THE EFFECT OF REPELLENT TREATED SURFACES ON INSECT BEHAVIOR

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THE EFFECT OF REPELLENT TREATED SURFACES ON INSECT BEHAVIOR

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PREFACE

While engaged in graduate studies at Oklahoma State University, the writer was given the opportunity to work on a part-time basis with an experiment station study to find suitable repellent sprays for dairy cattle. This experience, together with previous work on animal systemic insecticides, aroused interest in the many complications involved in the development of chemical insect repellents. This interest led to the development of the present study.

A review of the literature shows that research on repellents has been stimulated in several categories. The development of repellents for crop protection, for use against insects of medical importance, for use against biting flies of cattle, and studies on the nature of insect repellency are considered by the writer to be the more important of these studies. These categories can of course be broken down into the many facets which comprise them.

As one can readily appreciate, this situation offers extremely broad opportunities for research. This being the case, the writer has limited this study to laboratory and field experiments that could be integrated and interpreted to aid in the selection and development of suitable repellents. This integration and interpretation has been the prime target of this study, with the hope that it will help explain some of the many questions on how insect behavior is affected by repellent treated surfaces.

The development of chemical insect repellents for the protection of man and animals against biting insects is one of the more important lines of entomological research today.

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CHAPTER I

INTRODUCTION

Although from the earliest times, man has used repellents of some sort for providing protection against arthropods, chemical insect repellents were not improved to any extent until the late 1930's and the beginning of the Second World War. This development was a result of synthesis of many thousands of chemicals which were given screening tests as mosquito repellents. After their repellency to mosquitoes had been demonstrated, smaller numbers were tested against other insects. Later, when the federal food and drug administration listed only a small number of toxicants as safe for spraying dairy cattle for fly control, an added stimulus was given to the field of insect repellency. This made the development of repellents necessary from both a medical and veterinary standpoint.

The emphasis on repellent studies has been placed on the development of non-toxic, nonirritating repellents which are long lasting and repel mosquitoes, biting flies, ticks, fleas and chiggers efficiently. Unfortunately much of this development has had to rely mainly on screening tests run on field or laboratory designs. Nevertheless, many fine repellents have been developed by this approach. There are, however, many unanswered questions regarding the exact nature of action of chemical insect repellents.

Scientists from the earliest times have recognized that the study

of the chemical senses was quite a fertile field for research. Therefore, from the time of Aristotle, who made observations on the way in which insects carefully select their food, to the present, there has been a steady accumulation of knowledge on the chemical senses of both vertebrates and invertebrates (Parker, 1922).

The Chemical Senses - In the vertebrates the chemical senses may be classified into three main headings. These are smell, taste and the general chemical sense, all of which can be subdivided into many more divisions. These three divisions in the vertebrates are thought to be distinct so that what is perceived by one of these senses will not stimulate the other two (Parker, 1922).

Parker has also shown that in some of the vertebrates, the chemical sense has a threshold value two or three times higher than that of taste, which is 24,000 times as strong as the threshold value for smell. No work of a similar nature has been done with insects, although the three main divisions of the vertebrate chemical senses have been taken to apply by some workers. McIndoo (1914) however, feels that these divisions are non-existent. He believes that taste is a less sensitive form of smell. In the vertebrates the three senses are all stimulated by means of a chemically active substance in solution, coming into direct physical contact with an exposed sensory cell while with insects the chemically active substance must pass through a thin membrane covering a sense receptor.

The organs which perceive this form of stimulation are found on various parts of the insect body. The antennae, the tarsi, and the maxillary or labial palpi are common sites. Three types of receptors have been identified (Wigglesworth, 1939). They are as follows: 1) pore plates, 2) thin walled cones and pegs, and 3) thin walled cones and pegs

which are sunk into pits. All of these have a cuticular covering which may be partially thin walled. Some may be completely thin walled, however. All are innervated by one or more bipolar sense cells situated on the distal process of a neurone. Some workers have found that the olfactory organs of insects are not equally responsive by demonstrating that thresholds for the antennal organs are probably lower than those for the palpi and maxillae. This suggests dual ranges of sensitivity and response but it has not been adequately demonstrated. Further work of this nature will be discussed in the review of the literature.

CHAPTER II

REVIEW OF THE LITERATURE

Introduction and Early Views of Chemoreception

Location of Taste Organs. Biologists have long known that the study of chemoreception was an attractive avenue of research. The earliest conjectures as to the location of the taste organs were based upon the belief that these organs must be in or near the mouth (Lehman, 1798). He also thought that it was possible that the palpi were probably the seat of this sense in insects and many other workers suggested various other parts of the insect body. Nagel (1894) reviewed the literature and on that basis and his own experiments he propounded some generalizations which are still applicable. He believed that the olfactory and gustatory organs are similar in function and structure and that one might function for the other, thus, stating a generalization which has only recently become generally accepted. He further classified the organs of taste into inner and outer types; the inner organs being present in all insects but being the only ones present in the mandibulate insects. Outer taste-organs, he believed were found only in sucking insects and in aquatic groups. These were to take the place of the sense of smell. The outer taste organs, he stated, are usually at the base of the palpi, tip of the hypopharynx and the tip of the labrum.

Location of Olfactory Organs. Lehmann (1799) propounded the theory that the olfactory organs were located in the stigmata or tracheae;

Lehmann compared the spiracles of insects with those of the vertebrates. Rosenthal (1811) located the seat of olfaction in the folds of chitin between the antennae and the palpi; this was suggested as the center for the olfactory organs by several other workers. Other early writers attributed olfactory perception to most parts of the insect body, of course, depending largely upon their own personal bias.

It was noted by Von Frisch (1919) that bees, whose antennae had been removed, appeared to react normally to color stimuli. He also stated that their other habits appeared to be normal. Thus, he postulated that antennal amputation probably should not result in abnormality sufficient to prejudice olfactory experiments.

Correlations between the number of sensory organs on the antennae and the olfactory requirements of different insects is also used to demonstrate the seat of olfaction in some insects. Finally, recent work supports the view that the olfactory organs are located on the antennae, and also the possibility that the olfactory organs may act as receptors of contact chemical stimuli (Marshall, 1935).

Effect of Stimulation. It is extremely difficult to isolate the effect of any one stimulus upon any one sense organ. The observed response is often the result of many stimuli, or it is the response to stimuli other than the one believed to be operating; therefore, neither the experimental nor the morphological studies made so far will allow unequivocal generalizations with respect to either position or structure of the chemoreceptors of insects. Further, differences in the loci and appearance of chemoreceptors have been found in insects from the same order, such as the roach and the grasshopper. Much further experimental work is necessary, followed by morphological and histological studies of

organs shown experimentally to be chemoreceptors, before further generalizations will be possible.

The Development of Chemical Insect Repellents

The history of the development of repellents has been reviewed by several authors, Dethier (1947, 1956 and 1960) and Shambaugh et al. (1957) being among the most notable. These reviews also attempt to evaluate and integrate the results of the many variables encountered in experimental designs and theoretical approaches to the nature of repellency. The more important findings along these lines are discussed below under three categories: 1. repellents for use against insects of medical importance 2. repellents for use against biting flies of cattle 3. studies on the nature of insect repellency.

Repellents For Use Against Insects of Medical Importance

Screening Methods. These investigations represent efforts to selectively screen out suitable materials for mosquito repellents. Granett (1940) reported a method of performing this type of test and of evaluating test results. His procedure consisted of application of the test material to one arm or leg of a human subject and determination of the protection time. This time was presented in minutes to the first bite and was designated as the repellent time of the material under test. Determination was also made of the insect biting frequency during the test by observations of the bites per minute on a corresponding untreated area. It was found that with an increase in biting rate there was a decrease in repellent time, but that for any given pair of repellents the ratio of repellent time at a given biting rate was approximately constant throughout the

entire biting range.

For comparative purposes a product developed during the repellent investigations was used as a reference standard. The repellent rating of any given material was then determined by dividing its repellent time by the repellent time of the reference standard obtained at the same biting rate as the compared material. If the compared product was tested at several biting levels the repellent ratings at the different levels were averaged to give a single comparative value.

Linduska et al. (1946) described a screening method for the determination of suitable flea repellents. Smith and Gouck (1946) presented information of field tests with repellents suitable for ticks. They found that a moderately high degree of protection from ticks was obtained with indalone and dimethyl phthalate by treating the clothing.

Kennedy (1947) studied the excitant and repellent effects of DDT on mosquitoes. This was done by observing the numbers of Aedes aegypti L. which settled on and departed from DDT treated paper. Linduska (1947) was interested in determining the repellency of solid chemicals to mosquitoes. He considered that the various characteristics of most solid chemicals, principally their lower vapor pressure and lower rate of absorption by the skin would make them useful as repellents. Morton et al. (1947) summarized the results of screening tests with materials evaluated as repellents at the Orlando laboratory from 1942 to 1947. McCulloch and Waterhouse (1947) continued studies of mosquito repellency with laboratory and field tests which were of the same type as previous screening tests.

In 1951 further tests of dibutyl adipate as a tick repellent were conducted by impregnation of coveralls and trousers, also aerosol

treatment of coveralls, trousers and dogs was done to aid in determining its worth and the easiest method of application that would still give good results.

Sarkaria (1951) divided liquid repellents into two classes on the basis of their mode of affording protection: those like citronella which may be designated as vapor repellents and those like indalone which are so slightly volatile that they allow the insects to approach and touch the treated skin, and are considered to be mainly contact repellents. He considered compounds of the latter class deficient in that they fail to remove the psychological hazard of swarming attack, and allow biting to occur on skin areas left uncovered by the application. Compounds of the former group were thought deficient because they are rapidly lost by evaporation from the skin.

Goodhue and Linnard (1952) made determinations of the repellent action of chemicals to the American cockroach by laboratory screening. They concluded that a good repellent would be useful in the control of those pests especially in warm climates where the source of infestation is always present.

Kasman et al. (1953) studied and evaluated the methods of testing insect repellents. They discussed the fact that synthesis of chemicals of biological interest is frequently hindered by the delays and the crudities of bio-assays. In an effort to correlate the functional groups in organic chemicals with their insect-repellent properites, many authors have encountered such difficulties. For example, the human arm test, in which the forearm is covered with one gram of repellent and exposed to biting insects, could be carried out economically at the rate at which pure chemicals can be synthesized for testing. The ultimate evaluation

of a repellent designed to protect humans against biting insects, especially mosquitoes, must be carried out with human subjects. However, it does not follow that the interim testing during which the successful repellent is being devised should be carried out with humans. Therefore, it was proposed by Kasman that a method of screening compounds as insect repellents with guinea pigs would be the most efficient way of testing. In comparisons with human arm tests, those with the guinea pig gave approximately the same results (Kasman et al., 1953).

Relation of Physical Characteristics of Repellents to Insect Repellency. Rhodehouse (1953) carried on laboratory studies on insect repellency with A. aegypti (L.) This information was the result of screening materials of homologous series of aldehydes, ketones and esters. He found that aldehydes and ketones were poor repellents while α-hydroxy esters with a boiling point range of 230° to 260° C. and cyclic monoalcohols with a boiling point near 260° C. were fairly effective repellents. He, therefore, presumed that chemicals with this range maintain a vapor concentration that is repellent to mosquitoes. Rhodehouse believed that an oxygenated linkage such as hydroxy or carbonyl is most important from the standpoint of repellency, although these linkages are not the only ones associated with repellency.

Determination of Repellent Residuals from Treated Surfaces. In determining the evaporation of repellents from skin and cloth, Gouck et al. (1957) applied dimethyl phthalate and diethyltoluamide to measured areas of the forearm of a human subject, the shaved ventral surface of a guinea pig, or a swatch of cloth. The treated surfaces were confined in glass vessels that were connected to a system of traps containing ethanol. An air stream was passed through the system at a constant rate and the amount

of repellent collected in the traps in 2 hours was determined by ultraviolet spectrophotometry. Dimethyl phthalate evaporated at twice the rate of diethyltoluamide. With both repellents the evaporation rate was highest from the guinea pigs, less from the forearm and least from the warmed cloth.

Schmidt et al. (1958) used an ultraviolet method of analysis to determine repellent in cloth and on glass plates. The absorbance of a sample in ethanol was read on a spectrophotometer at 230mu. with the slit width maintained constant. The concentration was then determined from a calibration curve relating absorbance to concentration.

Absorption spectra of the purified, ortho, meta, and para isomers were found to have no peaks, but at constant slit width their absorbance at 230mu. obeyed Beer's Law. Insufficient differences in the spectra of the three isomers preclude a satisfactory mathamatical determination of the individual isomers.

Schmidt et al. (1959) applied C^{14} labeled diethyltoluamide to the skin of guinea pigs at 6.97-7.11 mg. per sq. in. After 6 hours 0.96-0.98 mg. per sq. in. had been lost by evaporation and 1.32-3.40 mg. per sq. in. by absorption. The remaining repellent was removed. The radioactivity in the urine reached a peak within 12 hours after application, and over 80 percent of the absorbed dose was excreted in the urine. Only 0.75 percent of the absorbed dose was excreted in the feces during eight days, whereas 93 percent appeared in the urine. Very small amounts of radioactivity were found in the blood, skin and hair.

Repellents For Use Against Biting Flies of Cattle

Prior to the extensive use of DDT when the fly problem was severe and there were no chlorinated hydrocarbon or organic phosphate insecticides, repellent mixtures were experimented with in a limited manner. After development of DDT and the other insecticides which were effective in fly control, repellent studies of this nature were neglected. With the development of resistance, however, adequate fly control was no longer possible. This brought about a return to repellent investigations. The ruling of the Pure Food and Drug Administration which prohibits the use of many toxicants on dairy herds and sets tolerances on beef animals, has also stimulated that return to repellent investigations.

Laboratory Testing. Starnes et al. (1953) designed a laboratory method for testing repellents against biting flies. They used rabbits as bait and candidate repellents were screened as 5 percent solutions in acetone. Two 1-foot squares of cheesecloth were placed in a dish containing 30 mls. of the 5 percent solution. The cheesecloth absorbed the total 30 mls. and was then hung on a rack to dry at 70° F. Initial tests were conducted 24 hours later.

Plastic or glass cylinders 2 inches in diameter by 5 inches in length were used as test cages. One end of the tube was covered with a 7-inch square of treated cheesecloth and a clean untreated square was placed over this; both were held in place by a rubber band. Approximately 5 to 8-day-old stable flies were released into this cylinder. The open end was covered with another untreated piece of cheesecloth. To feed on the rabbit, the flies had to alight and bite through the treated cheesecloth. This gave indalone a rating of 92 percent repellency after 4 days and Crag fly repellent 49 percent repellency.

Eddy and McGregor (1949) used white mice as the host animals in conducting tests against S. calcitrans (L.). Each mouse weighed approximately 25 grams and was sprayed with 2.5ml. of a 0.25 percent acetone solution of the chemical. Application was made with a bulb-type hand atomizer that delivered coarse droplets so that the loss of chemical due to misting was minimized. Each mouse was confined in a 4-inch hardware-cloth cage. This was rotated by hand during spraying. After spraying, the mouse was placed in a small screen cage for about an hour to permit the acetone to evaporate, and then in a clean hardware-cloth cage for testing. The cage containing the mouse was placed in a widemouth quart fruit jar and 30 to 40 female S. calcitrans (L.) were introduced into each jar.

During exposure of the mice to the flies, constant observations were made for landing and biting. As soon as a bite was observed, the test was terminated. If a bite was not obtained in 45 to 60 minutes, the test was stopped for an hour or longer and then the mouse was exposed to other flies.

Field Testing With Cattle. Howell and Fenton (1944) found that an oil base cattle spray with 6 percent toxicants including pyrethrum and two grades of a thiocyanate (Lethane) applied to cows at rates of 0.5cc., 1cc. and 2cc. per 3.23 sq. ft. of body surface was repellent to the horn fly for a period up to 10.5 hours after spraying. Similar application rates were less repellent to the stable fly both as to amount and duration. Little repellency against this species was observed 4 to 5 hours following spraying. As the time interval following spraying increased, the amount of repellency to both species decreased. In parallel tests, 2cc. of spray per 3.23 sq. ft. of surface area was more repellent to the

hornfly than 0.55cc. for 7 hours after the morning sprayings. At other times no significant differences were noted. The heavier application was more repellent to the stable fly only for 4 hours after morning spraying and 2 hours after evening spraying. The hornfly infestation was greater in the morning than during the afternoon, but the stable fly infestation was greater in the evening.

Fryer et al. (1948) compared methods for testing repellent-type fly sprays. They comment that experimental work with cattle fly sprays under field conditions is an extremely complex type of research if the many variables are taken into account. The difficulties have been increased recently by the introduction of sprays which are primarily toxicants rather than primarily repellents, especially if attempts are made to compare sprays of both types. The toxicants tend to reduce the fly population and therefore add to an already complex picture of a changing population of flies about the cows. Fryer et al. further make comparisons between the whole cow method of treatment and the half-cow method.

