VISUAL AND OLFACTORY RESPONSES OF THE HORN FLY, *Haematobia irritans* (Linn.)

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LOUIE THOMAS HARGETT

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APPROVED: Redacted for privacy

Assistant Professor of Entomology

In Charge of Major

Redacted for privacy

Chairman of Department of Entomology

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

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Typed by Eloise Allison

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If we use excessively elaborate apparatus to examine simple natural phenomena Nature herself may escape us.

Karl Von Frisch

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VISUAL AND OLFACTORY RESPONSES OF THE HORN FLY, *Haematobia irritans* (Linn.)

I. INTRODUCTION

In our constant struggle against harmful parasitic insects it is no longer sufficient to know only the life cycle of the insect, rather the whole complex pattern of the biology of parasites must be investigated. Biochemical, physiological, ecological and behavior studies are of equal importance in understanding parasites and initiating adequate control measures against them. It is for this reason that the research which follows was designed to investigate more completely the behavior of the horn fly, *Haematobia irritans* (linn.).

The horn fly is an obligate cattle parasite which was introduced into the United States during the latter part of the last century. It rapidly spread from the point of entry, apparently Philadelphia, throughout the rest of the country. By the turn of the century it was found in almost every state as well as Puerto Rico and Canada. A similar pattern of distribution is now occurring with the face fly *Musca autumnalis* DeGeer.

The adult horn fly leaves the host only to oviposit on fresh manure (possible exceptions will be discussed later). Eggs hatch within 24 hours and the larvae pass through three instars, pupating in the third instar larval skin in

3-5 days. Adult horn flies emerge within 8-21 days after oviposition, with the largest portion emerging in 10-15 days, depending on the temperature. This parasite apparently overwinters in the southern United States as an adult, while in the northern United States and Canada it overwinters as a diapausing third instar larva or as a pupa. For a complete review of the biology of the horn fly see Hammer (26, p. 141-393) and McLintock and Depner (39).

The specific aims of this research are: (1) to develop a technique for rearing horn flies throughout the winter as well as summer months; (2) to determine if visual and olfactory stimuli are important in directing the insect to its host; and (3) to determine what other factors might be important in directing the horn fly to its host. The research will be presented in the order given above.

II. LITERATURE REVIEW

Rearing Technique

The economic importance of the horn fly led to efforts to rear it under artificial conditions. Glaser (21)(22) obtained first generation horn flies by allowing wild flies to oviposit on fresh cow manure. These flies were fed twice daily on fresh beef blood and lived from 2-25 days. No mating or oviposition was observed at any time among laboratory reared flies. Bruce (6) fed adult horn flies defibrogenated blood heated to 25-40°C., however, he was apparently unable to rear them. Cow manure dropped any time of the year was a suitable larva rearing medium (40). Attempts to rear the flies in cages on the back of the host did not prove practical, as many flies died and most eggs were lost. The eggs which were recovered did not hatch. Laboratory reared flies were obtained by Stirrat, McLintock, Schwindt and Depner (50) by placing eggs from wild flies on manure which was placed in battery jars. They did not attempt to rear second generation flies. Lewis (33) obtained laboratory reared flies by collecting eggs from wild flies and putting them on fresh manure and holding at 80°F until pupation. The pupæ were then placed in a screened enclosure with the host and the adults were

allowed to emerge for three days. No references were noted where anyone successfully reared horn flies on artificial media or where they reared them throughout the winter where they overwinter as larvae or pupae.

Visual Responses

The study of the visual responses of insects has proceeded in three main directions: (1) the response of insects to various objects; (2) the response of insects to different wavelengths of light; (3) the response of insects to light of different intensities. The first of these probably gives us more insight into the relationship between vision and behavior of the insect. The second is more important in explaining the physiology of vision and correlating it with the behavior of the insect. The last of these is important in understanding the limitations and results of the first and second.

The tsetse fly, Glossina swynnertoni, Austin, was stimulated by the sight of oxen at 110 and 180 feet but not at 300 feet (2). The honey bee is able to distinguish between different objects, especially those resembling blossoms (52, p. 63-84). Sippell (48) demonstrated that Aedes mosquitoes were attracted to a clear sealed plastic box containing a mouse rather than to an empty control box. Aedes mosquitoes suspended from a silk thread oriented

toward and followed a moving black stripe on a white background. Moreover, the mosquitoes chose moving black stripes over stationary stripes (31).

Two basic techniques have been used to study the response of insects to light of different wave lengths. The first, behavioristic studies, involves the measurement of some motor response of an insect after it has received a light stimulus. The second, electrophysiological studies, involves the measurement and recording of electric potentials between two electrodes, one inserted into the receptor and the other inserted into another region of the insect.

The behavioristic technique was used by Von Frisch (52, p. 63-99) in training honey bees to find food placed on colored papers. When food was placed on colored papers as well as on gray papers of the same shade, bees chose the food on the color on which they had been trained. From this and similar experiments he found that bees were sensitive to ultraviolet, blue, blue-green and yellow light but not to red light. Mazokhin-Porshniakov (37) used a similar method and found that bees and wasps could discriminate between green, yellow and orange colored papers, irrespective of their brightness. Colored papers were also used by DeWilde and Pet (17) to find out that larvae of Leptinotarsa decemlineata Say responded to colors, showing a preference for green.

Mast (34) found the regions of the spectrum which were the most stimulative to fifteen different species of animals. The larva of the blow fly was one of the animals studied. A wavelength of 503 mu had the greatest stimulating effect on this insect. The test animals were given the choice of two intersecting beams of light of equal intensity but different quality. One beam consisted of white light and the other of light of the desired wavelength. After the insects were given time to make a choice, counts were made and the intensity of the white light was changed so that an equal number of insects went to both beams of light. In this manner the relative attractiveness of the test light waves could be compared with the white light. Larvae of Lucilia species avoided light in the blue region of the spectrum and congregated at the red region of the spectrum (28). This reaction could be expected with negatively phototactic insects because most insects cannot distinguish red from black. The two previously mentioned workers used color filters to obtain the desired wavelengths.

Hylamorpha elegans and Brachysternus prasinus were attracted to colored lights; red, ruby-red, blue and dark green being the most attractive colors. The degree of attraction was proportional to the intensity of the light (41). Bertholf (3)(4) used a box with light of the

desired wavelength entering at one area and white light of the same intensity entering at another area. With this arrangement he concluded that the upper limit of the spectrum for the honey bee was 677 mu and the lower limit was 313 mu. The wavelengths which had the greatest stimulating effect on the bee were 365 mu and 553 mu, the former being about four and one-half times as strong as the latter. A multiple choice filter arrangement was used by Weiss (60) in determining the spectral responses of seven species of adult Coleoptera and sixteen species of coleopterous and lepidopterous larvae. In all but two instances the maximum response was at 365 mu and a slightly smaller one was at 490 mu. The larvae had a choice of any one of ten different spectral colors and ten dark areas between the spectral colors. Dorner and Mulla (18) compared a filter box and a spectrophotometer in measuring the response of the eye gnat, Hippelates collusor Townsend, to various regions of the spectrum. The gnats were placed in a cell of the instrument and as the insects collected in front of the light beam the deflection of the indicator was used as an indication of their response. The results were very similar even though light of an intensity of 0.1 lumen was used in the filter box and light of an intensity of 0.001 lumen was used in the spectrophotometer. The maximal response of the gnats was at 440 mu with the spectrophotometer and at 440

and 550 mu with the filter box.

Fingerman (19) used a Y-type apparatus in conjunction with a monochromator to study the response of Drosophila melanogaster Meigen to light of different wavelengths. He found that a maximal response occurred at 366 mu and a second one occurred at 400 mu. A Y-type apparatus was also used by Ballard (1) to study the spectral response of the stable fly, Stomoxy calcitrans (Linn.). The desired wavelengths, however, were obtained by the use of filters and proper lights. The stable fly responded most readily to the ultraviolet waves with a second peak being reached at 640 mu. Similar results were obtained from male and female flies. Tests conducted at 0.8 and 48 footcandles gave similar response curves. Motor responses of Calliphora to a revolving striped drum illuminated with light of different wavelengths and different intensities were used by Schneider (46) to determine the fly's response to the various regions of the spectrum. Responses were shown for the region from 480 mu to 631 mu with the maximum at 480 mu. The response curve of the eye was similar for dim and bright light.

Electrophysiological techniques were used by Jahn (30) in studies on the visual responses of Melanoplus. From these studies he concluded that the maximum response was at a wavelength of 500 mu, falling off sharply in the red

region and only slightly in the violet region. Walther (55)(56)(57) used similar techniques and found that the eyes of Calliphora erythrocephala (Meigen) and Periplaneta americana (Linn.) gave maximal spectral responses at 341-369 mu and at 507 mu. The electroretinogram for both insects was similar. In the ventral portion of the eye of the cockroach he recorded a single spectral sensitivity peak at 507 mu. Tests in the dorsal part of the eye revealed the two normal peaks, one in the ultraviolet and the other in the blue. This could explain why Jahn (30) obtained only a single peak which was in the blue region of the spectrum. Goldsmith (23)(24) obtained similar electroretinograms for the honey bee and the blow fly, Phormia regina (Meigen). The spectral sensitivity peaks for the honey bee were at wavelengths of 535 and 345 mu. Electrophysiological studies on the blow fly indicated a single maximal sensitivity at 500-510 mu, however behavior studies indicated two areas of maximal sensitivity, one at 365 mu and the other at 500-510 mu.

Most research on the visual threshold of insects to white light has been conducted in conjunction with experiments on the spectral sensitivity of insects. Larvae of Lucilia caesar Linn. and Calliphora vomitoria Linn. did not respond to light of an intensity below 0.00007 meter candles (28). Bertholf (3) found that a straight line

relationship exists between the response of the honey bee and the logarithm of the intensity, until a maximum is reached. Similar results were obtained by Goldsmith (23) and Heintz (27) who also used the honey bee as a test animal. The same relationship existed for all regions of the spectrum tested. An increase in light intensity did not appreciably alter the response of Leptinotarsa decemlineata Say (17) or the stable fly (1). Fingerman (19) observed that with Drosophila melanogaster as the light intensity decreased the response of the flies to the light also decreased. The apparent conflict just mentioned is probably because DeWild (17) and Ballard (1) were conducting their experiments using light intensities above the point where the maximum response of the insects would occur while Fingerman (19) was using light intensities approaching the visual threshold of the insect.

