THE SENSORY RESPONSES OF THE HOUSE FLY, MUSCA DOMESTICA LINN., TO ATTRACTANTS

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1949

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1955

Submitted to the Faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
May, 1965

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PREFACE

It was my privilege, while a member of the United States Air Force, to be assigned to Oklahoma State University to work toward an advanced degree in entomology. Since the control of house flies is one of the principal entomological problems in the Air Force, a problem of this type was sought. A problem was envisioned when Dr. D. E. Howell, Professor of Entomology and Head of the Entomology Department, Oklahoma State University, suggested to me during the fall of 1963, that house fly attractants should be investigated. Studies are needed to find suitable attractants that will lure house flies to poison bait preparations and to determine the sensory responses used in their allurement. This I have endeavored to do by a series of tests to determine the degree of olfactory response, the attractiveness to different colors and the stimulation of senses used by house flies in locating different attractants.

My sincere appreciation is expressed to Drs. D. E. Howell, my major advisor, whose kind, untiring assistance and cooperation have proven invaluable in helping me carry out this problem, R. R. Walton, Professor of Entomology, H. L. Chada, United States Department of Agriculture and E. D. Besch, Professor and Head, Department of Veterinary Parasitology and Public Health, for their constructive criticism and suggestions in this work. My sincere gratitude is expressed to the United States Air Force, who made this study possible, to the Phillips Petroleum Company, who furnished many of the candidate attractants, C. Dayton Steelman,

Paul Sterling and Donald L. Bailey, graduate students, for their assistance with methods and materials.

Appreciation is also extended to Mrs. Verna Duckwall, Secretary for the Entomology Department for typing this dissertation and to my wife, Margaret for her encouragement throughout the study.

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INTRODUCTION

The house fly, Musca domestica Linn., has been suspected of being involved in the transmission of disease since Biblical times. It has attained vast public health importance in the spread of such diseases as amoebic dysentery, cholera, yaws, typhoid fever and infantile diarrhea. The house fly has been able to establish itself successfully as a vector because of its rapid rate of reproduction, of the anatomy of its mouthparts and of its omnivorous feeding habits.

According to Howard (1911), it has been known for many years that good sanitation and prevention of breeding were our best protection against the house fly. However, due principally to negligence and to other reasons, these measures cannot always be carried out and they must be supplemented with chemical control.

Some of the earlier chemical control measures consisted of poison baits containing attractants to lure flies to their doom. Later, residual and contact insecticides played a major role in controlling house flies but these substances, in time, proved to be less effective for flies developed a resistance to them. The more modern methods of control that are being practiced today include radioactive sterilization, chemosterilization and the continuing search for more suitable attractants.

Insects have waged a struggle with man for survival for centuries. During this long period they have developed several supersensitive faculties to guide them to their food and mates. Among the most important of faculties is olfaction, using the sense of smell to detect chemical sub-

stances that are a source of attractive odors. Now, after millions of generations this keen sense may lead an insect to its doom instead of being a vital aid to survival of the past (Anonymous, 1963).

Steiner et al. (1958) reported that a poisoned-bait spray containing malathion and one of several types of protein hydrolysates gave good control of the oriental fruit fly, the melon fly and the Mediterranean fruit fly for as long as two weeks. The fruit flies were attracted to the protein hydrolysate, which contained nutrients essential for their sexual development, and they quickly ingested enough of the insecticide to kill them. In 1956, the Mediterranean fruit fly was eradicated in Florida by spraying this material over 800,000 acres.

Christenson (1963) stated that in a test of the male annihilation technique in the Western Pacific, aerial distribution of Celotex wafers impregnated with methyleugenol containing 3% Dibrom reduced the oriental fruit fly to only 28 males per 1000 trap days on Chichi Jima in 12 months. Male attractants for other tropical fruit flies were strong enough to warrant consideration as possible male annihilation agents.

These are only isolated instances of insect control with attractants as lures. Much research is now being conducted to synthesize food attractants so they may be used to lure destructive insects to their doom. Oviposition and sex attractants are being investigated for their attraction to insects; the latter has been isolated from roaches and is highly attractive to other roaches.

Discovery of additional attractants for use with poisons and in traps would lessen the amounts of insecticides needed and in turn lessen the dangers attributed to their use.

REVIEW OF LITERATURE

The review of literature in this dissertation will be limited to the discussion of the responses of house flies to attractants and instruments to measure the responses. Dethier (1947), Frings and Frings (1949), West (1951), Hodgson (1958), Green et al. (1960) and Beroza and Green (1963a) have presented comprehensive reviews on house fly attractants and chemoreception in insects.

Sensory Responses of Insects to Stimuli

Olfaction may be an important sensory response to an insect in its selection of food materials and oviposition sites. There may be other responses or a combination of these responses that are just as important to the insect's survival. The use and location of sensory organs of insects have been the object of much research and speculation by scientists.

Tragardh (1913) defined chemotropism as the automatic orientation of animals to any olfactory sensation in such a manner that both sides of the body are struck at the same angle by the lines of diffusion. Hewitt (1917) stated that chemotropism was the reaction to stimuli of a chemical nature perceived through the olfactory sense. McIndoo (1928) referred to the reaction of plants to stimuli as chemotropic and animals as chemotactic. In regard to attractive baits, smell and taste were both used, but McIndoo believed that insects do not have a true sense of taste. The reactions of insects to attractive and repellent odors are so different from that of man that human sensory responses cannot be used as a basis for study.

Thomssen and Doner (1942) recognized three functions that could be attributed to the chemical senses of insects (1) food selection (2) food selection for oviposition and (3) sexual attraction.

Lodge (1918) determined that flies whose eyes were painted with varnish and India ink, paid very little attention to baits, but sometimes would feed. Amputation of the antennae did not prevent flies from detecting the difference in foods; however, flies treated in this manner had less interest in food than normal flies. Lodge stated that curiosity apparently is an important factor in causing flies to come to baits, although the sense of smell and, to a lesser extent, the sense of sight are involved in this response. Kuzina and Kyenha (1940) tested the house fly's response to dung by obscuring the eyes and removing the antennae and proboscis. They reported that the flies responded to a suitable medium for oviposition almost entirely by smell, however, they considered taste to be necessary to stimulate oviposition itself. McIndoo (1933) conducted olfactometer tests and determined the response by three species of blow flies, with the antennae removed or intact. Approximately the same number of flies with and without antennae responded to the odors. He concluded that the antennae of blow flies did not bear the olfactory organs. Wiesmann (1960), in comparing the responses of normal house flies to various stimuli with those of house flies with the antennae removed, found that the antennae bore organs sensitive to odor, humidity, heat, shock and air disturbances. He did not find contact chemoreceptors on the antennae, and there were fewer olfactory receptors on the house fly flagella than on closely related species of flies. He found that the attraction to food on which other flies were feeding was the result of visual perception. Furthermore, since house flies found food and breeding

places in relatively confined areas, such as houses and animal quarters, he concluded that the sense of smell in this species was not very highly developed.

Abbott (1928) stated that screwworm flies could distinguish between acceptable and unacceptable substances as food through chemoreceptors located on the tarsi, because they avoided urea when the tarsi were placed in the fluid. Deonier and Richardson (1935) determined that the tarsal segments of the house fly were sensitive to solutions made of sucrose and levulose, but less so to the latter. Minnich (1926) demonstrated that the tarsi of blow flies were sensitive to water and sucrose solutions, since they extended the proboscis when the tarsi came in contact with these substances. This response indicated that the tarsal chemoreceptors serve as taste organs. However, the chemoreceptors of the proboscis oral lobes were more sensitive to 1 M sucrose solutions than were those of the tarsi. McIndoo (1934) challenged the work of Minnich when he reported that when the tarsi of blow flies were touched to a piece of screen wire 3 mm above a sugar solution, a positive proboscis response was invoked. During this test the tarsi of the fly were not in contact with the sugar water and McIndoo theorized that the proboscis response was due to combined olfactory and tactile stimuli. Because Deonier (1938) using house flies could not duplicate the results of McIndoo, he concluded that since there was no olfactory response the tarsal receptors were contact chemoreceptors. Abbott (1936) reported that in the presence of odorous substances the motor reactions and the proboscis response of flies do not always depend on the same factors. Flies may give a proboscis response to substances which either attract or repel them. The chemical substances that attract insects, in most cases, prob-

ably consist of mixtures. Efforts of several workers to demonstrate a positive response in flies with specific chemical compounds has in most cases proved futile.

Hayes and Liu (1947) found that the chemoreceptive sensilla were located lateroventral on the second to fifth tarsal segments and not on the dorsal side of those segments nor in the first tarsal segment. There are several kinds of appendages located on the cuticula, such as spines, fixed hairs, tactile setae, tenent hairs and chemoreceptive setae. Frings and Frings (1949) revealed that on the labella of the house fly the long trichoid sensilla were tactile and the shorter trichoids were contact chemoreceptors. The pseudotracheal papillae were found to be possible receptors. Chemoreceptors were located on all the tarsi and especially on the four terminal tarsal segments.

Crombie (1944) reported that the response of adult blow flies to the odor of menthol was modified after exposing the larval stages immediately after emergence. The memory of an experience in the larval stage apparently survived metamorphosis and ultimately affected the behavior of the adult flies. Various groups of habituated flies responded differently when exposed to menthol; some were indifferent to the exposure, some were repelled and some were attracted to it.

Wright (1958) observed that the mechanism whereby the odor guides the insect to its goal is not known, nor is it self evident, for olfaction differs from sight and hearing in being a non-directional sense. An insect flying at random and entering a cloud of odor would perceive the odor not as a continuous sensation of gradually increasing strength, but rather as a series of pulses or alternations of high and low odor intensity as it passed through the many odor trails that make up the cloud.

In work with the olfactory guidance of flying insects, Kellogg et al. (1962) stated that in the absence of odor, Drosophila flight was in a straight pattern. Very little flying occurred in the dark and the insects stayed close to the ground. In a uniformly diffused odor with no wind, they landed on the nearest object and crawled about.

Olfactometers

Olfaction was one of the first sensory reactions to be used by insects in locating their food and oviposition sites. Much research work was initiated to determine the extent of the olfactory sense and assist in the definition of this response.

McIndoo (1926) constructed an olfactometer consisting of dark and light chambers connected by a "y" tube. A suction apparatus drew the odors through the tube from the dark chamber to the light chamber. Insects were allowed to pass from the dark chamber, were attracted to light near the free ends of the forks and were forced to make a choice between an attractant, repellent or a control. Snapp and Swingle (1929a) used the McIndoo olfactometer to check the attractiveness of portions of peach plants to the plum curculio.

