

FACTORS INFLUENCING MEMBRANE FEEDING BY
THE STABLE FLY, STOMOXYS CALCITRANS (L.)
(DIPTERA: MUSCIDAE)

by 3235

JOHNSON OLATUNJI ARCKOYO

B.S., Kansas State University, 1969

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

1970

Approved by:

C. W. Pitts
Major Professor

LD
2668
T4
1970
A74
C.2

TABLE OF CONTENTS

| | |
|---|----|
| INTRODUCTION | 1 |
| MATERIALS AND METHODS | 3 |
| Experimental Insects | 3 |
| Membrane Feeder | 4 |
| Membranes | 4 |
| RESULTS AND DISCUSSION | 5 |
| Blood Temperature | 5 |
| Length of Exposure to Membrane Feeder | 7 |
| "Conditioning" | 8 |
| Response of Males and Females | 9 |
| Fly Age | 10 |
| Membranes | 11 |
| CONCLUSIONS AND SUMMARY | 16 |
| ACKNOWLEDGMENTS | 17 |
| LITERATURE CITED | 18 |
| APPENDIX | 20 |

INTRODUCTION

The stable fly, Stomoxys calcitrans, (Linnaeus) is a cosmopolitan haematophagous dipteran of economic importance.

Losses due to the stable fly have not been accurately determined, but nevertheless, are believed to be considerable. According to the USDA (1965), the annual economic loss caused by stable flies alone has been estimated at 74 million dollars in meat production and 68 million dollars in milk production in 1965. The stable fly is also important as the mechanical carrier of Trypanosoma evansi, which causes surra in horses and mules (Chandler and Reed, 1960). The fly has been found to be important as an intermediate host of the parasitic nematodes Habronema microstoma and Setaria cervi.

The first successful feeding of a blood-sucking arthropod was done by Rodhain et al. (1912) who fed tsetse flies on blood through a rat skin membrane. Much of the work that has been done in membrane feeding techniques involved studying of dietary requirements, factors affecting feeding behavior, disease transmission potentials of haematophagous arthropods, and screening of drugs and insecticides for possible use in chemotherapeutic control of parasites. The principal arthropods that have been used in membrane feeding studies are fleas, ticks, and mosquitoes (Tarshis, 1958).

Collins et al. (1964) infected mosquitoes by allowing them to feed through a Baudruche membrane (prepared commercially from bovine intestines, Long and Long Company, Belleville, N.J.) in

order to determine infection and transmission thresholds of Semliki forest virus in Anopheles quadrimaculatus and A. albimanus.

Rivnay (1930) studied the tropisms of Cimex lectularius by membrane feeding while De Meillon and Goldberg (1947) studied the nutritional requirements of the bed bug using the same technique.

Very few membrane studies have been done with stable flies. Ferris and Hanson (1952) studied the virus-transmitting ability of stable flies (and mosquitoes) by putting the insects on the inner inanimate egg shell membrane. This membrane had to be pierced by the insects in order to reach the highly vascular network of the chorioallantoic membrane. The investigators reported that some of the stable flies engorged fully, others punctured the membrane without feeding. Some of the test insects did not pierce the shell membrane even though sterilized cattle hair and droplets of blood were placed on the membrane as inducements. Granett (1960) used silverlight (derived from the ox caecum, commercially available as Silverlight, Julius Schnid, Inc., New York City) to evaluate chemicals as repellents against the stable fly. Another membrane study with stable flies was by Totze (1934) who used cellophane as an artificial membrane.

Devices used in the membrane feeding of haematophagous arthropods vary greatly, ranging from simple floating cages to complicated equipment made with machined parts and with waterbaths or incubators (Rutledge et al., 1964).

Many varied membranes have been utilized. Tarshis (1958)

screened as many as 25 membranes of both synthetic and of animal origin, for flea feeding. Many are now available commercially.

This research studied the effects of blood temperature, membrane origin, thickness, color, sex, and fly age on membrane feeding by the stable fly.

MATERIALS AND METHODS

Experimental Insects

The stable fly colony was established from pupae obtained from C.M. Jones of USDA, Lincoln, Nebraska. A modified version of McGregor and Dreiss (1955) rearing procedure was utilized. Flies were fed on citrated-saline bovine blood (1 part citrate-saline/9 parts blood).

All rearing and experiments were conducted under constant temperature and relative humidity (26°C and 50% RH respectively).

Twenty adult stable flies placed in a plastic cylindrical cage (13.9 cm. high and 11.7 cm. in diameter) were used in each trial except in the comparative studies, in which 30 flies were used in a rectangular plastic cage (38.1 cm. x 27.9 cm. x 21.2 cm.). The membrane feeder was then mounted over an opening in the top of the cages. Flies were exposed to the membranes for 30 minutes unless stated otherwise. They were then anesthetized with carbon dioxide, frozen and examined for evidence of feeding.

All flies were starved for 18 hours prior to testing and all experiments were conducted between 8:00 a.m. and 12:00 noon.