For a more complete review on the visual responses of insects see the following authors: Mast (34), Bertholf (3), Weiss (58)(59), Dethier (15), Roeder (44, p. 448-522), Ballard (1) and Von Frisch (53, p. 19-40).

Olfactory Responses

Olfactory responses of insects have not been as widely studied as have the visual responses, however, they have been more widely exploited for control of harmful

insects than have the visual responses. The tsetse fly, Glossina swynnertoni was able to detect the odor of an ox at 110 and 180 feet but not at 300 feet (2). The flies were placed in a cage in the center of an enclosure and their motor responses were recorded as the oxen passed in front of the flies. The tests were performed when the wind was blowing toward the flies and the oxen were behind the enclosure. Aedes mosquitoes were attracted to an opaque perforated plastic box which contained a mouse rather than a similar box which did not contain a mouse (48). Von Frisch (52, p. 44-62) was able to train honey bees to distinguish certain odors by placing an odorous substance in the food then after a period of time placing this food with foods of different odors. The bees would choose the food with the odor on which they had been trained.

The buffalo fly, Lyperosia exigua (de Meij.) was attracted to the odor of buffalo manure, cow manure and horse manure, in that order (32). The flies were placed in a cage containing two tubes which entered from opposite sides of the cage. One tube contained the test substance and the other was the control. The flies were also attracted to fresh manure which had been covered with a cloth, thus, verifying the results obtained with the choice chamber. Cragg (12) and Cragg and Cole (13) found that a substance in wool, the "wool factor", was an attractant

for Lucilia sericata (Meigen). They used a choice chamber constructed so that the temperature under each half could be controlled. McIndoo (38) used a choice chamber modified so that the air flow and concentration of the attractant could be regulated. With this apparatus he found that blow flies were attracted to milk, lactic acid, lactose and casein. Aedes mosquitoes (5) were found to be attracted to low concentrations of sweat but repelled by high concentrations. The technique used was similar to the one used by McIndoo (38).

A modified Y-type olfactometer with temperature, light and humidity controls was designed by Schwertferger (47) to test the olfactory responses of the stable fly. The flies were attracted to secretions from 12 of the 21 hosts tested. No correlation was found between the actual number of flies on the host and the attraction of their secretions to the insect. Wigglesworth (61) used the split area test in studying the olfactory response of the human louse, Pediculus humanus corporis DeGeer. The area beneath a glass container was divided in half by two boxes, in one box was placed the control material and in the second was placed the test material. The position of the lice was noted and recorded after an interval of time. Odor of a human was preferred over the control, as was the odor of a rabbit. Human odor, however, was preferred over rabbit odor. By a

similar technique female Aedes mosquitoes were found to be attracted to fresh cattle blood (7). For a more complete review on the olfactory responses of insects see: Wigglesworth (61), Dethier (14)(15) and Roeder (44, p. 544-576).

III. REARING TECHNIQUE FOR HORN FLIES

An attempt to establish a colony of horn flies during the summer of 1958 met with failure. On August 11, 1958 a Jersey heifer with at least 500 horn flies was placed in a screened enclosure. The freshly dropped manure in which the flies had oviposited was collected and placed on moist sand. The number of adult horn flies gradually decreased to 80 adult flies by August 25, on August 27 there were no adult flies. New emergence increased the number of flies to 35 by October 2, these flies, however, failed to establish a colony. No attempt was made to control the photoperiod during this period. The temperature was maintained at about 75 degrees with a daily high of about 90 and a low at night of about 60°F.

A second attempt to establish a horn fly colony met with somewhat better results during the summer of 1959. A small colony was established July 5, 1959, reached a maximum of 700 flies on August 7, and completely disappeared by October 11, 1959. A second colony was established September 19, 1959 on a host in an animal isolation room. The first adults began emerging 15 days later. This colony was destroyed by insecticide contamination and attempts to reestablish it with wild flies failed. Manure in which the wild flies had oviposited was collected and placed on moist

sand. This manure was retained throughout the winter. On January 1, 1960 a single adult horn fly emerged. The flies continued to emerge in small numbers until April 1, 1960 when the last fly emerged. The colony did not become re-established during this time. Temperature had been controlled in previous attempts to establish colonies, however, photoperiod had not. This provided the needed clue for successful colonization of horn flies. The probable reason why a winter colony had not been established was that the short photoperiod induced the insects to go into diapause. This was substantiated in the first successful attempt to establish a laboratory colony of horn flies the following year.

A shipment of horn fly larvae was received from the USDA Entomology Research Laboratory in Texas, April 20, 1960. These were placed in the enclosed room with the host. The first adult emerged on May 2 and 20 adults emerged each day for the following two days. Mating was observed on the fourth day after the first fly appeared. Oviposition was observed on the fifth day after the first fly appeared. The manure in which the flies had oviposited was collected and placed on a heating cable which was covered with a half inch layer of moist sand. Temperature of the heating cable was controlled by a thermostat located on the floor.

The temperature of the manure remained approximately 85°F. First generation flies began emerging 20 days later. In forty-two days the size of the colony increased from one to 150 adult horn flies.

During the above time a special rearing room was constructed. The room was 12 feet wide by 16 feet long, 8½ feet high in the front and 7½ feet high in the rear. The top half of the room was covered with screen and plastic, the bottom half was covered with half inch exterior plywood (Figure 1). A floor 60 inches wide was put across one end of the room opposite the space allowed for two animals. Two General Electric 40 watt power groove fixtures, containing two cool white power groove lamps each (F 48 PG17 CW) were hung over the space allowed for each animal. Between these two fixtures was placed a rack containing four 100 watt and two 200 watt incandescent lamps. All lights were connected through a timer which was adjusted to give 19 hours of light and 5 hours of dark. The room was heated by two 1650 watt automatically controlled electric heaters. An intake and an exhaust fan were connected to an ether wafer thermostat to cool the room when the temperature went above 85°F. In extremely hot weather a sprinkler was placed on top of the building in order to keep the temperature of the room below 100°F.

July 16, 1960 the horn fly colony along with the host

Figure 1. Horn fly rearing room.

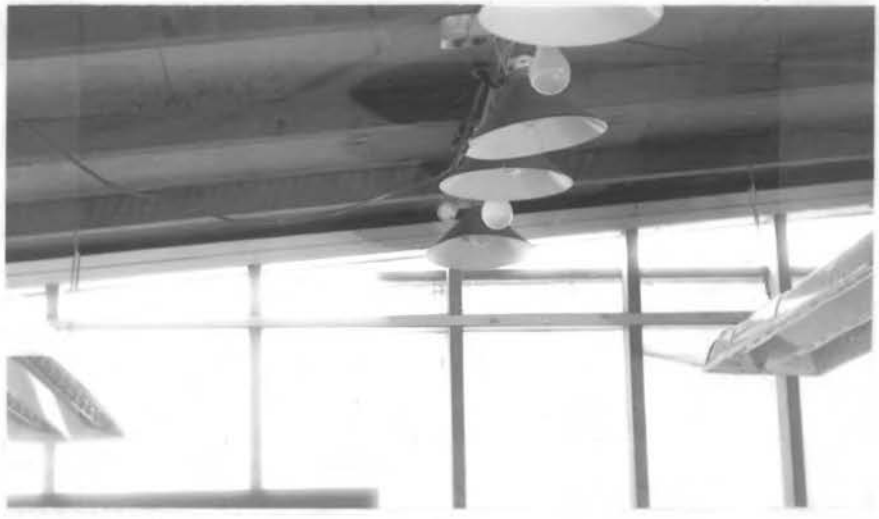

Figure 2. Light arrangement in rearing room.

was transferred to the new rearing room. The manure that the flies had oviposited in was collected and placed on moist sand above a thermostatically controlled heating cable. A vigorous colony of horn flies with a maximum number of 1500 was maintained until early October when it diminished to a low of 100 flies. The apparent reason for the decline was that an extremely large number of Phalangids and spiders had built up and were feeding on the adults as soon as they emerged, as well as when they oviposited or rested on the walls.

At the time of the above decline it was found that horn fly larvae could be reared in gallon jars which had about an inch and a half of saw dust in the bottom. Immediately all the manure which was collected was placed in gallon jars which were put on the heat cables. The fresh manure in which the flies had just oviposited was taken to a different building where it was placed on heat cables (Figure 3), the older manure was left in the rearing room (Figure 4). Within three weeks after this method was initiated the fly colony had increased to almost 2000 flies. This method offered greater control over the flies as those which emerged could be retained and counted. It also made it possible to obtain unfed flies of known age to be used later in the behavior experiments.

Temperature, humidity and emergence records were

Figure 3. Jars containing larvae infested manure which was collected immediately after it was dropped.

Figure 4. Jars containing larvae infested manure on heating cables in rearing room.

maintained from the time the new rearing method was initiated, October 18, 1960, until May 7, 1961. An average maximum temperature of 81°F and an average minimum temperature of 63°F was maintained. The average of the maximum and minimum temperatures was 72°F. The average of the maximum humidity was 74 per cent while the average minimum humidity was 61 per cent. The average of the maximum and minimum humidity was 67 per cent.

Effect of ambient temperatures on the length of the developmental period of the horn fly is shown in Table 1. During cooler weeks, 1-11, the day of maximum emergence ranged from the twelfth day through the sixteenth day after oviposition. During warmer weeks, 12-25, the day of maximum emergence ranged from the tenth day through the twelfth day after oviposition. It is also shown in Table 1 that warmer temperatures shortened the emergence period while cooler temperatures lengthened it.

