STUDIES ON THE HOST ORIENTATION AND FEEDING BEHAVIOUR OF SOME HEMATOPHAGOUS FLIES WITH SPECIAL REFERENCE TO TABANIDAE AND *STOMOXYS CALCITRANS* (LINN.)

A

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by

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ABSTRACT

by

Garth K. Bracken

STUDIES ON THE HOST ORIENTATION AND FEEDING BEHAVIOUR OF SOME HEMATOPHAGOUS FLIES WITH SPECIAL REFERENCE TO TABANIDAE AND *STOMOXYS CALCITRANS* (LINN.)

The host orientation of tabanids was studied by comparing the numbers taken in modified forms of the helio-thermal trap and the numbers captured on sticky objects of different color, shape and altitude. Traps which presented a larger dark silhouette at rest or in motion were the most effective modifications. The results suggest that visual stimuli are of prime importance in the orientation of tabanids to their hosts from some distance.

Thirty species of Tabanidae, which represents three-quarters of the total number recorded in Manitoba by taxonomists, were identified from the captured specimens. The modified trap is therefore a valuable instrument for the study of both the population density and seasonal distribution of species as well as their orientation behaviour.

The descending order of attractiveness of colored objects for tabanids was black, red, blue, green, white and yellow. Objects which were positioned within five feet of the ground were more attractive than objects at a higher altitude. There is some indication that spherical objects are preferred to cylindrical or flat shapes. This preference might also be accounted for by the varying degrees of motion imposed by wind on the

differently shaped objects.

Observations on the feeding behaviour of tabanids revealed that whereas water or sugar is readily accepted by captive flies, blood under the same conditions is refused. The sensory preconditioning required to elicit a response to blood requires further investigation.

Studies were conducted on the relative palatability of blood fractions to the stable fly. Plasma or serum is usually less palatable than whole blood. Centrifuged blood cells reconstituted either in saline or plasma were substantially as acceptable as blood. Therefore the most active factors are contained in the blood cells. Hemolysis of the cells does not reduce their palatability in either blood or in saline but cells hemolized in water were less acceptable. The individual substances, saline, glucose, adenosine monophosphate and adenosine diphosphate, did not by themselves account for the normal response of the stable fly to whole blood.

INTRODUCTION

Everyone is well acquainted with the biting activities of hematophagous flies. The question of how the insects arrive at their host and the sensory stimuli to which they are responding when feeding is often forgotten in the urge either to escape the insects or to launch expensive programs for their control.

Most biting flies are oviparous and the immature stages of these species develop in soil, ground water or decomposing organic matter. Newly emerged adults are not assured of finding a host in the immediate vicinity. Host location therefore may involve preliminary random dispersal. The eventual successful finding of the host must require some degree of orientation of the insect through sensory perception of the host. The senses that could subserve are visual, olfactory and thermal.

The feeding process which follows has largely been taken for granted. However, recent findings in the feeding behaviour of phytophagous insects have shown that feeding is regulated by rather specific chemicals in the host plant. It is highly probable that a similar pattern is present in the gustatory behaviour of biting flies.

These two aspects of biting fly behaviour form the central problem in this investigation.

<u>The scope of the study</u>. This study had two objectives: (1) to examine the effective stimuli in the host orientation of the Tabanidae; (2) to determine the components in blood which stimulate feeding to satiation. Although taxonomic information was not the primary purpose

of the study much of the material taken in traps was identified to species and this information is included.

The organization of thesis. The thesis is divided into two sections. The first section will cover the work done on host orientation and will be restricted chiefly to observations of the Tabanidae. The second section will describe the work done on feeding behaviour. The greater part of this work concerns the stable fly, *Stomoxys calcitrans* Linn. since it could be easily reared, permitting experiments to be carried on in the winter months. Some preliminary observations of tabanid feeding behaviour are entered at the beginning of this section. The sections will be summarized and concluded together.

SECTION I. HOST ORIENTATION

Review of Literature

A review of the earlier literature including several significant observations of adult horse fly behaviour was done by Philip (1931). The attraction of tabanids to automobiles was noted by several earlier authors and Philip refers to the use of an auto for collecting. This method of collecting is referred to again by Hearle (1938) and more recently by Snow *et al* (1957). Philip concluded that heat was the chief attraction (a postulation earlier put forth by Cameron (1926)) since tabanids seek the warmest parts of the car. It was also noted by this author that a moving object will cause tabanids in flight to hesitate for investigation and that darker objects and darker cattle in a herd were especially attractive. Odour also was considered important by Philip as he observed individuals to be attracted to a cloth which had been used to rub down cattle.

The attractiveness of dark colors has been noted by several authors. Hansen (1947) records that individuals wearing blue denims were visited and bitten more frequently than those wearing white. Bromely (1952) reported that *Tabanus atratus* preferred black cattle and would attack the black patches of Holstiens more frequently than the white. Tashiro and Schwardt (1953) observed that the general condition of the animal, its size and color all effected its attractiveness to attack; animals of poor health being attacked more than healthier ones and darker more often than lighter.

From the foregoing observations it should be possible to make a device for the capture of horse flies by constructing it to duplicate one or more attributes of the host; e.g. color, silhouette, motion, heat and odour. Hansen (1951) constructed a simple device consisting of two square foot piece of black masonite placed back to back, staked two feet from the ground and smeared with tanglefoot to capture the flies. The same device was used by this author for the capture of stable flies, Hansen (1952). Brown and Morrison (1955) used tents of black cloth to attract Tabanidae and evaluated their relative numbers in sprayed and unsprayed areas by counting the landing rates on these structures. An interesting trap for the capture of tse-tse flies was constructed by Morris and Morris (1949). This very briefly was a rotund-shaped frame covered with dark cloth and open to the bottom. The tse-tse flies, attracted to this structure, swept underneath where they moved upward toward light coming from a slot at the top which led to a no-return chamber. Haufe (1960), describes a trap designed to exploit the optomotor responses of mosquitoes. He reports also the capture of Tabanidae but does not comment on the effectiveness of the trap for this group.

Tabanidae have also been taken in traps designed for the capture of other insects and which exibited no obvious attribute of the host. Frost (1936) captured Tabanidae in a trap designed for the oriental fruit moth. This trap was baited with sugar, water and small amounts of other chemical substances. The same author (1953) reported the capture of 17 species in a single light trap. Although none of these was represented in large numbers their occurrence in light trap catches indicates nocturnal activity.

A recent design of trap for the capture of horse flies is the helio-thermal trap described by Thorsteinson (1958). This trap and modifications of it were used extensively in this present study and will be further described under materials and methods.

Materials and Methods

The orientation behaviour of Tabanidae was studied by two methods: (1) by recording the numbers of horse flies captured in different modifications of the helio-thermal trap (Thorsteinson 1958); (2) by recording the numbers of Tabanidae attracted to objects of different color, shape, size and altitude. These objects were smeared with tangle foot which ensnared the insects striking them.

The helio-thermal trap was modified by placing a no-return at the apex of the tripod continuous with the canopy (Fig. 1 A), use of a black canopy in lieu of a translucent one, introduction of a black body below the canopy and further changes in the height and shape of this black body.

The no-return unit used in 1958 was constructed from a glass jar with an inverted funnel at the mouth. In 1959 this was replaced by a larger improved unit made from plastic. The black body was furnished by a laundry tub, (bushel size) painted black. This was inverted and placed on the ground under the canopy or in some cases it was raised slightly by supports to be visible above the tall grass. The moving black body was constructed from a tub of equal size fixed to a bicycle hub. The hub was fitted to a shaft; the other end of which was screwed into a heavy metal base. Four white anemometer cups were fastened to the sides of the tub and these in the presence of light breezes caught sufficient air to rotate the tub gently. (Fig. 1 D). This unit was raised in some cases by placing another black tub of shallower design underneath the base (Fig. 1 E). Another form of black body was supplied by a black cylinder 24" in diameter x 36" deep constructed from corrugated cardboard and suspended from the tripod so that about two thirds of its surface was exposed below the base of the canopy (Fig. 1 F). Since it was suspended loosely, light breezes would set it into a swaying motion. Further modifications were supplied by using black rather than translucent polyethylene for the canopy (Fig. 1 G). To provide motion in this trap a black cylinder was suspended from the tripod in the same manner as described for Type F (Fig. 1 H).

