

BIOLOGY AND CONTROL OF MOSQUITOES IN THE
GREATER WINNIPEG AREA

A

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by
Reinhart Albert Brust

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ABSTRACT

by

Reinhart Albert Brust

BIOLOGY AND CONTROL OF MOSQUITOES IN THE GREATER WINNIPEG AREA

Larvae and pupae of Aedes vexans, Culiseta inornata, Aedes spencerii and Aedes aegypti responded to short wave lengths of light at low light intensities, but to long wave lengths at only high light intensities.

Adult mosquitoes native to Manitoba were able to distinguish between red and blue light. When subjected to white light at different light intensities the mosquitoes were attracted to the light of greatest intensity.

Further experiments were conducted using the laboratory reared adults of Aedes aegypti. In feeding trials whole blood was preferred to corpuscles washed in 0.15% saline. Serum or plasma was just as attractive as whole blood and was preferred to 0.15% saline. When host stimuli were investigated, temperatures of 36-40°C. were found to be preferred; moisture was found to be highly attractive; colours black and dark blue were visited in large numbers while lighter hues attracted very few mosquitoes. A horizontally placed cylinder, 3" in diameter and 18" long was found to be three times as attractive as a vertically placed one.

Plant flowers of sweet clover, red clover and mustard were found to be attractive to unfed female and male mosquitoes. Rose plant extracts produced a positive olfactory stimulus but extracts of lilac and strawberry were found to be repellent.

Adult females of Aedes aegypti (Linnaeus) preferred coarse textured surfaces for oviposition. Culiseta inornata (Williston), a species most abundant in the light collections, was not found in "biting" collections.

Tests have indicated that the top minnow, Gambusia affinis, which was earlier presumed not to be cold-hardy, can overwinter in this climate.

Tests have shown that no resistance to DDT has developed in the mosquitoes of the Winnipeg area.

CHAPTER I

INTRODUCTION

Among the earliest references in the literature to the mosquito are certain passages in Aristotle (384-322 B. C.) referring to "empis" (signifying the mosquito) as being among certain creatures which, coming from putrifying liquids, are neither produced out of other animals nor do they copulate, i.e., they were thought to arise by spontaneous generation of life, a belief held in regard to the mosquito larva which still persisted some two thousand years later (Christophers, 1960). The name mosquito was used from early times in North America and the Oxford English Dictionary quotes Whitbourne (1623) writing of Newfoundland as saying, "There is a little nimble fly which is called the musketo".

This long history shows how successful mosquitoes have been in adapting themselves to countless habitats ranging from the tropics well into arctic regions. The habit of breeding in small bodies of standing water in areas susceptible to drought and living in climates where freezing temperatures predominate six months of the year has not reduced their part in the production of human disease, the lowering of industrial efficiency, the retarding of agriculture and the lowering of real estate values. In the tropics and subtropics the pathogens of malaria, denque, yellow fever, filariasis and equine encephalomyelitis are transmitted by mosquitoes and kill many millions of people each year (Chandler, 1956).

Mosquito populations reach a much greater density in colder climates

than they do in tropical regions where breeding is continuous throughout the year, giving a constant level of infestation (Chandler, 1956). Mosquitoes in temperate regions such as Winnipeg have a cold-resistant stage, usually in the egg, which can survive the winter and hatching occurs with the return of warm weather and surface water. Development to the adult is rapid, eggs are laid and the whole life cycle is completed in a short time.

Many different methods have been devised to control the mosquitoes throughout the world. The methods used in one area are not necessarily successful in another and they must be tested to see if they can meet the problems presented by climatic conditions, environment and the species present in an area. Mosquito control in Greater Winnipeg was first begun in 1927 when an organization called the Greater Winnipeg Anti-Mosquito Campaign was formed. Control operations by this group were financed by voluntary contributions until 1953 when the Greater Winnipeg Mosquito Abatement District was formed and today this still represents the only incorporated mosquito control group in Canada. In the fall of 1960 it, undoubtedly, will be incorporated into the Metropolitan Government Administration for Winnipeg. The control area covers 225 square miles and benefits more than half a million people.

THE PROBLEM

This study was conducted during 1959 and 1960 in the Greater Winnipeg area. The main investigation was concerned with the following aspects:

I. Mosquito Biology and Behaviour

- (a) sensory and visual stimuli
- (b) the colour sense of mosquitoes
- (c) classification and relative abundance of mosquito species in the Greater Winnipeg area
- (d) flight behaviour patterns of native Manitoba species

II. Effectiveness of Mosquito Control in Greater Winnipeg

- (a) larval and pupal survey inside and outside the district boundaries
- (b) adult light trap collections inside and outside the district

IMPORTANCE OF THE PROBLEM

A complete knowledge of the life cycle and behaviour of any insect is basic for the establishment of an effective program of pest control. For this reason efforts were directed toward biological studies of native mosquito species. In the winter of 1959-60, when prairie mosquitoes were not available, laboratory cultures of Aedes aegypti were used.

From the standpoint of mosquito abatement, a knowledge of mosquito species is invaluable. Not only are the necessary control methods determined by the habits of the particular species but one cannot reach a true estimate of the results of the work without this knowledge. Since mosquitoes fly, or are wind-borne, often for some distance, locality is

not decisively a determining factor. If the mosquitoes have been breeding outside the control area, a knowledge of that fact would prevent the useless expenditure of time and public funds on control of local breeding. McLintock (1944) was the first to list the mosquito species found in the Greater Winnipeg area. In the summer of 1959 and 1960, five mosquito species not recorded by McLintock, were identified from areas in and around Winnipeg. These species have long been found in regions of Minnesota and Wisconsin. The inference could be made that these species are exceeding their former boundaries and are infiltrating the more northern parts of the continent.

Clarke (1937) reports that some mosquitoes are able to fly great distances under favourable conditions and supports his statement with evidence that only two hours after tagging, a specimen of Aedes vexans was recaptured six miles from the point of release. Specimens of Aedes vexans were captured 14 miles from the release point after one night.

Investigations on flight behaviour of mosquitoes around Winnipeg were conducted to determine their flight distance and orientation.

PART A. MOSQUITO BIOLOGY AND CONTROL

CHAPTER II

MOSQUITO BIOLOGY

Oviposition

Mosquitoes can, in general, be divided into two classes depending on whether or not their eggs enter into a period of dormancy.

In Manitoba those species laying "non-dormant" eggs are included under the genera Anopheles, Culex, Culiseta, Mansonia. Aedes species, 25 of which have been recorded on the prairies (Rempel, 1953), lay eggs which become "dormant".

Dormant eggs are much more resistant to drying and freezing temperatures; these being two reasons why the genus Aedes is the most abundant in and around Winnipeg.

Development

"Non-dormant" eggs are laid directly on the water and stay afloat until they hatch. Larvae appear 48-60 hours after oviposition at a temperature of 22°C. and 8-11 days at 11°C. (Barr, 1958). The larvae may require from 6-24 days to complete their development under normal conditions, but if they are subjected to continuous low temperatures development may be prolonged for 35-40 days and under these conditions high mortality is incurred.

The pupal stage lasts about 2 days at normal outside temperatures but persisting low temperatures may delay adult emergence for 4-5 days.

The adult female overwinters in barns, outhouses, granaries, caves, houses and other sheltered situations. In early spring the female leaves its hibernating quarters to seek a blood meal. The first generation larvae are found during the latter part of May in the Greater Winnipeg area.

Aedes species, laying "dormant eggs", usually have only one generation a year, however, this may vary according to the species, the season or both. Species that characteristically hatch from eggs in very cold water have a generation in the spring and another in the fall (Barr, 1958). Aedes dorsalis and Aedes vexans belong to this group. Other Aedes species found in northern regions produce eggs requiring cold temperatures during the incubation period (Barr, 1958). It is the egg that overwinters and hatching occurs in spring run-off water. The first larvae found in and around Winnipeg in 1959 and 1960 have been Aedes spencerii and Aedes dorsalis.

Mating

Male mosquitoes in a given population practically always begin emerging a day or two before the females. The shortening in development time amongst the males occurs in the fourth larval instar. Due to this shorter period of development, pupae and adult males are correspondingly smaller in size (Barr, 1958).

Mating of most mosquitoes takes place in a "swarm" formation. Under certain conditions males begin a peculiar dancing flight which seems to be necessary for most species (Bassler, 1958). Male swarms may vary in size from a few to several thousand and form at low light intensities in the evening or in the morning. The female usually pairs immediately

upon entering the swarm; copulation requiring only a minute or so, although it may be prolonged for hours in some species (Culiseta inornata) (Bassler, 1958).

CHAPTER III

LABORATORY EXPERIMENTS WITH Aedes aegyptiAdult Feeding BehaviourIntroduction

The blood-sucking habit is almost universal among female mosquitoes, and in most cases blood is necessary for egg maturation. Exceptions to this are Aedes atrapalpus, Aedes communis and Culex pipiens, all of which can lay eggs "autogenously" (without blood) but will take blood on occasions (Barr, 1958).

The female of Aedes aegypti has a diurnal biting cycle with two peaks of major activity. The first peak occurs 3 hours after sunrise, and the second one hour before sunset (McClelland, 1960). As early as 1905 it had been noted that adults of Aedes aegypti fed readily in artificial light, but would not feed in total darkness (Horsfall, 1955). The writer's experience has been that 80% of the females that fed under optimum light conditions also took blood in periods of total darkness. A subdued light was effective but the best feeding was obtained with a red light. The time allotted to feeding was usually five minutes, and one blood meal was sufficient for egg laying. Mosquitoes fed on human blood laid an average of 88 eggs after the first meal and 55 after the second (Barr, 1958).

It was observed that when the females were allowed to engorge themselves, they were unable to fly and would consequently drop to the floor. This is not very surprising when it is considered that they are

capable of ingesting 1.5 times their own weight in blood, calculated from Tarshis, 1960. Culex tarsalis consumed on the average 2.44 mgs. of outdated human blood and Aedes aegypti 2.29 mgs.

Burgess and Brown (1957) and Laarman (1955) demonstrated that mosquitoes are attracted to beef blood and rabbit blood respectively. By the olfactometer method, whole blood was 3-4 times more attractive than water, while plasma was preferred 4-5 times. When whole blood was compared to washed corpuscles suspended in 0.9% saline, blood was 2-3 times as attractive (Burgess and Brown, 1957). Hosoi (1959), by employing voluntary and forced feeding methods, found erythrocytes to be more attractive than plasma. He went even further and identified the attractive element as adenosine -5' - phosphates. These can be extracted from the blood corpuscles by paper chromatography.

Prior to Hosoi's publications, the writer attempted quantitative feeding of blood and its various components. A feeding stimulus involves both olfactory and gustatory responses and both are necessary to determine the palatability of an ingestible component. For this reason a feeding method was preferred to the olfactometer.

Tarshis (1959) found that Culex tarsalis fed well through animal derived membranes. Silverlight which was derived from the caecum of an ox, gave the best feeding results. The writer obtained this from Julius Schmid, Inc., New York, N. Y. for use in the experiments which follow.

Method

Outdated human blood was used for all experiments. The attractiveness

of whole blood was compared with washed corpuscles, serum or plasma and 0.15% saline.

The feeding cage, 12" x 12" x 14" consisted of two compartments: the upper part enclosed and maintained at 38°C. by a small heater; the lower part maintained at room temperature of 25-26°C. and 50-60°F. (Figure 1). Two ports connected the upper and lower sections of the cage where test substances were offered. Two small screen cylinders were fastened to the ports from below and into these were inserted equal numbers of female mosquitoes. The test substance was emptied into the thistle tube when the upper part of the cage reached 38°C. The large end of the tube was covered with a membrane and placed into the port. Rhodamine B dye, which fluoresces under ultraviolet light, was added to the test fraction for identification of the fed mosquitoes. Feeding time allotted to the experiment was two hours.

Results

Figure 2. gives the percentage of mosquitoes that fed on whole blood compared to the percentage that fed on blood fractions and saline. Whole blood was preferred to washed corpuscles and 0.15% saline. Plasma was found to be just as attractive as blood and preferred to 0.15% saline.

Host Factors Attractive to Mosquitoes

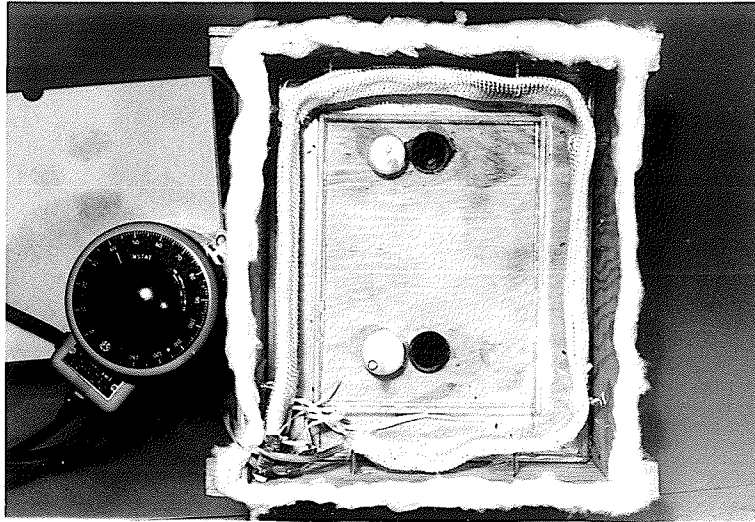
The realization that the adult female mosquito approaches its animal host as a response to certain token stimuli that the host introduces into the environment has prompted the study of individual host factors that might constitute stimuli. The attractiveness of carbon dioxide,

Figure 1. Adult Feeding Cage, Aedes aegypti

A. Top View

B. Side View

A



JUL • 60

B

09 • 707 •

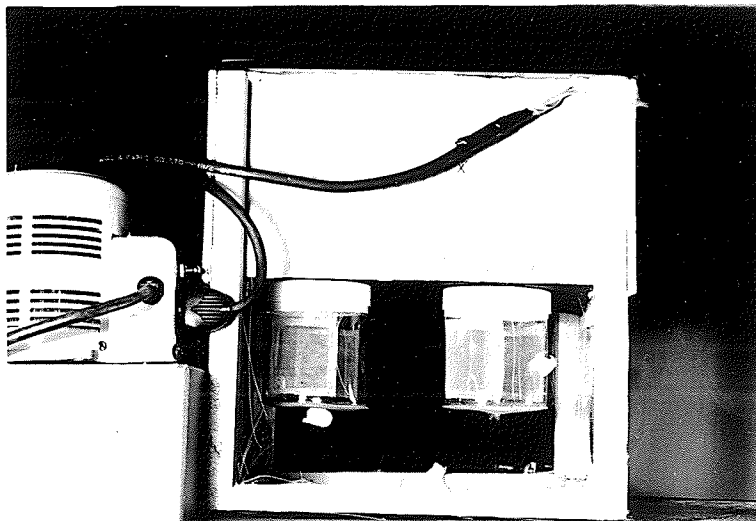
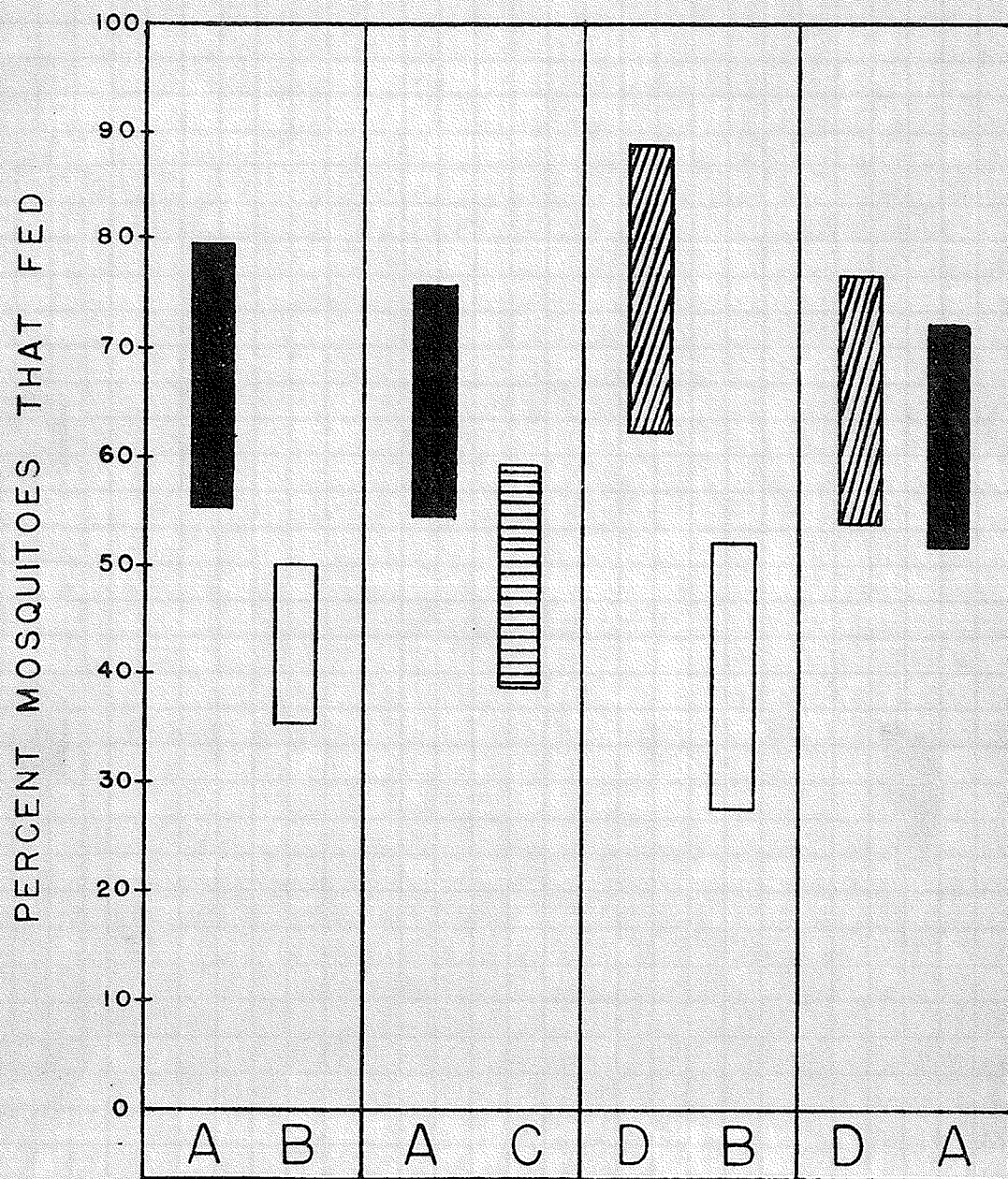


