	53
5% crotoxyphos Vaseline®	11.3	100	91	72	52	59	55
		100	87	83	68	68	61
5% malathion Vaseline®	13.0	100	100	72	69	28	18
		100	100	100	56	53	13
Untreated	11.0	+10	+30	-2	-29	+2	-1

*/ Based on 7 horses.

Appendix L. Blood meal weights determined from $\bar{d}$ analysis for female _Simulium_ _vittatum_.

Trial	Difference (mg)
1	2.82
2	2.90
3	2.21
4	3.15
5	2.21
6	2.02
7	2.32
8	2.75
9	3.05
10	2.20
11	2.02
12	2.32
13	2.34
14	2.14
15	2.46
16	2.15
17	2.57
18	2.69
19	2.46
20	2.57

$\bar{X} = 2.46 \pm 0.34$ mg

The vita has been removed from this piece

FEEDING ACTIVITY, A STUDY OF CONTROL MEASURES, AND A SURVEY OF BLACK FLY PESTS (DIPTERA: SIMULIIDAE) OF HORSES IN VIRGINIA

by

Lee Hill Townsend, Jr.

(ABSTRACT)

A survey of Virginia veterinarians and horse owners indicated black flies (Diptera: Simuliidae) were a significant nuisance to pastured horses in northern and southwestern Virginia. Simulium (Phosterodoros) prob. jenningsi Malloch and S. vittatum Zetterstedt were the only species collected from horses during the study. S. vittatum was the predominant pest along the New River near Radford, Virginia. This species, which usually feeds in the ear, caused the horses to be head-shy and difficult to handle. Vaseline® petroleum jelly, applied at the rate of 3 gm per ear, was found to be the most satisfactory protectant. Its performance was superior to a 3% stirofos dust formulation, a 1% stirofos oil wipe-on formulation, and a commercial wipe-on fly repellent for horses. The addition of 2% weight-dosages of malathion, diazinon, or crotoxyphos, 5% malathion or crotoxyphos, or 10% methoxychlor did not enhance the effectiveness of the gel. A 2% stirofos gel formulation did provide longer

protection periods than Vaseline®. This was due to the lower viscosity of the base rather than the insecticide present. Vaseline® acted as a physical barrier to the black flies. Eight female Simulium vittatum observed feeding on a pastured pony required an average of 10.9 minutes to engorge. Mean blood meal volume for these flies was 2.6 ± 0.34 mm^3. The presence of eight simuliids per ear is proposed as a treatment threshold, based on the extent of scabbing produced.