# EVALUATION OF THE RESPONSE OF *DROSOPHILA MELANOGASTER* TO CHEMICAL STIMULI

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# EVALUATION OF THE RESPONSE OF DROSOPHILA MELANOGASTER TO CHEMICAL STIMULI

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## PREFACE

The need for a suitable repellent against Drosophila flies which cause a serious economic problem to the tomato canning industry, was indicated by Dr. D. E. Howell, Professor and Head of the Department of Entomology. The author selected this as a thesis problem and studied the response of Drosophila flies to the chemical stimulation of more than 200 chemicals with the objective of finding a suitable repellent out of this lot.

The author wishes to express his sincere appreciation to his major adviser, Dr. D. E. Howell, for his thoughtful guidance and encouragement throughout the experimentation and in the writing of this manuscript. Sincere appreciation is expressed to Dr. R. R. Walton, Professor of Entomology, and Dr. John E. Thomas, Professor of Botany and Plant Pathology, for their advice and constructive criticism of the thesis manuscript. Also special thanks are expressed to Dr. L. D. Goodhue of the Phillips Petroleum Company for providing the candidate chemicals used in different tests.

Indebtedness is expressed to the following who assisted in this project: Dr. G. A. Mount and Dr. Richard G. Price, former Entomology graduate students, for assistance in setting up the tests; Dr. L. H. Bruneau, Associate Professor of Zoology, for supplying the fly stock; Mr. Donald M. McCroddan, graduate student of Entomology, for assistance in taking photographs; Mr. Mohamed Abdel Razig, graduate student

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# INTRODUCTION

*Drosophila* spp. often called pomace flies, vinegar flies or fruit flies pose a serious economic problem to the tomato canning industry. They cause heavy losses each year by contaminating tomato products with their eggs and maggots. Such contaminated products are considered unfit for food by the Food and Drug Administration and are subject to seizure and condemnation. Several species of *Drosophila* flies infest tomatoes; the one usually most prevalent is *Drosophila melanogaster* Meigen (Ditman and Bickley, 1952; Collins, 1956). Bickley (1956) concluded that, for the entire tomato harvesting period, it was estimated that *D. melanogaster* constituted at least 95% of the *Drosophila* associated with tomatoes. When tomatoes are ripe in the fields, infestation by these flies is common and many eggs are laid in cracks and the stem area of the fruit. Even after fruits are loaded in trucks, more severe infestations result from continued oviposition on the fruits. Collins (1956) remarked that control of this pest was complicated by such factors as enormous populations both in the field and at the processing plant, other numerous sources of infestation, and the limitation of insecticide use because of residue hazard.

All of these factors indicated that *Drosophila melanogaster* Meigen was worthy of investigation.

The objective of the investigations reported in this thesis was to find a suitable repellent which could be used during and after tomato

harvest to protect the fruit from egg deposition. Three different types of tests were conducted proceeding from simple screening procedures to more sophisticated olfactometer tests. Over 200 chemicals which had shown repellency or attractancy to some arthropods were evaluated as repellents, toxicants or attractants. The effectiveness of the final selected materials was evaluated after they had shown positive results in all the tests.

# REVIEW OF LITERATURE

## TOXICANTS

FIELD CONTROL. - One of the early articles on Drosophila control with toxicants by Ditman et al. (1936) indicated that pyrethrin dusts were of little value in reducing the number of Drosophila eggs deposited on tomato fruits; but pyrethrin sprays were quite effective around washing sheds.

Bickley and Ditman (1953), Pepper et al. (1953), Michelbacher and Middlekauff (1954), and Mason (1956) conducted experiments on the direct control of Drosophila in tomato fields with different insecticides but failed to get promising results. Davis (1960) applied Diazinon at the rate of one-half pound per acre and obtained a satisfactory reduction of Drosophila activity. He further reported that malathion and Phosdrin applications were effective for 2 to 3 days. Ronnel and Dibrom compared favorably with Diazinon. Mason et al. (1959) reported that one application of aldrin, dieldrin, ethion, heptachlor, chlordane, methoxychlor, ronnel, malathion, dicapthon, Diazinon, or Dipterex gave good control of Drosophila breeding in piles of cull tomatoes at Beltsville, Maryland.

Control of the fly after the fruits had been picked has also been studied. Bickley and Ditman (1953) reported that frequent applications of pyrethrum as an aerosol or mist applied with electric atomizing sprayer reduced the adult population of Drosophila. Pepper et al. (1953) reported that pyrethrum concentrates, when applied with a fog-generator,

were effective in controlling the adults. Bickley et al. (1956) investigated a pyrethrum spray synergized with piperonyl butoxide and found it effective in reducing egg-deposition on tomatoes in baskets. Collins (1956) reported that tests were made on the effectiveness of Dyna-Fog applications of pyrethrum and allethrin formulations, as well as DDT and lindane, for the control of Drosophila on truck loads of tomatoes. Lindane appeared to offer the most promise. Stombler et al. (1957) undertook a series of experiments to determine the full effectiveness of pyrethrum in protecting picked tomatoes from infestations by Drosophila. He reported that a pyrethrum dust containing 0.11% pyrethrins applied to boxes of stacked tomatoes in the field, or applied to pallets of tomatoes at the receiving station, or at the cannery, afforded excellent protection from egg-deposition by Drosophila for a period of approximately 24 hours.

Mason and Dorst (1962), ARS, USDA, recommended the following chemicals for the field control of Drosophila: aldrin (one-half pound active material per acre), Diazinon (one and one-half pounds active material as a dust or granules, three-fourths pound active material as emulsifiable concentrate or wettable powder per acre), and malathion (two pounds active material per acre) with the minimum days requirements of 1, 3, and 1 days respectively from last application to harvest. They also recommended the application of a mixture of 0.1% pyrethrins and 1.0% piperonyl butoxide at the rate of 8-16 ounces of freshly mixed stabilized dust to one ton of harvested tomatoes.

LABORATORY CONTROL. - Ebeling (1958) made an extensive laboratory evaluation of 36 insecticides and 19 inert dust diluents to study their immediate and residual effects on the control of D. melanogaster. In his experiments, the most effective insecticides -- Lethane 384 and TEPP

resulted in the death of all the flies in 1 and 3 minutes, respectively. Dust SG77 was the most effective inert dust and resulted in the 100% mortality in 28 minutes. In 1955, 1956, and 1957 laboratory screening tests were conducted at Logan, Utah, to furnish information on the effectiveness of about 40 insecticides at various strengths in killing adults of D. melanogaster (Dorst, 1959). The results indicated that DDT, pyrethrum, rotenone, Chlorthion, ryania, Guthion, Perthane, toxaphene, piperonyl butoxide, TDE, Sevin, Chlorbenzilate, Strobane, methoxychlor, Karathane, or allethrin at 2% gave less than 50% mortality after 24 hours. Twenty-one of the total materials tested at 2% showed 100% mortality after the first 24 hours. Malathion and Diazinon, both of low mammalian toxicity, were effective about 2 weeks; lindane and BHC for 7 to 10 days and heptachlor for 30 days.

## REPELLENTS

Although from the earliest time, man had 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 (Taylor, 1960). This improvement was due to the screening of many thousands of chemicals, mainly against mosquitoes and some other medically important insects.

A great deal of work has been conducted with repellents against the pests of man and animals but very little, by comparison, on the pests attacking plants. Investigations of possible repellents probably started by testing extracts of plants known to be immune to the attack of one or more species of insects. One of the early extensive studies of this

type was that of Metzger and Grant (1932). In an attempt to find a repellent for the Japanese beetle, extracts were made of 390 species of plants not attacked by the Japanese beetle.

Reed (1938) reported that ethyl alcohol and acetic acid above 25% and 5%, respectively, acted as repellents to D. melanogaster Meigen. Wieting and Hoskins (1939) reported that mixed groups of house flies having a sex ratio of approximate unity were repelled by ammonia and ethyl alcohol at concentrations greater than 0.03% and 0.05% by volume, respectively. Chamberlain (1956) used a modified olfactometer in testing repellents. His results with three species of insects (fourth and fifth instar nymphs of Melanoplus femur-rubrum Deg., adults of Heliothis zea Boddie, and adults of Thyanta custator Fab.) showed that more volatile compounds like alpha pinene, amyl acetate, the two "solvonel" fractions, and dipenetene were among the most repellent materials.

Bickley et al. (1956) reported that some small scale experiments in 1956 showed that butoxy polypropylene glycol was effective in repelling Drosophila adults. Baskets of tomatoes were sprayed with a mixture of 1 pint in 9 pints of water and egg counts were made 18 hours later. The number of eggs per square inch of exposed flesh averaged only one on sprayed tomatoes in contrast to 1000 on unsprayed fruits. Stombler et al. (1957) reported that pyrethrum protection from egg deposition by Drosophila flies appeared to be due mostly to a repellent action rather than to killing of the flies. Johnson and Hofmaster (1961) reported that stabilized pyrethrins plus piperonyl butoxide in a one to ten ratio applied on tomatoes repelled Drosophila for about 6 hours. Gojmerac and Fox (1962) reported that Dibrom at 4 and 8 ounces per 100,000 cubic feet, vaporized on a hot plate, had given excellent control of Drosophila spp. in fruit

storage rooms of lemon packing houses.

## ATTRACTANTS

There appears to be no record of the use of insect attractants by primitive man. The earliest report was that of Pliny in which he suggested that a fish be hung adjacent to trees to lure ants away from the foliage. The attractiveness of honey to bees and of light to myriads of other insects is proverbial (Dethier, 1947).

