ECOLOGY AND CONTROL OF MOSQUITOES OF THE WINNIPEG AREA, WITH SPECIAL REFERENCE TO *AEDES VEXANS* (MEIGEN)

A

Thesis Presented To

The Department of Entomology

Faculty of Agriculture and Home Economics

The University of Manitoba

Winnipeg

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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January 1967

ACKNOWLEDGEMENTS

The writer wishes to express sincere appreciation to his advisor, Dr. R. A. Brust, Assistant Professor, Department of Entomology, University of Manitoba, for his helpful suggestions, valued criticisms and guidance throughout the study and in the preparation of this thesis. Grateful acknowledgement is also extended to Dr. A. J. Thorsteinson, Professor and Head, Department of Entomology, University of Manitoba, for his helpful criticisms.

Further appreciation is extended to Mr. Peter Belski, Field Manager, Metropolitan Winnipeg Mosquito Abatement Branch, for his continual interest during these investigations.

The writer is also grateful to Mr. John Giardino for the photographic work in this study.

Thanks are due to Mr. R. Dixon and Mr. R. Phillips, Student Assistants, and to Miss Casey Johannesson, Laboratory Technician, for their assistance during the course of this study.

The investigations were made possible by a grant from the Metropolitan Corporation of Greater Winnipeg, Mosquito Abatement Branch, to the Department of Entomology, University of Manitoba.

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ABSTRACT

The distribution of Aedes vexans (Meigen) in the province of Manitoba appears to be correlated with suitable developmental sites, as well as climatic factors.

Viability of mosquito eggs in storage was best maintained at low temperatures and high humidities. The temperature at which eggs were conditioned and hatched influenced their hatchability. Relative humidity of pre-hatch conditions affected the viability and the rate of egg hatch. Eggs, four weeks old, lost moisture at a slower rate in an unsaturated atmosphere than eggs two weeks old.

Laboratory experiments showed that Aedes vexans adults could not survive exposure to 40°F for 24 hours. Mosquitoes given water or honey survived longer than unfed adults. Female mosquitoes lived longer than male mosquitoes, and relative humidity had no effect on adult longevity when adults were given a source of water.

Aedes vexans is the most abundant species in the Winnipeg area and most of the adult mosquitoes occurring inside the controlled area originated in breeding sites outside the area.

Western encephalitis investigations in Manitoba in 1965 and 1966 found no trace of the virus in mosquitoes collected from different parts of the province.

Fewer mosquitoes were found at 40 feet than at 5 feet above ground level. Swarms of male mosquitoes occurred periodically at the

40 foot level.

Pre-season insecticide applications were most effective when applied to permanent pools. DDT had a longer residual effect than other chemicals tested. DDT impregnated on vermiculite granules was stored up to two years with no deterioration. The mosquitoes of the Winnipeg area have not developed resistance to DDT, as determined by the World Health Organization tests.

CHAPTER I

INTRODUCTION

There are 39 known species of mosquitoes in Manitoba (Carpenter and La Casse, 1955; Kalpage, 1966), of which 25 have been collected in the Winnipeg area. The primary importance of mosquitoes at the present time lies in their nuisance value rather than as vectors of disease. The entire province is plagued with these pests during the spring and summer months, and the resulting annoyance can lower agricultural and industrial efficiency and decrease real estate values.

Mosquito abatement operations in Manitoba are at present confined to Metropolitan Winnipeg. The control area covers 256 square miles and benefits over half a million people. Control measures are carried on throughout the year. Fall and winter applications of insecticide are applied to the areas known to produce mosquitoes each year to kill the young larvae as they hatch in the spring. Spring and summer spraying and fogging are used to eradicate mosquitoes which fly in from areas outside the control district.

In order to obtain information about mosquito populations inside and outside the control area, and to get comparisons of mosquito populations from year to year, which are important in determining the efficiency of a mosquito abatement program, the Department of Entomology at the University of Manitoba operates a number of light traps throughout the spring and summer.

A number of environmental factors must be considered in the interpretation of data resulting from surveys designed to sample insect populations. Among these factors are wind, temperature, relative humidity, and precipitation. Each is subject to a considerable degree of variability from year to year. Variability in precipitation, especially, results in fluctuations in the mosquito population. If sufficient knowledge can be gained on how the other factors of the environment affect mosquito populations, we may be better prepared to forecast the population levels to be expected, leading to more efficient control measures.

The light trap survey of adult mosquito populations has been in operation since 1957, and has revealed that Aedes vexans is the most abundant species in the Winnipeg area (Smith 1959, Brust 1960, Brandt 1964), comprising up to 85% of the mosquito population. A survey made by McLintock in 1944 also reported Aedes vexans to be the most abundant species.

Additional knowledge of the life history of Aedes vexans in Manitoba was gained by larval and adult surveys and field observations. Laboratory experiments were performed on the egg and adult stages of Aedes vexans, providing information about the physiology and ecology of this species.

In recent years there has been an increased incidence of western encephalitis in horses in Manitoba. A W.E. investigation program was initiated on a preliminary basis in 1965 and expanded in 1966. The primary objective is to discover the virus in mosquitoes before horses and humans

are affected, so preventive measures can be taken.

Fall and winter application of insecticides has been carried out in the Winnipeg area since 1956. Periodic evaluations are necessary to assay the continued effectiveness of this type of control. Evaluation may be carried out by bioassays of the treated areas, by experiments which simulate natural conditions, and by tests to determine whether resistance has developed.

The Objectives of the Study

The objectives of this study were threefold: (1) to investigate the ecology of mosquito species occurring in the Winnipeg area, especially Aedes vexans Meigen; (2) to study some physiological aspects of Aedes vexans in order to gain more knowledge of the biology of this species and to improve culture techniques; (3) to examine the short and long term effectiveness of control measures in the Winnipeg area.

Organization of the Thesis

The thesis is made up of ten chapters, in which three main aspects are covered. Chapter IV, V, and VI cover laboratory studies of Aedes vexans. In chapters VII, VIII, and IX, field studies of mosquitoes are described. Chapter X outlines investigations made on chemicals now in use for mosquito control, and on chemicals under consideration for future use.

CHAPTER II

REVIEW OF LITERATURE

Borg and Horsfall (1953) demonstrated that the basic hatching stimulus for mosquito eggs is a decrease in the oxygen content of the hatching medium. Horsfall (1956) showed that the hatching of eggs in a suitable medium was influenced by the "conditioning" factors of temperature and relative humidity. Beckel (1958) studying Aedes hexodontus Dyar, found that the permeability of the egg cuticle to both loss and uptake of water became less as the egg aged. Meola (1964) found that a reduced rate of transpiration, best achieved at low temperatures and high humidities, was necessary to maintain viability of eggs of Aedes aegypti L. during prolonged storage.

Clarke (1943), using aniline dyes to label mosquitoes for flight and longevity studies, reported recapturing a female Aedes vexans 37 days after staining and release. The longevity of adult Aedes aegypti is influenced by mating and association of the sexes, according to Liles (1965). Working with Culex tarsalis Coquillett, Anderson and Harwood (1966) found the survival of this species at temperatures around $0^{\circ}C$ was increased by cold preconditioning. Clements (1963) reported that at moderate temperatures there does not appear to be any direct relationship between longevity and relative humidity.

Smith (1959), Brust (1960), and Brandt(1964), found that the mosquito populations, as measured by light traps, are greater outside the

Metro. Winnipeg abatement district than inside. Collett et al (1964) state that both light trap collections and larval sampling are valid methods of estimating population changes and differences.

McLintock (1944), Smith (1959), Brust (1960), and Brandt (1964), found Aedes vexans the most abundant species in the Winnipeg area. A number of studies have been made on the ecology of this species. Horsfall (1954) reported on Aedes vexans as a migratory species. Horsfall (1962) studied the vertical distribution of eggs of floodwater mosquitoes, particularly Aedes vexans. Gunstream and Chew (1963), found that Aedes vexans adults remain relatively inactive for two days after emergence. The relationship between precipitation and abundance of Aedes vexans was emphasized by Price (1964).

The first investigation of western encephalitis in mosquitoes of Manitoba was made from 1942 to 1945 by McLintock (1946). No further W.E. studies on mosquitoes were made in this province until 1965, but the occurence of W.E. in horses and humans was recorded by the Manitoba Department of Health. Investigations by Burton (1965) in Saskatchewan revealed that many aedine species may harbour the virus, but Culex tarsalis is still regarded as the principal transmitter of W.E. in Saskatchewan.

MacCreary (1941) compared the density of mosquitoes at ground level and at 100 feet, and found that greater numbers of mosquitoes occurred at the lower level. Burgess and Haufe (1960), and Main et al (1966), independently found that greater numbers of mosquitoes occur at ground level than at 25 feet above ground level in prairie locations, but in forest locations equal or greater numbers of adults occur at 25 feet

as compared with ground level. Brandt(1964) caught large numbers of Aedes vexans males on several occasions in a trap with an entrance 40 feet above ground level.

Sutherland and Mazurkewicz (1963) found that the solvent used in DDT impregnation of granules, and the type and size of the granules impregnated, had a great influence on the release of DDT from these granules. Chapman (1966) evaluated different chemicals as larvicides in the laboratory by use of a modification of World Health Organization resistance test techniques.

The use of radioisotope tracers for insecticide studies is a relatively new and highly promising technique. Casida (1962) studied the metabolism of organophosphate insecticides in plants using P32 as a label. Plapp and Lindquist (1963) used radiosotopes to study the fate of insecticides applied to animals and plants. Heath (1963) described the use of radioisotopes to study insecticide residues when chemical or biochemical methods are not adequate.

Smith (1959), Brust (1960), and Brandt(1964) found that no resistance to DDT had developed in the mosquitoes of the Winnipeg area.

CHAPTER III

LIFE HISTORY STUDIES

The thirty-nine species of mosquitoes known to occur in Manitoba are distributed among six genera (Table I).

TABLE I

MOSQUITO SPECIES IN MANITOBA

Aedes	abserratus
A.	aurifer
A.	barri
A.	campestris
A.	canadensis
A.	decticus
A.	cinereus
A.	communis
A.	diantaeus
A.	dorsalis
A.	excrucians
A.	fitchii
A.	flavescens
A.	hexodontus
A.	impiger
A.	implicatus
A.	intrudens
A.	nigripes
A.	nigromaculis
A.	pionips
A.	punctor
A.	riparius
A.	spencerii
A.	sticticus
A.	stimulans
A.	trichuris
A.	triseriatus
A.	vexans
Anopheles	earlei
A.	punctipennis
A.	walkeri
Culiseta	alaskaensis
C.	impatiens
C.	inornata
C.	morsitans
Culex	restuans
C.	tarsalis
Mansonia	perturbans
Wyeomyia	smithii

Anopheles, Culex, and Culiseta species over winter as adults. The inseminated females spend the winter in cellars, caves, outbuildings, rabbit burrows, hollow trees, and other well protected locations. The females leave these areas in the spring, obtain a blood meal and oviposit in permanent pools. The eggs hatch within 4-7 days after oviposition. In southern Manitoba larvae of Culiseta and Culex species are first observed in early June. Larvae are found throughout the summer, often until the middle of September.

Mansonia perturbans and Wyeomyia smithii overwinter as larvae in mud at the bottom of permanent pools. They pupate in spring or early summer and the adults emerge almost a week later. Little work has been done on the biology of these two species in Manitoba due to their rare occurence. Mansonia larvae obtain oxygen from submerged plant stems and roots by siphonal penetration. In larvae of Wyeomyia, respiration is largely cutaneous and they are rarely found at the surface.

Mosquitoes of the genus Aedes are the most important species in Manitoba, in terms of numbers and human annoyance. In contrast to Culex, Culiseta, and Anopheles, aedine species overwinter in the egg stage. The eggs are insulated from extreme sub-zero temperatures by snow.

Classification of Manitoba Aedine Species

Aedine mosquitoes can be divided into three classes: univoltine, multi-voltine, and a mixture of the two (Table II). Univoltine species have an obligatory embryonic diapause; multivoltine species have facultative diapause which can be arrested at will; still other species consist of a multivoltine and univoltine strain, but not much is known to date about

the species involved in the last group.

TABLE II

CLASSIFICATION OF MANITOBA AEDINE SPECIES

Univoltine

Aedes abserratus	A. diantaeus	A. impiger	A. punctor
A. aurifer	A. excrucians	A. intrudens	A. riparius
A. barri	A. fitchii	A. implicatus	A. stimulans
A. communis	A. flavescens	A. nigripes	A. trichurus
A. decticus	A. hexodontus	A. pionips	

Multivoltine

Aedes campestris	A. sticticus
A. dorsalis	A. triseriatus
A. nigromaculis	A. vexans

Univoltine and Multivoltine

Aedes cinereus

A. canadensis

A. spencerii

Univoltine species can have only one generation a year since the eggs must go through several months of cold conditioning before they hatch. The eggs of univoltine species usually hatch in pools formed by melting snow. Following development, the adults lay their eggs in early summer and the eggs remain in diapause until the following spring.

Multivoltine species have as many generations a year as climatic conditions (primarily precipitation and temperature) will allow. The eggs of

multivoltine species do not enter diapause and therefore do not require chilling prior to hatching.

Aedes spencerii, A. cinereus and A. canadensis in Manitoba each consist of multivoltine and univoltine fractions (Table II). These three species have previously been considered as univoltine, but a portion of the egg population of each species, which oviposited in the laboratory, hatched without cold treatment. In A. spencerii, the fraction hatching without cold treatment out of three replicates of 30 eggs each was 69%. In A. cinereus and A. canadensis, only one test was conducted, and of 319 eggs of A. cinereus, 156 hatched without cold treatment (48%). Of 162 eggs of A. canadensis, 51 hatched without cold treatment (32%). In all three of these species further investigation needs to be done, firstly, to determine if an individual female lays both univoltine and multivoltine eggs or only one type. Secondly, adults of each species could be collected throughout the spring and summer to determine whether there is any change in the proportion of multivoltine and univoltine eggs laid with successive broods. Thirdly, the multivoltine fraction could be selected for several generations to determine whether multivoltinism is a genetic character that can be isolated from univoltism in the same species.

Spring mosquito populations are made up largely of univoltine species in the Winnipeg area (Fig. 1). By early summer, multivoltine species appear in increasing numbers and by July these species are predominant. By the end of August few univoltine adults remain as they lay their eggs and die generally before the middle of the month.

Egg and Larval Sites of Aedes vexans

Aedes vexans in Manitoba is a floodwater species primarily found

in grassy ditches, pastures, and aspen forests, where small pools form after a heavy rain. Unlike univoltine aedine species, A. vexans does not select muskeg or a coniferous forest habitat where pools are lined with moss.

A province-wide adult biting survey has revealed that the percentage of A. vexans decreases from south to north in the province (Fig. 2). Climatic conditions, while very important, are not the only factors affecting the distribution of this species. In some cases lack of suitable oviposition sites may limit its occurrence.

Around Winnipeg, where the A. vexans population is high, farmland and grassy ditches are abundant. Some farmland also occurs near The Pas where A. vexans makes up about 50% of the biting population. In the Flin Flon area, only 100 miles from The Pas and on the edge of the Canadian shield, the terrain consists of precambrian rock outcrops (Weir, T. R., 1960 Economic Atlas of Manitoba) and bog. This type of surface deposit does not provide suitable oviposition sites for A. vexans, and at Flin Flon this species makes up only about 3% of the mosquito population. West and north-west of Flin Flon, where clay and silt occur as well as bog and rock outcrops, A. vexans again becomes numerous. Aspen stands are associated usually with clay and silt deposits in the area, and where aspen stands were abundant A. vexans was also numerous. In the Churchill area A. vexans is extremely rare. The climate at Churchill is certainly more severe than in the central parts of the province, but it is unlikely that this has much effect on A. vexans. The winter soil temperatures at Churchill are not very different from those at Winnipeg and are not low

FIGURE 1: Relative Abundance of Adults of Univoltine and Multivoltine Aedine Mosquitoes in the Winnipeg Area, 1965 Survey.

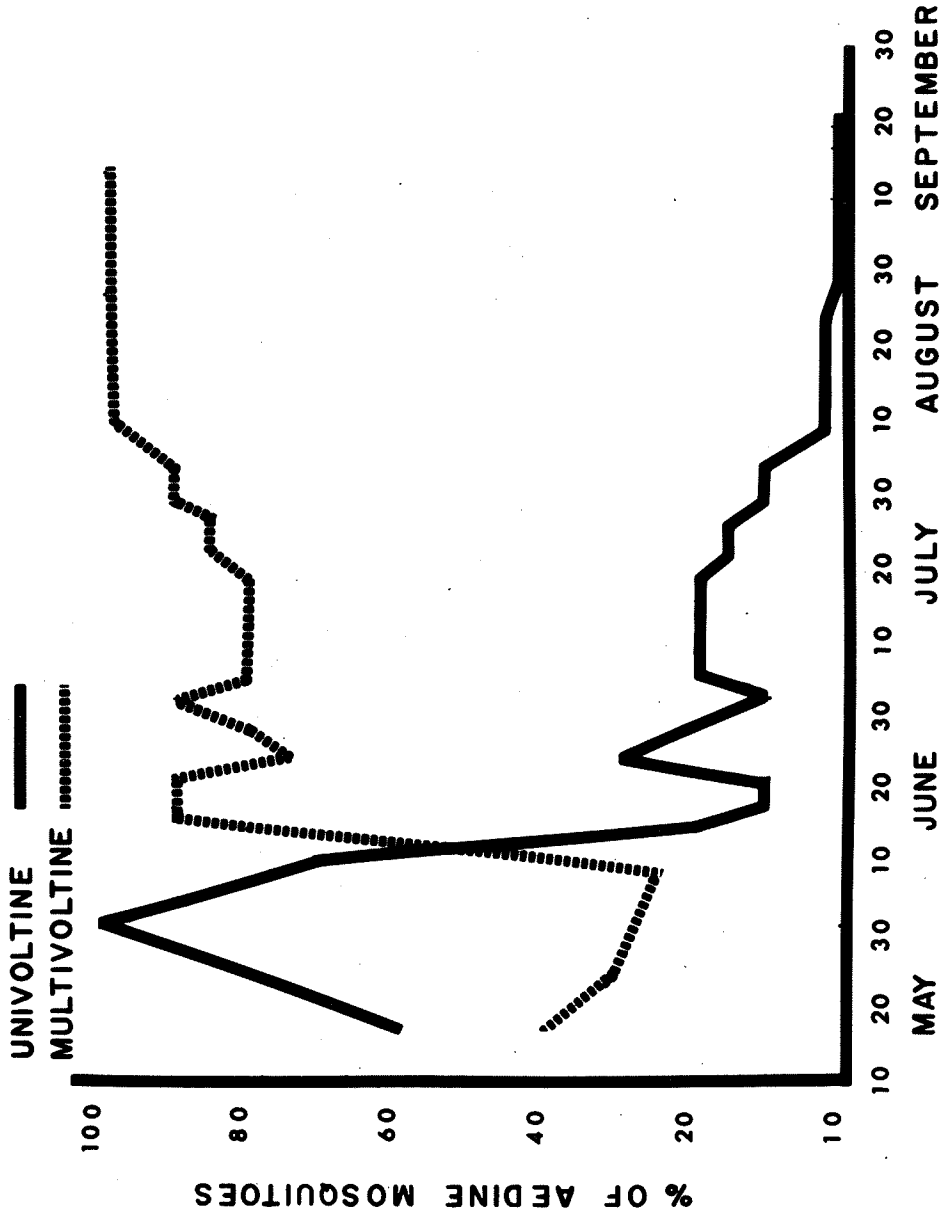

UNIVOLTINE
MULTIVOLTINE
% OF AEDINE MOSQUITOES
100
80
60
40
20
10
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30
MAY JUNE JULY AUGUST SEPTEMBER

Figure 1

enough to cause the eggs any harm. The only apparent reason for the small number of A. vexans, 0.1% of the adult biting population in July, is the lack of clay or silt deposits. The area is historically marine with extensive bogs and coarse alluvial deposits (Weir, T. R., 1960).