Figure 2. Comparative Percentages of Mosquitoes that Fed on Various Preparations of Outdated Human Blood

- A. Whole blood
- B. 0.15% NaCl
- C. Corpuscles suspended in 0.15% NaCl
- D. Plasma or serum

Confidence limits of the percentages were taken at 0.05 significance level.



moisture, warmth and rabbit blood vapours have been investigated by Laarman (1955) and Brown (1956). It was demonstrated that moist air was more attractive than dry air, and that carbon dioxide increased the attractiveness of dry air but not moist air.

The writer confirmed the above findings of Laarman (1955) and Brown (1956) by using outdated human blood and comparing its attractiveness to water. Further investigations were concerned with the warmth, moisture, colour and position of an inanimate object and whether these factors affected the attractiveness of the object.

Method

For the tests 500-600 mosquitoes were placed in an experimental cage, 24" x 24" by 36". The adults were maintained on 10% sugar solution until 48 hours prior to the experiments. This solution was then replaced with distilled water until 12 hours before the tests.

In order to compare the attractiveness of blood and water, 5 mls. of blood were placed in a petri-dish and covered with cotton wool. One dish containing blood and the other containing 5 mls. of water, were placed side by side in the cage. The number of adults landing on the cotton wool over the test substances were counted every 30 seconds for a period of 5 minutes.

A glass cylinder 18" high and 3" in diameter was used in the second set of experiments. Warm cylinders were compared to cold; white compared to black; and a horizontal position compared to a vertical. Coloured balloons were introduced into the mosquito cage to

compare various hues.

Results

In Table I it was observed that moisture increases the attractiveness of an object 3-4 times. Blood vapour was found to be more attractive than water in accordance with the findings of Burgess and Brown (1957).

A warm cylinder (between 36-40°C.) was found to be nearly twice as attractive as a cold one and female mosquitoes, upon alighting, were observed to probe the warm cylinder excitedly thus indicating that warmth is an important probing stimulus (Table II).

The black cylinder being compared with a white one was preferred in all cases whether warm, moist or dry (Table III). When balloons of different colours were tested; green, dark blue, and black were preferred. No mosquitoes landed on the yellow or orange balloons.

A cylinder suspended in the horizontal position attracted 2-3 times more mosquitoes than the same cylinder suspended in a vertical position.

TABLE I

RESPONSE OF Aedes Aegypti TO MOISTURE AND BLOOD STIMULI

Experiment No.	Number of Mosquitoes Aggregating on Dishes		
	Blood	Water	- t -
1	77	67	1.3
2	82	65	1.6
3	<u>81</u>	<u>62</u>	<u>1.7</u>
Total	240	194	2.85*
	Moist Cotton	Dry Cotton	
1	73	14	2.9*
2	95	14	3.2**
3	140	28	3.3**
4	<u>123</u>	<u>33</u>	<u>3.2**</u>
Total	431	89	6.6**

* 5% level of significance

** 1% level of significance

Each experiment consisted of 10 replicates

TABLE II
THERMAL RESPONSE OF Aedes Aegypti

Experiment No.	No. of Mosquitoes Landing on the Cylinder		- t -
	Warm Cylinder	Cold Cylinder	
1	176	122	2.9
2	282	160	3.2**
3	<u>98</u>	<u>32</u>	<u>3.4**</u>
Total	556	314	3.1**

** 1% level of significance

Each experiment consisted of 10 replicates

TABLE III
VISUAL RESPONSE OF AEDES AEGYPTI

Number of Mosquitoes Landing on the Cylinder			
Experiment No.	Black	White	- t -
1	185	28	3.8**
2	222	36	3.7**
3	<u>304</u>	<u>40</u>	<u>4.2**</u>
Total	711	104	5.4**

	Horizontal	Vertical	
1	236	102	3.3**
2	212	120	3.1**
3	<u>318</u>	<u>164</u>	<u>3.2**</u>
Total	766	386	3.1**

** 1% level of significance

Each experiment consisted of 10 replicates

Plant Odours Attractive to Mosquitoes

Female mosquitoes have been observed to visit and probe several species of flowering plants for nectar and juices (Barr, 1958). Under laboratory conditions Aedes communis males and females visit flowers and ingest plant juices and nectars as shown by their accumulation of P^{32} from activated flowering plants (West, 1951).

In laboratory experiments the writer found that mosquitoes lived for 24-48 hours without water and four to five days without food. The longevity of the average mosquito is 60-80 days in the laboratory and 30-40 days in the field (Horsfall, 1955). Mosquitoes not able to obtain a blood meal after four to five days must therefore depend on other food sources.

Investigations were concerned with some plant flowers native to Manitoba and whether these serve as possible food sources to the mosquito.

Method

In the first series of laboratory experiments, olfactory stimuli of plant flowers were investigated. Sweetclover, red clover and mustard plants were selected and their flowers removed for the tests. The flower parts were placed in petri dishes and covered with cotton wool in order to test the olfactory response. Water was found to produce a standard response and was therefore used as a control. Better results were obtained from starved than from fed mosquitoes hence adults were given no food for 24 hours before the trials. The test dish together with its control were placed simultaneously into the cage. The mosquitoes

aggregating on the dishes were counted every 30 seconds for a five minute period. Responses to sweet clover and buckwheat honey were investigated and compared to a water stimulus.

In the second series of experiments extracts of plant flowers were obtained from a perfume distributor¹. (These products were manufactured from raw materials by a Butane gas cold extraction process). Olfactory responses to the perfumes were tested in a similar manner as described above.

All experiments were conducted at a room temperature of 25-26°C. and 50-60% humidity.

The figures presented in Tables I, II and III are the experiment totals. Each consists of ten replicates. A Student -t- experimental design was used to compare the treatment means.

Results

The experiment totals show that there was an olfactory response to the plant flowers (Table IV). Sweet clover and buckwheat honey were more than twice as attractive as water. This response was not due to the sugar content in honey since tests with ten percent glucose and ten percent sucrose showed that these were not attractive (Table V).

Extracts of rose² plant flowers were attractive to mosquitoes but extracts of lilac³ and strawberry⁴ plant flowers were repellent (Table VI).

¹P. Robertet, Inc., 221 Fourth Avenue, New York 3, N.Y.

²Rose Maroc (1323 J) and Rose (3700)

³Lilac (3717)

⁴Strawberry (3709)

(Numbers listed are for the manufacturers identification)

TABLE IV
RESPONSE OF AEDES AEGYPTI TO OLFACTORY STIMULI

Number of Mosquitoes Aggregating on Dishes			
Experiment No.	Sweet Clover	Water	- t -
1	120	73	3.3**
2	148	135	1.4
3	<u>311</u>	<u>227</u>	<u>3.0</u>
Total	579	435	3.1**

	Red Clover	Water	
1	136	121	2.5
2	<u>86</u>	<u>68</u>	<u>3.0</u>
Total	222	189	3.2*

	Mustard	Water	
1	142	130	2.6
2	<u>161</u>	<u>155</u>	<u>1.1</u>
Total	303	285	2.6*

** Significant at the 1% level

* Significant at the 5% level

10 replicates for each experiment

TABLE V

RESPONSE OF Aedes Aegypti TO OLFACTORY AND CHEMICAL STIMULI

Experiment No.	Number of Mosquitoes Aggregating on Dishes		
	10% Buckwheat Honey	Water	- t -
1	84	17	3.3**
2	368	64	4.8**
3	<u>464</u>	<u>71</u>	<u>6.8**</u>
Total	916	152	5.6**
	10% Sweet Clover Honey		Water
1	74	41	3.3**
2	86	48	3.0**
3	<u>132</u>	<u>58</u>	<u>4.2**</u>
Total	292	147	5.3**
	10% Sucrose		Water
1	55	51	0.4
2	68	69	0.3
3	<u>49</u>	<u>53</u>	<u>0.3</u>
Total	172	173	0.23
	10% Glucose		Water
1	84	79	0.5
2	59	55	0.4
3	<u>96</u>	<u>101</u>	<u>0.6</u>
Total	239	235	0.65

** Significant at the 1% level

* Significant at the 5% level

10 replicates for each experiment

TABLE VI

RESPONSE OF AEDES AEGYPTI TO PLANT FLOWER EXTRACTS

Experiment No.	Rose Maroc (1323 J)	Water	T - C	- t -
1	65	30	35	2.8
2	<u>78</u>	<u>53</u>	<u>25</u>	<u>1.9</u>
Total	143	83	60	2.5*
	Rose (3700)	Water	T - C	
1	109	88	21	1.6
2	<u>94</u>	<u>35</u>	<u>59</u>	<u>2.4</u>
Total	203	123	80	2.3*
	Strawberry (3709)	Water	T - C	
1	10	55	-46	3.6**
2	<u>16</u>	<u>88</u>	<u>-72</u>	<u>3.7**</u>
Total	26	143	-118	3.6**
	Lilac (3717)	Water	T - C	
1	11	35	-24	3.3**
2	<u>13</u>	<u>43</u>	<u>-30</u>	<u>3.2**</u>
Total	24	78	-54	3.2**

** Significant at the 1% level

* Significant at the 5% level

10 replicates for each experiment

T - Treatment; C - Control

Influence of Surface Textures on Oviposition

Introduction

In laboratory rearing of Aedes aegypti, eggs were laid on filter paper which was immersed in water. It was observed that eggs were deposited in small depressions of the filter paper. Gupta and Thorsteinson (1960) working with the diamond-back moth Plutella maculipennis found that coarse textured oviposition surfaces were preferred to smooth ones.

In investigations on the mosquitoes, different textured filter paper and white paper towelling was used to study oviposition responses to the texture of substrate surfaces.

Method

Filter paper shaped in the form of a cone was placed in a water filled petri-dish. A coarse-textured filter paper was compared: (a) to fine filter paper, (b) to white paper towelling. Each experiment consisted of two replicates.

Result

The course filter paper averaged 648 eggs and the fine filter paper 212 eggs per night. When the course filter was compared to paper towelling, the paper towelling received an average of 8280 eggs and the filter paper 620 (Figure 3).

Figure 3. Influence of Surface Texture on Oviposition of

Aedes aegypti

1. White filter paper
2. White paper towelling



The Effect of Adult Age on Light Response

In 1959 the mosquito flight peaks extended over a period of a few days to a week, according to collections taken in the New Jersey Mosquito light trap (Figure 17). This may be due to two possible reasons:

(1) the average life of mosquito may be one week, (2) that mosquitoes may fly less readily after one week. However, the average life of our most common mosquito, Aedes vexans and Culiseta inornata is 30-40 days (Barr, 1958). This led to the study of the effect of age on mosquito light response.

Method

A screened cage, 3' x 3' x 6' long, containing a large window on each side was used for the light experiments. Native mosquito species were not available during the winter and the laboratory culture of Aedes aegypti was used. Pupae were placed in the cage and 250 adults (60 percent females) emerged within 24 hours. The remaining pupae were removed. The adults used in the experiments were maintained on ten percent sugar solution. A 60 watt lamp was set at one end of the cage and mosquitoes preferring the bright end were counted as "light attracted". The adults were dispersed in total darkness and by means of a subdued red light the number aggregating on the end of the cage were recorded. The cage light was turned on and after one hour counts were made of the light attracted mosquitoes. By shaking the cage the mosquitoes were disturbed and after the second hour another count was made. The light was then moved to the opposite end of the cage where two more counts were made in a similar manner.

The figures presented in the table show the daily response to light over a 30 day period (appendix I).

Results

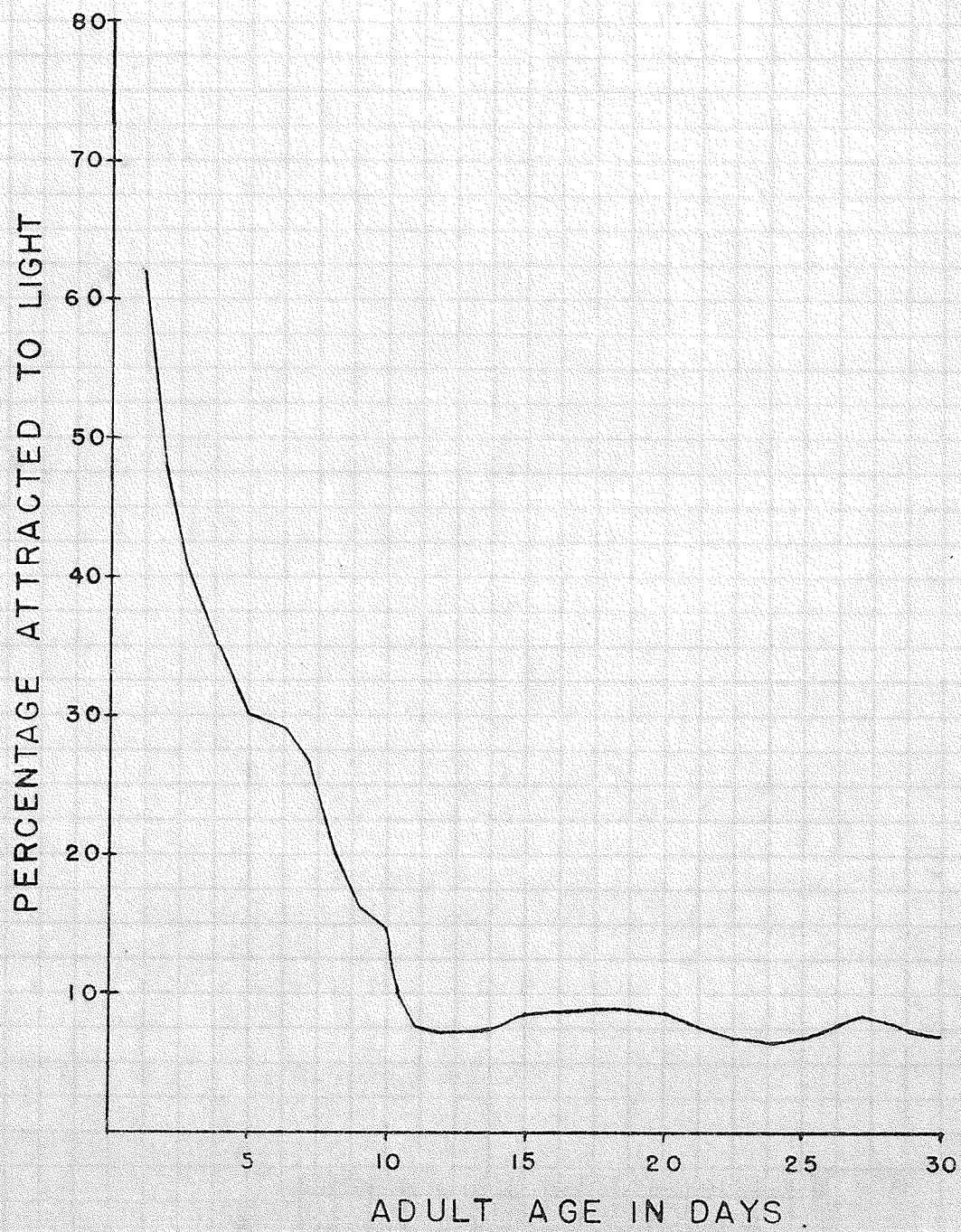
On the first day 62 percent were light attracted and on the second day 47.5 percent. The number of mosquitoes attracted to light decreased with each succeeding day and on the eleventh day 7.1 percent selected the bright end (Figure 4).