Results were analyzed using a one way analysis of variance at 0.05 level and Fisher's LSD test.

Citrated-saline bovine blood was used in all the experiments. Blood temperature in the membrane feeder was maintained at 35°C unless otherwise stated. Blood not used within a week was kept frozen at 0°C in plastic bottles.

Membrane Feeder

A slightly modified version of the mosquito membrane feeder designed by Rutledge et al. (1964) was used as the membrane feeder in all experiments. The feeder was in the form of a long-necked inverted funnel. The inverted mouth and lower portion of the neck tubing were surrounded with an outer cylindrical water jacket. Water from a constant temperature bath was circulated in the outer jacket through a lower inlet and an upper outlet. The membrane was attached over the open end of the cylinder and securely held in place by a hose clamp. Six ml. of blood was introduced into the feeder from the top at the beginning of each experiment. The top was then corked and a thermometer put in place. The area of the feeding surface was approximately 28.3 sq. cm.

Membranes

Seven membranes, 6 of animal (rabbit and cow) origin and 1 synthetic were studied for their suitability in stable fly membrane feeding. These membranes were first exposed singly with flies in the small cylindrical feeding cages. Then two different

membranes were exposed simultaneously using the large rectangular feeding cages to determine preference.

All membranes of animal origin were prepared on the night prior to the first feeding trials and then refrigerated after each day's experiments. The subcutaneous layers of fat and membranous tissue were removed from the skins, hairs slightly clipped and then attached to the feeders. Except where otherwise stated, a membrane was used for several trials before it was discarded, however, no membrane was used in more than one experiment. Membranes for each experiment were prepared from the same animals except in the preference studies. In the preference experiments where two membranes were simultaneously exposed, 0.5% fluorescent dye (Blancophor SV conc., Gen. Aniline and Film Corporation, New York, N.Y.) was incorporated with the blood placed in one of the membrane feeders. Flies that fed from this feeder could then be separated from flies that fed from the other by examining them under ultraviolet light.

Membranes prepared from black rabbit skins were used for all the experiments except in the studies evaluating other membranes.

RESULTS AND DISCUSSION

Blood Temperature

Flies were fed through a black rabbit skin membrane on blood at different temperatures to determine the optimum feeding temperature (Table 1). It was found that most flies (88.75%) fed

when the blood temperature was 35°C and the ambient temperature was 26°C. This was the optimum blood temperature and consequently was used for all subsequent experiments. Flies were found to feed at blood temperatures as high as 50°C and as low as 15°C.

Table 1. The Effect of Blood Temperature on Feeding of Stable Flies (2-6 days old) through a Black Rabbit Membrane.

| Blood Temp. ¹ (°C) | Total Flies Exposed | Total Flies Fed | Average Fed | Percent Fed |
|----------------------------------|------------------------|--------------------|----------------|----------------|
| 55 ^(a) | 80 | 0 | 0.0 | 0.0 |
| 50 ^(b) | 80 | 25 | 6.3 | 31.3 |
| 45 ^(c) | 80 | 60 | 15.0 | 75.0 |
| 40 ^(c) | 80 | 68 | 17.0 | 85.0 |
| 35 ^(c) | 80 | 71 | 17.8 | 88.8 |
| 30 ^(c) | 80 | 56 | 14.0 | 70.0 |
| 20 ^(a) | 80 | 1 | 0.3 | 1.5 |
| 15 ^(a) | 80 | 3 | 0.8 | 3.8 |

¹ANOVA and Fisher's LSD. Temperatures followed by an identical letter are not significantly different ($p=0.05$).

²Four replicates of 20 flies each.

In preliminary studies it was noted that varying ambient temperatures gave erratic results. This concurred with Bishop (1913), who found decreased feeding activity by stable flies at

temperatures below 15°C and no feeding below 12°C . Ticks, fleas, and mosquitoes have been reported to feed poorly when ambient temperature rose about 30°C (Tarshis, 1958).

It was found in this study that the membrane (feeding surface) temperature (32.7°C average) was always lower than the actual blood temperature (35°C) by a few degrees.

Length of Exposure to Membrane Feeder

The adult stable fly moves about several times on its host before it settles to engorge, and often it will fly to another animal to complete the meal. This feeding behavior was also observed during the experiments. Thus the stable fly is potentially capable of mechanical transmission of pathogenic organisms due to its feeding habits.

Significant differences ($p=0.05$) were found between the means of the number of flies which fed at some of the time intervals (Table 2). However, since no significant differences were found between 45 minutes and 30 minutes and both gave satisfactory results, the later time was chosen for subsequent experiments.

Several flies were observed to have fed from the membrane in as short an interval as 3 minutes. This agreed with earlier reports which stated that it takes an average of 2-5 minutes (Bishop, 1913) for a fly to feed to repletion. Observations by Mitzmain (1913) showed that a starved stable fly would be fully engorged in 3-4 minutes if undisturbed.