FIGURE 2: Percentage of *Aedes vexans* in the Adult Mosquito Biting Population in the Winnipeg, The Pas, Flin Flon and Churchill areas, 1965 survey.

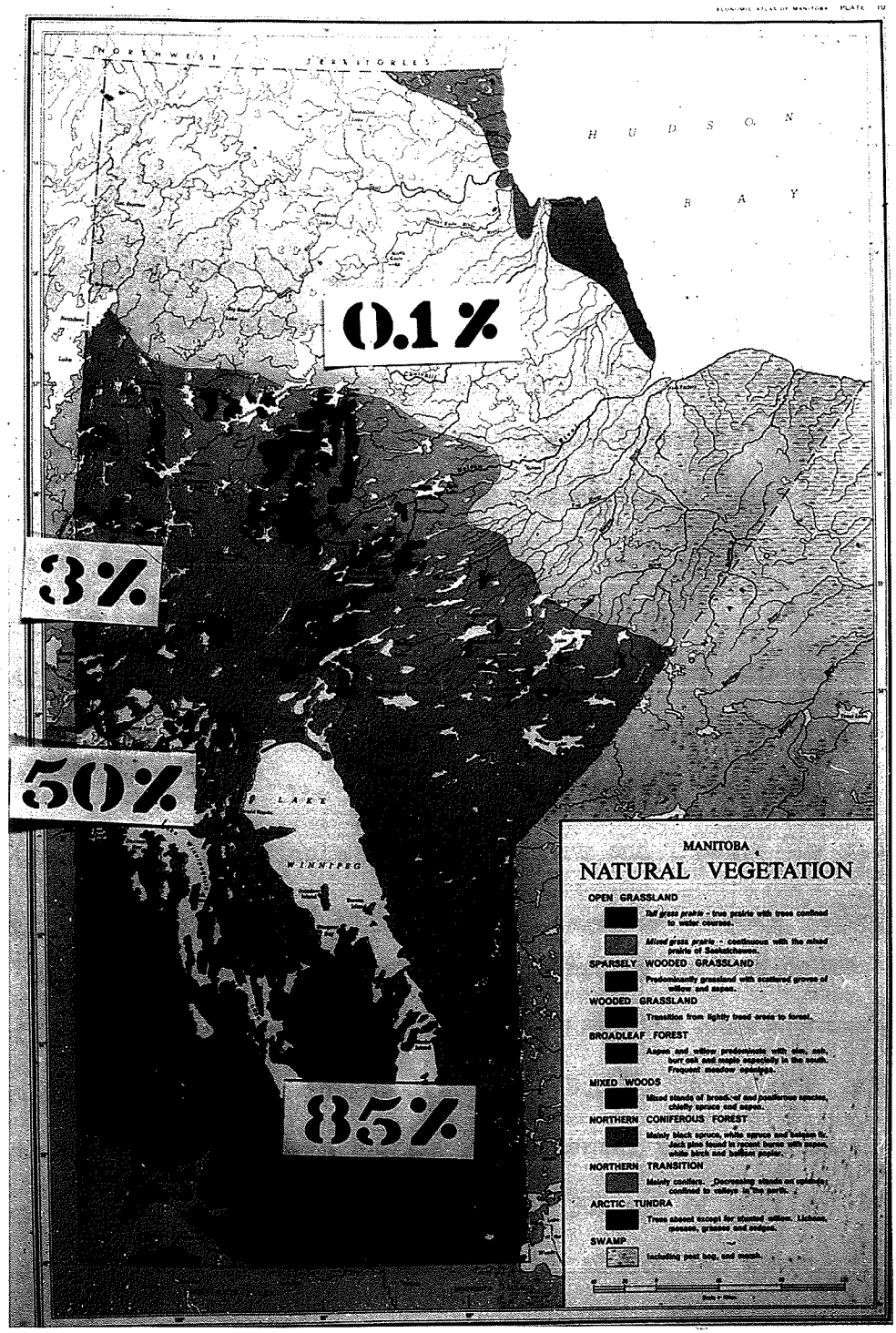

0.1%
3%
50%
85%
MANITOBA
NATURAL VEGETATION
OPEN GRASSLAND
SPARSELY WOODED GRASSLAND
WOODED GRASSLAND
BROADLEAF FOREST
MIXED WOODS
NORTHERN CONIFEROUS FOREST
NORTHERN TRANSITION
ARCTIC TUNDRA
SWAMP
HUDSON BAY
WINNIPEG

Figure 2

CHAPTER IV

THE INFLUENCE OF TEMPERATURE AND HUMIDITY ON VIABILITY AND HATCHING OF AEDES VEXANS EGGS

A number of experiments were carried out on eggs of A. vexans. The influence of temperature and humidity on egg viability and hatching was studied (1) to obtain information on laboratory techniques for storage, conditioning, and hatching, (2) to gain information useful in ecological studies, and (3) to better understand the physiology of the egg stage of A. vexans.

Method and Equipment

All eggs used were obtained from field collected females. A test for viability was made by placing the eggs in a saturated solution of Na Cl and water. The non-viable eggs **shrivel** and sink to the bottom, whereas the viable eggs remain firm and float. The viable eggs were then collected and washed.

The relative humidites used were obtained by various concentrations of potassium hydroxide solutions. The humidity chambers were desiccators with a 10 inch inside diameter. B.O.D. incubators, accurate to 1.8° F, were used to get the experimental temperatures. The hatching solution consisted of approximately 300 mg. of nutrient broth (Bacto-Beef Extract and Bacto-Peptone) in 250 mls of tap water.

Effect of Different Temperatures and Relative Humidities on Viability and Hatching of Eggs

The influence of temperature and relative humidity on egg viability and hatchability was studied by subjecting eggs to different pre-hatch temperatures and humidities and different hatching temperatures.

Method

The eggs were divided into two groups, one stored at 35°F, the other at 70°F. After five months storage the eggs were placed at 70°F at one of four relative humidities, 20, 50, 80, or 100%. After two weeks the eggs were placed in hatching solutions at 40°, 50°, 60°, 70°, or 80°F.

Results

The percentage of eggs hatching after different treatments is given in Table III.

The statistical analysis of this data is given in Table IV, A and B. The analysis of variance (Table IV-A) indicated significant differences in all treatments. To find more precisely where these differences occurred, the method of finding the least significant differences was employed (Table IV-B). With this method, for two treatments to be significantly different, the difference between their means must exceed the calculated least significant difference (Steel and Torrie, 1960).

The percentage of eggs hatching at 40°F at all treatments was significantly lower than the percentage hatching at the other temperatures. There was no significant difference between the mean number of eggs that hatched at the other four temperatures, 50°, 60°, 70°, and 80°F, although the time

TABLE III

THE PERCENTAGE OF EGGS HATCHING AFTER DIFFERENT TREATMENTS

Eggs stored at 35°F or 70°F for 5 months were pre-conditioned for hatching by placing them at 70°F and 20, 50, 80, or 100 per cent R.H. for 2 weeks. Eggs were then hatched at 40°, 50°, 60°, 70°, or 80°F.

Pre-Hatch Conditioning 2 weeks at 70°F and the following RH levels	Hatching Temperature	% Hatch[1] A[2]	% Hatch[1] B[3]
20	40°F	0	0
50		4	1
80		28	8
100		44	4
20	50°F	0	0
50		50	22
80		79	11
100		88	50
20	60°F	0	0
50		36	20
80		77	23
100		98	68
20	70°F	0	0
50		47	13
80		87	30
100		94	46
20	80°F	0	0
50		58	59
80		67	67
100		81	62

1 mean of 3 reps. of 30 eggs each
2 A-eggs stored at 35°F
3 B-eggs stored at 70°F

TABLE IV-A

ANALYSIS OF VARIANCE OF TABLE III

Source of Variation	D.F.	S.S.	M.S.	F
Hatching Temperature	4	6614.19	1653.54	6.13 *
Pre-Hatch Humidity	3	22133.30	7377.77	24.78 *
Storing Temperature	1	5152.90	5152.90	19.10 *
Residual	31	8363.55	259.79	
TOTAL	39	42263.90		

* significant at 5% level

TABLE IV-B

TEST SIGNIFICANT DIFFERENCE ANALYSIS OF MEAN PERCENTAGE HATCH

Hatching Temperatures

	40°F	50°F	60°F	70°F	80°F
Mean	11.13	37.50	40.25	39.63	49.25

$$LSD = \frac{2\,Se^2}{r} \times t\ (5\%,\ 31\ df)$$

$$= \frac{2 \times 269.79}{8} \times 2.04 = 16.75$$

Pre-Hatch Humidity

	20% RH	50% RH	80% RH	100% RH
Mean	0	31.00	47.70	63.50

$$LSD = \frac{2 \times 269.79}{10} \times 2.04 = 14.97$$

Storing Temperature

	35°F	70°F
Mean	49.90	24.20

$$LSD = \frac{2 \times 269.79}{20} \times 2.04 = 10.59$$

taken for the eggs to hatch at the lower temperatures was much greater than at higher temperatures.

Each pre-hatch relative humidity tested was significantly more favourable to those below it. The largest number of larvae obtained at all hatching temperatures were from eggs kept at 100% R.H. during pre-hatch conditioning, while no larvae were obtained from eggs kept at 20% R.H. pre-hatch conditioning (Table III).

At all treatments, significantly greater numbers of eggs hatched out of those stored for 5 months at 35°F than those stored for 5 months at 70°F (Table III).

Discussion

The difference in hatchability of eggs subjected to different temperatures and relative humidities indicates a relationship between transpiration and embryonic longevity. The increase in hatchability at low temperatures and high humidities illustrates that reduction of transpiration is a prerequisite to prolonged egg storage.

It has been the practice to place *A. vexans* eggs at 35°F when they are stored for a number of months. This experiment confirmed that this temperature is advantageous in maintaining viability during storage, as reported by Meola (1964) for *A. aegypti*.

The rate of transpiration increases with a decrease in relative humidity. Meola (1964) found that the decline in hatchability of *A. aegypti* eggs at lower relative humidities was nearly directly proportional to water loss. Reduced transpiration is necessary for embryonic survival,

so high relative humidities are required for storage. High relative humidities result in occasional hatching and encourage fungal growth which can be harmful, so there is a limit as to how long eggs can be stored even at reduced temperatures. Low R.H. levels cause desiccation and death of the embryos, so some fungal growth must be tolerated if eggs are to be stored over an extended period.

The low hatching percentage occurring at 40°F coincides with what is known of the ecology of A. vexans. The eggs of many aedine species hatch in early spring in water from melted snow, but A. vexans does not. Larvae of this species are seldom found until after the first heavy spring rainfall, when the pool temperatures are higher. This experiment indicates that to hatch more than half of A. vexans eggs a temperature of at least 50°F is required. The conditioning temperature of 70°F used in this experiment is higher than the conditioning of eggs hatching in the spring, but is nearer to the conditioning which subsequent generations would undergo.

Influence of Temperature on the Desiccating Effect of Low Humidities

Method

Different lots of A. vexans eggs were conditioned at 20% R.H. for 16 days at a constant temperature at 40°, 50°, 60°, or 70°F. They were then transferred to 100% R.H. and 70°F for 24 hours and hatched at 70°F.

Results:

TABLE V

PER CENT HATCH OF A. VEXANS EGGS AFTER DIFFERENT TREATMENTS

Pre-hatch treatment		
% RH	Temp. (°F)	% Hatch
20	40	41
20	50	37
20	60	2
20	70	0

* means of 3 reps. of 30 eggs each

About 40% of the eggs kept at the lower temperature of 40°F and 50°F were viable, whereas at 60°F only 2% of the eggs were viable, and none at 70°F. The higher rate of transpiration at 60°F and 70°F resulted in the mortality of almost all the eggs, while at 40°F and 50°F, the water lost through transpiration killed only 60% of the eggs.

Discussion

The results show that low relative humidities may be used in combination with low temperatures for egg storage. Also if fungal growth in stored eggs becomes a serious problem, the eggs could be placed in a less than saturated atmosphere at a low temperature for a short time to reduce

fungal growth and still maintain egg viability. Low temperature lessens the desiccating effect on the eggs.

The results of the above laboratory experiment also apply to conditions which occur in nature. In winter the soil near the ground surface where mosquito eggs occur is frozen, making its moisture unavailable to the eggs. Also, the moisture in the snow above the eggs is in the form of ice crystals. It appears probable that mosquito eggs overwinter in an atmosphere of less than 100% relative humidity, but are able to survive because of the low temperature resulting in decreased transpiration.

Influence of Conditioning and Hatching Temperature on Hatchability of Eggs

Eggs were taken from storage at 35°F and divided into two groups: one at 40°F, the other at 70°F. After two weeks they were transferred directly into hatching solutions at one of 40°, 50°, 60° or 70° F.

Results:

TABLE VI

THE PERCENTAGE OF A. VEXANS EGGS HATCHING AFTER DIFFERENT TREATMENTS

One group of A. vexans eggs was conditioned at 40°F, the other at 70°F, for 2 weeks before hatching at 40°, 50°, 60°, and 70°F.

Hatching temp. (°F)	% Hatch *	
	40°F	70°F
40	0	44
50	2	88
60	8	98
70	56	94

* mean of 3 reps. of 30 eggs each

The eggs conditioned at 70°F hatched better at all temperatures tested. Of the eggs conditioned at 40°F, only half hatched at the relatively high temperature of 70°F, 8% hatched at 60°F, 2% hatched at 50°F, and none hatched at 40°F. However, of the eggs conditioned at 70°F, about half hatched at the low temperature of 40°F, and approximately 90% hatched at 50°, 60°, and 70°F.

Discussion

Horsfall (1956) found that the percentage of A. vexans eggs hatching at 77°F, after being maintained at 40°F, increased as the number of days they were exposed to 77°F increased. The present study confirms these results and extends it to apply at different hatching temperatures.

The temperature at which eggs are conditioned can have a great effect on the number of eggs hatching at a particular temperature, and hence laboratory experiments designed to simulate hatching of eggs in nature must take conditioning into consideration.

Hatching of Aedes vexans and Aedes abserratus at Different Temperatures

Method

Eggs of A. vexans and A. abserratus (F. ♂ Y) were taken from storage at 35°F and immediately placed in hatching solutions at 35°, 40°, 50°, 60°, or 70°F.

Results:

TABLE VII. Percentage hatch of A. vexans and A. abserratus. Eggs taken from storage at 35°F and placed in hatching solutions at 35°, 40°, 50°, 60° and 70°F.

Hatching temp. (°F)	% Hatch *	
	A. abserratus	A. vexans
35	1	0
40	27	0
50	47	2
60	53	8
70	4	56

* mean of 3 reps. of 30 eggs.

A greater percentage of A. abserratus eggs hatched at low temperatures than A. vexans eggs. At 35°F, 1% of the A. abserratus eggs hatched, and no A. vexans eggs hatched. At 40°F, a 27% hatch of the A. abserratus eggs occurred, compared with none for A. vexans. About half the A. abserratus eggs hatched at 40° and 50°F, while less than 10% hatching of the A. vexans eggs occurred. At 70°F, the percentage hatching of A. abserratus eggs dropped to 4%, and of A. vexans eggs rose to 56%.

Discussion

The conditioning which the eggs received in this experiment was similar to the conditioning they would receive in early spring as the snow melted. The hatching of A. abserratus at a temperature as low as 40°F indicates that hatching could occur in pools resulting from melted snow. This is indeed the case in Manitoba, as A. abserratus larvae are found as early as April.

The higher temperatures required to hatch A. vexans eggs explains why A. vexans larvae are not found in melted snow pools. Such pools usually dry up before becoming sufficiently warm to hatch A. vexans eggs, and these eggs remain unhatched until a heavy late spring or early summer rainfall occurs.

Influence of Temperature and Relative Humidity on Conditioning and Rate of Hatching

Method

A. vexans eggs were taken out of storage at 35°F and placed at 40°, 50°, 60°, and 70°F. Within each temperature the eggs were kept at 20%, 50%, 80% and 100% relative humidity. After two weeks the eggs were placed in hatching solutions at the same temperature at which they had been conditioned. Counts on the number hatching were taken at half hourly intervals.

Results

Table VIII. Percentage and rate of hatching of A. vexans at different temperatures.

Eggs of A. vexans taken from storage at 35°F were conditioned at 40°, 50°, 60°, or 70°F at 20%, 50%, 80%, or 100% R.H. for 2 weeks, then hatched at the conditioning temperature.

Conditioning and Hatching Temp (°F)	Conditioning Relative Humidity							
	20%	50%	80%	100%	20%	50%	80%	100%
	% Hatch				Hrs. for 50% of Hatch to Occur*			
40	-	-	-	-	-	-	-	-
50	50	52	68	67	78	60	54	30
60	1	34	92	70	-	35	32	20
70	-	47	87	94	-	10	9	9

* Mean of 3 reps. of 30 eggs

The highest hatching percentages occurred at the highest conditioning relative humidity (80% and 100%) and at the highest conditioning and hatching temperatures (60°F and 70°F). No hatching occurred at the 40°F treatment. At 50°F, half the eggs subjected to 20% and 50% R.H. hatched, and two-thirds of the eggs kept at 80% and 100% R.H. hatched. The desiccating effect of the 20% R.H. was greatest at 60°F and 70°F, as shown by the almost negligible hatch at these conditions (Table VIII). The eggs subjected to low humidities survived fairly well when also subjected to low temperatures. No eggs hatched at 20% RH and 70°F, while 50% hatched at 20% RH and 50°F.

Within the same temperature, the percentage of eggs hatching increased as the relative humidity was increased. At 70°F, no hatching occurred in eggs subjected to 20% RH, 47% hatching occurred from the 50% RH treatment, and 87% and 94% hatching occurred after the 80% and 100% RH treatments.

The eggs, after different conditioning and hatching treatments, hatched at noticeably different rates. Hatching occurred sooner at the higher temperatures. At 70°F, 50% of the eggs had hatched within 10 hours, while at 60°F, it took 2 to 3 times as long for 50% of the hatching to occur, and at 50°F hatching took as much as 5 to 6 times as long.

Within each temperature treatment, eggs conditioned at high relative humidities hatched more rapidly than eggs conditioned at low relative humidities. This was especially noticeable at the 50°F temperature, as the time for 50% of the hatch to occur dropped from 78 hours at 20% RH to 30 hours at 100% R.H.

Discussion

The conditioning and hatching temperatures tested in this experiment simulated the conditions eggs are exposed to in nature. The conditioning and hatching temperature of 40°F, at which no hatching occurred, approximates the conditions the eggs are exposed to in early Spring. Temperatures of 50°F and 60°F, where two-thirds of the eggs kept at 100% RH hatched, closely simulate natural conditioning occurring in late spring, when A. vexans larvae are first observed. Over 90% hatching occurred at the conditioning and hatching temperature of 70°F, which are the conditions most similar to summer conditions in pools when the second generation of A. vexans occurs.

The higher rate of hatching at the higher temperature was due to the condition of the hatching medium. The basic hatching stimulus for conditioned eggs is provided by a decrease in the dissolved oxygen content of a medium (Horsfall, 1956), and the rate of bacterial action which results in decreased oxygen content increases with temperature.

The reduced rate of hatching of eggs conditioned at lower humidities may be due to the more desiccated embryos having to replace some of their lost water before they could hatch. Also the porous egg micropyle may have had to replace lost moisture before hatching could occur.

Summary

Viability of eggs during prolonged storage is best maintained at hig

relative humidities and low temperatures, as these condtions result in a low rate of transpiration and hence low water loss. Pre-hatch conditioning temperatures influence the ability of eggs to hatch. In nature, hatching of A. vexans eggs occur at temperatures above 50°F, while A. abserratus eggs hatch readily at temperatures as low as 40°F. Pre-hatch relative humidity as well as temperature can affect the rate of hatching of eggs.