The traps were positioned at Harperville, Lake Francis, and Raeburn in 1958 and at Lake Francis, Raeburn, La Salle and the Whiteshell in 1959. The Whiteshell area is east of Winnipeg close to the Manitoba-Ontario border. It is a wooded area spotted by many lakes. All the other areas were pasture lands to the west and north of Winnipeg. At Raeburn, La Salle and Harperville the traps were placed in the open pasture. At Lake Francis there are numerous patches of trees and sloughs spotted over the area which gave a certain degree of variation to the ecology of different chosen sites.

Studies of the attractiveness to tabanids of objects of different size, shape, color and altitude were carried out in the Lake Francis area in 1959. A spherical shape was provided by a meteorological balloon

Figure 1. Sketches of the different modifications of the helio-thermal trap.

A. The original helio-thermal trap described by Thorsteinson consists essentially of an aluminum tripod supported by two aluminum rings. A black funnel with a killing bottle at the base is suspended from the tripod. The tripod is covered by a translucent polyethylene canopy from the apex to the lower ring.

B. A no-return unit is placed at the axpex of the canopy.

C. A black body (painted laundry tub) is placed below the canopy, the funnel is removed.

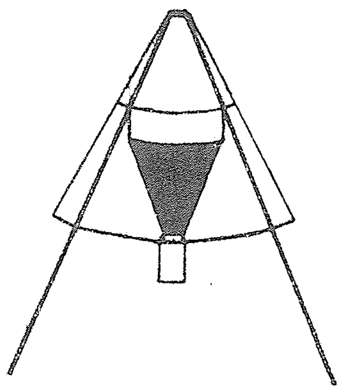
D. A rotating black body (white anemometer cups) placed under the canopy.

E. The rotating black body raised by placing on top of a shallow tub.

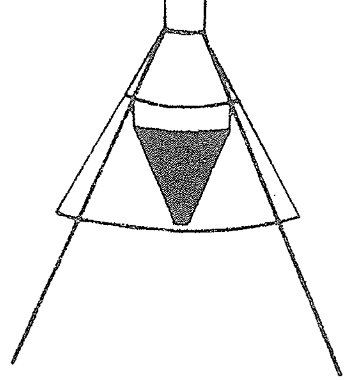
F. A black cylinder suspended from the tripod.

G. Black polyethylene used for the canopy instead of translucent polyethylene.

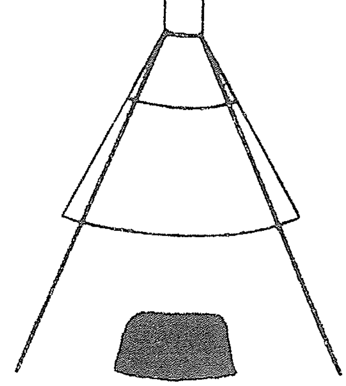
H. A cylinder suspended from the tripod of Type G in the same manner as type F.



A



B



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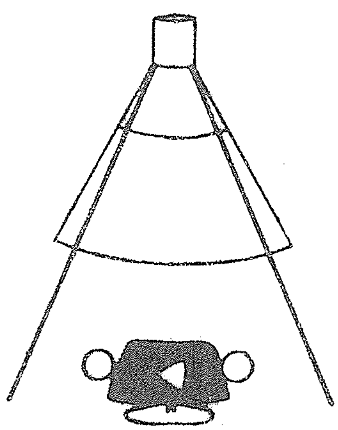

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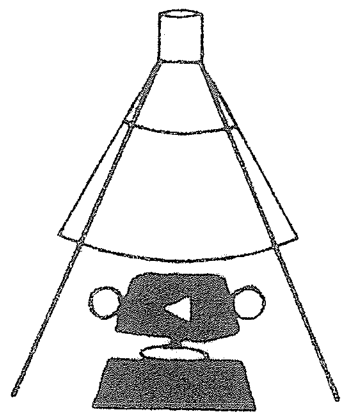

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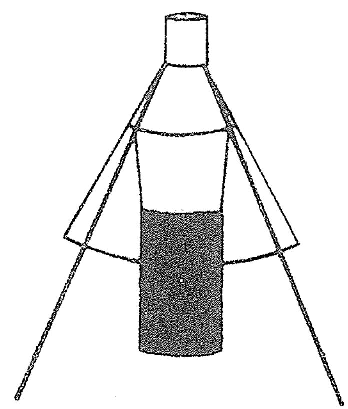
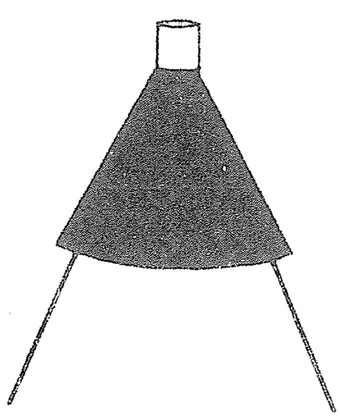
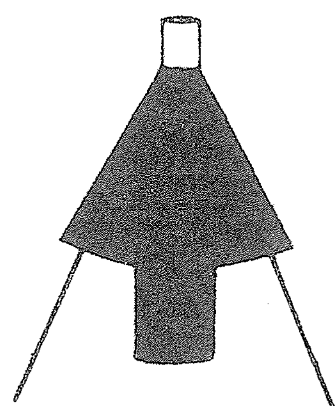


F



G



H

or a beach ball inflated to a diameter of 24 inches. These were painted the appropriate color and suspended by a suitable support. Cylindrical shapes were supplied by cardboard cartons 8 inches in diameter by 10 inches deep or larger ones 24 inches in diameter by 36 inches deep were constructed from corrugated cardboard. Flat surfaces were supplied from pasteboard supported by wooden strips.

The effect of color was studied by comparing the catches on spheres, cylinders and plaques of six different colors viz. black, red, green, blue, yellow and white. These were suspended in random order in a straight line two feet apart and three feet from the ground. The effect of altitude was studied by suspending red spheres at 4 ft., 10 ft. and 20 ft. from the ground. This was carried out in sheltered areas on days when the wind was not over 5 m.p.h. The effect of shape on the orientation of horse flies was studied by comparing the catches on spheres, plaques and cylinders of the same color and surface area in competition with each other.

The material was cleared from the traps every 5 to 7 days. The Tabanidae were classified to species, other biting flies to family and the remainder to order. Where suspended objects were used counts were made in unit time thus arriving at the number caught per hour. Only tabanids were counted and no attempt was made to recover the insects for species determination.