Bruce and Decker (1957) applied several new repellents to cattle to determine their relative merits in protecting against stable flies. They brought out in their study the fact that much confusion has arisen from published reports on tests involving repellents and mixed populations of stable and horn flies. During the course of their study, populations of horn flies comprised approximately 90 percent of the flies. They, therefore, found that when a repellent with some toxicant such as activated pyrethrum was applied to cattle, killing action resulted in reduced fly populations. Thus, if the results of such experiments are reported as repellency to horn flies and stable flies, the data are unreliable.

Actually such data may represent only the killing action of the toxicant on the horn fly and no repellency whatsoever to the stable fly. This may have been a factor contributing to the results obtained by Granett and Haynes (1955) and Granett and Hansens (1956).

Granett and Hansens (1956) found that R-326 and Tabatrex gave better control of the stable fly than activated pyrethrums. Tabatrex gave 1 to 6 days residual repellency. Thus they concluded that the first day or two it was an olfactory repellent while the residual 3 or 4 days it was a gustatory repellent.

Cutkomp and Harvey (1958) studied weight responses of beef cattle in relation to control of horn and stable flies. They used repellent-insecticide oil formulations in treadle sprayers and found significant weight gains on treated cattle in 1954 and 1955, however in 1956 there was no significant weight gain in treated animals. The control of horn-flies was about 95 percent and stable flies 70 percent. Pyrethrins and piperonyl butoxide or MGK-264 gave good results. Individual animals, within a breed had greater variation in fly numbers than between breeds. No significant differences in fly populations were due to breed or sex of animals.

Studies on the Nature of Insect Repellency

The response to chemical stimulation with insects has been measured most frequently on the basis of behavior of the insect as influenced by environmental differences and internal physiological conditions of the test insects. Thus, temperature and humidity should alter the response by affecting the over-all activity of the animal. Absolute thresholds of response for insects so affected have not been established. Many

chemoreception studies have been conducted on the basis of observation of the response of individuals to various materials offered. Other studies of a basic nature have been less frequent as is indicated in the following review.

Behavior Studies. Willis (1947) studied the olfactory responses of female mosquitoes with an olfactometer. He stated that although it has long been taken for granted that mosquitoes are attracted by animal odor, the proof of this has never been conclusive. When the attraction of mosquitoes to host odor is studied in situ, an interpretation of their reactions is often complicated by a strong response to body heat. It has been known for many years that females of many species of mosquitoes will be attracted to a source of heat. Since the problems of determining whether host odors are attractive to mosquitoes involves their responses to olfactory stimuli, the use of an insect olfactometer was indicated. In this type of apparatus odors were presented to the insects under controlled conditions in such a way that the attraction or repellency of the odor was evident from the reactions of the insects. It is practically impossible to investigate the responses of insects to a single stimulus, such as host odor, without at the same time introducing other factors which may influence the reactions of the insects. A suitably designed olfactometer permits close regulation of some of these factors such as temperature, humidity, light, sound, air velocity and contaminating odors. As a result the effects of any additional responses of the insects may be divorced from the action of the odor under investigation.

Frings and Hamrum (1950) explored the possibility of the proboscis, palpi and antennae of adult yellow-fever mosquitoes, Aedes aegypti (L.) having contact chemoreceptors. This was done by mounting them alive on

paper strips fastened on the ends of glass rods and bringing to them sucrose and NH_4Cl solutions. The labella of both males and females were found to have these receptors, which were probably medium-sized curved hairs. The palpi, antennae and parts of the proboscis other than the labella were thought not to have contact chemoreceptors. Tests with mounted insects indicated that males possessed tarsal receptors, which females do not. Tests with mosquitoes allowed to walk freely on drops of distilled water and sugar water, however, showed clearly that both sexes possess tarsal receptors on the fore and middle tarsi at least. The end organs on the tarsi may be hairs similar to the labellar hairs which are probably active but no direct evidence about this was obtained. Results of tests on the oviposition of females were inconclusive. NH_4Cl acted as a non-orienting repellent substance for this species.

Roth (1951) showed that the antennae and palpi are the chief organs used by A. aegypti females in locating the host. He stated that the antennae function as directional distance thermoreceptors and probably chemoreceptors as well, while the palpi receive stimuli when the insect is on or near the skin of the host. Temperature receptors were also thought to be on the palpi. Females whose antennae were removed, were not attracted to man, and combined antennae removal and palpi removal in almost all cases abolished probing. Other activities, however, appeared to be normal.

Wallis (1954) made initial observations on oviposition of mosquitoes. These indicated that the legs were involved in detecting chemical differences of the water, however, some of the movements of these insects were executed with great speed and were probably invisible to the observer. Therefore, direct observations were supported by physically removing or

nullifying suspected sensory areas in various combinations and then testing the mosquito for its oviposition reactions. If the insect retained its ability to distinguish between a tolerated and a repellent solution, other sensory areas were blocked until there was no reaction to a repellent concentration.

Chemical Structure and Repellency. Chadwick and Dethier (1947) studied the relationship between chemical structure and the response of the blowfly to tarsal stimulation by aliphatic acids. This was accomplished by removing the antennae and labella from 1 to 3-day-old flies, suspending them from glass rods and offering them the test solutions in an ascending series of concentrations. In 0.1 M. sucrose, contact of the tarsi with an acceptable solution elicited an extension of the proboscis, and the minimum concentration of test substance which would prevent this response was recorded as the threshold for rejection.

Dethier (1951) published one of his most significant works on the comparative effectiveness of organic compounds of homologous series in producing some given physiological phenomena with a wide variety of living systems. In the majority of cases there is a logarthithmic increase in effectiveness as the carbon chain increases in length. It is of interest, therefore, that studies of the relative effectiveness of homologous compounds in stimulating certain chemoreceptors should reveal a modification of this. Dethier noted that the curves for different series occurred at increasing chain lengths in passing from the less to the more water-soluble compounds. In addition, the break in each series occurred consistently near the point which marked the division between those members which are miscible in water in all proportions and those with finite solubilities in water. This stimulating effectiveness of the latter members was shown to be inversely proportional in their molar

solubility in water. These facts prompted Chadwick and Dethier (1949) to postulate a two phase system for the limiting mechanism in contact chemoreception in the blowfly. According to this hypothesis the smaller molecules gain access to the receptors in part through an aqueous phase while the larger aliphatic molecules penetrate through or accumulate in a lipoid phase. It is this tendency of all properties to change in a relatively orderly manner within such a series which may have imposed limitations on attempts to assign to any particular properties a major role in stimulation. Of all chemical properties examined, solubility alone agrees consistently with the accumulated data. The fact that the threshold values for individual compounds are frequently different from those which would be expected, solely on the basis of the correlation between threshold and solubility in water suggests that other factors which have not been identified are also concerned in stimulation.

Threshold Values. Dethier and Chadwick (1948) defined an acceptance or rejection threshold as the least concentration of a chemical required to cause or prevent some response selected by the investigator and interpreted as acceptance or refusal by that investigator. Despite precautions taken in the determination it is commonly observed that not all individuals of a given species respond alike to a single concentration of the test agent. Over a certain critical range, at least, some specimens will accept while others will reject. With a small group of individuals it is usually possible to extend the range in both directions (unless solubility interferes) until 100 percent acceptance or refusal is obtained, but increasing the number of insects sampled generally requires a further extension of range in order to achieve 100 percent response.

Wallis (1954) devised a method of testing an experimentally treated mosquito for loss of reaction. It was based on the selection of an oviposition site when only two choices were available. One contained distilled water and the other a known repellent. With this choice of sites available, a normal control mosquito would always deposit eggs on the distilled water. An experimentally treated one, with all sensory areas blocked or eliminated would oviposit indiscriminately on either site or both. After the localization procedure was carried out and the extent of the sensitive areas among the individual tarsomeres determined, these sensitive areas were compared to surface structures such as spines, hairs, etc. that were found on nonsensitive areas of the legs. Then, such structures found present on the sensitive areas and absent from the nonsensitive areas, were further examined by histological methods for evidence of direct innervation with fibers of bipolar sensory neurons.

Histological Studies. Hays and Liu (1947) investigated tarsal chemoreceptors of the housefly and their possible relationship to DDT toxicity. They found chemoreceptive sensilla associated with the function of taste and smell. These sensilla were found on various parts of the body such as antennae, palpi and tarsi. Those on the tarsi have usually been considered to be gustatory in function and in many species of insects have been shown to be more sensitive to sugars. Chemoreceptive sensilla are of various types. Those associated with small, slender hairs which have delicate walls are supposed to be receptive to odors and are considered chemoreceptive hairs. They fall into the category of sensilla called sensilla trichodea. Such hairs are innervated by a group of sense cells as are the trichodea sensilla that are regarded as organs of touch. According to Wigglesworth (1939) these thin-walled sensilla have no

socket or trichopore at their base. The tormogen cell is often absent although the trichogen cell is usually large and secretes a product that fills the thin walled hair. The sense cells are usually many in number and may form clusters of 20 or 30. They are enclosed in a nucleated coat which is continuous with the neurolemma of the attached nerves. The proximal nerve forms the afferent process and the distal nerve forms the terminal filament.

After establishing the chemoreceptive sensibility in various species of insects, Eltringham (1933) and Hays (1947) worked with histological methods and described the structure of chemoreceptors in various insects.

Hays and Liu then did an histological study on the tarsi of three species of insects which included the adult housefly, Musca domestica L., the adult German cockroach, Blatella germanica (L.) and both the adult and larva of the Mexican bean beetle Epilachna varivestis Muls.

The histology of the tarsi of these species is as follows: The cuticle, epidermis and basement membrane form the wall while the tracheae, an ungitractoral tendon and a pair of nerves occur within and run throughout the tarsal segments. On the cuticula are several kinds of appendages such as spines, fixed hairs, tactile setae, tenant hairs and chemoreceptive setae.

Among the three species studied, the chemoreceptive sensilla were found only in the tarsi of the house fly (figures 1, 2, 3, 4 & 5). They are located lateroventrad on the second to fifth tarsal segments and have not been found either on the dorsal side of those segments or in the first tarsal segment. The chemoreceptive organ is composed of a group of sense cells located in a sub-epidermal position and covered by a nucleated neurolemma continuous with that of the longitudional nerve. The indivi-

dual cells are more or less spindle shaped. The distal end of the sensilla is attached to a long, thin walled chemoreceptive seta.

Grabowske and Dethier (1954) found thin walled tarsal hairs which were associated with groups of sense cells and considered them to be chemoreceptors. These innervated hairs, found on the tarsi and proboscis of the fly Calliphora erythrocephala (Meigen), contained a large eccentrically located cavity and a smaller cavity in the heavy portion of the wall. Tinbergen (1939) described them and postulated on the basis of the structure and distribution of these sensilla that they were chemoreceptors. A type sensillum identical to that described was found on the legs and proboscis of Phormis regina by Grabowske and Dethier.

Lewis (1954) stated that each chemoreceptor is a flexible hollow seta characteristically innervated by a spindle-shaped group of six to eight sensory cells. He described the cuticle of each receptor as differentiated longitudinally into a thicker, opaque posterior wall. The frontal membrane extends the full length of the receptor for about one-fourth the total receptor surface.

There was little doubt in his mind that the frontal membrane, a fraction of a micron thick was the boundary across which molecules stimulating a chemoreceptor diffuse. There appeared to be a continuous lipoid epicuticle present which affects the interpretation of data concerning the stimulation of receptors. In referring back to Dethier (1948), Lewis followed the hypothesis that the rejection thresholds of aqueous solutions of organic substances and of inorganic electrolytes appears to be inversely related to lipid solubility or to oil-water distribution coefficients.

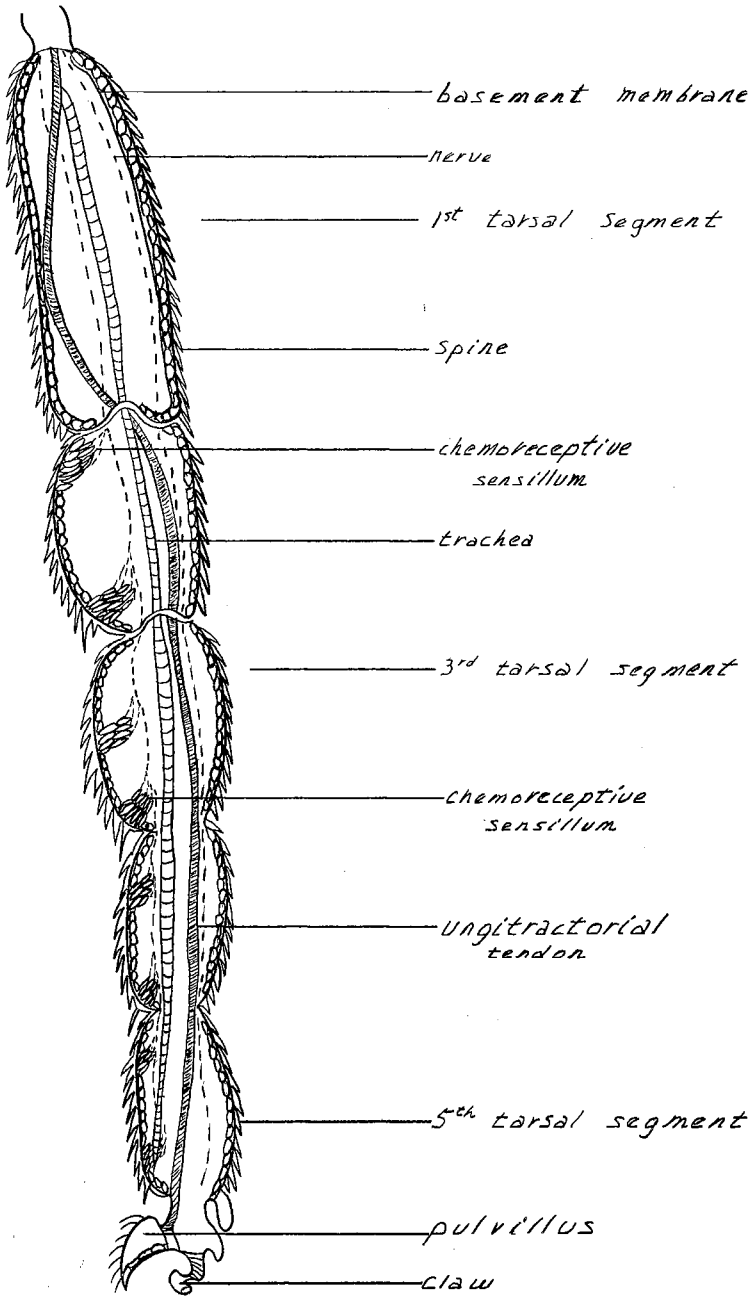

Figure 1. Cross Section of Tarsus (M. domestica L.)
(Redrawn after Hays and Liu)

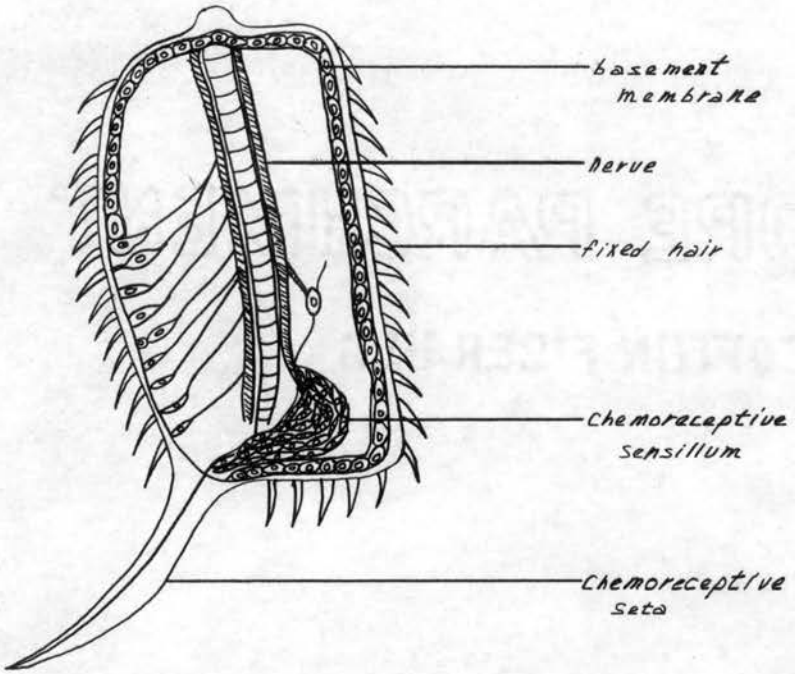

Figure 2. Fourth Tarsal Segment (Sagittal Section) (M. domestica L.) (Redrawn after Hays and Liu)