CHAPTER V

ABSORPTION AND LOSS OF WATER BY A. VEXANS EGGS

Water absorption and loss by A. vexans eggs at different treatments was studied.

Method and Equipment

All eggs used were obtained from field collected A. vexans females. A test for viability was made by placing the eggs in a solution of Na Cl and water.

The relative humidities used were obtained by various concentrations of potassium hydroxide solutions. The humidity chambers were desiccators with a 25 cm. inside diameter. B.O.D. incubators, accurate to 1.8°F, were used to get the experimental temperatures.

In experiments where weight change of eggs was determined, a Cahn Electrobalance, Model M-10, was used. To facilitate handling and weighing, the eggs were placed in small aluminum foil trays.

The hatching medium consisted of approximately 300 mg. of nutrient broth in 250 mls. of tap water.

Ability of A. vexans Eggs to Absorb Moisture

Method

Eggs of known weight were divided into two groups, half at 80% R.H. for 3 days and half at 50% for one day. Both were kept at 70°F. The weight

loss was first determined and then they were transferred to 100% R.H. for three days. After 3 days at 100% R.H. the eggs were weighed again.

Results

TABLE IX. Weight change in eggs of A. vexans at different relative humidities. Eggs were transferred from 50% or 80% R.H. at 70°F to 100% RH at 70°F.

A - Eggs placed at 50% RH, 70°F, for 1 day.

B - Eggs placed at 80% RH, 70°F, for 3 days.

(all weights in milligrams)

	Original Weight	Weight After Low R.H. Treatment	Weight Change	Weight After 3 Days At 100% R.H.	Weight Change
A	0.36*	0.15	-0.21	0.22	+0.07
B	0.32*	0.21	-0.11	0.31	+0.10

* Mean of 3 Reps. of 10 Eggs Each

Some of the moisture lost by the eggs at the low humidities was regained in the saturated atmosphere (Table IX).

The eggs placed at 50% RH lost a greater amount (58%) of their moisture than the eggs placed at 80% RH (35%). The eggs that had lost moisture at 80% RH regained almost all the lost moisture when placed at 100% R.H., but the eggs that had lost moisture at 50% RH regained only a third of the lost moisture.

Subsequent weighings showed no further weight gain.

Discussion

The greater loss in weight at 50% RH than at 80% RH indicates the rate of transpiration was higher at the lower relative humidity.

The ability of the eggs subjected to 80% R.H. to replace more of the lost moisture than the eggs subjected to 50% R.H. may be due to more of the embryos dying due to desiccation at the lower humidity, and hence being unable to assimilate moisture.

The process of moisture assimilation by eggs is not known but the results suggest it is an active biological process.

Water Loss and Viability in A. vexans Eggs

Method

Aedes vexans eggs were divided into 2 groups: one group for weighing and one group for hatching.

The eggs were placed at 50% R.H. and 80% R.H. at 50°F and 70°F. Small aluminum foil discs were used to hold the eggs. At twelve hour intervals, three discs of 10 eggs were weighed and three discs of 10 eggs were hatched from each treatment combination.

Results

The eggs subjected to different temperatures and relative humidities lost different amounts of moisture and showed different rates of water loss (Table X).

Most of the weight loss occurred in the first six hours. The eggs subjected to 80% RH showed no further weight loss after the first 24 hours. At 50% RH the eggs continued to lose weight up to 72 hours, but at a decreasing rate.

TABLE X. Percent weight loss and percent hatch of A. vexans eggs after treatment to different relative humidities and different temperatures.
Eggs were subjected to 50° or 70°F and 50% or 80% R.H. and weighed at 12 hour intervals. Other eggs under the same conditions were hatched at 70°F at the time of each weighing.

A* Per cent Weight Loss
B* Per cent Hatch

Temp.(°F)	% RH		Hours After Being Placed In The Treatment 6	12	24	36	48	60	72	84	
50	50	A	35	35	35	40	54	54	57	57	Mean % Hatch
		B	33	13	10	23	27	17	53	33	- - - 26
	80	A	41	43	43	43	43	43	43	43	
		B	37	23	3	67	83	67	47	47	- - - 47
70	50	A	54	57	61	61	63	69	65	65	
		B	37	33	23	23	23	17	3	3	- - - 20
	80	A	23	26	28	28	28	28	28	28	
		B	23	23	13	37	60	23	67	53	- - - 37

* Mean of 3 replicates of 10 eggs each.

The greatest loss in weight occurred at 70°F and 50% R.H., the treatment with the highest temperature and lowest relative humidity. This is the condition resulting in the highest rate of transpiration.

There was no apparent regularity in the change of viability of the eggs kept at the different treatments. However, a comparison of the mean per cent hatch occurring at the various treatments indicates that eggs remained viable longer at 50°F than at 70°F and at 80% R.H. than at 50% R.H. (Table X). This is presumed to be due to the greater transpiration occurring at high temperature and low humidities.

Discussion

The attempt to correlate water loss and viability was unsuccessful due to the irregularity of hatching. Other investigators have been successful in doing this. Meola (1964) found a nearly directly proportional relationship between water loss and embryonic viability in A. aegypti. However, he worked with greater numbers of eggs, and his hatching and weighing intervals were greater.

The declining rate of water loss indicates that after the initial water loss, the remaining moisture is less likely to be given up by the egg.

The greatest moisture losses occurred under the conditions which would result in the highest rate of transpiration. The lowest hatching rates were also associated with these conditions.

Influence of Age on the Ability of Eggs to Resist Desiccation

Method

A. vexans eggs were obtained from field collected adults. Oviposition was obtained by placing the oviposition cages on moist cheesecloth pads. The cages were moved each day and the date of oviposition recorded. Two and four week old eggs were used for the experiments. The eggs were placed at 50% or 80% relative humidities and 50° or 70°F for three days. They were weighed every 12 hours.

Results

The four-week-old eggs lost weight less rapidly than the two-week-old eggs (Table XI).

Table XI. Percent weight loss of eggs of different ages kept at different relative humidities and different temperatures. 2 and 4 week old eggs were kept at 50% or 80% RH and 50° or 70°F.

Temp.	R.H.	Age	Hrs. after being placed in treatment 12	24	36	48	60	72
			Per Cent Weight Loss *					
50°F	50%	2 weeks	26	29	39	39	39	39
		4 weeks	5	5	5	25	28	28
50°F	80%	2 weeks	0	7	20	20	20	20
		4 weeks	2	5	5	5	5	5
70°F	50%	2 weeks	47	53	67	67	67	67
		4 weeks	12	12	12	12	12	12
70°F	80%	2 weeks	28	56	67	67	67	67
		4 weeks	3	8	8	8	8	13

* Mean of 3 replicates of 10 eggs each.

Under all temperature-relative humidity treatments, the older eggs had both a lower rate of weight loss and a smaller percentage weight loss.

The younger eggs appeared to be particularly susceptible to moisture loss at 70°F, as they lost 67% of their weight while the older eggs lost only 12 and 13 per cent of their weight at 70°F after 72 hours.

At 50°F the difference in percentage weight lost after 72 hours by old and young eggs was not as great, 11% at 50% RH and 15% at 80% RH.

Discussion

The ability of the older eggs to resist desiccation may have been due to a more impermeable shell, a more mature embryo, or a combination of these. Further investigation is required to determine this.

Summary

A. vexans eggs are able to absorb moisture, apparently by an active biological process, under certain conditions. After an initial loss in moistures by eggs, the remaining moisture is lost at a declining rate. The amount and rate of water loss by eggs is highest at high temperatures and low humidities, lowest at low temperatures and high humidities. Age is a factor in the ability of eggs to withstand desiccation.

CHAPTER VI

LONGEVITY OF ADULTS OF A. VEXANS UNDER CONTROLLED CONDITIONS

The survival of mosquitoes under laboratory conditions was investigated under different temperatures, humidities, and adult nutrition. The effect of these conditions on males and females was investigated.

Method and Equipment

The adults used were 12 to 24 hours old when placed in each treatment. Three replicates were carried out at each of the 27 different conditions. Each replicate consisted of 20 adults, 10 females and 10 males.

The cages used were 6 inches by 1 inch by 1 inch. Two opposing sides were of nylon mesh, the other two sides and ends were of clear 1/8 inch perspex. One end had a corked opening.

The desired humidities were obtained by using different concentrations of potassium hydroxide solutions (Solomon, 1951). Three relative humidities were tested, 20%, 50% and 80%, in combination with three selected temperatures, 40°F, 55°F, and 70°F.

Along with each temperature and humidity combination, there were three nutritional treatments. Some adults were fed on honey, some on water and others were not fed. The honey and water was provided by placing strips of cotton batten on the mesh side of the cages (Fig. 3).

The cages containing the mosquitoes were placed in the humidity chamber (desiccators of 10 inch inside diameter) on plastic trays (Fig. 3). The mosquitoes were kept in total darkness throughout the experiment. Mortality counts were taken every twelve hours.

Results

The results of this experiment are given in Table XII.

FIGURE 3. Desicator With Caged Mosquitoes, 2 With Honey and Water, 2 With Water Only, and 2 Unfed.

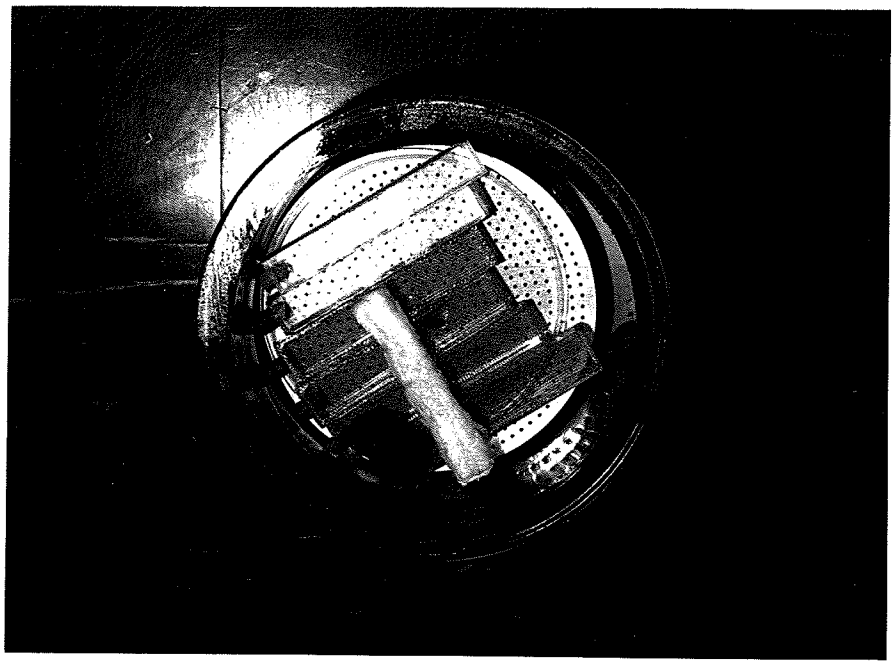


Figure 3

TABLE XII. Longevity of Caged A. vexans Adults At Different Temperatures, Relative Humidites And On Different Foods

Temp.	Food Source	20% RH		50% RH		80% RH	
		♀	♂	♀	♂	♀	♂
40°F	Honey	1	1	1	1	1	1
	Water	1	1	1	1	1	1
	Nothing	1	1	1	1	1	1
55°F	Honey	17.2[1](49)[2]	13.0(33)	25.5(68)	11.4(29)	18.8(49)	10.2(30)
	Water	19.3 (45)	10.9(39)	27.9(82)	13.0(45)	13.9(58)	7.4(23)
	Nothing	4.6 (14)	1.9(25)	4.0(17)	1.9(9)	11.6(42)	4.6(19)
70°F	Honey	16.7 (49)	11.7(30)	12.3(41)	7.0(15)	16.9(40)	25.3(62)
	Water	37.4 (76)	5.7(13)	19.5(49)	8.1(24)	12.1(68)	8.4(23)
	Nothing	1.4 (3)	0.8(1)	2.1(6)	0.8(4)	1.9(9)	0.9(6)

1. av. of 3 reps.

2. maximum no. of days an individual survived.

At 40°F death occurred within one day at all treatments. At 55°F and 70°F, adults survived for various lengths of time depending on the treatment conditions.

Adult survival was greater when nourishment, either honey or water, was provided than when no food was provided.

There appeared to be little difference in longevity at the different humidities.

Females lived longer than males at all treatments, and the maximum number of days an individual survived was greater for females than for males at all treatments.

An analysis of variance was performed of this data. (Table XIII).

TABLE XIII. Analysis of Variance of Data in Table XII.

Source of Variation	D.F.	SS	M.S.	F
Temperature	2	21.94	10.97	.33
Relative Humidity	2	3.52	1.76	.49
Food Source	2	1222.07	611.04	16.97 **
Sex	1	400.67	400.67	11.13 **
Residual	28	1008.00	36.00	
Total	35	2696.00		

** significant at 5% level.

Exposure to 40°F for 24 hours was lethal, and therefore the 40°F data was not used in the analysis.

The analysis of variance indicated no significant difference in longevity of adults at 55°F and 70°F, or at the different relative humidities. Significance was indicated in nutrition and between males and females.

Least significant difference techniques (Steel and Torrie, 1960) were used to locate more precisely where the differences occurred (Table XIV).

TABLE XIV. Least Significant Difference Analysis

Food Source	Honey		Water	Nothing
Mean	15.5		15.3	3.0
Difference		0.2		12.3
LSD =	$\frac{2 S_e^2}{r} \times t$ (5%, 28 d.f.) = $\frac{2 \times 36}{2} \times 2.048 = 5.02$			
Sex	Female	Male		
Mean	14.6	7.9		LSD = 4.10
Difference		6.7		
Temperature		55°F		70°F
Mean		12.1		10.5 LSD = 4.10
Difference			1.6	
Relative Humidity	20%		50%	80%
Mean	11.7		11.1	11.0
Difference		0.6		0.1
LSD =		5.02		

The L.S.D. analysis indicates, as did the analysis of variance, that there is no significant difference in longevity at 55°F and 70°F or at the different humidities, and that there is a significant difference in the longevity of males and females. This analysis also revealed that adults lived as long on water as on honey, but lived a significantly shorter time when unfed.

Discussion

At 40°F death occurred within one day. Different conditioning or use of older adults may have resulted in greater longevity, but under the conditions of this experiment, 40°F for 24 hours was lethal. This in itself is worth noting, as the disappearance of A. vexans adults could be anticipated if the air and ground surface temperature dropped to 40°F for 24 hours. Adults of this species seldom occur before June in Manitoba (Chapter VII, Fig. 4), and usually are not abundant until later in this month, so in nature the adults are seldom exposed to 40°F. If the air temperature drops to 40°F, but the ground temperatures do not drop, the mosquitoes find shelter in the surface vegetation.

There was no significant difference in longevity between mosquitoes kept at 55°F and those kept at 70°F. Both these temperatures occur frequently at the time of year when A. vexans is abundant in nature.

The ability of the mosquitoes to survive as long on water alone as on both honey and water may be partially due to the relatively low activity possible in the test cages. If more activity had been allowed, the mosquitoes given honey may have shown greater longevity, but under conditions of restricted activity water alone appeared to be adequate. The significant difference in the longevity of those mosquitoes given water and those given none was to be expected, as water is lost in respiration and must be replaced.

The females showed significantly greater longevity than the males. In this case laboratory results can probably be applied to nature. At present the reason for the shorter longevity of the adult

male mosquito is not known.

There were no significant differences in survival at the three relative humidities tested. It appears that mosquitoes can survive relative humidities as low as 20% if they have a moisture source available to replenish water loss due to respiration and transpiration. Relative humidities as low as 20% are uncommon in the Winnipeg area, but do occur occasionally for short periods of time. This experiment indicates that A. vexans could survive such conditions with no difficulty.

It is not suggested that the results of these experiments indicate that longevity of mosquitoes in the laboratory can be translated directly to survival in the field, but the data may prove useful in the interpretation of survey data as well as provide useful information on culture techniques.

CHAPTER VII

SURVEY OF MOSQUITO POPULATIONS IN THE WINNIPEG AREA

Adult Survey

A number of New Jersey light traps were operated in and around Winnipeg during the spring and summer months of 1965 and 1966. They were controlled by electric timing devices and operated from 7 pm to 7 am. The catches were collected twice weekly and the adults sorted to sex and counted. In 1965, eight traps were operated inside Metro Winnipeg. These were located at the Legislative greenhouse, Kildonan Park, Assiniboine Park, Brookside Cemetary, Charleswood Sewage Lagoon, University of Manitoba, Windsor Golf Course, and Lot 62, St. Vital. As a comparison, four traps were operated outside of the area treated and fogged by the abatement district. These were located at the Glenlea Research Station, Oak Bluff, Lilyfield, and La Barrier Park. In 1966 an additional trap was operated at Transcona, inside the control area.

(a) Species Classification and Relative Abundance

Twenty-five species were identified from survey operations in the Winnipeg area in 1965 and 1966. Table XV gives the species identified, and their relative abundance.

TABLE XV. Relative Abundance of Adult Mosquito Species in Light Traps.

	1965			1966		
	Outside City	Inside City	Charleswood Lagoon	Outside City	Inside City	Charleswood Lagoon
Aedes vexans	54.2%	55.6%	36.6%	39.3%	37.0%	18.2%
Culiseta inornata	30.3	28.4	30.1	20.3	23.3	23.8
Culex restuans	0.2	0.35	-	5.8	5.2	3.3
Culex tarsalis	1.8	3.5	22.4	2.1	3.9	8.1
Aedes flavescens	1.3	1.6	2.9	7.3	7.9	9.1
Aedes stimulans	3.3	0.8	0.7	6.2	4.8	4.6
Aedes riparius	1.5	1.0	-	4.8	3.7	1.6
Aedes spencerii	2.0	2.2	3.6	3.4	4.0	19.2
Aedes fitchii	0.7	0.05	0.4	3.0	3.1	1.6
Aedes excrucians	0.2	0.4	-	2.5	1.3	0.3
Aedes nigromaculis	0.2	-	0.4	2.0	0.7	2.6
Aedes dorsalis	0.6	0.8	1.8	1.2	1.3	4.6
Aedes implicatus	0.2	0.1	-	0.9	0.8	1.0
Aedes cinereus	0.6	0.9	0.4	0.4	0.8	0.3
Aedes intrudens	-	0.1	-	0.2	0.5	0.3
Aedes sticticus	-	0.2	-	0.1	0.1	0.3
Aedes campestris	-	0.005	-	0.1	0.1	-
Aedes canadensis	-	0.05	-	-	0.1	-
Aedes communis	0.9	1.1	-	-	0.1	-
Anopheles spp.	0.2	0.7	-	0.3	0.5	-
Culiseta morsitans	-	0.05	-	0.1	0.6	-
Mansonia perturbans	-	-	-	0.1	0.1	-

The species of adults and the proportion of each species occurring inside the control area was similar to the species and proportion of each occurring outside this area. This suggests a possible infiltration into the control area from the outside.

With regard to location, the Charleswood Sewage Lagoon trap was considered separately as it was subjected to a different environment than the other traps. This will be discussed later in the chapter.

Aedes vexans was found to be the most abundant species in the Winnipeg area. At times A. vexans comprised a greater percentage of the mosquito population than is indicated in Table XV. In Table XV the figures given are average figures for the entire period of operation, May to September. Figure 4 shows more clearly the seasonal abundance of A. vexans. During the summer months of 1965 this species comprised up to 85% of the mosquito population. The importance of A. vexans in terms of human annoyance is emphasized by the fact that Culiseta inornata, the second most common species, is primarily a bird feeder and seldom attacks man.