A "U" type olfactometer was developed by Eagleson (1939) to determine the olfactory response of flies to chemical stimulation. Flies were introduced into one arm of the "U" while the other contained the attractant or repellent to be tested. Counts were made of the flies resting on a screen that closed the arms of the "U".

Ingle (1943) attempted to determine the response of house flies to different substances on wire screens. To accomplish this, he developed an apparatus in which a blue light was used to attract house flies and

subjected the flies to test materials in the same state used for field tests. Data recorded from these tests represent a summation of the fly's receptor system.

Chamberlain (1956) designed an olfactometer that consisted of a large rectangular stainless-steel box enclosing a smaller cage containing the test insects. The smaller test cage was designed in such a manner that the insects had a choice to leave by a left or right port. The design allowed for the quantitative control of the concentration of the test chemical.

An olfactometer was reported to be used in Hawaii for screening new attractants using fruit flies as the test insects. In a screened room, fruit flies were exposed to 12 materials that were mounted on a slowly rotating wheel. The numbers of insects drawn to each material were counted after a specified period of time and the attractant qualities were determined in this manner (Anonymous, 1957).

Howell and Goodhue (1964) develped an olfactometer that was composed of two cylindrical chambers to hold test flies. Each chamber was divided into two equal vertical compartments by a cardboard partition. Solutions of the test materials were placed on Kleenex-covered, flared, glass tubes located beneath each compartment. Air was drawn through the chambers by suction from water pressure. The numbers of flies were counted that were found resting on the attractant or on the diluent sides of the cylinder.

Chemical Attractants

Attractants were among the earliest means of luring insects to poison baits. A suitable attractant to lure the insects to the bait was equally as important as the toxicant to kill them.

Dethier et al. (1960) defined an attractant as a chemical which causes insects to make oriented movements toward the source of the chemical. He stated that an arrestant was a chemical which caused insects to aggregate in contact with it, the mechanism of aggregation being kinetic or having a kinetic component.

Smith (1911) was able to clear buildings of house flies in 36 to 48 hours with a formalin bait mixed with water or milk. Weiss (1912) reported good results in controlling house flies with a bait prepared from corrosive sublimate, arsenic trisulfide and quassia. Morrill (1914) stated that vinegar was attractive to house flies and when used with sugar or bread the attractiveness was increased. An attractive bait for house flies was found by Buck (1915) to be one of light bread and buttermilk to which 7% formalin and a little sugar or syrup were added. McIndoo (1927) reported that the first successful poisoned bait used in the United States (California, 1885) was an attractive bait for grasshoppers consisting of bran, arsenic, sugar and water. Good house fly control with a wet bait made from trisodium arsenite and arsenic acid dissolved in cane sugar was reported by Pearson and Richardson (1933). Fenton and Bieberdorf (1936) reported good results in killing large numbers of house flies with a formalin, milk, water and molasses bait. Frost (1936) was successful in capturing a liberal number of Muscidae in traps baited with sweet baits, amylacetate and syrup being the most attractive, although traps baited with citric and malic acids caught large numbers of Diptera. Gahan et al. (1953) obtained effective house fly control in dairy barns by using blackstrap molasses or malt as an attractant in wet baits containing tepp, sodium fluoroacetate or sodium arsenate. Good house fly control in barns was obtained by Langford (1954) and Keller et al. (1956)

using baits with sugar as the attractant and Bayer 1359 as the toxicant.

Richardson (1916a) stated that house flies are attracted to fermenting organic substances principally by the odor of ammonia. The ammonia odor attracted mainly the females and they oviposited in organic substances where food was available for larval development. In tests of the compounds which occur as products of fermentation in barnyard manure, Richardson (1916.) found that butyric acid and to some extent valerianic acid, when added to moist ammoniated cotton, augmented the oviposition response of the house fly. Ammonium carbonate and moist cotton without the aid of these acids brought forth almost no response.

Richardson (1917), in work with baited traps, reported that lactose and dextrin were more attractive to house flies than glucose, fructose, maltose, sucrose and starch. Sucrose gave consistently poor results. Four percent amylic alcohol gave better results than 4 to 10% ethyl alcohol or acetic acid, and better than 10% amylic alcohol. Four percent ethyl alcohol was more attractive than 10% but the reverse was true of 4 and 10% acetic acid.

Imms and Husain (1920) stated that ethyl alcohol alone was not attractive to house flies but the addition of small amounts of butyric, valerianic or acetic acid made powerful attractant stimulants. House flies were attracted to banana during fermentation, but its attractiveness decreased as the putrescent mass dried, according to Speyer (1920). Valerianic acid, amyl acetate and amyl alcohol were attractive to house flies in that order. Saturated compounds contained in fermenting vegetable substances and containing the molecular group $CH_3(CH_2)_x$ may have produced the stimuli by which house flies were guided to their food.

Awaiti and Swaminati (1920) found that house flies were attracted to the odor when certain substances were allowed to ferment and putrefy. Eggs, meat and fish had greater attractive properties than rice, wheat and pulses. The essential attractant in these substances was either ammonia, sulphuretted hydrogen or compounds of phosphorus.

Crumb and Lyon (1921) reported sodium carbonate to be attractive to house flies for oviposition. The house fly was attracted by decaying organic matter. The degree of attractiveness was proportional to the amount of carbonic and acetic acids liberated in the process of fermentation.

Richardson and Richardson (1922) stated that bran, which volatilized the products of decomposition of ammonium carbonate in an aqueous solution, attracted house flies and induced oviposition. Since carbon dioxide and water did not produce oviposition, it was believed that ammonia released during decomposition was largely responsible for the attraction to ammonium carbonate. Carbon dioxide and water when volatilized by bran would not produce oviposition.

Atkins (1921) related that a mixture consisting of acetyl cellulose, acetone, methyl acetate, methyl alcohol, ethyl alcohol and benzene was attractive to house flies. Methyl acetate probably was the principal attractant. Butyl acetate also was demonstrated to attract large numbers of house flies.

Yates (1951) determined that the addition of 20% ammonium carbonate to bran and other protein feeds provided a more attractive bait to female than to male flies. Baits composed of these substances were more attractive to flies that were over five days old than to younger flies.

Bishop et al. (1923) stated that acetone and amyl butyrate were found to be good attractants for the house fly. Furfural, safrol and salicylic aldehyde, along with several essential oils, such as anise, cassia, clove, citronella, fennel and sassafras, various pine oils, certain camphor oils and artificial mustard were demonstrated as being repellent to house flies. Morgan and Crumb (1928) discussed data that indicated carbon dioxide, acetic acid, sodium hydroxide, formic acid, proprionic acid, sodium sulfate and butyric acid to be attractive to house flies. Laake et al. (1931) reported that house flies were attracted to geraniol, bromoform, ethyl mercaptan, chloroform, butyraldehyde, formaldehyde and arsenic solution. Hobson (1936) showed that blow flies were attracted to indole and skatole applied to sheep fleece. The attractant properties of mixtures of ammonium carbonate and indole appeared to be equal to, or slightly greater than, the sum of those of the compounds separately. Larval excreta were very attractive in the moist state. None of the materials were attractive when not on the sheep.

Brown et al. (1961), in tests of chemical attractants for the house fly, determined that no single compound was so active that its attractiveness could not be enhanced by an admixture. The most attractive material used consisted of a combination, in aqueous solution, of malt extract 5%, ethyl alcohol 0.5%, skatole 0.12%, acetaldehyde 1% and acetal 1%. It was significant that the addition of 1% acetal to the mixture increased the attractiveness to house flies.

Organic chemicals of the paraffin series were found to be attractive to house flies by Cook (1926). There appeared to be an optimum concentration for each compound used in his study. The optimum concentration was

related to the boiling point of the compound and became smaller as the boiling point increased. The relative attractiveness of the paraffin alcohols and esters was reported to be related to the boiling point of the compounds.

Wieting and Hoskins (1939) demonstrated that a substance attractive to house flies at a low concentration may be repellent at a high concentration. Mixed groups of flies, in a 50:50 sex ratio, were attracted to ammonia at a concentration of 0.012% by volume and repelled at concentrations greater than 0.03% by volume. Carbon dioxide had no effect on up to about 2% by volume and ethyl alcohol had little attraction at 0.02% and repelled at concentrations above 0.05%. The females were more attracted to ammonia and the males were more attracted to the alcohol.

Lineva et al. (1960), in the Soviet Union, found technical trichlorophon (Khlorofos), when used in solution to impregnate paper sheets for control purposes, to have an odor so attractive to house flies that no other attractant was necessary. Mason and Henneberry (1963) showed that when lindane wettable powder was added to a bait, consisting of 1% apple cider vinegar, 4% active dry yeast and 10% granulated sugar, the attractiveness of the bait was increased for fruit flies. The addition of lindane emulsifiable concentrate decreased the attractiveness of the bait.

Beroza and Green (1963) conducted an extensive program in which a large number of chemical attractants were screened against large numbers of insects, including the house fly. The standard used for comparison purposes in the tests was Edamin, an enzymatic milk digest. Materials 5 to 20 times as attractive to house flies as the standard are as follows:

Butyric acid, 2-ethyl,-2-(2-butorylethoxy)ethyl ester.

4, 7-Methanoinden-6-ol, 3a, 4, 5, 6, 7, 7a-hexanydro,-formate.

m-Toluic acid, 2-(2-butoxyethoxy) ethyl ester.

m-Toluic acid, 2-methoxyethyl ester.

m-Dioxane, 2-benzyl-4,-6-dimethyl-.

m-Dioxane, 5, 5-dimethyl-2-p-tolyl-.

1, 3-Propanediol, 1-(3, 4-methylenedioxy-phenyl)-2-phenyl-.

Pyran, tetrahydro-2-(2-propynyloxy)-.

Staley's Bait #2.

Staley's Bait #7.

Attractants - Fly Factor

It has been noted that during the feeding process, house flies contribute something to the food that makes the food attractive to other flies. The substance contributed to the food by the house flies is thought to be secretions, excretions or a combination of both and has been termed fly factor. Fly factor was collected by saturating fed-upon sugar with a solvent. The material collected not only made sugar more attractive to flies but also appeared on the spectrophotometer, absorbing a certain length of ultra-violet light. It was unstable in light, air and high temperatures (Anonymous, 1955).

Using the Petri-dish tests, Barnhart and Chadwick (1953) observed that flies which visited a bait contributed to it some substance which enhanced its attractiveness to the species. Dethier (1955) found that aggregations of flies, to exposed dishes of solid or dissolved sugar, were brought together by ortho-kinesis. Sugar-baited traps became attractive by the presence of the flies themselves. Flies emerging from traps left a volatile substance on the outside of the trap. Sugar has

been called an attractant because flies gather around it in great numbers. Sugar causes flies to stop all exploratory operations and once stopped, flies tend to accumulate around the sugar supply. From the practical standpoint any material like sugar which is accepted (acceptant) is less efficacious than a true attractant because it retains the insects which have blundered there. The true attractant has properties which stimulate the chemoreceptors of the insect and cause the insect to be oriented toward the attractant.