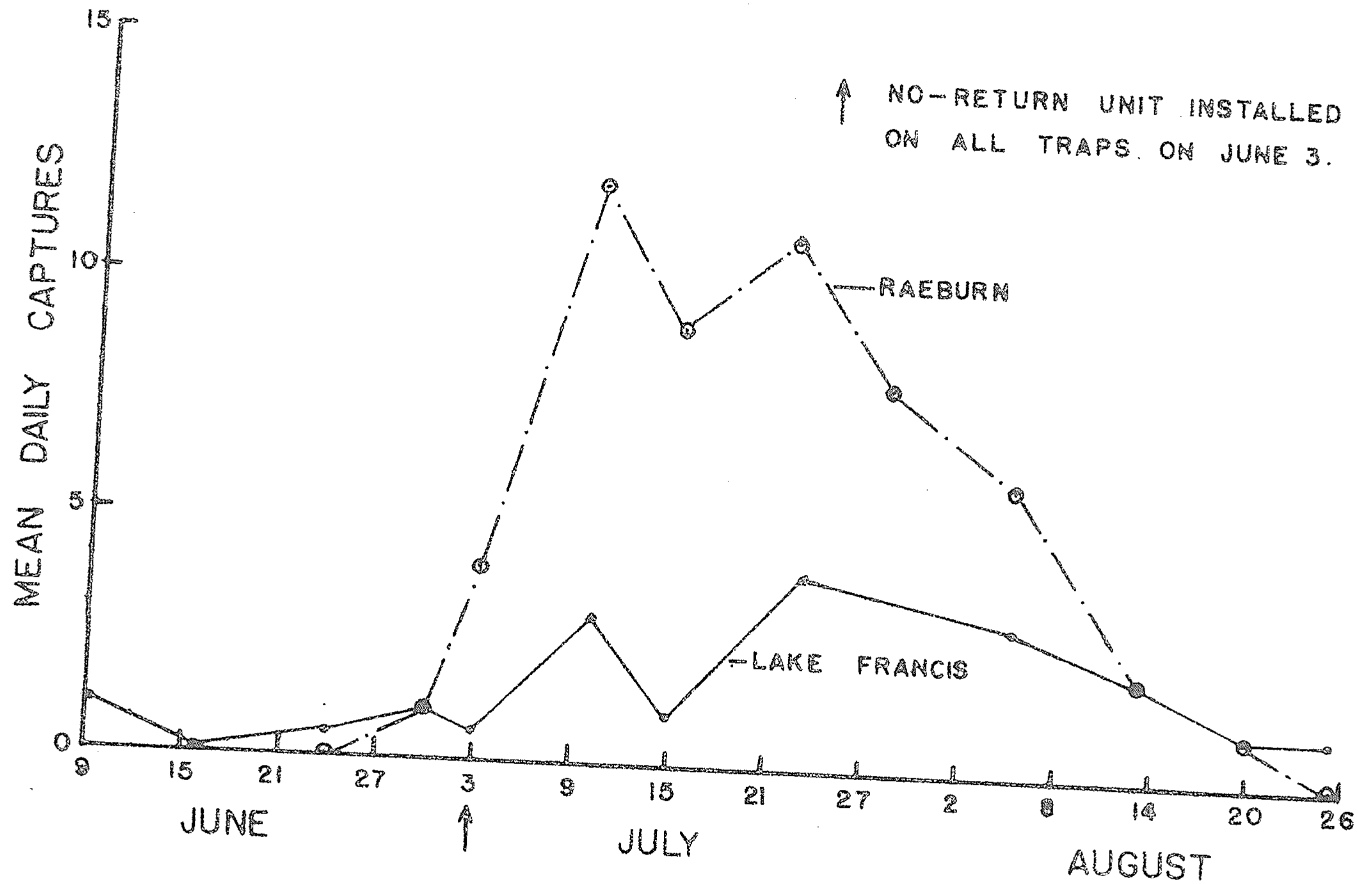
Results and Discussion

The captures for six traps at Raeburn and six traps at Lake Francis during the summer of 1958 are shown in graph form in Fig. 2. Initially these were all of the helio-thermal type. It can be seen that in this form the trap was unsuccessful in capturing large numbers. The obvious deficiency was the failure of the temperature within the canopy to rise sufficiently to cause heat prostration to the insects captured therein. The introduction of the no-return unit at the apex of the canopy trapped the flies here and the captures were substantially increased.

At this stage the trap was still considered a helio-thermal trap since its success was attributed to the attractiveness of the heat generated by the black funnel within the canopy. It must be remembered however that any trap presents a silhouette. This consideration became more important as the study progressed.

Late in the season of 1958 a black body was introduced under the canopy of two traps (Type C) at Lake Francis. This modification increased the catches by a factor of 9 to 1 (Table I, comparison B:C). The relative effectiveness of further trap designs employed in 1959 is shown in Table I. The total captures of Tabanidae for identical periods are compared as ratios. With the exception of those marked by an asterisk the traps compared were in the same ecological site in competition with each other.

Figure 2. The mean daily captures for six traps at Lake Francis and at Raeburn in 1958.



NO-RETURN UNIT INSTALLED ON ALL TRAPS ON JUNE 3.
RAEBURN
LAKE FRANCIS
MEAN DAILY CAPTURES
0
5
10
15
9
15
21
27
3
9
15
21
27
2
8
14
20
26
JUNE
JULY
AUGUST

Table I.

Total Captures of Modified Forms of the Helio-Thermal Trap

Compared as Ratios

Area	B:C	C:G	C:E	G:E	F:H	G:H
Whiteshell		1:7			1:5	1:13
La Salle		1:9				
Lake Francis	1:9+	1:1.7*	1:1*	1:3		
			1:3			

\+ Comparison made in 1958

* Ecological site of traps not the same.

Traps with black canopies were more effective than traps with translucent canopies (Comparison C:G). When motion was provided in the form of a rotating black body to a translucent canopy type (Type E), it then proved more effective than Type C and Type G both of which presented no motion. However, motion provided in the form of a black suspended cylinder in a trap with a black canopy (Type H) proved superior to Type F which had a translucent canopy and a similar black body and to Type G which presented no motion.

Fully evident in the results is the importance of a dark body and the increased attractiveness of traps presenting motion. The relative importance of thermal and visual stimulation provided by the black body remains to be resolved.

All of the effective traps presented a dark object in the form of a funnel, tub, cylinder or canopy. These black objects absorb solar energy and emit heat. As well as emiting heat the dark objects also present a silhouette which can be perceived visually.

The compound eyes of tabanids are well developed. Presumably they are endowed with color vision and can certainly distinguish dark from light objects. The increased response to traps presenting motion clearly implicates the importance of vision since a black body in motion will not become hotter than a stationary one.

If the color vision of tabanids resembles that of other insects that have been studied critically they probably do not perceive infra-red radiation by the eyes. However, responses to thermal radiation have been clearly demonstrated for Rhodnius, (also a hematophagous insect) the locus of perception being the antennae (Wigglesworth 1950). It is therefore not improbable that thermal stimuli are important for host orientation of tabanids, at least at short range. To test this possibility it will be necessary in some way to cool the outer surface of a dark body and compare its effectiveness to a warm one.

It will be noted from Table I that when the ecological site of the traps compared was not the same, some of the foregoing results did not hold. To illustrate this, the mean daily captures for two traps (Nos. 4 & 3) at Lake Francis are plotted against date in Fig. 3. Trap No. 3 (Type D) was sheltered to the north by trees but open to the south. Trap No. 4 (Type C) was positioned in a small clearing surrounded by trees about 30 yards from No. 3. Type D performed poorly compared to

Type C and on July 14th it was changed to Type E. For the remainder of the season this design proved more effective than Type C. The raising of the moving body could have been entirely responsible for the improved performance. However, the population density of horse flies may be higher in sheltered areas and/or some species may prefer certain trap types. In trap No. 4, 67% of the total tabanids captured were *Tabanus lineola scutellaris* whereas in trap No. 3 only 13% of the total were of this species. As the seasonal abundance of this species was replaced by *T. liorhinus*, *T. frontalis* and *T. frontalis septentrionalis*, trap No. 3 captured more of the latter species. Again it is interesting that in this area only traps which displayed motion captured *T. liorhinus*.

The effect of color on the orientation of Tabanidae is summarized in Table II. These results are compiled from the numbers of Tabanidae captured on spheres, cylinders and plaques of different colors. From this table it appears that the most attractive color is red. The results need some clarification however. The same number of tests was not done with all colors. This is important since the fly activity was not the same on all days. Also the black balloons which were used for spherical shapes became overheated with the result that the rubber became cracked, air was lost and the balloons shrank in size. It must be stated that until deflation of the black spheres occurred and when both colors were compared on the same day that black was equally as effective as red. Lighter colors, especially white and yellow were very ineffective and there is little doubt that black, red and blue are much more attractive.