During the experiment the adult population decreased from 250 to 180.

Conclusions

If our native mosquito species become less attracted to lights as they grow older, as does Aedes aegypti, the interpretation of light trap survey data might require revision. The very short duration of "peaks" indicated by the light trap data suggest that our native mosquitoes are strongly attracted to lights only when the adults are young. On the other hand, mortality is possibly much greater in Northern areas and the decline in numbers indicated by light traps may reflect some real reduction in population.

Figure 4. Percentage of Caged Adult Aedes aegypti Attracted to
Light Over a 30 Day Period



CHAPTER IV

COLOUR PERCEPTION

Larval and Pupal Reactions to Coloured Light

Those familiar with the habits of mosquito larvae and pupae will recall one stimulus, that of negative phototaxis, as especially characteristic. Christophers (1960) in describing the larvae of Aedes aegypti notes their extreme sensitiveness to light.

Omardeen (1957) observed that second and third instar larvae of Aedes aegypti showed positive phototaxis but this was reversed in fourth instar larvae and in the pupal stage. The writer extended these studies to investigations of fourth instar larvae and pupae of Aedes aegypti, Aedes spencerii, Aedes vexans and Culiseta inornata, and their reaction to coloured light.

Materials and Methods

Monochromatic light filters⁽¹⁾ consisted of 2" of 2" square glass with wave lengths ranging from 405 m μ to 700 m μ . An ultra violet fluorescent lamp was used to obtain light reactions below 405 millimicra.

In each test 200 larvae were placed in a clear perspex trough 2" x 1" by 2' long. A lamp to which the filters were fitted was set up at each end of the trough and a rheostat connected to each lamp permitted

¹ Obtained from Canadian Laboratory Supplies, Ltd., 288 William Avenue, Winnipeg.

a selection of light intensities (Figure 5). The larval reaction to light at a wave length of 700 m μ was obtained by dispersing the larvae in complete darkness for three minutes. The light intensity was then increased until 90 percent of the larvae were repelled as far as the center of the trough. In this first trial with red light only one lamp was used. No light source was provided at the other end of the trough. The lowest intensity of red light (700 m μ) effective for eliciting a negative phototactic response under these conditions was established. This standard red light remained at one end of the trough in all subsequent trials with Aedes aegypti. By means of this minimal stimulus all the larvae could be assembled at the opposite end of the trough. At the larval-concentrated end another lamp was placed to which various filters were fitted as required. By means of the rheostat the intensity of light at each wave length was gradually increased until the larvae were repelled beyond the center line toward the red light. The wave lengths tested in this way included 405, 425, 445, 475, 500, ---, 670 m μ . The minimal intensities required to achieve this are presented in table VII. Experiments involving the other mosquito species, Aedes spencerii, Culiseta inornata and Aedes vexans were conducted in the same manner.

Pupae were subjected to coloured light in the manner described for the larvae. Preliminary colour experiments with larvae and pupae of Aedes aegypti were conducted with red, orange, yellow, green and blue perspex filters. In these experiments only one lamp was used and no light source was provided at the other end of the trough.



Results

The larvae and pupae react to short light waves at very low intensities but are sensitive only to higher intensities of longer wave lengths (Figure 6). Violet light (405 m μ) at a light intensity of 15 foot candles appeared to produce the same response as red light (700 m μ) at 680 foot candles (Table VII, Aedes aegypti). Light waves of 475-500 m μ , referred to by von Frisch (1950) as "blue-green", elicit a greater response from mosquito larvae and pupae than blue and violet. Ultraviolet caused a phototactic response at 1-2 foot candles with larvae and 3-4 foot candles with pupae.

Pupae of all species under investigation were less sensitive than larvae to visual stimuli (Table VII) with the exception of preliminary experiments with Aedes aegypti. In these experiments pupae were more sensitive than larvae to coloured light and higher light intensities were required to give a phototactic response with the perspex filters (Figure 7). However, referring to the method above, a different procedure had been adopted with the perspex filters. This may in part account for the reversal in the pupal reactions as shown in figure 6 and figure 7.

Conclusions

Mosquito larvae and pupae are sensitive to ultraviolet light. This was demonstrated by their negative phototactic response. In the visible spectrum (400-700 m μ) larvae and pupae are more sensitive to violet, blue and green light. Red light (700 m μ) is only detected at relatively

Figure 6. Coloured Light Responses of Aedes aegypti.

Minimal Light Intensities Required To Repel the
Larvae and Pupae.

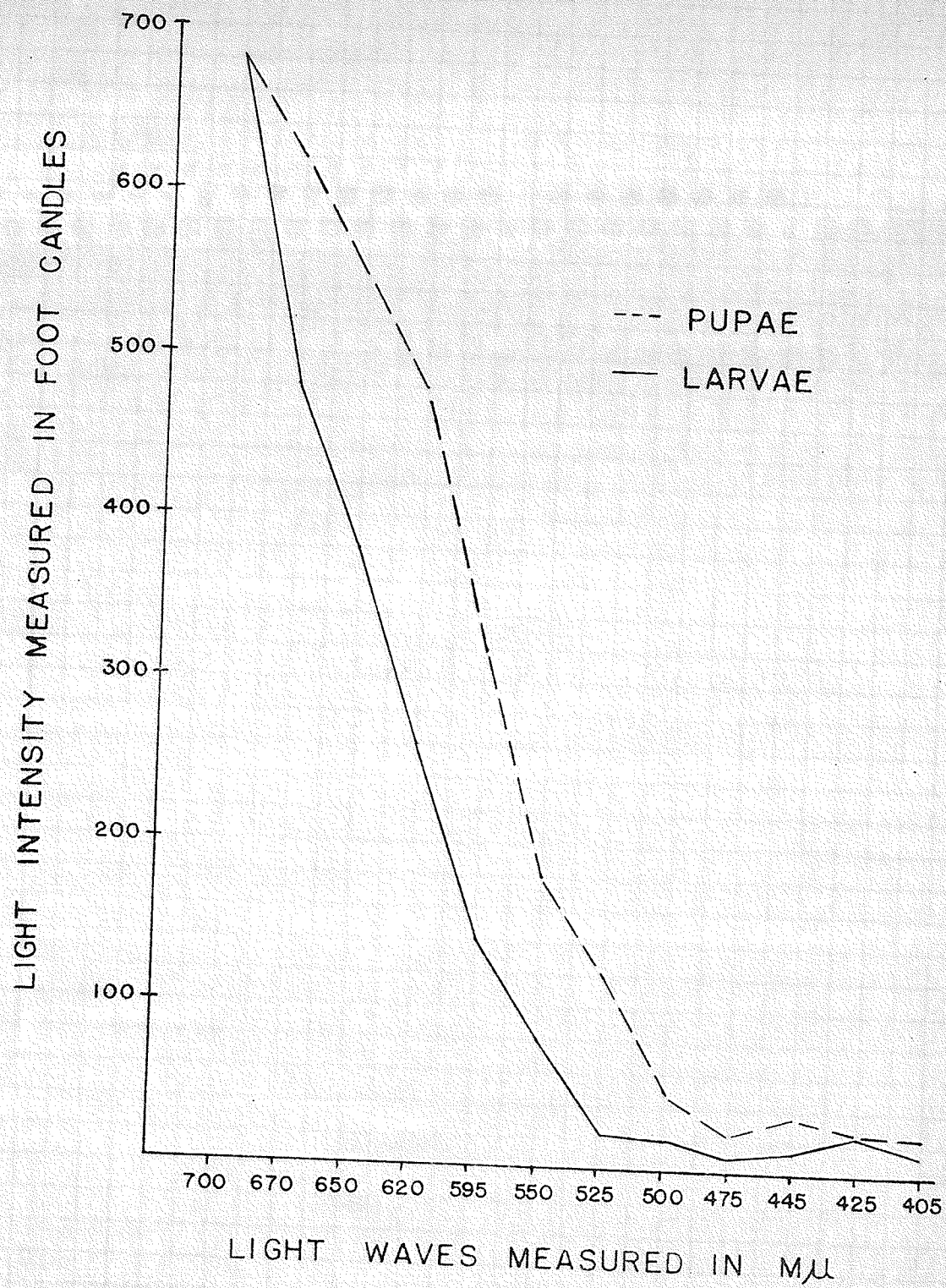


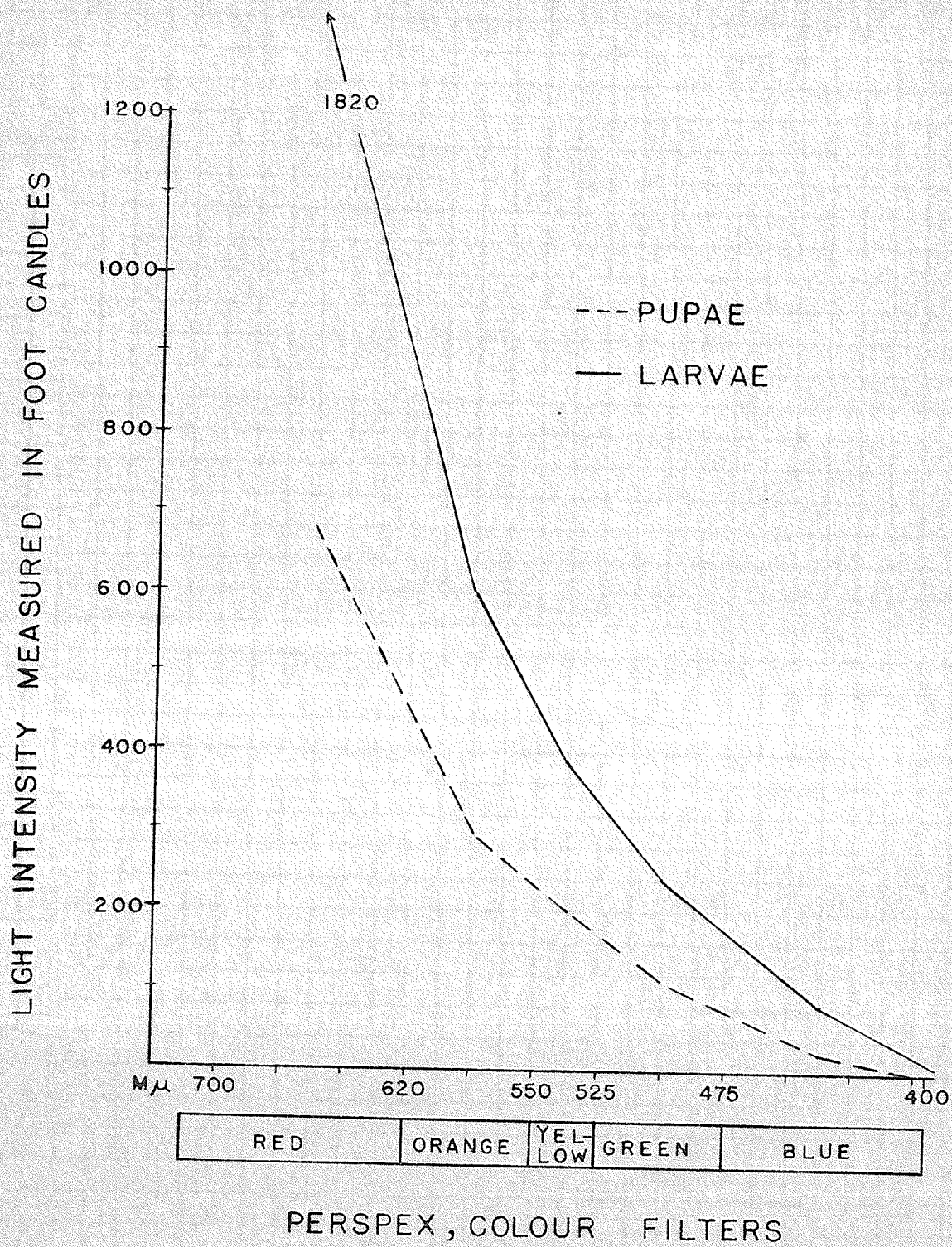
TABLE VII

THE REACTION OF PUPAE AND FOURTH INSTAR LARVAE TO
MONOCHROMATIC LIGHT⁽¹⁾

Colour	Wave Length in m μ	Mosquito Species							
		<u>A. aegypti</u>		<u>A. spencerii</u>		<u>C. inornata</u>		<u>A. vexans</u>	
		Lar.	pupa	Lar.	pupa	Lar.	pupa	Lar.	pupa
		Light Intensity in Foot Candles							
Red	700	680	680	520	520	510	510	400	400
	670	500	620	380	460	420	450	320	360
	650	400	560	290	400	300	380	280	340
	620	250	480	240	310	250	290	250	290
Orange	595	150	290	180	280	180	220	160	275
Green	550	80	160	120	220	140	165	150	240
	525	25	110	75	160	80	110	90	180
Blue-Green	500	15	55	50	140	45	40	60	160
	475	10	20	25	30	10	20	16	20
Blue	445	20	30	45	100	40	25	50	98
	425	25	25	30	55	20	30	42	76
Violet	405	15	25	30	35	15	25	18	20
Ultra-violet	Ultra-v fluorescent lamp	1	3	1	4	1	3	1	2

(1) Figures presented in the table are minimal light intensities required to produce a negative phototactic response at the given light intensities.

Figure 7. Preliminary Experiments with Aedes aegypti.
Minimal Intensities of Coloured Light Required to
Repel the Larvae and Pupae.



high light intensities (680 foot candles). Larvae are more sensitive to "blue-green" light than to blue and violet. This effect is displayed better in the table than in the graph.

In tests with chromatic filters, pupae were less sensitive to light than larvae, but in preliminary experiments with perspex filters, this comparison was reversed. This may be due to one or two reasons:

- (1) pupal reactions may change with age as occurs in larvae (Omardeen, 1957),
- (2) the different experimental procedure and criteria of response in the two series of experiments may involve different behaviour patterns.

ADULT MOSQUITO REACTION TO COLOURED LIGHT

Introduction

In 1910 a famous ophthalmologist, Professor C. von Hess investigated the colour sense of fishes, insects and other lower animals. He tested them while they were in the positive phototactic condition (attracted to light) and found that in a light spectrum the animals always collected in the green and yellow-green region. This is the brightest part of the spectrum for the colour-blind human eye. Therefore von Hess asserted, fishes and invertebrates and in particular insects are totally colour-blind (von Frisch, 1950).