Table 2 shows the estimated number of flies which emerged in the rearing room during these studies and the number emerging on each of the indicated days after oviposition. Most flies emerged in the 10-15 day period after oviposition. Approximately 150,000 flies emerged during the 187 days for which records were kept. Average emergence was about 800 flies per day. An average of 377 additional flies per day, or a total of 72,499 in 192 days,

Table 1. Effect of temperature on length of horn fly development in fly rearing room, October 2, 1960--April 25, 1961.

Week	Average temperature[a]	Days after oviposition when maximum, first and last emergence occurred		
		Maximum	First	Last
1	66	--	--	--
2	66	--	--	--
3	74	13	12	18
4	70	14	12	17
5	68	15	12	18
6	68	14	11	18
7	70	13	12	21
8	67	16	11	19
9	72	14	12	19
10	70	12	10	15
11	72	13	8	17
12	71	--	--	--
13	71	12	10	15
14	71	10	9	13
15	72	11	9	16
16	74	11	9	14
17	72	11	9	14
18	77	11	10	15
19	73	11	9	14
20	72	11	9	15
21	72	12	9	15
22	72	12	9	16
23	70	11	9	14
24	72	12	9	15
25[b]	74	12	9	16
26	75	13	10	19
27	72	13	11	19
28	73	13	11	18
29	73	14	11	17

[a]The average of the total maximum and minimum temperature for each week.

[b]Jars were not placed on the heating cables after the 25th week until pupation had occurred.

Table 2. Estimated number of horn flies which emerged in rearing room. October 18, 1960--April 25, 1961.

Days after oviposition	Total number of flies emerging each day
8	5
9	1,910
10	12,505
11	28,545
12	35,649
13	32,228
14	21,775
15	11,095
16	4,611
17	1,924
18	590
19	315
20	55
21	35
Total	151,242[a]

[a]Average for 187 days was 809 flies per day.

emerged from the fresh manure which was kept in a separate building. This latter group of flies was used in the behavior studies then released on the host while the former group was released immediately on the host. Altogether approximately 220,000 flies or an average of over 1,000 flies per day emerged during the period for which records were kept. During this period the number of flies on the host ranged from an estimated low of 1,000 to an estimated high of 10,000, the average being about 3,700 flies (Figure 5).

Figure 5. Host supporting horn fly colony, January 1961.

Several unique problems arose during the colonization of the horn fly. As previously mentioned when the larvae were allowed to develop in the manure, on the sand, a large population of spiders and phalangids had built up and were feeding on the adults as soon as they emerged, as well as when they oviposited or rested on the walls or floor. When the manure was moved to the jars the phalangid population disappeared, however, the spider population still presented a problem because as many as 200 adults would become caught in the webs at night. In order to minimize this problem the room was swept daily to remove spider webs.

During the week of December 11-17, 1961, many dead larvae were observed in the jars, in fact in some jars there were no living larvae. These larvae were examined by an insect pathologist who could find no pathogens. The probable cause of the mortality was lack of oxygen. When a jar was half filled with liquid manure the only exposed surface area was the top of the manure. Only those larvae remaining in the top survived. After this the manure was placed loosely in the jars which were only half filled. No further problem of this nature was encountered. Another problem of an entirely different and unexpected nature developed which was attributed to the behavior of the host. The chain holding the steer came loose and the animal was not fastened as tightly as before. Previous to the chain

coming loose he was unable to lick his back, lower sides or hind legs. After the chain was replaced he could easily reach these places to lick. The fly colony dropped from 10,500 to 2,000 flies within three days after he was able to reach these regions. The animal was seen licking flies from his back, hind legs and sides, and on one occasion he licked off about 300 flies just released on his back. On three occasions when the chain became loose a large reduction in the size of the colony occurred.

The host did not appear to be extremely irritated by the large number of flies on him. The only signs of irritibility noticed were head tossing and the kicking of saw dust. Some physical damage caused by the feeding of the horn fly on the host was apparent. The first sign of injury was a red irritation somewhat resembling a rash. Scabs formed shortly after the irritation was apparent. The overall appearance of the feeding injury was that of an abrasion over which a thick scab had formed. It was noted that the flies seemed to feed where the hair was the shortest, for this reason one side of the host was closely clipped, practically all injury was restricted to the clipped side. The animal was clipped on the opposite side when the hair had grown out on the shaved side. This allowed the other side time to heal without much feeding damage. Several small areas where the animal was not

clipped also had feeding injury, especially the dewlap and the area between the front leg and the body. For this reason the injury could not be attributed to shaving irritation.

One host animal should support a colony containing well over 10,000 adult horn flies with at least 50,000 new adults emerging each day. In order to accomplish this the following steps should be taken: (1) Put the host on a free choice feed schedule; (2) Manure should be collected at least twice daily; (3) The host should be fastened so that it cannot lick the flies off; (4) Manure must be placed in the jars so that the maximum surface area is exposed to the air; (5) Temperature should be maintained at a constant 85°F; (6) Parasites and predators should be kept to a minimum; (7) A proper photoperiod must be maintained to prevent diapause.

The successful colonization of the horn fly throughout the year has made this insect available for investigations which would otherwise not be possible. It is now feasible to conduct biological and toxicological studies on the horn fly without interruption. Disease transmission studies may be initiated under strictly controlled conditions at any time of the year. The way is clear for investigating the possibility of controlling this insect pest using sterilization techniques.

IV. VISUAL RESPONSES OF HORN FLIES

Field Studies on Visual Responses of Horn Flies

Research was begun during the summer of 1959 to determine what factors were responsible for attracting the horn fly to its host. During this series of experiments the role of vision was investigated to the exclusion of odor. Other factors were controlled as far as field conditions would permit. The experiments were designed so that the flies could choose between a test object and no test object. The analysis of variance with individual degree of freedom was used to analyze the results.

A circle with a radius of 14 feet was marked off in the center of a pasture. (Figure 6) Steel fence posts were placed around the circumference of the circle so that 4 x 8 foot plywood panels could be inserted to make a uniform background of the desired color. One side of the panel was painted white, the other black. The black side of the panel faced the center of the circle during this series of experiments because in previous investigations higher F values were obtained with a black background than with a white one (Table 3).

A separator, made from three sheets of 4 x 8 foot plywood panels, hinged together at one end, was placed so that the hinged end of the separator was at the center of the

Table 3. The attractiveness of various objects to the horn fly, Haematobia irritans (Linn.)

Object	Total number of flies to quadrant containing object	Total number of flies to vacant quadrant	F[a] Value	Number trials
Steer (black background)	944	344	84.3638	12
	288	78	44.310	3
Steer (white background)	374	123	20.037	3
Sheep	148	122	1.5550	3
	215	199	0.3926	3
Man	177	168	0.3111	3
	299	241	0.0357	3
Artificial object	218	211	0.1068	3
	193	194	0.0036	3

[a]d.f. = 1 and 33, F.05 = 4.1450
d.f. = 1 and 6, F.05 = 5.9874

circle (Figure 6). The leaves of the separator were adjusted to bisect the circle and make two quadrants. The separator was adjusted after each trial so that the center leaf was perpendicular to the direction of the sun and each quadrant received approximately the same amount of light. The right quadrant received slightly more illumination than the left due to the movement of the sun during each trial. Statistical analysis, however, confirmed that the left and right quadrants were not significantly different.

The object to be tested for its attractiveness to the

Figure 6. Apparatus used for testing the visual responses of the horn fly.

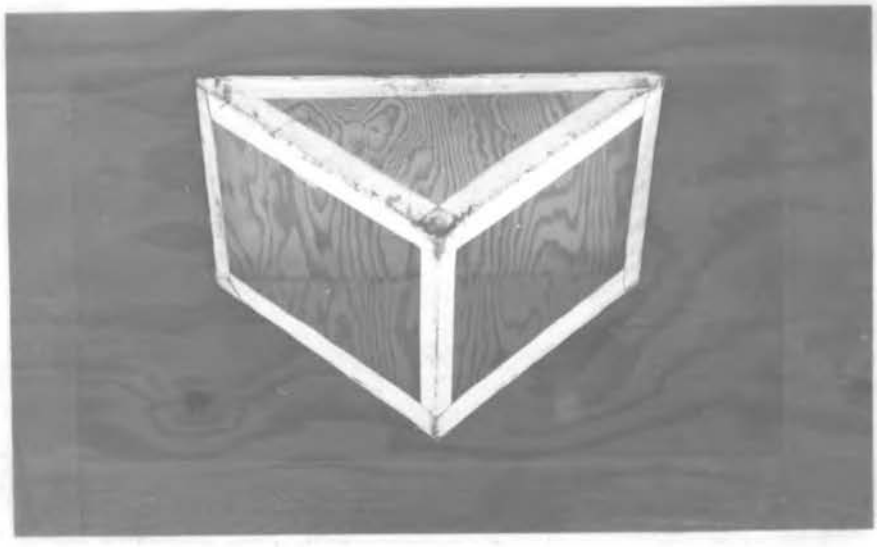

Figure 7. Box used for testing the visual responses of the horn fly.

horn fly was placed next to and parallel to the fence in one quadrant and the other quadrant was left vacant. A box, shaped like a right triangle with the sides opposite the 45 degree angles made of glass, was placed on the separator at the hinged end, one glass side facing each quadrant. (See Figure 7).

Twenty horn flies, which had been collected from the host, were released into the vision box which was then sealed to eliminate odor and humidity as influencing factors. The box was then shaken to prevent orientation before it was put into position. The flies were allowed one minute to orient after being put into position and the number on each window was recorded every two minutes thereafter for a total of ten observations. After the tenth observation the box was removed, agitated and turned upside down. The test object was moved to the other quadrant and the box containing the same twenty flies was replaced and the same procedure was repeated. At the end of this trial the flies were released and twenty fresh flies were placed in the box which was again sealed. The same procedure was followed for a total of three complete trials. The entire experiment was repeated for each test object at a later time. Test objects used included a year and a half old white Shorthorn steer, a year old Hampshire X Romany sheep, a seventeen year old boy dressed in white clothes and a white

movable plywood object with a lateral surface area approximating the lateral surface area of the steer (Figure 8).