The earliest recorded use of attractants for economic purposes was Coquillett's attempt in 1885 to control grasshoppers in California by means of attractive poisoned baits (Dethier, 1947). Without a doubt the greatest impetus given in this trend of investigation stemmed from Peterson's early work in 1925 with molasses-yeast baits for peach moths. Within the next few years Yetter (1925), Peterson (1927), Frost (1927) and others, tested hundreds of aromatic compounds in a search for one that was more attractant to orchard insects than their natural food.

The most extensive early work on the olfactory sense of Drosophila spp. was that of Barrows (1907). He made some preliminary experiments with a trap to determine substances which caused positive reactions, and later, conducted experiments with a specially developed olfactometer (described in olfactometer section). From these experiments, Barrows concluded that Drosophila flies were positively chemotropic to amyl and, especially, ethyl alcohol, acetic and lactic acids, and acetic ether. The optimum concentrations of ethyl alcohol and acetic acid were determined by the number of positive reactions given to 20% and 5%, respectively.

Reed (1938) studied the reactions of Drosophila melanogaster Meigen to solutions of ethyl alcohol and acetic acid. His experimental set up consisted of a belljar on a sand-blasted glass plate, containing a trap with the odor solution and a watch glass of cotton soaked with distilled water to regulate humidity. His results indicated that Drosophila females were strongly attracted to solutions of ethyl alcohol up to 25%, with a maximum response at 10-15%; and Drosophila males responded to ethyl alcohol solutions up to 15% with a maximum response at or below 5%. Drosophila females attracted to acetic acid concentrations up to 1%, with a maximum response at 0.4% while Drosophila males reacted to acetic acid in concentrations up to 1%, with a maximum response at 0.2%.

Ditman et al. (1936) used jar traps baited with banana and tomatoes in tomato fields to reduce Drosophila fly infestations. Though many flies were caught in the trap, there was no apparent reduction of flies in the field. Hutner et al. (1937) reported that diacetyl, acetyldehyde, and indol were especially attractive. Dorsey and Carson (1956) reported that an artificial bait consisting of equal parts of vinegar, molasses, and water was a most effective attractant for wild Drosophila melanogaster and D. immigrans.

Gow (1954) conducted field and olfactometer tests to find a more effective bait for the oriental fruit fly, Dacus dorsalis Hendel. He reported that ammonia was only mildly attractive. Yeast, soy, lactal-bumin, casein hydrolysates, and a vitamin-B preparation called Lederplex were all attractive to this fruit fly, soy hydrolysate being somewhat superior to the others. Some of the other noteworthy examples of responses to specific chemical stimuli were the attraction of the sheep blow-fly, Lucilia sericata to indol, skatol, and ammonium carbonate

(Hobson, 1936); attraction of the Colorado potato beetle, Leptinotarsa decemlineata by the steam distillate of solanaceous plants (McIndoo, 1926), and the attraction of fruit flies of the genus Dacus by methyl eugenol (Howlett, 1912; Steiner, 1952). Wieting and Hoskin (1939) reported that the mixed group of house flies having a sex ratio of approximately unity were attracted to ammonia and ethyl alcohol at concentrations of 0.012% by volume.

OLFACTOMETER STUDIES. - An olfactometer was used to study Drosophila fly reaction to odor by Barrows (1907). He introduced the simple Y-tube in this experiment in which the insects were introduced at the base of the Y and at the fork the insects could make a choice between the arms. One of the thorough pieces of research conducted with this type of olfactometer was that of McIndoo (1926) on the reactions of potato beetles. A disadvantage of the Y-tube olfactometer is that some source of strong attraction is needed to obtain the active participation of the insects in a test.

Another type of olfactometer developed by Folsom (1931) was called a chemotropometer. It consisted of a simple straight tube and served very well for the purpose of studying attractants. An olfactometer that required the active participation of nearly all the individuals and could be used with a variety of insects was used by Wieting and Hoskins (1939) to study responses of house flies to different concentrations of ammonia, carbon dioxide, and ethyl alcohol. The apparatus might be described as a box in one side of which were two closely adjacent circular holes covered with wire screen, called the test and check areas. Beyond these were two long funnels which served as inlets for two streams of air, one of ordinary composition and the other containing a known concentration of

the gas which was being studied. Concentrations of the substances to be tested were controlled by the use of flowmeters and saturating chambers. The incoming gases were withdrawn through outlets in the floor near the entry ports to avoid contaminating the whole chamber and decreasing the concentration gradient. Beyond the funnels a light attracted house flies to the ports.

An olfactometer modified from the above was used by Willis and Roth (1950) in testing humidity reactions of Tribolium casteneum Herbst and by Chamberlain (1956) in testing repellents against agriculturally important insects. The essential part of this equipment consisted of a rectangular stainless steel box with a smaller inserted cage containing the test insects. In the smaller test cage, the insects had a choice of the right or left port.

Gow (1954) described an olfactometer used at the Honolulu fruit fly laboratory of the U. S. Department of Agriculture. The olfactometer consisted of the testing materials which were contained in small glass invaginated traps suspended from a slowly rotating, motor-driven wheel mounted horizontally on a bearing through the roof of a cage. The cage was 9 feet square and 8 feet high well stocked with a large population of Oriental fruit flies, Dacus dorsalis Hendel. Usually, three replications of four baits could be tested at the same time with this olfactometer.

## MATERIALS AND METHODS

TEST INSECT. - Drosophila melanogaster was the test insect in these experiments. Two laboratory strains of this fly, Stephenville and Oregon, were reared in the insectary and 2-3-day-old flies of both strains were used in all the tests. The physiological state of the test insects was assumed to be similar because they were all reared on one kind of rearing medium under constant temperature of 77 F in a temperature cabinet. Moreover, after temporary inactivation, flies were allowed 2 hours of rest in all cases before subjecting them to any test.

METHOD OF REARING. - The flies were reared on a standard rearing medium which contained the following ingredients:

| Ingredients | Quantity |
|---|---|
| Water | 250 ml |
| Brewer's yeast | 8 g |
| Agar | 5 g |
| Karo syrup | 1 tablespoon |
| Malt extract | 1 tablespoon |
| Crushed ripe banana | 65 g |
| Water and propionic acid | 25 ml and 0.8 ml respectively |

The ingredients were mixed in above proportions, heated to boiling with constant stirring and poured in 1-pint milk bottles. Later, the milk bottles were infested with fresh flies and kept in a temperature cabinet at 77 F. The milk bottles were closed with paper lids punched

by a fine needle for ventilation.

METHODS OF INACTIVATION AND SORTING. - Two methods of temporary inactivation were practiced during these experiments. One was the use of ice-cold water and the other ethyl ether. Flies were collected in an empty milk bottle and later, the bottle was plunged up to the neck in ice-cold water. In 5-10 minutes all the flies were inactivated. When using ethyl ether a cotton plug soaked in the chemical was held over the mouth of the milk bottle for 3-5 minutes. Recovery of the flies in both cases was very satisfactory but, flies inactivated by ice-cold water recovered faster.

In the olfactometer tests, flies were inactiviated by only ice-cold water. In the remaining tests, two replications of each treatment were tested with flies inactivated by ice-cold water and two by flies inactivated by ethyl ether.

Inactivated male and female flies were sorted into eight dram shell vials with the aid of a camel hair brush.

CHEMICALS. - A total of 202 different chemicals was tested in these experiments against D. melanogaster. Out of these, 184 chemicals which had shown some degree of repellency to some arthropod, were supplied by the Phillips Petroleum Company and they belong to 23 different chemical groupings as classified in Table 1. There were four sulfur and nitrogen chemicals, 35 sulfides, 19 sulfoxides, seven thioethers, one thioamide, one mercaptan, seven sulfones, five organic acid salts, three aldehydes, 12 amines and amides, two olefins, one ketone, five nitriles, two esters, two hydrocarbons, four halogenated hydrocarbons, five nitro chemicals, one peroxide, 62 pyridine and closely related chemicals, two alcohols and phenols, one organic acid, one aromatic ester and two

miscellaneous chemicals dissolved in acetone or water.

The remaining 18 chemicals were well known insect repellents and had been studied by many workers. The chemical names or formulations of these repellents were taken from the Entomological Society of America Bulletin, June 1960 and from the individual articles. The chemicals are listed below:

R-55 = tert-Butylsulfenyl diemthyldithiocarbamate.

MGK 933 = N-Octyl bicycloheptene dicarboximide 5%, oil 90.5%, piperonyl butoxide 3%, pyrethrins 1.5%.

175RC = 20% pyrethrum and 80% petroleum distillate.

R-874 = 2-hydroxyethyl n-octyl sulfide.

R-326 = di-n-propyl 2,5-pyridinedicar = boxylate.

R-1207 = 3-chloropropyl n-octyl sulfoxide.

AR-55 = Aromatic hydrocarbon (A.P.I. Gravity C 60F. 19.0-22.0

M-1960 = N-butyl acetanilide 30%, 2-butyl-2-ethyl-1,3-propanediol 30%, Benzyl benzoate 30% and emulsifier 10%.

MGK 264 = N-(2-ethylhexyl)-bicyclo=(2.2.1)hept-5-ene-2,3-dicarboximide.

R-11 = 1,5a,6,0a,0b-hexahydro-4a(4H)-dibenzofuran=carboxaldehyde.

R-612 = 2-ethyl-1,2-hexanediol.

Tabutrex = Dibutyl succinate.

Crag fly repellent = Butoxy polypropylene glycol.

N-butylacetanilide, N-butyl Adipate, Piperonyl butoxide, and N,N-diethyl-m-toluamide.