A colour-blind person, for example, can distinguish between a red and blue surface because they reflect different degrees of light and appear as shades of grey. Von Hess (1910) did not investigate whether his animals responded to a colour sense or to light intensity. Von Frisch, however, working on the colour sense in bees compared different coloured cards to a series of grey cards. The reflectance of red, orange, yellow, green, blue and ultraviolet were compared to an equivalent reflectance in grey. On the basis of bee behaviour he concluded that bees could distinguish at least four colours: yellow, blue-green, blue and ultraviolet.

The colour sense of field mosquitoes was investigated by the writer, and light was used as the attractant. Experiments were designed to determine whether mosquitoes responded to different wave lengths of light or to light intensity.

Method

New Jersey light traps were used for the colour experiments and fitted with white and coloured 25 watt bulbs¹. Red, yellow², orange, green and blue lamps were selected. By means of rheostats the light intensity (output) per bulb was standardized at 100 foot candles. With light intensity for all bulbs being equal, the only apparent variable to which the mosquitoes were subjected, was light wave length. A 6 x 6 latin square experimental design was used. The light bulbs were rotated from trap to trap to eliminate the "trap location" effects. The records for three nights are presented in table VIII and the treatment means were compared by Keul's method.

Results

Collections from blue and green lamps were similar, but captures from either one were higher than from other colours. Traps with red, yellow and orange lamps captured very few mosquitoes. The yellow lamp (insect repellent) was found to be more attractive than red or orange lamps (Table VIII).

Conclusion

Mosquitoes were able to distinguish at least two colours; blue and red. The reaction to white light was a combined response to red and blue. The blue light consistently attracted higher numbers than

¹Westinghouse Electric Company Ltd.

²"Bug-Away" lamp

TABLE VIII

ADULT MOSQUITO ATTRACTION TO COLOURED AND WHITE LIGHT AT
EQUAL LIGHT INTENSITIES

Date	Blue	Green	White	Yellow	Orange	Red
Number of Mosquitoes Collected per Trap						
June 20	164	152	46	29	24	16
23	130	146	38	27	16	10
27	98	118	36	18	10	9
30	172	151	59	33	27	18
July 4	149	168	51	22	22	14
7	<u>151</u>	<u>106</u>	<u>27</u>	<u>20</u>	<u>25</u>	<u>18</u>
Total	864	841	257	142	124	85
$\sqrt{X}$ Trans- formation of Trap counts	71.69	70.7	39.1	29.8	26.9	22.3
Treatment Mean $\sqrt{X}/6$	11.95	11.45	6.52*	4.97	4.48	3.72**
Grouping of Like Means		11.20**	6.52*		4.39*	

** Significant at the 1% level

* Significant at the 5% level

white light. The efficiency of the New Jersey Light Traps could be increased by using blue lamps instead of the standard white lamp. This is provided that the blue lamp emits light at the same intensity as the white lamp.

ADULT MOSQUITO REACTION TO WHITE LIGHT AT DIFFERENT LIGHT INTENSITIES

Mosquitoes common to northern regions find shelter from the sun in different types of vegetation. They become active during the evening and on warm days when the sun's rays are hidden by clouds. In the laboratory they are repelled by intense light but are attracted to low light intensities.

Method

Investigations were concerned with the reaction to increased light intensities in the New Jersey Mosquito Light Trap. White frosted filament type bulbs, 25, 60, 100 and 150 watt were used in the tests and the trap hoods raised three inches to permit full view of the lamps. Each lamp was set at an equal height from the ground. A 4 x 4 latin square experimental design was used and the lamps were rotated from trap to trap to avoid "trap position effects". The figures in the table represent a two night collection and Keul's method of comparing treatment means was used in the analysis.

Results

The light trap collections increased directly with the light intensity in foot candles (Table IX). The 150 watt lamp, being 2.8 times brighter than the 25 watt lamp, collected 2.5 times as many mosquitoes. The trap location differences were not significant at the 5% level. The data are not included but when 25 watt lamps were used it was observed that the light traps were more effective when the trap hoods were raised than when they were lowered.

TABLE IX

ADULT MOSQUITO ATTRACTION TO WHITE LIGHT AT DIFFERENT
LIGHT INTENSITIES

Wattage	25	60	100	150
Light Intensity (Foot Candles)	2000	3600	5200	5600
Collection Date	Number of Mosquitoes Captured per Trap			
May 30, 1960	16	32	48	92
June 1, 1960	26	55	81	93
June 3, 1960	10	15	17	26
June 5, 1960	<u>22</u>	<u>113</u>	<u>146</u>	<u>252</u>
Total	74	215	292	463
$\sqrt{X}$ Transformation	16.9	27.4	32.1	40.05
Treatment Means $\sqrt{X}/4$	4.22	6.85	8.02	10.10*

* Significant at the 5% level



Conclusions

The efficiency of the New Jersey Mosquito light traps was increased by: (1) using an increased light source, (2) raising the trap hoods to allow full view of the lamp.

MOSQUITO RESPONSE TO BLACK-LIGHT

Introduction

Although extensive studies have been made to indicate the response of insects to light intensities, including ultraviolet; little information is available concerning the reaction of insects to black-light with wave lengths in the range 300-400 millimicrons. Frost (1953) investigated the number of insect families attracted to fluorescent light compared to white filament type lamps.

Ultraviolet or Black-Light Mosquito Trap

Frost (1953) compared a 2 watt black-light lamp to a 7 and a 15 watt white filament lamp. He found black-light to be more attractive than white to numerous insect families, but the white bulb attracted more mosquitoes.

Investigations carried out by the writer were concerned with the attraction of northern mosquitoes to black-light.

Method and Material

The New Jersey Light Trap was used but the standard 25 watt filament type lamp was replaced by a 15 watt fluorescent black-light (15 TBLB)⁽¹⁾ (Figure 8). Two black-light traps were constructed in this way and compared to Standard New Jersey Traps at Oak Bluff and Kildonan Park. These traps stood 10 feet apart and were operated from July 10th to September 3rd, 1959. The lights were turned on by a time

⁽¹⁾ General Electric Co., Ltd.

Figure 8. Ultraviolet or Black-light Trap

• JUL • 60



clock at 7 p.m. and off at 7 a.m. Collections were made semi-weekly. The data presented in Figure 18 represent equal time intervals.

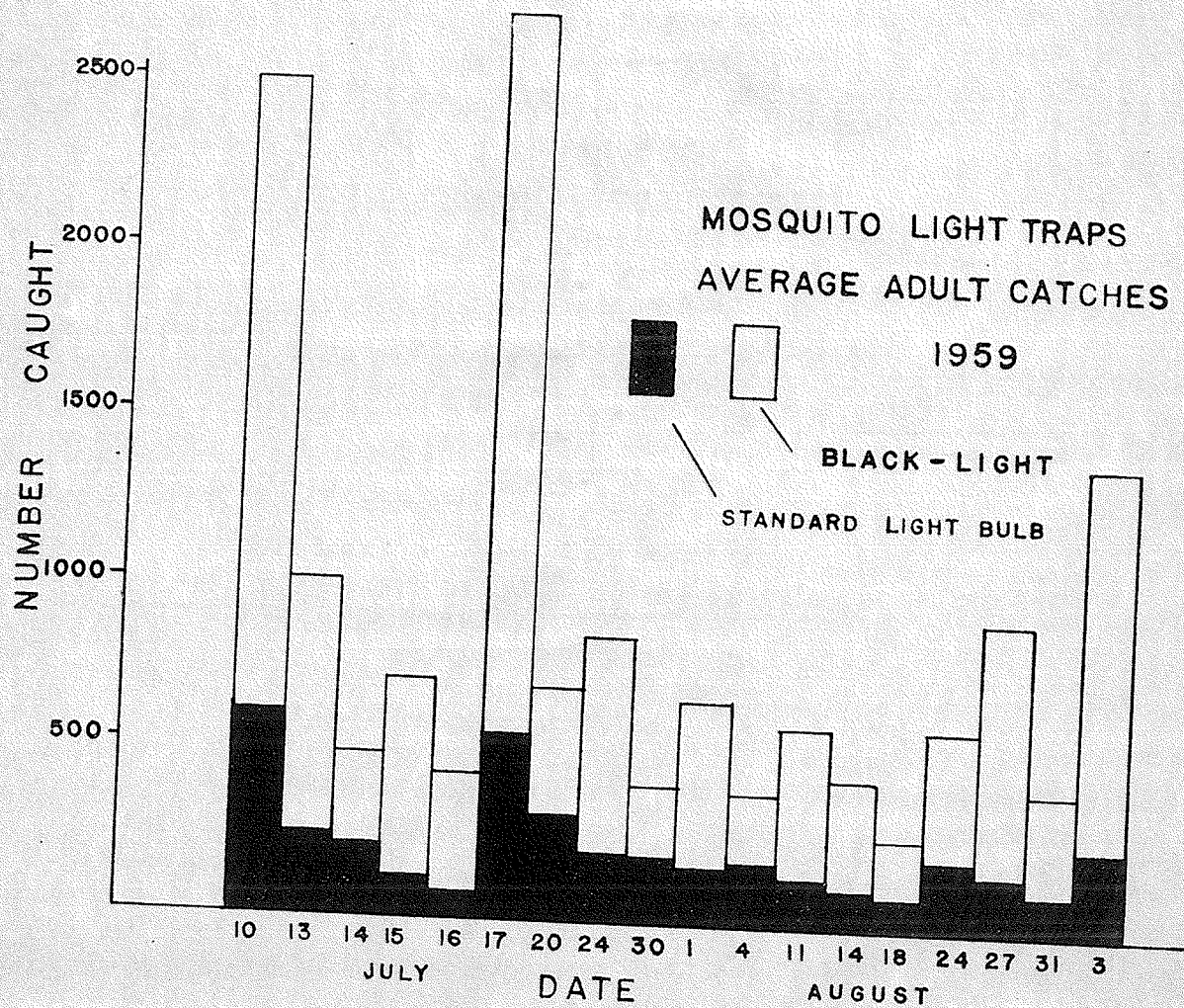
Results

The ultraviolet trap collected 4-5 times more mosquitoes than the Standard New Jersey Trap (Figure 9).

Conclusion

Due to the higher catches obtained from the ultraviolet light traps these traps would be useful in recovering tagged adult mosquitoes. The ultraviolet light trap was found to perform relatively well in highly "competitive" areas, i.e., where traps were surrounded by distracting city or farm lights.

Figure 9. Ultraviolet or Black-light trap Catches Compared to Those Taken in the Standard New Jersey Incandescent Light Traps.



CHAPTER V

MOSQUITO SPECIES TAKEN IN THE GREATER WINNIPEG AREA

1959 AND 1960

Classification and Relative Abundance

A knowledge of mosquito species is of taxonomic and practical importance. Species information is necessary to evaluate: (1) the level of migration and, (2) the abundance of disease vectors in an area. From the standpoint of mosquito abatement, control methods are determined by blood appetite, oviposition preferences and longevity of the adult female. The data concerning Culiseta inornata and Culex tarsalis, presented later, illustrate the significance of identification.

During 1960 larvae were collected from breeding areas in and around Winnipeg. A major part of the collection was made during the larval and pupal survey conducted on these areas. In order to obtain a random sample, 200 larvae were taken from each breeding area whenever possible.

Adult mosquitoes were collected in Standard New Jersey Mosquito light traps. Three traps were operated "outside"⁽¹⁾ and five "inside" the district boundaries during 1960.

Results and Discussion

Collections of larvae consisted of 14 species (Table XI).

(1) Outside the area controlled by the Greater Winnipeg Mosquito Abatement District (GWMAD).

Collections of adults consisted of 25 species. All major species occurred in both larval and adult collections. Throughout 1960, Culiseta inornata occurred consistently in larger numbers. In May and June, this species was represented only by females which had overwintered. Males of this species were found on July 7. This indicated that a new generation had begun. Aedes vexans was the most abundant species in the larval collections but represented a lower percentage of the adult collection (Table X). The reason for this was that breeding areas dried up before the larvae reached maturity. Most of the larval collections were made therefore during May and June, and the figures presented in the tables record larval, adult and "biting" collections from May 2 to July 18. Aedes vexans made up a larger part of the adult collection during the latter part of July (not shown in the tables).

Aedes dorsalis made up a large part of the larval collections but occurred in very low numbers in the adult collection (Table X). This species favoured breeding locations high in organic matter and were found at the Sugar Beet Factory and the Union Stockyards. The adults appear to be less attracted to light than other species.

Culex tarsalis, an important vector of encephalomyelitis was not found in large numbers. It represented 0.9 percent of the adult collection and no larvae were found. This means that the Winnipeg area is relatively free from virus carriers of this species.

In the biting collections, Culiseta inornata was not recorded in spite of its large numbers taken in the adult trap collections.

A COMPARISON OF THE RELATIVE ABUNDANCE OF THE MOSQUITO SPECIES OCCURRING IN THREE TYPES OF SURVEYS 1960

Larval Collections *	Light Trap Collections *	"Biting" Collections *
Total 22,040	Total 4,812	Total 122
Aedes vexans	42.5%	Culiseta inornata 35.2%
Aedes dorsalis	29.1%	Aedes vexans 30.5%
Culiseta inornata	5.73%	Aedes riparius 11.1%
Aedes spencerii	5.3%	Aedes flavescens 8.8%
Aedes riparius	5.15%	Aedes dorsalis 2.9%
Aedes idahoensis	3.91%	Aedes spencerii 2.0%
Aedes trichuris	3.68%	Anopheles earlei 1.8%
Aedes stimulans	2.23%	Aedes fitchii 1.1%
Aedes canadensis	1.13%	Aedes intrudens 1.0%
Culiseta minnesotae	0.05%	Aedes barri 0.8%
Aedes flavescens	0.04%	Culex tarsalis 0.8%
Aedes fitchii	0.04%	Aedes trichuris 0.7%
Culex apicalis	0.04%	Culex restuans 0.7%
Aedes sticticus	0.02%	Aedes excrucians 0.5%
		Mansonia perturbans 0.3%
		Aedes punctator 0.3%
		Others 1.14%

* Mosquito species are listed in order of their decreasing numbers

Barr (1958) reports that Culiseta inornata may bite humans in early spring but is reluctant to bite later in the summer.

Figures presented in Table XI show the relative abundance of mosquito species taken from outside and inside collections. Those species occurring in large numbers outside the district were also represented in high numbers in inside catches. If there was an influx of adults from outside areas, it was only slight.

MOSQUITO SPECIES AND RELATIVE ABUNDANCE INSIDE AND OUTSIDE THE GREATER WINNIPEG MOSQUITO ABATEMENT DISTRICT, 1960

Larval Collections *	INSIDE		OUTSIDE				
	Adult Trap Collections *	Larval Collections *	Adult Trap Collections *	Adult Trap Collections *			
Aedes vexans	48.3%	Culiseta inornata	42.6%	Aedes vexans	48.1%	Culiseta inornata	38.2%
Aedes dorsalis	30.6%	Aedes vexans	21.4%	Culiseta inornata	16.5%	Aedes vexans	17.6%
Culiseta inornata	12.9%	Aedes riparius	21.2%	Aedes spencerii	15.1%	Aedes flavescens	12.3%
Aedes spencerii	3.1%	Aedes flavescens	2.1%	Aedes riparius	14.2%	Anopheles earlei	6.5%
Aedes flavescens	1.1%	Anopheles earlei	1.7%	Aedes idahoensis	3.1%	Aedes riparius	4.4%
Aedes stimulans	1.1%	Aedes barri	1.6%	Aedes trichuris	3.0%	Aedes fitchii	3.8%
Aedes canadensis	0.94%	Aedes punctor	1.5%	Culex apicalis	0.4%	Aedes barri	3.7%
Aedes riparius	0.7%	Culex restuans	1.4%	Aedes sticticus	0.2%	Aedes punctor	2.1%
Culiseta minnesotae	0.6%	Aedes triseriatus	1.3%			Aedes spencerii	1.7%
Aedes fitchii	0.5%	Culex territans	1.2%			Aedes intrudens	1.7%
		Aedes dorsalis	1.1%			Aedes dorsalis	1.5%
		Aedes spencerii	1.1%			Aedes trichuris	1.1%
		Aedes fitchii	0.9%			Aedes diantaeus	0.8%
		Aedes intrudens	0.9%			Aedes excrucians	0.8%
		Culex tarsalis	0.9%			Anopheles walkeri	0.6%
		Others	2.7%			Others	2.5%

* Mosquito species are listed in order of their decreasing numbers

PART B - MOSQUITO CONTROL

CHAPTER VI

MOSQUITO CONTROL OPERATIONS OF THE GREATER WINNIPEG MOSQUITO

ABATEMENT DISTRICT

Pre-Season Applications of DDT

Pre-seasonal control of mosquitoes with oils began experimentally in the Winnipeg area in the year 1947. This consists of a winter spraying program designed to prevent the hatching of eggs or to kill the larvae as they hatched in the spring. Currently the Greater Winnipeg Mosquito Abatement District uses air and ground sprays between seasons over an area of 225 square miles.