The results of these experiments are shown in Table 3. The number of flies attracted toward the steer was approximately three times the number attracted toward the vacant quadrant. F values for these data show that they are highly significant. In contrast there was little difference between the number of flies attracted toward the sheep and the vacant quadrant; the man and the vacant quadrant and the artificial object and the vacant quadrant. Significant results were not obtained for any of the last three objects. As previously mentioned, the left and right quadrants, in the absence of the test objects, were not significantly different, therefore, it may be assumed that the conditions under which these results were obtained were equal except for the presence or absence of the test object.

From these results it can be concluded that radiation is a significant factor in attracting the horn fly to the steer. Whether the radiation was from the visible or invisible regions of the spectrum was not determined in this investigation. The horn fly is able to distinguish its host from other objects at a distance of at least 12 feet when odor and humidity are eliminated as influencing factors. Mass and motion are apparently not significant factors in attracting the horn fly at close range.

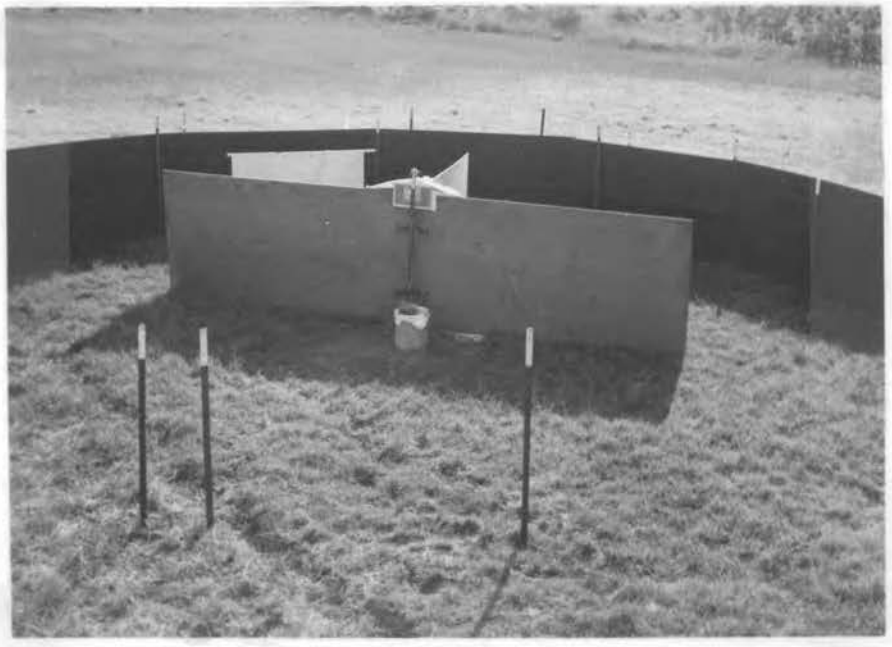

Figure 8. Artificial object used in testing the visual response of horn flies.

Response of Horn Flies to White Light of Different Intensities

It was evident from preliminary experiments and casual observations that horn flies were positively phototactic. The intensity of light to which they responded, however, was not known. In order to determine the horn fly's threshold of response to light, methods were developed to subject the insect to white light of different intensities.

A triangular box approximating a right triangle was constructed so that the side opposite the right angle was about 16 inches long and the sides opposite the 45 degree angles were 11.5 inches long. The box was 5.5 inches deep and the top of the box was hinged to form a lid. Black velvet ribbon was glued to the edges of the top as well as the edges of the box so that a light-tight seal would be formed. A hole one inch in diameter was drilled in the center of each 11.5 inch side (Figure 9). A trap made from 15/16 inch glass tubing two inches long and wrapped with black plastic tape was inserted into each hole. A funnel shaped piece of glass with the smallest end having an opening 1/8 inch in diameter was inserted into the glass tube so that the large opening faced the inside of the box and the small opening faced the outside of the box (Figure 10). This funnel prevented the flies from returning to the main portion of the box once a choice had been made. A number 0

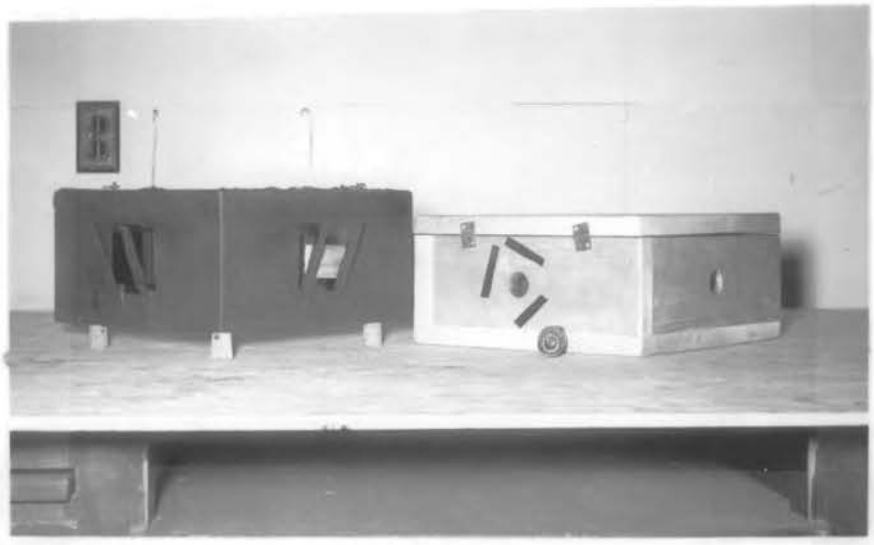

Figure 9. Right, box used to study response of horn flies to white light of different intensities; left, box used to study response of horn flies to spectral light.

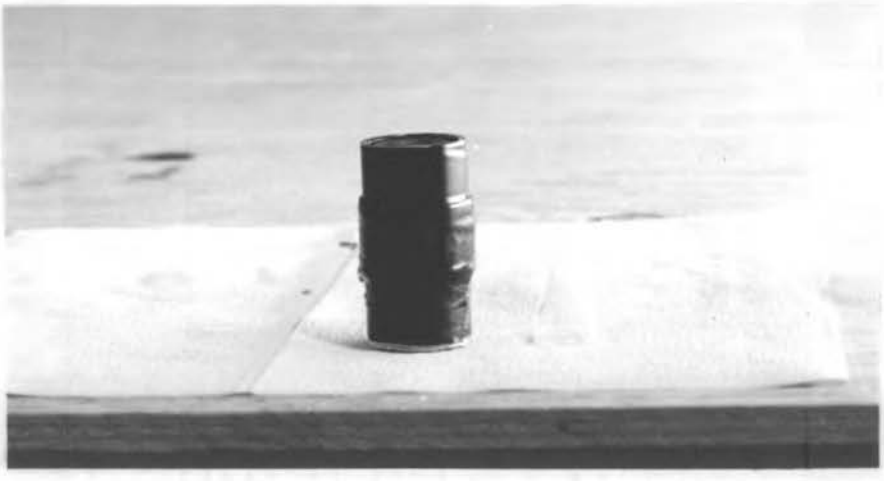

Figure 10. Trap used in retaining flies in intensity studies.

cover glass was taped over the outside of the hole to prevent the flies from escaping from the trap to the light.

The following sources of light were used: General Electric 7.5, 40, 75, 100 watt frosted incandescent lamps and a 40 watt daylight fluorescent lamp. A General Electric uncorrected footcandle meter was used to measure the intensity of the light. Desired light intensities were obtained by varying the distance between the light source and the test box. Relative accuracy of the meter was checked by comparing it with other footcandle meters. A water filled aquarium 5½ inches deep by 6½ inches high and 9½ inches wide was placed between the light source and the window of the box to act as a heat filter (Figure 11). All experiments were conducted in a dark room.

The box was placed at a distance from the light source necessary to obtain the desired intensity at the window. A vial containing twenty freshly emerged horn flies which had previously been fed honey water was placed upside down in the box.[1] The lid of the box was sealed and one window of the box was turned toward the light source, the other window was covered with a piece of opaque plastic and served as the control. When all adjustments had been made the box

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[1]Flies fed honey water were more vigorous and lived longer than unfed flies.

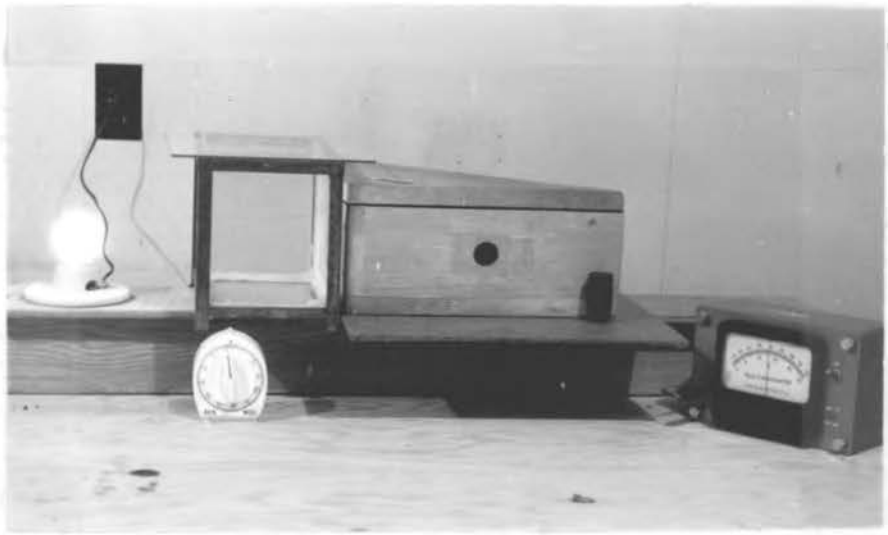


Figure 11. Apparatus used in studying the response of horn flies to white light of different intensities.

was tilted so that the flies were released from the vial. Flies trapped in the test and control traps were counted at the end of ten minutes. These flies were removed and 20 fresh flies were placed in the box and the procedure was repeated for a total of five times. At the end of the fifth replicate the box was turned so that the control window now faced the light, the opaque plastic was placed over the former test window. The same procedure was again repeated five times giving a total of ten replicates. Light intensities within the range of 0.004 to 98.0 footcandles were tested.