These 18 chemicals were all dissolved in acetone. The above 202 chemicals were tested as 1% solutions in all tests except otherwise mentioned.

LABORATORY PROCEDURES

Shell Vial Test. - Small plastic Petri dishes of two sizes, one 3.5 cm diameter by 0.8 cm high and the other 3.9 cm diamater by 0.5 cm high were used as containers for the rearing medium. The Petri dishes were filled to the brim with hot medium and, after the medium cooled, an area 2.3 cm diameter was marked out by an eight-dram shell vial with its open end pushed through the medium until it touched the bottom of the Petri dish. The delineated surface of the medium was treated with 0.07 ml of the chemical solution and left open for 1 hour to insure complete evaporation of acetone. After that, one eight-dram shell vial containing five male and five female flies was inverted over the treated medium (Fig. 1). The flies could move freely in the vial and had free access to the treated surface.

Only 184 chemicals supplied by the Phillips Petroleum Company were tested in this test. The number of eggs laid on the treated medium and the flies "down" were noted after 8 and 24 hours. Egg counting was done with the aid of a binocular microscope. Flies unable to produce co-ordinated movements were considered "down". Each treatment was replicated four times. Checks of untreated media were also simultaneously run to evaluate the effect of the chemicals.

Carton Test I. - Petri dishes similar to those used above were also used in this test as containers for the rearing medium. Only that portion of the medium marked by the open end of a vial was kept on the Petri dishes and the rest was discarded. This made the surrounding sides of the medium available for chemical treatment. The top surfaces and the sides of these discs of media, 2.3 cm diameter by 0.8 cm high and 2.3 cm diameter by 0.5 cm high, on the two types of Petri dishes were subjected

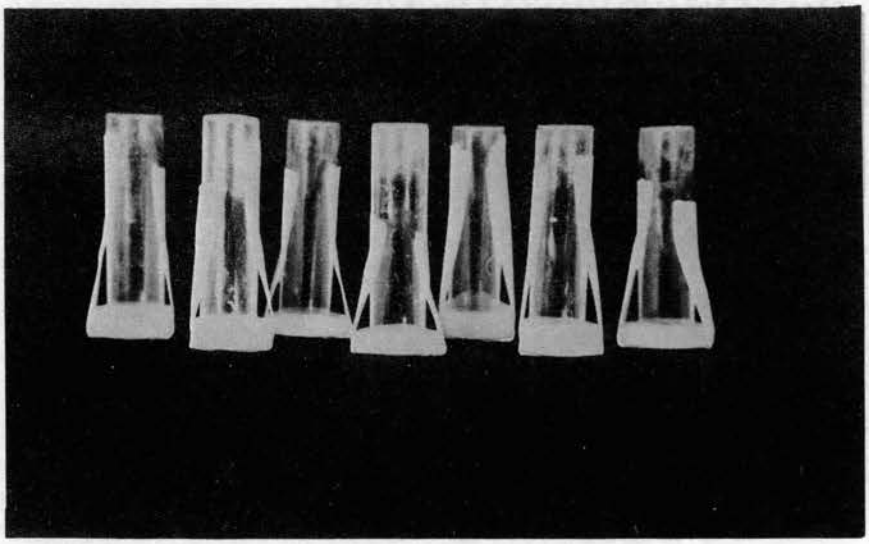

Figure 1. Equiptment for Shell vial Tests.

to chemical treatments. Each disc of this medium* was treated with 0.23 ml of 1% chemical. The same quantity of solvent was applied to the check medium.# The lower surface of the medium was neither treated nor available to the flies. Check and treated dishes were attached to the bottom of 1-quart Dixie paper cartons with Scotch tape (Fig. 2) and 25 male and 25 female flies were introduced into the container where they were secured by fine nylon netting. Before releasing the flies, the treated and the check media were left exposed for one and a half hour to ensure complete evaporation of acetone. Each carton represented one replication of a treatment and each treatment was replicated four times. The tests were placed in a temperature and humidity controlled cabinet where 77 F and 80%-85% relative humidity were maintained. The duration of the test was 30 hours.

Series A. The following categories of chemicals were selected from the shell vial test for testing in Series A: those whose application resulted in the death of eight or more flies and/or had less than five eggs in 24 hours, and all others whose application resulted in the death of not more than two flies and had more than 50 eggs (except 19500).

Series B. The responses of D. melanogaster to 18 known chemical insect repellents were tested in Series B. The chemicals are listed in Table 3. The methods of treatment and the concentration of chemicals etc. were all same as described in Series A.

Carton Test II. This test was essentially the same as described in Carton Test I. The only major difference was that, in all its series except Series B, only treated medium was exposed to 25 pairs of flies in each carton without check. The other differences were described in each

*Henceforth referred to as treated medium.
#Henceforth referred to as check, untreated or check medium.

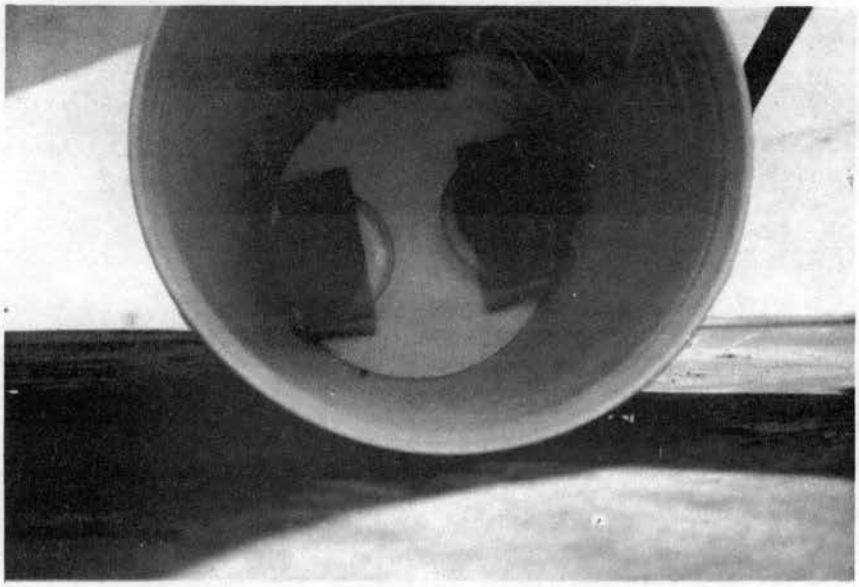

Figure 2. Arrangement of Petri dishes in Carton Tests.

series.

Series A. The chemicals in Carton Test I (Series A and B) which resulted in less than five eggs on each treated medium were tested in this series. The chemicals are listed in Table 4. The concentration of the chemicals and other procedures were the same as described in Carton Test I. Each treatment was replicated four times. The duration of this test was 30 hours.

Series B. Chemicals 2147, 3100, 1971, 2153, 3272, and 175RC which proved effective in Series A were tested in Series B (Table 5). The concentrations of the chemicals were 0.5% and 0.1%. Duration of the test was 24 hours. Each treatment was replicated four times.

Series C. Chemicals 2147, 3100, 1971, 2153, 3272, and 175RC were further tested in this series (Table 6) at 0.5% and 0.1% concentrations but the difference of this series from Series B was that in this series only treated medium was used in each carton while in Series B, both treated and untreated media were used. Duration of the test was 24 hours. Each treatment was replicated four times.

Series D. Chemicals 2147, 3100, 2153, and 175RC which proved still effective in Series C were further tested in Series D (Table 7). The object of this test was to see how long these chemicals would repel and/or inactivate Drosophila flies when the media were treated 30, 48, and 72 hours before exposure to flies. Duration of the test was 24 hours and each treatment was replicated four times.

Olfactometer Test. - This apparatus (Fig. 3) was developed to study the responses of D. melanogaster to the vapor phase of chemicals. The flies were exposed in two glass cylinders, each 9.5 cm diameter by 20 cm high. A fine screen 9.5 cm diameter was placed horizontally inside

Figure 3. Equipment used in the Olfactometer Tests.

the cylinder 12 cm from its base. A cardboard 12 cm by 9.5 cm was inserted below this screen to divide the cylinder in two equal parts. This partition separated treated from untreated air.

A 9.5 cm piston ring was used to form a tight seal inside the cylinder 0.7 cm above the horizontal screen. Another fine screen 9.5 cm diameter was placed over the piston ring to check escape of test flies from the cylinder.

The apparatus was supported on a wooden frame having one horizontal platform in the middle. This platform had six holes, two to draw fresh air through the flow meters and four to accommodate four flared glass tubes connected with the flow meter system. The cylinder was placed on the horizontal platform encircling two glass tubes. The cardboard inside the glass cylinder formed a partition wall between the two flared glass tubes.

Air was introduced into each side of the cylinders by being drawn through funnels covered by sheets of Kleenex paper tissue. A standard amount of the candidate material was added to the paper tissue on one funnel, an equal amount of the solvent alone might be used on the other. The air flow was controlled by stop cocks, flow meters, and manometers. The entire system was made air tight by the use of ground glass joints and stop cock grease. A water vacuum pump produced negative pressure to pull the air through the olfactometer and to remove the contaminated air from the system.

Flies placed in the small area between the two horizontal screens were able to move freely in the limited space. The effectiveness of the repellent was determined by comparing the number of flies in the area exposed to the vapor phase with those in the check area. Cylinders

were changed and carefully cleaned between tests and position of the repellents was rotated. Cylinders were wrapped in black cloth to exclude side lights.