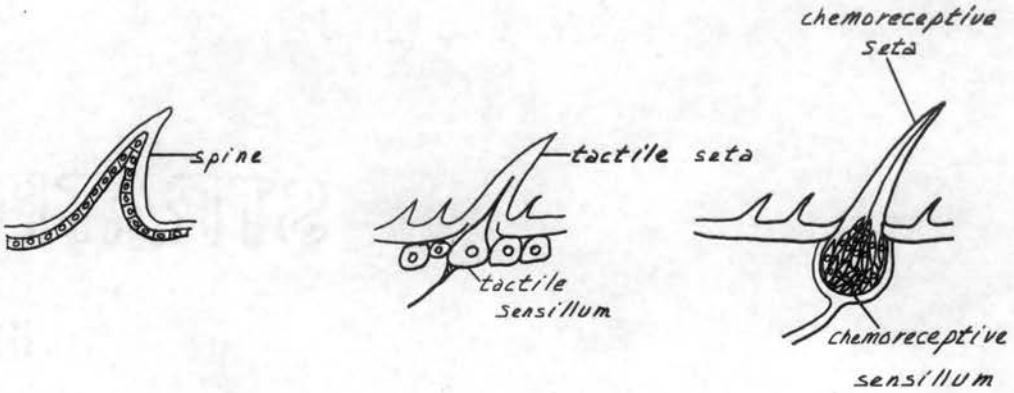

Figure 3. Sagittal Section of a Spine and a Tactile Seta. Cross Section of a Chemoreceptive Seta. (M. domestica L.) (Redrawn after Hays and Liu)

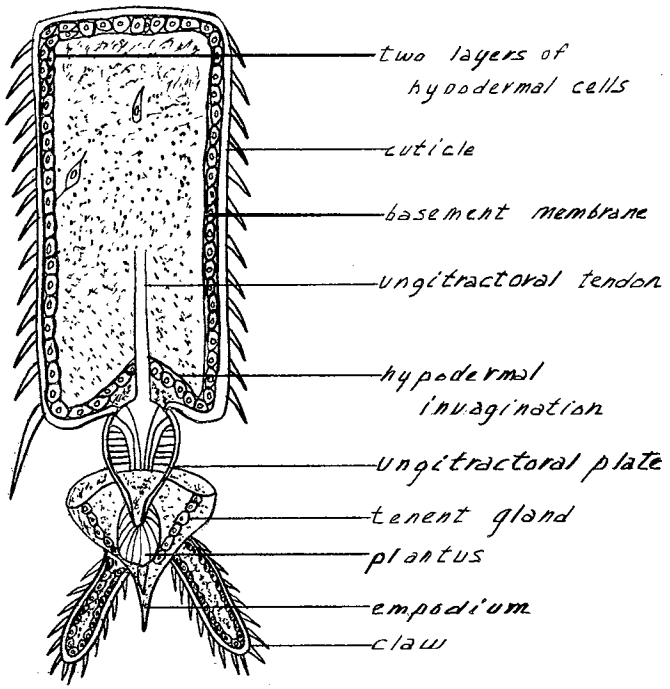

Figure 4. Frontal Section of Fifth Tarsal Segment (M. domestica L.)
(Redrawn after Hays and Liu)

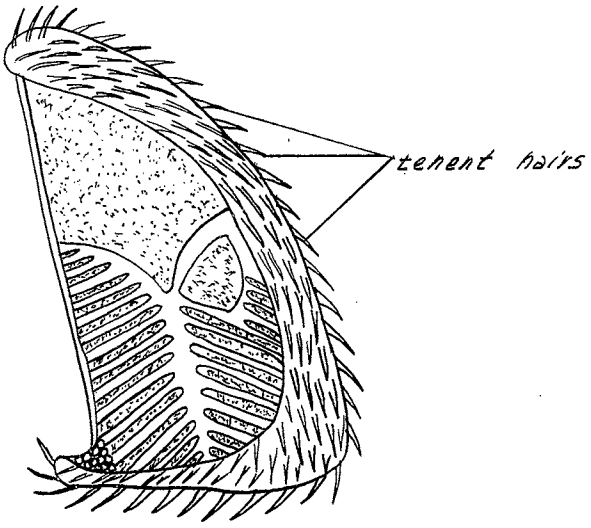

Figure 5. Frontal Section of Pulvillus (M. domestica L.)
(Redrawn after Hays and Liu)

Stimulation of End Organs. The processes involved in the chemical stimulation of an end organ are: 1. the penetration of the receptor wall and 2. the irritation of the sense cells by the molecules which penetrate. Therefore, differences in threshold concentration may be a measure of their ability to penetrate a lipoid barrier in the receptor membrane rather than a measure of differences in the sensitivity of the sense cells to the substances (Lewis 1954).

Progress in the physiology of chemoreception has been hampered by the fact that in most reported experiments the criteria of sensory excitation have been limited to behavioral responses of the animals studied. These studies leave in doubt the nature of the response of the primary receptor surface to specific chemical stimuli. Hodgson (1955) found that potential change between the small localized sensory surface and the body of the animal could be conveniently recorded on an oscilloscope. He found the electric response from a single hair consisted either of one or both of two series of spike potentials, each series clearly originating in a single neuron. The larger spike predominated when the electrode contained a sugar solution with only a trace of electrolyte. Stimuli that evoked the smaller spike resulted in a positive feeding response (proboscis extension) in the intact fly, and stimuli that evoked the larger spike caused a rejection reaction. This may then be direct evidence of a peripheral discrimination mechanism in each chemosensory hair as postulated by Dethier.

A third neuron is associated with the hair though it does not send a process to the tip. Potentials from this third neuron were not recognized.

Slifer (1955) has found that the permeable basiconic pegs do not

function as hygroceptors as was suggested earlier and that although they may be stimulated by certain odors they are not affected by others. The evidence collected so far, in these and earlier studies suggests that the permeable basiconic pegs may be the receptors for the common chemical sense. Silfer used grasshoppers in this study.

Wright (1957) proposed a theory of olfaction and of the action of mosquito repellents. He considered vibrational modes or frequencies such as airborne-water vapor, CO_2 and convective heat, movement, contour and reflectivity capable of setting off trigger molecules of pigment in the olfactory end organs. Melting points below about 50° C. were thought to possibly be volatile enough to have a distant effect. However, if the repellent is neither markedly acid or alkaline and not noticeably irritating they are not likely to act on the common chemical sense.

CHAPTER III

MATERIALS AND METHODS

Chemical Materials for Surface Treatment. Twelve chemicals were used in the laboratory and field investigations of this study. These chemicals are listed below with their chemical formulae and some of their physical characteristics. The formulations depended upon the test for which they were used. The specific formulations used are given with each test description.

1. Pyrocide 175 ®- is a brown liquid containing 20 percent pyrethrins and 80 percent petroleum distillate.

2. Tabatrex ®-(di-n-butyl succinate) is a water white chemical having a specific gravity of D 0/4=0.9963 or 8.31 lbs./gal., a melting point of -29.25°C., boiling point of 108°C. at 4mm. and a flash point of 275°F. by the Cleveland open cup method.

3. Crag ®-(butoxypropanediol polymer) is a water white chemical having a specific gravity of 0.990 at 20°C., a molecular weight of 800, vapor pressure of 1×10^{-3} at 30°C. and a flash point of 420°F.

4. MGK-264 ®-(N-(-ethylhexyl)-bicyclo-[2.2.1]-5-heptene-2,3-dicarboximide) is a clear viscid liquid having a boiling point of 158°C. at 2mm. and is miscible with petroleum oils and other organic solvents as are all of the compounds listed here.

5. CP 16226-(3)-(Bis(2,3,3,3-tetrachloropropyl) ether) is a light yellow liquid having a specific gravity at 25/15.6°C. of 1.6216, a flash

point of 400°C., viscosity of 28.52 centistokes at 25°C. and 46.25 at 5°C., boiling point of 137.5°C. at 0.8mm. and a refractive index of 1.5263 (n_D^{25}).

6. MGK-R-11 ®-(2,3,4,5-Bis($\triangle^2$-butenylene) tetrahydrofurfural) is a brown viscid liquid having a molecular weight of 204.271, specific gravity of 1.20, boiling point of 115°C. at $b_{1.0}$ and a refractive index of 1.5240 at n_D^{20}.

7. MGK-R-326 ®-(Di-n-propyl isocinchomeronate) is a brown viscid liquid having a molecular weight of 251.287 and a boiling point of 124°C., $b_{0.2}$.

8. 949-(2-Hydroxypropyl n-octyl sulfide) is a clear viscid liquid having a molecular weight of 204.379, boiling point $b_{0.2}$ 102-103°C. and a refractive index n_D^{20} of 1.4709.

9. 1113-(n-propyl n-octyl sulfoxide) is a clear to white solid with a molecular weight of 20r.379 and a melting point of 39-41°C.

10. 1207-(3-Chloropropyl n-octyl sulfoxide) is a clear to white solid having a molecular weight of 238.831, a melting point of 41-42°C. and a refractive index of 1.4748 at n_D^{50}.

11. 1345-(2-Methyl-2-propenyl n-octyl sulfoxide) is a clear to white solid having a molecular weight of 216.390, a melting point of 29-30°C. and a refractive index of 1.4810 at n_D^{50}.

12. 1357-(Allyl n-octyl sulfoxide) is clear to white solid having a molecular weight of 202.363, a melting point of 38-39°C. and a refractive index of n_D^{50} 1.4692.

Dairy Cattle Sprays. The following repellent formulations were given practical trial against fly populations found on dairy animals.

1. Tabatrex-2	Pyrethrins	0.025	percent weight/volume
	Tabatrex	1.0	percent weight/volume
	MGK-264	2.0	percent weight/volume
	In APCO-467		
2. 1207-35	Pyrethrins	0.025	percent weight/volume
	1207	1.0	percent weight/volume
	MGK-264	2.0	percent weight/volume
	In APCO-467		
3. 1207-37	Pyrethrins	0.025	percent weight/volume
	1207	0.05	percent weight/volume
	MGK-264	2.0	percent weight/volume
	In APCO-467		

Test Animals

Five species of arthropods were used in the laboratory investigations. Musca domestica L. and Stomoxys calcitrans (L.) were obtained as pupae from the Phillips Petroleum Company's Research Laboratory through the courtesy of Dr. L. D. Goodhue. These two species were supplied regularly by Dr. Goodhue and in addition, were reared at the insectary of Oklahoma State University. Standard rearing techniques were used on these species (Goodhue, 1958).

Aedes aegypti (L.) eggs were obtained from the United States Public Health Service Laboratories at Savannah, Georgia and reared according to the procedure set forth by Peterson.

Xenopsylla cheopis (Rothchild) pupae were obtained from the United States Department of Agriculture Laboratory at Orlando, Florida and reared according to methods prescribed by Smith and Eddy (1953).

Rhipicephalus sanguineus (Latreille) nymphs were obtained from wards of the Oklahoma State University, Veterinary Medicine Small Animal Clinic. The nymphs were kept in a 15 gallon lard can with sand in the bottom approximately 1/4 inch deep. The nymphs were then used in testing as needed.

Laboratory Experimentation

1. Alightment Tests. Alightment tests were done with 1 to 3-day-old M. domestica adults, 3 to 5-day-old S. calcitrans adults, and 3 to 5-day-old A. aegypti adults. This type of test was designed to evaluate the intrinsic repellency of candidate repellents. For this test, brown wrapping paper of 3 by 5-inch dimensions was used. One-half of the paper was treated with the candidate repellent while the other half was left untreated to serve as check. Each of the candidate repellents was tried in the following repellent-xylene formulations: 0.1 percent, 0.25 percent, 1 percent, 5 percent, 10 percent, 25 percent, and 50 percent. After treatment, the papers were allowed to dry for 24 hours before beginning the tests.

These treated papers were then placed, one at a time, from low dilutions to high, in a cage with a high density of insects and left for a total of 12 minutes. The first 2 minutes were to allow for settling of the insects and the subsequent 10 minutes were used to observe their behavior. The cages were placed so that there was an opaque background and the lighting about the cage was diffused so that it was lighted relatively uniformly. The tests were run at a temperature of 70° to 75° F.

These tests were run 1, 5, 10 and 20 days after treatment of the paper. The percent repellency was arrived at in each case by dividing the mean number of insects alighting on the treated side by the mean number of insects alighting on the untreated side. This product was then subtracted from 100.

2. Patch Tests. Patch tests were done with 3 to 5-day-old adult X. cheopis and R. sanguineus nymphs. For this type of test which tests

the intrinsic repellency of a compound as does the alightment test, a 2-inch square muslin patch was treated with the candidate repellent and a line bisecting the cloth was made. Each of the candidate repellents was used in repellent-xylene formulations of 0.1 percent 0.25 percent, 1 percent, 5 percent, 10 percent, 25 percent and 50 percent. The patches were then used in testing 1, 5, 10, and 20 days after treatment.

For conducting the tests, a pan 9 inches in diameter and 4 inches deep was used as the test chamber. The sides of the pan had an angle of approximately 95°. To insure against loss of the test animals from the chamber, a ring of petrolatum was applied to the top of the pan. This ring was 1/2 inch deep. The size of this chamber allowed two chemicals to be evaluated at one time. This was done by placing the patches from low dilution to high around the inside of the pan with an untreated patch between the low dilution of one of the chemicals and the high dilution of the other chemical. This provided for two check patches. The patches were placed so that they touched the bottom of the container. They were held in place with masking tape. Next, the test insects were put into the container and the relative numbers found on each of the patches were used to arrive at the percent repellency (figure 6).

Approximately 200 X. cheopis adults were put into the container and allowed to settle about 10 minutes before counting took place.

R. sanguineus nymphs were used in the same way as were X. cheopis. Fifty to one-hundred of these test animals were placed in the center of the pan and allowed to disperse to the sides of the pan before counting. The negative geotrophism of this arthropod is of particular value in this type of test. Counts were made during the following five-minute period to determine the percent repellency of each of the chemicals and the

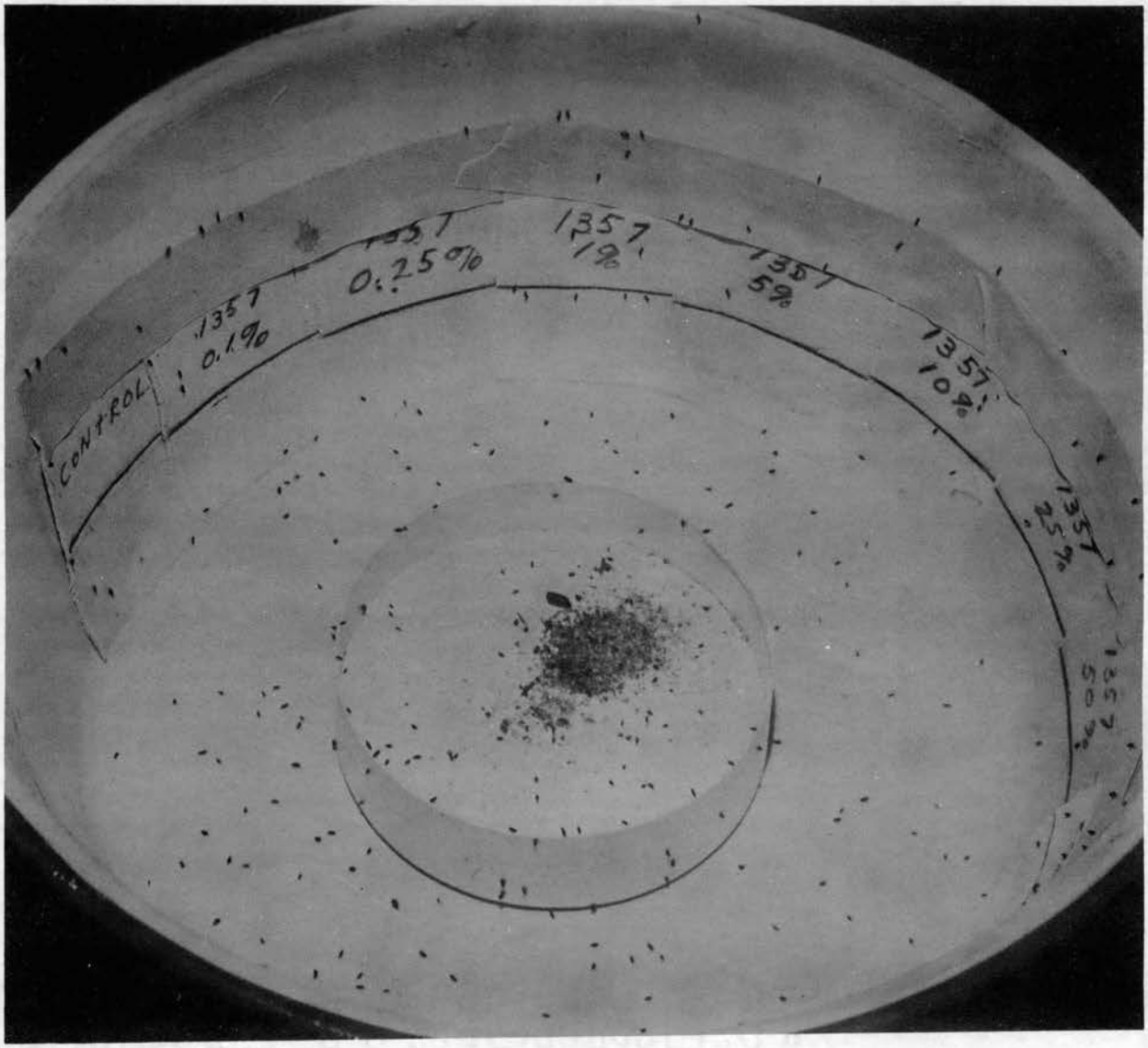

Figure 6. The Test Chamber for the Patch Test.

differences between dilutions. Those test animals which crossed over the line bisecting the patch were the ones counted in this test.