Pre-seasonal control started in earnest in 1955 when 150 acres were sprayed with DDT granules from a truck. In 1956-57 the area covered from the air was 6,100 acres. In 1958-59, pre-season operations on the ground and in the air covered more than 18,000 acres (Table XII).

Tobacco stem granules impregnated with 15% DDT were used in 1955, but when tobacco stems were no longer available the District turned to vermiculite. Vermiculite impregnated with 15% DDT proved as effective as DDT in tobacco stem base (or carrier). Vermiculite granules impregnated with 30% DDT were used experimentally in 1957. Due to the greater payload in the aerial operations, the cost per acre to the District was considerably reduced. The survey conducted by Smith (1959) showed that the pre-season applications of 30% DDT in vermiculite controlled breeding of larvae throughout one entire season. The granules were

TABLE XII

PRE-SEASON ACREAGES TREATED BY THE GREATER WINNIPEG MOSQUITO
ABATEMENT DISTRICT

Year	Aircraft Application 30% DDT Impregnated Granules	Truck Spray 15% DDT in No. 2 Fuel Oil	Total Acreage
1960	8,536	4,639	13,175
1959	10,856	7,699	18,555
1958	13,917	3,160	17,077
1957	6,117	1,811	7,928
1956	564	1,327	1,891
1955	0	150	150

deposited at the rate of 1.1 lbs. of active ingredient per acre. A single-engined Piper Cub was used for aerial applications and DDT granules were effectively spread over bushland areas inaccessible to ground driven equipment. Roadside ditches and accessible low lying areas were controlled with truck mounted aero-mist⁽¹⁾ blowers using 15% DDT in oil at a rate of 1.1 lbs. of DDT per acre (Figure 10, A).

Summer Spraying of Flood Waters and Rain Pools

The largest part of the summer operation was done by surface crews and aero-mist blowers. Surface crews operated portable compressed air sprayers to apply 1.7% DDT and .25% Pyrethrin in water (Table XIII). No. 2 fuel oil was used experimentally instead of water in 1960 and appeared to provide a longer period of control. Ornamental pools, rain barrels and small permanent pools were controlled with DDT and lindane bombs. Where insecticide could not be used, Top Minnows Gambusia affinis were placed in the water (Table XIII).

Roadside ditches and semi-permanent pools accessible to truck-operated blowers were controlled with 7% DDT in water (Figure 11). Due to susceptibility of trees and shrubs to insecticide during the summer, the DDT percentage was decreased to 6 and later to 5% in water. River and rain-flooded areas were controlled in spring and summer by aerial sprays of 5% DDT in water. An area of 2,020 acres was treated in 1956, 3588 acres in 1957 and 2,357 acres in 1960.

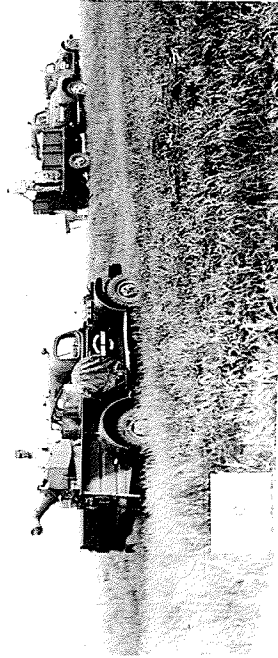
¹Turbine Liquid Atomizer

Figure 10. Pre-season Operations for Larval Control:

- A. "Aero-Mist" blower - Liquid applications of DDT
- B. Aircraft applications - DDT impregnated granules

Summer Adult Control Operations:

- C. Vegetation "Barrier Spray"
- D. Roadside Fogging



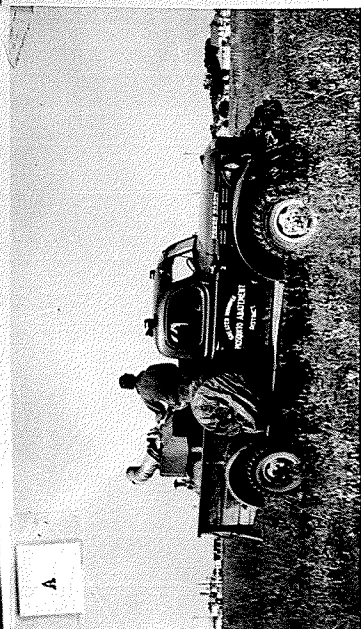
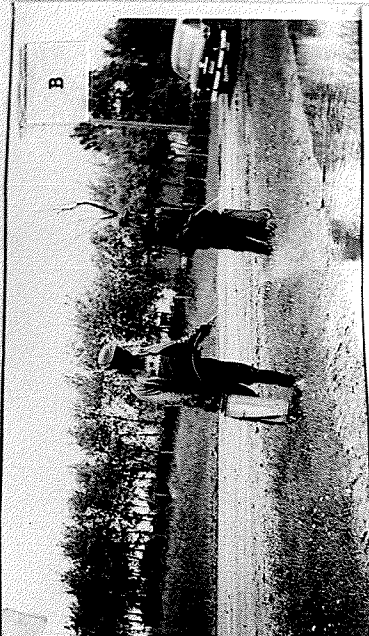
EQUIPMENT AND INSECTICIDES FOR CONTROLLING MOSQUITO LARVAE ACCORDING TO SITUATIONS 1959 AND 1960

Situation	Equipment Owned and Operated by the Greater Winnipeg Mosquito Abatement District	Insecticide	DOSAGE	
			Quantity per acre	Active in- gredient per acre
Any Breeding Area in Late	Aircraft	Piper Cub 30% DDT in Vermicu- lite (rented)	3 1/4 lbs.	1 lb.
Fall or Early Spring before	Lawrence Aero-Mist Blower	15% DDT in No. 2 fuel oil - Fall only	1 gal.	1.3 lbs.
Eggs Hatch. "Pre-season		7% DDT in No. 2 fuel oil - Spring work	1 1/2 gal.	1.1 lbs.
Operation".	Portable Compressed air Sprayer	2% DDT in No. 2 fuel oil	3 gals.	.6 lbs.
Flood waters, Rain Pools,	Lawrence Aero-Mist Blower	DDT in Water: 7% early spring; 6% early summer; 5% mid- summer.	1 1/2 gal.	1.1, 0.9 and 0.75 lbs. respective- ly
Permanent Pools, Roadside Ditches and Catch Basins		0.25% Pyrethrin is added to control pupae.		
"In-Season Operation"	Aircraft	5% DDT in Water	1.6 qts.	0.2 lbs.
	Portable Compressed-air Sprayer	1.7% DDT in No. 2 fuel oil + 0.25% pyrethrin	2 gals.	0.34 lbs.
	DDT and Lindane Bombs	12.5% DDT + 4.5% Lindane		0.2 lbs.
	Fish - <u>Gambusia affinis</u>	used in 4 ponds		
Ornamental ponds and	DDT and Lindane Bombs	12.5% DDT + 4.5% Lindane		0.2 lbs.
Rain Barrels	Hand Sprayers	0.25% pyrethrin + 1.7% DDT in water		0.2 lbs.
	<u>Gambusia affinis</u>			

Drainage: Cleaning,
Culverts, opening ditches,
Hand Equipment, draining
low lying areas, etc.

Figure 11. In-season Larval Control Operations

- A. "Aero-Mist" blower
- B. Portable compressed-air sprayers
- C. Airplane spraying operations
- D. Control of catch basin breeding



The Greater Winnipeg Mosquito Abatement District was not responsible for drainage work. However, when such work was neglected by the local authorities, ground crews opened ditches, culverts and drained low lying areas.

Space Fogging Operations

Fogging operations were conducted by the Greater Winnipeg Mosquito Abatement District to destroy adult mosquitoes in residential areas, parks and playgrounds (Figure 12). Fogging in parks was done during the day but residential areas were treated at night. The night crew began at 11 P.M. and ended at 8 A.M. Traffic on the city streets during these hours, was at a minimum. The entire district was covered in 10-11 days after which the operation was repeated if necessary. The equipment and insecticides used for fogging are presented in Table XIV).

In 1959, fogging of parks and residential areas began on June 18 and ended August 19.

In 1960, due to complaints of mosquito annoyance early in the season, fogging of parks began May 30 and in residential areas on June 6.

Figure 12. Adult Fogging Operations

- A. Portable "Swing Fogs" operated in city parks
- B. "Harry M", Fogging of tree areas on the banks
of the Red River
- C. Fog delivered by truck-mounted "Tifa" fogger;
view is of the grandstand at the Winnipeg Stadium
- D. Swing Fogs used in areas inaccessible to vehicles

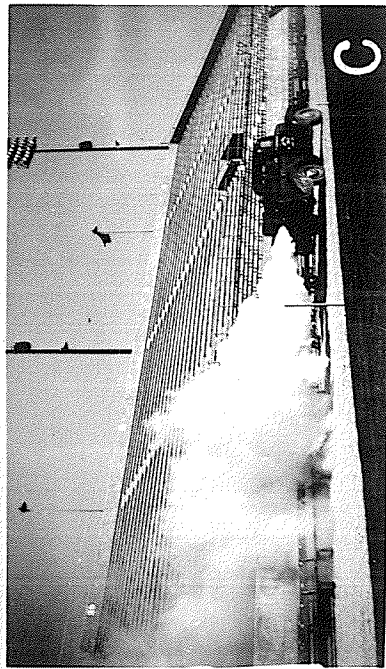
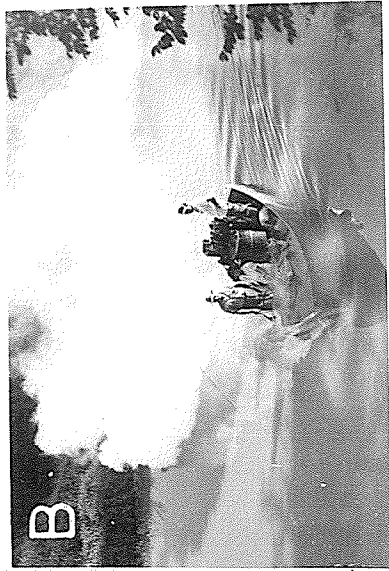
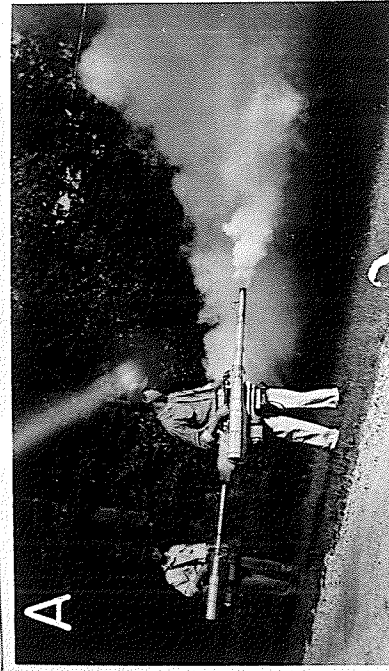


TABLE XIV

EQUIPMENT AND DDT FORMULATIONS FOR THE CONTROL OF ADULT MOSQUITOES IN VARIOUS SITUATIONS, 1959 AND 1960

Situation	Equipment Owned and Operated by the Greater Winnipeg Mosquito Abatement District		Insecticide	Dosage Active Ingredient per Acre
	District			
Space Fogging: Residential areas, Playgrounds, Parks, Golf Courses, Picnic Areas, Service Clubs, Community Clubs, Banks of the Red Assiniboine and Seine Rivers.	Truck - Mounted "Tifa" Foggers	6	7% DDT in No. 2 Fuel and 0.25% Pyrethrin (pyrethrin for parks only)	1 lb.
	"Harry M" (Tifa Fogger mounted on the mosquito boat)	1	7% DDT in No. 2 Fuel oil	1 lb.
	Portable powered "Swing Fogs"	2	7% DDT in No. 2 Fuel oil and 0.25% pyrethrin (pyrethrin for parks only)	1 lb.
Residual Spray on Vegetation	Aircraft	1 (rented)	5% DDT in Water	0.2 lb.
	"Barrier" Ground Spray	Lawrence Aero-mist Blowers	2	7% DDT in No. 2 Fuel oil

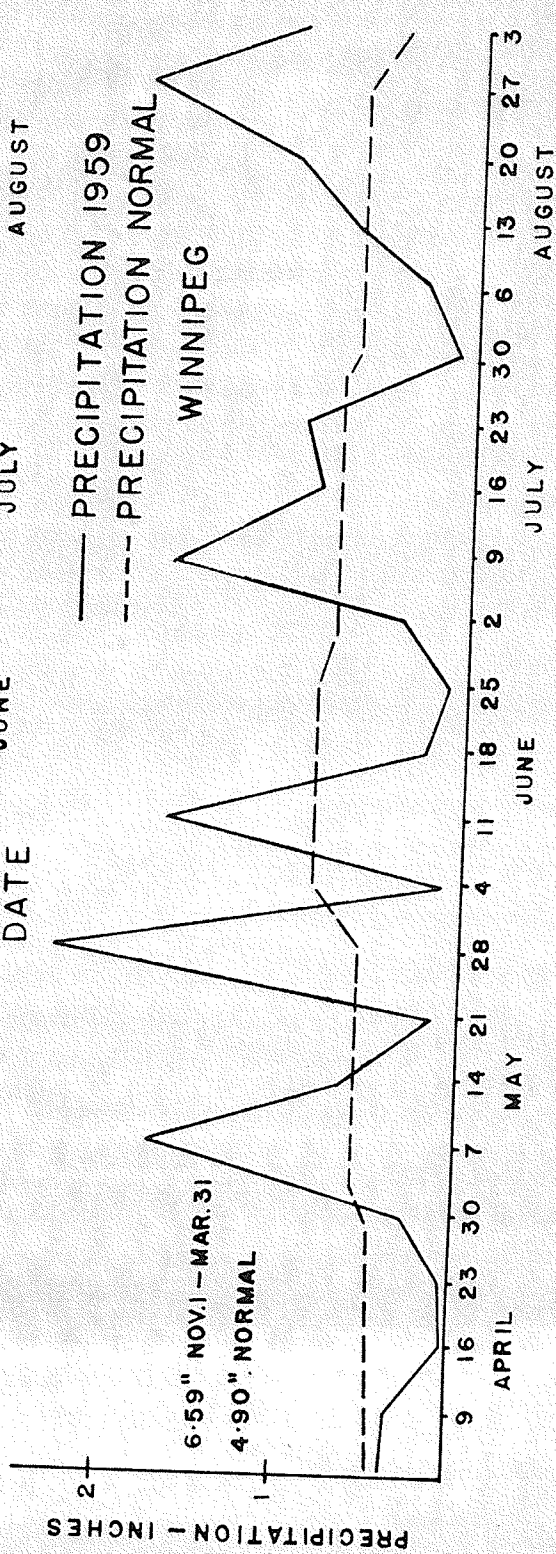
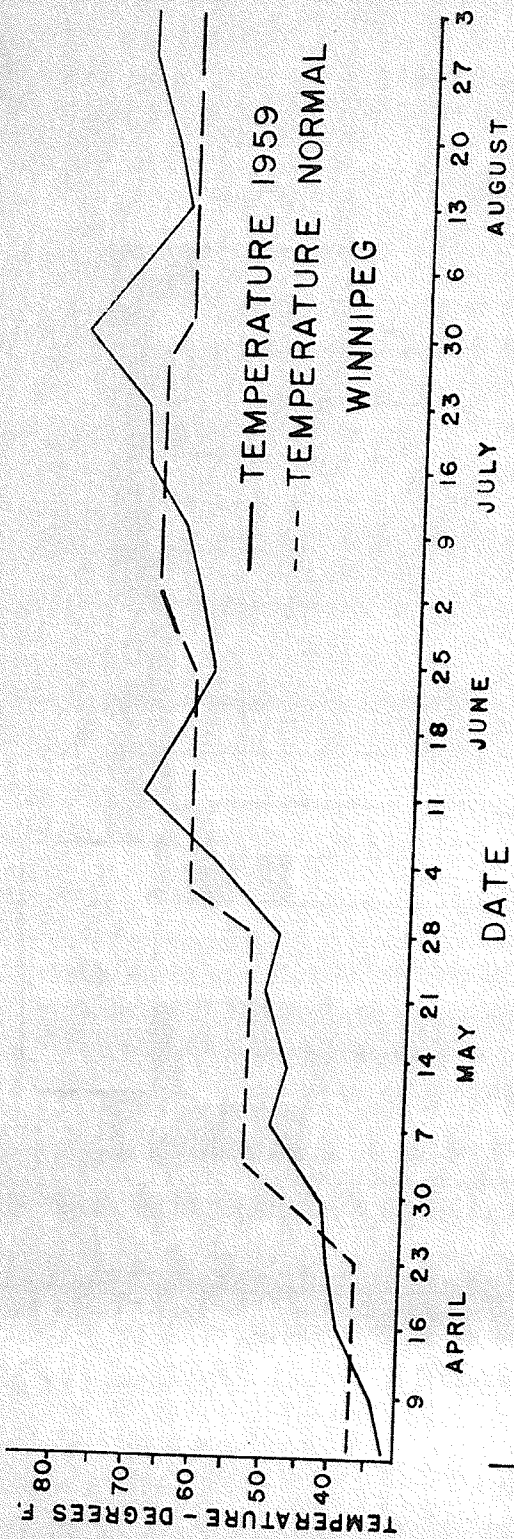
Precipitation in the Greater Winnipeg Area 1959 and 1960

Due to the flat terrain and heavy soil in and around Winnipeg, drainage from low lying areas is poor. Permanent and semi-permanent rain and flood pools are ideal places for mosquitoes to breed. Heavy precipitation during the summer fills many of the low lying areas and this results in higher mosquito populations. Headlee (1945) states that there is no doubt that species of mosquitoes show greater success during seasons of heavy and well distributed rainfall, because that type of rainfall not only creates an enormous increase in number and size of pools but maintains them over a period of sufficient length to permit the completion of brood developments.