Figure 3. Comparison of the mean daily captures of two traps in different ecological sites at Lake Francis 1959.

Trap # 3 Type D ____.____ Type E ___..___..___

Trap # 4 Type C __________

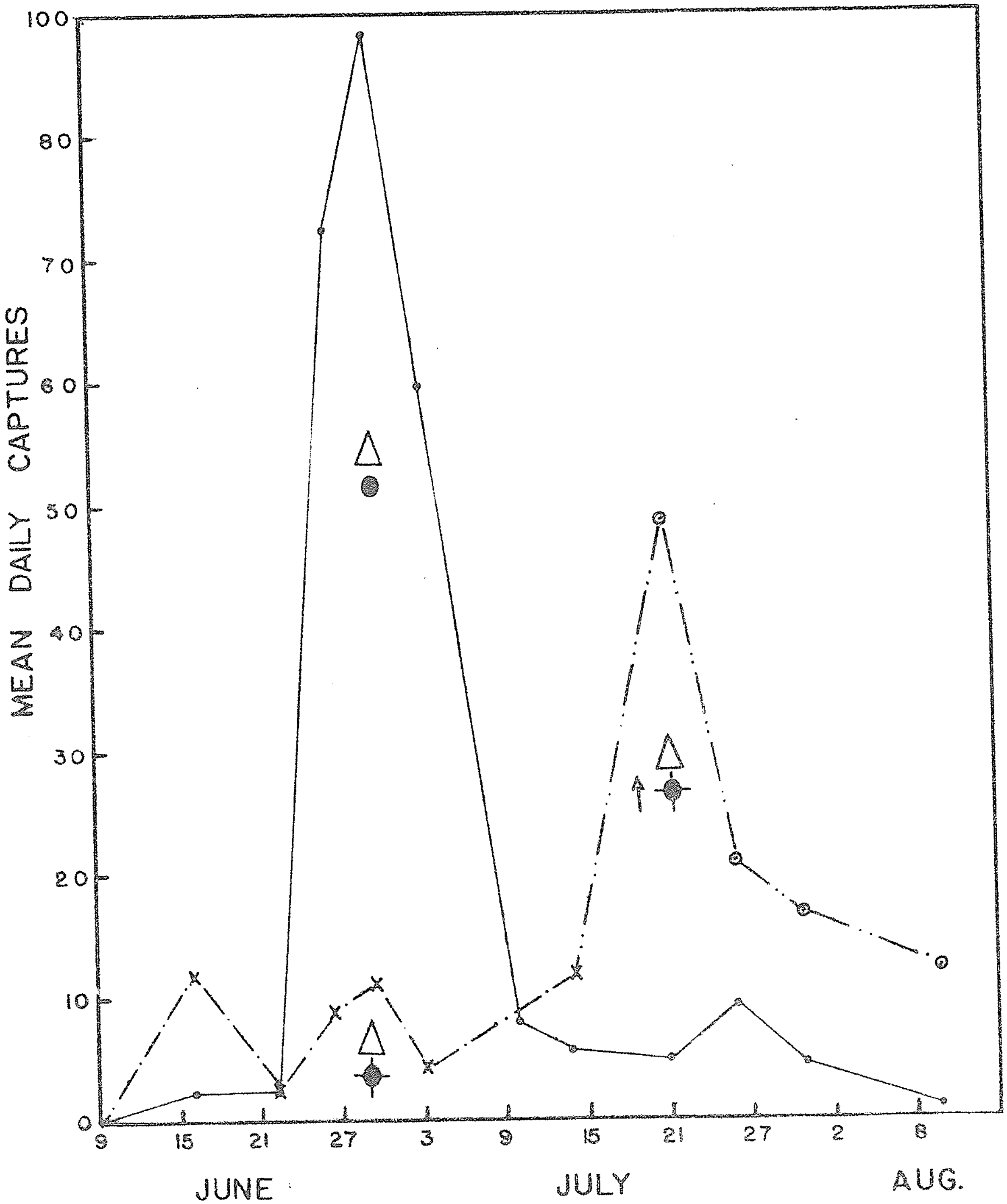

MEAN DAILY CAPTURES
100
80
70
60
50
40
30
20
10
0
9
15
21
27
3
9
15
21
27
2
8
JUNE
JULY
AUG.

Table II.

The Number of Horse Flies Caught Per Hour on Objects of Different Color

Color	No. of tests	Mean No. of Horse Flies caught per hour
Black	4	29.3
Red	7	39.1
Blue	4	9.6
Green	5	1.1
Yellow	5	0.2
White	5	0.73

Table III. shows the orientation of Tabanidae to spheres at different altitude. The most attractive altitude was 3 - 4 ft. from the terrain. The ineffectiveness of the spheres at higher altitude can not be attributed to wind since the trials were conducted in sheltered areas on days when the wind velocity did not exceed 5 m.p.h.

Table III.

The Number of Horse Flies Caught at Different Altitudes

Altitude*	No. of tests	Mean No. of Horse Flies caught per hour
3 - 4 feet	3	35.4
10 feet	3	3.3
20 feet	2	0.0

* Height from the ground to the bottom of the sphere.

The investigations of the effect of shape have not proceeded sufficiently to be conclusive. There are indications from the limited tests done that certain shapes were more attractive than others. In the comparison of a sphere, cylinder and plaque (all black) of the same surface area, the catches in two replications were as follows:

Sphere	Cylinder	Plaque
234	40	17
288	58	32

The preference of a cylinder to a plaque is not great and when a similar experiment of smaller objects of these two shapes were compared, (200 sq. inches) no preference was found. The spherical shape however is undoubtedly more attractive than either cylinder or plaque. The highest catch recorded on a suspended sphere was 401 in a period of $2\frac{1}{2}$ hours; a much higher catch than has been recorded by an other trapping device used in this study.

It has been shown that the presentation of motion increases the effectiveness of the modified helio-thermal traps. Since motion of varying type and degree is imparted by wind to the different shaped objects, their relative effectiveness might not be due to shape alone. More careful investigation will be required to make certain of the relative effectiveness of shape.

Taxonomic Data Yielded by Traps

The tabanids caught in the traps in four areas were identified and classified taxonomically through the assistance of Dr. L. L. Pechuman. Table IV shows the species identified and their frequencies as a percentage of the total captures. By far the largest number of species were taken in the Whiteshell area, especially of the *Chrysops* genus. In the other three areas only one species of this genus viz. *aestuans* (not taken in the Whiteshell area) was captured. At La Salle and Raeburn *C. aestuans* made up a high percentage of the captures. *Tabanus lineola scutellaris*, *T. liorhinus* and *Atylotus incisuralis* also seem restricted to the prairie regions whereas *T. typhus* which made up almost 50% of the total captures in the Whiteshell did not appear in these areas at all.

Collections could not be made regularily because of the excessive travelling involved. However, regular collections were made at Lake Francis and the seasonal distribution of the five most abundant species in this area is shown in Fig. 4.