3. Feeding Tests. Feeding tests were conducted with 1 to 3-day-old M. domestica adults, 3 to 5-day old S. calcitrans adults and 3 to 5-day-old A. aegypti adults. This procedure was designed to demonstrate any masking effect the candidate repellents might have.

M. domestica feeding tests consisted of a modified sandwich-bait method. This was made up by placing two strips of creme-honey, 20cm. wide and 30 cm. apart on a 3 by 5-inch index card; these strips were 100cm. long. The strips were then allowed to dry for approximately 8 hours. This produced a glossy surface. One strip was then treated with the repellent formulation and the other strip remained untreated to serve as check. The treatment was painted on the glossy surface with a camel's hair brush.

The degree of repellency was determined by a comparison between the number of flies attempting to feed on the untreated side as compared to that number trying to feed on the treated side. Treatments were checked 1, 5, 10, and 20 days after the repellents were applied in repellent-xylene formulations of 0.1 percent, 0.25 percent, 1 percent, 5 percent, 10 percent, 25 percent and 50 percent.

S. calcitrans and A. aegypti feeding responses were checked by observing the percent of insects which would feed on warmed citrated beef blood, through a hot gut membrane. The membranes were prepared by stripping them from the gut, rolling them out on a glass surface and allowing them to dry. They were then cut into 25cm. squares and soaked off the glass when needed for the tests. For the tests, 2cc's. of blood was put into a 10cc. serology tube. The top of the tube was then covered with the

membrane which was held in place with masking tape. After this, the tubes were refrigerated until the membranes dried out; this normally took about 5 minutes. When the membranes had dried, they were painted with dilutions of 1 percent and 5 percent of each of the candidate repellents and again refrigerated for 3 to 5 hours so that the xylene would evaporate. The tubes of blood were then heated to 120°F., turned upside down and placed in the feeding rack (figure 7). The tubes were then placed into a cage of hungry stable flies about 3 to 5 days old. Another series of these tubes was placed in a cage of 3 to 5-day-old A. aegypti. Included in the tube rack was a control tube against which percent repellency was arrived at. Counts of flies and mosquitoes feeding during a 2-minute period after placing the rack in the cage were made. After about 2 minutes, the tubes would cool and a clot would form under the membrane which would inhibit feeding.

4. Behavioral Tests. Close observation of each species of insect as affected by various repellent treated surfaces is important to the understanding of the nature of repellency. This type of work was carried on once screening techniques established that a chemical had repellent properties.

For this study, a glass observation cell 20 by 20mm. by 5mm. deep was constructed from lucite. The cell was glued onto the surface of a slide and holes 1.5mm. in diameter were made on two sides of the cell. To one hole was attached a capillary pipette which was attached to a vacuum line. The vacuum was created by the emptying of a five-gallon bottle of water (figure 8). This would move approximately five gallons of air per hour. A glass coverslip was then treated with a 50 percent solution of the candidate repellent; a paper treatment of the same con-

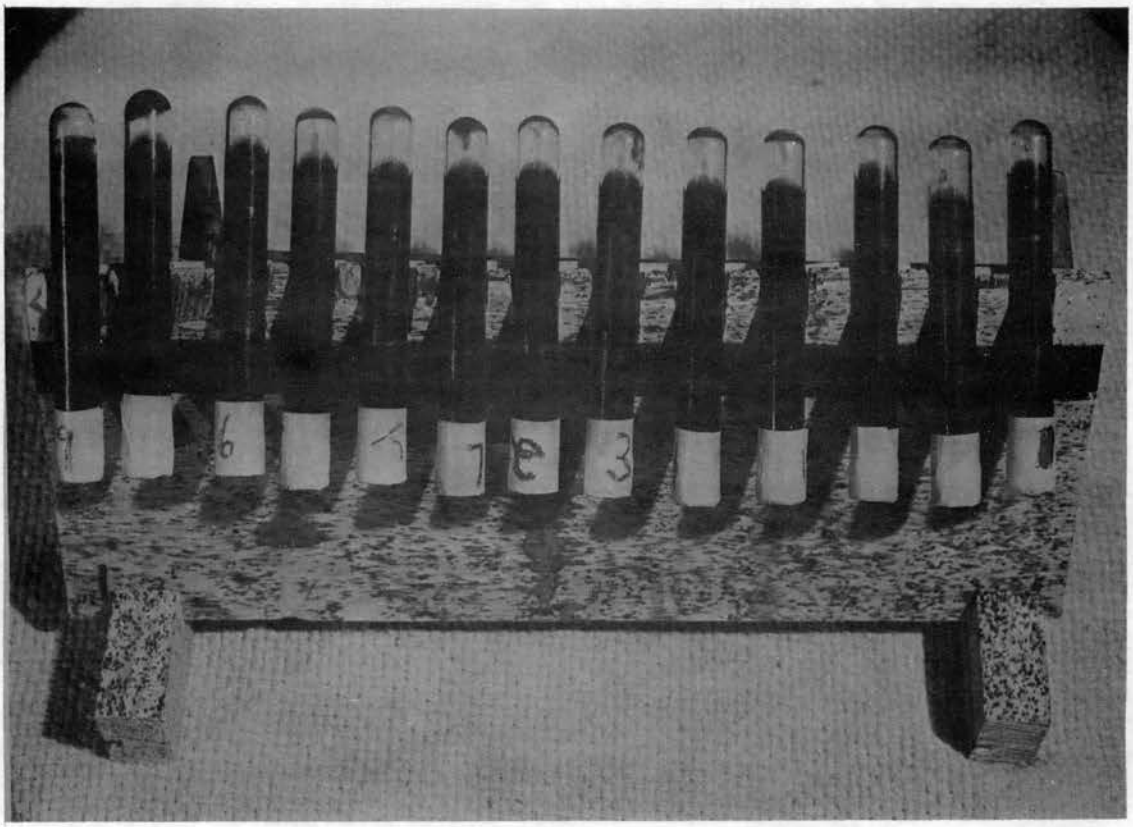

Figure 7. Feeding Rack Holding Serology Tubes of Citrated Blood for S. calcitrans and A. aegypti Feeding.

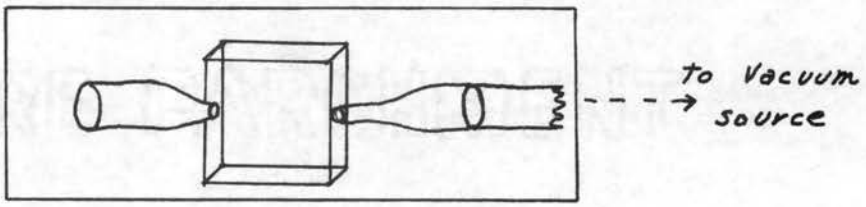

Figure 8. Glass Observation Cell for Behavioral Studies.

centration was also used. The paper was then placed in the bottom of the cell, a test insect placed in the cell and the coverslip placed on top. The test insects were manipulated easily with the aid of CO_2 anaesthesia. After placing the coverslip on the top of the cell, it was placed under a binocular microscope for close observation. Approximately 2 minutes after recovery from anaesthesia, the insects reactions to the surfaces were noted.

5. Physiological Tests. This procedure was designed to give information on the fundamental excitation processes in chemoreception and the mechanisms by which impulses from chemoreceptors initiate integrated patterns of motor activity. Repellent chemicals were applied to intact M. domestica and S. calcitrans and their electrical activity was amplified and monitored with the aid of an oscilloscope and recorded on the physiograph. The physiograph will handle three different specimens simultaneously.

The test insects were slowed by refrigeration for approximately 5 minutes, then three specimens were imbedded, ventral side up, in wax. Care was taken in handling the insects and imbedding them so that they would not be damaged. Particular care was taken to see that the wax was not too hot when imbedding took place.

The imbedded insects were then pierced in the head and the posterior portion of the abdomen with minuten electrodes (figure 9).

After the electrodes were placed, the insects were allowed to rest for approximately 5 minutes until typical trains recurred with regularity. During this time the amplification used with each preparation was standarized as much as possible so that comparative data could be obtained. After standardization was accomplished, the electric activity

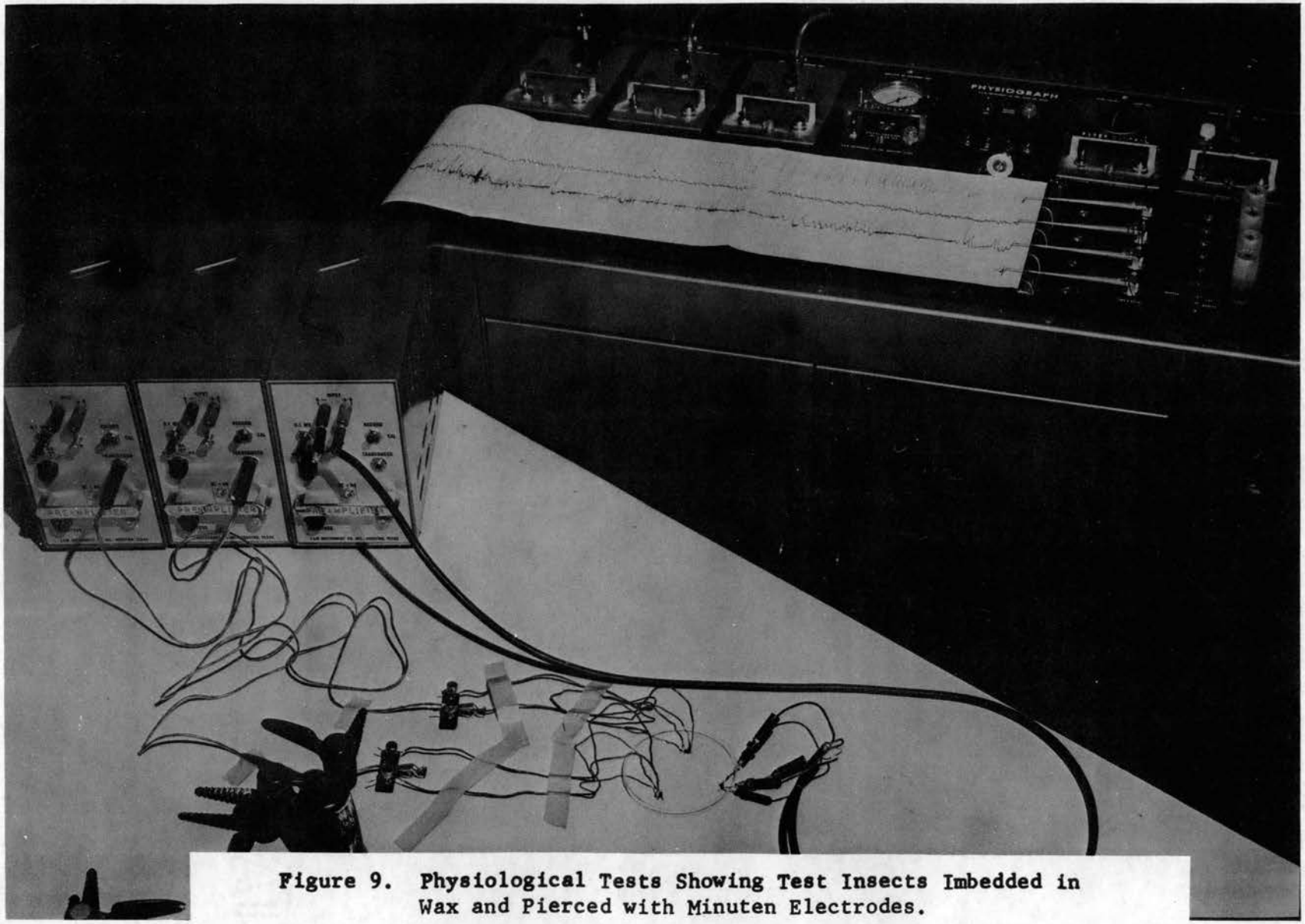

Figure 9. Physiological Tests Showing Test Insects Imbedded in Wax and Pierced with Minuten Electrodes.

was recorded for 2 minutes, then the repellent was applied to each specimen. The application was made with a cotton tipped stick which had been dipped into the repellent and allowed to dry for 2 to 3 hours. The cotton tipped stick was then brought into contact with the test insect's legs for 2 seconds, after which the electrical activity was recorded for 2 minutes.

All the materials were applied as 100 percent except the solids, 1113, 1207, 1345 and 1357 which had been dissolved in xylene to a 50 percent concentration.

6. Chemical Tests. To determine the penetrating ability of each of the candidate repellents a paper chromatography type of experiment was used. Five-inch strips of brown wrapping paper were treated with 50 percent concentrations of each of the materials to a line bisecting the strip. For each treatment 0.5ml. of chemical was used. The strips were then hung, treatment side down for 24 hours after which the length of travel of each material was measured.

Field Experimentation

1. Surface Repellents. As a result of laboratory experimentation which indicated that certain of the chemicals under test had repellent properties, it was thought to be advantageous to conduct correlative field research. This would then enable integration with laboratory work so that the final selection of suitable chemical insect repellents would be more accurate. Since the results of work by the writer and other authors indicated that background activity from many mechanoreceptors stimulated in unison as by a smooth surface might contribute to an overall reaction, these candidate repellents were used on several suitable surfaces.

The work with insects in the laboratory indicated that several of the chemicals in question were adequate repellents in high concentrations but resulted in sub-threshold activity at low concentrations. Consequently, it was deemed advisable to use 50 percent concentrations of the candidate repellent on field surface treatments. This would then insure that the repellent thresholds of each material would be reached. To obtain the most accurate analysis of the worth of these candidate repellents, the treated surfaces were placed in a dairy barn which had an extremely high fly population. This dairy was located at 63rd. and Memorial Drive, Tulsa, Oklahoma. The treated surfaces were hung in series at the top of the barn where the flies rested in very high numbers. The surfaces were treated with the repellents in a completely random design and replicated eight times. The surfaces used were all 5 1/2 inches long by 1/4 inch diameter. They were glass tubing, Tygon plastic tubing, galvanized wire, cotton string, paper string, unpainted wood doweling and painted wood doweling. The paint used was a non-gloss, oil-base white.

The treatment was applied to the bottom 4 inches of each surface so that the effect of space repellency could be measured; this left 1 inch of untreated surface at the top. Figure 10 shows the method of hanging the treatment surfaces in the dairy barn. The surfaces were treated by dipping them into test tubes 1cm. in diameter by 100cm. in length until the surface was treated up to the 4-inch mark. There was some absorption with the string and unpainted surfaces, so that their treatment was made at somewhat less than 4 inches so there would still be an untreated top inch.