Table 2. Effect of Length of Exposure on the Feeding of Stable Flies (3-6 days old) through a Black Rabbit Membrane.

| Time ¹ (Minutes) | Total Flies Exposed ² | Total Flies Fed | Average Fed | Percent Fed |
|--------------------------------|-------------------------------------|--------------------|----------------|----------------|
| 45 ^(a) | 80 | 72 | 18.0 | 90.0 |
| 30 ^(a) | 80 | 71 | 17.8 | 88.8 |
| 25 ^(a) | 80 | 68 | 17.0 | 85.0 |
| 20 ^(a) | 80 | 56 | 14.0 | 70.0 |
| 15 ^{(a)(d)} | 80 | 57 | 14.3 | 71.3 |
| 10 ^(b) | 80 | 38 | 9.5 | 47.5 |
| 5 ^{(b)(d)} | 80 | 42 | 10.3 | 51.3 |
| 3 ^(c) | 80 | 24 | 6.0 | 30.0 |

¹Times followed by identical letters are not significantly different ($p=0.05$).

²Four replicates of 20 flies each.

"Conditioning"

Flies previously fed on 5.0% sugar solution (unconditioned flies), and then membrane-fed on blood, showed less feeding response than those maintained on blood (conditioned flies) (Table 3). However, there were no statistical differences between the means of the two types of flies.

Table 3. Effect of Conditioning on the Feeding Response of Stable Flies (3-6 days old) Fed through a Black Rabbit Membrane.

| Type of Flies ¹ | Total Exposed ² | Total Fed | Average Fed | Percent Fed |
|------------------------------|----------------------------|-----------|-------------|-------------|
| Conditioned ^(a) | 200 | 140 | 14.0 | 70.0 |
| Unconditioned ^(a) | 200 | 82 | 8.2 | 41.0 |

¹Types of flies followed by an identical letter are not significantly different ($p=0.05$).

²Ten replicates of 20 flies each.

It is not clearly known how a prior diet affects arthropods in membrane feeding. It was observed, however, that the activities of conditioned flies were more vigorous than those of the unconditioned flies.

Response of Males and Females

Both male and female flies responded equally to membrane feeding (Table 4). There were no statistical differences between the means of the number of flies of each sex that fed (Table 4). Tarshis (1958) made the same observations working with fleas.

Table 4. Feeding Response of Male and Female Stable Flies
(3-5 days old) Fed through a Black Rabbit Membrane.

| Sex ¹ | Total Flies Exposed ² | Total Fed | Average Fed | Percent Fed |
|------------------|-------------------------------------|--------------|----------------|----------------|
| Male | 200 | 156 | 15.6 | 78.0 |
| Female | 200 | 163 | 16.3 | 81.5 |

¹No significant difference between males and females
($p=0.05$).

²Ten replicates of 20 flies each.

Fly Age

It was observed that the vigor of the fly could be a factor in membrane feeding (Table 5). Many one-day-old flies held in the feeding cages were observed to fall on their backs and had great difficulty righting themselves. Many also were unable to pierce the membranes. These accounted for the low feeding response in one-day-old flies. There was a significant difference between the means of one-day-old flies that fed and all the other ages observed (Table 5). It has been stated by both Mitzmain (1913) and Newstead (1906) that stable flies in nature would feed as early as an hour after emergence. The three- and five-day-old flies were much more vigorous and active than the other groups. Some of the eight-day-old flies were much more vigorous and active than the other groups. Some of the eight-day-old flies were observed to have lost part of their wings and consequently walked around in the feeding cages.

Table 5. The Effect of Fly Age on the Feeding of Stable Flies through a Black Rabbit Membrane.

| Fly Age ¹ (Days) | Total Flies Exposed ² | Total Fed | Average Fed | Percent Fed |
|--------------------------------|-------------------------------------|--------------|----------------|----------------|
| 1(b) | 200 | 83 | 8.3 | 41.5 |
| 3(a) | 200 | 155 | 15.5 | 77.6 |
| 5(a) | 200 | 152 | 15.2 | 76.0 |
| 8(a) | 200 | 139 | 13.9 | 69.6 |

¹Fly ages followed by an identical letter are not significantly different ($p=0.05$).

²Ten replicates of 20 flies each.

Membranes

Six membranes of animal origin and one synthetic were evaluated for their suitability in membrane feeding of stable flies. Membrane factors found to be important in feeding were thickness, color, and origin.

Testing the membranes separately, it was found that feeding from the white and black rabbit membranes of about equal thickness was relatively high while it was very low for both the cow-leg and cow-back membranes which were thicker (Table 6). Although no significant difference was found between the two types of rabbit membranes, a statistical difference ($p=0.05$) was found between these and all the other membranes evaluated separately (Table 6). Despite repeated and vigorous probings on the

cow membranes only very few flies were able to pierce it and obtain blood.