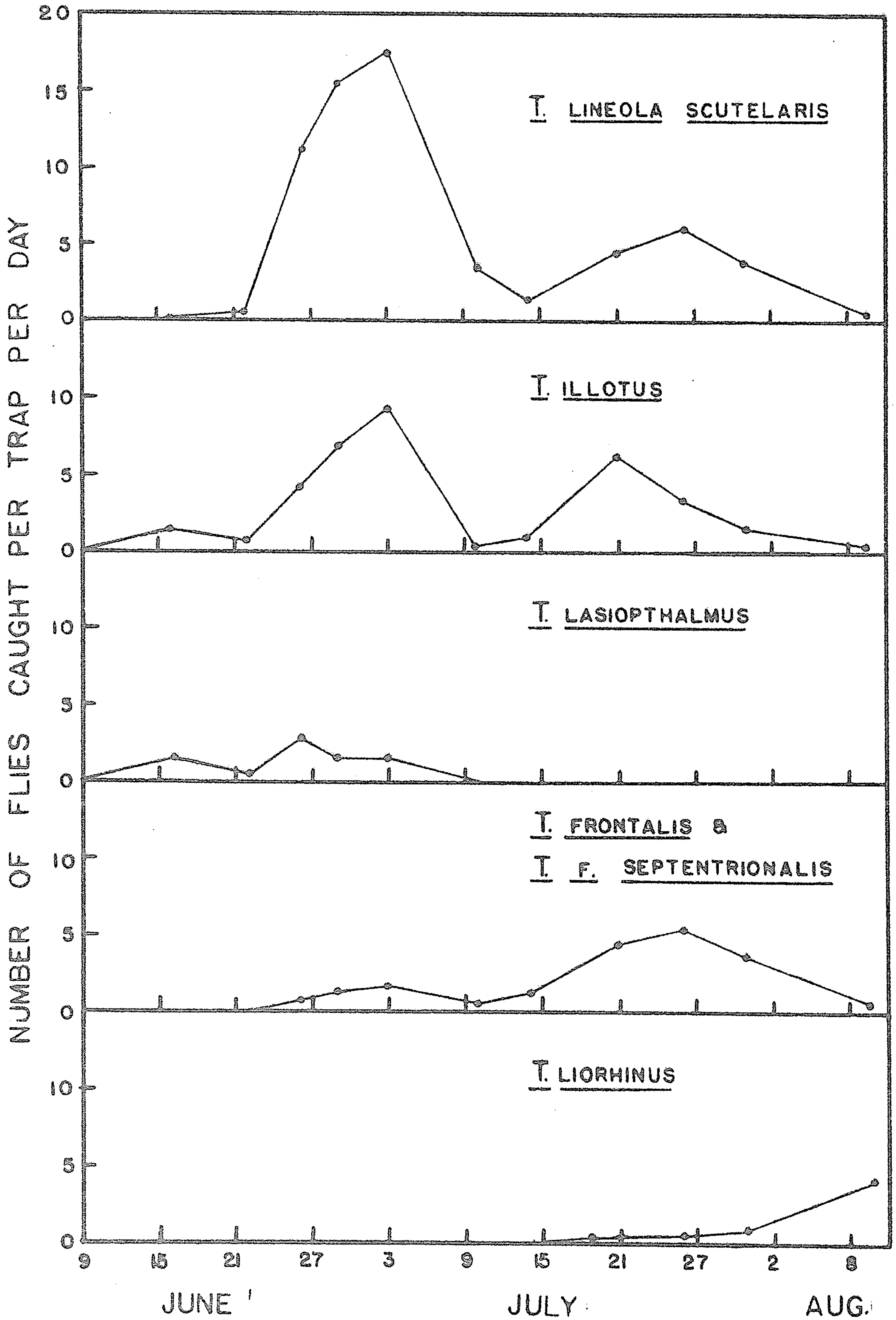
A total of 30 species has been taken in the traps. This does not represent all the species reported from Manitoba but it is possible that the missing ones do not occur in the areas covered. As previously mentioned there is some indication of species preference for different forms of the trap but this is bound to occur with any survey device. Another taxonomic disadvantage of the traps is that they collect females only. Overall however, there is good reason to conclude that the more effective forms of the trap will be valuable both for estimation of population density and relative abundance of species.

Table IV.

Species of Tabanidae Captured in Traps (Manitoba 1959)

Species	Whiteshell	La Salle	Raeburn	Lake Francis
Tabanus (Hybomitra) affinis Kirby	0.9	--	--	--
" " epistates O.S.	4.7	--	0.8	3.5
" " frontalis Wlk. & F. septentrionalis Lw.	0.1	5.1	27.4	16.4
" " gracilipalpis Hine	2.0	--	--	--
" " illotus O.S.	2.9	1.6	1.7	--
" " lasiopthalmus Macq.	3.1	4.1	0.7	27.1
" " lineola scutellaris Wlk.	--	33.9	7.3	4.8
" " liorhinus Philip	0.4	--	0.1	38.0
" " microcphalis O.S.	0.1	--	--	6.3
" " nudus McD.	4.4	--	--	--
" " trepidus McD.	0.4	--	--	1.7
" " typhus Whitn.	47.3	--	--	--
" " zonalis Kirby	0.4	--	--	--
Tabanus (Tabanus) marginalis Fab.	1.6	--	--	--
" " fulvicallis Philip	0.1	--	--	--
Chrysops aestuans Wulp.	--	55.3	44.8	1.1
" dawsoni Philip	0.1	--	--	--
" excitans Wlk.	4.4	--	--	--
" frigita O.S.	10.0	--	--	--
" furcata Wlk.	0.3	--	--	--
" inda O.S.	11.2	--	--	--
" montana O.S.	2.3	--	--	--
" mitus O.S.	0.5	--	--	--
" nigripes Wied.	2.3	--	--	--
" sackeni Hine	0.1	--	--	--
" venus Philip	0.4	--	--	--
Chrysozona americana	0.1	--	--	--
Atylotus incisuralis (Macq.)	--	--	17.5	1.5
TOTALS	1386	506	1009	2088
No. of Traps	4	2	3	4

Figure 4. The seasonal distribution of the five most abundant species of Tabanidae captured at Lake Francis 1959.



NUMBER OF FLIES CAUGHT PER TRAP PER DAY
T. LINEOLA SCUTELARIS
T. ILLOTUS
T. LASIOPTHALMUS
T. FRONTALIS &
T. F. SEPTENTRIONALIS
T. LIORHINUS
0
5
10
15
20
9
15
21
27
3
9
15
21
27
2
8
JUNE
JULY
AUG.

SECTION II. FEEDING BEHAVIOUR

Review of Literature

The study of gustatory behaviour per se in hematophagous insects has received little attention. Much of the work on chemosensory factors has been devoted to the identification of attractive vapours emanating from the host. Krijgsman (1930) found that moisture, CO_2, heat, animal odour, blood odour and plasma odour were attractive to the stable fly *Stomoxys calcitrans* Linn. to some degree. He observed also that many of these substances stimulated a piercing response and that serum elicited feeding. Schaeffenburg and Kupka (1951), recognizing the fact that blood was itself attractive, reported the isolation of a "blood factor" which was highly attractive to both the stable fly and to the mosquito *Culex pipiens*. Unfortunately the method of extraction of the substance was not described and no further publications on the subject have appeared.

An intensive study of the factors stimulating host approach of *Aedes aegypti*, has been carried out by Peterson and Brown (1951), Smart and Brown (1956) and Burgess and Brown (1957). Here again CO_2, heat, moisture, sweat, blood and plasma were found to be attractive. Significant in the findings of Burgess and Brown was the fact that plasma was attractive whereas washed corpuscles were not, indicating that an attractive factor is contained in the plasma.

None of these investigations has been specifically concerned with gustatory behaviour however. Although feeding on sugar solutions has been reported for *Tabanus sulfricons* by Frings (1936) and for *T. affinis* by Hocking (1953) no mention of the acceptability of blood is made by these authors. Difficulty in the feeding of captive tabanids on the host is reported by Webb and Wells (1924) and more recently by Miller (1951). Webb and Wells observed tabanids resting on a fresh pelt hung out to dry but whether these were feeding on blood or not was not mentioned.

In the artificial feeding of mosquitoes many workers have found it necessary to add sugar or honey to the blood in order to induce feeding (McLintock 1952, Bruchan *et al* 1956). Tashirus (1959) has been successful in obtaining a high percentage of blood feeding with *Aedes aegypti* through animal membranes. Here it was necessary to maintain the blood close to body temperature. In this regard the stable fly is much more responsive and feeds readily on free blood at room temperature.