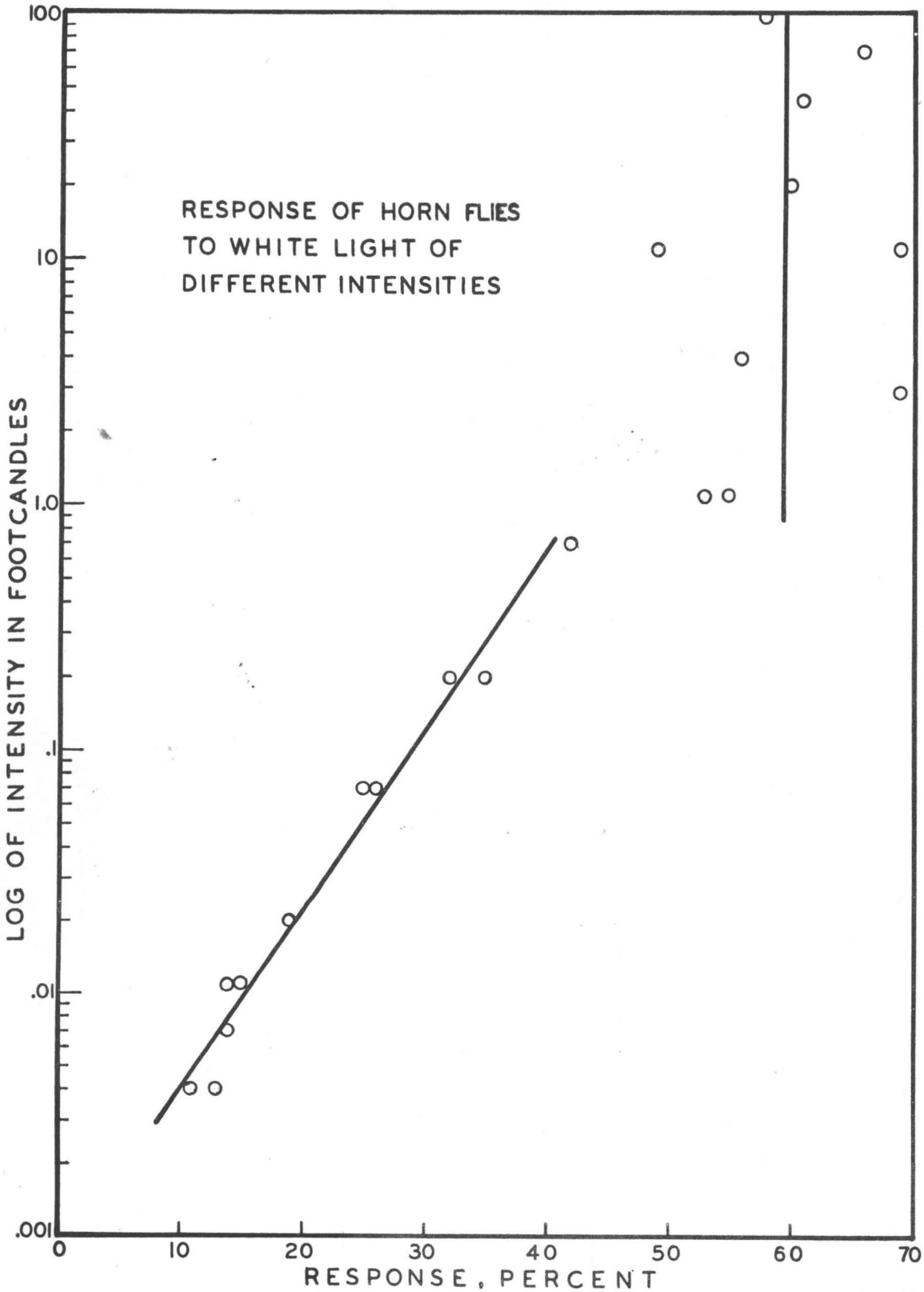
Results of these experiments are given in Table 4. The threshold of response of the horn fly to white light is below 0.004 footcandles. This is in agreement with Herms (28) who found the threshold of Lucilia and Caliphora larvae to be 0.0007 and 0.00007 footcandles, respectively. DeWilde (17) found the threshold of Leptinotarsa decemlineata Say to be below 0.009 footcandles. A linear relationship exists between the logs of the intensities and the per cent response of horn flies in the intensity range of 0.004 to 0.7 footcandles. Above 0.7 footcandles a point is reached where an increase in intensity does not cause a corresponding increase in response (Figure 12). These results are in agreement with Bertholf (3), Goldsmith (23),

DeWilde (17) and Heintz (27) for other insects.

Table 4. The response of horn flies to white light of different intensities.

Illumination in foot-candles	Per cent response	Type of light used
.004	13	G.E. 7.5 watt white bulb
.004	11	" " " " "
.007	14	" " " " "
.011	15	" " " " "
.011	14	" " " " "
.020	19	" " " " "
.070	25	" " " " "
.070	26	" " " " "
.200	35	" " " " "
.200	32	" " " " "
.700	42	" " " " "
1.100	53	" " " " "
1.100	55	" " " " "
2.900	69	G.E. 40 watt daylight fluorescent
5.000	56	G.E. 40 watt frosted incandescent
11.000	49	G.E. 40 watt daylight fluorescent
11.000	69	G.E. 40 watt daylight fluorescent
20.000	60	G.E. 75 watt frosted incandescent
44.000	61	G.E. 40 watt daylight fluorescent
70.000	66	G.E. 100 watt frosted incandescent
98.000	58	G.E. 100 watt frosted incandescent

Figure 12. Response of horn flies to white light of different intensities.


RESPONSE OF HORN FLIES
TO WHITE LIGHT OF
DIFFERENT INTENSITIES
LOG OF INTENSITY IN FOOTCANDLES
100
10
1.0
.1
.01
.001
0
10
20
30
40
50
60
70
RESPONSE, PERCENT

Response of Horn Flies to Light of Different Wavelengths

Spectral studies were initiated to determine the quality of light to which the horn fly would respond. A box of the same dimensions as the one described in the previous section was constructed with several modifications. (See Figure 9) The lid was made to open from the back rather than the top and legs were put at each angle of the box. The inside and outside of the box was painted flat black. Sliding door traps which could be operated from the outside of the box were constructed for each window. Springs were attached to the outside of the windows to hold the filters tight against the box.

Light of the desired wavelengths was obtained by the use of narrow-band-pass filters and an appropriate light source. Filter numbers, transmission range and the midpoint of the transmission range are given in Table 5. Ultraviolet radiation was obtained by using a General Electric 40 watt blacklight BL fluorescent lamp as the source. Radiation in the visible region was obtained by using a General Electric 40 watt daylight fluorescent lamp as a source. Far red and infrared radiation was obtained by using a 100 watt frosted General Electric incandescent lamp. Light intensities were measured with a General Electric uncorrected footcandle meter. As light passed

Table 5. Response of horn flies to light of different wavelengths but same intensity.[a]

Filter number	Transmission range in mu	Mid-point of range in mu	Per cent response
Corning 7-83	350-390	370	62.4
Kletts 42	400-450	425	62.1
47	445-505	475	59.2
52	485-550	518	59.6
54	520-580	550	57.6
56	540-590	565	34.4
59	565-630	598	33.5
62	590-660	625	28.7
Corning 2-106	670-infrared	720	1.7[b]

[a]An intensity of 2.5 footcandles was used for each filter.
[b]Control trap contained 1.2 per cent of flies.

into the box the intensity was reduced by a factor of 2.2 thus all readings of the light meter were corrected by this amount. Light intensities were determined by placing the filter to be used in front of the meter, taking the reading and dividing by the correction factor. The box was then placed so that the filter was in the same location at which the intensity was determined. An intensity of 2.5

footcandles was used for each wavelength because it was well above the point at which white light caused maximum response.

When an experiment was to be conducted the box was placed with one window facing the light source and the other facing at a right angle to it. (Figure 13) A filter giving the desired wavelength was placed over the window facing the light. The other window was blacked out by a piece of black felt. A vial containing 20 freshly emerged horn flies which had been fed honey water was placed top down inside the box. The box was then sealed, the traps opened and the flies released. At the end of ten minutes the traps were closed and the flies in them were removed with an aspirator and counted. This procedure was repeated five times using fresh flies for each replicate. When the fifth replicate was completed the box was turned so that the former control window was in the position where the test window had been. The filter was placed in front of the new test window and the new control window was covered with the black felt. Fresh horn flies were released into the box and the procedure was repeated for a total of ten replications. The experiment was repeated at another time for each wavelength giving a total of 20 replications.