Precipitation (measured in inches of water) during the winter of 1958-59 was two inches above normal. After the spring thaw all permanent and semi-permanent pools were filled with water. In May, more than five inches were added to the already superfluous moisture and precipitation continued above normal each succeeding month to September (Figure 13). Mosquito breeding in non-treated areas was abundant throughout the summer.

Precipitation during the winter of 1959-60 was 1.5 inches below normal. In contrast to the previous year, rainfall recorded during May was very slight and remained below normal for June. The month of July was a record month for low precipitation and breeding areas largely disappeared (Figure 14). Mosquito populations during June and July were low in Winnipeg and the surrounding areas.

Figure 13. Temperature and Precipitation 1959



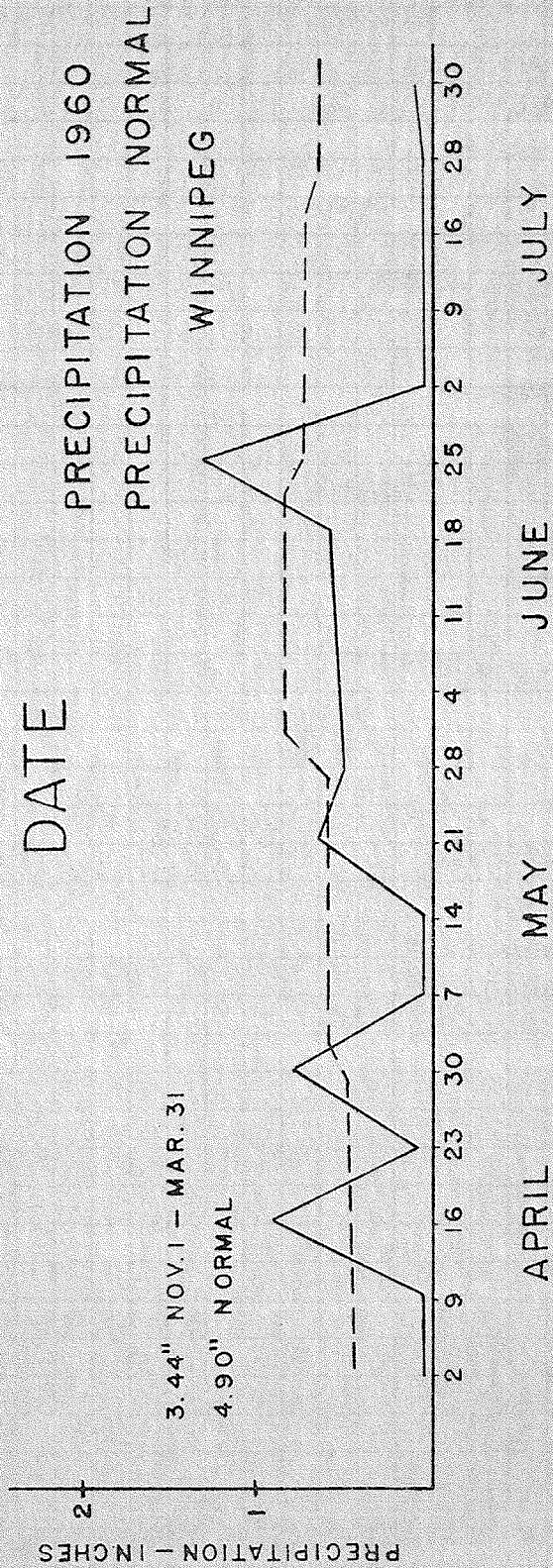
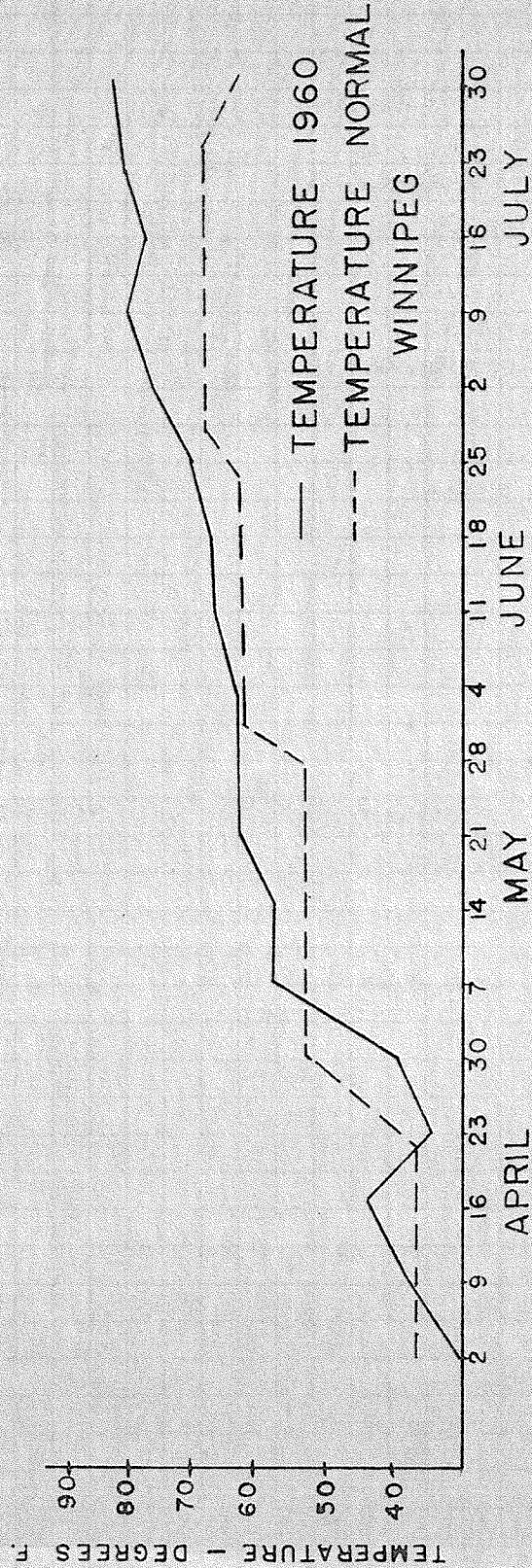
Temperatures in the Greater Winnipeg Area 1959 and 1960

Seasonal temperatures play an important part in mosquito breeding and development. Low temperatures decrease the number of eggs hatching and slow down the development of larvae and pupae while higher temperatures increase the rate of hatching and development. For this reason large flights of mosquitoes never occurred in the Winnipeg area with persisting low temperatures in spite of heavy precipitation. A precipitation peak, followed by high temperatures, usually results in a large flight of mosquitoes in areas around Winnipeg.

In 1959 the proper conditions of temperature and precipitation existed early in the season and a major peak of mosquito flight was reached on June 15 (Figure 13). This was more than a month earlier than seasonal peaks in 1957 and 1958 (Smith, 1959).

In 1960 temperatures were above normal during May, June and July (Figure 14). This, together with low precipitation, caused breeding areas to dry up before the larvae matured. As a result no major flight of mosquitoes was recorded during June or July as had occurred in previous years.

Figure 14. Temperature and Precipitation 1960



CHAPTER VII

ADULT POPULATION SURVEY

New Jersey Light Trap Catches

In order to obtain a true picture of the mosquito population, it was necessary to have some means of measuring the cross section of an area. Firstly, such measurements could be made by counting the "biting" or "landing" rates on human hosts. Data obtained in this way would give a true estimate of the troublesome mosquitoes in a particular location. However, if human hosts were to be used for a large survey area, there were several disadvantages: (1) a certain number of human hosts were required, (2) some humans were attacked more readily than others. A second method of sampling a mosquito population was by mechanical trapping. Collections of this order contained a high percentage of adults which did not attack humans (Culiseta inornata, Chapter V). On the other hand, trap collecting had certain advantages: (1) traps could be standardized to give an unbiased record (2) light traps were particularly effective in the evening when mosquito activity was at its "peak". For these reasons light traps were used for the adult mosquito survey.

Method and Equipment

The type of light trap used in this survey was the Standard New Jersey Light Trap (Figure 15). They were supported on tripods so that they hung approximately four feet from the ground. The traps were connected to electric timers which automatically turned the power on at

Figure 15. Standard New Jersey Mosquito Light Trap

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7 p.m. and off at 7 a.m. the next morning. The catches were collected twice weekly, sorted as to sex and counted.

In 1959 five light traps were operated inside and three outside the Greater Winnipeg Mosquito Abatement District (G.W.M.A.D.) boundaries. The "inside" traps were located at Kildonan Golf Course, Windsor Golf Course, Assiniboine Park, the University of Manitoba and the Government Greenhouse near the Legislative Buildings. The "outside" traps were located at Oak Bluff, west of the city, Lilyfield, north of the city and Navin School, east of the city (Figure 16). The light traps were operated from May 14 to September 3 in 1959.

In 1960 the light traps were returned to the same locations with the exception of Navin School. An additional trap was operated "inside" and was located in St. Vital north of the St. Boniface Sanitorium. The light trap data presented in the graph covers the period from May 2 to July 18 in 1960.

The locations of the traps were selected to provide similar conditions of environment and to give the best possible coverage of the Winnipeg area.

Results

In 1959, due to excessive moisture and high temperatures during May and June, mosquito flight activity increased rapidly (Figure 17). The major "peak" of mosquito flight was reached on June 15, one month earlier than the two previous years. Major flight "peaks" occurred on July 12, 1957 and July 22, 1958. Three smaller peaks occurred on June 25, July 27, and August 27, 1959. The mosquito flight peaks followed peaks of precipitation by approximately two weeks. On June 15, light

Figure 16. Locations of the New Jersey Light Traps 1959 - 1960

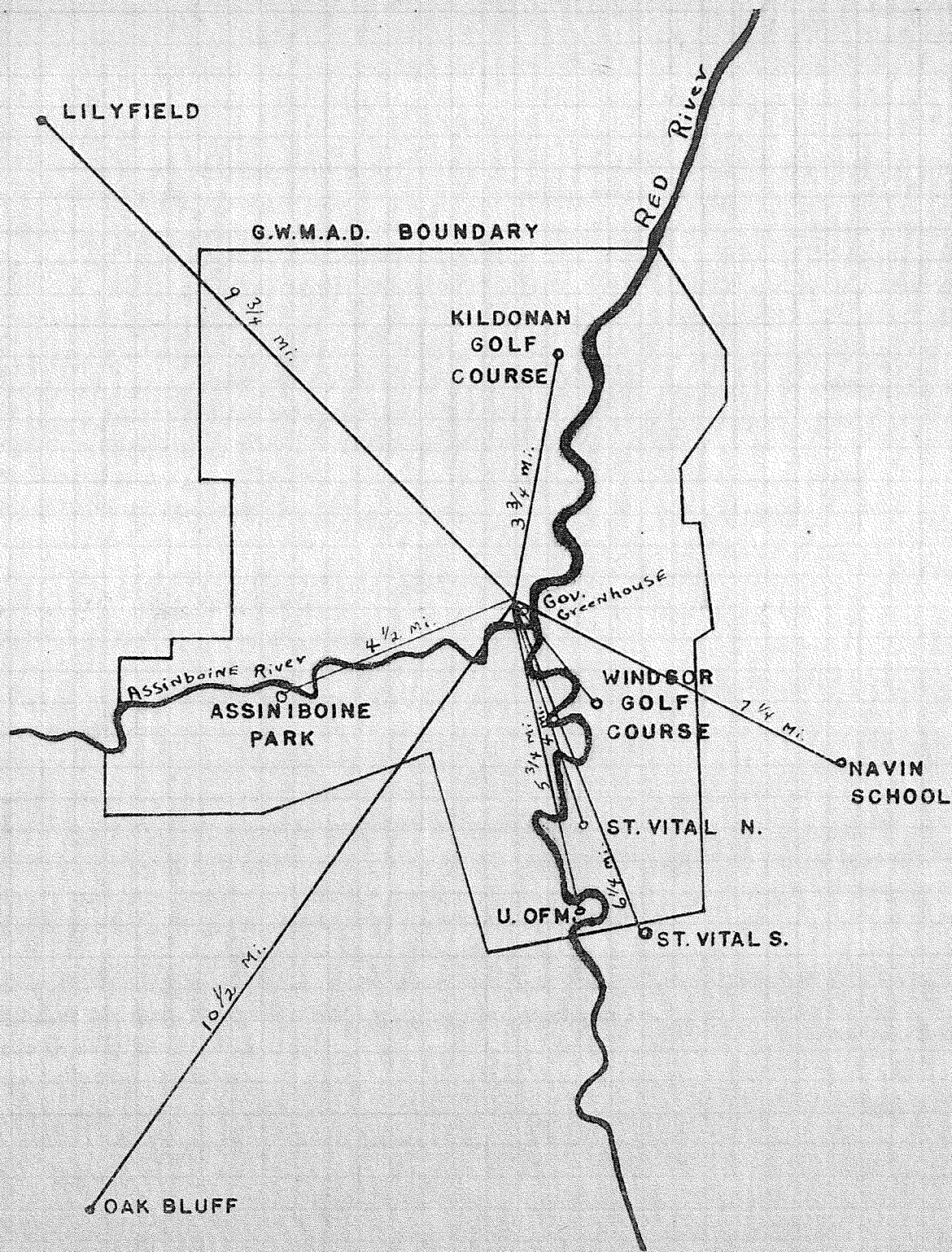
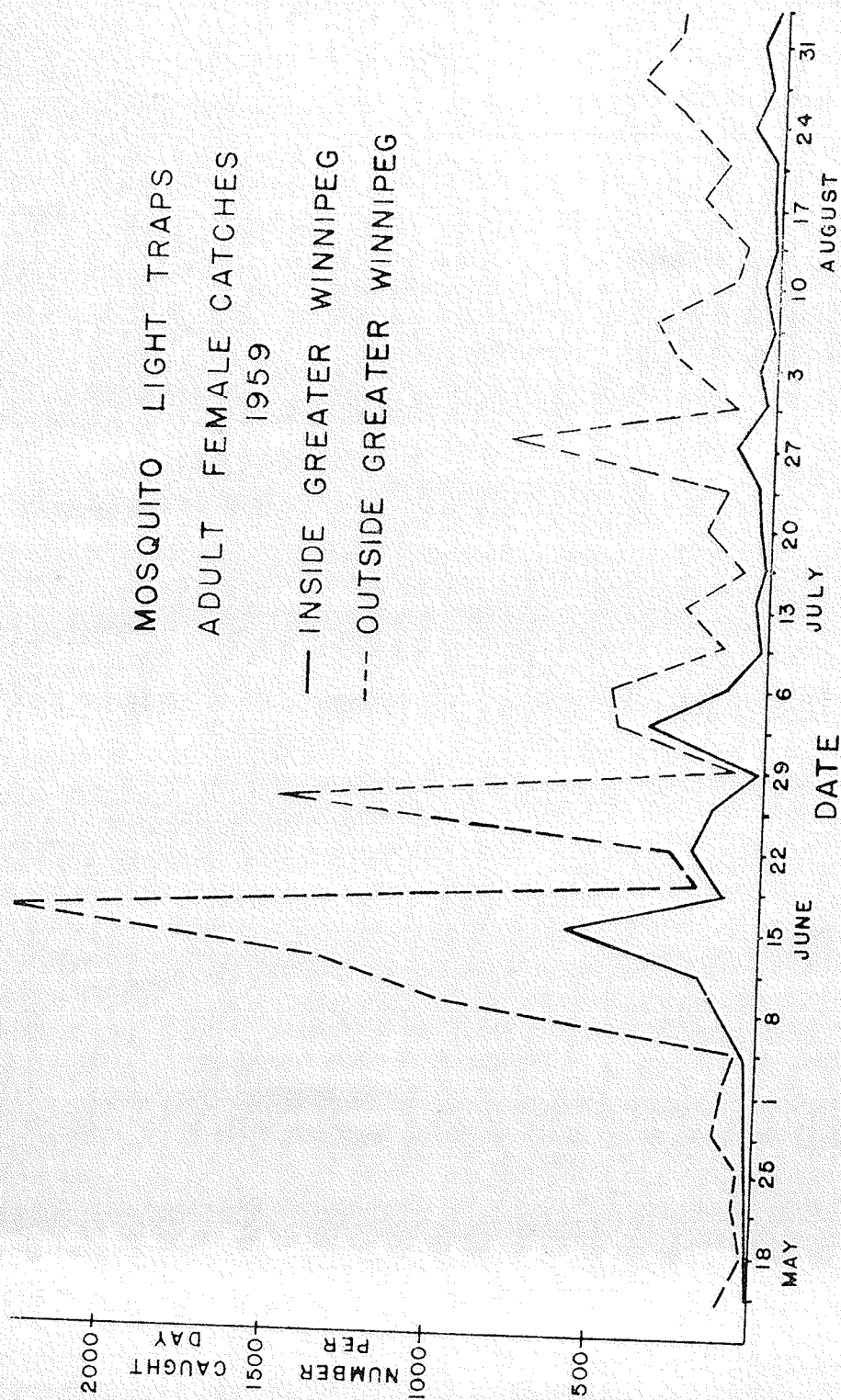