Fly counting was done as efficiently and accurately as possible but due to the movement of a few flies, exact numbers could not be obtained. A few flies hanging on side walls were excluded from the count. Two hundred flies (100 male and 100 female) were used in each replication. In most cases, unless otherwise mentioned, flies once used were discarded. In all the series of this test, flies were exposed for 5 minutes in each replication.

Series A. The effect of ammonium hydroxide (reagent grade), ethanol, glacial acetic acid, fermenting banana, imitation banana extract (ethyl alcohol 40%, amyl acetate, and other artificial flavors, certified color, and water), and a mixture of glacial acetic acid, water and molasses (1 ml : 10 ml : 3 drops, respectively) on Drosophila flies were studied in Series A. The Kleenex tissue was treated with 0.1 ml of candidate substance. Fermenting banana, prepared by exposing a mixture of crushed ripe banana, dry yeast, and molasses for 24 hours, was placed on the Kleenex tissue. The test was run immediately after application of the material on the tissue and after being tested for 5 minutes, the treated tissue was left exposed for half an hour and then, the same batch of flies was again exposed to it. After 6 hours a fresh batch of flies was exposed to the same treated tissue.

Series B. The responses of D. melanogaster to different concentrations of ammonium hydroxide (reagent grade), ethanol, glacial acetic acid, imitation banana extract were studied in Series B. The intervals of exposure were the same as mentioned in Series A.

Series C. The candidate chemicals whose treatment in Carton Test I resulted in less than five eggs or more than 100 eggs on the treated medium, were tested for their repellency or attractancy in Series C. Each replication was treated with 0.23 ml of a 1% solution. The test flies were exposed to the vapor 15 minutes after application to ensure complete evaporation of acetone. Check Kleenex tissues were also treated at the same time with 0.23 ml of solvent only and exposed for 15 minutes before use.

In this series, treated Kleenex was exposed to flies only once.

Series D. The chemicals 2147 and 3272 which repelled D. melanogaster in Series C were further tested in Series D at 1%, 0.5%, and 0.1% concentrations. The Kleenex tissues after the applications of 0.23 ml of chemical solutions at each concentration, were left exposed for 6 hours. During this time, at intervals of 15 minutes, ½ an hour, 1 hour, and 6 hours, flies were exposed to these tissues in the olfactometer. Duration of each exposure was 5 minutes only. During 2-hour and 6-hour intervals, fresh flies were used.

## RESULTS

Shell Vial Test. - Preliminary screening in the shell vial tests was conducted with 184 candidate chemicals. The results tabulated in Table 1 indicate that some chemical groups are more toxic to the flies and produce more knock down than others. Knock down after 24 hours is only slightly greater than after 8 hours but egg deposition increased greatly during this interval. Due to the limitation of space, the detailed individual chemical stimulation on the flies could not be furnished. But the results indicate that some chemicals had inactivated all the ten flies in 24 hours and had allowed only few eggs to be laid by the flies. One chemical, 3272 (coded in Carton Test I) had no toxicity but repelled flies from laying eggs. Some chemicals had little effect on the mortality and oviposition. On the other hand, some were attractants and increased feeding and egg deposition (evident from the number of eggs at the end of the range). These apparent toxic, repellent, and attractant chemicals were selected and run extensively in carton and olfactometer tests. The individual code numbers of these selected chemicals are porvided in Carton Test I, Series A.

Carton Test I.

Series A. - The chemicals which promised to be either toxicants and/or repellents or attractants in the shell vial tests were tested in Series A. The results in Table 2 indicate the toxicity of three chemicals, repellency and/or toxicity of 13 chemicals, and attractancy of 13 other chemicals.

Table 1. Response of Drosophila melanogaster to chemical stimuli in shell vial tests.*

| Group Code[a] | No. Tested | Exposure Period: 8 Hours Ave. No. Eggs | 8 Hours Range of No. Eggs | 8 Hours Ave. Fly K.D. | 8 Hours Range of K.D. | 24 Hours Ave. No. Eggs | 24 Hours Range of No. Eggs | 24 Hours Ave. Fly K.D. | 24 Hours Range of K.D. |
|---|---|---|---|---|---|---|---|---|---|
| SN | 4 | 10 | 1-20 | 6 | 0-10 | 13 | 1-25 | 7 | 0-10 |
| S | 35 | 13 | 0-38 | 4 | 0-10 | 23 | 1-58 | 5 | 0-10 |
| SO | 19 | 20 | 1-50 | 1.4 | 0-8 | 33 | 2-62 | 2.8 | 0-10 |
| SE | 7 | 16 | 1-46 | 3.4 | 0-10 | 24 | 1-60 | 5 | 0-10 |
| SA | 1 | 19 | -- | 1 | -- | 35 | -- | 1 | -- |
| SH | 1 | 30 | -- | 0 | -- | 42 | -- | 1 | -- |
| $SO_2$ | 7 | 19 | 8-27 | 0 | -- | 34 | 20-42 | 0.43 | 0-2 |
| Salt | 5 | 18 | 10-30 | 0.6 | 0-2 | 32 | 15-40 | 1.6 | 0-4 |
| CHO | 3 | 28 | 8-45 | 1.7 | 0-4 | 35 | 18-52 | 2.7 | 2-4 |
| A | 12 | 14 | 0-30 | 3.6 | 0-10 | 22 | 0-45 | 4 | 0-10 |
| C=C | 2 | 24 | 18-30 | 0 | -- | 40 | 0 | 0 | -- |
| CO | 1 | 20 | -- | 0 | -- | 35 | -- | 0 | -- |
| CN | 5 | 23 | 5-35 | 1 | 0-3 | 30 | 10-41 | 4 | 0-9 |
| E | 2 | 10 | 5-15 | 5.5 | 1-10 | 21.5 | 5-38 | 6.5 | 3-10 |
| H | 2 | 18 | 16-20 | 0 | -- | 28 | 36-40 | 0 | -- |
| HH | 4 | 10 | 0-25 | 5 | 0-10 | 19 | 0-42 | 5.3 | 0-10 |
| $NO_2$ | 5 | 12 | 7-18 | 0.8 | 0-3 | 24 | 15-29 | 2 | 0-8 |
| PO | 1 | 15 | -- | 0 | -- | 35 | -- | 0 | -- |
| PY | 62 | 20.3 | 2-50 | 1.18 | 0-10 | 35 | 2-60 | 2 | 0-10 |
| OH | 2 | 29 | 28-30 | 0 | -- | 41 | 36-46 | 1 | 0-2 |
| COOH | 1 | 20 | -- | 1 | -- | 52 | -- | 1 | -- |
| AES | 1 | 30 | -- | 0 | -- | 39 | -- | 0 | -- |
| M | 2 | 24 | 20-28 | 0 | -- | 41 | 40-42 | 0.5 | 0-1 |
| Check | -- | 23 | 20-31 | 0 | -- | 45 | 35-60 | 0 | -- |

*Each candidate chemical was replicated four times; average of all the replications of all chemicals in each group is presented in the table.

[a]S=Sulfides; SO=Sulfoxides; SN=Sulfur and nitrogen chemicals; $SO_2$= Sulfones; SA=Thioamides; SE=Thioethers; SH=Mercaptan; A=Amides and Amines; AES=Aromatic esters; OH=Alcohols and Phenols; CN=Nitriles; E=Esters; H=Hydrocarbons; HH=Halogenated hydrocarbons; C=C =Olefins; CO=Ketones; CHO=Aldehydes; COOH=Organic acid; $NO_2$=Nitro chemicals; PO=Peroxides; PY=Pyridine and closely related chemicals; Salt=Organic acid salt; M=Miscellaneous.

Table 2. Effectiveness of the chemicals selected from shell vial tests in protecting treated medium (Carton Test I).*

| Code No. of Chem. | Group Code[a] | Treated Ave. No. Eggs | Treated Ave. No. Larvae | Check Ave. No. Eggs | Check Ave. No. Larvae | Ave. Fly Knock Down |
|---|---|---|---|---|---|---|
| 290 | SN | 20 | 2 | 118 | 40 | 15 |
| 5625 | SN | 25 | 4 | 99 | 20 | 3 |
| 14380 | SN | 25 | 3 | 100 | 50 | 15 |
| 1829$ | S | 0 | 0 | 140 | 45 | 2 |
| 2954$ | S | 0 | 0 | 125 | 35 | 3 |
| 1971$ | S | 0 | 0 | 140 | 32 | 3 |
| 9016$ | S | 3 | 0 | 125 | 35 | 10 |
| 1701$ | S | 4 | 0 | 130 | 38 | 3 |
| 8739 | S | 10 | 1 | 95 | 30 | 10 |
| 16924 | S | 15 | 1 | 120 | 45 | 18 |
| 20069 | S | 16 | 0 | 115 | 45 | 6 |
| 17926 | S | 19 | 1 | 90 | 25 | 12 |
| 8706 | S | 30 | 3 | 125 | 30 | 5 |
| 16702 | S | 32 | 12 | 100 | 35 | 5 |
| 8788 | S | 38 | 5 | 75 | 15 | 9 |
| 16530 | S | 46 | 10 | 55 | 22 | 15 |
| 8800 | S | 50 | 7 | 75 | 20 | 8 |
| 16223 | S | 75 | 12 | 127 | 60 | 5 |
| 16931# | S | 82 | 30 | 105 | 50 | 6 |
| 19191# | S | 100 | 50 | 45 | 22 | 3 |
| 10564# | S | 110 | 48 | 45 | 10 | 0 |
| 1361$ | SO | 3 | 0 | 101 | 15 | 4 |
| 10994 | SO | 12 | 0 | 100 | 18 | 4 |
| 14542 | SO | 18 | 1 | 74 | 18 | 3 |
| 11511 | SO | 20 | 1 | 75 | 20 | 3 |
| 11516 | SO | 21 | 1 | 75 | 20 | 2 |
| 10583# | SO | 102 | 50 | 55 | 11 | 0 |
| 10681# | SO | 105 | 38 | 46 | 15 | 1 |
| 10921# | SO | 108 | 36 | 60 | 22 | 1 |
| 10920 # | SO | 130 | 50 | 60 | 15 | 2 |
| 3100$ | SE | 2 | 0 | 91 | 22 | 6 |
| 16569 | SE | 17 | 2 | 60 | 25 | 15 |
| 17223¢ | SE | 40 | 0 | 120 | 60 | 34 |
| 8511 | SE | 100 | 40 | 95 | 40 | 2 |
| 6149 | CHO | 75 | 30 | 70 | 30 | 3 |
| 1805$ | A | 0 | 0 | 80 | 20 | 2 |
| 2918$ | A | 0 | 0 | 125 | 35 | 2 |
| 19599¢ | A | 10 | 0 | 22 | 3 | 44 |
| 17582 | A | 25 | 1 | 92 | 30 | 9 |
| 17484 | CN | 15 | 1 | 125 | 35 | 15 |
| 8817¢ | E | 34 | 12 | 75 | 20 | 35 |
| 16220 | HH | 35 | 1 | 96 | 42 | 15 |
| 16216 | HH | 40 | 1 | 95 | 45 | 4 |
| 2097$ | $NO_2$ | 4 | 0 | 165 | 20 | 2 |
| 2147$ | PY | 0 | 0 | 95 | 15 | 3 |