(b) Adult Population Levels Inside and Outside the Control Area

Figure 5 and Figure 6 illustrate the adult mosquito population levels occurring inside and outside the city in 1965 and 1966, as measured by light traps. As expected, the greatest numbers of mosquitoes were trapped in locations outside the control area. (See Appendix I and II for individual trap data.)

Two population peaks occurred inside and outside the control area in both 1965 and 1966. The first occurred near the end of June and the second four to six weeks later. The June population was first observed

FIGURE 4. Numbers of *Aedes vexans*, *Culiseta inornata*, and Other Species Trapped in 1965 Survey.

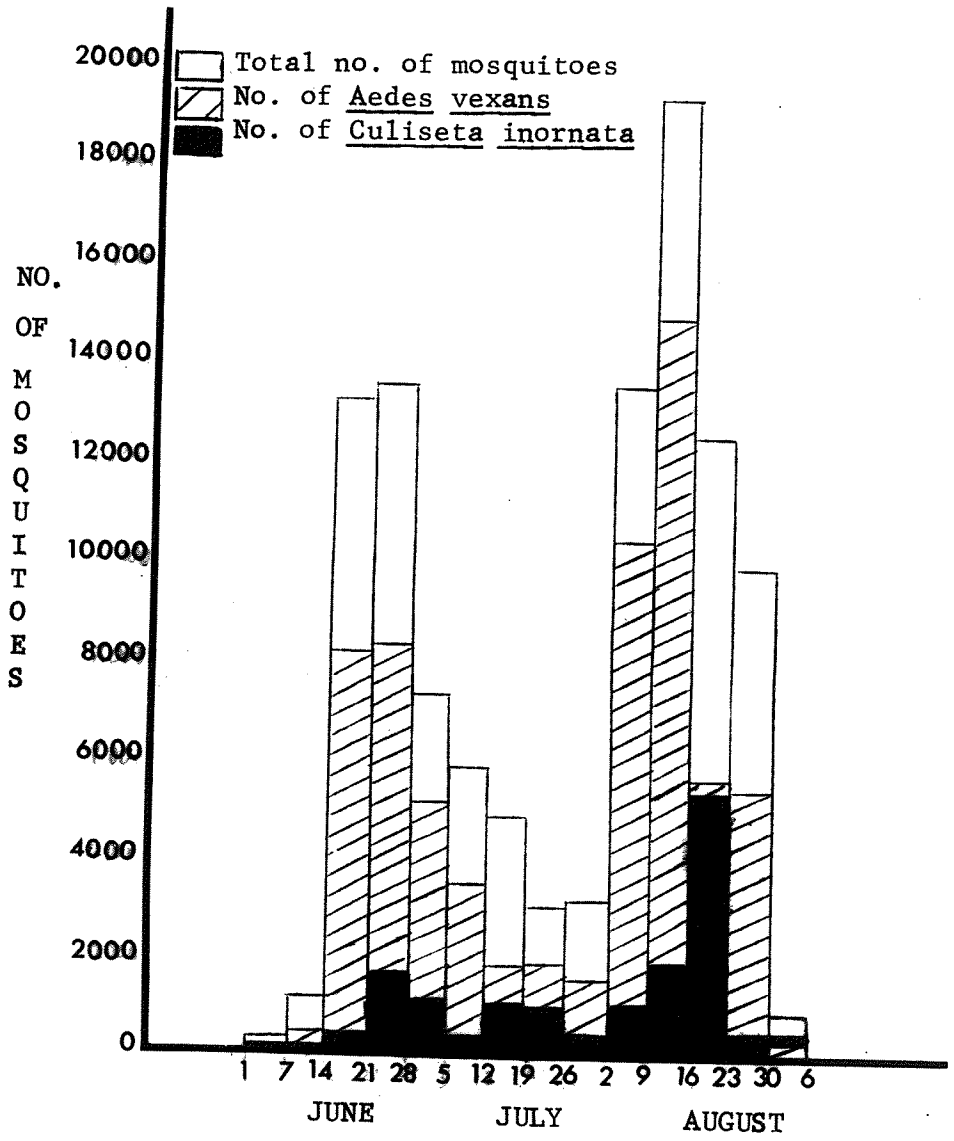

Total no. of mosquitoes
No. of Aedes vexans
No. of Culiseta inornata
NO. OF MOSQUITOES
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
1 7 14 21 28 5 12 19 26 2 9 16 23 30 6
JUNE
JULY
AUGUST

Figure 4

in the traps outside of Metro Winnipeg, then in the traps inside Metro, 4 or 5 days later. This has been the trend for most years since trapping was begun in 1957. This indicated that mosquitoes produced outside of Metro Winnipeg move in some days later.

In 1965, the first population peak inside Metro Winnipeg was later but larger than the first peak occurring outside the city (Fig. 5). In 1966, the population peak occurring about July 15th was larger inside the control district than outside it (Fig. 6). This suggests that infiltration of mosquitoes into Winnipeg was not only from the area immediately outside it, but that a migration and convergence on the city from farther off occurred. Migration and convergence of other populations later in the summer are not apparent from the graphs (Figs. 5 and 6), but they may have occurred over an extended period so that the effect was not shown in the light trap catches.

(c) Daily Catches of Male and Female Aedes vexans Adults

Three traps outside the control area were serviced daily from June 17th to July 30th, 1965. These traps were located at Oak Bluff, La Barriere Park, and the Glenlea Research Station. The daily catches are recorded in Appendix III.

As shown in Figure 5, a population peak occurred during the last 2 weeks of June, 1965. The daily catches of males and females from three traps during this period are shown in Table XVI.

FIGURE 5. Numbers of Adults Trapped Inside and Outside Metro Winnipeg in 1965 Survey.

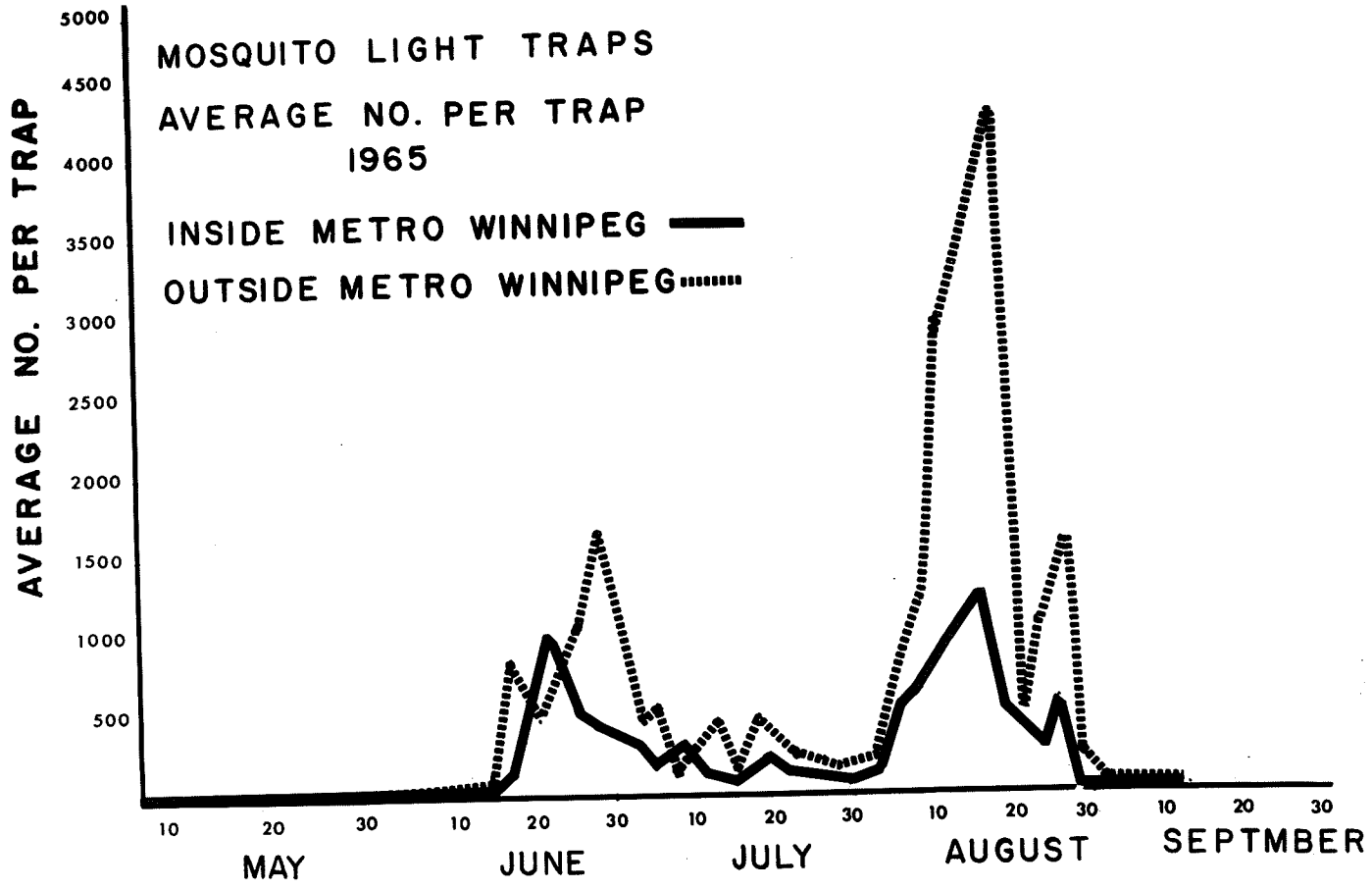

MOSQUITO LIGHT TRAPS
AVERAGE NO. PER TRAP
1965
INSIDE METRO WINNIPEG
OUTSIDE METRO WINNIPEG
AVERAGE NO. PER TRAP
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
10
20
30
MAY
JUNE
JULY
AUGUST
SEPTMBER

Figure 5

FIGURE 6. Numbers of Adults Trapped Inside and Outside Metro Winnipeg in 1966 Survey.

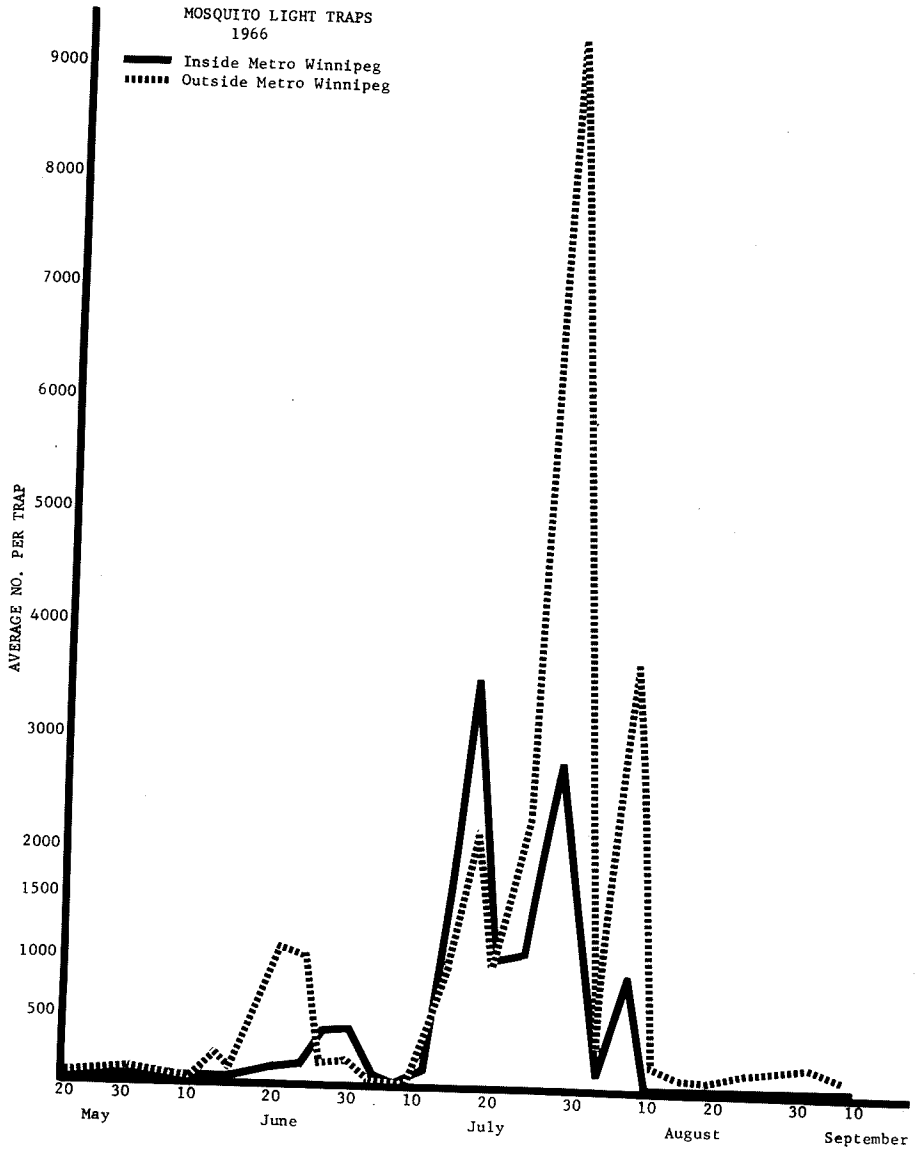

MOSQUITO LIGHT TRAPS
1966
Inside Metro Winnipeg
Outside Metro Winnipeg
AVERAGE NO. PER TRAP
9000
8000
7000
6000
5000
4000
3000
2000
1500
1000
500
20
30
10
20
30
10
20
30
10
20
30
10
May
June
July
August
September

Figure 6

TABLE XVI. No. of Adult *Aedes vexans* Trapped at 3 Locations Outside the Control Area, 1965.

Date	Oak Bluff		La Barriere		Glenlea		Total	
	♀	♂	♀	♂	♀	♂	♀	♂
June 17	160	0	7	0	4	21	171	21
18	80	140	0	7	54	21	134	161
19	150	110	7	20	40	30	197	160
20	50	70	132	132	70	70	252	272
22	100	80	20	60	8	4	128	144
23	80	280	6	9	9	4	95	293
24	75	50	0	0	45	40	120	90
25	1000	100	70	30	270	90	1340	220
26	0	0	1	2	15	6	16	8
27	60	15	0	0	30	0	90	15
28	45	30	2	0	4	0	51	30
29	66	33	44	22	120	0	230	55
30	110	30	4	0	11	2	125	32

During the early stages of the increase in population, from June 18th to June 23rd, more male than female mosquitoes were trapped (Table XVI). From June 23rd on, greater numbers of females were trapped. The predominance of males at the start of a population rise is due to the shorter developmental period of males.

In rearing *Aedes vexans* in the laboratory, it has been found that males have a shorter developmental time, from 1st instar to adult, than females. Females usually do not emerge until 2-3 days after male emergence, depending upon the rearing temperature. Also, female mosquitoes live longer than males and progressively make up more of the subsequent population.

The appearance of large numbers of males in survey traps may possibly be used as an indicator of population increases. No work has been published to date on sex ratios in field populations of mosquitoes and trap catches.

(d) Number and Ratio of Females and Males of All Species "Inside" and "Outside" During Population Peak, 1965

The ratio and average number per trap of female and male adult mosquitoes trapped inside and outside the control area during the first population peak of 1965 (Fig. 5) is given in Table XVII.

TABLE XVII. Number of Adults Per Trap and Ratio of Females to Males of all Species Trapped Inside and Outside the Control Area, 1965.

Collection Period	Inside Control Area			Outside Control Area		
	No. Caught Per Trap F	M	F:M Ratio	No. Caught Per Trap F	M	F:M Ratio
June 1-June 3	7	1	7:1	10	2	5:1
" 4- " 7	9	1	9:1	20	5	4:1
" 8- " 10	2	1	2:1	14	1	14:1
" 11- " 13	66	28	2.36:1	92	99	1:1.08
" 14- " 16	98	41	2.39:1	695	647	1.07:1
" 17- " 20	727	331	2.20:1	273	247	1.11:1
" 21- " 24	422	46	9.17:1	351	296	1.19:1
" 24- " 28	394	34	11.59:1	942	498	1.89:1
" 28-July 2	357	31	11.52:1	360	117	3.08:1
July 3- " 5	187	17	11.00:1	494	70	7.06:1

The mosquitoes trapped in the first three collection periods were mostly Culiseta inornata females which had left their overwintering sites. This accounts for the large female to male ratio occurring up to June 10 (Table XVII).

The female to male ratios outside the control areas stayed relatively constant at about 1:1 from June 11 to June 24 (Table XVII). After this, the F:M ratio gradually changed to favor females, and by July 3 it was 7:1 females to males. This was due most likely to the greater longevity of females (Aedes females normally live about twice as long as males in the laboratory). Inside the control area, the F:M ratio was about 2:1 at the beginning of the population peak (June 14-16). During the next three days, June 21-24, the inside trap caught 9 times as many females as males and this ratio went up to 11:1 (F:M) on June 24-28. During the same period, June 17-24, the F:M ratio in the outside traps stayed at 1:1 (Table XVII) and only climbed to 2:1 by June 24-28.

The sudden change in the F:M ratio (2:1 to 11:1) occurring around June 20 inside the city was due to three possible factors: (1) more females than males migrated into the city, (2) males may have been more adversely affected by DDT fogging being carried on in the city, and (3) males migrating into the city may not find a proper food source and may die within a few days, never reaching the traps. All of these factors should be studied in much more detail in the future.

(e) Effect of Precipitation On Adult Population Levels

Precipitation is the most important environmental variable affecting mosquito populations. This is shown in Figure 7 and Figure 8, which relate adult population levels to precipitation in the years 1964 and 1965.

FIGURE 7. Relation of Adult Mosquito Population to Precipitation, 1965.

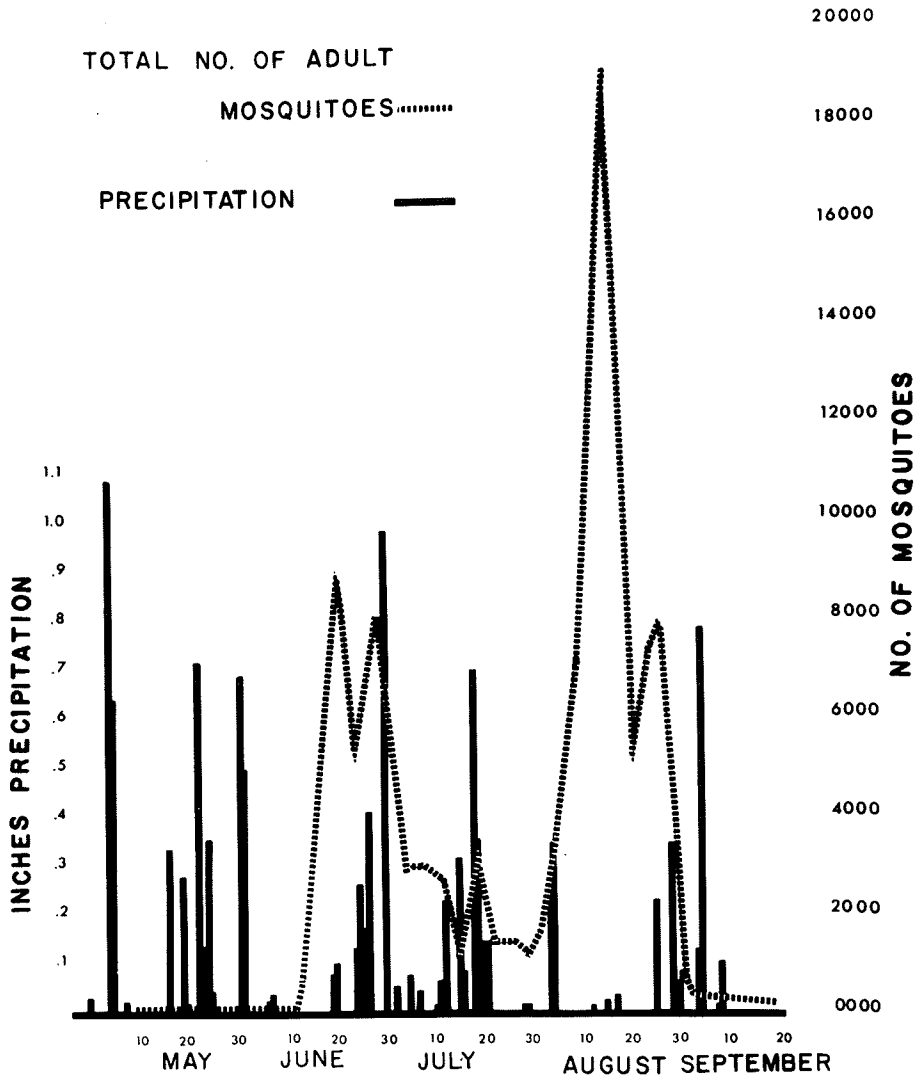

TOTAL NO. OF ADULT
MOSQUITOES
PRECIPITATION
INCHES PRECIPITATION
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
NO. OF MOSQUITOES
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0000
10 20 30 10 20 30 10 20 30 10 20 30 10 20
MAY JUNE JULY AUGUST SEPTEMBER

Figure 7

FIGURE 8. Relation of Adult Mosquito Population to Precipitation, 1966.