Dethier (1957) pointed out that there were two categories of agents which were truly attractants: the products of the female scent glands and the unknown materials or interactions by which some insects enhance the attractiveness of food upon which they have fed.

Acree et al. (1959) reported that fly factor was mostly moisture. The flies responded, not to the moisture itself, but to the difference between the relative humidities generated by the samples undergoing test and the relative humidity of the surrounding atmosphere. Fly factor was a low grade attractant. Wiesmann (1962) stated that flies were attracted to food already fed on. They were also attracted to dishes containing dead flies and bits of black paper. He concluded that the attraction of flies to food that was being fed on by other flies was due to the formation of attractive sugar solutions on the baits by the action of the saliva of the flies, combined with the visual aggregative instinct of the flies themselves.

Attractants - Color, Light and Shapes of Objects

According to Galaine and Houlbert (1916) house flies were restless and then inactive in the presence of blue light. When blue window panes

were used in buildings, flies would not enter from the outside. In experiments with different colors in artificial light and daylight, Awaiti (1920) showed that yellow had the greatest attractiveness to flies, red and violet the least, and blue, green and orange were intermediate. The response was identical by day or night.

Freeborn and Berry (1935) used a checkerboard design to determine the preferred resting places of house flies in relation to color. They found that there was a sharp division in color preference between the pale colors beginning with primrose and including ivory, foam green, coral and white. Intermediate groups were light blue, jade green and aluminum. Orange was apparently not distinguished by the flies from five other colors ranging from light blue, through jade green, aluminum, canary yellow and light gray.

Atkeson et al. (1943), in color tests with house flies in dairy barns, showed that the flies preferred to rest on the darker colored surfaces. The trapping effect of screens and the tendency of flies to migrate to light was shown by the fact that five times as many flies were found on screens as on the walls in the bedded barn, while more than eleven times as many were found on the screens in a clean barn. Waterhouse (1948) also demonstrated that house flies preferred the darker colored resting surfaces. The most attractive color was red and the least attractive white. The order of preference was due largely to the intensity of the light reflected by the colored surfaces. Harsham (1946) discovered, in contrast to the belief of grocers that house flies preferred to rest on yellow wrappers and foil wrappers, that foil was the most attractive, yellow was moderately attractive and purple was the least attractive.

Howell (1961) found that when different colored dusts were added to baits, tan baits killed the most flies, followed by yellow, brown and pink. These were more attractive than uncolored baits. Black, dark blue, red and deep orange were the least attractive and reduced the effectiveness of the baits as much as 50%. Mount (1962) used ten different colored baits on black and on white plates to determine the color preference of house flies. Black gave the highest mean fly kill for all factors; however there was no significant difference in plates at the 5% level.

Cameron (1938) reported that the house fly was much more strongly stimulated by ultra-violet light of wave length 3656 A. than by any other part of the spectrum tested. The influence decreased as the longer wave lengths were reached and on the short wave length side of the peak. Weiss et al. (1943) found that Drosophila melanogaster reacted with a peak response in the ultra-violet wave length band with a secondary in the blue-green area. Weiss (1943) stated that the responses to wave lengths which we interpret as responses to color, were simply manifestations of the effect of radiant energy (wave length and intensity) upon living organisms and although insects may or may not have color sensations, to us they frequently behave as if they do, whether they are benefited or not. Weiss (1944) revealed that it appeared that both the electrical responses of the insect eye and the motor responses of the insect to different colors of equal intensity were due to the differences in sensitivity to the absorption of light, which varied with wave length, by the primary photosensitive substance of the visual sense cells and were not the effects of wave length itself. Weiss (1946) concluded that of the two inseparable constituents, wave length and intensity the latter

seems to be the most important in producing reactions.

Smirnov and Chuvakhina (1956) found that house flies were attracted to sugar baits when small black paper triangles resembling house flies were added to the baits. In tests with small squares and rectangles the former were more attractive. They observed that the reactions of the house flies were manifestations of the instinct that caused flies to be more intensely attracted by food if it was already frequented by other flies.

METHODS AND MATERIALS

Tests were conducted in 1964 and 1965 to determine the sensory responses of the house fly, Musca domestica Linn., to several types of chemical attractants. Over 40 chemical materials were tested as attractants for house flies in the Howell-Goodhue and the hemispheric olfactometers. Tests with sight, sound, smell, color, and light were conducted to determine their importance to house flies in selecting attractants.

The Howell-Goodhue olfactometer tests were performed in the insectary. The remaining tests were conducted in a 6 x 6 x 6 ft Peet-Grady chamber located in the basement of the entomology laboratory. The light source for the latter tests, with the exception of the light tests, was 12 7-watt light bulbs, evenly spaced around the walls of the Peet-Grady chamber. Approximately 500 (24-hr starved) flies were released into the chamber for each test.

To decrease the variance of position of test materials, the experiments were conducted on a 36-inch diameter revolving turntable (1 rpm). The turntable was covered with white filter paper for all tests performed in the Peet-Grady chamber, with the exception of the color tests, during which several different colored papers were used. Counts of flies in tests when the turntable was used were taken as the turntable passed a fixed point; the flies were disturbed after each count. The temperature ranged from 80 to 86°F and the relative humidity from 60 to 70% inside the Peet-Grady chamber.

An attempt was made to control the temperature and relative humidity

by the use of an evaporative cooler and an electric heater.

Rearing

A colony of house flies was established from wild house flies collected from the barns of Oklahoma State University in the summer of 1964. Adult flies were maintained in 24- x 24- x 24-inch wire- or plastic-screened holding cages. The emergence and holding cages are shown in Figure 1. The flies were collected from the emergence cages by aspiration from a vacuum cleaner and transferred to the holding cages for transport to the test sites. The flies were sustained on dry skimmed milk, sugar cubes, and water, each of which were placed in separate containers in the emergence cages. Tischler (1931) found that bread in milk without the addition of yeast, meat broth, or sugar was an adequate diet for adult house flies.

When the adult flies were 2 to 4 days old, they were allowed to oviposit on moist paper towels wrapped around a portion of Chemical Specialties Manufacturers Association (CSMA) medium (Ralston Purina Company). The eggs were removed from the paper towels with a camel's-hair brush and placed in 25 cc of water in a 50 ml beaker. The supernatant fluid was decanted and the eggs were introduced into a 10 ml graduated cylinder. Peterson (1959) stated that a 6- x 8-inch-battery jar two-thirds filled with medium, will support approximately 2100 well-developed larvae. To obtain this number the medium was seeded with 2800 eggs, approximately the amount contained in 0.4 ml in a graduated centrifuge tube. The above procedure was followed in seeding eggs to obtain the approximate numbers of house flies needed for the tests. Richardson (1932) formulated the first CSMA medium and it was later standardized by

the Chemical Specialties Manufacturers Association. The medium consisted of a mixture of bran, alfalfa meal, yeast and Diamalt. The bran and alfalfa meal were mixed together and constituted the dry part of the mixture. The dry mixture was mixed with a suspension of yeast and Diamalt in water to complete the formula. Only the dry part of the formulation of the CSMA medium, mixed at the rate of 340 g to 750 ml of water, was used to prepare the medium for this study. There appeared to be no adverse effects to the life stages of the house flies reared on the CSMA medium devoid of yeast and Diamalt. The house-fly larvae rearing containers are shown in Figure 2.

Three or four days after seeding the medium with eggs, two inches of vermiculite were added to the surface of the medium and the larvae migrated into the drier and cooler area to pupate. After pupation the vermiculite was separated from the pupae by sifting in a 10-mesh hardware cloth cage. Incho (1954) described this same procedure except that he separated the vermiculite from the pupae by an air blast.

Cummings et al. (1964) constructed a cylindrical 8 1/4- x 8 3/4-inch cage for rearing smaller numbers of flies. A larval tub was attached to the bottom of the cage to hold the larval medium and an adapter ring was installed in the middle with an emergence hole in the center leading to the cage. His method was used for this study except the adapter ring in the middle was omitted and after pupation the adults went directly into the cage.

Anesthetization Tests

The adult house flies used for the Howell-Goodhue olfactometer tests were anesthetized before they were subjected to the olfactometer.

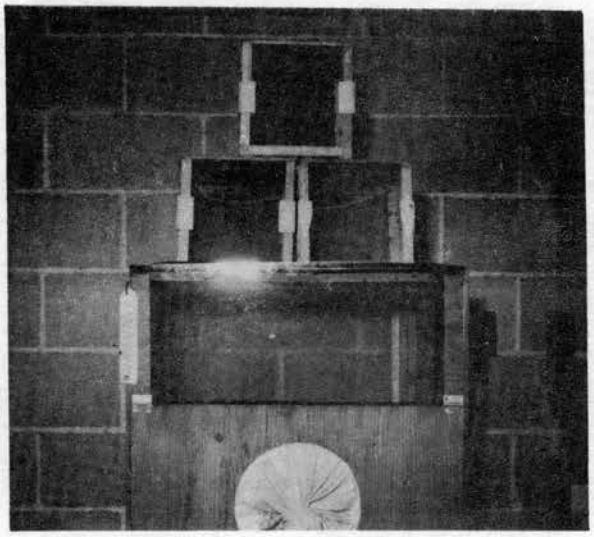

Figure 1. The emergence and holding cages used for adult house flies.

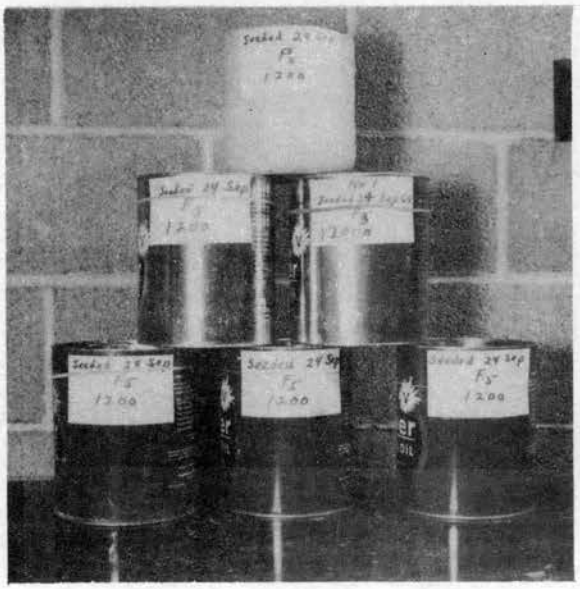

Figure 2. The containers used for rearing house fly larvae.