An interesting investigation has been carried out by Hosoi (1959) concerning the relative palatability of blood fractions to the mosquito *Culex pipiens* var. *pallens* (Coquillett). This worker found that plasma was not accepted by the mosquito but that washed corpuscles in physiological saline were fed on to satiation. He then proceeded to fractionate the corpuscles, isolating an active fraction which he found to be rich in nucleotides. The author reports that 49% of the mosquitoes exposed fed to satiation on a 0.0001 M concentration of adenosine-5-phosphate in 0.15 M saline. Lower percentages were obtained with the related substances adenosine diphosphate and adenosine triphosphate. This work will be referred to further in the discussion of the feeding results obtained with the stable fly.

Observations on the Feeding Behaviour of Tabanidae

Female Tabanidae were captured in the field and brought to the laboratory for study. Individuals tethered to a flight mill accepted water and sugar solutions readily but rejected citrated blood, human blood (fresh) and mixtures of blood and sugar. Additional flight exercise on the mill did not induce blood acceptance but more sugar solution or water was then taken.

In the field females of _Tabanus lineola scutellaris_ that had commenced piercing the arm accepted citrated blood from a capillary tube which was presented carefully to the mouthparts as the insect pierced. If the individuals were disturbed or confined they could not be induced to continue feeding.

Caged individuals were induced to feed on sugar from a capillary tube held to the screen. If, when feeding had commenced this was replaced by a tube containing blood the insect would move from the food source and refuse to feed. However by breathing lightly on the insect it could in some cases be induced to make piercing movements with the mandibles. If it could be so stimulated and a capillary tube containing blood touched to the mouthparts at the same time, the insect would commence to feed, proceeding rapidly to satiation.

Apparent from these observations is the fact that the previous excitation of piercing movements is a pre-requisite to blood acceptance. On the other hand water and sugar solutions require no external preconditioning stimuli. The presence of the critical stimuli which stimulate piercing must lower the threshold so that the receptors can mediate the response to blood.

It is difficult to be certain of the factors which stimulate piercing in these insects. The odour of blood which has been found to evoke piercing in the mosquito Aedes aegypti and the stable fly does not do so with the Tabanidae. The odours and presumably the CO_2 in breath does evoke this reaction in tabanids. A piece of paper towelling that had been rubbed over the body of a cow also was found to induce piercing in a limited number of individuals. Thus it appears that CO_2 and odours of the body present the important stimuli. However a large number of individuals especially T. lineola scutellaris have been observed to pierce the napped lining of the interior of an automobile as it stood in the field. In this instance no perceptible odour was present and apparently warmth and texture in the absence of host odour can, under these conditions, provide adequate stimulus to evoke piercing.

Studies on the Feeding Behaviour of the Stable Fly Stomoxys calcitrans. Linn.

Materials and Methods. Stable flies were reared using methods which closely followed those described by Campeau et al (1953) and Champlain et al (1954). The adults were maintained in cages 13" square by 11½" high, screened on the top and two sides. A solution of approximately 10% sucrose was provided for the nourishment of the adults. Blood meals were provided once daily using out-dated human blood obtained from the Canadian Red Cross. The blood was soaked up on a wad of cotton batting which was placed on the top screen of the cage. The adults fed readily from the cotton through the screen from below. A small petri dish was placed over the cotton to prevent excessive drying of the blood.

Breeding medium for the larvae was prepared from a mixture of chopped cereal grains and supplements. The mixture was moistened and fermented with Bakers' yeast until the fermentation had stopped. Wood shavings were mixed with the medium to absorb excess moisture and improve aeration. The prepared medium was placed in large evaporating dishes (6.75" in diam. 3.25") to within one inch of the top. This was placed in the cage and the adults deposited their eggs directly in the medium. It was found necessary to prevent overcrowding of the larvae otherwise the resulting pupae and adults were undersize.

Due to the occurrence of a mite (predacious on the adults) which often built up to injurious numbers, the pupae were removed frequently from the medium and transferred to a clean container for emergence. For the most part this parasite was not present in sufficiently large numbers to warrant this procedure and the pupae were allowed to remain where they were formed.

Radiation from a sun lamp* was provided over the cage containing the adults for a period of six hours daily. This greatly improved mating and egg laying. The temperature in the rearing room was 80°F; the relative humidity varied from 65 to 80%

Capillary tubes were used to obtain volumetric measures of feeding. The inner diameter of the tubes was determined and from this the cross

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* Sylvania Sun Lamp 110-115 V. A.C. 275 W.

sectional area computed. This factor was multiplied by the displacement of the fluid in the tube during feeding giving the amount consumed in lambda.

Tethering of the insects and presenting the capillary tube to the mouth parts was not favoured since it is time consuming and injury to the insects may occur during the process. Instead the insects were placed in an opaque chamber into which the open ends of the capillary tubes extended and were permitted to find the food source on their own. A circular transparent area was provided around the openings of the capillary tubes. The stable fly, attracted to light, frequents this area and its chances of finding the food source are increased. A diagram of the apparatus is shown in Fig. 5. The box is constructed from black plastic and has a removable top. A partition divides the box into two chambers each being served by a transparent area which has two small holes for the insertion of capillary tubes. Twelve such boxes were constructed giving a total of 24 chambers.

Flies six to ten days old were used in the tests. Individuals which had fed to satiation on blood were transferred to a separate cage, supplied with water and tested twenty four hours later. The tests were conducted using one insect per chamber. The time for feeding was restricted to ten minutes in most experiments to minimize evaporation error. Since blood and some of the fractions crusted over in four to five minutes, two presentations of these substances were necessary to bring the time they were available up to the ten minute standard.

Not all of the insects fed even when whole blood was presented. This was often due to the failure of the insects to find the food source

Figure 5. A sketch of the apparatus used to determine individual volumes consumed by the stable fly.

A - capillary tubes

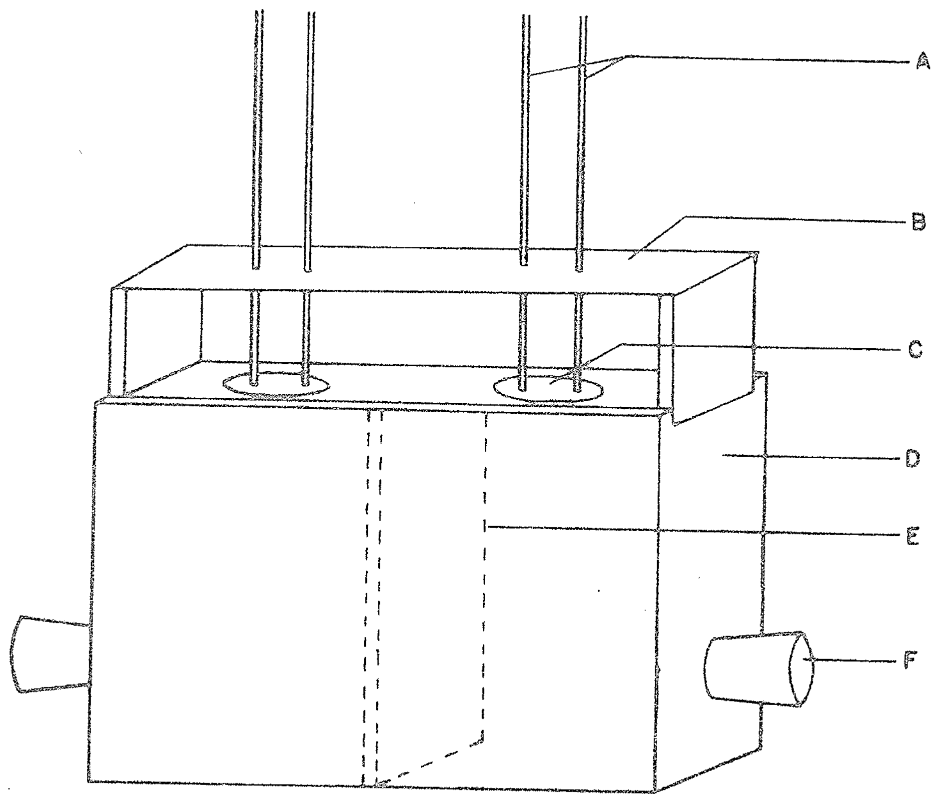
B - holder

C - transparent area

D - opaque chamber

E - partition

F - cork covering entry hole


A
B
C
D
E
F

rather than its rejection. Some individuals were not as active as others, preferring to rest on the sides of the chamber to the investigation of the clear area where the food was presented. The result is that zero values occur in the data. To treat the data statistically all readings have been transformed viz. $X_i = \sqrt{0.5 + x_i}$ to avoid skewness and zero values (Goulden 1945). This transformation has been made in all cases where statistical analysis is used.