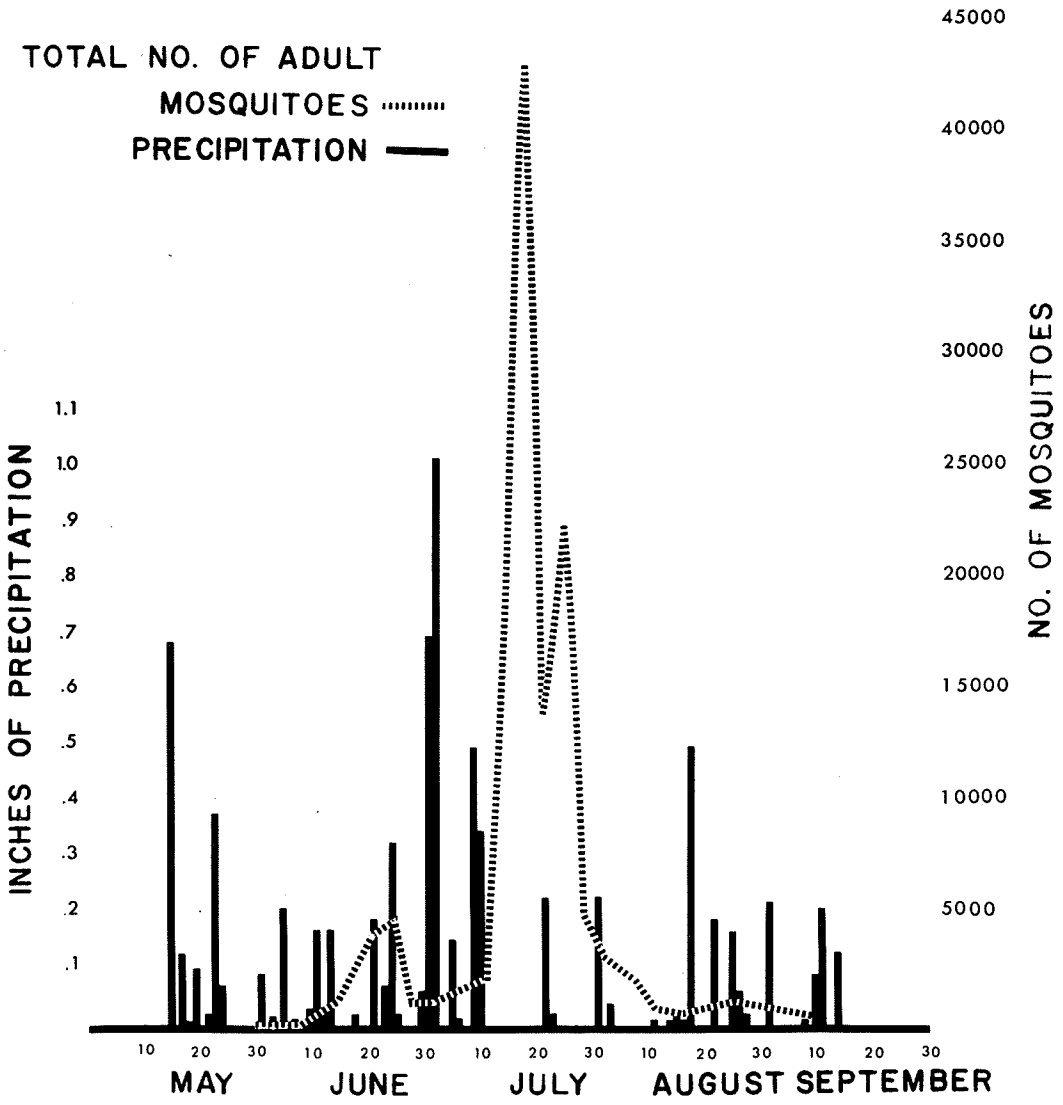

TOTAL NO. OF ADULT
MOSQUITOES
PRECIPITATION
INCHES OF PRECIPITATION
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
NO. OF MOSQUITOES
45000
40000
35000
30000
25000
20000
15000
10000
5000
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30
MAY
JUNE
JULY
AUGUST
SEPTEMBER

Figure 8

The ground water resulting from melting snow in early spring did not result in any appreciable numbers of adult mosquitoes, as can be seen in the graphs. The first rise in the adult population occurred in the first part of June of both years. This was three to four weeks after the first heavy spring rain, the time required for mosquito eggs to hatch and the life cycle to be completed. Similarly, the second population peak occurred after heavy rains in late June and July. The interval between a heavy rainfall and a population increase was shorter for the second population because of higher temperatures. The two population peaks were made up mainly of A. vexans, a multi-generation species whose over-wintering eggs do not hatch during the spring run-off, but hatch later when the water temperature rises to an appropriate level.

Larval Survey

Mosquito larval surveys were conducted throughout the Spring and Summer of 1965 and 1966, usually prompted by heavy rainfall. The species collected and their date of collection are given in Appendix IV and V. Table XVIII shows the number of developmental sites of each species sampled, and the month in which the collections were made. The univoltine aedine species occurred primarily in the spring, whereas the multivoltine aedine species were found throughout the summer. The larvae resulting from oviposition by overwintering females of Culex and Culiseta species were not found in appreciable numbers until July. A. vexans was found more frequently than any other species(Table XVIII).

The larval surveys of 1965 and 1966 revealed 92 developmental sites outside the control area and 33 within it. The survey was largely restricted to areas easily accessible by road, so many developmental sites outside the

TABLE XVIII. No. of Mosquito Developmental Sites Found.

1965

	APRIL	MAY	JUNE	JULY	AUGUST	TOTAL
Aedes campestris		1				1
Aedes dorsalis				6		6
Aedes excrucians		1				1
Aedes fitchii		3				3
Aedes implicatus		6	1			7
Aedes nigromaculis			2			2
Aedes spencerii	1	1				3
Aedes sticticus			1			1
Aedes stimulans			2			2
Aedes trichurus	1	2				3
Aedes vexans		6	10	10		26
Culex restuans					1	1
Culex tarsalis			1	6		7
Culiseta inornata			2	3		5
						68

1966

	MAY	JUNE	JULY	AUGUST	SEPTEMBER	TOTAL
Aedes campestris	1					1
Aedes communis	1					1
Aedes dorsalis			3			3
Aedes flavescens			1			1
Aedes fitchii	3					3
Aedes implicatus	2		1			3
Aedes nigromaculis			1	4		5
Aedes riparius	1					1
Aedes spencerii	2		3			5
Aedes stimulans	1					1
Aedes vexans			5	7	1	13
Culex restuans			4	3	1	8
Culex tarsalis			4	3	1	8
Culiseta inornata		2	2	2		6
						59

city were possibly overlooked. Usually developmental sites in the city were small and contained relatively small larval populations, whereas the outside sites were large and hence supported greater numbers of larvae. The large number of larvae found outside the city combined with the few larvae found inside the city again indicates that many of the adult mosquitoes found within the control area were produced outside.

Survey of Charleswood Sewage Lagoon

Sewage lagoons may be considered possible health hazards due to both their function as purification units of human wastes and their potential as mosquito production sites. Because of this a light trap was operated and an extensive larval survey was made at the Charleswood Lagoon, on the outskirts of Metro Winnipeg.

In both 1965 and 1966 greater percentages of Culex tarsalis, considered the primary encephalitis vector (Horsfall, 1962), were trapped in the lagoon area than at the other trap locations (Table XIX).

FIGURE 9. (1) Shore of Charleswood Sewage Lagoon Cell Free of Vegetation.

(2) Shore of Charleswood Sewage Lagoon With Vegetation, Making it More Suitable For Mosquito Oviposition.

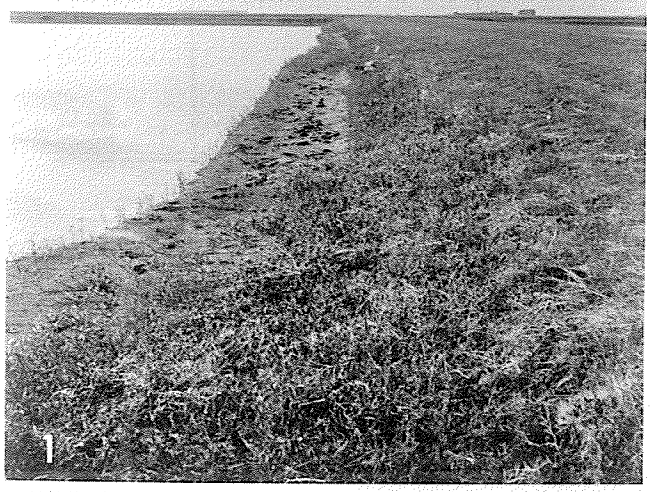


Figure 9

TABLE XIX. Relative Abundance of Major Mosquito Species.

	1965			1966		
	Outside City	Inside City	Charles-wood Lagoon	Outside City	Inside City	Charles-wood Lagoon
Aedes vexans	54.2%	55.6%	36.6%	39.3%	37.0%	18.2%
Culiseta inornata	30.3	28.4	30.1	20.3	23.2	23.8
Culex tarsalis	1.8	3.5	22.4	2.1	3.9	8.1

It is known that Culex species can develop in permanent pools and lagoons, and hence a weekly larval survey was conducted from May to September in 1965 and 1966. Three of the five cells making up the lagoon were examined each week. Samples of the water at the edge of the cells were taken every 10 yards with dippers, and the cells were also given close visual examination. No mosquito larvae were observed, but it is possible that in the vast expanse of water making up the lagoon, several hundred larvae could have gone unnoticed.

In 1965, fewer adults were caught in the lagoon trap than at any of the other traps (Appendix I). This may have been due to the grass in the area being kept short and the lagoon edges being kept clear of debris (Figure 9-1). In 1966, the trap at the lagoon rated fourth in the number of mosquitoes caught of the thirteen traps operated. In 1966, vegetation was allowed to grow around the edges of some of the cells (Figures 9-2) and this vegetation may have contributed to the large adult population in the area by providing shelter for adults and suitable oviposition sites for aedine species as well as Culex and Culiseta species. In spite of the large

adult population, no larvae or egg masses were observed in the lagoon cells.

CHAPTER VIII

WESTERN ENCEPHALITIS INVESTIGATIONS IN MANITOBA

The western equine encephalitis virus causes inflammation of cells found in the central nervous system of horses, primates and numerous birds. In man its effect is usually mild and short lived, but occasionally permanent damage may be done to areas of the central nervous system and death, although uncommon, may occur. In 1941 an epidemic struck man in Southern Manitoba and neighbouring parts of North Dakota and Minnesota, and over 3,000 cases were reported with a mortality of 8 - 15 per cent (Horsfall, 1962). No epidemics have been reported in recent years, but isolated outbreaks among horses and occasional human cases have occurred. The occurrence of western encephalitis in Manitoba in the past 25 years is shown in Table XX.

The first investigations on western encephalitis in Manitoba were done by Dr. J. McLintock from 1942 to 1945. The methods of collecting and handling mosquitoes used by McLintock (1946), together with his later developments in Saskatchewan, provided the basis for the present program in Manitoba.

The present program began in 1965 on a preliminary basis. Lack of funds and personnel limited the number of mosquitoes which could be properly handled, and dictated the use of sub-optimal techniques. In 1966, increased funds and personnel were available, permitting more mosquitoes to be handled and permitting the use of more reliable techniques. The wes-

TABLE XX. Incidence of Western Encephalitis in Manitoba Over The Past 25 Years

YEAR	HUMAN		HORSE	
	CASES	DEATHS	CASES	DEATHS
1941	521	79		
1942	41			
1943	13			
1944	8			
1945	8			
1946	6	5		
1947	81	14		
1948	4	7		
1949	37	6		
1950	2	2		
1951	4	1		
1952	6			
1953	11	4		
1954	5	3	32	
1955	1	1	3	
1956	8	1	3	
1957	8	1		
1958	8	1	5	
1959	5	2	6	
1960	3	1		
1961				
1962	4	2		
1963	9		95	
1964			18	
1965	9		75	

tern encephalitis project in Manitoba is expected to expand in 1967 due to increased financial support and knowledge gained from past experience.

The investigation is being carried out with the assistance and cooperation of the Manitoba Department of Health, and the Winnipeg General Hospital Virus Laboratory.

Design and Operation of Western Encephalitis Survey Trap

The trap used at present in Manitoba is a modified New Jersey trap (Fig. 10).

The trap consists of three units: (1) a cylinder containing an electric motor and fan, (2) a canopy carrying a light, and (3) a screen cage that fits into the cylinder to hold the insects. The fan is located at the base of the cylinder below the level of the screen cage. This draws the mosquitoes down into the cage without having them pass through the fan blades. The canopy has an attached fixture for a black ultraviolet light. Ultraviolet light was found to be more attractive to mosquitoes than white incandescent light (MSc thesis, Brust, 1960). This is the major difference from the trap used by McLintock (1946). The cage consists of a screened funnel leading to a cylinder, both of 16 mesh screening. The base of the cylinder is a removable metal cap by which the insects are removed. The cage is supported by three metal tabs which attach at the top of the trap cylinder (Fig. 10-1).

The trap was started and stopped by an electric timer. It was

started at 8 pm and stopped at 10 am daily. The trap was emptied as soon as possible after daybreak to avoid the heat of the sun and its subsequent desiccating effect on the mosquitoes. While the fan was running, a sponge stopper was inserted into the mouth of the funnel to prevent any of the insects from escaping. The screen cylinder was replaced with an empty one. The catch was placed in a styrifoam picnic cooler together with a refreezable ice pack and a moisture plug, and taken to the Winnipeg laboratory.

1965 Investigation

Four traps were operated in 1965. Two were operated at the Delta Wildfowl Research station, another on a farm near Gladstone, and a fourth on a farm at Oak Bluff, near Winnipeg.

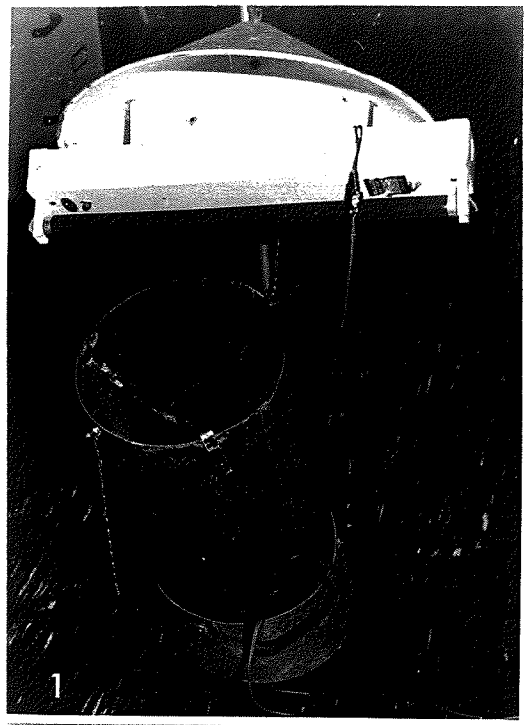
The traps were serviced daily and the cages with the insects were immediately placed in a freezer at -20°C. This immobilized the insects sufficiently to transfer them to another container in the freezer. Due to lack of personnel, the mosquitoes were not separated from other insects. The daily catches of all insects caught for a three week period at Oak Bluff were pooled in a single container, and the same procedure was used for the Gladstone catches. Each day's catch from Delta was kept separately. The insects were kept at -20°C until they were transported to the virus laboratory for analysis. The analysis technique consisted basically of grinding up samples of the insects and forming a suspension to use as an inoculum for mouse and tissue culture tests.

All attempts to isolate the virus were negative (Appendix VI). How-

FIGURE 10.

10-1 Encephalitis Trap, Showing Collecting Cage.

10-2 Encephalitis Trap in the Field.



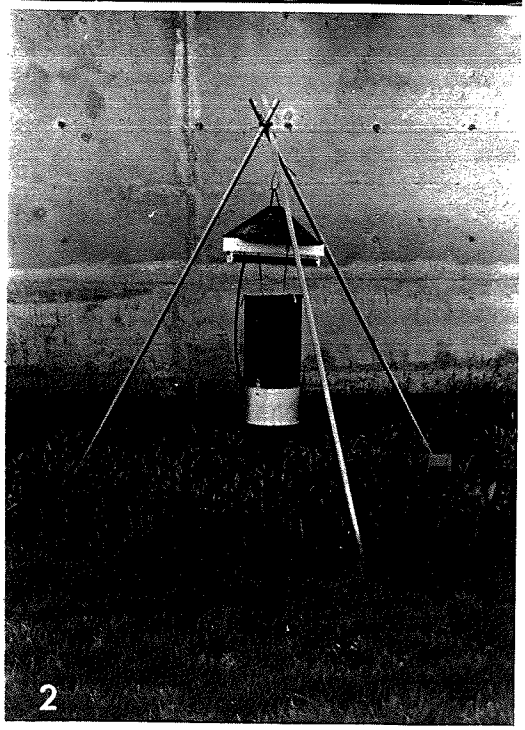


Figure 10

ever the techniques used in handling the mosquitoes, particularly leaving them unseparated from other insects, was unsatisfactory and may possibly have contributed to the negative results.

1966 Investigation

The encephalitis project was operated on a larger budget in 1966. This allowed the purchase of necessary materials and permitted daily shipment of mosquitoes by bus from rural trap locations. Equally important was the operation of collecting and handling of mosquitoes which became the full responsibility of Mr. J. MacKay.

Four traps were operated in 1966, and these were located at Delta, Brandon, Assiniboine Park, and the Charleswood Sewage lagoon. Several factors were considered in choosing a trap location. (1) Personnel must be available to service the trap and send the catch to Winnipeg. (2) The trap must be located near a bus route to have the catch reach Winnipeg rapidly. (3) Electrical power must be available. (4) The trap should be located in areas with large concentrations of waterfowl or domestic animals. Charleswood and Delta were chosen because of the large concentration of migrating waterfowl which serve as hosts to mosquito species commonly found to transmit WE . Brandon was selected because of the high incidence of WE in horses over the past years. Assiniboine Park in Winnipeg was convenient as a trap location and the zoo animals attracted a large number of mosquitoes to the area.

Immediately upon removal from the trap, the cage containing the insects was placed in a styrifoam cooler containing an icepack and wet rags to avoid desiccation in transport. From the Brandon and Delta loca-

tions, the cooler was sent by bus to Winnipeg. The time between servicing of the trap and the time the insects reached the laboratory was seldom more than four hours. The traps at Charleswood and Assiniboine Park were emptied in the morning and the catch brought directly to the laboratory.