A study was undertaken to determine if there were any visible detrimental effects on the flies from anesthetization when compared to normal flies. Flies were collected from the emergence cages by aspiration and were placed in the freezer compartment of a refrigerator. Different batches of flies were placed in 1 1/2- x 5-inch plastic tubes and exposed to a temperature of 0^{o} F for periods of 7, 10, 15, 20, and 30 minutes. The flies were removed after the required exposure time and held in the plastic tubes in groups of 25 flies per tube. Data were recorded on the mortality of the flies at intervals of 6 min, 1 hr, 12 hr, and 24 hours. Each test consisted of four replicates and a check involving unexposed flies. After having been exposed to the low temperature, data were recorded for the 7- and 10-min exposure groups regarding time elapsed before first signs of activity, and the time elapsed before revival was complete. The arrangement of the plastic holding tubes containing the flies used in the anesthetization tests is shown in Figure 3.

Aspiration Tests

The house flies used for attractant tests were collected from emergence cages with a vacuum cleaner and a study was undertaken to determine if any visual detrimental effects were caused to occur in flies due to aspiration. The vacuum cleaner was of the canister type. A rubber juice cup with a hole cut in the bottom and fitted over the nozzle of the vacuum cleaner hose was used as an adapter. The other end of the juice cup was inserted into a hole in the bottom of a one-quart container. A wire disc was fitted into the paper carton to prevent the flies from being aspirated into the canister of the vacuum cleaner. The air velocity at the vacuum cleaner nozzle, measured with an anemometer, was 840 feet per min-

ute. During collection of the flies from the emergence cages, the different groups were subjected to aspiration for periods of 6 sec, 1 min, 10 min, and 30 minutes. After collection the flies were held in 1 1/2- x 5-inch plastic tubes for periods of 6 min, 1 hr, 12 hr, and 24 hours. Mortality determinations were recorded at the end of each period. Each test consisted of six replicates and a check with flies collected by hand was included. Each plastic tube used in the tests contained 70 flies.

Olfactometers

Howell-Goodhue

The Howell-Goodhue olfactometer has been described in the review of literature. The procedure for testing the candidate attractants for their attractiveness to house flies in the olfactometer was as follows: One bacteriological loop (1/100 ml) of the attractant was added to the Kleenex covering one glass flared tube while an equal amount of the diluent (acetone or water) used in the attractant was added to the Kleenex covering the other flared tube. The attractant materials were tested at the 0.1%, 1% and 10% concentration levels and each test was replicated four times. The test concentrations were prepared from 5% solutions and were mixed in a teaspoon. The teaspoon and the bacteriolgical loop were washed and heat sterilized after each mixture.

After the test solutions had been applied to the Kleenex the water was turned on to create a vacuum in the olfactometer and the flies were introduced into the cylinders. After two minutes the numbers of flies resting on the attractant side and numbers resting on the diluent side of the wire screen were counted and recorded. A downward probing of the proboscis by the flies was interpreted as a measure of attractiveness.

The numbers of flies demonstrating this response on the attractant side of the cylinder were recorded as a positive response and the numbers moving freely to either side of the cylinder as a negative response. When over 50% of the flies moved to the diluent side of the cylinder and remained there a repellent response was recorded.

The flies were collected from the emergence cages using the detachable cylinders of the Howell-Goodhue olfactometer or by aspiration supplied by a vacuum cleaner. After the test flies were collected they were anesthetized at 0^{o} F for seven minutes in the freezer compartment of a refrigerator and introduced into the test chambers. Ten to 25 flies were used for each test and they were allowed to rest one hour or longer between tests. The Howell-Goodhue olfactometer is shown in Figure 4.

Square Cage and the Hemispheric Attractant Selector

An attempt was made to construct an olfactometer for testing the attractiveness of chemical attractants to house flies in such a manner that the flies, when selecting an attractant, would become trapped and could be counted later. The first olfactometer of this type that was constructed consisted of a square panel inserted into a square plastic screened cage. The panel contained holes with screen wire cone traps and plastic tubes containing the attractants. The flies were released in the front of the cage and collected in the tubes attached to the back of the panel. This design proved unsatisfactory because the thigmotropic response of the flies caused higher counts of flies in the tubes near the sides of the cage. For this reason the cage olfactometer was abandoned in favor of the hemispheric olfactometer.

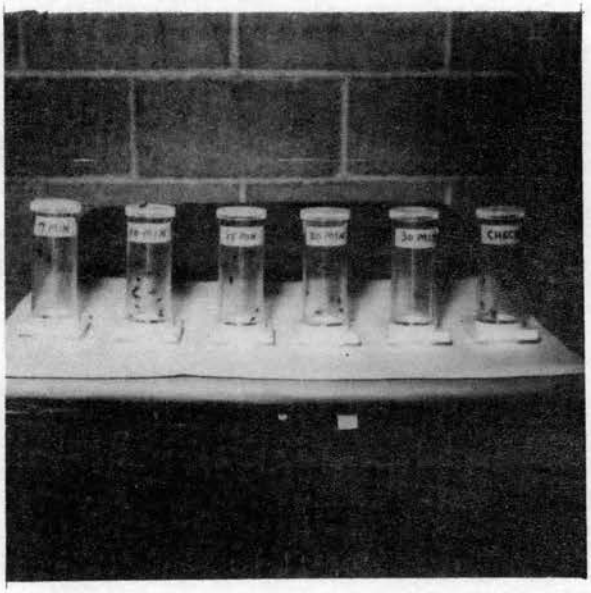

Figure 3. The arrangement of plastic holding tubes used in the anesthetization tests.

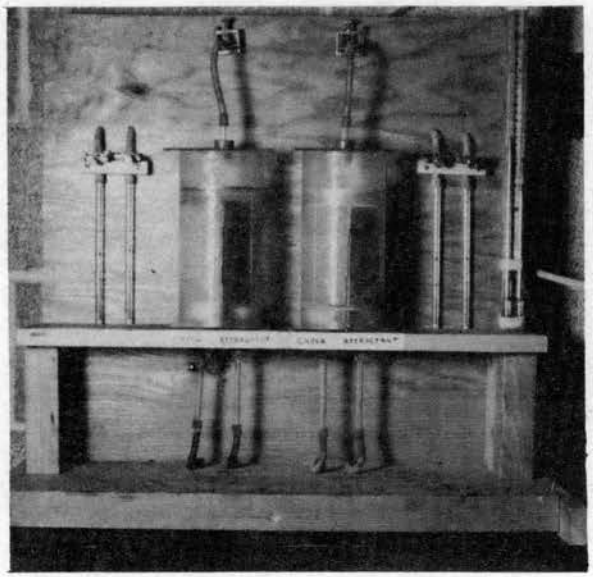

Figure 4. The Howell-Goodhue olfactometer.

The hemispheric olfactometer was constructed from one-half of a 36-inch plastic globe. Ten holes, 1 3/4 inches in diameter and spaced 9 to 10 inches apart, were drilled around the 30° latitude line of the hemisphere. The inside and outside of the hemisphere were painted white. Plastic tubes, 1 11/16 x 4 inches, were constructed and used as inserts in the holes to collect the flies during the tests. One end of the plastic tube was covered by wire screen and the other end contained a wire screen cone trap to prevent the escape of flies. A 12.7 mm penicillin paper pad soaked in a 1% solution of the attractant was placed in the plastic tube between the two screens and the tubes were inserted from the inside, into the holes of the olfactometer. The olfactometer was placed on the 36-inch turntable in the Peet-Grady chamber. After a period of time, the tubes were removed and the numbers of flies were counted and recorded. Ten tests constituted a replicate with each tube occupying a different hole position in the olfactometer each time. The tests were replicated four times. The diluent used was acetone. An outside view of the hemispheric olfactometer is shown in Figure 5 and an inside view with the collection tubes inserted is shown in Figure 6.

Light Tests

Tests were conducted to determine the importance of light to house flies in locating attractants. They were conducted in the presence of light and in total darkness. Small plastic bottle caps containing equal amounts of fresh hog manure used as the standard attractant were placed between the screen cone and the end screen of the previously-described, specially-constructed plastic tubes. The tubes were then inserted into the ten holes of the hemispheric olfactometer from the inside and the

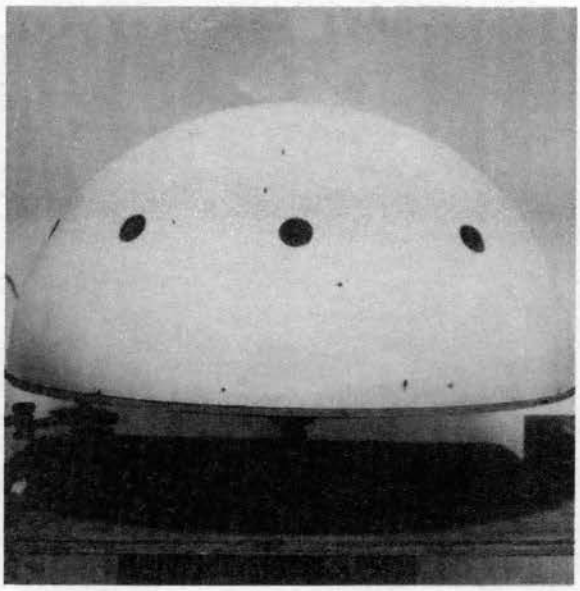

Figure 5. The hemispheric attractant selector (olfactometer), outside view.

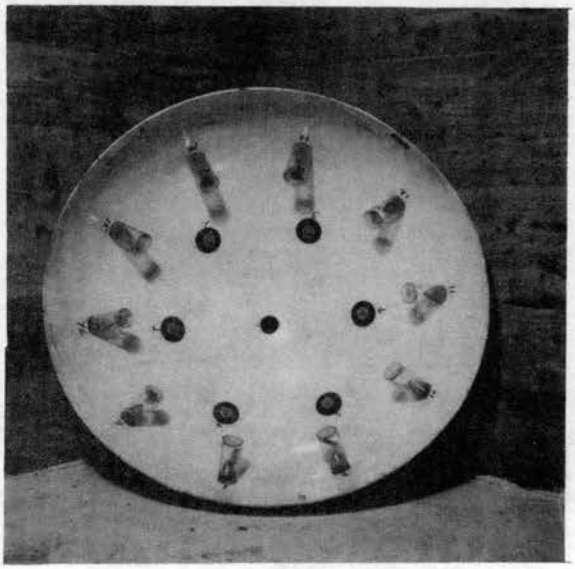

Figure 6. The hemispheric attractant selector (olfactometer), inside view showing collection tubes inserted.

olfactometer was placed on the revolving turntable in the Peet-Grady chamber. The tubes were collected four hours later and the numbers of flies in each tube were recorded. The tests were replicated five times and a different tube occupied a different hole each time. Fresh hog manure was provided for each test.