Trials with the baudruche was discontinued since the membrane was too thin (0.02 mm.) and leaked each time it was pierced by a fly. However, Hoffman (1965) using a different apparatus (feeding cage was put on top of membrane with blood beneath) was able to achieve satisfactory results using the same membrane for stable flies.

Table 6. Feeding Response of Stable Flies (2-7 days old) through Different Kinds of Membranes.

| Membrane ¹ | Membrane ² Thickness (mm.) | Total Exposed | Replicates (20 each) | Total Fed | Ave. Fed | Percent Fed |
|------------------------|---|------------------|-------------------------|--------------|-------------|----------------|
| Black Rabbit (a) | 0.927 | 100 | 5 | 93 | 18.6 | 93.0 |
| White Rabbit (a) | 0.904 | 100 | 5 | 81 | 16.2 | 81.0 |
| Cow (Leg) (c) | 1.226 | 100 | 5 | 7 | 1.4 | 7.0 |
| Cow (Back) (c)(b) | 1.231 | 100 | 5 | 19 | 3.8 | 19.0 |
| Parafilm M (c) | 0.116 | 200 | 10 | 27 | 2.7 | 13.5 |
| Baudruche ³ | 0.020 | 40 | 2 | -- | -- | -- |
| Calf (Back) (b) | 1.181 | 200 | 10 | 63 | 6.3 | 31.5 |

¹Membranes followed by an identical letter are not significantly different ($p=0.05$).

²Measurement of unstretched membrane (± 0.0254 mm.) with a micrometer.

³Discontinued after second replicate.

The thickness of the membrane (maximum and minimum) used in feeding is a critical factor in this technique (Langley and Maly, 1965). Fuller et al. (1949) found that the thickness of a membrane may be a determining factor in the feeding of lice. Langley and Maly (1958) showed that tsetse flies would ingest blood more frequently when the membrane used was about 2 mm. than if thinner. Similarly, Tarshis (1958) reported that membranes more than 0.3302 mm. thick were unsatisfactory for feeding fleas.

Table 7. Color Preference in the Feeding of Stable Flies (4-5 days old) Exposed to Two Animal Membranes Simultaneously.

| | Total No. of Flies | MEMBRANES | |
|-------------|-----------------------|--------------|--------------|
| | | Black Rabbit | White Rabbit |
| Exposed | 240 ¹ | | |
| Fed | 126 | 119 | 7 |
| Ave. Fed | 15.8 | 14.9 | 0.8 |
| Percent Fed | 52.5 | 49.6 | 2.9 |

¹Thirty flies in 8 replicates.

From the results (Table 6) a membrane thicker than 0.94 mm. is not satisfactory for eliciting the feeding response in the stable fly in the laboratory.

The stable fly exhibited a color preference when exposed to two membranes of different colors simultaneously (Tables 7 and 8). Although 81% of the flies fed from a white rabbit membrane when used alone (Table 6), only 2.9% (out of a total of 52.5%) fed from the same membrane when an alternative, a black rabbit membrane, was presented (Table 7). There was a significant difference between the means of the number of flies feeding on the black membrane and a white membrane when both were used simultaneously (Table 7).

Table 8. Color Preference in the Feeding of Stable Flies (4-5 days old) Exposed to Two Animal Membranes Simultaneously.

| | Total No. of Flies | MEMBRANES | |
|-------------|-----------------------|--------------|------------|
| | | White Rabbit | Black Calf |
| Exposed | 240 ¹ | | |
| Fed | 68 | 6 | 62 |
| Ave. Fed | 8.5 | 0.8 | 7.8 |
| Percent Fed | 28.3 | 2.5 | 25.8 |

¹Thirty flies in 8 replicates.

Observations showed that most of the flies in this experiment congregated on or around the black membrane. These observations were also made when white rabbit and black calf membranes were used. These findings are in agreement with Parr's (1962)

studies of stable flies in East Africa. He concluded that dark-colored animals were more seriously affected by stable flies than light-colored ones. More flies actually fed from the black calf membrane which was slightly thicker (1.2 mm.) than from the white rabbit (1.04 mm.) membrane (Table 8). Burns et al. (1962) and Frank et al. (1964) all reported similar color preference in Haematobia irritans on beef cattle.

The general assertion, as indicated in the literature, that membranes of animal origin are better than synthetic ones (Tarshis, 1958) was also confirmed in this study. However, there was the problem of getting a consistent membrane thickness from different animals of the same age group. There was comparatively very low feeding (Table 6) from unstretched Parafilm M (American Can Company, Neenah, Wisconsin) a wax synthetic membrane. Several flies were observed probing the membrane but very few were able to pierce and obtain a meal. The durability of the rabbit membranes was found to be good with refrigeration. The cow membranes on the other hand, dried out quickly even with refrigeration. Except for the commercially available Baundruche, preparation of all the membranes of animal origin was both difficult and messy.

CONCLUSIONS AND SUMMARY

Although flies fed on blood at all the blood temperatures (15° - 50° C) examined, the optimum temperature was found to be 35° C.