All fractions except serum (obtained from freshly drawn blood) were prepared from out-dated Red Cross blood. This blood is treated by the Red Cross with A.C.D.* solution for preservation.

Results and Discussion. The stable fly differs from most hematophagous insects in that both sexes feed on blood. If the sexes differ in their response this would cause additional variability in the results. In a preliminary experiment, 48 individuals were fed on blood, after which they were killed and the sex determined. Considering only those insects which fed, there was no significant difference between the mean volume taken by either sex (males 10.0 lambda, females 12.4 lambda). A slightly higher percentage of the males fed (males 52%, females 44%) but since tests flies are selected at random both sexes have an equal chance of occurring in any given treatment. It was therefore felt that sexing of the flies was unnecessary, at least for the generalized experiments conducted in this study.

* A.C.D. solution contains 1.76 grams dextrose 1.58 grams sodium citrate and 528 mgms. of anhydrous citric acid disolved in 120 cc. of water. This is added to 380 cc. of blood.

The importance of the presence and integrity of the formed elements in blood (i.e. the blood cells) was first investigated. Whole blood, plasma, reconstituted blood and hemolyzed blood were compared. The blood was reconstituted by adding the cells back to the plasma. Hemolyzed blood was prepared by freezing to fracture the cells. The transformed means are shown in Table V. exp. 1. The responses to whole blood, reconstituted blood and hemolyzed blood do not differ significantly but all are more acceptable than the plasma. These results indicate that the particulate nature of the cells is not an important factor but that some other property, presumably chemosensory, is essential for a maximal frequency of normal feeding responses.

Although the mean feeding responses to plasma is less than to blood, some of the responses to plasma are equal to the best responses obtained for whole blood. This implies that plasma provides an adequate stimulus to some of the individuals possibly those whose response thresholds have, at the time, assumed low levels. However the chemosensory stimulus provided by plasma is deficient in some respect necessary to elicit feeding from flies having higher thresholds.

Further experiments were carried out with the plasma by testing fractions which contained reduced protein concentration. A comparison of plasma and serum showed no significant difference thus eliminating the possible importance of the clotting protein fibrinogen (Table V. exp. 2). The proteins were precipitated from the plasma with acetone. After removal of the acetone the supernatant, readjusted for volume with distilled water, was compared to plasma. The responses were significantly

Table V.

Feeding Responses of the Stable Fly to Blood Fractions

Exp.	Treatment	Treatment means*	No. flies per treatment	L.S.D 5%	L.S.D 1%
1	blood	2.97			
	plasma	2.03			
	reconstituted blood	3.02	24	5%	1%
	hemolized blood	2.95		0.50	0.66
2	plasma	2.41			
	serum	2.46	24	0.40	0.54
3	plasma	2.26			
	acetone extract of plasma	1.62	24	0.41	0.55
4	blood	2.74			
	cells in 0.15 M saline	2.24			
	cells in H_2O hemolized	1.82			
	cells in 0.15 M saline (hem.)	2.19	36	5% 0.44	1% 0.58

* Means of transformed data

lower than to normal plasma (Table V. exp. 3). Since this preparation would be almost completely free of protein it would seem that proteins may be of some significance in the response to plasma. This is not conclusive however since other active substances may have been precipitated by acetone.

Turning to the formed elements an experiment was conducted to investigate the palatability of cells. Blood cells washed four times with saline then suspended in 0.15 M fresh saline and compared with whole blood. Also compared in this experiment were cells suspended as previously described but hemolyzed and cells washed with saline four times and suspended in water. Since the cells hemolyze immediately when placed in water this preparation is similar in this respect to the hemolyzed cells in saline.

The results (Table V. exp. 4) show all of the cell fractions to be less acceptable than whole blood, but the cells hemolyzed in water rather than saline are least palatable. Again no difference is shown between the intact and fractured cells when saline is present. The possible importance of the presence of saline will be considered later.

Examination of the individual responses to washed cells in saline shows them to be similar to those for whole blood (see Table VI). A higher frequency of zero values occurs in this treatment and the reason for this has not yet been investigated. From a gustatory standpoint however, highly effective factors appear to be associated with the cells.

Since the intact cells are accepted and since this palatability is not lost when the cells are fractured the question of whether attractive substances diffuse from the cells into the surrounding medium comes to mind. A preliminary experiment was conducted to test this hypothesis. Cells washed three times with saline then suspended in fresh saline were compared with the saline from the third washing and with blood as a positive control. The complete results are shown in Table VI. It can be seen that the washings are much less palatable than the other two substances. However four responses approach those commonly obtained for blood indicating that stimulating substances do diffuse from the cells into the saline.

Hematophagous flies generally respond to saccharine fluids such as the nectar of plants. The sugar glucose is known to occur in the blood at a concentration of the order of 0.005 M. A series of glucose concentrations were tested. Six concentrations beginning at 0.5 M and decreasing by one fifth each time to 0.00016 M were used. The results shown in Fig. 6 suggest a bimodal response to this substance (Beck 1956). However it appears to be least effective at the molar concentration found in blood. Glucose therefore does not account for the insect's response to blood, but it remains to be seen whether it interacts with other chemosensory stimuli.

Comparing the foregoing results with those obtained by Hosoi (1959) for the mosquito, an obvious difference is the response to plasma. The stable fly shows irregular but in some cases full responses to plasma whereas the mosquito does not respond to this fraction at all. In both insects the particulate nature of the cells in regard to palatability is not significant but hemolyzed cells in saline are more acceptable

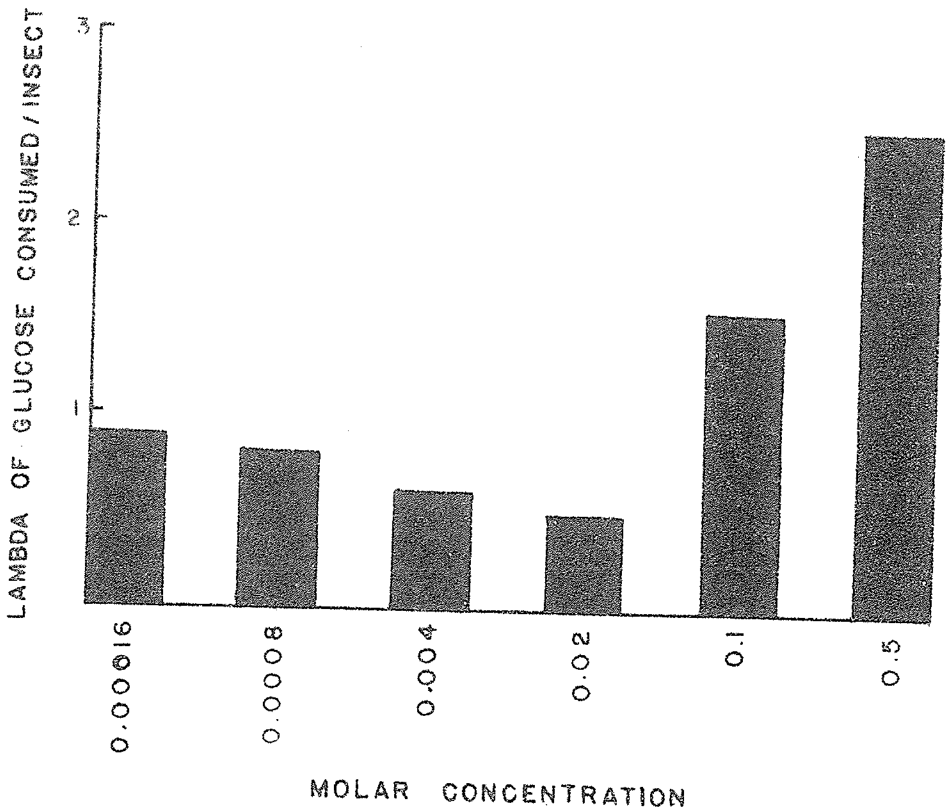
Table VI.