Sight and Sound Tests

The sight and sound tests were designed to determine the attractiveness of the sight, sound or smell of house flies to other house flies. These tests included active flies in cages, inactive flies in cages, and empty cages. The cages were 1 x 1 x 1 ft in size and constructed of plastic screen. Six cages were used: one covered and one uncovered containing active flies, one covered and one uncovered containing inactive flies, and one covered and one uncovered containing no flies. White bond paper was used on the cages to obscure the view of the flies inside the covered cages. The cages were placed on the turntable and counts were taken of the flies resting on the top and three sides of the cages. Six tests constituted one replicate with each cage occupying each of the six positions on the turntable during each replicate. The experiment was replicated five times. The flies in the two active fly cages were activated by jiggling the cages between counts; inactive flies were not disturbed during the tests. The cages were thoroughly scrubbed between tests to remove any trace of fly deposits. The arrangement of the cages on the turntable for the sight and sound tests is shown in Figure 7.

Fly Factor Tests

Tests were conducted to determine the attractiveness of fly factor to house flies. Six combinations of sugar cubes and fly factor were used

with 75 mm paper filter papers and the bottom sections of perforated plastic 35 x 10 mm plastic Petri dishes. The Petri dishes were perforated by drilling 19 5/64-inch diameter holes in the tops and sides of the dishes. Ten live flies were placed in each Petri dish. Fly factor filter papers were used on which flies had regurgitated and excreted, and facsimilies were made to resemble the fly factor papers by stipling plain filter papers with pencil lead. Plain sugar cubes, previously unexposed to flies, were used and are hereafter known as plain cubes. Simulated sugar cubes were made by wrapping plain cubes with Scotch tape. Fed-on sugar cubes were obtained by allowing flies to feed on them previous to the tests. The combinations of fly factor, flies and sugar cubes were arranged in such a manner that visually each appeared the same. The combinations used during the tests were (1) fly factor paper with plain sugar cubes, (2) fly factor paper with simulated sugar cubes, (3) fly factor paper with fed-on sugar cubes, (4) plain stipled filter paper with plain sugar cubes, (5) plain stipled filter paper with simulated sugar cubes and (6) plain stipled filter paper with fed-on sugar cubes.

In conducting the test, the filter papers were placed on the turntable and the perforated, Petri dishes containing the live flies were placed in an inverted position in the center of the papers. A cube of sugar was placed on the filter pads on each side of the Petri dishes. The first complete test was conducted with flies obtained by aspiration from the emergence cages. The same test was duplicated with flies which had not been subjected to aspiration to determine if there was a difference in their response to the potential attractants.

Counts of the numbers of flies resting on the sugar cubes and the Petri dishes were taken simultaneously. Six tests were conducted with

combinations of attractants occupying a different position on the turntable each time. The tests were replicated four times. The filter papers, Petri dishes and sugar cubes were renewed after every ten counts to preclude any build up of fly factor. The arrangement of the fly factor tests is shown in Figure 8.

Tests With the Metabolic Products of House Flies as Attractants

A test was made to determine if an odor was emitted from the metabolic products of house flies which was attractive to other house flies. Ten live flies were placed in separate 35 x 10 mm plastic Petri dishes. One set of Petri dishes was perforated as previously described to permit house fly odors to escape, and the other set was non-perforated thereby confining the body odors. Two perforated and two non-perforated Petri dishes containing live flies were inverted and placed on 75 mm plain filter papers near the border of each quarter of the turntable. A cube of sugar was placed on the filter papers on each side of the Petri dishes.

The two types of dishes were arranged alternately around the border of the turntable. Fifty counts were taken of the flies resting on the Petri dishes and sugar cubes. The Petri dishes were then rearranged so that the two perforated dishes were followed by the two non-perforated ones. Different filter papers, Petri dishes, flies and sugar cubes were added after every ten counts. The tests were replicated four times.

Tests With Live Flies, Dead Flies and Simulated Flies as Attractants

Tests were conducted to determine if the presence of live flies, dead flies or facsimilies of flies around food material would attract other flies to the food. Ten specimens of live flies, dead flies, and simulated flies were placed in separate 35 x 10 mm perforated, plastic Petri dishes.

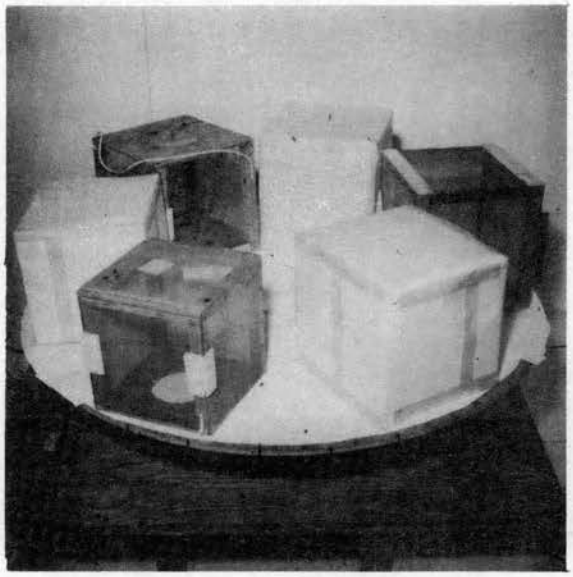

Figure 7. The arrangement of the cages on the turntable used in the sight and sound tests.

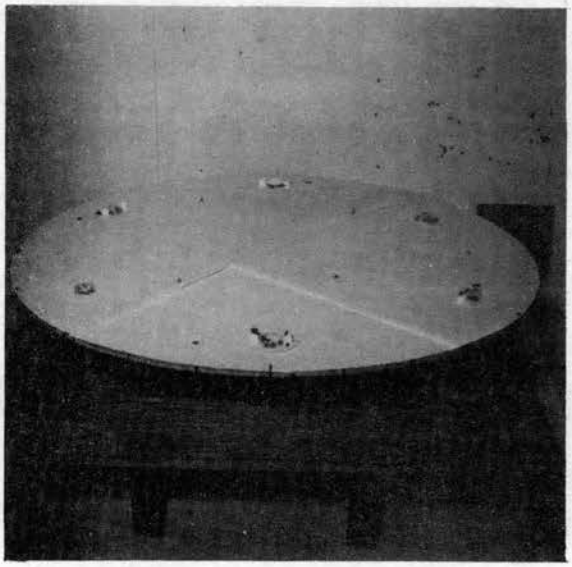

Figure 8. The arrangement of the Petri dishes used in the fly factor tests.

Simulated flies were made from black shoestring knots, each knot approximating the size and color of one fly.

The filter papers holding the Petri dishes, flies and sugar cubes were placed in a 1, 2, 3 sequence around the border of the turntable. The combinations were then changed to a 2, 1, 3 arrangement. Fifty counts were taken of the flies resting on the Petri dishes and sugar cubes each time. The counts were taken simultaneously on all three combinations. New filter papers, Petri dishes, and sugar cubes were added after every ten counts. The tests were replicated five times.

Dyed Filter Paper Color Tests

Two different experiments were conducted to determine the attractiveness of colors to house flies. The colors tested in both experiments were black, blue, brown, green, grey, purple, red, white and yellow. To determine the response to color by house flies, 75 mm filter papers were dyed in a solution of prepared dye and granulated sugar. Putnam dye was used for the purple color and Rit dye for the remaining colors. The dyes were prepared according to the directions on the labels, and sugar was dissolved in the dye when it reached the boiling point, in proportions of four parts dye to one part sugar by volume.

The filter papers were colored by soaking them in the dye-sugar solution for ten minutes, and they were hung to drip dry. Background colors on the turntable were also tested with the same colors, and the paper covering the turntable was dyed in the same manner as the filter papers, except that the dye contained no sugar.

In the first test the colored, sweetened filter papers were taped to the bottoms of inverted 90 x 15 mm Petri dishes. Three counts were made

of the flies resting on the filter papers, and then the Petri dishes and papers were shifted to a different position on the turntable. This procedure was repeated until each color had been placed in every position on the turntable, and each color had been situated beside every other color. This procedure was repeated with a different colored background on the turntable until all nine colors had been tested. Counts were made of the numbers of flies resting on the colored, sweetened filter papers, and the papers were changed after each replicate. The tests were replicated four times.

In the second experiment, the Petri dishes were placed in an open position with the dyed filter papers underneath the dishes. Flies were exposed to the color of the papers but could not come into contact with the paper. Granulated sugar (13 mg) was placed in each Petri dish. The sugar was measured with a scoop made from a cross section of a soda straw glued to a toothpick and calibrated to hold 13 mg of sugar. The arrangement of the dyed filter paper color test is shown in Figure 9.

Colored Light Tests

Tests were conducted to determine the attractiveness of house flies to the colors and intensities of light that were projected through different Wratten light filters. To test the attractiveness of colored light to house flies, the different filters used in these tests were calibrated to produce the same intensity of light. A Weston light meter (Model 735) and several neutral density filters were used in the calibration of the intensity of light. To test the response of the house flies to light color at full intensity, the neutral density filters were eliminated and the brightness of the light color was determined by the density

of the Wratten filter. The filter numbers and colors, in the order of decreasing density, were as follows: No. 35 - violet, No. 47 - blue, No. 45 - blue, No. 29 - red, No. 58 - green, No. 25 - red, No. 11 - green, No. 22 - orange and No. 15 - yellow. Short wave (2540 A) and long wave (3600 A) ultra-violet light also were tested at the same calibrated intensity as that of the Wratten filters.

The color filters and the neutral density filters were inserted into a 500-watt projector located over the window atop the Peet-Grady chamber. A shaving mirror was attached to the projector and adjusted to a 45° angle in front of the lens to direct the beam of colored light downward to the turntable located 68 inches below in the chamber. The projector fitted with the mirror is shown in Figure 10.

The projector was turned on and counts were made of the flies landing on the turntable. A period of 15 minutes was allowed to elapse between counts and the flies were disturbed immediately after the projector was turned on for each color. One count was taken for each filter color and the test was replicated five times using a different filter sequence each time.