After hanging each replicate, fly counts were made daily; those flies resting on the untreated control surfaces were used as the basis

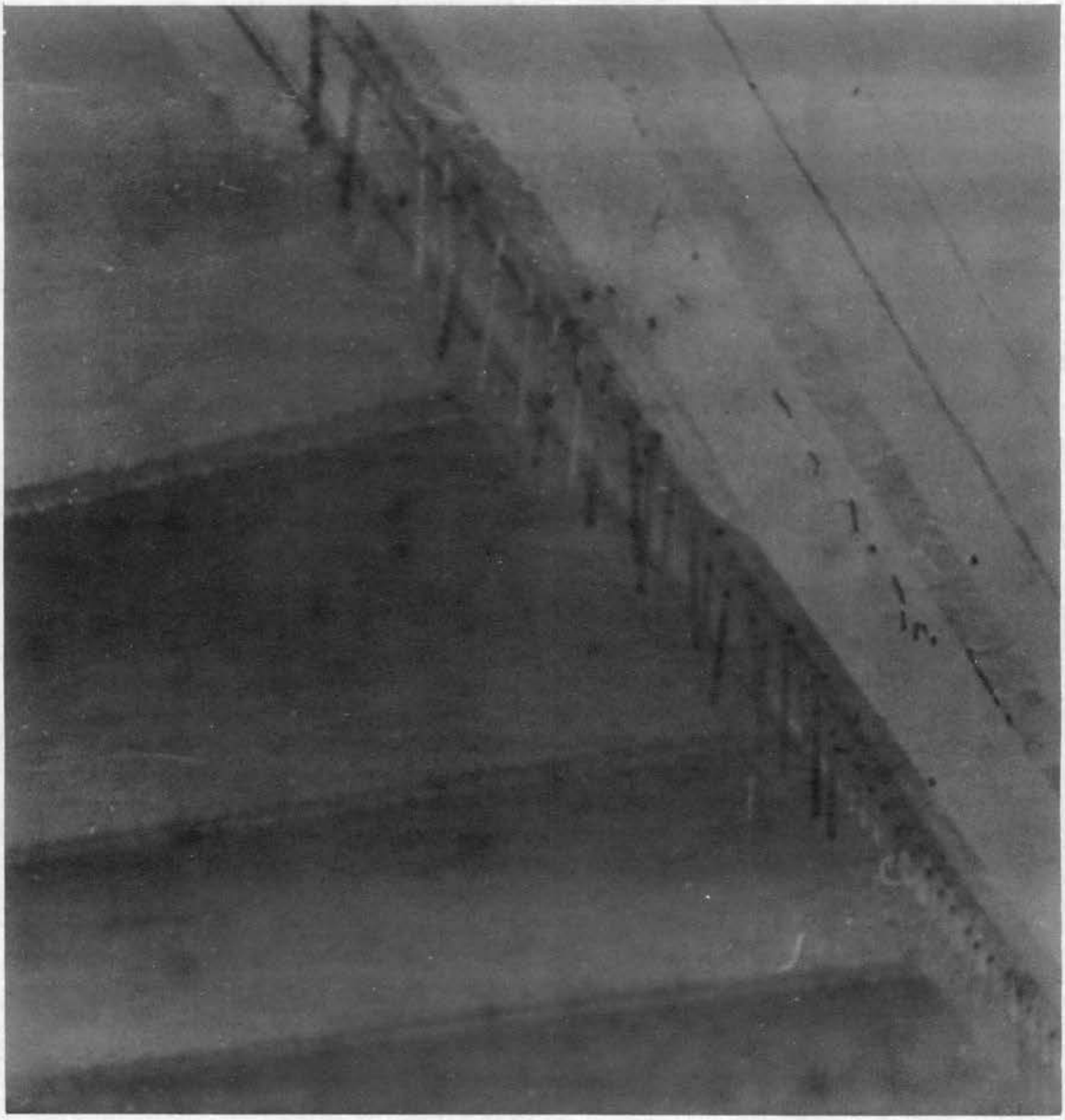

Figure 10. The Method of Hanging Treatment Surfaces in the Dairy Barn.

for determination of percent repellency. The replicates were taken down at different intervals so that variations in fly deposits found after various lengths of exposure to a high density fly population could be noted. Those intervals were 10, 20, 25 and 30 days.

All of the candidate repellents were used in the field surface repellency experimentation.

2. Dairy Cattle Repellent Sprays. The ultimate evaluation of a repellent which has the capability of protecting against biting insects must be carried out in the field with the animals to be protected. The following design has been used by many workers in the field evaluation of repellent chemicals and has been shown to be reasonably sound statistically, even though there may be variables which have not been taken into consideration at this time. The spray formulations listed on page 27 contained amounts of materials which had been studied under laboratory conditions and found to have repellent properties. It was thought that these should therefore, be of value in protecting dairy cattle from biting insects. These formulations were made up by personnel of the Phillips Petroleum Company Research Laboratory through the courtesy of Dr. L. D. Goodhue. Dr. Goodhue's research team had given these formulations and many other spray formulations field evaluation in the Bartlesville, Oklahoma area. The Oklahoma Agricultural Experiment Station has also given these formulations field trials. Their work indicated varying degrees of repellency, consequently more information was sought on these materials. The opportunity to try these formulations on a commercial dairy herd was thus quite fortunate, in that this type of field trial would fairly accurately indicate the results which the dairy farmer might obtain in use of repellent spray formulations.

Out of this dairy herd of approximately 60 animals, 20 Holsteins were chosen for study. The fly populations on these animals were studied for a week by taking morning and afternoon fly counts. These fly counts were then averaged. This seemed to give a fairly good indication of the individual susceptibility of each animal to the attacks of M. domestica, S. calcitrans and S. irritans. On completion of this preliminary study, four groups of five animals each were selected. The animals varied from low to high susceptibility to fly populations. They were therefore grouped so that each group would have relatively uniform fly counts. This meant that each group would have animals with varying degrees of susceptibility.

Group #1 received a daily spray treatment with Tabatrex-2, Group #2 received a daily spraying with 1207-35, Group #3 received a daily spraying with 1207-37 and Group #4 was left as an untreated check.

Fly counts were made in the morning at approximately 10:00 A.M. Evening fly counts were made at approximately 4:00 P.M. just prior to milking time.

Three gallon Hudson Hand Sprayers were used with a mist nozzle which had a cone spray pattern. The nozzle had an .026 inch orifice diameter and delivered approximately 2.2 gallons per hour at 30 pounds per square inch pressure. Approximately 2 to 3 ounces of spray was applied per animal; this of course depended upon the size of the animal. In any case adequate coverage of the entire animal with the exception of the head was attempted.

CHAPTER IV

RESULTS

Laboratory Experimentation

1. Alightment Tests. Tests with M. domestica were done using surfaces which were not made attractive by the addition of a bait. Those surfaces were of brown wrapping paper which had been treated with the candidate repellents. Observations of housefly behavior were then made to determine the intrinsic repellency of each of the materials tested.

The results as tabulated in Table I indicate on a percentage basis the relative position of each material. In all cases the surfaces were tested in cages of high fly density so that the percentages are based on the actual resting of from 25 to 50 flies on each individual surface.

Pyrocide evidently was not particularly repellent to the test insects since only 15 percent repellency was noted with the 50 percent material.

Tabatrex gave good results at the higher concentrations of 25 percent and 50 percent but its repellency was lost quickly and after only 5 days there was no detectable repellency. Crag was much the same as Tabatrex but not quite as active. It, too, was not residual enough to last as much as 5 days.

MGK-264, although of known value for its synergistic action, was found to be repellent in high concentrations and to last up to 10 days on this type of surface. Erratic results at the lower concentrations

seemed to indicate that MGK-264 does not have sufficient repellent action to reach the repellent threshold of the housefly from a chemical stimulation basis only. It is possible that the combination of mechanical and chemical stimulation of this material was responsible for these results. CP 16226-(3) gave results much like those of MGK-264.

R-11 was a good repellent at even lower concentrations. R-326 gave comparable results although its residual strength appeared to last somewhat longer.

949 gave good results at the higher concentrations but had no residual activity.

1113, 1207, 1345 and 1357 gave results which were much the same in the amount required to elicit a response but 1207 was outstanding in its ability to repel at the lower concentrations even after 20 days aging.

Tests with S. calcitrans were conducted using the same procedure as was used with M. domestica. The results of this test were presented in Table II. Here pyrocide appeared to have some repellent activity at the higher concentration, however, it was lost quickly.

Tabatrex and Crag gave identical results in this test. They were quite repellent at high concentrations but only for short periods of time.

MGK-264 and CP 16226-(3) were again quite similar in activity, MGK-264 being slightly more active and residual.

R-11 and R-326 were quite similar in activity, however R-11 was somewhat lower in activity and its effectiveness was short lived.

949 appeared to be effective at the higher concentration only and its drop to 68 percent repellency with the 50 percent material, after 5 days was dramatic proof of its ineffectiveness in this series of tests.

1113, 1207, 1345 and 1357 were quite effective and long lasting. They were almost indistinguishable in their activity at the higher con-

centrations, however at the lower concentrations the effectiveness of 1207 was outstanding.

Tests with A. aegypti as summarized in Table III indicated that there was generally a higher degree of repellency arrived at with this species. This was probably due to the often observed, more acute sensitivity which mosquitoes demonstrate.

Pyrocide gave fairly good results 1 day after treatment but that was short lived. Tabatrex and Crag gave fair repellency 1 day after treatment, but results were erratic and their residual effectiveness was poor.

MGK-264 and CP 16226-(3) were fairly good repellents at the higher concentrations and had considerable residual effectiveness but at the lower concentrations of 10 percent and under their effectiveness was negligable.

R-11, R-326, 1113, 1207, 1345 and 1357 were all quite effective, even in concentrations of 0.1 percent. Here, 1207 was again outstanding in that it was residually effective at the 0.1 percent concentration for 20 days.

2. Patch Tests. These tests were done with X. cheopis and R. sanguineus using muslin patches which had been treated with several concentrations of the candidate repellents. This type surface was not particularly attractive to these arthropods but they were placed so as to take advantage of the negative geotropism innate to these species.

With X. cheopis as shown in Table IV, the effect of pyrocide could not be measured on a repellent basis because there was enough material present to cause knockdown of all the test insects.

Tabatrex and Crag were quite effective at the higher concentrations but lower concentrations were only effective for between 1 and 5 days.

MGK-264 was quite effective and demonstrated residual activity at concentrations of 1 percent. CP 16226-(3) was effective at the higher concentrations for up to 5 days but it was ineffective after that time.

R-11 was extremely effective for as long as 20 days at as low a concentration as 1 percent.

R-326 was quite effective up to 10 days at the higher concentrations but it was relatively ineffective at the lower concentrations.

949 was quite effective for up to 20 days in as low a concentration as 0.1 percent.

1113 was quite effective but the lower concentrations began to lose their effectiveness after 5 days.

1207 was fairly effective but in comparison with the other materials it was not so outstanding against flies and mosquitoes.

1345 and 1357 were quite effective and the residual activity was great, however, 1357 began to lose its activity in the 0.1 percent and 0.25 percent concentrations after 5 days.

Results obtained with R. sanguineus were much the same as those with X. cheopis except that R. sanguineus appeared to be able to tolerate the repellent chemicals to a greater degree. These results are presented in Table V.

3. Feeding Tests. These tests were done with M. domestica, S. calcitrans and A. aegypti in order to obtain information on the effect of repellent treatment on a normally attractive surface.

The results of this procedure as used with M. domestica are presented in Table VI.

Pyrocide resulted in considerable repellent activity for up to 5 days in all concentrations. After that time this repellency was quickly

lost. Flies would land on this surface randomly, but they would not attempt to feed and would quickly move over to the more attractive untreated surface for feeding.

Tabatrex gave results similar to those of pyrocide with a 50 percent drop in repellency of the 5 percent concentrations after 5 days.

Crag gave good results in all concentrations for up to 20 days. MGK-264 gave similar results but repellency in the lower concentrations was lost after 5 days.

CP 16226-(3) gave good results in the higher concentrations for up to 20 days but the lower concentrations were relatively ineffective from the beginning of the tests.

R-11 and 949 gave quite similar results in amount and length of effectiveness. R-11 lost its effectiveness somewhat earlier than 949 however.

R-326, 1113, 1207 and 1345 were extremely effective in all concentrations for up to 20 days.

1357 was quite effective in concentrations of 10 percent and over for up to 20 days but the lower concentrations became ineffective after the first day.

Tests conducted with S. calcitrans were somewhat limited in that it was not possible to study the effect of aging on the repellent treated surface. However, much information was gained from the use of 1 percent and 5 percent concentrations of the repellent chemicals. This data is presented in Table VII. The percentages are based on the number of flies feeding on the treated surfaces as compared with those feeding on the untreated control surfaces. In all cases there were approximately 50 flies feeding on the control surface during a two-minute period.

Pyrocide was ineffective as a repellent in both 1 percent and 5 percent concentrations. Tabatrex and Crag gave fairly good results in both concentrations although they were not quite as good as some of the other materials.

MGK-264 and CP 16226-(3) were similar in effectiveness at the 1 percent concentration but MGK-264 was considerably better at the 5 percent concentration while CP 16226-(3) was not much better at this concentration than at the 1 percent concentration.

R-11 and 1113 were excellent repellents in this test, giving 100 percent repellency at both the 1 percent and 5 percent concentrations.

R-326, 949, 1207 and 1345 were all equally active at the 1 percent and 5 percent concentrations, giving excellent results with the 5 percent concentration.

1357 was distinctive in that it gave very poor results at the 1 percent concentration and fair results at the 5 percent concentration.

Tests of this nature were tried with A. aegypti but treatment of the membrane with repellents even in concentrations as low as 0.1 percent would inhibit feeding. A. aegypti would feed through the untreated membrane but in such low numbers that no practical data of a comparative nature could be obtained.

4. Behavioral Tests. This procedure was conducted to determine the reactions of each of the test insects to repellent treated surfaces.

By a close look at the test insects it was possible to see that the repellent surfaces stimulated an increase in the general irritability of the test insects. In this observation cell it was impossible, however, to determine whether there was repellent action, i.e. stimulating the test animal to move away from a source. Observations were made with all

of the test animals available but no distinguishable behavior was noted during the studies with A. aegypti, X. cheopis and R. sanguineus other than an increase in irritability which could not be made into interpretable data.

M. domestica and S. calcitrans appeared to react almost identically to the repellent surfaces in this type of observation cell. These reactions are presented below.

Pyrocide appeared to excite the test insects almost immediately with knockdown following in 1 to 3 minutes.

Tabatrex stimulated the test insects to a state of excitation in 10 to 15 seconds. This was followed by extension of the mouthparts and ovipositor extension.

Crag too, appeared to stimulate the flies almost immediately. This consisted of mouthpart and ovipositor extension and very pronounced excitation. Crag appeared to be one of the most irritating of the compounds tested.

MGK-264 and CP 16226-(3) produced no detectable stimulation or general irritability.

R-11 produced immediate cleaning and avoiding reactions. This compound appeared to produce a burning sensation in the test insects.

R-326 produced the cleaning reaction in 2 to 3 seconds. This was followed by general irritability and attempts to get out of the cell.

949 and 1113 stimulated the mouthparts and general irritability after about 2 minutes. This stimulation was very slow, however, and would stop immediately upon withdrawal of the surface.

1207, 1345 and 1357 did not produce any detectable irritation when the flies came into contact with them. It was noted, however, that when

the flies were upside down and trying to cling to the surface of the coverslip, that the pulvilli were unable to keep them up, consequently there was a frantic effort on the part of the flies to try not to fall from that surface. It appeared that the repellents made the normal suction cup apparatus (the pulvilli) unable to function properly. This was also noted with MGK-264 and CP 16226-(3).

5. Physiological Tests. These tests were done in order to give information on the fundamental excitation processes involved in the insects behavior as affected by a repellent treated surface. The more important factors involved in this study were thought to be the amount of time necessary for the insect to get the message that there was some undesirable chemically treated surface and the amount of increase in nervous activity due to that stimulation.

The results of this test were quite similar with the two species of test insects used. These results are presented in Table VIII for M. domestica and Table IX for S. calcitrans. From this data it can be seen that Crag and R-326 were quick to stimulate but did not result in a very noticable increase in nervous activity. Tabatrex was slow to act on the nervous system but created somewhat more of an increase in activity.

MGK-264 and CP 16226-(3) appeared to be slow to act but caused much more of an increase in activity than some of the other compounds. Perhaps this is an explanation of their role as synergists. 949 was also slow to act but the nervous activity was not affected nearly so much as it was by MGK-264 and 16226-(3).

R-11, 1113, 1207, 1345 and 1357 had similar reaction times but R-11 and 1357 did not seem to affect the nervous activity as greatly as the other compounds.

6. Chemical Tests. Brown wrapping paper was treated with each of the repellent materials to measure their penetrating ability. 1357 and Tabatrex did not migrate up the paper to any measurable extent. 1113 migrated 2mm., Pyrocide 4mm. and 1207 to 8mm. R-11 and MGK-264 migrated 20mm., 949 migrated 35mm., Crag 44mm., CP 16226-(3) 45mm., R-326 51mm. and 1345 55mm.

Field Experimentation

1. Surface Repellents. Seven types of surfaces were treated with 50 percent concentrations of the candidate repellents and hung in a dairy barn having a high fly population. Fly counts were then made daily for 30-day periods and the percent repellency derived from these data. Tables X through XVI present this data. During the course of this study, the flies were predominately M. domestica, however, S. calcitrans were frequently noted in small numbers. S. calcitrans, however, did not make up more than 10 percent of the total population. It was hoped that by leaving a portion of each surface untreated, it would be possible to distinguish some space repellent effect by noting flies on the untreated portion if there was no space effect and no flies on the untreated portion if there were some space effect. Unfortunately, it was not possible to distinguish any space effect.