Feeding Responses of the Stable Fly in Lambda to Whole Blood, Cells in Saline and Saline Washings

Whole blood	Cells in saline	3rd saline washings
10.4	14.1	2.2
18.5	0.0	1.5
12.6	0.0	3.0
5.2	0.0	0.0
11.1	17.0	5.2*
19.2	14.8	11.1*
21.5	0.0	5.9*
0.0	0.0	0.0
16.3	0.0	3.7
9.6	0.0	0.0
14.1	14.1	2.2
11.8	0.0	0.0
14.1	0.0	1.5
0.0	14.1	0.0
20.0	12.6	0.0
0.0	0.0	0.7
11.8	15.5	0.0
0.0	0.0	2.2
19.2	5.2	1.5
6.7	0.0	0.0
11.1	11.1	1.5
0.0	0.0	0.0
11.1	14.9	5.2*
17.0	12.6	0.0
Means		
10.9	6.1	1.6

* Values which approach the responses to whole blood

Figure 6. The responses of the stable fly to varying concentrations of glucose.


LAMBDA OF GLUCOSE CONSUMED / INSECT
3
2
1
0.00016
0.0008
0.004
0.02
0.1
0.5
MOLAR CONCENTRATION

than cells in water. Hosoi suggests that this effect is due to the suitable osmotic pressure supplied by saline at the physiological concentration. He found also that 0.15 M saline by itself exhibited a slight degree of blood like stimulation.

Physiological saline was tested to see if it was stimulatory to the stable fly. Saline was compared with distilled water in two paired tests. The transformed means were as follows: saline 0.85, 0.97, water 0.86, 0.92. The means do not differ significantly and both are very low compared to the usual response to blood. Saline itself cannot therefore be considered stimulatory to the stable fly.

The most striking result obtained by Hosoi was his demonstration that adenosine monophosphate at a concentration of 0.0001 M and dissolved in saline can account for the entire response of the mosquito to whole blood. Similar preparations of the related nucleotides adenosine di- and triphosphate were somewhat less effective.

A preliminary test was conducted to see if these effects might apply equally to the stable fly. AMP and ADP at the same concentrations effective for the mosquito were dissolved in saline and compared with saline alone and blood. The results shown in Table VII indicate that the response to the nucleotides is little better than that of saline and falls far short of that obtained for blood. It appears that the stimuli which induce feeding to satiation by the stable fly differ from those found by Hosoi for the mosquito _C. pipiens_ var. _pallens_.

Table VII.

Feeding Responses of the Stable Fly in Lambda to Adenosine Monophosphate (AMP), Adenosine Diphosphate (ADP), Saline and Whole Blood

AMP* 0.0001 M	ADP* 0.0001 M	0.15 M NaCl	Blood
3.0	0.7	0.0	7.4
0.7	3.0	0.7	9.6
0.0	0.0	1.5	14.8
0.0	1.5	0.0	9.6
0.7	0.7	0.0	8.9
0.7	0.0	0.0	7.4
0.7	0.0	0.0	16.3
3.0	3.0	0.7	12.5
1.5	4.4	0.0	0.0
0.7	0.0	1.5	0.0
1.5	0.0	0.7	11.1
0.7	0.0	0.0	10.4
0.0	0.0	2.2	8.1
1.5	0.0	2.2	11.1
3.7	1.5	0.0	9.6
0.0	1.5	1.5	5.9
2.2	0.0	0.0	11.1
0.0	3.0	0.0	6.7
Means			
1.1	1.1	0.6	8.9

* These substances were disolved in 0.15 M NaCl

SUMMARY

The host orientation of tabanids was studied by comparing the numbers taken in modified forms of the helio-thermal trap and the numbers captured on sticky objects of different color, altitude and shape. Traps which presented a dark silhouette in the form of a black canopy or in the form of a black body placed under a translucent canopy proved more effective than the original design. Motion supplied by a rotating black body placed below the canopy or by a black cylinder suspended from the tripod so that two-thirds of its surface was visible below the canopy increased the effectiveness further. The results indicate that visual stimuli are of prime importance in the host orientation of tabanids from a distance although thermal stimuli may be important at close range.

There are indications that some trap designs are more attractive to certain species than to others. Supporting this contention is the fact that *T. liorhinus* was taken predominately in traps which presented motion whereas *T. lineola scutellaris* was taken in large numbers in traps which presented no motion.

The Tabanidae taken in the traps from four areas of Manitoba were identified. Thirty species were determined which represents three-quarters of the total number previously reported by taxonomists for Manitoba. Considering the relatively small area sampled there is reason to believe that the modified form of the trap will be attractive to additional species in this province. As such it is a valuable instrument for the evaluation of the seasonal abundance and the population density

of tabanids as well as for the study of their orientation behaviour.

Objects colored red, black or blue were more attractive to horse flies than those colored green, yellow and white. Objects suspended within five feet of the terrain are more attractive than objects at a higher altitude. Spherical shapes were highly preferred to cylindrical or flat shapes. This preference might also be accounted for by varying degrees of motion imposed by wind on the different shapes.

The limited studies on the feeding behaviour of tabanids showed that although individuals in captivity will accept water or sugar solutions, blood presented under the same conditions is refused. If the insects are first stimulated by CO_2 and odour to make piercing movements with the mandibles, blood is then accepted. Horse flies apparently require certain preconditioning stimuli to elicit a feeding response to the chemosensory stimuli of blood.

The feeding responses of the stable fly to blood fractions was studied by measuring the actual volumes consumed by individual insects. The mean value for plasma is less than that for whole blood but it does stimulate some insects to feed to satiation. No difference was found between serum and plasma but if the protein of the plasma is precipitated by acetone much of the palatability is lost. This does not prove conclusively that protein is responsible for the acceptability of plasma since other stimulating substances may have been precipitated by the acetone.

Centrifuged cells reconstituted in plasma or in saline were substantially as acceptable as whole blood. The more attractive factors appear to be associated with the blood cells. Hemolysis of the cells does not reduce their effectiveness either in saline or plasma but hemolyzed cells when presented in water rather than saline are less acceptable. The presence of saline therefore is in some way contributary to the palatability of the cells. However, saline tested alone is no more attractive than distilled water.

Glucose was presented to the stable fly in six different concentrations. It was found to be least effective at the concentration at which it occurs in blood. Glucose itself cannot therefore account for the response of the stable fly to blood although it may well interact with other constitutents.

Hosoi reports that the mosquito Culex pipiens var. pallens Coquillett does not accept plasma and in this respect the gustatory behaviour of the stable fly differs. The nucleotides adenosine monophosphate and adenosine diphosphate which were shown by Hosoi to account for the gustation of blood by the mosquito were not found to be stimulatory for the stable fly. Further investigations must be conducted to determine whether the feeding response of the stable fly is regulated by one specific substance as in this mosquito or by a combination of chemosensory stimuli.

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