Table 2. (Continued)

| Code No. of Chem. | Group Code[a] | Treated Ave. No. Eggs | Treated Ave. No. Larvae | Check Ave. No. Eggs | Check Ave. No. Larvae | Ave. Fly Knock Down |
|---|---|---|---|---|---|---|
| 2153$ | PY | 0 | 0 | 160 | 50 | 2 |
| 3272$ | PY | 3 | 0 | 150 | 49 | 2 |
| 19429 | PY | 8 | 0 | 150 | 40 | 2 |
| 20516 | PY | 20 | 1 | 110 | 50 | 5 |
| 20505 | PY | 40 | 13 | 60 | 25 | 12 |
| 18482 | PY | 95 | 35 | 70 | 20 | 2 |
| 19500# | PY | 101 | 22 | 55 | 15 | 18 |
| 18477# | PY | 105 | 38 | 55 | 22 | 1 |
| 20502# | PY | 105 | 45 | 75 | 40 | 2 |
| 19479# | PY | 105 | 35 | 45 | 15 | 0 |
| 19472# | PY | 120 | 48 | 60 | 30 | 1 |
| 20500# | PY | 130 | 48 | 65 | 30 | 1 |
| 19681# | COOH | 115 | 52 | 45 | 16 | 4 |

* - Average of four replications.
a - These group codes have been clarified in the foot note of Table 1.
$ - Repellent and/or toxicant.
# - Attractants.
¢ - Toxicants.

Series B. - The response of D. melanogaster to some known chemical insect repellents was tested in Series B. The results tabulated in Table 3 indicate that out of 18 repellents tested, only four repelled or inactivated the flies.

Carton Test II.

Series A. - The chemicals which were shown to be either repellents and/or toxicants in Carton Test I (Table 2 and 3) were further tested in this series. The results tabulated in Table 4 demonstrate that only 1971, 3100, 2147, 2153, and 175RC either repel and/or inactivate a high percentage of the flies with no or few eggs on the medium.

Series B. - In this series, the chemicals 1971, 3100, 2147, 2153, and 175RC which proved effective in Series A and 3272 which repelled flies in olfactometer were tested at 0.5% and 0.1% in the presence of

Table 3. Response of D. melanogaster to some known chemical insect repellents.#

| Chemicals | Treated | | Check | | |
|---|---|---|---|---|---|
| | Average No. Eggs | Average No. Larvae | Average No. Eggs | Average No. Larvae | Average Fly Knock Down |
| *175RC | 0 | 0 | 50 | 3 | 46 |
| *R-55 | 2 | 0 | 110 | 40 | 5 |
| *R-874 | 2 | 0 | 158 | 50 | 4 |
| *MGK 933 | 3 | 0 | 65 | 5 | 6 |
| Piperonyl butoxide | 7 | 0 | 95 | 23 | 5 |
| R-326 | 12 | 0 | 95 | 22 | 10 |
| R-1207 | 19 | 2 | 110 | 38 | 4 |
| N-butyl Acetanilide | 24 | 1 | 150 | 48 | 2 |
| AR-55 | 29 | 2 | 105 | 18 | 4 |
| MGK 264 & R-11 | 30 | 2 | 150 | 50 | 2 |
| MGK-264 | 50 | 10 | 85 | 20 | 4 |
| R-612 | 50 | 10 | 40 | 8 | 1 |
| M-1960 | 52 | 22 | 95 | 35 | 2 |
| N-butyl Adipate | 55 | 8 | 90 | 20 | 3 |
| N-N-diethyl-m-toluamide | 58 | 9 | 80 | 25 | 2 |
| Crag fly repellent | 59 | 15 | 75 | 18 | 2 |
| Tabutrex | 70 | 15 | 115 | 20 | 3 |
| R-11 | 80 | 20 | 90 | 25 | 3 |

*Repellent and/or toxicants.
#Average of four replications.

untreated checks. The results in Table 5 indicate that 2147, 3272, and 175RC are effective at 0.5% in reducing the number of eggs on the treated medium. The chemicals 3100, 1971, and 2153 are partially effective at 0.5%. At 0.1% none of the chemicals are effective in reducing the number of eggs on the treated medium except 3272 which is only partially effective.

Series C. - Effectiveness of 1971, 2147, 3100, 2153, 175RC, and 3272 at 0.5% and 0.1% in reducing the number of eggs on the treated medium in absence of untreated checks was determined in this series. The results in Table 6 indicate that only 175RC at 0.5% is still effective, and 2147 and 2153 are partially effective at 0.5%.

Table 4. Effectiveness of the chemicals selected from Carton Test I in repelling or inactivating D. melanogaster.*

| Code No. of Chemicals | Treated | | Average Fly Knock Down |
|---|---|---|---|
| | Average No. Eggs | Average No. Larvae | |
| 175RC | 0 | 0 | 50 |
| 2147 | 1 | 0 | 48 |
| 3100 | 1 | 0 | 44 |
| 1971 | 5 | 0 | 33 |
| 2153 | 5 | 0 | 35 |
| 2918 | 14 | 1 | 30 |
| 2954 | 27 | 2 | 15 |
| MGK 933 | 30 | 1 | 43 |
| 2097 | 30 | 5 | 25 |
| 1805 | 30 | 6 | 22 |
| 9016 | 50 | 0 | 15 |
| R-55 | 62 | 8 | 9 |
| 1701 | 65 | 3 | 4 |
| 3272 | 70 | 3 | 3 |
| 1361 | 80 | 1 | 7 |
| 1829 | 90 | 4 | 12 |
| R-874 | 100 | 2 | 6 |
| Untreated check | 175 | 55 | 0 |
| Without food | -- | -- | 40 |

*Average of four replications.

Table 5. Effectiveness of the chemicals selected from Carton Test II, Series A at 0.5% and 0.1% in repelling and/or inactivating D. melanogaster.*

| Code No. of Chemicals | Treated | | Check | | Ave. Fly Knock Down |
|---|---|---|---|---|---|
| | Ave. No. Eggs | Ave. No. Larvae | Ave. No. Eggs | Ave. No. Larvae | |
| 2147 (0.5%) | 0 | 0 | 150 | 8 | 3 |
| 175RC (0.5%) | 1 | 0 | 100 | 4 | 45 |
| 3272 (0.5%) | 2 | 0 | 175 | 30 | 2 |
| 3272 (0.1%) | 6 | 0 | 180 | 35 | 1 |
| 3100 (0.5%) | 9 | 0 | 140 | 9 | 2 |
| 2153 (0.5%) | 10 | 0 | 150 | 18 | 2 |
| 1971 (0.5%) | 12 | 0 | 155 | 10 | 2 |
| 175RC (0.1%) | 32 | 1 | 136 | 12 | 10 |
| 2147 (0.1%) | 35 | 1 | 160 | 12 | 0 |
| 3100 (0.1%) | 70 | 3 | 100 | 13 | 2 |
| 1971 (0.1%) | 80 | 2 | 130 | 25 | 1 |
| 2153 (0.1%) | 95 | 2 | 137 | 30 | 0 |

*Average of four replications.

Table 6. Effectiveness of the chemicals selected from Carton Test II, Series A, at 0.5% and 0.1% in repelling and/or inactivating D. melanogaster in absence of untreated check.*

| Code No. of Chemicals | Treated | | Average Fly Knock Down |
|---|---|---|---|
| | Average No. Eggs | Average No. Larvae | |
| 175RC (0.5%) | 0 | 0 | 49 |
| 2147 (0.5%) | 12 | 0 | 15 |
| 2153 (0.5%) | 12 | 0 | 35 |
| 3100 (0.5%) | 25 | 0 | 18 |
| 3272 (0.5%) | 50 | 2 | 3 |
| 175RC (0.1%) | 60 | 7 | 34 |
| 2153 (0.1%) | 80 | 1 | 20 |
| 1971 (0.5%) | 85 | 0 | 4 |
| 3100 (0.1%) | 90 | 4 | 1 |
| 2147 (0.1%) | 95 | 6 | 2 |
| 3272 (0.1%) | 160 | 18 | 2 |
| 1971 (0.1%) | 170 | 5 | 2 |

*Average of four replications.