Behavioral Studies

Aggregation Studies

This test was designed to determine the attractiveness of flies resting on sugar cubes in attracting other flies to the same cubes. A sugar cube was placed on the turntable and observations were made of the time elapsed before the first, second, third, fourth, etc., house fly landed on it during a period of ten minutes. Observations were also made of the frequency with which flies landed on the same sugar cube after the first

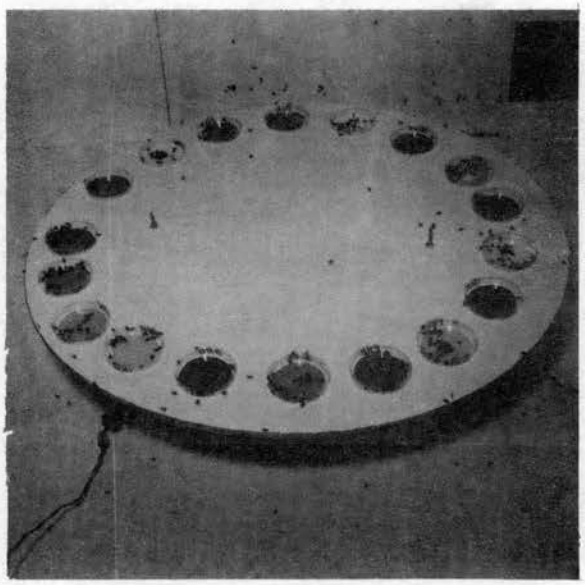

Figure 9. The arrangement of the dyed filter papers used in the color tests.

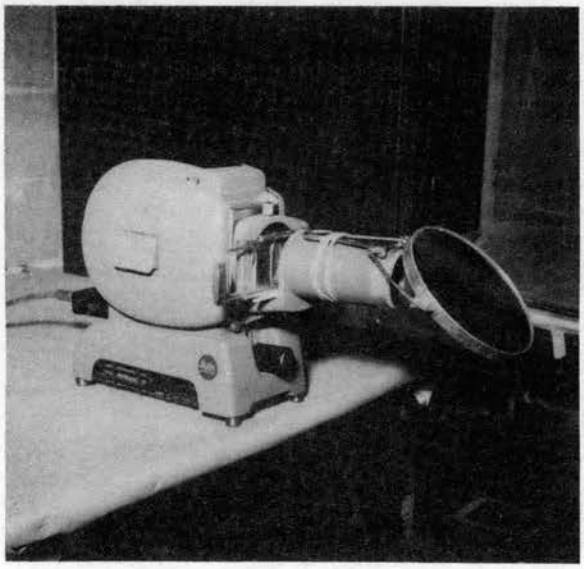

Figure 10. The projector fitted with mirror used in the colored light tests.

fly had landed than landed on other cubes. The flies were disturbed at one-minute intervals after each count.

Live and Simulated Flies as Attractants

As has been previously stated an experiment was conducted to determine the difference in the attractiveness of live, dead and simulated house flies to other house flies. The observations in this test were made to determine if live or simulated flies in perforated Petri dishes were more attractive to other house flies than the perforated dishes containing no flies. The inverted Petri dishes containing the test flies were placed on the turntable and a sugar cube was placed on top of each Petri dish. An equal number of empty Petri dishes, each with a sugar cube on top, were used to compare the results with the dishes containing the live and simulated flies. Counts were taken of the flies landing on the sugar cubes and the Petri dishes.

Tracking Patterns and Feeding Observations

To observe the tracking patterns of house flies in approaching a food medium, 3- x 3-inch glass plates were smoked and placed in a 1- x 1- x 1-ft plastic screened cage. A sugar cube was placed in the center of the glass plate and one to several flies were introduced into the cage, and feeding observations were made.

Fly Factor Studies

Observations were made of fly deposits on the walls of the Peet-Grady chamber to determine which were vomitus and which were excrement. Studies were also made of the importance of moisture as the attractant in fly factor. Three sugar cubes, one dry, one to which two drops of sputum were added and one to which two drops of water were added were placed in a cage of flies. Observations were made of the numbers of flies feeding

on the different cubes during a specified period of time.

RESULTS AND DISCUSSION

Anesthetization Tests

The results of the anesthetization tests with house flies are presented in Table 1. The data showed that there were no visible detrimental effects to the flies chilled by exposure to a temperature of 0° F

Table 1. The effects of anesthetization on house flies exposed for different periods of time to a temperature of 0° F.

Time subjected to anesthetization (min)	Average time after exposure before activity noted (min)	Percent mortality after holding periods of:			
		6 min	1 hr	12 hr	24 hr
7	6	0	0	1	3
10	9	0	0	0	0
15	16	-	32	32	39
20	19	-	66	66	68
30	-	-	100	100	100
Unexposed flies	-	0	0	1	2

for up to ten minutes. Mortality levels increased as exposure was extended beyond ten minutes. At an exposure time of 15 minutes 32 to 39% of the flies failed to recover and at an exposure time of 20 minutes 66 to 68% failed to recover. Exposure to 0° F for 30 minutes caused complete mortality to all flies. If flies were exposed for less than five minutes they were immobilized temporarily but were still able to

cling to the walls of the exposure tubes. At an exposure of 7 to 10 min the flies were completely anesthetized, fell to the bottom of the tubes and could be conveniently dispensed into the hand or other containers for counting and subsequent disposition. This provided from 6 to 9 min before the flies were sufficiently revived to crawl out of the tubes.

Aspiration Tests

The results of the aspiration tests with house flies are presented in Table 2. The data showed that there were no appreciable detrimental effects to flies subjected to aspiration for 6 sec to 30 min and held for as long as one hour. However, mortality increased with the exposure time and holding period. The mortality rate for house flies subjected to aspiration for 6 sec to 30 min ranged from 1.8 to 4.2% when held for 12 hr and 9.5 to 28% when held for 24 hours. Part of the mortality to the flies held for 24 hr could be attributed to overcrowding, since the

Table 2. The effects of aspiration on house flies collected from emergence cages with a vacuum cleaner.

Time subjected to aspiration	Percent mortality after holding periods of:			
	6 min	1 hr	12 hr	24 hr
6 sec	0.0	0.0	1.8	9.5
1 min	0.0	0.0	2.4	13.0
10 min	0.0	1.1	4.2	20.0
30 min	0.0	0.0	4.0	28.0
Unexposed	0.0	0.0	2.3	17.0

holding tubes contained an average of 70 flies per tube. The mortality to the flies not subjected to aspiration was 17% at the end of the 24 hr holding period. However, greater mortality was noted when the flies were

subjected to aspiration for periods of 10 to 30 minutes. The mortality ranged from 20 to 28% at the end of the 24 hr holding period.

Other causes of mortality to flies were probably loss of body moisture and mechanical injury to flies on the way into the aspiration cup during collection from emergence cages. All flies used in the other tests were subjected to aspiration for no longer than one minute and used for test purposes within one hour.

Howell-Goodhue Olfactometer Tests

Of the 44 chemical materials tested for attractiveness to house flies in the Howell-Goodhue olfactometer, only two elicited a response in all replicates that could be considered attractive to house flies. Odors from candidate attractants 5-carbamyl-3-cyano-4,4-dimethyl-6-amino-2-piperidone and 4-Pyridine carboxaldehyde, at the 10% concentration, caused a downward probing of the proboscis by the flies on the attractant side of the cylinder. With the test material 5-carbamyl-3-cyano-4,4-dimethyl-6-amino-2-piperidone approximately 75% of the flies moved to the attractant side of the cylinder and 25% stayed on the diluent side during the two minute observation period, after the olfactometer was placed in operation. With test material 4-Pyridine carboxaldehyde the reaction by the flies to the odor was less marked with a majority of the flies staying on the attractant side of the cylinder but frequently moving back and forth from one side to the other.

N-nitroso-2-methyl-5-ethyl piperidine was definitely repellent to flies. Immediately after the flies were introduced into the chamber and the olfactometer was placed in operation the flies moved to the diluent side of the cylinder and stayed there during the two-minute observation period. When the position of the Kleenex-covered flared glass tubes

containing the repellent and the diluent were reversed, the flies immediately moved to the diluent side of the cylinder and remained there.

The reactions of the flies to the odor of the remaining chemical materials tested could not be measured as a definite attractant or repellent response. After the flies were introduced into the cylinders and the olfactometer was placed in operation, the flies were in a continuous state of turmoil, and expressed in anthropomorphic terms, appeared to be apathetic toward the whole situation. It would be difficult to record a definite attractive response by house flies in this olfactometer unless the material was strongly attractive to the flies. With a strong repellent odor the response of the flies was easier to observe; they would immediately move to the diluent side of the cylinder and remain there.

Hemispheric Attractant Selector Tests

Data on the test materials that showed attractiveness or repellency to house flies in the hemispheric attractant selector are presented in Table 3. Hog manure as the standard attractant, acetone as the diluent and eight of the chemical materials that showed signs of attractiveness and repellency to house flies in the Howell-Goodhue olfactometer were tested for their attractiveness or repellency to flies in the hemispheric attractant selector. The materials tested in the hemispheric attractant selector were as follows: (1) Hog manure, (2) N,N'-3-Thiapentamethylene bis(pyrrolidone), (3) 5-Carbamyl-3-cyano-4,4-dimethyl-6-amino-2-piperidone, (4) 4-Nitroaminopyridine, (5) Chlorodiisopropyl benzene, (6) 4-Pyridine carboxaldehyde, (7) Scopolamine, (8) Acetone, (9) Dimethylane, and (10) N-nitroso-2-methyl-5-ethyl piperidine.

The data showed (1), the standard attractant, to be the most attrac-

Table 3. The attractiveness or repellency to house flies of certain test materials in the hemispheric attractant selector.

Test material	Replicate 1 No flies	Rank	2 No. flies	Rank	3 No. flies	Rank	4 No. flies	Rank	Total flies	Final rank
1	254	1	293	1	281	1	276	1	1104	1
2	201	3	212	2	233	2	186	3	832	2
3	208	2	191	3	214	3	207	2	820	3
4	150	4	163	4	129	4	122	4	564	4
5	63	5-6	74	5	104	5	47	7	288	5
6	63	5-6	70	6	51	6	88	5	272	6
7	41	8	57	7	27	9	59	6	184	7
8	47	7	46	8	23	10	40	8	156	8
9	32	9	42	9	49	7	29	9	152	9
10	26	10	15	10	28	8	15	10	84	10

tive material to house flies tested in the hemispheric attractant selector. Test material (3), which elicited an attractive response from house flies in the Howell-Goodhue olfactometer tests, was about 75% as attractive as (1) in the hemispheric attractant selector. Test material (2) attracted slightly more flies than (3) but showed very little attractiveness in the Howell-Goodhue tests. This has not been explained. The remaining test materials attracted considerably less flies than materials (1), (2), or (3).