Of the surfaces tested, string retained the highest repellency for 30 days. This was noted with all of the materials under test except R-11. R-11 lost much of its repellency after 10 days. Plastic ran a close second while paper string was third. Paper string showed high repellency after 30 days with all the materials except Tabatrex, Crag and R-11. Glass was the fourth least attractive surface, however, high repellency

after 30 days was noted with some of the candidate repellents. Tabatrex, CP 16226-(3), R-11, R-326, 1113, 1345 and 1357 were relatively ineffective after 20 to 30 days while Pyrocide, Crag, MGK-264, 949 and 1207 remained quite effective for up to 30 days.

Wire was the next least attractive surface with only Crag and 1207 standing up for 30 days. Painted wood was next in line and unpainted wood was evidently the most attractive of all the surfaces to the flies. Possibly both of these surfaces were equally attractive because of their resemblance to the barn surface but the repellents might have gone into the unpainted wood somewhat more, thus leaving less repellent on the surface.

Of the repellents tested, there was considerable difference between them on some of the surfaces, but in the main there was consistency in percent repellency between chemicals and between surfaces. Pyrocide was quite effective overall but most of its repellency was spent after 20 days.

Tabatrex and R-11 were much alike in overall repellency, however, much of their effectiveness was lost after 10 days.

MGK-264 and CP 16226-(3) were almost identical in their activity, MGK-264 retained its effectiveness somewhat longer however.

1207 was most effective on all surfaces with Crag running a close second in overall repellency.

R-326 had the next most effective overall repellency which was 89 percent. 949, 1113, 1345 and 1357 were much the same in their activity, however 1357 was notably poor on the wire and wood surfaces.

2. Dairy Cattle Repellent Sprays. To evaluate a repellent which has the capability of protecting against biting insects, animals in a dairy

herd were sprayed daily during the fly season with three types of spray formulations. Fly counts were made daily and the results of this project are presented in Table XVII and Figures 11, 12 and 13.

Table XVII is an analysis of variance which shows that there was a significant difference in the populations of S. irritans, S. calcitrans and M. domestica because of treatment with repellent sprays. Figure 11 demonstrates the effect of formulations on S. irritans; it seems that all treatments were equally effective in repelling horn flies. Figure 12 and figure 13 demonstrate that 1207-35 affected the number of S. calcitrans and M. domestica while the other two formulations and the control group had fly populations which were so close that no significant differences were noted.

CHAPTER V

DISCUSSION

Screening Techniques. During the course of these experiments many of the types of tests and observations made could be regarded as excellent screening techniques, though the primary purpose of several of the tests has been to bring to light some of the intricacies involved in insect behavior as affected by repellent treated surfaces.

In work with M. domestica one of the screening methods offering the broadest study opportunities was found to be a field technique which permitted the evaluation of a number of candidate repellents on several types of surfaces at the same time. The running of this type of test against natural populations is also of advantage because the results obtained appear to be more in line with what might be expected in practical application. There are of course more variables encountered such as wind, dust, variations in temperature and humidity, etc. but with randomized and replicated test series, such as those accomplished during this study, one can obtain a fairly accurate evaluation of candidate materials.

In the laboratory, alightment and feeding tests are excellent methods of determining the relative repellency of various compounds. The feeding tests however, are somewhat easier to run because after the settling period, the test insects stay in one spot much longer than if the surface did not have an attractive base. This facilitates counting and improves the accuracy of the test.

Evaluation of materials for use against S. calcitrans is somewhat more difficult than with M. domestica. Normally the field populations are not high enough to allow evaluation of surface treatments and in the laboratory, their rearing and maintainence requires more time and effort. However, with alightment tests and feeding tests, a fairly accurate index of a candidate repellent's effectiveness can be obtained.

The feeding tests require more preparation but appear to be more satisfactory in that the repellent is tested against an attractive surface which will hold the flies. The alightment tests with S. calcitrans however, are considerably easier to run than with M. domestica because of the resting habits of S. calcitrans. After S. calcitrans initially settle, they do not move about as randomly as do M. domestica. This habit facilitates accurate counting.

Alightment tests with A. aegypti are also quite satisfactory in that they too, will settle about two minutes after disturbance and thereafter make fewer random moves than M. domestica.

Patch tests as conducted with X. cheopis and R. sanguineus were modified by the writer from screening techniques of earlier workers. This modification was to take advantage of the negative geotropic behavior of these species. It would appear to be more satisfactory to test the chemicals against a known attractant, but for screening techniques this would involve much too much time with results that are not much more comparative.

Behavioral tests were used to note the general irritating effects that a compound might have on test insects. Close observation of this type does not allow the use of quantative data for classing the various compounds. Threshold values are more easily obtained by the use of well designed experiments which enable observation of the behavior of large

populations of insects rather than a close look at a few specimens. Behavioral tests, can however give valuable information on the location of chemoreceptors and fundamental problems of the excitation process.

Behavior Studies. The behavior studies that were accomplished during the course of this work were valuable in that some of these observations helped fill the gap between the stimulation of a test insect by a repellent treated surface and the internal nervous activity created or modified by that stimulation. The behavioral observations made during this study were of course not confined to close observations in a cell but extended through all of the experimental procedure. Many of these observations were then of a quantative nature. This enables statements to which can be attributed a greater probability.

To help explain the nature of each of the chemicals under test, the information obtained from these observations was most valuable in that it pointed out whether there was any outstanding excitation or behavior as a result of coming into contact with a repellent. The quantative data obtained from the screening type tests, the physiological tests and chemical tests could then be integrated with these observations so that possible explanations of the mode of action of each of the chemicals could be presented.

The work of Hays and Liu (1947) on the histology of the housefly tarsi and that of Smyth and Roys (1955) and Wallis (1954) on location of chemoreceptors in insects give sufficient background on the location and innervation of chemoreceptors. Lewis (1954) who says that there is little doubt that the frontal membrane of chemoreceptors, a fraction of a micron thick, is the boundry through which chemicals which cause some stimulation, may penetrate also postulates that this is a lipoid barrier

through which chemicals in the lipoid phase may penetrate.

The preceding literature information points to the possibility of there being several ways in which repellents bring about a modification of insect behavior. The experimental data gathered during this study further substantiates these hypothesis. Repellents may penetrate in varying degrees, affect the nervous system as a poison, affect the nervous system so that a repellent action is set up, stimulate the nervous system mechanically as would petrolatum or a surface to which the insect could not cling or all or any combination of these factors may be working at the same time.

Surface Variations. It appears that the surfaces studied result in variations in length of effectiveness of individual repellents because of the amount of material which the surfaces can absorb and hold, string being a prime example of a surface on which a high degree of repellency was noted some time after repellency on other surfaces was lost. The length of effectiveness was affected by the volatility of the compounds used. The solid compounds were notably more effective for longer periods of time while those of a higher volatility were dissipated more quickly. It appeared, in the case of galvanized wire, that possibly some of the materials might have been changed chemically so that their repellent effect was lost more quickly.

The data obtained from chemical tests which measured the amount and length of penetration of materials points to another factor involved in the length of effectiveness of a compound.

Repellent variations. Pyrethrins appeared to cause a repellent effect possibly because of the quick knockdown achieved with the 50 percent concentration. MGK-264 and CP 16226-(3) which have been used

in formulations as synergists, also appear to poison the test insects. Intense nervous activity is set up when they have received a dose of these materials. This factor together with mechanical stimulation noted in many cases may account for their being fairly good repellents when applied in high concentrations.

MGK-264 and CP 16226-(3) penetrated the paper surface to a considerable extent, however, in physiological tests, it took some time for increased nervous activity to occur. This may be a variation in penetration ability or it might indicate that these materials act as poisons because no almost instantaneous repellent reaction is set up. Mechanical stimulation may also be a big factor in the repellent action of these materials.

Tabatrex and Crag have consistently given similar results except that Tabatrex has demonstrated a degree of repellency somewhat lower than Crag. Tabatrex did not penetrate the paper surface to any extent while Crag had considerable penetration ability. This sort of information may explain why, during physiological tests, Crag stimulated an increase in nervous activity almost immediately while Tabatrex took somewhat longer to stimulate an increase. The increase with Tabatrex was greater, however, possibly due to the amount of material concentrated in the tissue at the same time.

R-11, R-326 and 949 have given similar results in many of the tests. They have approximately the same volatility, penetration ability and potential to stimulate nervous activity. R-326 penetrates more deeply and is ranked first of these three materials in overall repellency. 949 penetrates to a lesser degree and is second. R-11 penetrates somewhat less and is ranked third. This may mean that not as much R-11

is taken up by the treated surface and that it is, in addition, completely exposed to evaporation by the atmosphere.

1113, 1207, 1345 and 1357 have demonstrated similar repellent activity throughout these tests. 1357 had the lowest degree of repellency of the four, 1113 and 1345 were only slightly better. 1357 and 1113 did not appear to have much of an ability to penetrate; this factor may have been the cause of an earlier loss of repellency. 1345 was notably better and more long lasting than any of the other compounds tested. Since many of the other factors involved in the repellency of these four compounds were found to be essentially the same, it is possible that the chlorine atom, which is a part of 1207, has resulted in that compound being somewhat toxic to the test insects. This would then make the action of 1207 much like that of MGK-264 and CP 16226-(3).

Possibly these materials are such that the insect walking over a surface treated with them gets a dose sufficient to change it's behavior pattern so that it will stay away from that surface in the future and thus result in observations which point out fewer insects on the treated surface than on the untreated surface. This would point to a learned behavior. This seems logical considering the observed fact that 1207, MGK-264 and CP 16226-(3) do not appear to excite or irritate the test insects. There has also been noted the mechanical effect of these compounds which may not give the insects enough traction. This factor may bring up the degree of repellency.

It is not possible to say just how many of the factors such as poison, repellent activity, mechanical stimulation or any combination of these factors are involved in the repellent effect of each of the chemicals. However, it appears that with Pyrethrins, MGK-264,

CP 16226-(3) and 1207 at least, all of these factors may be working. This then would account for the observed effects on population distribution.

Dairy Cattle Repellent Sprays. In evaluating the capability of protecting animals against biting insects with repellents, the same principles of stimulation and toxic effects of the compounds are present. In addition, there is the involvement of the many variables introduced by the host animals.

In the series of tests conducted on dairy animals, these many host factors probably resulted in the just barely significant population differences noted after treating the animals daily with repellent sprays.

There was of course much less material used in this type of spray and the changes in fly population were notable on the animals. This population fluctuation makes it difficult to obtain high figures in percent repellency. However, the flies found on treated animals were not feeding in many cases; perhaps, the materials were then, in effect, still protecting the animals. From this standpoint, the criterion of repellency which has been deemed by many to be a percent repellency of around 80% or more would not be a valid criterion. This type of evaluation would make repellency and control synonymous.

A significant difference in populations that is found on treated animals should certainly be an indication that repellent chemicals are affecting the population distribution. In any case the use of 1207-35 which had 1 percent 1207 in it, resulted in a significant difference in the population of S. calcitrans and M. domestica found on those animals as compared to the other three groups of animals.

The difference in S. irritans population was interesting in that all three materials gave a reduction in flies. This may be attributed to the toxic effect of Pyrethrins, MGK-264 and possibly of 1207 which has some insecticidal properties.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Laboratory and field experimentation was conducted with the hope that the results could be integrated and interpreted to aid in the selection and development of suitable repellents and to help explain some of the many questions on how insect behavior is affected by repellent treated surfaces.

In the laboratory, alightment, patch, and feeding tests enabled determination of the amount of repellent material necessary to elicit response in five species of arthropods. In addition these tests showed how long the repellents would last when applied in various concentrations. This, in effect, established a threshold value for each material and each species of test animal. These results are summarized in table I.

In the field, repellent chemicals were evaluated on glass, plastic, metal, cotton, paper, unpainted and painted wood surfaces. The repellent concentrations used were 50 percent. Field surface repellent tests are summarized in table II.

The quantitative results of these series of experiments as interpreted with the supportive information obtained by close observation of each species of test animal, physiological experimentation and chemical tests, together with evaluation of previous literature and knowledge of the chemical formulae and physical characteristics of each of the compounds, suggest possible explanations of the mode of action of several

TABLE II

STANDARD DEVIATION OF FIELD SURFACE TESTING OBSERVATIONS
20 DAYS AFTER TREATMENT

Repellents	Surfaces						
	Glass	Plastic	Wire	String	Paper	Wood	Ptd.Wood
1. Pyrocide	6.8(3)*	0.0(2)	15.6(3)	0.0(3)	0.0(2)	2.0(3)	23.5(3)
Mean	93%	100%	85%	99%	95%	98%	63%
2. Tabatrex	37.0(5)	46.0(2)	36.1(5)	22.4(5)	0.0(5)	22.4(5)	25.5(5)
Mean	38%	54%	16%	90%	58%	17%	38%
3. Crag	14.4(5)	0.0(2)	2.5(5)	3.5(5)	3.0(2)	31.6(5)	31.2(5)
Mean	92%	100%	98%	97%	95%	72%	78%
4. MGK-264	14.3(8)	2.0(2)	23.8(8)	7.7(8)	1.0(2)	22.3(8)	35.2(8)
	90%	93%	76%	95%	92%	77%	71%
5. CP 16226-(3)	4.5(3)	0.0(2)	2.6(3)	2.6(3)	3.4(2)	2.8(3)	25.1(3)
Mean	81%	100%	28%	97%	96%	85%	57%
6. R-11	0.0(3)	8.5(2)	2.6(3)	11.0(3)	1.0(2)	0.0(3)	28.3(3)
Mean	0%	62%	46%	56%	67%	0%	36%
7. R-326	28.9(8)	0.0(2)	18.4(8)	0.0(8)	0.0(2)	7.9(8)	31.8(8)
Mean	75%	95%	81%	100%	100%	93%	73%
8. 949	0.3(8)	5.0(2)	14.2(8)	0.0(8)	8.6(2)	5.0(8)	27.3(8)
Mean	99%	92%	78%	100%	65%	1%	35%
9. 1113	20.2(8)	6.5(2)	25.5(8)	7.5(8)	0.0(2)	27.1(8)	28.6(8)
Mean	75%	93%	33%	96%	100%	28%	61%
10. 1207	0.0(8)	0.0(2)	9.0(8)	0.0(8)	0.0(2)	13.3(8)	9.0(8)
Mean	100%	100%	95%	100%	100%	88%	91%
11. 1345	22.2(8)	10.1(2)	11.2(8)	0.0(8)	0.0(2)	27.3(8)	29.7(8)
Mean	61%	89%	43%	100%	99%	36%	47%
12. 1357	24.8(8)	10.1(2)	11.7(8)	0.0(8)	0.0(2)	22.5(8)	27.8(8)
Mean	61%	84%	16%	99%	99%	25%	44%

*Numbers in parenthesis indicate replications.

of the materials which were evaluated. The effect of the repellent materials on treatment surfaces made possible an explanation of length of effectiveness variations between surfaces.

Surface variations or differences in degree of effectiveness and residual value were deemed to be a result of the porosity of the surface linked with the penetrating ability of the compound and its volatility.

Repellent variations were found to be many. Pyrethrins, MGK-264, CP 16226-(3) and 1207 were found to act as poisons, stimulate repellent activity and cause a mechanical stimulation of the test insects. These factors seem to adequately account for their effectiveness relative to other compounds tested. Possibly that poison activity is the result of a sub-lethal dose which changes the behavior pattern of the test insect, causing it to avoid the surface in the future. In the case of Pyrethrins, that insecticidal activity may cause quick knockdown and thus fewer insects on the treatment surface.

The other materials, Tabatrex, Crag, R-11, R-326, 949, 1113, 1345, and 1357 demonstrated different degrees of repellency depending on the surface tested. The innate ability of the individual compounds to stimulate repellent action is not overlooked, however. This is taken into account in explaining the difference in threshold demonstrated by each of the compounds.

Physiological tests have pointed out that there is a definite difference in the amount of time necessary for the chemical to initiate a response and the degree of change in nervous activity brought about by a repellent chemical. It is proposed that this be attributed to the ability of the individual compounds to penetrate the lipoid barrier. Other writers have indicated that this possibility exists. This factor

may be one of the more important ones producing differences in repellent thresholds within the same population of insects. The variation between insect species may be a result of differences in this lipoid barrier.

Dairy cattle repellent sprays were tested in an attempt to evaluate repellent formulations which have the capability of protecting animals against biting insects. This resulted in a low percentage repellency however, it is proposed that the criterion of repellency be based not on percentage repellency but on whether or not the mixtures caused a significant change in population distribution. This would be a more accurate method of evaluation because there are many variables involved in evaluation of chemical repellents on host animals.

Although there are still many unanswered questions in regard to insect behavior as affected by repellent treated surfaces, it is hoped that this behavioral information together with some of the laboratory and field study techniques will enable other workers to go forward with synthesis and development of newer and better chemical insect repellents.