Horn flies responded most strongly to the ultraviolet and violet and only slightly less strongly to the blue and

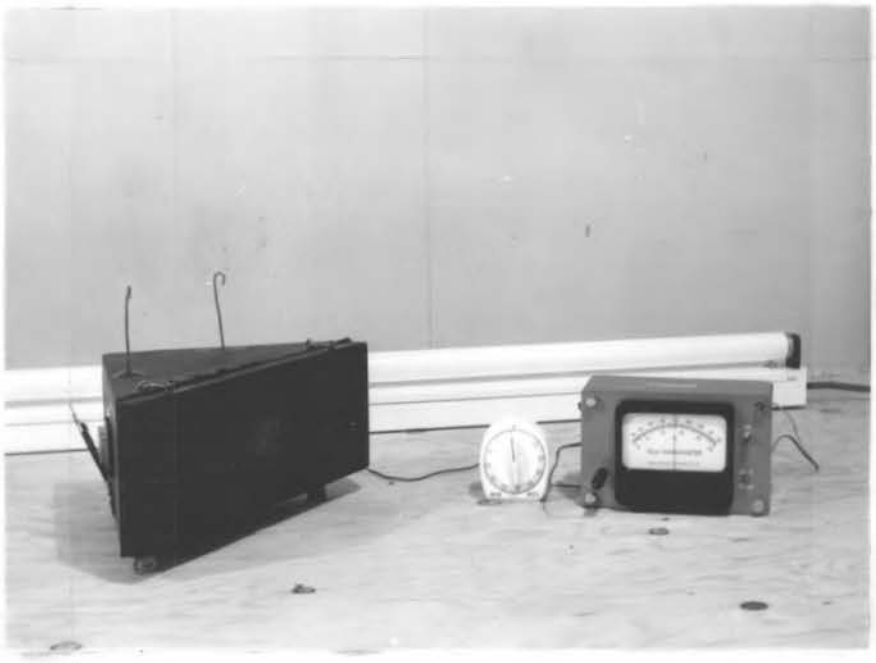


Figure 13. Apparatus used in studying the response of horn flies to light of different wavelengths.

green regions of the spectrum. Yellow and orange were only about half as attractive to the horn fly as the ultraviolet through green regions. Red and infrared were not attractive to the horn fly (Figure 14). These results are in agreement with the results of Von Frisch (53, p. 19-40) with the honey bee, Ballard (1) with the stable fly, Goldsmith (23) with the blow fly (Phromia regina), Mazokhin-Porshniakov (36) with the dragon-fly and others.

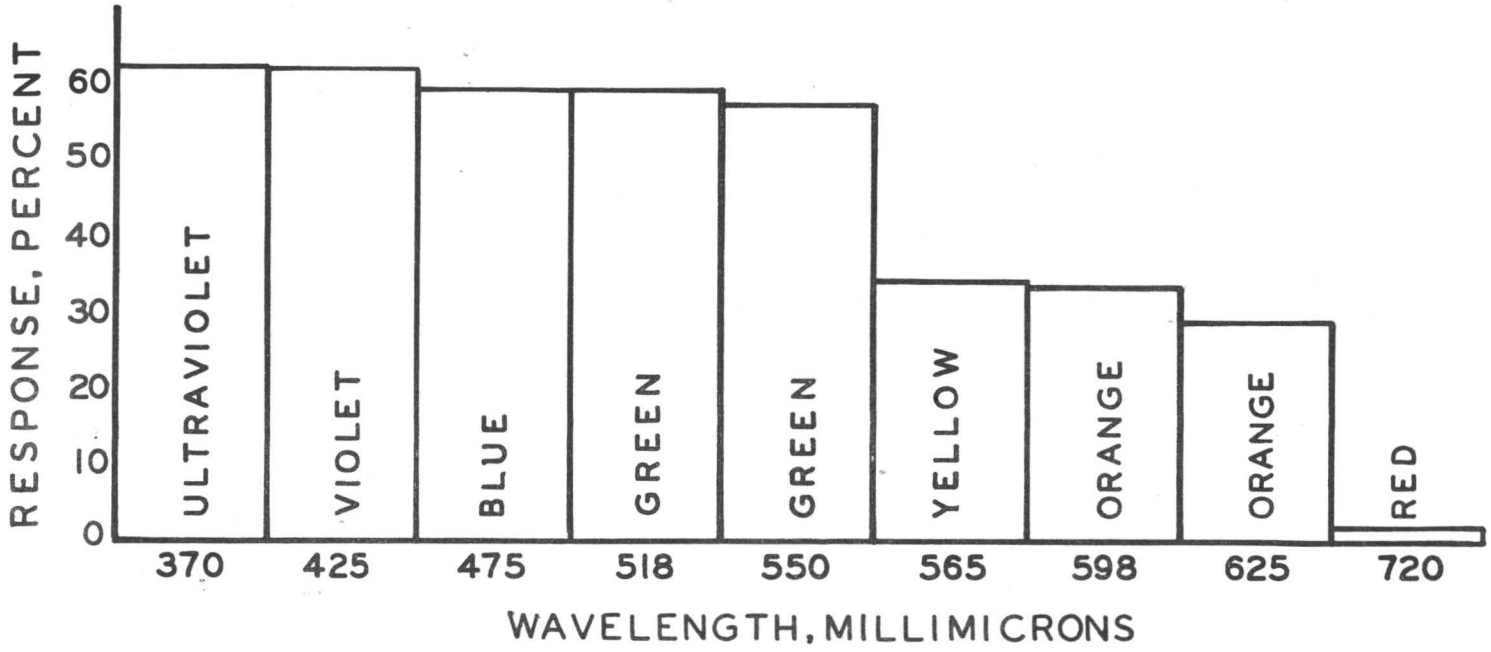
It is possible that the response in the yellow-orange region represents the response of one sex and the absence of response in the other sex. This is unlikely, however, as Ballard (1) and Dorner and Mulla (18) obtained similar response curves for both sexes of Stomoxy calcitrans and Hippelates collusor, respectively.

Response of Horn Flies to Areas of Contrast

Preliminary observations indicated that horn flies were attracted to dark areas. In order to verify these observations methods were developed to measure the response of these insects to areas of contrast. Containers to hold the flies were constructed from wide mouth gallon jars which were cut in half. The bottom half was discarded leaving the top part with the two openings. Cheese cloth was stretched over the end opposite the lid and fastened to the jar with plastic cement (Figure 15). Three types

Figure 14. Response of horn flies to light of different wavelengths.

RESPONSE OF HORN FLIES TO LIGHT OF DIFFERENT WAVE-LENGTHS--INTENSITY OF 2.5 FOOTCANDLES



of patterns with contrasting areas were made from cover glasses which were 1 3/4 inches wide by 2 7/8 inches long: (1) A cover glass covered with black plastic tape was used as the test pattern while a clear cover glass was used as the control; (2) Black plastic tape was put around the edges of a cover glass with the center area remaining as a clear control area; (3) White paper was glued to one-half of a cover glass and black plastic tape was placed over the other half. In one experiment the cover glass with the clear center was placed on 1/8 inch high clear plastic legs in order to eliminate any effect of contact with the glass surface. In all other experiments the cover glasses were placed glass side down on the cheese cloth.

Fifty freshly emerged adult horn flies which had been fed honey water were placed in each of two containers. One of the containers was placed on a table about 36 inches below a 32 watt General Electric circline fluorescent lamp. Patterns to be tested were placed on the center of the cheese cloth top. Counts of the number of flies on the control and test areas were made every minute for ten minutes. At the end of ten minutes this container was removed and the second container was placed under the light and the procedure was repeated. When this ten minutes was up the first container was placed under the light and so on until

the end of the experiments. Position of the pattern was alternated for each experiment so that bias due to position would be reduced to a minimum. In order to determine if horn flies were attracted to an area of contrast from the side, a jar was turned on its side and the pattern with the clear center was taped to the cheese cloth. Light was also directed from the side, behind the pattern.

Results of the experiments to determine the response of horn flies to contrasting areas are given in Table 6. In all patterns tested black was much more attractive to the flies than clear or white areas (Figure 16). Black was least attractive when it was compared with white rather than clear area. This was to be expected, however, because the translucent white cast a slight shadow while the clear areas of the slides formed no detectable shadow. Horn flies were still attracted to black when there was no contact involved. Flies were attracted to black patterns whether the patterns were in a vertical or horizontal position provided the light was behind the patterns. These results are in agreement with previous results where it was found that horn flies are attracted in greater numbers to a white steer against a black background than to a white steer against a white background.

Figure 15. Container used in studying the response of horn flies to areas of contrast, heat and odor.

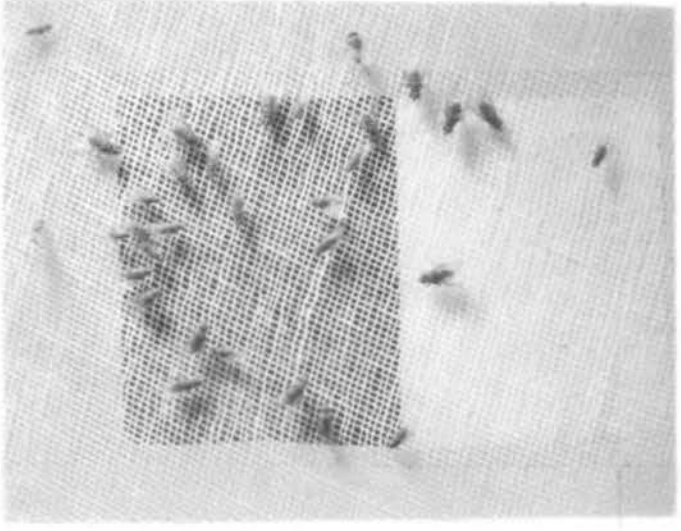


Figure 16. Response of horn flies to area of contrast.

Table 6. Response of horn flies to areas of contrast.

Replication	Type of contrast	Number of flies on black[a]	Number of flies on control
	Clear slide vs. black slide		
1		86	26
2		173	84
3		183	4
4		315	16
	Clear area vs. black area		
1		97	4
2		128	2
3		275	22
4		238	0
	Clear area vs. black area, no contact		
1		56	8
2		101	0
3		86	1
4		133	4
5		25	0
6		72	1
	White vs. black		
1		60	1
2		88	5
3		90	5
4		90	49
5		89	9
6		65	20
	Clear area vs. black area, light and pattern on side		
1		127	13
2		183	11

[a]Each total represents 10 observations.

V. OLFACTORY RESPONSES OF HORN FLIES

Attempts to investigate the olfactory responses of horn flies with the aid of a Y olfactometer did not succeed. It was decided that a modification of the procedure used in the previous section might be useful in studying responses of this nature. The same glass containers described in that section were used in the olfactory experiments (Figure 15).

Aluminum cups about 1/8 the size of a one cup measure were used as the container for the odorous substance and for the control. One hundred freshly emerged horn flies which had been fed honey water were put in each of two glass containers. Test cups were prepared by placing the odorous material in the bottom of one cup and the control material in the bottom of the other cup. If the material to be tested was black hair it was placed in the bottom of the test cup and black thread was placed in the bottom of the control cup. If the material to be tested was white hair white spun glass was placed in the control cup. When extracts made by washing bovine hair was used it was placed on white filter paper and put in the bottom of the test cup, a piece of filter paper with no material on it was placed in the control cup. A portion of the material used in the control was always put in the test cup along with

the test material. After the material had been placed in the bottom of the test and control cups, a false bottom made from two layers of cheese cloth was put in the middle of each cup.