Series D. Effectiveness of 2147, 3100, 2153, and 175RC at 1% in reducing the number of eggs on treated medium after it had been exposed for 30, 48, and 72 hours, was studied in this series. The results in Table 7 indicate that 2147, 175RC, and 3100 are effective for the first 54 hours and 2153 is partially effective for the same duration. While the effectiveness of other chemicals gradually declines after 54 hours of exposure, 175RC is still effective up to 96 hours of duration.

Table 7. Effectiveness of 2147, 3100, 2153, and 175RC at 1% in repelling and/or inactivating D. melanogaster after the treated media were exposed for 30, 48, and 72 hours.*

| Code No. of Chemicals | After 30 Hr. Expos. | | | After 48 Hr. Expos. | | | After 72 Hr. Expos. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Eggs | Larvae | Knock Down | Eggs | Larvae | Knock Down | Eggs | Larvae | Knock Down |
| 175RC | 0 | 0 | 49 | 2 | 0 | 46 | 4 | 0 | 37 |
| 2147 | 0 | 0 | 10 | 18 | 3 | 7 | 100 | 30 | 2 |
| 3100 | 5 | 0 | 25 | 39 | 6 | 10 | 138 | 40 | 3 |
| 2153 | 8 | 0 | 35 | 50 | 5 | 18 | 130 | 38 | 5 |
| Untreated Check | 160 | 50 | 0 | 169 | 49 | 1 | 157 | 40 | 1 |

*Average of four replications.

Olfactometer Test.

Series A. - The responses of D. melanogaster to the vapors of ammonium hydroxide, ethanol, glacial acetic acid, fermenting banana, imitation banana extract, and a mixture of water, glacial acetic acid and molasses were studied in Series A. The results in Table 8 indicate that all substances repel flies for the first 5 minutes of the test. After half an hour, ammonium hydroxide and glacial acetic acid continue to be repellent but fermenting banana, acetic acid mixture and imitation banana extract switch to become attractants. After 6 hours the fermenting banana and acetic acid mixture continue to be attractive but ethanol, ammonium hydroxide, glacial acetic acid, and imitation banana extract have no effect on the responses of the flies.

Series B. - The responses of D. melanogaster to the vapor phase of ethanol, ammonium hydroxide, glacial acetic acid, and imitation banana extract at lower concentrations were studied in Series B. The results in Table 9 indicate that ethanol at 10% and 5%, glacial acetic acid at 2% and 1%, and imitation banana extract at 1% are attractive to the flies. Ammonium hydroxide at 1% is repellent to the flies. Ethanol at 20% and glacial acid at 5% are weak repellents. Ethanol at 1% does not stimulate the flies at all. After half an hour, glacial acetic acid at 2% and 1%, ethanol at 20%, and imitation banana extract at 1% become attractants to the flies but ethanol at 10% and 5%, glacial acetic acid at 5%, and ammonium hydroxide at 1% cease to stimulate the flies in any way. After 6 hours, all treatments cease to stimulate the flies in any direction.

Series C. - In this series, the chemicals which were attractants in Series A and repellents and/or toxicants in Series A and B of Carton Test I, were tested. During this test, it was found that only 2147 and 3272

Table 8. Response of D. melanogaster to the vapor phases of some chemicals in an olfactometer.*

| Chemicals | Per Cent on Treated Side | | |
|---|---|---|---|
| | Immediately After Treatment | After ½ Hr. of Treatment | After 6 Hr. of Treatment |
| Ammonium hydroxide | 2-5 | 11-25 | 45-55 |
| Ethanol | 2-5 | 45-55 | 45-55 |
| Glacial acetic acid | 2-5 | 11-25 | 45-55 |
| Fermenting banana | 20-30 | 70-80 | 70-80 |
| Acetic acid mixture | 11-20 | 70-80 | 70-80 |
| Imitation banana extract | 20-30 | 70-80 | 45-55 |

*Average of two replications.

Table 9. Response of D. melanogaster to the lower concentrations of some chemicals in an olfactometer.*

| Chemicals | Per Cent on Treated Side | | |
|---|---|---|---|
| | Immediately After Treatment | After ½ Hr. of Treatment | After 6 Hr. of Treatment |
| Ethanol (20%) | 36-44 | 70-80 | 45-55 |
| Ethanol (10%) | 75-85 | 45-55 | 45-55 |
| Ethanol (5%) | 70-80 | 45-55 | 45-55 |
| Ethanol (1%) | 45-55 | 45-55 | 45-55 |
| Glacial acetic acid (5%) | 25-35 | 45-55 | 45-55 |
| Glacial acetic acid (2%) | 80-90 | 70-80 | 45-55 |
| Glacial acetic acid (1%) | 80-90 | 70-80 | 45-55 |
| Ammonium hydroxide (1%) | 15-25 | 45-55 | 45-55 |
| Imitation banana extract (1%) | 80-85 | 70-80 | 45-55 |

*Average of two replications.

caused appreciable stimulation on Drosophila flies (Table 10). Since the other chemicals did not have any noticeable effect on the olfactory receptors of flies, they were not included in the table.

Series D. - The chemicals 2147 and 3272 which repelled flies in Series C were further studied in Series D at lower concentrations and for a longer period of time. The results in Table 11 indicate that 2147 at 1% is most effective in repelling Drosophila flies for a longer period of time. The chemical 3272 at different concentrations and 2147 at 0.5% and 0.1% gradually loose effectiveness as time passes and the concentrations decrease.

Table 10. Response of D. melanogaster in an olfactometer to the vapor phase of chemicals selected from Carton Test I.*

| Chemicals | Per Cent on Treated Side |
|---|---|
| 2147 | 0-1 |
| 3272 | 5-15 |
| Other chemicals | 45-55 |

*Average of two replications

Table 11. Effect of time and concentration on the effectiveness of 2147 and 3272 as a repellent for D. melanogaster tested in an olfactometer.*

| | Per Cent on Treated Side After | | | | |
|---|---|---|---|---|---|
| Chemicals | 15 Min. | ½ Hr. | 1 Hr. | 2 Hr. | 6 Hr. |
| 2147 (1%) | 0-1 | 5-10 | 5-10 | 20-30 | 45-55 |
| 2147 (0.5%) | 0-1 | 5-10 | 10-20 | 45-55 | 45-55 |
| 2147 (0.1%) | 20-30 | 45-55 | 45-55 | 45-55 | 45-55 |
| 3272 (1%) | 7-15 | 20-30 | 20-30 | 45-55 | 45-55 |
| 3272 (0.5%) | 7-15 | 20-30 | 45-55 | 45-55 | 45-55 |
| 3272 (0.1%) | 20-30 | 45-55 | 45-55 | 45-55 | 45-55 |

*Average of two replications.

## DISCUSSION

Three different types of tests were conducted in screening and evaluating the response of D. melanogaster to the chemical stimuli of 202 chemicals. The results of these tests are clarified and discussed in this section in detail and efforts are made to compare and correlate them with the findings of different authors.

Laboratory Techniques. The usual procedures for testing repellents consisted of determining the repelling power of pure substance applied on an artificial attractant or one to which the animal was normally subject in nature. Their ability to prevent feeding or oviposit on as the case may be, was a measure of their effectiveness as a repellent (Dethier, 1947). From this point of view, the shell vial test and the carton test were sound and reasonable for screening and evaluating the chemical stimuli of candidate chemicals against Drosophila. The presence of the treated and the untreated media in a carton provided free choice for feeding and oviposition. The total absence or presence of only a few eggs on some treated media clearly demonstrated that the flies did not select such media for oviposition. This was further substantiated by the approximate equal distribution of eggs on two untreated media in the check carton.

The idea that an insect's response to a substance in the gaseous state would vary with its concentrations was not new but little use of this concept had been evident in experimental work with D. melanogaster.

Though Barrows (1907) and Reed (1938) tested various concentrations of ethyl alcohol, acetic acid etc. as bait solutions for D. melanogaster, their methods gave no information on the concentrations of the compounds in the surrounding air, i.e., the concentration as a gas to which this fly reacted positively or negatively. In fact this concentration varies greatly depending upon distance from the solution and air movements. Since there was no work done on the response of D. melanogaster to different concentrations of substances in air stream, the author attempted to study their responses in a new type of olfactometer where control of air stream was possible. The support for this idea of using an olfactometer for screening repellents and attractants was available from Dethier's (1947) report in which he suggested that another means of testing for possible crop repellents and attractants was to conduct experiments with an olfactometer.

Shell Vial Test. - A standard rearing medium for D. melanogaster was treated with the candidate chemicals and exposed to the flies in the limited space of a shell vial to observe the amount of egg deposition. Out of the 184 chemicals in 23 groups screened in this test, 41 chemicals exhibited appreciable toxicity evidenced by 80% or more knock down in 8 hours. Another chemical, 3272, was a strong repellent with little toxicity to the flies. The 42 effective chemicals included three sulfur and nitrogen compounds, 16 sulfides, five sulfoxides, three thioethers, four amines and amides, one nitrile, one ester, two halogenated hydrocarbons, one nitro chemical, and six pyridine and closely realted chemicals. The chemicals in the groups of thioamides, mercaptan, most pyridines, sulfones, organic acid salt, aldehydes, olefins, ketones,

hydrocarbons, miscellaneous, peroxides, alcohol and phenols, and aromatic ester did not have much effect on the feeding and oviposition of Drosophila flies. Oviposition on untreated checks was essentially normal.