The black rabbit membrane was concluded to be the most suitable of the seven evaluated in this study.

Neither the fly age nor sex of flies was found to be a critical factor in membrane feeding. The most important factors were blood temperature, ambient temperature, membrane origin, color, and thickness and feeding intervals.

It was concluded that a combination of 35° C blood temperature and a feeding interval of 30 minutes using a black rabbit membrane would be ideally suitable for stable fly membrane feeding.

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. C.W. Pitts, for his tremendous assistance, invaluable suggestions and guidance prior to and during the course of this study.

Thanks are also due to Dr. T.L. Hopkins and Dr. M.F. Hansen who served on my supervisory committee.

I am especially indebted to Dr. Herbert Knutson, Head of the Entomology Department for personally requesting my government to extend my stay in the U.S. to enable me to do this study.

I also wish to thank Dr. Robert Mills, my academic adviser as an undergraduate for his advice and encouragement and also for recommending me to my government for graduate study.

My sincere gratitude to Dale Lind, Dairy husbandman, Dairy Science Dept., for supplying the blood used in this research.

I am also grateful to C.M. Jones, USDA, Lincoln, Nebraska, for supplying the pupae with which I established my colony.

Finally, I would like to extend my sincere thanks to Mary Danner for her wonderful support throughout this study and for typing this paper.

REFERENCES CITED

- Bishop, F.C. 1913. The Stable Fly. USDA Farmer's Bull. No. 540.
- Burns, E.C., B.H. Wilson, R.S. Temple and C.C. Phillips. 1962. Color preference of horn flies. Anim. Sci. Dept., Louisiana State Univ. Ag. Expt. Stat. Livestock Producers' Day Bull. 2:37-40.
- Chandler, A.S. and C.P. Read. 1961. Introduction to Parasitology. John Wiley and Sons, Inc., New York and London. p. 695.
- Collins, W.E., A.J. Harrison and J.C. Skinner. 1964. The use of a membrane feeding technique to determine infection and transmission threshold of Semliki forest virus in Anopheles quadramaculatus and Anopheles albimanus. Mosq. News 24:25-27.
- De Meillon, B., and L. Golberg. 1947. Preliminary studies on the nutritional requirements of the bedbug (Cimex lectularius L.) and the tick Ornithodoros moubata Murray. J. Exp. Biol. 24:41-63.
- Ferris, D.H. and R.P. Hanson. 1952. A technique employing embryonating egg to test virus-transmitting ability of certain insect vectors. Cor. Vet. 42:389-394.
- Frank, R.E., E.C. Burns and N.C. England. 1964. Color preference of the horn fly, H. irritans on beef cattle. J. Econ. Entomol. 57(3):371-372.
- Fuller, H.S., E.S. Murray, and J.C. Synder. 1949. Studies on human body lice Pediculus humanus corporis. 1. A method for feeding lice through membrane and experimental infection with Rickettsia prowazeki (R.). U.S. Public Health Serv. 64:1287.
- Granett, P. 1960. Use of animal membrane in the evaluation of chemical repellents against stable flies. J. Econ. Entomol. 53(3):432-435.
- Hoffman, R. 1965. A master's thesis. Oklahoma State Univ.
- Langley, P.A. and H. Maly. 1969. Membrane feeding technique for Tsetse flies (Glossina spp.). Nature 221:855-856.
- McGregor, W.S. and J.M. Dreiss. 1955. Rearing of stable flies (Stomoxys calcitrans) in the laboratory. J. Econ. Entomol. 48:327-328.

- Mitzmain, M.B. 1913. The bionomics of Stomoxys calcitrans (L.); a preliminary account. Phillippine J. Sci. 8 (Sec. B):29-48.
- Newstead, R. 1906. On the life history of Stomoxys calcitrans (Linn.). J. Econ. Biol. 1(4):157-166.
- Parr, H.C.M. 1962. Studies on Stomoxys calcitrans in Uganda, East Africa II--Notes on Life History and Behaviour. Bull. Ent. Res. 53(2):437-443.
- Rivnay, E. 1930. Technique in artificial feeding of the bedbug, Cimex lectularius. J. Parasitol. 16:246-249.
- Rodhain, J., C. Pons, J. Vandenbranden, and J. Bequaert. 1912. Contribution au mecanisme de la transmission des trypanosomes par les glossines. Arch. f. Schiffs. u. Trop. Hyg. 16:732-739.
- Rutledge, L.C., R.A. Ward and D.J. Gould. 1964. Studies on the feeding response of mosquitoes to nutritive solution in a membrane feeder. Mosq. News 24(4):409-419.
- Tarshis, Barry. 1958. Feeding techniques for blood-sucking arthropods. Proc. 10th Intern. Cong. Ent. 3:767-784.
- Totze, R. 1934. Blutsaugen unter rein experimentellen Bedingungen. (Blood-sucking under purely experimental conditions.) Zbl. Bakt. (1) Orig. 132(5-6):382-384.
- USDA. 1965. Losses in Agriculture. Agric. Handbook No. 291. Agric. Res. Ser. U.S. Dept. of Agric., Washington, D.C.