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A P P E N D I X

TABLE III

PERCENT REPELLENCY OF SEVERAL CHEMICALS USED IN ALIGHTMENT TESTS WITH M. DOMESTICA

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
1. Pyrocide	1	0	0	0	0	0	0	15
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
2. Tabatrex	1	0	0	0	0	0	96	100
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
3. Crag	1	0	0	0	0	0	57	80
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
4. MGK-264	1	0	54	0	52	5	63	81
	5	0	0	0	0	0	80	75
	10	0	0	0	0	0	40	30
	20	0	0	0	0	0	0	0
5. CP 16226-(3)	1	0	0	0	0	0	0	47
	5	0	0	0	0	0	0	30
	10	0	0	0	0	0	0	10
	20	0	0	0	0	0	0	0
6. R-11	1	33	40	63	75	88	92	93
	5	0	10	43	66	80	83	84
	10	0	0	0	31	55	80	88
	20	0	0	0	0	50	62	78
7. R-326	1	81	72	80	78	95	95	86
	5	0	0	50	90	95	95	95
	10	0	0	20	50	70	76	82
	20	0	0	10	30	46	33	85
8. 949	1	37	32	37	79	65	66	90
	5	0	0	0	30	44	38	32
	10	0	0	0	20	30	0	20
	20	0	0	0	0	0	0	10
9. 1113	1	82	57	96	82	82	88	97
	5	0	0	63	53	84	90	99
	10	0	0	50	50	60	86	95
	20	0	0	10	20	50	77	89

TABLE III (Cont'd.)

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
10. 1207	1	69	89	89	83	89	96	100
	5	71	77	95	87	95	97	99
	10	60	60	90	88	90	98	100
	20	20	60	95	90	90	95	100
11. 1345	1	42	56	75	92	92	91	96
	5	0	0	0	24	90	90	100
	10	0	0	0	10	60	80	90
	20	0	0	0	0	70	70	90
12. 1357	1	0	50	85	88	90	92	84
	5	0	0	0	42	60	88	95
	10	0	0	0	0	55	90	90
	20	0	0	0	0	55	90	90

TABLE IV

PERCENT REPELLENCY OF SEVERAL CHEMICALS USED IN ALIGHTMENT TESTS WITH S. CALCITRANS

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
1. Pyrocide	1	0	0	0	0	0	25	45
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
2. Tabatrex	1	0	0	0	0	85	95	100
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
3. Crag	1	0	0	0	0	55	95	100
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
4. MGK-264	1	0	0	0	0	53	66	80
	5	0	0	0	0	50	61	80
	10	0	0	0	0	23	50	75
	20	0	0	0	0	0	0	0
5. CP 16226-(3)	1	0	0	0	0	0	45	60
	5	0	0	0	0	0	0	60
	10	0	0	0	0	0	0	50
	20	0	0	0	0	0	0	0
6. R-11	1	20	20	35	70	85	100	100
	5	0	0	20	50	65	85	85
	10	0	0	0	0	45	80	78
	20	0	0	0	0	0	0	0
7. R-326	1	77	78	80	85	95	100	100
	5	0	0	60	78	87	95	98
	10	0	0	60	78	86	90	90
	20	0	0	0	77	80	82	88
8. 949	1	40	38	45	57	68	72	95
	5	0	0	0	33	52	61	68
	10	0	0	0	0	31	40	61
	20	0	0	0	0	0	0	0
9. 1113	1	82	84	94	95	95	100	100
	5	0	0	0	82	87	98	99
	10	0	0	0	73	83	97	97
	20	0	0	0	70	80	95	95

TABLE IV (Cont'd.)

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
10. 1207	1	52	53	73	88	95	100	100
	5	50	50	71	86	93	95	100
	10	0	0	68	86	93	93	95
	20	0	0	0	80	90	90	90
11. 1345	1	37	55	86	100	100	100	100
	5	0	0	66	95	100	100	100
	10	0	0	0	77	98	100	100
	20	0	0	0	0	0	100	100
12. 1357	1	0	55	88	88	95	99	100
	5	0	0	77	68	94	96	100
	10	0	0	0	63	90	96	100
	20	0	0	0	50	90	95	100

TABLE V

PERCENT REPELLENCY OF SEVERAL CHEMICALS USED IN ALIGHTMENT TESTS WITH *A. AEGYPTI*

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
1. Pyrocide	1	50	75	100	50	60	64	84
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
2. Tabatrex	1	67	67	91	90	95	100	100
	5	0	0	0	0	67	0	33
	10	0	0	0	0	42	66	60
	20	0	0	0	0	0	0	0
3. Crag	1	34	67	42	60	40	17	43
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
4. MGK-264	1	0	0	0	0	58	82	95
	5	0	0	0	0	50	79	90
	10	0	0	0	0	0	0	80
	20	0	0	0	0	0	0	70
5. CP 16226-(3)	1	30	100	55	80	67	87	90
	5	0	0	0	0	60	90	90
	10	0	0	0	0	10	30	80
	20	0	0	0	0	0	0	60
6. R-11	1	100	100	100	100	100	100	100
	5	0	0	55	89	95	95	100
	10	0	0	0	0	90	90	95
	20	0	0	0	0	50	75	95
7. R-326	1	100	100	100	100	100	100	100
	5	0	50	63	100	100	100	100
	10	0	30	42	90	95	95	94
	20	0	0	10	86	90	90	90
8. 949	1	100	100	100	100	100	100	100
	5	0	0	0	40	43	60	90
	10	0	0	0	10	15	10	30
	20	0	0	0	0	0	0	10
9. 1113	1	100	100	100	100	100	100	100
	5	0	0	100	100	100	100	100
	10	0	0	50	60	70	90	98
	20	0	0	30	50	75	86	91

TABLE V (Cont'd.)

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
10. 1207	1	100	100	100	100	100	100	100
	5	80	90	100	100	100	100	100
	10	77	90	95	94	96	98	98
	20	70	81	92	90	98	98	97
11. 1345	1	100	100	100	100	100	100	100
	5	0	0	0	50	100	100	100
	10	0	0	0	40	76	91	94
	20	0	0	0	30	70	88	90
12. 1357	1	100	100	100	100	100	100	100
	5	0	0	67	100	100	100	100
	10	0	0	0	57	90	95	98
	20	0	0	0	0	75	90	95

TABLE VI

PERCENT REPELLENCY OF SEVERAL CHEMICALS USED IN PATCH TESTS WITH X. CHEOPIS

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
1. Pyrocide	1	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
2. Tabatrex	1	70	75	77	94	100	100	100
	5	67	71	70	88	98	98	99
	10	0	0	0	61	73	82	85
	20	0	0	0	23	56	58	57
3. Crag	1	77	77	88	88	88	94	94
	5	0	51	73	76	84	88	90
	10	0	0	59	73	81	85	89
	20	0	0	42	58	71	85	86
4. MGK-264	1	91	94	95	97	97	97	97
	5	72	71	83	81	90	90	92
	10	0	0	58	81	89	88	91
	20	0	0	43	72	85	87	88
5. CP 16226-(3)	1	85	87	96	96	98	98	100
	5	0	0	0	90	92	93	98
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
6. R-11	1	85	87	96	96	98	98	100
	5	80	83	91	93	100	100	100
	10	0	38	90	90	99	100	100
	20	0	31	88	86	100	100	100
7. R-326	1	84	92	100	100	100	100	100
	5	0	34	58	79	84	85	90
	10	0	31	53	81	82	85	88
	20	0	0	0	0	0	0	44
8. 949	1	86	84	92	90	96	100	100
	5	84	83	90	88	95	100	100
	10	85	80	88	83	96	100	100
	20	80	80	82	80	95	100	100
9. 1113	1	96	96	96	96	96	96	97
	5	82	88	90	94	95	97	98
	10	40	51	53	63	70	81	93
	20	0	0	0	0	50	67	90

TABLE VI (Con't.)

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
10. 1207	1	81	84	91	91	91	91	94
	5	0	61	69	73	91	91	94
	10	0	31	52	61	90	90	91
	20	0	0	33	40	89	90	92
11. 1345	1	100	100	100	100	100	100	100
	5	100	100	100	100	100	100	100
	10	98	98	98	98	100	100	100
	20	98	98	96	96	96	100	100
12. 1357	1	98	98	96	96	96	100	100
	5	70	71	82	97	99	100	100
	10	60	62	81	95	100	100	100
	20	0	0	50	95	100	100	100

TABLE VII

PERCENT REPELLENCY OF SEVERAL CHEMICALS USED IN PATCH TESTS WITH R. SANGUINEUS

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
1. Pyrocide	1	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
2. Tabatrex	1	90	90	95	100	100	100	100
	5	84	79	91	98	98	99	100
	10	67	77	78	88	90	91	93
	20	0	0	0	0	0	67	70
3. Crag	1	67	69	71	70	70	84	95
	5	0	0	0	0	0	65	89
	10	0	0	0	0	0	0	82
	20	0	0	0	0	0	0	73
4. MGK-264	1	93	86	93	100	100	100	100
	5	90	90	91	98	98	100	100
	10	80	81	88	93	95	95	97
	20	0	0	71	82	81	83	85
5. CP 16226-(3)	1	50	53	51	48	54	57	59
	5	0	0	0	0	0	45	51
	10	0	0	0	0	0	0	48
	20	0	0	0	0	0	0	0
6. R-11	1	100	100	100	100	100	100	100
	5	98	90	94	100	100	100	100
	10	90	88	88	90	93	95	100
	20	0	0	65	71	88	92	100
7. R-326	1	90	90	90	100	100	100	100
	5	90	91	89	95	98	98	100
	10	81	88	88	92	92	95	100
	20	0	53	57	60	90	91	94
8. 949	1	57	91	96	100	100	100	100
	5	42	53	91	94	95	100	100
	10	0	0	32	44	89	91	94
	20	0	0	0	0	73	89	91
9. 1113	1	95	95	100	100	100	100	100
	5	93	94	95	95	98	100	100
	10	39	41	44	89	90	94	99
	20	0	0	0	0	72	81	89

TABLE VII (Cont'd.)

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
10. 1207	1	62	93	93	93	93	94	95
	5	64	91	92	90	93	93	94
	10	31	44	68	71	91	90	93
	20	30	40	50	61	84	91	91
11. 1345	1	90	90	90	100	100	100	100
	5	88	88	90	98	97	99	100
	10	81	80	83	85	95	97	95
	20	32	38	54	67	83	85	89
12. 1357	1	100	100	100	100	100	100	100
	5	83	84	84	91	94	97	100
	10	73	78	78	80	88	91	96
	20	0	0	42	66	79	89	93

TABLE VIII

PERCENT REPELLENCY OF SEVERAL CHEMICALS USED IN FEEDING TESTS WITH M. DOMESTICA

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
1. Pyrocide	1	98	99	100	100	100	100	100
	5	95	98	98	99	99	99	99
	10	0	0	0	0	70	83	85
	20	0	0	0	0	0	0	0
2. Tabatrex	1	50	80	99	100	100	100	100
	5	40	40	40	50	98	100	100
	10	0	0	0	0	43	88	90
	20	0	0	0	0	0	0	80
3. Crag	1	98	99	100	100	100	100	100
	5	98	99	100	100	100	100	100
	10	81	88	91	93	100	100	100
	20	62	60	88	90	100	100	100
4. MGK-264	1	97	98	98	98	98	99	100
	5	83	85	89	93	96	98	100
	10	41	43	51	66	82	99	100
	20	0	0	0	21	80	99	100
5. CP 16226-(3)	1	50	50	75	100	100	100	100
	5	0	20	30	98	100	100	100
	10	0	0	0	83	100	100	100
	20	0	0	0	80	100	100	100
6. R-11	1	98	98	100	100	100	100	100
	5	0	80	80	100	100	100	100
	10	0	0	0	48	56	77	91
	20	0	0	0	0	0	0	0
7. R-326	1	99	100	100	100	100	100	100
	5	99	99	100	100	100	100	100
	10	80	86	97	100	100	100	100
	20	67	67	93	97	100	100	100
8. 949	1	75	85	100	100	100	100	100
	5	75	75	75	83	90	90	94
	10	43	40	45	53	68	73	70
	20	15	15	18	65	65	67	68
9. 1113	1	99	100	100	100	100	100	100
	5	94	99	100	100	100	100	100
	10	83	85	99	100	100	100	100
	20	71	82	90	98	100	100	100

TABLE VIII (Cont'd.)

Repellents	Days After Treatment	Percent Concentration						
		0.1	0.25	1	5	10	25	50
10. 1207	1	99	100	100	100	100	100	100
	5	75	85	98	100	100	100	100
	10	75	83	95	100	100	100	100
	20	75	80	90	100	100	100	100
11. 1345	1	98	99	100	100	100	100	100
	5	98	99	99	100	100	100	100
	10	88	86	91	94	98	100	100
	20	72	74	70	75	90	100	100
12. 1357	1	95	100	100	100	100	100	100
	5	0	0	0	0	90	100	100
	10	0	0	0	0	92	94	95
	20	0	0	0	0	85	90	90

TABLE IX

PERCENT REPELLENCY OF SEVERAL CHEMICALS USED IN FEEDING TESTS WITH S. CALCITRANS

Repellents	Percent Concentration and Percent Repellency	
	1	5
1. Pyrocide	0	0
2. Tabatrex	82	91
3. Crag	71	88
4. MGK-264	42	86
5. CP 16226-(3)	48	53
6. R-11	100	100
7. R-326	86	100
8. 949	61	100
9. 1113	100	100
10. 1207	83	100
11. 1345	94	100
12. 1357	26	86

TABLE X

INCREASE IN NERVOUS ACTIVITY OF M. DOMESTICA RESULTING FROM TREATMENT WITH REPELLENT CHEMICALS

Repellents	Time in Seconds to Increased Activity				Percent Increase in Overall Activity			
	Replicates				Replicates			
	1	2	3	Average	1	2	3	Average
1. Tabatrex	14.0	11.0	15.0	13.3	80	13	59	50.6
2. Crag	4.0	3.5	5.0	3.8	44	0	24	34.0
3. MGK-264	3.0	8.0	8.0	6.3	38	30	30	32.6
4. CP 16226-(3)	9.0	5.0	7.0	7.0	34	78	53	55.0
5. R-11	2.5	2.0	3.5	2.6	0	27	38	21.6
6. R-326	1.0	2.3	1.0	1.1	8	18	23	16.3
7. 949	13.0	9.0	17.0	13.0	24	20	8	17.3
8. 1113	12.0	13.0	10.0	11.6	43	73	28	48.0
9. 1207	6.0	4.0	7.0	5.6	54	5	72	43.6
10. 1345	6.0	4.5	3.0	4.5	87	0	74	53.3
11. 1357	5.0	4.5	4.5	4.6	46	9	22	25.6

TABLE XI

INCREASE IN NERVOUS ACTIVITY OF S. CALCITRANS RESULTING FROM TREATMENT WITH REPELLENT CHEMICALS

Repellents	Time in Seconds to Increased Activity				Percent Increase in Overall Activity			
	Replicates				Replicates			
	1	2	3	Average	1	2	3	Average
1. Tabatrex	19.0	28.0	21.0	23.5	60	55	61	57.5
2. Crag	5.0	3.0	2.0	3.3	34	20	42	36.0
3. MGK-264	10.0	10.0	18.0	12.6	89	88	59	78.6
4. CP 16226-(3)	16.0	13.0	18.0	15.6	80	50	60	63.3
5. R-11	4.0	3.0	3.0	3.3	88	60	57	68.3
6. R-326	2.5	2.0	3.0	2.5	10	20	19	16.3
7. 949	16.0	15.0	15.5	15.5	34	34	35	34.3
8. 1113	2.5	2.0	3.5	2.6	0	29	25	18.0
9. 1207	7.5	8.0	6.0	7.1	34	50	20	34.6
10. 1345	4.0	3.5	3.0	3.5	27	20	21	22.6
11. 1357	3.0	2.0	4.5	3.1	9	20	23	24.0

TABLE XII

PERCENT REPELLENCY OF SEVERAL CHEMICALS APPLIED TO WIRE SURFACES

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
1. Pyrocide	1						100	100	100	100
	10						100	100	100	100
	20						95	97	63	85
	30						33	62	0	31
2. Tabatrex	1				100	100	100	100	100	100
	10				100	0	79	0	0	17
	20				83	0	0	0	0	16
	30				45	0	0	0	0	9
3. Crag	1				100	100	100	100	100	100
	10				100	100	100	100	100	100
	20				100	100	100	94	100	98
	30				91	100	100	100	100	98
4. MGK-264	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	100	100	82	54	48	87	39	100	76
	30	40	67	0	0	0	0	0	100	25
5. CP 16226-(3)	1						100	100	100	100
	10						84	71	100	85
	20						27	25	31	28
	30						22	50	0	24
6. R-11	1						100	100	100	100
	10						78	73	76	76
	20						44	46	50	47
	30						42	55	44	47
7. R-326	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	93	100	100	100	100	99
	20	80	97	65	41	93	99	92	79	81
	30	52	67	0	0	0	83	63	65	41
8. 949	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	50	93
	20	81	88	89	62	72	99	57	84	78
	30	60	94	0	0	0	100	87	0	42
9. 1113	1	100	100	100	100	100	100	100	100	100
	10	92	100	49	81	82	96	100	50	81
	20	41	77	0	29	62	25	31	0	33
	30	31	48	0	0	0	33	75	0	23

TABLE XII (Cont'd.)