Thirteen chemicals showed promise as attractants as media treated with these chemicals had more eggs than the untreated checks. This was evident from the range of the number of eggs in each group. Two sulfides, four sulfones, one thioether, one aldehyde, four pyridines, and one organic acid fell in this group of attractants. The rest of the chemicals showed little attractancy to the flies.

Carton Test I. - Results in table 2, Carton Test I, Series A demonstrated that 8817, 17223, and 19599 were toxic to the flies but without repellent properites. Most of the flies in the carton were knocked down even in the presence of untreated medium. Dethier (1956) remarked that there is no good reason that a toxicant should also be a repellent a priori, for example CO is not repellent to man, boric acid to Blattella, or formaldehyde to flies. The above statement supports the above findings. But, as the author was interested in chemicals which would repel flies from the treated surface or act as inhibitors of egg laying, an arbitrary limit of five eggs was set as the level of effectiveness. So, these chemicals were not further investigated. Moreover, the number of eggs on the media treated with these chemicals was well above the level which demonstrated that though the chemicals were toxic, the flies had adequate opportunity to lay eggs before being knocked down. This made these chemicals unimportant from the standpoint of the objectives of these experiments.

While 13 chemicals were shown to be repellents in this test, only

9016 proved to be both toxic and repellent to the flies. The results of this test did not completely agree with the vial test because in the vial test, flies were put in a very limited space without an alternate untreated medium and when the flies attempted to feed on the treated medium, they picked enough chemicals which caused their knock down. In the carton test, the flies had enough space to move and an alternate untreated medium on which to feed and lay eggs. If the treated medium in Carton Test I would not have repelled the flies, the flies would have fed and laid eggs on it too, or at least would have attempted to do so, when they could pick enough chemicals to knock them down as had been proved in the shell vial test where the knock down of more than eight flies occured in most of the cases in 24 hours. The distribution of approximately equal numbers of eggs on the untreated media in the check cartons provided additional evidence for this opinion of the repellency of the chemicals. Also 8817, 17223, and 19599 proved further that the chemicals which were only toxicants without repellent properties were visited by the flies and thereby, the flies picked enough chemicals to knock them down.

The chemical names and group codes of these repellents are listed below:

| Chemicals | Group Code | Chemical Names |
|---|---|---|
| 9016 | S | 2-Hydroxyethyl decyl sulfide |
| 1701 | S | Octylmercaptobutyonitrile |
| 1829 | S | 2-Hydroxybutyl octyl sulfide |
| 2954 | S | Octylmercaptobutyl methyl ketone |
| 1971 | S | 3,3'-Dichlorodipropyl disulfide |
| 1361 | SO | Butyl octyl sulfoxide |
| 3100 | SE | Ethylene trithiocarbonate |
| 2097 | $NO_2$ | 2,4-Dinitrophenol |
| 1805 | A | N-Vinyl phthalimide |
| 2918 | A | Amyl succinimide |
| 2147 | PY | 2=Chloro-5-nitropyridine |
| 3272 | PY | 3-Nitro-2,6-lutidine |
| 2153 | PY | 2-n-Butoxy-5-nitropyridine |

Also in Table 2, 13 other chemicals which proved to be attractants in the shell vial tests further demonstrated their attractancy even in the presence of untreated checks. Chemical 19500, apart from being attractant, was also toxic to the flies. The nature of their attractancy was clarified and explained in the olfactometer test.

Results in Table 3 indicate that out of the 18 insect repellents tested, only R-55, R-874, and MGK 933 proved to be repellents with lesser toxicity to the flies and 175RC showed to be a strong toxicant.

Olfactometer Test. - The response of D. melanogaster to a certain concentration of chemical vapor in the air-stream has not been extensively studied before. The author first undertook this experiment to evaluate the response of these flies to the vapor phases of selected repellents and attractants screened in the Carton Test I. A new type of olfactometer was used in this test. Since it was not known whether the flies would respond to the chemical vapors in this olfactometer, a trial study was made with the known repellents and attractants of D. melanogaster. From the works of Barrows (1907) and Reed (1938), it is known that Drosophila flies respond positively to the ethyl alcohol and acetic acid bait solutions. So, ethyl alcohol, acetic acid, fermenting banana, imitation banana extract, and ammonium hydroxide were first tried in this test.

Preliminary observations of the distribution of flies in the olfactometer without any treatment revealed that flies were uniformly distributed on the screen ranging from 45%-55% on either side. This distribution from 45% to 55% was considered to be normal.

The results in Series A and Series B showed that the flies did respond positively to the chemical vapors and the results were similar

to those of Reed (1938) and Barrows (1907). Limitations in the degree of response between the previous findings and the present experiment were mainly due to the differences in the methods of testing. Ethanol in the concentrated form and as a 20% solution first acted as repellents but after half an hour, the 20% solution became an attractant to the flies due to the evaporation of enough alcohol from the Kleenex tissue which provided an optimum concentration in the air-stream. The concentrated ethanol still had enough alcohol on the Kleenex tissue to create neither repellency nor attractancy to the flies. After 6 hours neither formulation provided any response, apparently due to the complete evaporation of alcohol. Ethanol at 10% and 5% in Table 9 at first provided such concentration in the air stream that it became attractive to the flies. A similar result was reported by Reed (1938).

In the case of glacial acetic acid similar responses were observed although the concentrations were much lower than those of ethanol. Ten percent acetic acid solution mixed with molasses was at first repellent to the flies but after half an hour, it became attractive. The attractancy of this mixture even after 6 hours was probably due to the presence of molasses.

Imitation banana extract at first repelled Drosophila flies, perhaps due to the presence of 40% alcohol in it but after half an hour, it became attractive possibly due to the evaporation of enough alcohol from it. At 1% solution, banana extract was attractive to the flies immediately after the application on Kleenex tissue and also, after half an hour interval which implied that amyl acetate was attractive to the flies because, the alcohol concentration in the air stream even from 1% ethanol treated Kleenex tissue was not sufficient to create an attractive stimulation on Drosophila flies (Table 9).

From the above observations it can be remarked that the concentrations of the chemicals and the duration of their exposures after the application on the Kleenex tissue had tremendous influence in maintaining their concentrations in the air stream to create effective stimulation on Drosophila flies.

At first it sounds incredible that fermenting banana which is very attractive to the Drosophila flies even from a long distance repelled flies in the first 5 minutes test in the olfactometer (Table 8). Von Loesecke (1929) reported that continued fermentation of banana produces 6.55% to 10.12% ethyl alcohol and 5.72% acetic acid. This report indicates why fermenting banana became repellent (i.e. due to the presence of 5.72% acetic acid which is repellent to the flies.). After half an hour, the banana became attractive to the flies. The fermenting banana test indicated that the air stream under the conditions of the olfactometer test picked up little more chemical vapors from the treated surface than would have been in the case of normal flow of air-current in nature.

Ammonium hydroxide was repellent to the flies so long as its presence in the air stream was maintained.

The above results proved beyond doubt that the new apparatus was efficient enough to evaluate olfactory repellent and attractant vapors against D. melanogaster.

The results in Series C (Table 10) revealed that only two chemicals, 2=Chloro-5-nitropyridine (2147) and 3-Nitro-2,6-pyridine (3272) were olfactory repellents. In Series D (Table 11), it was demonstrated that 2=Chloro-5-nitropyridine was more efficient in repelling Drosophila flies for a longer period of time than 3-Nitro-2,6-pyridine. Even at

0.1% 2=Chloro-5-nitropyridine was effective in repelling the flies for the first 5 minutes of the test.

The results in Table 10 also demonstrated that no attractants selected from the Carton Test I were olfactory in nature. Since an attractant by definition is something which causes an insect to perform directive locomotory responses toward the source of stimulation (Dethier, 1947), the results in the Carton Test I did not indicate any such directive stimulation toward the source of the chemicals. It showed that the flies found the treated medium by chance, liked it, and laid more eggs than on the untreated checks. In other words, the chemicals might have acted as ovipository contact stimulants or taste attractants. According to Dethier (1957) who termed sugar, around which flies gather, as an "acceptant" rather than an attractant; these so called attractants might also be called "acceptants". Had it been the case that these chemicals acted as attractants, comparatively few eggs would have been encountered on the untreated checks. Out of these 13 "acceptants", only one chemical 19500 proved to be simultaneously toxic to the flies. Natural as well as synthetic lures are sometimes toxic to insects, eg. kerosene is both attractive and toxic to the Mediterranean fruit fly.

Carton Test II. - Results in Table 4 demonstrated that the chemicals 9016, 1701, 1829, 2954, 1361, 1805, 2918, 2097, 3272, R-55, R-874, and MGK933 which clearly proved to be repellents in Table 2 and 3 did not repel Drosophila under the conditions of this test. A similar result was reported by Chamberlain and Hoskins (1949) in which they remarked that DDT was repellent to termites when a choice between a treated and an untreated surface was possible. In other situations,

repellency could not be demonstrated. These chemicals evidently allowed the flies to feed and lay eggs and during exposure, many of the flies were inactivated due to the toxicity of the chemicals. The toxicity of all the chemicals except 3272 had been demonstrated in the Shell Vial Test. So, the reason for the presence of some eggs on the treated media might be either only toxicity, or mild toxicity coupled with poor or short term repellency of the chemicals. The opinion of strong toxicity and mild repellency would hold only for MGK 933 where mortality was high. Repellency of MGK 933 had been proved in Table 3. Also in Table 4, apart from its toxicity, repellency was demonstrated by the presence of only one larva in 24 hours which proved that the flies laid their eggs much later. The opinion of only strong toxicity for MGK 933, without any repellent property would not hold because in that case, all the flies were expected to be knocked down as was the case with 175RC. The support for this idea of mild repellency was found in John and Hofmaster's (1961) report in which they remarked that stabilized pyrethrins plus piperonyl butoxide in a one to ten ratio applied on tomatoes repelled Drosophila for about 6 hours.