APPENDIX

Table 9. The Effect of Blood Temperature on Feeding of Stable Flies (2-6 days old) through a Black Rabbit Membrane.

| Replicates | TEMPERATURE (°C) | | | | | |
|------------|------------------|------------|----------------|------------|----------------|------------|
| | 55° | | 50° | | 45° | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 0 | 20 | 5 | 20 | 17 |
| 2. | 20 | 0 | 20 | 10 | 20 | 10 |
| 3. | 20 | 0 | 20 | 5 | 20 | 18 |
| 4. | 20 | 0 | 20 | 5 | 20 | 15 |
| Total | 80 | 0 | 80 | 25 | 80 | 60 |
| Average | 20 | 0 | 20 | 6.3 | 20 | 15 |
| Range | | | | 5-10 | | 10-18 |
| Percent | | 0.0 | | 31.3 | | 75.0 |

| Replicates | 40° | | 35° | | 30° | |
|------------|----------------|------------|----------------|------------|----------------|------------|
| | No. Exposed | No. Fed | No. Exposed | No. Fed | No. Exposed | No. Fed |
| | No. Exposed | No. Fed | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 17 | 20 | 16 | 20 | 10 |
| 2. | 20 | 18 | 20 | 20 | 20 | 16 |
| 3. | 20 | 14 | 20 | 18 | 20 | 17 |
| 4. | 20 | 19 | 20 | 17 | 20 | 13 |
| Total | 80 | 68 | 80 | 71 | 80 | 56 |
| Average | 20 | 17 | 20 | 17.8 | 20 | 14 |
| Range | | 14-19 | | 16-20 | | 10-17 |
| Percent | | 85.0 | | 88.8 | | 70.0 |

Table 9 (Continued).

| Replicates | TEMPERATURE (°C) | | | |
|------------|------------------|------------|----------------|------------|
| | 20° | | 15° | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 0 | 20 | 0 |
| 2. | 20 | 1 | 20 | 3 |
| 3. | 20 | 0 | 20 | 0 |
| 4. | 20 | 0 | 20 | 0 |
| Total | 80 | 1 | 80 | 3 |
| Average | 20 | 0.3 | 20 | 0.8 |
| Range | | 0-1 | | 0-3 |
| Percent | | 1.3 | | 3.8 |

Table 10. Effect of Length of Exposure on the Feeding of Stable Flies (3-6 days old) through a Black Rabbit Membrane.

| <u>Replicates</u> | TIME IN MINUTES | | | | | |
|-------------------|------------------------|--------------------|------------------------|--------------------|------------------------|--------------------|
| | 45 | | 30 | | 25 | |
| | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Exposed</u> | <u>No. Fed</u> |
| 1. | 20 | 19 | 20 | 16 | 20 | 18 |
| 2. | 20 | 17 | 20 | 20 | 20 | 18 |
| 3. | 20 | 18 | 20 | 17 | 20 | 15 |
| 4. | 20 | 18 | 20 | 18 | 20 | 17 |
| Total | 80 | 72 | 80 | 71 | 80 | 68 |
| Average | 20 | 18 | 20 | 17.8 | 20 | 17 |
| Range | | 17-19 | | 16-20 | | 15-18 |
| Percent | | 90.0 | | 88.8 | | 85.0 |

| <u>Replicates</u> | 20 | | 15 | | 10 | |
|-------------------|------------------------|--------------------|------------------------|--------------------|------------------------|--------------------|
| | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Exposed</u> | <u>No. Fed</u> |
| | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Exposed</u> | <u>No. Fed</u> |
| 1. | 20 | 16 | 20 | 15 | 20 | 12 |
| 2. | 20 | 16 | 20 | 16 | 20 | 11 |
| 3. | 20 | 9 | 20 | 11 | 20 | 8 |
| 4. | 20 | 15 | 20 | 15 | 20 | 7 |
| Total | 80 | 56 | 80 | 57 | 80 | 38 |
| Average | 20 | 14 | 20 | 14.3 | 20 | 9.5 |
| Range | | 9-16 | | 11-16 | | 7-12 |
| Percent | | 70.0 | | 71.3 | | 47.5 |

Table 10 (Continued).

| Replicates | TIME IN MINUTES | | | |
|------------|-----------------|------------|----------------|------------|
| | 5 | | 3 | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 13 | 20 | 9 |
| 2. | 20 | 9 | 20 | 2 |
| 3. | 20 | 8 | 20 | 6 |
| 4. | 20 | 12 | 20 | 7 |
| Total | 80 | 42 | 80 | 24 |
| Average | 20 | 10.3 | 20 | 6 |
| Range | | 8-13 | | 2-9 |
| Percent | | 51.3 | | 30.0 |