The extract tested was made by washing bovine hair with a solution containing 95 per cent ethyl alcohol and one per cent ethyl ether. The solution was poured into a petri dish and evaporated until all traces of alcohol and ether were removed. Hair used in all experiments was obtained by clipping different areas of the host with unoiled hand clippers and mixing the hair.

One of the containers with the flies was centered beneath the 32 watt circline fluorescent lamp described in the previous section. Light intensities for all areas of the top of the container which were tested were the same. Cups containing the test material and the control were placed opposite each other on the top of the container with the bottoms up (Figure 15). In order to eliminate bias in making the observations the cups were marked A and B and the observer did not know which was the test or control cup until the end of the experiment. After the cups were placed on top of the container the number of flies under each was recorded at the end of two minutes. Horn flies were then scattered by blowing on the top of the container

after the cups were removed. Test and control cups were replaced on the top, 45 degrees clockwise from the former position. This procedure was continued until each cup had been at each of the four positions three times so that a total of 12 observations were made for each replication. At the end of the 12 observations this container was removed and the second container was put in its place. The same procedure was followed with the new container. Six replications with 12 observations each were made for each experiment. All experiments were repeated twice except the experiment in which the extract from bovine hair clippings was tested.

Some factor associated with bovine hair clippings is an olfactory stimulus to the horn fly. Results given in Tables 8 and 9 show that horn flies were attracted to hair removed from a black and a white steer, respectively (Figure 17). In a statistical analysis of the data using the F test it was found that the number of flies attracted to hair clippings was significantly greater than the number attracted to the control. This attractive substance may be removed from the hair with a solution of ethyl alcohol and ether. When the alcohol and ether were evaporated the substance which remained was also a strong olfactory stimulus to the horn fly (Table 10). A statistical analysis of the

Table 8. Response of horn flies to odor of hair clippings from black steer.

Replication	Total number of flies to clippings[a]	Total number of flies to control
1	148	137
2	308	182
3	281	164
4	351	193
5	284	176
6	362	217
7	240	207
8	249	159
9	220	146
10	218	161
11	215	136
12	181	131

F = 80.5476, d.f. = 1 and 11, F.05 = 4.8443

[a]Each total represents the sum of 12 observations.

Table 9. Response of horn flies to odor of hair clippings from white steer.

Replication	Total number of flies to clippings[a]	Total number of flies to control
1	271	210
2	272	279
3	309	234
4	294	280
5	286	259
6	289	250
7	275	270
8	277	233
9	281	216
10	289	209
11	249	200
12	233	191

F = 27.101, d.f. = 1 and 11, F.05 = 4.8443

[a]Each total represents the sum of 12 observations.

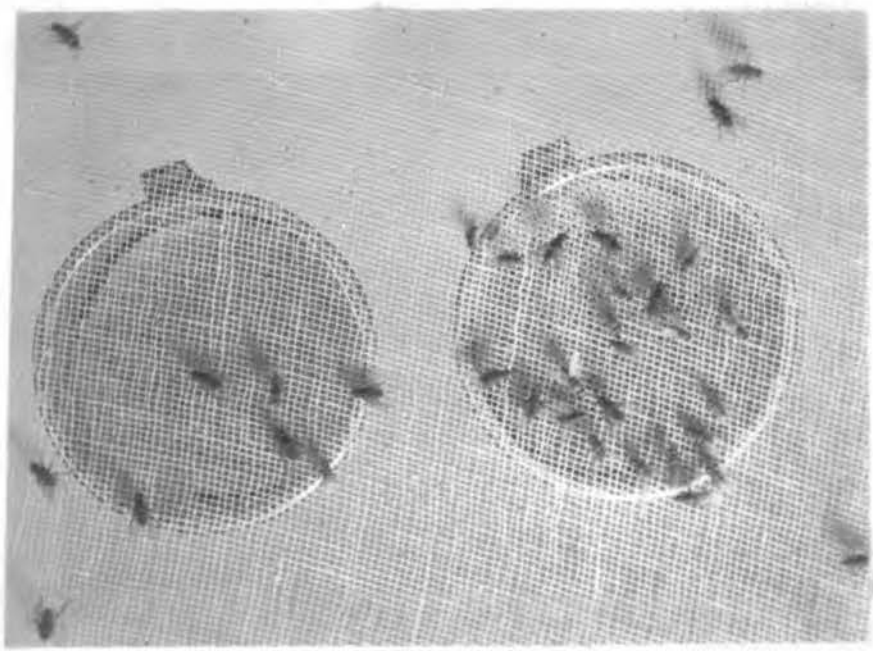


Figure 17. Response of horn flies to odor; right hair clippings, left control.

Table 10. Response of horn flies to extract from bovine hair clippings.

Replication	Total number of flies to extract[a]	Total number of flies to control
1	324	285
2	183	170
3	255	208
4	206	167
5	229	175
6	180	123

$F = 41.2924$, d.f. = 1 and 5, $F_{.05} = 6.6079$

[a]Each total represents the sum of 12 observations.

data confirmed that significantly more flies were attracted to the extract than to the control. It should be pointed out that the flies were not attracted to either the clippings or the control when they were placed inside the container in the opposite direction of the light. It would thus seem that light is the main stimulus and that odor is a secondary stimulus.

VI. OTHER RESPONSES OF HORN FLIES

Response to Heat

Horn flies were observed resting on the black spots on a Holstein steer while the animal was lying on the ground with the spots facing the sun. The observations were made early in the mornings while the air temperatures were still fairly cool. During cool mornings and late evenings horn flies were also observed clustered on the base of their host's horns or on horn scars. In order to determine if this could have been a response to different temperatures, laboratory experiments were conducted to find out if horn flies were attracted to or repelled by heat, and if so the temperature ranges to which they were attracted or repelled.

Apparatus used in the olfactory experiments were also used in the heat studies. In addition a YSI Thermistemp tele-thermometer with surface probe number 409 was used to measure the surface temperature of the two aluminum cups. Two experimental designs were used to investigate the response of horn flies to heat. The first design compared the response of the flies to a 70°F control and a cooling heated surface. The second design compared the response of the flies to a 70°F control and a series of constant

surface temperatures. Each will be discussed in the order given.

Fifty freshly emerged horn flies which had been fed honey water were placed in each of four containers. The container of flies to be tested first was placed under the circline fluorescent lamp previously described. A thermister probe was taped to the side of each aluminum cup near the bottom. Each cup was partially filled with cold water, then enough hot water was added to bring the surface of one cup to 70°F and the other to 125°F. Both cups were placed on the cheese cloth top of the container about an inch apart. Position of the test and control cups were alternated for each replication. The number of flies under each cup was recorded as the temperature of the test cup dropped through a range of 120°F to 75°F. After the last observation the first container of flies was removed and a container of fresh flies was placed under the light. The same procedure was repeated for a total of four replications.

The second design was similar to that of the first except that a range of constant temperatures was compared with the 70°F control. Temperatures compared to the control were 70, 75, 85, and 95°F. Both test and control cups were placed on top of the container in the same manner as

previously described. The number of flies under each cup was recorded after one minute, the time required for the thermister probe to stabilize. When the counts were made both cups were removed and enough hot water was added to bring the surface temperature of the cups to the desired temperature. This was repeated a total of five times, then fresh flies were used and the whole procedure was repeated for a total of three times. Thus for each temperature combination there were three replications with five observations each.

Horn flies were attracted to heated surfaces provided the temperature of the surface did not go to 110°F or above (Table 11)(Figure 18). Flies were repelled by temperatures 110°F and above. Where the temperature was 110°F and above the flies congregated at the edge of the cup and probed extensively, however, they would not walk on the heated surface. When given a choice between two surfaces heated to 70°F approximately the same number of flies went to each surface. If given the choice of 70°F and a warmer surface most flies chose the warmer surface (Table 12). The threshold of the response of the horn fly is apparently very narrow as the fly is attracted to a surface heated to 107°F but not to one heated to 110°F. In preliminary experiments there seemed to be no difference in the number of flies

Table 11. Response of horn flies to heated surfaces.

Degrees F	Total number to heat[a]	Total number to control[b]	Per cent to heat[c]	Per cent to control
120	0	0	0	0
115	0	1	0	.5
110	2	1	1	.5
107	28	6	14	3.0
105	36	5	18	2.5
103	59	7	30	3.5
100	72	9	36	4.5
97	66	6	33	3.0
95	76	6	38	3.0
90	82	3	41	1.5
85	58	7	29	3.5
80	64	4	32	2.0
75	64	9	32	4.5

[a]Each total is the sum of four replicates.
[b]Temperature of control was 70°F.
[c]Per cent of total number of flies for four replicates.

Table 12. Number of horn flies which responded to heated surfaces.

Replication[a]	Temperatures tested (F°)							
	70 vs 70		70 vs 75		70 vs 85		70 vs 95	
1	73	71	56	96	29	115	29	115
2	21	32	21	67	12	83	12	77
3	53	59	23	89	22	115	36	103
Total:	147	162	100	252	63	313	77	295

[a]Each replication represents five observations.