Less flies were collected in the tubes containing the repellent material (10) than from the other tubes. This material was repellent to house flies in the Howell-Goodhue tests. The fact that 84 flies were

taken from the tubes containing the repellent was probably due to the exploratory habits of house flies, whereby, they ventured through the cone traps in the tubes and could not retreat.

Uniformity trials were conducted with the hemispheric attractant selector previous to this test to determine if there was a preference to the flies for certain holes. The data showed that although the variance was high at times for different holes, the greatest and least numbers of flies were never consistently taken from the same holes and all holes caught the greatest and least numbers of flies at one time or another. However, with the hemispheric attractant selector it was noted that some of the individual counts within the replicates were quite high and this was probably due to aggregation by the flies. Thus, if a few flies ventured into a hole first, then other flies would possibly have been attracted to the flies in the tubes, entered and become entrapped.

Light Tests

The results of the light tests with house flies are presented in Table 4. The data showed that the mean percentage of flies that entered the attractant selector during the four-hour period in the presence of light was 79.9% compared to 29.9% that entered during darkness. Converting the total flies released to a uniform number for both light and darkness the average flies collected per tube for the five replicates was five in the lighted chamber and two in the dark. It has been stated that flies fly very little in the dark; the reason for this is not fully known, although it could be connected with their inability to see in the dark. In these tests when the flies were released into the chamber they immediately sought the walls and ceiling to rest. In darkness they were reluctant to leave their resting places but when the lights were turned on they immedi-

Table 4. The attractiveness of light to house flies with the hemispheric attractant selector in the Peet-Grady chamber.

	Test chamber lighted			Test chamber in darkness		
Replicate	No. flies released	No. flies attracted	Percent	No. flies released	No. flies attracted	Percent
1	473	362	76.5	764	209	27.4
2	594	538	90.6	687	127	18.5
3	625	423	67.7	589	174	30.0
4	381	351	92.1	670	260	40.0
5	452	342	75.7	465	175	37.6
Total	2525	2016	79.9	3175	945	29.8

ately became active.

Sight and Sound Tests

Data showing the attractiveness of house flies in cages to other house flies are presented in Table 5. The numbers of house flies resting on the cages were higher on cages where active and inactive flies were

Table 5. The attractiveness to house flies of the sight and sound of other house flies in cages.

	Replicate					
	1	2	3	4	5	
Cages	No. flies	No. flies	No. flies	No. flies	No. flies	Total flies
Uncovered, active flies	166	171	97	122	167	723
Uncovered, inactive flies	111	135	61	90	126	523
Uncovered, no flies	114	98	62	73	84	431
Covered, active flies	87	92	61	55	80	375
Covered, inactive flies	73	91	47	59	73	343
Covered, no flies	71	82	47	53	75	328

visible to other flies than where the flies were obscured from view. Since more flies were counted on cages where active and inactive flies were visible than on the covered cage containing active flies, sight appeared to be more attractive to the flies than sound. Higher counts on the cages where the flies were visible and active further indicated that the movements of the flies were important as attractants to other flies. Also, since more flies were counted on the uncovered cages, screen could be considered preferable to paper as a resting surface for house flies.

Fly-factor Tests

Data showing the attractiveness of fly factor, sugar and simulated sugar combinations to house flies collected by aspiration and by hand are presented in Table 6. As has been previously stated, fly factor was something that flies contributed to the feeding medium while feeding that was attractive to other flies. With the house flies collected from emergence cages by aspiration, fly factor on filter papers with fed-on sugar, plain filter papers with fed-on sugar and fly factor on filter papers with plain sugar attracted over 77% of the flies counted. Fly-factor filter papers with simulated sugar attracted only 6.2% of the flies counted. The plain filter papers with fed-on sugar attracted 25% of the flies while fly factor on filter papers with simulated sugar attracted only 6.2% of the flies. The fed-on sugar appeared to be more attractive to the flies than fly factor on the filter papers. This was probably due to the volatility of the fly factor. The fed-on sugar cubes were taken from cages where flies had recently fed on the sugar and although the fly-factor filter papers were taken from cages where flies had regurgitated and excreted on the papers the moisture content was not as high on the filter

Table 6. The attractiveness of various combinations of fly factor and sugar to house flies.

Fly factor combination	Flies collected by aspiration	Percent of collection	Flies collected by hand	Percent of collection
Fly-factor paper, fed-on sugar	238	32.9	114	27.3
Plain filter paper, fed-on sugar	181	25.0	99	23.7
Fly-factor paper, plain sugar	148	20.0	77	18.5
Plain filter paper, plain sugar	81	11.0	77	18.5
Fly-factor paper, simulated sugar	45	6.2	28	6.7
Plain filter paper, simulated sugar	30	4.1	22	5.3
Total	723	99.2	417	100.0

papers as on the fed-on sugar cubes. Fly factor on sugar cubes from flies feeding on the sugar appeared to be very attractive to the flies. However, if the sugar was allowed to dry it lost its attractiveness. The length of time for the fed-on sugar to lose its attractiveness was not determined. There was little difference in the attractiveness of fly factor, or any visible detrimental effects to the flies collected by aspiration, since the data showed that the counts closely paralleled each other for the flies collected by aspiration and by hand.

It was shown that the flies preferred plain sugar to fly factor on filter papers with simulated sugar cubes. They preferred fed-on sugar to plain sugar and they preferred fed-on sugar on fly factor papers to any combination of fly factor filter paper, plain sugar or simulated sugar.

With fed-on sugar on fly-factor papers there was actually a double fly-factor value since fly factor was on the filter papers and the sugar too.

Tests With Metabolic Products of House Flies as Attractants

Data comparing the attractiveness to house flies of odors from the metabolic products of house flies are presented in Table 7. There was very little difference in the numbers of flies attracted to live flies in perforated Petri dishes, where the odors were emitted, and to live flies in closed Petri dishes. Evidently the flies were attracted to the sight and activity of the flies in the Petri dishes but the odor, if any, emitted from the metabolic products of the flies was not a factor in attractiveness to the flies. The total number of flies counted on the perforated Petri dishes was 246 and that on the non-perforated dishes was 234.

Table 7. The attractiveness to house flies of the odors from the metabolic products of house flies.

	Numbers of flies counted on dishes	
Replicate	Perforated Petri dishes containing live flies	Non-perforated Petri dishes containing live flies
1	65	62
2	53	58
3	62	46
4	66	68
Total	246	234

Tests With Live Flies, Dead Flies and Simulated Flies as Attractants

Records on the attractiveness to house flies of live, dead or simulated flies are presented in Table 8. House flies demonstrated very little preference between live, dead or simulated flies as attractants.

Flies tended to land on the sugar cubes and Petri dishes containing the flies and simulated flies at random. A preference was shown for live or simulated flies when compared with empty Petri dishes, as will be discussed later. The number of flies attracted to the live flies was 225, to the dead flies 222, and to the simulated flies 228.

Table 8. The attractiveness to house flies of live, dead or simulated house flies.

Replicate	Numbers of flies counted on dishes containing sugar cubes and:		
	Live flies	Dead flies	Simulated flies
1	45	53	43
2	49	34	40
3	44	51	46
4	42	41	48
5	45	43	51
Total	225	222	228

Dyed Filter Paper Color Tests

Data on the attractiveness to house flies of different colors on sugar-treated dyed filter papers and dyed filter papers are presented in Table 9. The data showed that more flies were attracted to purple (15.9%) in the sugar-treated, dyed filter paper test and more were attracted to brown (14.4%) when they were exposed to the colors of the papers only. Green (7.9 and 9.1%), blue (8.2 and 10.6%) and yellow (10.2 and 10.0%) were least attractive to the flies.

The percentage of flies attracted to the different colors in both tests closely paralleled each other, although the ranking of the colors

was different. The darker colors, purple, brown, and black, with the exception of grey in the sugar-treated test and red in the dyed-filter-paper test, attracted the most flies (39.8% in the dyed-filter-paper test and 38.9% in the sugar-treated dyed-filter-paper test).

Table 9. The attractiveness of dyed filter papers and sugar-treated dyed filter papers to house flies.

Flies exposed to dyed filter papers only			Flies exposed to sugar-treated dyed filter papers		
Color	No. flies	Percent of total	Color	No. flies	Percent of total
Brown	4666	14.4	Purple	8505	15.9
Black	4450	13.8	Grey	6998	13.1
Red	3951	12.2	Black	6228	11.7
Purple	3756	11.6	Brown	6011	11.3
Grey	3459	10.7	White	5812	10.9
Blue	3416	10.6	Red	5727	10.7
Yellow	3246	10.0	Yellow	5442	10.2
Green	2943	9.1	Blue	4380	8.2
White	2444	7.5	Green	4221	7.9
Total	32331	99.9		53324	99.9

Various authors have alluded to different colors as being the most attractive to house flies. The majority of these workers do not agree on which color was the most attractive to house flies. Some authors believed color preference by house flies to be linked to wave length of light or to background color or to light reflection from different surfaces. From results obtained in this study it can be concluded that the

flies apparently prefer the darker colors.

The purple dye used in these tests was manufactured by Putnam, all the remaining dyes were manufactured by Rit. What effect the brands had on the results of the tests was not determined.

Data showing the attractiveness of different colored filter papers and backgrounds to house flies are presented in Table 10. The data showed that a purple filter paper on a yellow background gave the highest fly counts. The fly counts were intermediate when the filter papers and background colors were reversed. A white filter paper on a brown background gave the lowest fly counts. The fly counts were intermediate when the filter paper and background colors were reversed. For all replicates a yellow background color with purple, grey, white, black or red filter paper was more attractive to flies than any other color combination. A brown background consistently attracted less flies than other background colors.

Table 10. The attractiveness of different colored filter papers and backgrounds to house flies.

Most attractive colors			Least attractive colors		
Color of filter pad	Background color	No. flies	Color of filter pad	Background color	No. flies
Purple	Yellow	1249	White	Brown	303
Grey	Yellow	1060	Blue	Black	306
White	Yellow	993	Green	Grey	358
Black	Yellow	885	Red	Brown	404
Red	Yellow	841	Brown	Red	480
Brown	Blue	814	Yellow	Black	504
Yellow	Red	722	Black	Brown	528
Green	Black	658	Grey	Brown	576
Blue	Grey	655	Purple	Brown	675

The numbers of flies present in the chamber when the tests were conducted varied from 200 to 500. This accounted for the counts being higher or lower throughout the replicates. Thus, the reason for a higher count (Table 10) for a purple filter paper on a brown background in the least attractive column than for a blue filter paper on a grey background in the most attractive column was that the lowest count on the purple background was higher than the highest count on the grey background. For all replicates a total of 108 fly counts were made of each colored filter paper and background color combination. Data presented in Table 10 depicts only the combinations with the highest and lowest counts. By and large, most color combinations were intermediary and could not be distinguished as particularly attractive to the house flies.