In the laboratory the contents of the screen cages were transferred to separate perspex cages. To do this the base of the screen cage was inserted through a sleeved opening and the metal cap removed. The female mosquitoes were then taken individually from the perspex cage with an aspirator and identified to genus (Aedes, Culex, Culiseta, Anopheles). Mosquitoes of the same genus and location were placed in World Health Organization adult testing tubes. These tubes were shaken vigorously to stun the mosquitoes. The mosquitoes were then placed in 10 cc ampoules and the ampoules labelled as to date, location, genus of the specimens, and number of mosquitoes. The ampoules were heat sealed and immediately placed at -65°C on dry ice. They were subsequently stored and transported on dry ice until analysis for the virus was carried out.

The technique for analysis consisted of grinding the samples with either a glass rod for small samples, or with a Tenbroek tissue grinder for the larger samples. Suspensions made in the diluent consisted of 199 with 30% foetal calf serum. Antibiotics used in the diluent consisted of 100 units Bactracin, 200 units Polymysin B Sulphate, and 0.2 mgs. of Neomycin Sulphate. Suspensions were kept frozen at -20°C until ready for testing, at which time they were thawed and centrifuged at 3000 R.P.M.. All attempted virus isolations were made in cultures of fibroblast tissue of chick embryos, and were conducted by the virus laboratories Winnipeg General Hospital.

The number of mosquitoes caught throughout the summer at the four trap locations is given in Appendix VII.

All attempts to isolate the virus from collected mosquitoes were negative (Appendix VIII). The incidence of WE in 1966, as determined from the number of clinical cases in horses and humans (8 suspect cases in horses, none in humans), was one of the lowest ever to occur in Manitoba.

CHAPTER IX

COLLECTION OF ADULT MOSQUITOES IN A 40 FOOT VERTICAL TAYLOR TRAP

Method and Equipment

A trap was put up by the Department of Entomology, University of Manitoba, to sample insect populations at 40 feet above ground level. The trap (Figure 11) consisted of a number of sections of 24 gauge galvanized piping, 10 inches in diameter, with a 4 inch flare at the top, extending vertically to 40 feet. Suction was applied at the base of this piping by a 13½ inch fan powered by a 3/4 horsepower electric motor. The motor was encased in plywood and was bolted to a cement foundation. No attractant was used. Insects sucked in at the apex of the trap were funneled into a bottle containing potassium cyanide.

The trap was located at the Glenlea Research station, 14 miles south of Winnipeg. In 1965 the trap was operated 24 hours a day and was serviced daily from May 1st to August 30th. The mosquitoes caught in this trap were separated by sex and identified.

Results

The number, sex, and species of mosquitoes caught are given in Appendix X.

Discussion

Aedes vexans and Culiseta inornata were the species trapped in the greatest number. They were also the main species caught by a light trap

operating in the same area, however the light trap caught greater numbers due to both the use of light as an attractant and the fact that it was closer to the ground.

The number of females of all species taken throughout the summer was small, indicating that the mosquito population at 40 feet was small. This was similar to the results reported by Burgess and Haufe (1960) in a study of stratification of prairie mosquitoes. They sampled mosquito populations at 5 feet, 25 feet, and 50 feet, and found that over the prairie there was a general decrease in the density of all species as height above ground increased.

On several occasions relatively large numbers of Aedes vexans males were caught. Burgess and Haufe (1960) also reported capturing large numbers of A. vexans males at the top level of a vertical series of traps. Possibly the trap had acted as a swarm marker. Male mosquitoes frequently swarm over tall trees, chimneys, church steeples, and other such objects which contrast with the background (Downes, 1958).

FIGURE 11. 40 Foot Taylor Trap

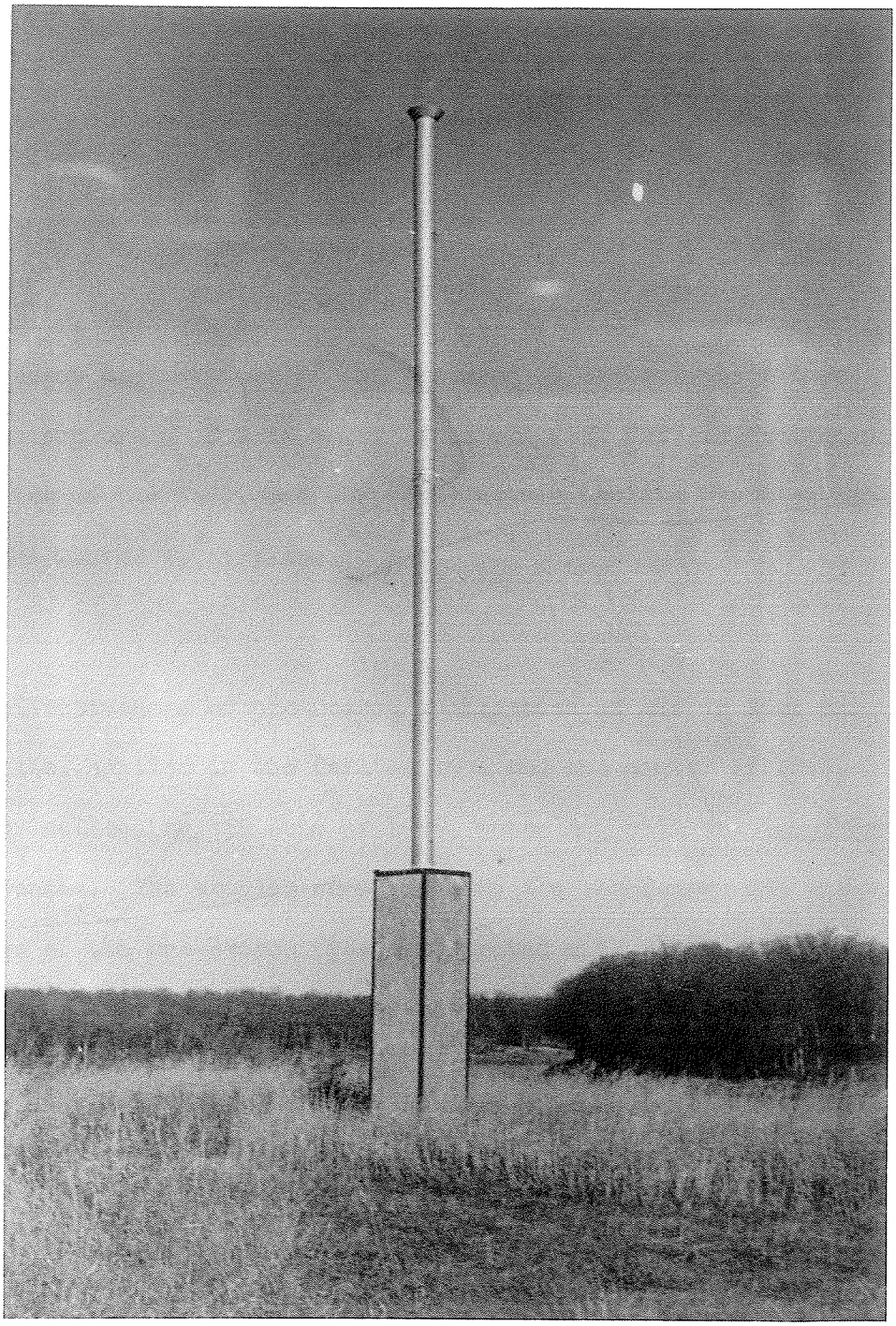


Figure 11

CHAPTER X

INSECTICIDE STUDIES

Testing the Effectiveness of the Pre-Season Treatments

Pre-season applications of DDT are used to control mosquitoes in Metro Winnipeg area. DDT in No. 2 fuel oil (15% DDT) is applied with ground vehicles in the fall, and DDT (30%) on vermiculite granules is applied in the winter by airplane.

Method

The effectiveness of pre-season application of DDT in fuel oil and vermiculite, applied in the fall of 1964 and the winter of 1965, was tested by collecting, in June of 1965, water and soil samples from the areas treated. The samples were taken to the laboratory and the effectiveness of the pre-season treatment tested with 4th instar *A. vexans* larvae. After 24 hours counts were taken of dead and moribund larvae.

Results

Table XXI A and B shows the results of this testing. The pre-season application of 15% DDT in No. 2 fuel oil remained effective until June only in permanent pools such as sloughs and ponds, or in ditches where no run-off occurred (Table XXI-A). Temporary pools, and ditches where run-off

TABLE XXI. Effectiveness of Pre-season Treatments for 1965.

A. Water samples of areas treated with 15% DDT in No. 2 Fuel oil in Fall of 1964, samples taken June, 1965.

Sample	Type of Water	% Mortality
A	Permanent	100
B	Temporary	32
C	Temporary	100
D	Permanent	100
E	Temporary	15
F	Temporary	12
G	Temporary	15
H	Temporary	15
I	Temporary	16
J	Temporary	10
K	Temporary	20
L	Permanent	100
M	Temporary	20
N	Temporary	30
O	Permanent	100
P	Temporary	20
Q	Temporary	4
R	Temporary	10
S	Permanent	100
T	Permanent	92
U	Temporary	10
V	Temporary	32
CONTROL		10

B. Samples from areas treated with 30% DDT Vermiculite granules by airplane in 1965.

The samples were taken in June 15, 1965 and June 30, 1965 and "2" of rainfall occurred between these dates.

Sample	Type of Water	% Mortality June 15	% Mortality June 30	Difference In % Mortality
A	Permanent	96	24	- 72
B	Permanent	100	100	0
C	Permanent	20	0	- 20
D	Permanent	100	100	0
E	Permanent	100	100	0
F	Temporary	16	24	+ 8
G	Permanent	100	100	0
H	Permanent	100	100	0
I	Temporary	100	3	- 97
J	Temporary	36	4	- 32
K	Permanent	100	24	- 76
CONTROL		8	12	+ 4

occurred, did not contain enough insecticide to kill larvae by June. With one exception (Sample C), only the samples taken from permanent pools resulted in at least 90% mortality in the test larvae. The samples taken from temporary pools generally resulted in less than 20% mortality. This indicates that fall treatment of temporary pools and ditches does not give control in June. The tests made on the winter application of 30% DDT vermiculite granules also indicates better control was obtained in permanent than in temporary pools (Table XXI-B). The apparent tendency of the insecticide impregnated granules to be carried away with run-off water suggests limiting the use of this material to permanent and semi-permanent pools.

Table XXI-B also shows the effect of a heavy rainfall on the effectiveness of pre-season insecticide application. The 2 inch rainfall occurring between sampling dates resulted in a decrease in effectiveness of the insecticide in half of the sample areas. This was possibly due to dilution of the insecticide and further loss by run-off. Additional rainfall throughout the summer would possibly result in a further decrease in the effectiveness of the insecticide.

Discussion

Pre-season application of insecticide should be limited to permanent or semi-permanent pools. A second treatment with insecticide should be considered before the summer is over. Larvae of Aedes vexans, the primary pest species in the Winnipeg area, do not occur until June and are found mainly in temporary pools. Therefore, it appears that a pre-season insecticide appli-

cation would have a limited effect in controlling this species.

Evaluation of Different Chemicals and Formulations

Method

An evaluation of various chemicals and formulations, both new and now in use, was started in the summer and fall of 1965 and continued until the fall of 1966. Plastic pans, 12 inches in diameter and 3 inches deep, were partially filled with soil (Figure 12). The materials being studied were applied at recommended field rates or, in the case of new insecticides, at varying rates, to the surface of the soil in the pans. The materials tested, their formulations and rates of application, are given in Table XXII.

Table XXII. Formulations and Rates of Application of Material Tested in 1965 and 1966.

Insecticide	Formulation	Concentration	Rate of Application
DDT	No. 2 Fuel Oil	7%	1 gal / acre
DDT	No. 2 Fuel Oil	15%	1 gal / acre
DDT	Bentonite Granules	10%	10 lbs / acre
DDT	Vermiculite Granules	30%	3 lbs / acre
BAYTEX	Granular	1%	20 lbs / acre
DURSBAN	Corn Cob Granules	1%	5 lbs / acre
DURSBAN	Corn Cob Granules	1%	10 lbs / acre
DURSBAN	Corn Cob Granules	1%	20 lbs / acre

To test the effectiveness of the insecticides, the pans were brought into the laboratory once a month and flooded with distilled water. Ten 4th

instar A. vexans larvae were placed in each pan and after 24 hours mortality counts were made.

Results

The loss in effectiveness of the different chemicals, as measured by the decrease in mortality of the test larvae, is shown in Figure 13. (All the curves on this graph start at 100% mortality.)

DDT, in its various formulations, retained its effectiveness better than the other chemicals. The two granular formulations of DDT, bentonite and vermiculite, killed at least 90% of the test larvae after a year. These forms retained their effectiveness throughout the winter, with a slight loss occurring in the summer of 1966. The 15% DDT in No. 2 fuel oil remained 90% effective after 17 months. The 7% DDT in fuel oil began to decrease in effectiveness after a month, and after 17 months it killed only 50% of the test larvae.

Dursban started to lose its effectiveness almost immediately at all three rates of application. After the first three months, the 5 pound per acre application killed only 5% of the test larvae. The 10 pound per acre application of dursban showed a continual decline in effectiveness until after 13 months it remained only 30% effective. Dursban at 20 pounds per acre was only slightly more effective (35%) after 13 months.

Baytex, at 20 pounds per acre, retained its effectiveness over the winter but declined rapidly during the summer to the point where it killed only 50% of the test larvae after 13 months.

FIGURE 12. Pans of Soil Containing Different Insecticidies Applied at Field Rates.

Figure 12

FIGURE 13. Mortality Due to Different Chemicals After Exposure To Natural Conditions.

Effectiveness of various chemicals after exposure to natural conditions (average of four replicates)

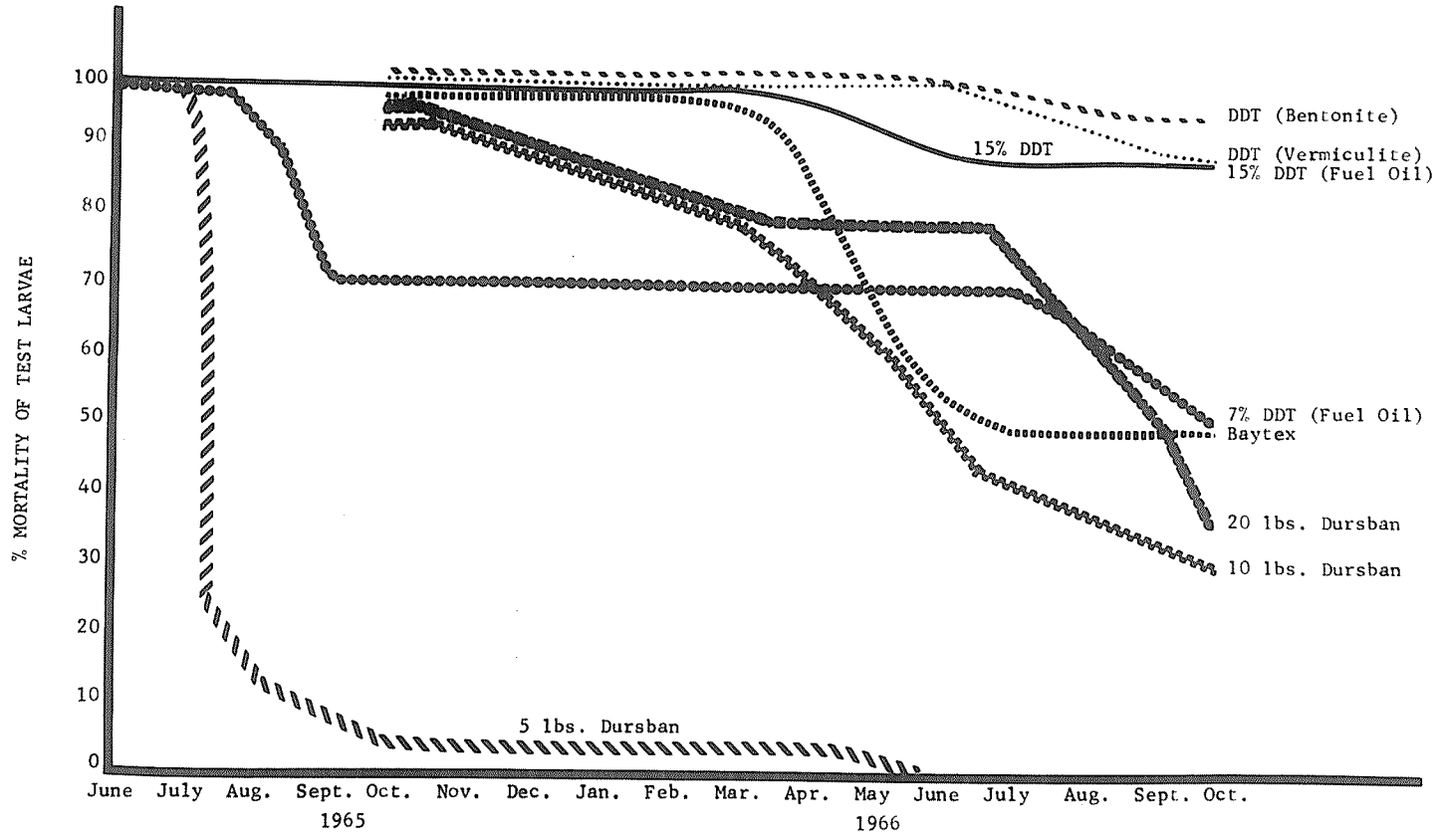


Figure 13

Discussion

These tests indicated that DDT in its various formulations has a longer residual effect than baytex or dursban and therefore is most suitable for pre-season insecticide application.

The validity of these comparisons in terms of longevity of these chemicals under natural conditions is not definite. The chemicals in this experiment were exposed to the same temperature fluctuations as those applied in the field, but the effect of precipitation may have been dissimilar. Chemicals in the field are affected by run-off in the spring and after heavy rains during the summer, while the chemicals in this experiment were only effected when sufficient precipitation occurred to cause the pans to overflow.

Actual DDT Loss By Granules Under Different Conditions

Method

The insecticides used were obtained from the Metro Winnipeg Mosquito Abatement Commission.

100 grams of bentonite and 30 grams of vermiculite, 1963 spring stock (29.30% DDT), were placed in 60 mesh, 9 inch diameter, brass screen discs (FIG. 14). These discs were placed outside on the ground from September, 1965, to October, 1966. Samples of the original material were kept in airtight containers at room temperatures as controls.

In May, 1966, one pint of vermiculite, 1966 spring stock (30.80% DDT), was placed in a 45cm. x 45cm., 90 mesh, nylon organdy bag and anchored below the surface of a permanent pool. It was left immersed until October, 1966. As a control, a sample was stored at room temperature in an airtight container.

All samples were tested by The National Testing Laboratories Ltd., Winnipeg, in November of 1966.

Results

The percentage of DDT by weight of the samples is given in Table XXIII.

TABLE XXIII. DDT Content of Granules Subjected to Various Outdoor Conditions, Tests Made November, 1966.

Material and Treatment	DDT Content % by WT
1965 Stock	
Vermiculite-Original *	29.30
Vermiculite-Control	30.48
Vermiculite-Treatment	29.52
- exposed in screen discs for 1 year	
Bentonite - Control	12.36
Bentonite - Treatment	12.67
- exposed in screen discs for 1 year	
1966 Stock	
Vermiculite-Original **	30.80
Vermiculite-Control	30.02
Vermiculite-Treatment	21.52
- in nylon bags at the bottom of a permanent pool	

* tested January 1965 ** tested January 1966

FIGURE 14. Screen Discs Used To Test DDT Impregnated Granules By Exposure to Natural Conditions. In test, top screen completely covered bottom screen continaining granules.