That 3272 was only a repellent without any toxicity was clarified in the later part of this discussion. In the case of other chemicals, the opinion of strong toxicity did not hold because if that was the case, more knock down would have occurred as with MGK 933. One might argue that most of the flies in these cases did not visit the treated media. But, this argument could not stand because the flies cannot live without food for 30 hours. The check carton without food demonstrated that 40 flies out of 50 died due to starvation during the period of this test.

The opinion that the chemicals were short-term repellents apart from their medium toxicity was strongly supported by the absence of a reasonable number of larvae on the treated media (Table 4) which demonstrated that the flies fed and laid eggs much later when they were very hungry and the repellency of the chemicals was no longer effective. Of course, during this feeding time, some of the flies were inactivated due to the toxicity of the chemicals. During this 30-hour test, there were many larvae on the untreated media in the check cartons. This proved that if the flies had fed and laid eggs earlier on the treated media, enough larvae were expected to be present on them too. Of course, this opinion will not hold if it is assumed that the flies did feed and lay eggs much earlier than it is thought, but all the chemicals acted adversely on the egg to hatching time.

The possibility of poor repellency of these chemicals was also considered. This was supported by the fact that the chemicals in the presence of alternate checks (Table 2) acted as repellents but when there were no alternate media (Table 4) and the flies were hungry, their poor repellency was not longer able to inhibit the hungry flies and feeding and oviposition occurred. During this act of feeding, some flies were inactivated by the toxicity of the chemicals. That many repellents are also effective insecticides is supported by the following examples. Dethier (1947) reported that the odor of oil of cloves which is repellent to ants also kills ants very quickly. Steelman (1963) reported that many of his test animals (ticks) used in testing the repellency of some repellents were killed by the repellent materials. He suggested that the candidate repellents in his tests were effective toxicants as well as repellents.

The failure of 9016, 1701, 1829, 2954, 1361, 1805, 2918, 2097, R-55, R-874, and MGK 933 to stimulate Drosophila flies in the olfactometer demonstrated that the chemicals were contact repellents. Dethier (1956) remarked that it is abundantly clear from the work of Frings (1946), Frings and Frings (1947), Frings and O'Neal (1946), Chadwick and Dethier (1949), Dethier and Chadwick (1950) and Dethier (1951) that contact repellents act upon specialized chemoreceptors which are not normally sensitive to vapors.

Out of the five effective chemicals (175RC, 1971, 3100, 2147, and 2153) in Table 4, 175RC still proved to be a toxicant. It's application resulted in no eggs on the treated medium which demonstrated that the chemical was so strong that the flies had no chance to lay any eggs. The author noticed during the test that within 2 hours about 15-20 test flies were inactivated in the carton by 175RC which further proved its strong toxicity and lack of repellency. This was also demonstated in Table 3 where an alternate untreated check was available to the flies. The Olfactometer Test did not prove any olfactory repellency of 175RC. Bickley et al. (1956) remarked that pyrethrum sprays have little or no repellent action. As to the toxicity of pyrethrum, Bickley and Ditman (1953) and Pepper et al. (1953) reported that pyrethrum afforded excellent protection for the picked fruits by controlling Drosophila adults. The Olfactometer (Table 10) and Carton Test I, Series A (Table 2) had shown 2147 to be an effective olfactory repellent. The Shell Vial Test proved it to be a toxicant. The results in Table 4 proved that 2147 was a strong olfactory repellent for which flies were repelled to starvation and subsequently to knock down. The support for this opinion was provided by the knock down of 40 flies out of 50 in the

carton without food. The additional knock down of eight flies might be attributed to the toxicity of the chemical which was visited by the hungry flies while they tried to feed on the treated medium. The argument that the knock down of 48 flies was only due to the toxicity of the chemical could not hold because substances which were only toxic like 8817, 17223, and 19599 took time to kill the flies during which flies had some chance to lay certain number of eggs on the treated media. The case of 175RC was different because it proved in all the tests to be only a strong toxicant without any repellent property.

The chemicals 1971, 3100, and 2153 proved to be toxicants in the Shell Vial Test and repellents in the Carton Test I. The nature of their repellency was determined to be contact because they could not stimulate the flies in the Olfactometer Test. The degree of the repellency and the toxicity of 1971 and 2153 was less than for 3100. This was proved by the number of inactivated flies and eggs in each of these cases. The suggestion that these chemicals might be only toxicants and not contact repellents was disproved by the fact that they definitely proved to be repellents in the Carton Test I and did not knock down flies in that test, unlike 8817, 17223, and 19599 which were proved to be only toxicants.

The chemical 3272, despite its lack of efficiency in either repelling or intoxicating the flies (Table 4), was included in Tables 5 and 6 because it proved to be an olfactory repellent in the Olfactometer Test and its efficiency in repelling the flies in Table 3 was well demonstrated. The results in Table 5 proved that 3272 was still an

effective olfactory repellent at 0.5% and 0.1%, without any toxicity. But in Table 6, it did not prove effective as was also the case in Table 4. This indicated that this chemical is an effective repellent when there is an alternate medium, but its repellency does not work when there is no alternate medium for the flies to feed. Similar results were observed in the Olfactometer Test (Table 11) where its degree and the duration of repellency were less than that of 2147.

Formulation 175RC was still effective at 0.5% in both the Tables 5 and 6. Chemical 2147 at 0.5% was also effective as an olfactory repellent in Table 5. Repellents 3100, 1971, and 2153 were not effective in either concentrations.

In Table 7, 175RC proved very effective in protecting the treated media for 96 hours; 2147 was also found effective for 54 hours; and 3100 and 2153 were partially effective for 54 hours.

## SUMMARY AND CONCLUSIONS

Three different laboratory experiments were conducted to screen 202 chemicals which had shown some repellency to an arthropod and to evaluate the response of D. melanogaster to them. The objective of these experiments was to find a suitable repellent which would repel the flies from a treated surface and thus protect it from being contaminated with the fly eggs. The response of the flies to the chemical stimuli of these chemicals was compared and contrasted to evaluate their effectiveness in achieving the objective of the experiment.

Laboratory experiments utilizing three screening techniques, shell vial, carton, and olfactometer tests were conducted to study the response of the flies. In the Shell Vial Test, 41 chemicals seemed to be toxicants which prevented most oviposition on the treated medium. Another chemical proved to be a repellent.

In the Carton Test I, three chemicals 17223, a thioether; 19599, an amine; and 8817, as ester, proved to be strong toxicants but they did not protect the medium from the contamination by fly eggs. Formulation 175RC which contains pyrethrum and petroleum distillate was also proved to be a strong toxicant against Drosophila flies in this and other tests, and it was found to protect the treated medium from fly oviposition. Thirteen chemicals including two sulfides, four sulfones, one thioether, one aldehyde, four pyridine and related compounds, and one organic acid were found to be attractive to the flies in the Shell

Vial and Carton Test I. But the data in these tests and the results in the Olfactometer Test proved that these chemicals were not olfactory attractants.

Also in Carton Test I, 13 chemicals and three known insect repellents were shown to be repellents. They include 2-Hydroxyethyl decyl sulfide (9016), Butyl octyl sulfoxide (1361), Octylmercaptobutyonitrile (1701), N-Vinyl phthalimide (1805), Amyl succinimide (2918), 2-Hydroxybutyl octyl sulfide (1829), Octylmercaptobutyl methyl ketone (2954), Ethylene trithiocarbonate (3100), 2,4-Dinitrophenol (2097), 2=Chloro-5-nitropyridine (2147), 3,3'-Dichlorodipropyl disulfide (1971), 2-n-Butoxy-5-nitropyridine (2153), 3-Nitro-2,6-lutidine (3272), R-55, MGK 933, and R-874. After checking and evaluating the responses of Drosophila to these 16 chemicals in the Olfactometer and in the Carton Test II, it was concluded that 2=Chloro-5-nitropyridine and 3-Nitro-2, 6-lutidine were the only two olfactory repellents and the rest of the 14 chemicals were contact repellents. All the above chemicals except 3-Nitro-2,6-lutidine were also found to be toxic to the flies. In fact, it was concluded that these 15 chemicals were both repellents and toxicants. The expression of these properties depended on the nature of the test conducted. The degree of the repellency and/or toxicity varied considerably from one chemical to another. Of the two olfactory repellents, 3-Nitro-2,6-lutidine was only a repellent without any toxicity, whereas, 2=Chloro-5-nitropyridine was both a repellent and a toxicant. The effectiveness of 2=Chloro-5-nitropyridine as an olfactory repellent was far greater than 3-Nitro-2,6-lutidine as demonstrated in both carton and olfactometer tests. Chemical 3-Nitro-2,6-lutidine was only an effective repellent when there was an alternate medium for the flies to feed

and lay eggs.

The results of these experiments, when interpreted with the supporting data from previous literature, indicate that 2=Chloro-5-nitropyridine is the best repellent which can protect the treated medium from being contaminated by Drosophila eggs for 54 hours. Also 175RC, which is a toxicant without any repellent property, can protect the treated medium from being contaminated with Drosophila eggs for 96 hours. At lower concentrations, 175RC is even better than 2=Chloro-5-nitropyridine in protecting the treated media.

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