Colored Light Tests

Data showing the attractiveness of different colored light to house flies are presented in Table 11. The data showed short wave (2540 A) and long wave (3600 A) ultra-violet light to be the most attractive to house flies. At equal intensities the short and long wave ultra-violet lights attracted more flies (50.3%) than the combined totals of all the remaining colors (49.8). Of the remaining filter colors tested violet was the most attractive (9.8%) and yellow the least attractive (1.7%). All other light colors were intermediate and attracted from 3.1 to 7.0% of the total number of flies.

With full light intensity, short wave ultra-violet attracted 16.0% of the flies, long wave ultra-violet attracted 15.7% and violet attracted 13.8% and again were the most attractive to flies. Of the remaining colors tested, red (9.0%) was the most attractive and green the least

attractive (2.8%). All other light colors were intermediate and attracted from 3.9 to 9.3% of the total number of flies.

Table 11. The attractiveness of different colored light at equal and full intensities to house flies.

Equal light intensity				Full light intensity			
Filter number	Light color	No. flies	Percent of total	Filter number	Light color	No. flies	Percent of total
--	Short wave ultra-violet (2540A)	91	25.6	--	Short wave ultra-violet (2540A)	75	16.0
--	Long wave ultra-violet (3600A)	88	24.7	--	Long wave ultra-violet (3600A)	72	15.7
35	Violet	35	9.8	35	Violet	63	13.8
47	Blue	25	7.0	25	Red	41	9.0
--	White	23	6.5	47	Blue	38	8.3
45	Blue	20	5.6	29	Red	34	7.4
29	Red	18	5.1	22	Orange	32	7.0
25	Red	16	4.5	45	Blue	27	5.9
11	Green	12	3.4	15	Yellow	23	5.0
58	Green	11	3.1	--	White	22	4.8
22	Orange	11	3.1	11	Green	18	3.9
15	Yellow	6	1.7	58	Green	13	2.8
Total		356	100.1			458	99.6

Light intensity did not appear to affect the attractiveness of the light colors to house flies, except that more flies were attracted to red when the intensity was increased. Green, yellow and orange were generally

less attractive to flies at both intensities than other colors while the ultra-violet colors were more attractive. The remaining light colors appeared to be intermediate in attractiveness at full or equal intensities. Blue was more attractive than red at equal intensity and the reverse was true at full intensity.

Behavioral Studies

Aggregation Studies

Observations showed that from 5 to 40 sec elapsed before the first fly landed on a sugar cube when several cubes were used. The greatest numbers of flies to land on a sugar cube in any one-minute period was six and on four occasions one minute elapsed before a fly landed on a sugar cube. The average flies per sugar cube per minute for the ten-minute period was two. There appeared to be no aggregation of flies during one-minute periods, since the counts ranged from six during the first minute to one for the last minute. The total counts were highest for the first, third, and fourth minutes and lowest for the seventh, eighth, and tenth minutes.

Observations with flies resting on sugar cubes attracting other flies revealed that immediately after the flies were disturbed and one fly landed on a sugar cube, another fly would approach in flight and land as if sensing the presence of the fly on the cube. Within a ten-minute period twice as many flies landed on the same sugar cube after the first fly had landed than landed on other cubes.

Live and Simulated Flies as Attractants

Observations showed that house flies preferred the presence of live or simulated flies in Petri dishes to Petri dishes with no flies. In a

ten-minute period, 48 flies were attracted to dishes containing live flies while 18 flies were counted on the empty dishes. Fifty flies were attracted to the Petri dishes containing simulated flies while 13 were counted on the empty dishes in the same period of time. The dishes were changed after each count to preclude fly factor. Flies were attracted to the presence of flies or facsimilies in dishes in contrast to empty dishes; however, as has been stated there was very little difference in the attractiveness of live or simulated flies to other flies.

Tracking Patterns and Feeding Studies

Observations of the tracking patterns of house flies in approaching feeding medium showed that there was no set pattern. The flies approached the medium by foot or by air. When approaching by foot they would sometimes crawl over the medium, go out a few millimeters, reverse their direction and return to feed. After exploring the medium with the labellum they would either stay and feed to satiation or leave the medium, make a 180^{o} turn and return to feed. They were observed to turn at sharp angles or in circles in either direction after leaving the medium before returning to feed. They appeared to be quite restless and curious in their actions. Undoubtedly the satiation of the flies played an important part in their actions toward the feeding medium. The 24-hr starved flies seldom left the medium to race about as was common with the partially satiated flies.

Observations of a house fly feeding on one granule of sugar showed that the fly immediately regurgitated on the granule of sugar and sucked up the liquid. Holding the granule between the forelegs the fly continued to regurgitate and feed as the granule hollowed from the inside.

When the granule was completely hollowed, the walls collapsed and the remaining sugar was liquefied by regurgitation and drawn up through the food channel.

Fly Factor Studies

Observations of residual fly deposits on the walls of the Peet-Grady chamber revealed that two kinds of material were present. One deposit was light brown in color and absorbed ultra-violet light, the other was medium brown in color with a small black speck of material in the center. The latter deposit did not absorb ultra-violet light. Observations of flies resting on the walls revealed that the light brown material was vomitus and the darker material with the black speck in the center was excrement. The vomitus appeared to be the more attractive to the house flies of the two materials. This was indicated when several flies were severed between the abdomen and the thorax and the liquids allowed to ooze out. The flies were immediately attracted to the material but the attractiveness was correlated with the moisture present. The material lost its attractiveness rapidly as it dried. This was also noted with fed-on sugar in that after the flies were disturbed from feeding on the sugar, it became unattractive very rapidly if the flies were not allowed to return to it immediately and resume feeding.

Acree et al. (1959) stated that fly factor was mostly moisture. Wiesmann (1962) stated that the attraction of flies to food that was being fed on by other flies was due to the formation of attractive sugar solutions on the baits by the action of the saliva of the flies combined with the visual aggregative instinct of the flies themselves.

This was found to be true but the moisture present did not necessarily have to be contributed by house flies. The abdomen of a spider was

punctured to release the liquid and placed in a cage of house flies. The material proved to be very attractive to the flies until it dried. Moisture alone may be attractive to flies if they are sufficiently unsatiated. When three sugar cubes (one plain, one to which was added two drops of human sputum and one to which was added two drops of water) were placed in a cage with house flies, the flies immediately swarmed over the cubes containing the sputum and water and ignored the plain dry cube.

After several minutes more flies were counted on the sputum-treated cube than on the water-treated. This was undoubtedly due to the lower volatility of the sputum. At no time during the ten-minute observation period did the flies prefer the dry sugar to the others.

GENERAL SUMMARY AND CONCLUSIONS

House flies anesthetized in a freezer compartment at 0^{o} F for up to ten minutes showed no detrimental effects. Mortality increased as exposure was extended beyond ten minutes and was complete after an exposure of 30 minutes.

House flies exposed to aspiration for 6 sec to 30 min showed no detrimental effects when held for as long as one hour. Mortality began to occur after one hour and increased with the holding period.

Of the 44 chemical materials tested in the Howell-Goodhue olfactometer the house flies were attracted to 4-Pyridine carboxaldehyde and 5-Carbamyl-3-cyano-4,4-dimethyl-6-amino-2-piperidone. In the hemispheric attractant selector the house flies were attracted to N,N'-3-Thiapentamethylene bis(pyrrolidone) and 5-Carbamyl-3-cyano-4,4-dimethyl-6-amino-2-piperidone, but were less attracted to 4-Pyridine carboxaldehyde. N-nitroso-2-methyl-5-ethyl-piperidine elicited a definite repellent response from the flies in both olfactometer tests.

The mean percentage of house flies that entered the hemispheric attractant selector during a four-hour period in the presence of light was 79.9% compared to 29.9% that entered during darkness. It has been stated that flies fly very little in the dark; the reason for this is not fully known, although it could be connected with their inability to see in the dark.

Greater numbers of house flies were attracted to the sight of flies

exposed in screened cages than to cages covered with paper in which the sound of flies was audible but flies were not visible. Since more flies were counted on exposed cages than on covered cages, sight appeared to be more attractive to house flies than sound.

Fly factor was defined as something that flies contributed to the feeding medium while feeding that was attractive to other flies. The fly-factor tests showed that the flies preferred plain sugar to fly factor on filter papers with simulated sugar cubes. They preferred fed-on sugar to plain sugar and they preferred fed-on sugar on fly-factor papers to any combination of fly-factor filter paper, plain sugar or simulated sugar. Fly factor on sugar cubes from flies feeding on the sugar appeared to be very attractive to the flies; however, if the sugar was allowed to dry it lost its attractiveness. The attractiveness of the fly factor appeared to be correlated with the moisture present.

Tests with metabolic products of house flies, showed that there was very little difference in the numbers of flies attracted to live flies in perforated Petri dishes, where the odors were emitted, and to live flies in closed Petri dishes. Odor, if any, from the metabolic products of house flies, evidently was not a factor in attractiveness to other house flies.

Tests with live flies, dead flies and simulated flies in perforated Petri dishes showed very little difference in their attractiveness to other house flies. However, when live flies and simulated flies in perforated Petri dishes were compared to empty dishes the flies and simulated flies were over twice as attractive as the empty dishes.

Tests with color showed that more flies were attracted to purple in the sugar-treated, dyed-filter-paper test and more were attracted to

brown when they were exposed to the colors of the papers only. Green, blue, and yellow were least attractive to the flies. The darker colors, purple, brown, and black, with the exception of grey in the sugar-treated test and red in the dyed-filter-paper test, attracted the most flies. A purple filter paper on a yellow background gave the highest fly counts and a white filter paper on a brown background gave the lowest counts.

To test light color at equal intensity, neutral density filters were used to equalize the light intensity for all the Wratten filters. To test the full light intensity and color, the neutral density filters were eliminated and the light intensity was proportional to the density rating of the individual filters. Short wave (2540A) and long wave (3600A) ultra-violet light and violet light were the most attractive to equal and full intensities to house flies. Yellow, orange, and green were the least attractive colors to house flies at equal intensity and green, white, and yellow were the least attractive at full intensity.

Aggregation studies showed that 5 to 40 sec elapsed before the first fly landed on a sugar cube when several cubes were used. There appeared to be no aggregation of flies during one-minute periods, since the counts ranged from six during the first minute to one for the last minute during a ten-minute period.

Tracking patterns and feeding observations showed that flies did not demonstrate a set pattern in approaching the feeding medium. Once in contact with the medium they would feed until satiated or feed for a few seconds, leave and return to resume feeding. The restless action of the flies occurred several times and was characterized by circular or angular movements.

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