Table 11. Effect of Conditioning on the Feeding Response of Stable Flies (3-6 days old) Fed through a Black Rabbit Membrane.

| Replicates | TYPE OF FLIES | | | |
|------------|----------------|------------|----------------|------------|
| | Conditioned | | Unconditioned | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 11 | 20 | 8 |
| 2. | 20 | 17 | 20 | 4 |
| 3. | 20 | 14 | 20 | 10 |
| 4. | 20 | 18 | 20 | 8 |
| 5. | 20 | 14 | 20 | 8 |
| 6. | 20 | 13 | 20 | 8 |
| 7. | 20 | 13 | 20 | 12 |
| 8. | 20 | 16 | 20 | 9 |
| 9. | 20 | 12 | 20 | 8 |
| 10. | 20 | 12 | 20 | 7 |
| Total | 200 | 140 | 200 | 82 |
| Average | 20 | 14.0 | 20 | 8.2 |
| Range | | 11-18 | | 4-12 |
| Percent | | 70.0 | | 41.0 |

Table 12. Feeding Response of Male and Female Stable Flies
(3-5 days old) Fed through a Black Rabbit Membrane.

| Replicates | SEX | | | |
|------------|----------------|------------|----------------|------------|
| | Male | | Female | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 12 | 20 | 17 |
| 2. | 20 | 19 | 20 | 18 |
| 3. | 20 | 11 | 20 | 20 |
| 4. | 20 | 13 | 20 | 14 |
| 5. | 20 | 8 | 20 | 17 |
| 6. | 20 | 19 | 20 | 14 |
| 7. | 20 | 20 | 20 | 17 |
| 8. | 20 | 20 | 20 | 17 |
| 9. | 20 | 17 | 20 | 15 |
| 10. | 20 | 17 | 20 | 14 |
| Total | 200 | 156 | 200 | 163 |
| Average | 20 | 15.6 | 20 | 16.3 |
| Range | | 11-20 | | 14-20 |
| Percent | | 78.0 | | 81.5 |

Table 13. The Effect of Fly Age on the Feeding of Stable Flies through a Black Rabbit Membrane.

| Replicates | AGE IN DAYS | | | |
|------------|----------------|------------|----------------|------------|
| | 1 | | 3 | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 7 | 20 | 12 |
| 2. | 20 | 5 | 20 | 17 |
| 3. | 20 | 10 | 20 | 16 |
| 4. | 20 | 7 | 20 | 18 |
| 5. | 20 | 6 | 20 | 12 |
| 6. | 20 | 9 | 20 | 12 |
| 7. | 20 | 8 | 20 | 19 |
| 8. | 20 | 6 | 20 | 16 |
| 9. | 20 | 11 | 20 | 14 |
| 10. | 20 | 14 | 20 | 19 |
| Total | 200 | 83 | 200 | 155 |
| Average | 20 | 8.3 | 20 | 15.5 |
| Range | | 5-14 | | 12-19 |
| Percent | | 41.5 | | 77.5 |

Table 13 (Continued).

| <u>Replicates</u> | AGE IN DAYS | | | |
|-------------------|------------------------|--------------------|------------------------|--------------------|
| | 5 | | 8 | |
| | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Exposed</u> | <u>No. Fed</u> |
| 1. | 20 | 14 | 20 | 19 |
| 2. | 20 | 15 | 20 | 18 |
| 3. | 20 | 11 | 20 | 16 |
| 4. | 20 | 17 | 20 | 10 |
| 5. | 20 | 17 | 20 | 14 |
| 6. | 20 | 17 | 20 | 10 |
| 7. | 20 | 17 | 20 | 13 |
| 8. | 20 | 17 | 20 | 15 |
| 9. | 20 | 12 | 20 | 16 |
| 10. | 20 | 15 | 20 | 8 |
| Total | 200 | 152 | 200 | 139 |
| Average | 20 | 15.2 | 20 | 13.9 |
| Range | | 11-17 | | 8-19 |
| Percent | | 76.0 | | 69.5 |

Table 14. Feeding Response of Stable Flies (2-7 days old) through Different Kinds of Membrane.

| Replicates | MEMBRANES | | | | | |
|------------|----------------|------------|----------------|------------|----------------|------------|
| | Black Rabbit | | White Rabbit | | Cow (Leg) | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 20 | 20 | 14 | 20 | 1 |
| 2. | 20 | 19 | 20 | 17 | 20 | 1 |
| 3. | 20 | 18 | 20 | 19 | 20 | 0 |
| 4. | 20 | 17 | 20 | 16 | 20 | 4 |
| 5. | 20 | 19 | 20 | 15 | 20 | 1 |
| Total | 100 | 93 | 100 | 81 | 100 | 7 |
| Average | 20 | 18.6 | 20 | 16.2 | 20 | 1.4 |
| Range | | 17-20 | | 14-19 | | 0-4 |
| Percent | | 93.0 | | 81.0 | | 7.0 |

| Replicates | Cow (Back) | | Baudruche | |
|------------|----------------|------------|---|------------|
| | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 0 | 20 | 14 |
| 2. | 20 | 6 | 20 | 19 |
| 3. | 20 | 4 | (Discontinued Because of Leakage) | |
| 4. | 20 | 6 | | |
| 5. | 20 | 3 | | |
| Total | 100 | 19 | | |
| Average | 20 | 3.8 | | |
| Range | | 0-6 | | |
| Percent | | 19.0 | | |