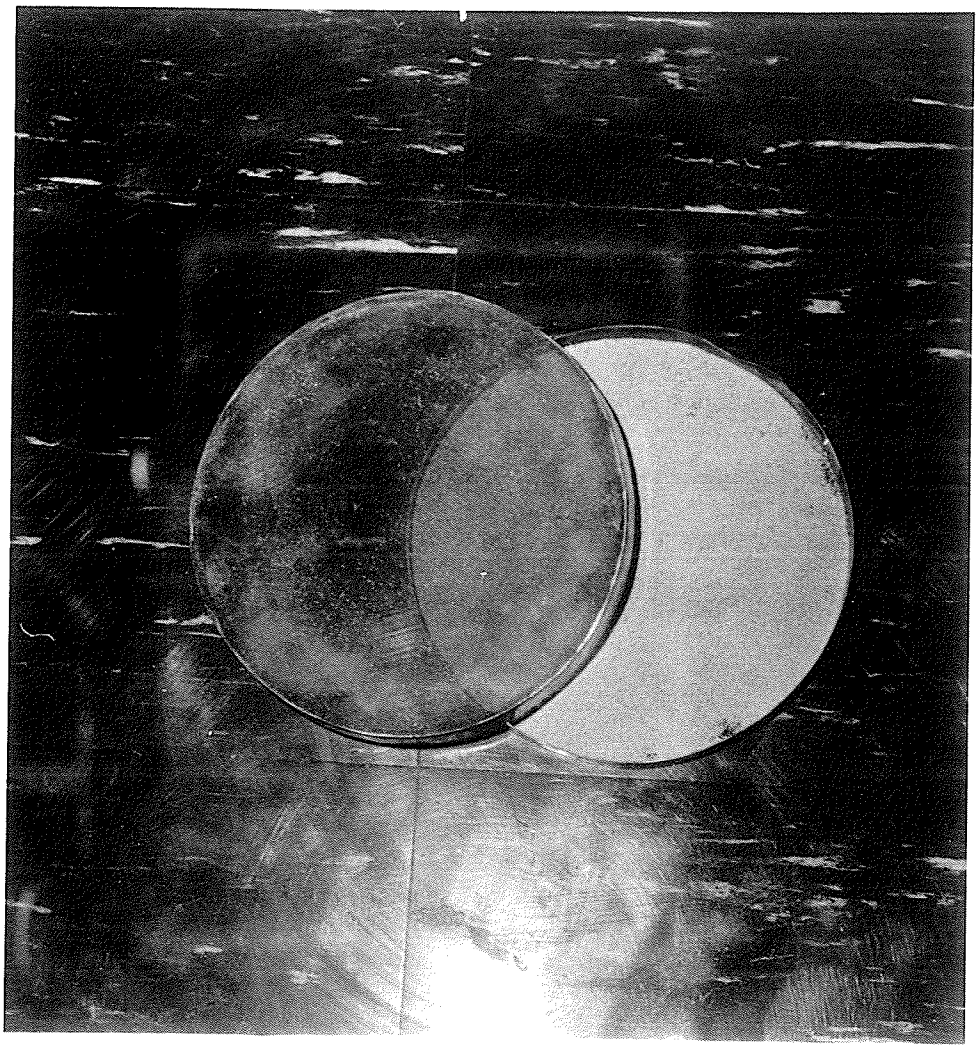


Figure 14

The vermiculite and bentonite granules lost none of their DDT content during a year's exposure to natural conditions in the screen discs. Neither of the two types of granules had a lower DDT content after exposure to the naturally occurring temperature fluctuations and precipitation than the control samples. The slight differences in DDT content of the samples can be attributed to variation in testing.

The vermiculite which was immersed in a pool for 6 months lost about 30% of its DDT content.

Discussion

The DDT content of vermiculite and bentonite granules remained constant during temperature fluctuations. It appears that rainfall had little effect on removal of DDT impregnated on the vermiculite and bentonite granules, although in the screen discs the larger rain drops were broken up before reaching the particles, and this may have diminished their erosive effect.

The relatively large loss (30%) in DDT content of the vermiculite which was immersed may have been due to the abrasive action of the water. However, the conditions which these particles were subjected to are unlike those which particles used in normal control procedures would receive, as vermiculite particles float and hence would not be exposed to the abrasive action of water to such an extent.

The fact that there was no loss of DDT content of the vermiculite (original test Jan. 1965 - 29.3%) after almost 2 years storage in paper bags at outdoor temperatures (Nov. 1966 - 30.02%) indicates that DDT im-

pregnated on vermiculite is stable and non-volatile, and retains its effectiveness for at least 2 years under normal storage conditions.

Use of C14 Labelled DDT in Field Studies

Knowledge of the long term effectiveness of insecticides is important in determining the rate, time, and frequency of their application. A study of DDT loss by DDT impregnated vermiculite and bentonite granules was made by using DDT labelled with the radioisotope C14. Vermiculite and bentonite granules were studied because these materials are commonly used in control operations.

Method

The required amounts of technical DDT were weighed and dissolved in acetone. To each of these solutions, one for vermiculite impregnation and one for bentonite impregnation, 1/5 ml. of C14 labelled DDT was added and the solutions well stirred. To impregnate the granules, these solutions were poured into beakers containing the granules and were well mixed to insure even distribution. They were then left until the acetone evaporated.

The vermiculite particles, 30-60 mesh size, were impregnated with DDT equal to 30% of the combined vermiculite-DDT weight, and the bentonite particles, also 30-60 mesh size, were impregnated with DDT amounting to 10% of the combined bentonite -DDT weight.

The dried vermiculite granules were divided into 50 samples, each weighing 13 mgs., and the bentonite was divided into 50 samples of 70 mgs. each. Each sample was placed in 2" x 2" bag of 90 mesh nylon organdy, which

was sewn with cotton thread.

Fifteen bags each of bentonite and vermiculite granules impregnated with radio-active DDT were placed at -20°C in the laboratory as controls. The remaining bags were placed outside, on April 4, 1966. These were tied to a cord connecting two stakes and were in contact with the ground.

Three bags each of vermiculite and bentonite granules were brought into the laboratory every two weeks until August 18, 1966. The granules were weighed and DDT extracted with benzene. The benzene containing the extracted DDT was then placed on copper discs and left to dry by evaporation. The radioactivity of the DDT residue left on the discs was measured by a nuclear-Chicago automatic read-out beta counter.

Results

The loss in DDT, as measured by the change in radioactivity, is given in Table XXIV. The changes occurring at two week intervals were small, so similar counts for a number of weeks were averaged. The June 27th reading of the exposed vermiculite was not used as it was so different from the other readings it seemed probable that some error had been made.

The percentage loss of DDT in the control samples was 16% for vermiculite, and 30% for bentonite, granules.

After 6 weeks exposure, the vermiculite had lost 12% of its DDT content in comparison with the original control, and after 18 weeks this had risen to 31% loss of DDT content. Since the control lost 16%

of its DDT content after 18 months, the loss of DDT due to exposure to weather was 15%.

TABLE XXIV. Radioactivity of Cl_4 Labelled DDT Extracted Vermiculite and Bentonite Granules Exposed in Nylon Bags (1966).

Radioactivity/mg./min.*

	Vermiculite			Bentonite		
Date	Control	Exposed		Control	Exposed	
April 4	10,212			3,037		
April 19		8,917			2,535	
May 2		9,226	8,981		2,362	2,449
May 16		8,800			2,767	
May 30		6,242			2,131	
June 13		7,601			1,887	
June 27		2,815**	7,015		1,556	
July 12		7,818			1,959	1,774
July 26		6,681		2,128	1,569	
Aug. 18	8,622	6,731			1,894	

* Av. of 3 Reps.

** Not used in getting average figure.

The loss of DDT content of the bentonite granules was 19% after 8 weeks and 42% after 18 weeks exposure. The loss in DDT of the control bentonite was 30%, so the DDT lost by bentonite due to natural conditions may be considered to be 12%.

Discussion

The greater loss in DDT content of the bentonite samples than the vermiculite samples indicates that DDT is held more strongly on vermiculite granules. This suggests that DDT on vermiculite would remain effective longer than DDT on bentonite.

The loss in DDT by the granules exposed to natural conditions was probably due to rain washing off the DDT from the granules.

Although the DDT content of the vermiculite and bentonite granules in this experiment was the same as those used by the Metro. Winnipeg Mosquito Abatement Commission for control work, the method of impregnation was different. Hence, the rate and amount of DDT loss observed in this experiment may not be the same as that occurring in granules used for control.

LD_{50} of Various Chemicals

Laboratory tests were carried out on Dursban, Baytex, and DDT, to find the concentrations required to kill 50% of Aedes vexans larvae placed in them.

The testing technique consisted of placing 20 fourth instar A. vexans larvae in 250 ml. beakers containing 100 ml. of the test solution. Distilled water was used as a diluent. Counts of dead larvae were taken after 24 hours.

The LD_{50} for Dursban was 0.009 ppm, for Baytex, 0.002 ppm, and for DDT, it was 0.002 ppm.

These results indicate that higher concentrations of Dursban are

needed than DDT and Baytex for larval control.

WHO Resistance Tests

Several tests were conducted on mosquito larvae and adults collected in the Winnipeg area to determine whether any resistance to DDT had developed.

Tests were made on adults of Aedes vexans and Culex restuans, and on larvae of Aedes vexans, Culex restuans, Culex tarsalis and Culiseta inornata. Standard World Health Organization kits were used for all tests.

DDT resistance is not suspected unless the LD_{50}, the dosage required to kill 50% of the mosquito sample, is greater than 0.1 parts per million for aedine species or 1.0 parts per million for Culex and Culiseta species. On this basis there was no resistance found in any of the species tested. (Appendix IX).

CHAPTER XI

SUMMARY

There are thirty-nine species of mosquitoes known to occur in Manitoba. Twenty-five species have been collected in the Winnipeg area, and of these, Aedes vexans (Meigen) is the most abundant. The distribution of A. vexans throughout the province is limited by lack of suitable developmental sites as well as climatic factors.

Experiments were carried out on eggs of A. vexans to study the influence of temperature and relative humidity on embryonic longevity and hatchability. It was found that the rate of water loss by eggs increased with temperature and decreased with relative humidity. Embryonic viability decreased as humidity decreased and temperature increased. Temperature and relative humidity pre-hatch conditioning influenced the percentage and rate of hatching of viable eggs. The higher temperatures required to hatch A. vexans eggs do not occur in nature until June. It was found that eggs which had lost moisture in a low relative humidity could re-absorb moisture in a saturated atmosphere. Four-week-old eggs lost less moisture in a less-than-saturated atmosphere than two-week-old eggs.

Exposure to 40°F for 24 hours was lethal to A. vexans adults.

Caged mosquitoes survived equally well on honey and water, but had a shorter life span when given neither. Female mosquitoes showed significantly greater longevity than the males, the females living an average of 7 days longer at all treatments. There were no significant differences in survival at the three relative humidities tested.

The infiltration of adult mosquitoes from outside the Metro Winnipeg control area to inside was indicated by (1) population peaks occurring outside the control area before they occur inside, (2) greater number of development sites being found outside the control area, (3) the very similar relative abundance of mosquito species trapped inside and outside the control area, and (4) the higher female to male ratio inside the control area than outside, assuming similar dispersal behaviour.

Culex tarsalis, the species considered the primary vector of western encephalitis, occurred in greater abundance at the Charleswood sewage lagoon than at other trap locations, but an extensive larval survey indicated that the lagoon itself was not a developmental site.

Western encephalitis investigations in Manitoba in 1965 and 1966 found no trace of the virus in mosquitoes collected from different parts of the province. Valuable experience was gained in collecting and handling mosquitoes, which will aid future W.E. investigations.

Fewer mosquitoes were found at 40 feet than at 5 feet above ground level. Male A. vexans apparently used the trap, which extended vertically to 40 feet, as a swarm marker.

Pre-season insecticide applications were found to be most effective

when applied to permanent pools. Tests of different chemicals indicated that DDT had the longest residual effect and therefore is most suitable for pre-season control measures. DDT impregnated on vermiculite granules remained stable in storage for two years. DDT was held more strongly by vermiculite than by bentonite granules.

The use of World Health Organization testing kits and techniques indicated that no resistance to DDT had developed in mosquitoes of the Winnipeg area.

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APPENDIX

APPENDIX I

LIGHT TRAP COLLECTIONS, MAY 10 - SEPTEMBER 13, 1965

Date		Lagoon		Honey House		Glenlea	
		♀	♂	♀	♂	♀	♂
May	10			1	0		
	13			1	0	0	0
	17	0	0	1	1	0	0
	20	0	0	0	0	0	0
	25	out of order		6	0	25	0
	31	0	0	11	0	11	1
June	3	0	0	9	0	2	0
	7	2	1	5	2	11	1
	10	1	0	4	0	2	0
	14	2	1	21	3	30	14
	17	0	0	108	92	107	101
	21	14	30	380	444	220	115
	24	30	6	162	45	102	96
	28	34	8	351	54	384	188
July	2	out of order		144	120	154	22
	5	11	1	188	16	582	14
	8	83	2	119	20	141	17
	12	12	0	93	21	49	59
	15	26	3	140	82	64	78
	19	37	8	208	93	64	61
	22	out of order		192	70	82	31
	26	39	3	88	72	95	37
	29	46	0	68	15	133	53
Aug.	3	102	6	270	50	175	102
	6	30	6	1584	308	696	144
	9	98	28	1509	256	936	464
	12	51	39	1312	496	928	640
	16	44	20	1478	1632	960	730
	19	28	6	287	626	136	544
	23	42	24	160	368	224	480
	26	104	24	976	720	288	730
	30	5	2	48	28	176	544
Sept.	2	14	0	17	5	52	480
	7	9	0	6	5	24	672
	10	out of order		11	5	12	214
	13	2	0	1	1	14	

APPENDIX I

LIGHT TRAP COLLECTIONS, MAY 10 - SEPTEMBER 13, 1965

Date		Oak Bluff		City Park		Brookside	
		♀	♂	♀	♂	♀	♂
May	10			1	0	0	0
	13			Bulb Burned		0	0
	17	2	1	2	0	5	2
	20	0	0	0	0	1	0
	25	49	2	8	0	3	0
	31	75	2	1	1	4	0
June	3	9	1	0	0	3	0
	6	30	5	2	0	2	2
	10	2	2	1	0	1	0
	14	206	274	4	0	4	0
	17	1496	684	36	10	28	3
	21	out of order		206	162	132	42
	24	400	523	144	160	120	20
	28	1333	378	55	2	46	12
July	2	516	190	120	4	48	3
	5	426	132	58	3	39	0
	8	338	84	93	14	30	3
	12	287	62	38	6	32	0
	15	108	77	28	2	60	2
	19	337	131	37	8	12	6
	22	249	58	27	12	11	4
	26	126	68	out of order		34	0
	29	62	30	27	19	out of order	
Aug.	3	181	83	28	12	27	4
	6	190	674	170	45	108	90
	9	726	252	176	64	32	8
	12	1648	656	out of order		138	72
	16	out of order		132	116	123	160
	19	208	392	out of order		21	5
	23	112	192	17	19	48	32
	26	out of order		out of order		56	6
	30	84	142	5	6	6	10
Sept.	2	62	60	12	4	6	1
	7	42	22	3	2	out of order	
	10	42	23	out of order		2	0
	13	8	9	1	0	5	1

APPENDIX I

LIGHT TRAP COLLECTIONS, MAY 10 - SEPTEMBER 13, 1965

Date		Lily Field		Kildonan		Legislative Bldg.	
		♀	♂	♀	♂	♀	♂
May	10			1	0	0	0
	13			1	0	1	0
	17	0	0	0	0	1	0
	20	1	0	0	0	0	0
	25	out of order		7	0	0	0
	31	16	2	5	0	1	0
June	3	20	6	21	6	1	0
	7	19	8	8	1	1	1
	10	9	0	2	1	0	1
	14	5	1	298	150	38	8
	17	97	76	220	70	125	38
	21	268	408	3680	1240	436	124
	24	520	240	1488	54	810	75
	28	1942	882	1638	118	328	8
July	2	278	91	1464	54	426	18
	5	472	64	652	64	267	30
	8	244	60	1136	80	365	32
	12	524	418	610	110	192	41
	15	195	82	255	57	102	26
	19	490	406	620	220	108	30
	22	180	272	356	82	27	7
	26	256	176	151	63	62	16
	29	110	9	230	60	64	54
Aug.	3	out of order		328	72	120	39
	6	out of order		out of order		312	168
	9	out of order		688	288	296	160
	12	3152	1104	832	298	608	280
	16	2208	4416	1008	1268	out of order	
	19	208	368	512	384	396	396
	23	1312	2272	714	304	out of order	
	26	1376	1056	696	174	408	176
	30	out of order		52	26	40	26
Sept.	2	out of order		102	102	21	11
	7	43	82	27	27	16	18
	10	23	103	26	26	9	5
	13	79	26	9	9	3	2
Total		26735					

APPENDIX I

LIGHT TRAP COLLECTIONS, MAY 10 - SEPTEMBER 13, 1965

Date		Windsor		Okolita		La Salle	
		♀	♂	♀	♂	♀	♂
May	10	0	0				
	13	0	0				
	17	1	2	3	0		
	20	0	0	0	0		
	25	1	0	14	0		
	31	4	0	17	0		
June	3	9	2	7	2		
	7	10	1	34	2		
	10	0	0	4	0		
	14	68	33	30	3	36	8
	17	110	118	60	21	385	81
	21	114	120	144	188	330	181
	24	120	20	57	12	31	28
	28	244	30	96	14	101	46
July	2	244	8	56	10	132	49
	5	78	1	24	6	2	0
	8	174	24	69	3	out of order	
	12	48	0	54	4	41	11
	15	56	2	26	2	70	45
	19	106	14	116	68	54	37
	22	out of order		105	45	51	14
	26	36	21	55	25	88	8
	29	38	10	48	12	65	27
Aug.	3	56	8	99	27	74	7
	6	out of order		250	115	984	288
	9	316	140	372	62	360	88
	12	188	60	1120	272	out of order	
	16	312	84	992	172	1416	1896
	19	80	255	96	12	102	192
	23	108	60	48	28	256	448
	26	184	72	186	18	336	406
	30	20	18	18	2	26	10
Sept.	2	out of order		16	2	5	3
	7	11	2	3	0	0	0
	10	1	0	7	2	out of order	
	13	1	0	5	3		
				3			

APPENDIX II

LIGHT TRAP COLLECTIONS, MAY 13 - SEPTEMBER 8, 1966

Date		City Park		Brookside		Lily Field		Kildonan	
		♀	♂	♀	♂	♀	♂	♀	♂
May	13			2				2	
	16			1					
	19	2		1					
	24	7	16	23	1	19	4	33	3
	26	8	5	26	7	14		6	1
	30		1	3		4	3	4	1
June	2	7	7	4	3	25	17	12	16
	6	3	1	4	1	21	25	6	2
	9	0	1	4	6	48	52	8	8
	13	0	0	30	19	198	201	29	10
	16	1	3	4	3	79	80	16	3
	20	8	14	28	23	990	1296	124	58
	24	15	18	32	21	1036	1566	45	15
	27	1	5	15	7	504	216	35	75
	30	13	7	36	3	216	190	5	2
July	4	3	5	8	0	81	5	1	2
	7	8	4	12	12	72	45	13	2
	11	20	42	16	18	240	50	17	0
	14	80	194	22	19	250	700	194	96
	18	488	1192	230	940	1500	4290	705	675
	21	102	81	41	19	634	2316	714	300
	25	468	456	27	18	1640	1800	630	153
	28	144	30	8	2	960	1060	80	16
Aug.	1	160	110	14	6	320	368	125	88
	4	78	47	18	9	155	376	60	35
	8	14	4	7	0	370	230	59	3
	11	9	5	5	0	43	159	36	9
	15	12	3	9	0	59	78	19	4
	18	out of order		6	1	128	14	9	3
	25	14	14	21	7	17	30	21	23
Sept.	1	3	6	8	4	21	23	4	2
	6	13	8	30	4	37	24	1	0
	8	15	12	0	0	20	56	4	3