Figure 17. New Jersey Mosquito Light Trap Collections 1959



traps inside the city averaged 700 females per night while outside traps averaged 2,000 females per night (Appendix II and III).

In 1960, due to extremely low precipitation and relatively high temperatures, mosquito populations were low throughout the season (Figure 18). In contrast with light trap data from previous years, no major flight "peak" was recorded during June or July. The average female collection inside the district was 24.4 per night while outside traps collected an average of 60.5 per night (Appendix IV and V).

Figure 18. New Jersey Mosquito Light Trap Collections 1960

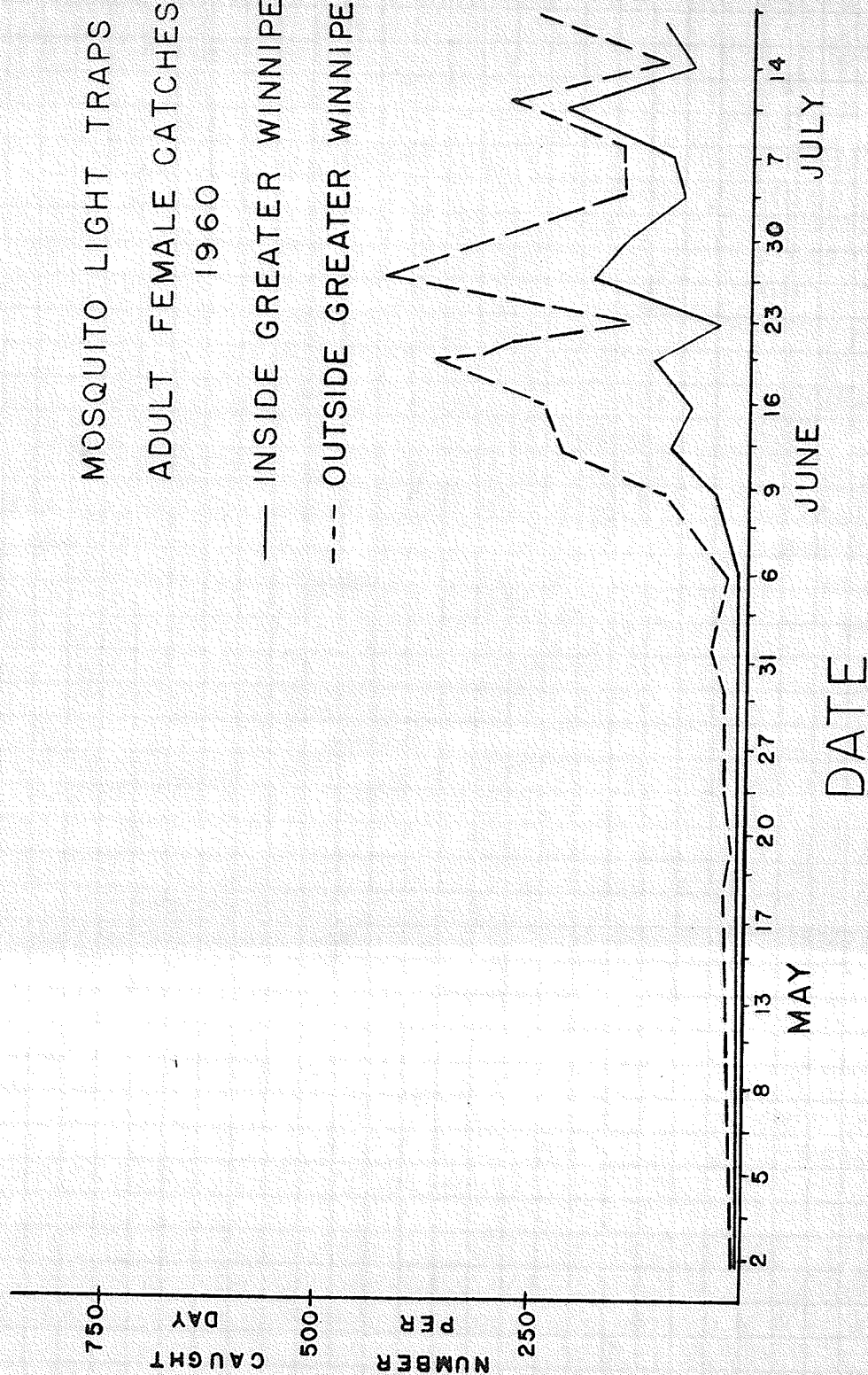
MOSQUITO LIGHT TRAPS

ADULT FEMALE CATCHES

1960

— INSIDE GREATER WINNIPEG

--- OUTSIDE GREATER WINNIPEG



CHAPTER VIII

LARVAL CONTROL BY PREDACEOUS MINNOWS

History of the Top Minnow, *Gambusia affinis* in Winnipeg

Due to its ability to adapt itself to various climatic conditions this minnow has been used in numerous areas to prey on mosquito larvae. In 1957 fifty minnows were placed in a pond 4 miles west of the University to determine whether they were sufficiently cold hardy to survive local winters. In the spring of 1958, fifty minnows were released in a pond just south of the University in King's Park. Rapid increase occurred during the summer and by the fall over 2000 minnows inhabited the pond. In May 1959, 30 minnows were released in the duck pond and 25 in the deer pond at the Assiniboine Park. In previous years mosquito breeding was found here most of the summer but during the 1959 season, although no insecticide had been used, no larvae were found.

Cold Hardiness

It was first presumed that *Gambusia affinis* could not survive the Manitoba winters. Out of fifty minnows released in a pond in October 1957, not one was found the following spring. Another attempt was made in May 1958 when fifty minnows were released in a large pond, 40' x 80' by 5' deep in King's Park. No minnows were found when the pond was inspected during February 1959. Repeated visits in the spring resulted in the discovery of ten *Gambusia affinis* on May 23, 1959. This was the first time that *Gambusia affinis* had overwintered in this latitude. Not

long afterwards mosquito control authorities at Minneapolis, Minnesota (200 miles south of Winnipeg) reported Gambusia affinis had survived in four ponds during the 1958-1959 winter.

In September 1959, Gambusia were released in four more ponds around Winnipeg. Approximately 350 minnows were removed with a minnow seine (Figure 19) from King's Park pond and distributed in the following locations: 100 at Oak Bluff, 12 miles west of Winnipeg; 91 in two ponds near Stony Mountain, 12 miles north of Winnipeg; 72 at Lilyfield, 6 miles north of the city and 120 in a pond 4 miles west of the University (Figure 20). Approximately 1000 were left to overwinter in the King's Park pond.

On June 3, 1960, Gambusia affinis were found in ponds at King's Park and Stony Mountain.

Conclusion

The top minnow Gambusia affinis was found to survive two winters out of doors in Manitoba. They have been useful in controlling larvae in many private and public ponds around Winnipeg but since such a small percentage of the population survive the winter they were not practical for a large mosquito control program in Winnipeg.

Figure 19. Larval Control by Predaceous Minnows

- A. Top Minnows, Gambusia affinis
- B. Placing Top Minnows in Ornamental Ponds
- C. King's Park Pond: Fishing for Top Minnows
- D. Common Sights around Urban Homes

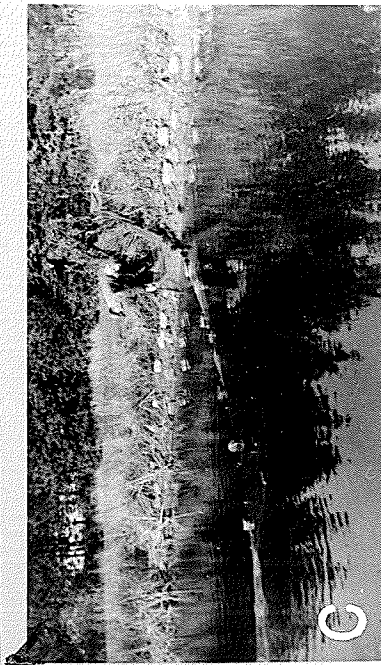
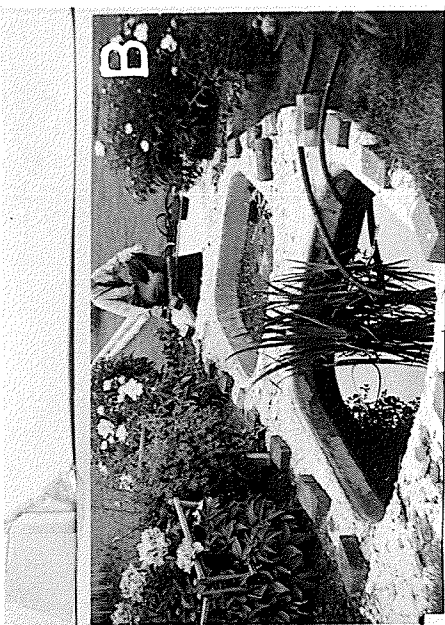
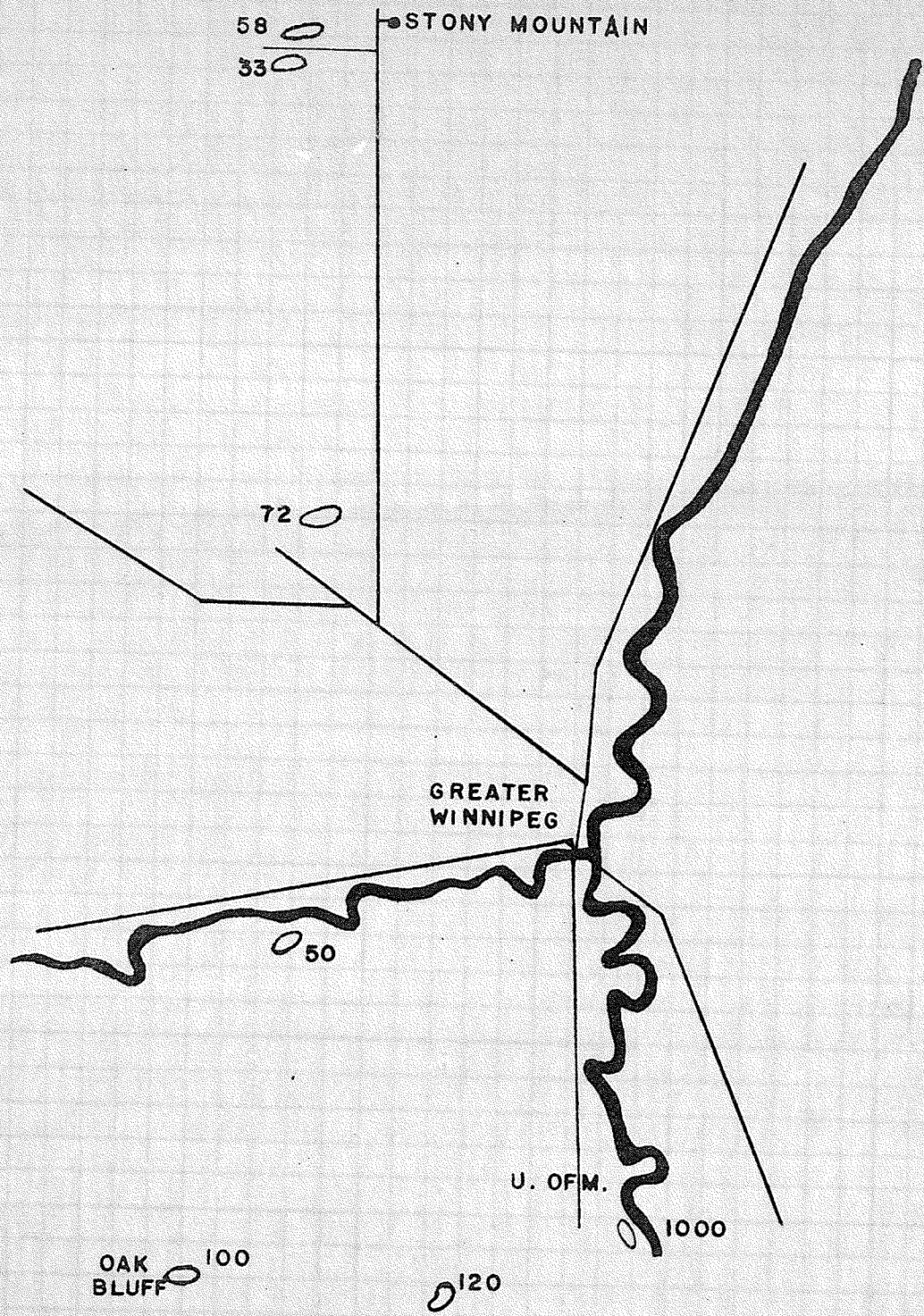


Figure 20. Locations and number of Gambusia affinis
Distributed around Winnipeg, Fall of 1959



CHAPTER IX

MOSQUITO DISPERSION: DDT BARRIER: INSECTICIDE RESISTANCE TESTS

Mosquito Dispersion 1959 and 1960

Investigations on mosquito dispersion have been under the direction of Dr. W. Hanec, Department of Entomology. Tagged mosquitoes have been released in an area north of Winnipeg in seasons 1959 and 1960. Larvae in the third and fourth instar were treated with radio-active phosphorus (P^{32}) and radiation counts per larvae up to 20,000 counts per minute were recorded with no apparent harm to the larvae. Adults emerged with 15,000 to 18,000 counts per minute.