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
10. 1207	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	88	100	100	100	97
	20	98	100	100	100	72	99	100	94	100
	30	93	100	100	27	87	100	100	90	87
11. 1345	1	100	100	100	100	100	100	100	100	100
	10	64	100	91	100	64	100	100	100	100
	20	46	48	50	54	33	25	54	36	43
	30	42	53	50	0	29	66	62	0	38
12. 1357	1	100	100	100	100	100	100	100	100	100
	10	71	100	31	56	82	97	85	100	77
	20	18	53	31	0	20	0	10	0	16
	30	0	0	0	0	0	0	0	0	0

TABLE XIII

PERCENT REPELLENCY OF SEVERAL CHEMICALS
APPLIED TO GLASS SURFACES

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
1. Pyrocide	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	100	100	100	100
	20	---	---	---	---	---	100	96	84	93
	30	---	---	---	---	---	100	100	50	83
2. Tabatrex	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	100	0	100	100	0	60
	20	---	---	---	62	0	33	96	0	38
	30	---	---	---	33	0	50	100	0	36
3. Crag	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	100	100	98	100	100	99
	20	---	---	---	100	100	64	100	100	92
	30	---	---	---	100	87	0	100	100	77
4. MGK-264	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	100	100	100	87	57	100	96	80	90
	30	76	68	100	58	0	100	100	100	75
5. CP 16226-(3)	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	100	100	100	100
	20	---	---	---	---	---	82	75	86	81
	30	---	---	---	---	---	40	100	0	46
6. R-11	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	40	0	15	18
	20	---	---	---	---	---	0	0	0	0
	30	---	---	---	---	---	0	0	0	0
7. R-326	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	91	81	92	50	91	96	92	8	75
	30	71	100	0	55	0	60	100	0	48
8. 949	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	98	100	100	100	100	100	96	100	99
	30	97	100	100	100	100	100	100	50	93
9. 1113	1	100	100	100	100	100	100	100	100	100
	10	80	100	76	87	100	33	100	100	84
	20	78	95	69	68	85	30	100	80	75
	30	51	28	0	0	62	0	0	50	23

TABLE XIII (Con't.)

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
10. 1207	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	100	100	100	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100	100
11. 1345	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	71	59	45	56	14	86	80	80	61
	30	50	79	0	22	0	50	0	0	25
12. 1357	1	100	100	100	100	100	100	100	100	100
	10	100	100	81	100	100	67	100	100	93
	20	47	9	57	93	71	50	75	85	61
	30	10	9	0	94	37	0	0	0	18

TABLE XIV

PERCENT REPELLENCY OF SEVERAL CHEMICALS
APPLIED TO STRING SURFACES

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
1. Pyrocide	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	100	100	100	100
	20	---	---	---	---	---	100	98	100	99
	30	---	---	---	---	---	100	100	100	100
2. Tabatrex	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	100	100	100	100	100	100
	20	---	---	---	52	100	100	100	100	90
	30	---	---	---	60	50	100	100	100	82
3. Crag	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	93	100	97	100	100	98
	20	---	---	---	100	91	98	100	100	97
	30	---	---	---	100	100	100	90	100	98
4. MGK-264	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	96	100	76	100	100	98	94	100	95
	30	98	79	100	95	100	100	100	100	96
5. CP 16226-(3)	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	100	100	100	100
	20	---	---	---	---	---	94	96	100	97
	30	---	---	---	---	---	98	100	100	99
6. R-11	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	100	100	100	100
	20	---	---	---	---	---	75	100	96	90
	30	---	---	---	---	---	0	0	0	0
7. R-326	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	100	100	100	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100	100
8. 949	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	100	100	100	100	100	100	100	100	100
	30	99	97	100	100	100	100	100	100	100
9. 1113	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	97	77	100	100	100	100	100	100	96
	30	90	100	100	100	100	100	100	100	98

TABLE XIV (Cont'd.)

Repellent	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
10. 1207	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	100	100	100	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100	100
11. 1345	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	100	100	100	100	100	100	100	100	100
	30	97	100	91	100	100	60	100	100	93
12. 1357	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	98	100	100	99
	20	99	100	100	100	100	98	100	100	99
	30	99	100	100	100	100	100	90	100	98

TABLE XV

PERCENT REPELLENCY OF SEVERAL CHEMICALS
APPLIED TO UNPAINTED WOOD SURFACES

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
1. Pyrocide	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	100	100	100	100
	20	---	---	---	---	---	98	96	100	98
	30	---	---	---	---	---	0	100	100	66
2. Tabatrex	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	0	57	40	100	0	38
	20	---	---	---	0	0	55	31	0	17
	30	---	---	---	0	0	66	0	0	13
3. Crag	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	25	100	77	85	100	77
	20	---	---	---	11	83	77	97	94	72
	30	---	---	---	0	95	100	100	80	75
4. MGK-264	1	100	100	100	100	100	100	100	100	100
	10	100	94	97	100	100	97	100	100	98
	20	90	91	82	88	88	33	100	47	77
	30	38	76	40	50	41	100	100	0	55
5. CP 16226-(3)	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	100	100	100	100
	20	---	---	---	---	---	82	84	89	85
	30	---	---	---	---	---	40	0	80	40
6. R-11	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	0	66	43	36
	20	---	---	---	---	---	0	0	0	0
	30	---	---	---	---	---	0	0	0	0
7. R-326	1	100	100	100	100	100	100	100	100	100
	10	87	100	100	100	100	100	100	100	98
	20	90	87	100	100	100	77	95	100	93
	30	67	74	100	85	90	100	100	100	89
8. 949	1	100	100	100	100	100	100	100	100	100
	10	0	83	0	0	0	0	57	100	30
	20	0	0	0	0	0	0	0	15	1
	30	0	0	0	0	0	0	0	0	0
9. 1113	1	100	100	100	100	100	100	100	100	100
	10	100	88	84	0	78	71	100	50	71
	20	32	4	69	0	66	11	42	0	28
	30	27	51	0	0	85	0	0	0	20

TABLE XV (Cont'd.)

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
10. 1207	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	100	100	100	100	100
	20	88	100	97	88	100	66	100	68	88
	30	63	100	100	75	79	33	50	65	70
11. 1345	1	100	100	100	100	100	100	100	100	100
	10	50	83	56	0	57	100	100	0	55
	20	30	62	36	0	0	99	62	0	36
	30	27	38	0	0	0	44	100	0	26
12. 1357	1	100	100	100	100	100	100	100	100	100
	10	75	94	91	25	14	45	100	100	68
	20	40	16	55	0	0	42	51	0	25
	30	0	31	0	0	0	0	0	0	3

TABLE XVI

PERCENT REPELLENCY OF SEVERAL CHEMICALS
APPLIED TO PAINTED WOOD SURFACES

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
1. Pyrocide	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	89	100	66	85
	20	---	---	---	---	---	96	42	52	63
	30	---	---	---	---	---	50	0	0	16
2. Tabatrex	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	86	71	72	0	0	46
	20	---	---	---	69	57	70	0	0	39
	30	---	---	---	0	42	0	0	0	8
3. Crag	1	---	---	---	100	100	100	100	100	100
	10	---	---	---	100	100	98	50	100	89
	20	---	---	---	100	100	100	0	94	78
	30	---	---	---	98	100	100	0	100	79
4. MGK-264	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	95	100	50	100	93
	20	76	100	85	100	89	95	26	0	71
	30	47	100	0	57	55	77	0	0	42
5. CP 16226-(3)	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	90	88	100	93
	20	---	---	---	---	---	79	93	0	57
	30	---	---	---	---	---	77	83	0	53
6. R-11	1	---	---	---	---	---	100	100	100	100
	10	---	---	---	---	---	0	91	89	60
	20	---	---	---	---	---	0	69	40	36
	30	---	---	---	---	---	0	59	0	19
7. R-326	1	100	100	100	100	100	100	100	100	100
	10	66	100	95	100	100	80	100	100	92
	20	90	88	91	94	89	0	90	42	73
	30	10	88	0	87	76	0	0	0	32
8. 949	1	100	100	100	100	100	100	100	100	100
	10	0	91	38	64	76	56	0	0	40
	20	0	96	75	58	55	0	0	0	35
	30	0	78	0	9	59	0	0	0	18
9. 1113	1	100	100	100	100	100	100	100	100	100
	10	66	100	85	100	100	80	100	66	87
	20	45	87	68	94	80	25	78	15	61
	30	37	76	0	55	60	0	0	0	28

TABLE XVI(Cont'd.)

Repellents	Days After Treatment	Replicates and Percent Repellency								
		1	2	3	4	5	6	7	8	Average
10. 1207	1	100	100	100	100	100	100	100	100	100
	10	100	100	100	100	97	100	100	82	97
	20	98	100	100	88	95	75	95	79	91
	30	93	100	60	62	95	0	100	70	72
11. 1345	1	100	100	100	100	100	100	100	100	100
	10	66	84	68	100	100	100	100	66	85
	20	57	67	42	66	57	0	88	0	47
	30	0	77	0	41	55	0	100	0	34
12. 1357	1	100	100	100	100	100	100	100	100	100
	10	66	94	62	86	92	98	100	100	87
	20	42	81	66	73	27	0	54	10	44
	30	20	82	0	23	0	0	0	0	15

TABLE XVII

PERCENT REPELLENCY OF SEVERAL CHEMICALS APPLIED TO PAPER AND PLASTIC SURFACES

Repellents	Days After Treatment	Replicates and Percent Repellency					
		Paper			Plastic		
		1	2	Average	1	2	Average
1. Pyrocide	1	100	100	100	100	100	100
	10	100	100	100	100	100	100
	20	96	95	95	100	100	100
	30	100	66	83	100	100	100
2. Tabatrex	1	100	100	100	100	100	100
	10	100	83	91	100	100	100
	20	57	58	58	8	100	54
	30	57	33	45	0	100	50
3. Crag	1	100	100	100	100	100	100
	10	100	100	100	100	100	100
	20	98	92	95	100	100	100
	30	100	33	66	100	100	100
4. MGK-264	1	100	100	100	100	100	100
	10	100	100	100	100	100	100
	20	94	91	92	91	95	93
	30	100	66	83	100	100	100
5. CP 16226-(3)	1	100	100	100	100	100	100
	10	88	100	94	100	100	100
	20	93	100	96	100	100	100
	30	83	100	91	100	100	100
6. R-11	1	100	100	100	100	100	100
	10	80	85	82	91	88	89
	20	66	69	67	71	54	62
	30	43	40	41	51	44	47
7. R-326	1	100	100	100	100	100	100
	10	100	83	91	100	100	100
	20	100	100	100	95	95	95
	30	100	100	100	100	100	100
8. 949	1	100	100	100	100	100	100
	10	100	83	91	100	100	100
	20	73	56	65	87	97	92
	30	33	66	50	66	100	83
9. 1113	1	100	100	100	100	100	100
	10	100	100	100	100	100	100
	20	100	100	100	87	100	93
	30	100	100	100	33	100	66

TABLE XVII (Cont'd.)

Repellents	Days After Treatment	Replicates and Percent Repellency					
		Paper			Plastic		
		1	2	Average	1	2	Average
10. 1207	1	100	100	100	100	100	100
	10	100	100	100	100	100	100
	20	100	100	100	100	100	100
	30	100	100	100	100	100	100
11. 1345	1	100	100	100	100	100	100
	10	100	100	100	100	100	100
	20	100	98	99	79	100	89
	30	100	100	100	33	100	66
12. 1357	1	100	100	100	100	100	100
	10	100	100	100	100	100	100
	20	99	100	99	74	95	84
	30	33	100	66	66	100	83

TABLE XVIII

AVERAGE PERCENT REPELLENCY OF SEVERAL CHEMICALS APPLIED TO SEVERAL TEST SURFACES

Repellents	Days After Treatment	Surfaces and Percent Repellency								
		Glass	Plastic	Wire String	Paper	Wood	Pt.Wd.	Ave.		
1. Pyrocide	1	100	100	100	100	100	100	100	100	
	10	100	100	100	100	100	100	100	100	
	20	93	100	85	99	95	98	63	90	
	30	83	100	31	100	83	66	16	68	
										89
2. Tabatrex	1	100	100	100	100	100	100	100	100	
	10	60	100	17	100	91	38	46	64	
	20	38	54	16	90	58	17	38	44	
	30	36	50	9	82	45	13	8	34	
										61
3. Crag	1	100	100	100	100	100	100	100	100	
	10	99	100	100	98	100	77	89	94	
	20	92	100	98	97	95	72	78	90	
	30	77	100	98	98	66	75	79	85	
										92
4. MGK-264	1	100	100	100	100	100	100	100	100	
	10	100	100	100	100	100	98	93	98	
	20	90	93	76	95	92	77	71	85	
	30	75	100	25	96	83	55	42	68	
										88
5. CP 16226-(3)	1	100	100	100	100	100	100	100	100	
	10	100	100	85	100	94	100	93	96	
	20	81	100	28	97	96	85	57	77	
	30	46	100	24	99	91	40	53	65	
										84
6. R-11	1	100	100	100	100	100	100	100	100	
	10	18	89	76	90	82	36	60	74	
	20	0	62	46	56	67	0	36	38	
	30	0	47	47	0	41	0	19	26	
										59
7. R-326	1	100	100	100	100	100	100	100	100	
	10	100	100	99	100	91	98	92	97	
	20	75	95	81	100	100	93	73	88	
	30	48	100	41	100	100	89	32	73	
										89
8. 949	1	100	100	100	100	100	100	100	100	
	10	100	100	93	100	91	30	40	79	
	20	99	92	78	100	65	1	35	67	
	30	93	83	42	99	50	0	18	55	
										75
9. 1113	1	100	100	100	100	100	100	100	100	
	10	84	100	81	100	100	71	87	89	
	20	75	93	33	96	100	28	61	69	
	30	23	66	23	98	100	20	28	51	
										77

TABLE XVIII (Cont'd.)

Repellents	Days After Treatment	Surfaces and Percent Repellency							
		Glass	Plastic	Wire	String	Paper	Wood	Pt.Wd.	Ave.
10. 1207	1	100	100	100	100	100	100	100	100
	10	100	100	97	100	100	100	97	99
	20	100	100	95	100	100	88	91	96
	30	100	100	97	100	100	70	72	90
									96
11. 1345	1	100	100	100	100	100	100	100	100
	10	100	100	80	100	100	55	85	88
	20	61	89	43	100	99	36	47	68
	30	25	66	38	93	100	26	34	54
									77
12. 1357	1	100	100	100	100	100	100	100	100
	10	93	100	77	99	100	68	87	89
	20	61	84	16	99	99	25	44	61
	30	18	83	0	98	66	3	15	40
									73
Averages		78.2	94.3	70.3	95.5	92.2	65.9	68.3	79.1

TABLE XIX

ANALYSIS OF VARIANCE
(Dairy Cattle Sprays)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F
		Siphona irritans		
Individual observations	168	1,269,813.84	7,558.41	38.20*
Repellents and control	3	866,248.51	288,749.50	
Total	171	2,136,062.35	12,491.59	
		Stomoxys calcitrans		
Individual observations	168	254.70	1.52	6.76*
Repellents and control	3	30.84	10.28	
Total	171	285.54	1.66	
		Musca domestica		
Individual observations	168	2,331.05	13.87	4.23*
Repellents and control	3	176.17	58.72	
Total	171	2,507.22	14.66	

*Denotes significant population difference at the 99% level.

Figure 11. The Multiple Range Test is Used Here to Demonstrate the the Effect of Repellent Spray Formulations on Populations of S. irritans.

	1207-35	Tabatrex-2	1207-37	Control
Mean Fly Count	77.23	99.23	128.33	260.06

*

Figure 12. The Multiple Range Test is Used Here to Demonstrate the Effect of Repellent Spray Formulations on Populations of S. calcitrans.

	1207-35	1207-37	Tabatrex-2	Control
Mean Fly Count	0.48	0.86	1.19	1.64

*

Figure 13. The Multiple Range Test is Used Here to Demonstrate the Effect of Repellent Spray Formulations on Populations of M. domestica.

	1207-35	Tabatrex-2	1207-37	Control
Mean Fly Count	2.71	4.13	4.21	5.57

*

*Mean numbers of insects not connected by the same horizontal line are significantly different from each other at the 99% level of probablity.

VITA

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