Table 14 (Continued).

| Replicates | MEMBRANES | | | |
|------------|----------------|------------|----------------|------------|
| | Calf (Back) | | Parafilm M | |
| | No. Exposed | No. Fed | No. Exposed | No. Fed |
| 1. | 20 | 5 | 20 | 6 |
| 2. | 20 | 3 | 20 | 0 |
| 3. | 20 | 4 | 20 | 12 |
| 4. | 20 | 11 | 20 | 0 |
| 5. | 20 | 7 | 20 | 0 |
| 6. | 20 | 3 | 20 | 8 |
| 7. | 20 | 10 | 20 | 0 |
| 8. | 20 | 10 | 20 | 1 |
| 9. | 20 | 2 | 20 | 0 |
| 10. | 20 | 8 | 20 | 0 |
| Total | 200 | 63 | 200 | 27 |
| Average | 20 | 6.3 | 20 | 2.7 |
| Range | | 2-11 | | 0-12 |
| Percent | | 31.5 | | 13.5 |

Table 15. Color Preference in the Feeding of Stable Flies
(4-5 days old) Exposed to Two Animal Membranes
Simultaneously.

| <u>Replicates</u> | <u>BLACK AND WHITE RABBIT MEMBRANES</u> | | | |
|-------------------|---|--------------------|--------------------------------------|--------------------------------------|
| | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Fed From Black Rabbit</u> | <u>No. Fed From White Rabbit</u> |
| 1. | 30 | 20 | 20 | 0 |
| 2. | 30 | 16 | 14 | 2 |
| 3. | 30 | 19 | 18 | 1 |
| 4. | 30 | 19 | 17 | 2 |
| 5. | 30 | 18 | 18 | 0 |
| 6. | 30 | 13 | 11 | 2 |
| 7. | 30 | 10 | 10 | 0 |
| 8. | 30 | 11 | 11 | 0 |
| Total | 240 | 126 | 119 | 7 |
| Average | 30 | 15.8 | 14.9 | 0.9 |
| Range | | 10-20 | 10-20 | 0-2 |
| Percent | | 52.5 | 49.6 | 0.9 |

| <u>Replicates</u> | <u>WHITE RABBIT AND CALF (BACK) MEMBRANES</u> | | | |
|-------------------|---|--------------------|--------------------------------------|-------------------------------------|
| | <u>No. Exposed</u> | <u>No. Fed</u> | <u>No. Fed From White Rabbit</u> | <u>No. Fed From Calf (Back)</u> |
| 1. | 30 | 8 | 0 | 8 |
| 2. | 30 | 7 | 0 | 7 |
| 3. | 30 | 10 | 2 | 8 |
| 4. | 30 | 4 | 0 | 4 |
| 5. | 30 | 10 | 2 | 8 |
| 6. | 30 | 8 | 0 | 8 |
| 7. | 30 | 12 | 0 | 12 |
| 8. | 30 | 9 | 2 | 7 |
| Total | 240 | 68 | 6 | 62 |
| Average | 20 | 8.5 | 0.75 | 7.75 |
| Range | | 4-12 | 0-2 | 4-12 |
| Percent | | 28.3 | 2.5 | 25.8 |

FACTORS INFLUENCING MEMBRANE FEEDING BY
THE STABLE FLY, STOMOXYS CALCITRANS (L.)
(DIPTERA: MUSCIDAE)

by

JOHNSON OLATUNJI AROKOYO

B.S., Kansas State University, 1969

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

1970

ABSTRACT

The effects of blood temperature, feeding interval, membrane properties, sex, age and conditioning on membrane feeding of Stomoxys calcitrans (L.) (Diptera: Muscidae) were studied at a constant temperature (26°C) and relative humidity (50% RH) room. Seven membranes, 6 of animal (rabbit and cow) origin and 1 synthetic, were evaluated for their suitability in this type of feeding technique.

The membranes of animal origin were found to be better than the synthetic one and the black rabbit skin was the one found the most suitable among the seven evaluated.

The most important factors in stable fly membrane feeding, were blood temperature, ambient temperature, membrane origin, color, and thickness, and feeding interval. Sex of the fly was not a critical factor.

A combination of 35°C blood temperature and a feeding interval of 30 minutes was the most satisfactory and convenient for membrane studies.

A membrane thickness over 0.94 mm. was unsatisfactory for membrane feeding by Stomoxys calcitrans in the laboratory.

The technique used was successful and would be useful in a variety of related studies.