In 1959 only one treatment was made. Twelve New Jersey Light traps were placed around the release area to recapture the tagged adults but no tagged specimens were taken that season.

In 1960 larger numbers were tagged and two releases made. For the first release in May, 200-300,000 larvae were treated. Five weeks later another release was made in which 100,000 larvae were treated. Twelve ultraviolet light traps were used to recapture the adults. These were placed within a radius of 6 miles from the release area. One tagged adult was captured four weeks after the first release showing 2000 counts per minute. Approximately 3 weeks after the second release, another tagged adult was captured showing 25,000 counts per minute. The tagged specimens were taken at one and two miles respectively from the release point.

Due to the low rainfall in 1960, larvae were not found in sufficient numbers to conduct further releases. At time of writing this report the work was still in progress.

DDT Barrier

In 1959 a barrier spray on vegetation was laid down north of Winnipeg. This so-called "insecticide carpet" was deposited on June 16, 1959 with ground equipment at the rate of 2 lbs. of DDT in No. 2 fuel oil per acre. The area covered was a strip 100 feet wide and 5 miles long. Mosquito light traps were located on both sides of the barrier, but due to the early peak of mosquito emergence that year, insufficient information was obtained to evaluate this type of protection.

Tests for Resistance

Several tests were conducted on the mosquito larvae collected in the Winnipeg area to determine whether any resistance to insecticides had developed. The tests were made for resistance to DDT since it has been used almost exclusively for mosquito control in this area for the past twelve years. A test kit provided by the World Health Organization was used and it consisted of five concentrations of DDT ranging from 0.004 p.p.m. to 2.50 p.p.m.

Methods

The larvae used in each test were collected in the field. Sufficient larvae were obtained so that 300 third or early fourth instar individuals of the same species could be selected. These larvae were placed in groups

of 25 in twelve containers. Test concentrations of 0.004, 0.02, 0.10, 0.05 and 2.50 parts per million were prepared from the kit. Two replicates of each concentration were used with two containers with distilled water as checks. The larvae were added to the test containers and after a period of 24 hours the number of dead, living and moribund individuals was recorded. Moribund larvae are here defined as those incapable of rising to the surface or showing the characteristic diving reaction when the water is disturbed.

Results and Discussion

In 1959 four tests for resistance were conducted on three local species. These were found breeding in areas treated previously that season with DDT. Two tests were conducted on Culiseta inornata but these showed no resistance to DDT (Appendix VI and IX). After 24 hours, 90% were dead in concentrations of 0.004 p.p.m. One test was conducted on Aedes dorsalis and one on Aedes vexans but no resistance was found (Appendix VII and VIII). DDT resistance is not suspected unless the LC_{50} is greater than .1 parts per million for Aedes or 1 p.p.m. for Culex species. (LC_{50} = dosage required to kill 50% of the mosquito sample). All the tests were satisfactory and the highest control mortality was 8%.

CHAPTER X

SUMMARY

In laboratory feeding experiments with Aedes aegypti whole blood was preferred to corpuscles washed in 0.15% saline. Serum or plasma was just as attractive as whole blood and was preferred to 0.15% saline.

Token stimuli consisting of warmth, moisture, colour and odour appeared to be the most important host factors attractive to female mosquitoes. Temperatures of 36-40°C. activated the highest response; a moist surface was found to be 3-4 times as attractive as a dry one; a black or a dark blue surface was preferred to the lighter hues. When blood was compared to water, blood "odour" was found to be more attractive to the female mosquito.

A horizontally placed cylinder, 18" long and 3" in diameter was found to be three times as attractive as a vertically placed one.

The flowers of sweet clover, red clover and mustard plants attracted female and male mosquitoes. Extracts of rose plant flowers were found to be attractive while lilac and strawberry were particularly repellent.

Adult females of Aedes aegypti preferred coarse surface textures for oviposition. The eggs were deposited in the small depressions of the paper towelling and thus fewer eggs were laid on the filter paper.

Short wave lengths of light (below 500 m μ) produced a negative phototactic response with larvae and pupae at relatively low light intensities. Long wave lengths of light (650-700 m μ) were stimulatory

only at high light intensities. Collections of adults were higher in traps equipped with blue lamps than in those equipped with red lamps. The lights were operated at equal light intensities with wave length of light being the only apparent variable. Adults were thus able to distinguish between the two colours. When white lamps were used in the traps, the collections increased directly with the light intensities.

Twenty-five species of mosquitoes were collected in the Winnipeg area during 1959 and 1960. The most important species in terms of annoyance were Aedes spencerii, Aedes riparius and Aedes vexans. Culiseta inornata was the most abundant species in the light trap collections but did not occur in the "biting" collections. Culex tarsalis, an important vector of encephalomyelitis, was not abundant in the Winnipeg area.

A survey of the adult mosquito population was carried out with the purpose of comparing the numbers of mosquitoes "inside" and "outside" the control area. In 1959 and 1960, light trap catches outside the district were higher than those inside. In 1959, an average catch per night in the outside traps was 95.1 and inside 20.2. In 1960, an average of 60.5 per night were caught outside and 24.4 inside.

Ultraviolet or black light was found to be 4-5 times more attractive than the standard white light used in the mosquito traps. The ultraviolet light traps were used to recapture tagged adults.

Top minnows, Gambusia affinis were found to overwinter in 1958-59, and 1959-60, in the King's Park pond just south of the University of Manitoba. The minnows survived the 1959-60 winter in another pond near

Stony Mountain, 12 miles north of Winnipeg.

In mosquito dispersion studies larvae were treated with P^{32} and adults emerged carrying the radio-active tracer. Two tagged adults were captured at one and two miles from the release point. One adult was captured four weeks after the release and retained 2500 counts per minute.

Experiments were carried out to determine whether mosquitoes in the Greater Winnipeg area had developed resistance to DDT. A kit supplied by the World Health Organization was used in the tests. Concentrations ranging from 0.004 p.p.m. to 2.50 p.p.m. were found to be fatal to mosquito larvae collected inside the district.

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APPENDIX I

DAILY LIGHT REACTIONS: CAGED ADULTS OF AEDES AEGYPTI

Age in Days	Percent on Illuminated End	Percent on Dark End
1	62	3.5
2	47.5	6.8
3	39.6	8.6
4	31.3	9.9
5	28.4	16.4
6	26.2	20.7
7	20.1	22.2
9	16.7	25.8
10	15.8	27.3
11	7.1	38.4
12	7.7	36.8
13	6.4	42.1
15	6.8	40.6
16	5.1	41.2
17	4.6	47.1
18	5.3	45.3
21	4.8	42.8
22	4.7	47.6
23	4.6	40.4
24	4.9	48.1
25	4.2	43.2
26	4.9	41.9
28	5.2	46.4
29	6.0	42.3
30	4.8	46.4

APPENDIX II

LIGHT TRAP COLLECTIONS, MAY 14 - SEPTEMBER 3, 1959

Date	Kildonan Park		Windsor Golf Course		Assiniboine Park		University Campus		Government Greenhouse	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
May 14	not	run	1	11	0	0	0	0	not	set up
18	0	0	0	0	0	0	0	0	"	"
21	0	0	0	0	0	0	0	0	"	"
25	0	0	0	0	0	3	0	6	"	"
June 1	0	7	0	1	0	15	0	2	"	"
4	0	0	0	0	4	17	2	5	"	"
8	69	101	0	0	3	81	7	11	8	41
11	32	34	2	10	12	14	1	2	3	6
15	146	331	73	168	7	24	44	83	27	183
18	102	94	not run		1	7	16	3	26	26
22	25	167	5	17	10	6	5	5	22	26
25	21	73	8	59	8	12	3	10	6	27
29	7	12	2	9	0	1	2	9	4	9
July 2	66	75	233	202	33	55	44	27	15	23
6	40	82	5	19	31	30	18	17	30	28
9	7	16	0	0	3	5	0	3	3	11
13	18	38	0	2	6	10	2	5	3	14
16	5	6	0	1	2	1	10	7	4	13
20	4	4	0	2	16	5	0	2	7	7
23	5	6	3	8	11	17	5	10	5	9
27	13	8	14	64	18	22	9	10	9	20
30	1	4	13	9	14	13	7	14	6	12
Aug. 3	3	8	4	5	17	22	77	26	7	14
6	4	8	0	1	64	4	15	18	20	34
10	4	6	0	4	2	2	2	2	6	10
13	7	7	1	2	1	2	0	1	1	0
17	0	3	1	2	0	1	1	1	5	2
20	4	6	30	4	2	4	9	6	13	9
24	3	10	12	24	2	30	3	12	10	36
27	4	8	16	23	4	16	6	14	8	27
31	10	21	39	21	5	8	12	6	13	18
Sept. 3	4	1	12	4	13	5	4	3	15	22
	<u>604</u>	<u>1108</u>	<u>472</u>	<u>672</u>	<u>289</u>	<u>430</u>	<u>312</u>	<u>313</u>	<u>276</u>	<u>627</u>
Total	32 Trap Nts.		32		33 Trap Nts.		33		26 Trap Nts.	
Ave. per Trap Nt.	18.9	34.6	14.7	22	8.7	12	9.5	9.5	10.6	24.1
Average per Trap night	12.5 ♂		20.2 ♀							

APPENDIX III

LIGHT TRAP COLLECTIONS, MAY 14 - SEPTEMBER 3, 1959

Date	Lilyfield		Oak Bluff		Navin School	
	♂	♀	♂	♀	♂	♀
May 14	0	70	0	16	0	0
18	0	0	0	0	0	0
21	0	10	0	0	0	0
25	0	0	0	2	1	3
29	0	93	0	29	0	5
June 1	1	32	1	84	0	0
4	21	37	4	17	0	9
8	60	609	3	81	15	21
11	not	run	12	14	0	0
15	533	1960	7	24	4	30
18	4	28	1	7	8	7
22	73	109	10	6	25	21
25	197	1305	8	12	17	9
29	31	61	0	1	16	13
July 2	118	251	31	50	111	17
6	65	236	33	30	9	29
9	15	84	3	5	0	14
13	30	48	6	10	36	30
16	26	34	21	13	34	23
20	1	10	122	165	8	10
23	4	32	22	88	11	21
27	23	26	245	500	143	280
30	21	41	38	98	12	35
Aug. 3	35	71	50	64	68	112
6	131	220	22	86	10	28
9	50	71	31	66	1	2
13	51	104	2	3	4	5
17	62	210	46	12	3	15
20	23	62	18	62	23	42
24	21	42	27	126	112	62
27	73	238	21	84	214	81
31	82	211	16	45	421	78
Sept. 3	38	54	79	64	166	95
	<u>1789</u>	<u>6365</u>	<u>879</u>	<u>1864</u>	<u>1472</u>	<u>1097</u>
Total	32 Trap nights		33 Trap nights		33 Trap nights	
Ave. per Trap night	55.9	198.9	26.6	56.5	44.6	33.2
Ave. per Trap night	42.2 ♂		95.1 ♀			

APPENDIX V

LIGHT TRAP COLLECTIONS, MAY 2 - JULY 18, 1960

Date	Lilyfield		Oak Bluff		St. Vital	
	♂	♀	♂	♀	♂	♀
May 2	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
8	0	0	0	0	0	0
12	0	0	0	3	0	0
13	0	0	0	2	0	1
16	0	9	0	4	0	2
17	0	4	2	23	0	4
18	0	6	4	9	2	5
20	6	3	0	5	0	1
24	8	9	26	13	2	3
27	27	9	29	18	4	6
28	28	12	18	22	12	10
31	58	33	104	36	1	0
June 3	52	53	33	17	2	5
6	9	6	14	4	8	20
7	24	40	32	64	17	32
9	38	75	65	78	24	43
13	223	244	140	108	41	72
16	180	176	51	195	14	64
20	48	138	144	332	64	240
23	18	52	42	141	32	80
27	44	232	174	360	36	212
30	44	204	48	180	4	120
July 4	96	160	64	82	24	62
7	19	65	65	164	4	44
11	42	193	83	196	42	112
14	1	14	30	47	8	46
18	29	141	33	151	6	130
Total	986	1876	1201	2254	347	1314
	30 Trap nights					
Average per Trap night	32.8	62.5	40	75.1	11.6	43.8
Average per Trap night	28.1	♂	60.5	♀		

APPENDIX VI

EFFECT OF DDT AT CONCENTRATIONS OF 0.004 - 2.50 P.P.M. ON
CULISETA INORNATA THIRD INSTAR LARVAE COLLECTED IN CHARLESWOOD,
 MANITOBA 1959

Concentration of insecticide p.p.m.	Replicate No.	Final condition of larvae after 24 hours				
		L	M	D	Total	% M + D*
0.004	1	0	0	10	10	100%
	2	0	0	10	10	100%
0.02	1	0	0	10	10	100%
	2	0	0	10	10	100%
0.10	1	0	0	10	10	100%
	2	0	0	10	10	100%
0.50	1	0	0	10	10	100%
	2	0	0	10	10	100%
2.50	1	0	0	10	10	100%
	2	0	0	10	10	100%
Control	1	10	0	0	10	0%
	2	10	0	0	10	0%

* L - living; M - moribund; D - dead.

APPENDIX VII

EFFECT OF DDT AT CONCENTRATIONS OF 0.004 - 2.50 P.P.M. ON
AEDES DORSALIS THIRD INSTAR LARVAE COLLECTED IN ST. BONIFACE,
 MANITOBA 1959

Concentration of insecticide p.p.m.	Replicate No.	Final condition of larvae after 24 hours				
		L	M	D	Total	% M + D*
0.004	1	0	0	25	25	100%
	2	0	0	25	25	100%
0.02	1	0	0	25	25	100%
	2	0	0	25	25	100%
0.10	1	0	0	25	25	100%
	2	0	0	25	25	100%
0.50	1	0	0	25	25	100%
	2	0	0	25	25	100%
0.50	1	0	0	25	25	100%
	2	0	0	25	25	100%
Control	1	23	0	2	25	.08%
	2	24	0	1	25	.04%

* L - living; M - moribund; D - dead.

APPENDIX VIII

EFFECT OF DDT AT CONCENTRATIONS OF 0.004 - 2.50 P.P.M. ON

AEDES VEXANS THIRD AND FOURTH INSTAR LARVAE COLLECTED IN WINDSOR PARK,

WINNIPEG, MANITOBA 1959

Concentration of insecticide p.p.m.	Replicate No.	Final condition of larvae after 24 hours				
		L	M	D	Total	% M + D*
0.004	1	0	0	25	25	100%
	2	0	0	25	25	100%
0.02	1	0	0	25	25	100%
	2	0	0	25	25	100%
0.1	1	0	0	25	25	100%
	2	0	0	25	25	100%
0.5	1	0	0	25	25	100%
	2	0	0	25	25	100%
2.50	1	0	0	25	25	100%
	2	0	0	25	25	100%
Control	1	24	0	1	25	.04%
	2	25	0	0	25	0%

* L - living; M - moribund; D - dead.

APPENDIX IX

EFFECT OF DDT AT CONCENTRATIONS OF 0.004 - 2.50 P.P.M. ON

CULISETA INORNATA LARVAE COLLECTED AT FORT ROUGE,

WINNIPEG, MANITOBA 1960

Concentration of insecticide p.p.m.	Replicate No.	Final condition of larvae after 24 hours				
		L	M	D	Total	% M + D*
0.004	1	1	0	9	10	90%
	2	0	0	10	10	100%
0.02	1	0	0	10	10	100%
	2	0	0	10	10	100%
0.10	1	0	0	10	10	100%
	2	0	0	10	10	100%
0.50	1	0	0	10	10	100%
	2	0	0	10	10	100%
2.50	1	0	0	10	10	100%
	2	0	0	10	10	100%
Control	1	0	0	1	10	10%
	2	10	0	0	0	0%

* L - living; M - moribund; D - dead.