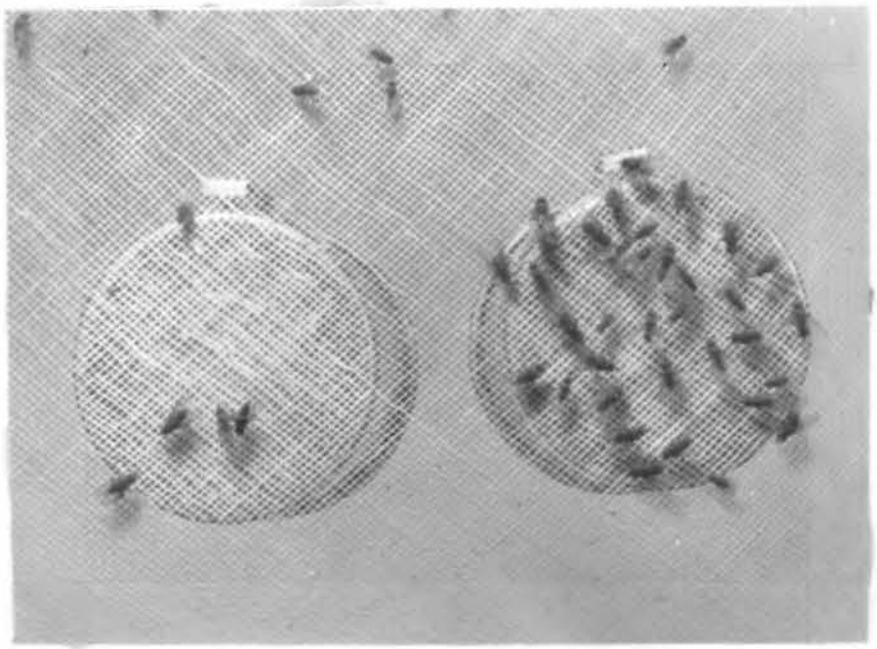


Figure 18. Response of horn flies to heat, right heated surface, left control.

attracted to a warm moist surface and a warm dry surface, therefore, all heat experiments described above were conducted using dry heat.

Horn flies were not attracted to heated surfaces which were placed in a direction away from the light. As with olfaction the heat stimulus seems to be a secondary stimulus and light a primary stimulus. Since heat, even 75°F, initiates probing, it may be considered to be at least a strong feeding stimulus.

Response to Moisture

Preliminary experiments were conducted to determine if horn flies were attracted to moisture. A Y-type olfactometer was used in these studies. Air into each arm was drawn through activated charcoal to remove odor and then through anhydrous calcium sulfate to remove moisture. Moisture was added to the air passed through one arm while air in the other arm remained dry. The humidity in the test arm was 88 per cent while the humidity in the control arm was 19 per cent. Temperature of the room and the water used to moisten the air was 72°F. Air flow was 250 cubic centimeters per minute. Tests were conducted to determine if one arm of the Y apparatus was more attractive to the horn flies than the other arm. In this test no moisture was added to either arm. There was no significant difference

between the arms. When the tests were conducted with moisture added to one arm and not the other, again there was no significant difference between the number of flies going into each arm. This is in agreement with the previous section where horn flies were found to respond to dry heat as readily as moist heat. This, however, should not be considered to mean that under other conditions horn flies would not respond to moisture. The flies used in these experiments were aged flies which had been recently removed from the host.

Response to Gravity

Experiments with the Y-type olfactometer indicated that the results would be biased for one arm if that arm were elevated slightly more than the other. An experiment was conducted to determine if this could have been a response of horn flies to gravity. Apparatus to study the response of these flies to gravity was constructed from two wide mouth gallon jars connected by taping their tops together (Figure 19). A hole about two inches in diameter was cut in the center of each top to allow movement of the flies from one jar to the other. Two containers of this type were constructed.

Fifty freshly emerged adult horn flies which had been fed honey water were placed in the top of each container.

Figure 19. Apparatus used in determining the response of horn flies to gravity.

The containers were placed in front of a fluorescent lamp located at the point where the jars were connected so that light would not direct the flies up or down. Immediately after the light was turned out one container was turned upside down. This resulted in the flies in one container being in the top jar and the flies in the other being in the bottom jar. At the end of ten minutes the light was turned on and the number of flies in the top and bottom of each container was recorded. This procedure was repeated five times, then the position of the containers was reversed and the procedure was repeated another five times.

In all instances more flies went from the bottom container to the top container than from the top container to the bottom container (Table 13). The number of flies going upward was significantly greater than the number going in the opposite direction. Bias encountered in the Y olfactometer may well have been due to a negative geotactic response of the horn fly.

Dispersal of Horn Flies

On every occasion when the horn fly colony was observed after the lights had gone off at night a large number of flies were seen away from the host. Many were seen resting on the walls and ceiling of the rearing room as well as crawling around on the floor. This lends

Table 13. Response of horn flies to gravity.

Replication	Number negatively geotropic	Number positively geotropic
1	15	5
2	15	5
3	20	3
4	20	6
5	17	2
6	34	1
7	41	1
8	25	3
9	39	0
10	35	1

$F = 39.426$, d.f. = 1 and 9, $F_{.05} = 5.1174$

support to the theory that vision is the main factor in leading the horn fly to its host. Apparently the flies were unable to locate the host in the absence of light. Olfactory and heat stimuli are probably unable to substitute for light in guiding the parasite to its host. When the light system was turned on the insects began flying back to the host, either directly or by first going to the light then to the host. When freshly emerged flies were released within a few inches of the host most flies flew directly to the light then back to the host rather than directly to it. This dispersal of flies from the host during dark might be an important factor in the distribution of horn flies between animals as well as between herds. This problem

should be thoroughly investigated to determine if this is a condition associated with the rearing technique or a general phenomenon.

VII. DISCUSSION OF RESULTS

The successful colonization of the horn fly has made it possible to study its behavior throughout the year. More intensive studies on its control, physiology and biology in general are now possible. While colonization of this parasite on the host is an important step and opens many new avenues for investigation it has many limitations. Strict control of the colony is not possible because it is still not feasible to remove flies from the host for a considerable period of time. The colony is completely dependent on the host, if it becomes sick or dies a new one must be found immediately. This rearing method is also time consuming and rather expensive because the host requires constant feeding and care. The ultimate in rearing the horn fly would be to develop a substitute for the manure to rear the larvae in and a substitute for a blood meal from the host to feed the adults. If this could be done a true laboratory colony of horn flies could be established.

The experiments with visual, olfactory and heat stimuli illustrated that horn flies responded to these stimuli, however, it was not determined at what distance from the stimulus the insect would cease to respond. This should be investigated especially with the olfactory and heat stimuli.

It would also be of interest to know at what distance the horn fly is visually stimulated by the host and if motion of the host is an added visual stimulus.

As previously noted all insects used in the laboratory experiments, except the experiments with moisture, were freshly emerged flies which had been fed honey water. Experiments should be conducted to determine if there is a difference in the responses of freshly emerged flies and flies which were removed from the host. As the adults reach sexual maturity certain behavior changes could be expected, however, these changes need not change the process of host selection.

Light meters used to measure the intensities of the light used in the visual experiments, while they gave consistent results, were not highly sensitive. Light intensities below one footcandle had to be calculated. A more sensitive meter would have eliminated this and would have made it possible to control the intensity of light by the use of diaphragms.

The results of this research support the contention that vision is the most important factor in directing the horn fly to its host. The probable series of events from emergence of the parasite to its feeding on the host are as follows: Light stimuli and negative geotaxis lead the fly from the site of emergence into the air. Once in the

air it flies toward the light until areas of contrast in front of it override the light stimulus and the fly flies toward them. When flying toward the areas of contrast, sight of the host stimulates a strong motor response and the fly goes more or less directly toward it. If all conditions are favorable, the insect lands on the host. Heat from the host stimulates the insect to probe and feed. After the horn fly has fed the odor and heat of the host keeps the fly near the animal. Once the fly leaves the animal vision must be used to relocate it. Olfaction and heat are apparently not adequate substitutes for visual stimuli.

VIII. ABSTRACT

Horn flies were reared under artificial conditions for a year beginning April 20, 1960. Adult flies were maintained on a steer in a specially constructed rearing room. Photoperiod in the room was controlled to 19 hours of light and 5 hours of dark. Average temperature in the room was 72°F and average humidity was 67 per cent. Larvae were reared in gallon jars which were placed on heating cables. An average of over 1,000 adults emerged during each of the 187 days for which records were kept.

Adult horn flies were able to distinguish visually between a steer and a control area at a distance of 12 feet. They were not able to distinguish between a sheep, man, or artificial object and a control area. The response threshold of the fly to white light was found to be below 0.004 footcandles. A straight line relationship exists between the per cent response of the flies and the log of the intensity up to .700 footcandles. Maximum response was obtained with an intensity of 1.1 footcandles and above.

By use of a choice box and appropriate narrow-band-pass filters with the proper light source, spectral responses of horn flies was investigated. Strongest responses were obtained with filters transmitting wavelengths of 550 mu and shorter. Responses to wavelengths between 565 and

625 mu were about half as great as the response to shorter wavelengths. There was no response to light of 670 mu and above. All wavelengths were tested at an intensity of 2.5 footcandles which was well above the point where maximum response of the horn flies was obtained with white light.

Horn flies were found to be attracted to areas of contrast. Large cover glasses with various patterns of contrasting areas were placed on the top of a container of flies. In all instances the flies congregated on the dark portion of the slide and not on the clear or white portion. More flies went to the white areas than to the clear areas. Contact with the contrasting areas was not an important factor in these experiments.

Odor from the hair of the host was attractive to the horn fly. Hair from two steers and an extract made from washing bovine hair were significantly more attractive to the flies than were the controls. The odorous substance and an appropriate control material were each contained in small aluminum cups placed upside down on a cheese cloth top of a container of horn flies. The number of flies going to each cup was used to indicate whether or not an olfactory response was present.

A procedure similar to the one used in studying olfactory responses of horn flies was also used to investigate the response of these insects to heat. Flies were

found to be attracted to the warmer of two surfaces from 70°F to 107°F. They were repelled by surface temperatures 110°F and above. Heat released a probing response in horn flies at all temperatures tested.

Tests conducted with a Y-type olfactometer indicated that horn flies were not attracted to moisture under the conditions described. Evidence is presented to show that these insects are negatively geotactic. The idea that horn flies remain on the host at all times except to oviposit is questioned because observations were made at night when many flies were found on the walls, ceiling and floor of the rearing room.

Evidence is given to support the contention that vision is the most important factor in leading the horn fly to its host.

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