APPENDIX II

LIGHT TRAP COLLECTIONS MAY 13 - SEPTEMBER 8, 1966

Date		Honey House		Glenlea		Oak Bluff		Lagoon	
		♀	♂	♀	♂	♀	♂	♀	♂
May	13	3				3			
	16								
	19	1							
	24	20	1			32	23	1	
	26	51	2			28	4	3	8
	30	7				1	1	32	0
June	2	22	9			72	53	1	0
	6	9	0	13	2	17	8	18	0
	9	1	1	8	4	10	5	6	0
	13	10	2	65	24	134	115	2	3
	16	25	9	10	1	27	19	10	0
	20	15	7	78	12	693	495	7	0
	24	85	92	144	63	369	329	0	0
	27	37	24	24	29	141	72	25	2
	30	60	43	15	8	56	49	43	1
July	4	8	0	13	29	18	4	53	0
	7	28	0	30	24	35	16	18	1
	11	120	120	16	10	194	236	18	4
	14	925	1100	360	1710	130	124	267	144
	18	6700	3460	0	0	422	874	5152	240
	21	240	780	56	261	no count		3450	12
	25	1494	486	420	324	1150	252	940	450
	28	316	84	161	72	375	140	3575	36
Aug.	1	85	23	360	208	120	200	340	0
	4	236	90	108	60	120	132	300	13
	8	104	20	120	170	58	105	309	2
	11	42	21	out of order		49	35	78	0
	15	39	11	42	8	17	29	58	9
	18	16	31	53	12	126	34	40	36
	25	25	21	24	53	29	306	0	0
Sept.	1	0	4	109	355	40	33	17	5
	6	2	6	6	63	63	50	53	1
	8	3	5	11	18		56	81	0
								54	
								all up one	

APPENDIX II

LIGHT TRAP COLLECTIONS MAY 13 - SEPTEMBER 8, 1966

Date		Legislative Bldg.		Windsor		Okolita		La Barriere	
		♀	♂	♀	♂	♀	♂	♀	♂
May	13	2				4			
	16					1			
	19	2							
	24	2		25	7				
	26	9	2	10	4			6	1
	30	2	2	4		11		9	8
June	2	15	24	31	36	104	56	6	0
	6	6	5	5	9	20	10	4	16
	9	6	6	7	2	8	12	1	0
	13	17	2	36	18	65	22	20	4
	16	35	9	17	0	48	18	207	54
	20	42	7	26	9	84	75	127	90
	24	11	7	66	49	108	135	33	13
	27	13	1	37	9	43	27	37	26
	30	5	2	17	3	79	53	5	1
July	4	2	0	6	2	6	2	7	2
	7	14	4	14	10	23	8	30	20
	11	18	10	32	36	105	360	4200	2000
	14	112	62	184	102	608	1792	1700	3450
	18	430	305	1020	1890	1875	4375	2700	480
	21	520	502	332	990	786	876	1440	500
	25	552	192	864	528	915	825	876	42
	28	102	57	100	232	54	26	power	failure
Aug.	1	185	116	192	100	132	20	140	95
	4	70	40	106	60	67	34	83	2
	8	36	19	72	47	60	12	36	2
	11	26	19	22	32	17	8	56	5
	15	12	9	15	3	16	5	51	2
	18	16	7	22	5	26	6	99	31
	25	23	27	3	0	25	12	10	34
Sept.	1	14	11	17	7	15	3	25	9
	6	22	10	14	16	21	3		14
	8	4	2	6	4	0	0		

APPENDIX II

LIGHT TRAP COLLECTIONS MAY 13 - SEPTEMBER 8, 1966

Date		Transcona ♀	Transcona ♂
June	24	84	9
	27	24	5
	30	26	18
July	4	4	0
	7	7	7
	11	21	10
	14	74	100
	18	705	2263
	21	369	207
	25	300	516
	28	31	11
Aug.	1	14	2
	4	22	5
	8	10	3
	11	8	4
	15	15	3
	18	6	2
	25	2	6
Sept.	1	5	0
	6	11	0
	8	0	0

APPENDIX III

NO. OF ADULT *AEDES VEXANS* CAUGHT DAILY AT THREE LOCATIONS OUTSIDE CONTROL AREA, 1965

Date		Oak Bluff		La Barriere		Glenlea		Total	
		♀	♂	♀	♂	♀	♂	♀	♂
June	17	160	0	7	0	4	21	171	21
	18	80	140	0	7	54	21	134	161
	19	150	110	7	20	40	30	197	160
	20	50	70	132	132	70	70	252	272
	22	100	80	20	60	8	4	128	144
	23	80	280	6	9	9	4	95	293
	24	75	50	0	0	45	40	120	90
	25	1000	100	70	30	270	90	1340	220
	26	0	0	1	2	15	6	16	8
	27	60	15	0	0	30	0	90	15
	28	45	30	2	0	4	0	51	30
	29	66	33	44	22	120	0	230	55
	30	110	30	4	0	11	2	125	32
July	1	150	30	15	5	54	0	219	35
	2	56	8	5	2	40	0	101	10
	3	320	40	1	0	60	10	381	50
	6	100	100	1	0	15	6	116	106
	7	65	13	0	0	24	0	89	13
	8	60	20	0	0	45	0	105	20
	9	12	0	3	0	6	0	21	0
	10	15	0	0	0	5	4	20	4
	11	7	0	3	0	45	30	55	30
	12	155	75	5	2	25	5	185	82
	13	9	4	36	0	30	18	75	22
	14	14	8	4	0	21	49	39	57
	15	150	130	0	0	7	17	157	147
	16	0	0	18	2	16	7	34	9
	17	120	0	0	0	27	12	147	12
	18	45	0	4	3	3	6	52	9
	19	0	0	5	8	1	1	6	9
	20	0	60	7	1	5	0	72	1
	21	70	0	15	0	9	1	94	1
	22	25	15	12	4	45	10	82	29
	23	50	5	70	0	50	5	170	10
	24	45	5	5	0	12	6	62	11
	25	0	0	10	10	4	1	14	11
	26	14	4	20	0	0	0	34	4
	27	0	0	6	1	20	10	26	11
	28	16	8	0	12	12	4	28	24
	29	6	2	2	1	0	25	8	28
	30	35	20	3	2	15	15	53	37

APPENDIX IV

LARVAL SURVEY 1965

Date	Species	Date	Species
April 13	Aedes spencerii	June 9	Culiseta inornata
15	Aedes spencerii	10	Aedes vexans
28	Aedes trichuris	16	Culex tarsalis
May 3	Aedes spencerii	16	Culiseta inornata
3	Aedes trichuris	22	Culiseta inornata
4	Aedes implicatus	25	Culex tarsalis
5	Aedes trichuris	25	Culiseta inornata
10	Aedes implicatus	July 3	Aedes vexans
12	Aedes implicatus	3	Aedes dorsalis
12	Aedes fitchii	3	Aedes vexans
13	Aedes implicatus	3	Aedes dorsalis
13	Aedes vexans	4	Aedes vexans
17	Aedes vexans	4	Aedes vexans
17	Aedes excrucians	5	Aedes dorsalis
17	Aedes vexans	5	Culex tarsalis
17	Aedes fitchii	5	Aedes vexans
17	Aedes vexan	5	Aedes vexans
17	Aedes implicatus	7	Aedes nigromaculis
18	Aedes vexans	7	Aedes dorsalis
19	Aedes vexans	7	Culex tarsalis
20	Aedes campestris	22	Aedes vexans
20	Aedes fitchii	22	Aedes vexans
20	Aedes fitchii	22	Aedes vexans
June 3	Aedes sticticus	22	Aedes vexans
3	Aedes vexans	22	Culex tarsalis
3	Aedes stimulans	22	Aedes vexans
3	Aedes vexans	27	Aedes dorsalis
7	Aedes vexans	27	Aedes dorsalis
7	Aedes vexans	27	Culiseta inornata
7	Aedes stimulans	27	Culex tarsalis
7	Aedes vexans	28	Culex tarsalis
7	Aedes vexans	August 15	Culex restuans
7	Aedes vexans		
7	Aedes implicatus		
8	Aedes vexans		
8	Aedes vexans		
8	Aedes nigromaculis		

APPENDIX V

LARVAL SURVEY 1966

Date	Species	Date	Species
May 5	Aedes implicatus	July 28	Culex tarsalis
5	Aedes fitchii	28	Culex restuans
9	Aedes implicatus	August 8	Culiseta inornata
10	Aedes spencerii	8	Culex tarsalis
10	Aedes riparius	8	Culex restuans
10	Aedes fitchii	12	Aedes vexans
11	Aedes campestris	12	Aedes nigromaculis
11	Aedes communis	12	Aedes vexans
25	Aedes spencerii	12	Aedes vexans
25	Aedes fitchii	12	Aedes vexans
June 7	Culiseta inornata	12	Aedes nigromaculis
9	Culiseta inornata	12	Culex tarsalis
July 8	Aedes stimulans	12	Aedes vexans
8	Aedes vexans	12	Aedes vexans
8	Aedes nigromaculis	12	Aedes nigromaculis
8	Aedes vexans	12	Aedes vexans
8	Aedes spencerii	12	Aedes nigromaculis
8	Aedes vexans	15	Culiseta inornata
8	Aedes spencerii	16	Culex restuans
8	Aedes dorsalis	17	Culex restuans
8	Aedes vexans	September 6	Aedes vexans
8	Aedes dorsalis	22	Culex restuans
8	Aedes spencerii	22	Culex tarsalis
8	Aedes vexans		
8	Aedes flavescens		
8	Aedes vexans		
8	Aedes implicatus		
8	Aedes dorsalis		
19	Culex tarsalis		
19	Culex restuans		
19	Culiseta inornata		
22	Culex restuans		
27	Culex tarsalis		
27	Culex tarsalis		
27	Culex restuans		
27	Culiseta inornata		

APPENDIX VI

ATTEMPTED WESTERN EQUINE ENCEPHALITIS ISOLATIONS 1965

Date		Location	Mouse Inoculation	Tissue Culture Inoculation
June	5	Delta	Negative	Negative
	6	Delta	Negative	Negative
	7	Delta	Negative	Negative
	8	Delta	Negative	Negative
	9	Delta	Negative	Negative
	10	Delta	Negative	Negative
	11	Delta	Negative	Negative
	11	Delta	Negative	Negative
	11	Delta	Negative	Negative
	12	Delta	Negative	Negative
	13	Delta	Negative	Negative
	14	Delta	Negative	Negative
July	12	Oak Bluff (Random)	Negative	Negative
	12	Oak Bluff (Random)	Negative	Negative
	12	Oak Bluff (Random)	Negative	Negative
	12	Oak Bluff (Random)	Negative	Negative

APPENDIX VII

MOSQUITOES CAUGHT IN THE TRAP LOCATED AT THE CHARLESWOOD SEWAGE LAGOON

Date 1966	Aedes	Culiseta	Culex	Anopheles
June 16-23	0	0	0	0
27	46	7	1	0
28	26	5	2	0
29	100	5	0	0
July 4	0	0	0	0
6	0	0	0	0
7	15	6	0	0
8	330	20	6	0
11	0	75	0	0
12	150	1	1	0
13	200	0	25	0
14	200	5	6	0
15	175	1	3	0
18	600	0	13	0
19	100	5	30	0
20	30	13	60	0
21	500	0	300	0
22	300	0	19	0
25	200	9	73	0
27	0	0	0	0
28	50	1	4	0
29	60	7	40	0
Aug. 2	16	15	30	0
3	30	50	41	0
4	0	0	0	0
5	15	15	20	0
8	0	0	0	0
9	0	8	0	0
10	14	10	3	0
11	2	8	3	0
12	25	20	15	0
15	30	25	3	2
16	30	2	2	0
17	10	4	6	-
19	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	4	0	0	0
25	28	5	0	1
26	50	6	0	0
29	4	1	0	0
30	30	10	1	0
31	0	0	0	0
Sept. 2	30	10	1	0
6	0	0	0	0
7	10	5	0	0
8	15	6	0	0
9	30	5	0	1
TOTAL	3455	365	708	4

MOSQUITOES CAUGHT IN THE TRAP LOCATED AT ASSINIBOINE PARK, WINNIPEG

Date 1966	Aedes	Culiseta	Culex	Anopheles
June 16-23	3	1	0	0
27	20	3	0	0
28	7	1	0	0
29	7	1	-	-
July 4	0	1	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
11	4	0	0	0
12	18	1	0	0
13	50	0	15	0
14	300	3	2	0
15	300	3	0	0
18	100	0	3	0
19	100	6	8	-
20	200	3	3	0
21	61	1	7	0
22	150	1	5	0
25	25	6	2	0
27	0	0	0	0
28	25	2	0	0
29	30	2	0	-
Aug. 2	0	0	0	0
3	40	2	0	0
4	30	0	0	0
5	15	-	-	-
8	3	3	3	0
9	0	-	-	0
10	7	2	0	0
11	0	0	0	0
12	11	4	1	0
15	5	1	1	0
16	30	0	0	0
17	4	0	0	-
18	-	0	0	0
19	-	0	0	0
22	-	0	0	0
23	-	0	0	0
24	2	0	0	0
25	5	0	0	0
26	20	0	0	1
29	5	0	2	1
30	3	1	0	0
31	0	0	0	0
Sept. 2	3	1	0	0
6	0	0	0	0
7	6	1	0	1
8	25	0	0	0
9	30	1	0	0
TOTAL	1644	51	52	3

MOSQUITOES CAUGHT IN THE TRAP LOCATED AT BRANDON, MANITOBA

Date 1966	Aedes	Culiseta	Culex	Anopheles
June 16-23	64	5	0	0
27	15	2	0	1
28	0	0	0	0
29	2	0	0	0
July 4	3	0	0	0
6	0	0	0	0
7	200	1	0	0
8	120	0	0	0
11	0	0	0	0
12	200	0	0	0
13	6	6	1	1
14	100	20	20	0
15	15	1	2	0
18	15	0	1	0
19	6	3	8	3
20	90	2	13	0
21	50	3	10	1
22	4	3	6	2
25	26	4	13	1
27	20	20	15	7
28	10	20	4	1
29	40	3	12	8
Aug. 2	0	0	0	0
3	50	2	5	3
4	4	0	3	1
5	0	0	0	0
8	2	30	12	0
9	2	10	5	1
10	3	3	4	2
11	4	4	10	1
12	5	4	4	2
15	4	6	7	0
16	5	12	2	3
17	15	40	4	-
18	2	1	3	1
19	12	4	4	1
22	0	24	0	0
23	5	18	0	0
24	3	5	3	-
25	4	18	0	9
26	15	3	0	7
29	30	1	0	2
30	20	3	1	0
31	200	0	0	2
Sept. 2	100	3	0	8
6	20	2	0	1
7	25	0	0	1
8	50	0	0	8
9	30	0	0	1
TOTAL	1596	286	172	79

MOSQUITOES CAUGHT IN THE TRAP LOCATED AT DELTA, MANITOBA

Date 1966	Aedes	Culiseta	Culex	Anopheles
June 16-23	no record	no record	no record	no record
27	119	15	50	8
28	0	0	0	0
29	0	0	0	0
July 4	12	15	5	2
6	15	35	0	0
7	0	0	0	0
8	50	75	0	0
11	0	0	0	0
12	20	4	0	0
13	0	0	0	0
14	1	1	0	0
15	10	6	6	0
18	3	3	6	0
19	20	12	20	2
20	11	9	26	5
21	2	1	1	0
22	13	3	7	0
25	1	0	7	0
27	50	11	40	8
28	10	9	15	0
29	15	10	5	0
Aug. 2	0	0	0	0
3	4	5	8	1
4	0	0	0	0
5	20	20	30	25
8	2	13	7	0
9	5	6	20	5
10	7	4	20	2
11	7	2	30	2
12	3	6	15	7
15	5	10	7	2
16	25	25	8	2
17	3	50	5	1
18	14	8	3	0
19	12	15	8	1
22	30	4	2	0
23	12	5	0	0
24	30	6	0	3
25	25	40	9	8
26	20	25	0	2
29	2	3	0	0
30	0	2	1	1
31	50	4	2	0
Sept. 2	0	0	0	0
6	4	1	1	1
7	0	1	0	0
8	3	0	1	0
9	15	1	0	1
TOTAL	650	465	365	89

APPENDIX VIII

ATTEMPTED ISOLATION OF WESTERN ENCEPHALITIS IN MANITOBA, 1966

Date	Genus	Locality	Number	W.E.E. Virus Isol'n
June 21	Aedes	City Park	20	Negative
21	Culiseta	Lagoon	1	Negative
22	Aedes	Brandon	1	Negative
23	Culiseta	Delta	4	Negative
24	Aedes	Brandon	34	Negative
24	Aedes	Delta	20	Negative
24	Aedes	Delta	30	Negative
24	Culex	Delta	1	Negative
24	Culiseta	Delta	30	Negative
27	Aedes	Delta	119	Negative
27	Aedes	City Park	20	Negative
27	Anopheles	Brandon	1	Negative
27	Culex	Lagoon	1	Negative
27	Culiseta	City Park	2	Negative
27	Culiseta	Lagoon	7	Negative
27	Culiseta	Brandon	2	Negative
28	Aedes	Brandon	11	Negative
28	Aedes	City Park	200	Negative
28	Anopheles	Brandon	1	Negative
28	Culex	City Park	1	Negative
29	Aedes	Delta	35	Negative
29	Aedes	Lagoon	100	Negative
July 4	Aedes	Brandon	3	Negative
4	Culiseta	Brandon	15	Negative
7	Aedes	Delta	65	Negative
7	Aedes	Brandon	150	Negative
7	Culiseta	Delta	35	Negative
8	Aedes	Brandon	120	Negative
8	Aedes	Delta	100	Negative
8	Culex	Delta	1	Negative
8	Culiseta	Delta	100	Negative
12	Aedes	Brandon	300	Negative
12	Culex	Lagoon	1	Negative
12	Culiseta	Park	1	Negative
12	Culiseta	Lagoon	1	Negative
13	Aedes	Brandon	1	Negative
13	Culiseta	Brandon	1	Negative

APPENDIX VIII

ATTEMPTED ISOLATION OF WESTERN ENCEPHALITIS IN MANITOBA, 1966

Date	Genus	Locality	Number	W.E.E. Virus Isol'n.
July 14	Culex	Lagoon	6	Negative
14	Culiseta	Delta	1	"
15	Aedes	Park	300	"
15	Aedes	Lagoon	175	"
15	Aedes	Park	300	"
15	Aedes	Lagoon	300	"
15	Aedes	Delta	20	"
15	Aedes	Delta	10	"
15	Aedes	Delta	13	"
15	Culex	Brandon	50	"
15	Culex	Brandon	2	"
15	Culex	Lagoon	3	"
15	Culiseta	Park	3	"
15	Culiseta	Lagoon	7	"
15	Culiseta	Delta	5	"
18	Aedes	Delta	3	"
18	Aedes	Brandon	50	"
18	Culex	Brandon	1	"
19	Aedes	Lagoon	100	"
19	Culex	Brandon	8	"
19	Culex	Park	8	"
19	Culiseta	Brandon	3	"
19	Culiseta	Lagoon	5	"
20	Aedes	Brandon	90	"
20	Aedes	Lagoon	30	"
20	Aedes	Park	200	"
20	Culex	Park	3	"
20	Culex	Brandon	13	"
20	Culex	Delta	26	"
21	Aedes	Brandon	50	"
21	Aedes	Lagoon	500	"
21	Culex	Lagoon	300	"
21	Culex	Delta	1	"
21	Culiseta	Delta	1	"
21	Culiseta	Park	1	"
22	Aedes	Delta	13	"
22	Aedes	Brandon	3	"
22	Aedes	Park	90	"
22	Aedes	Park	300	"
22	Anopheles	Brandon	2	"

APPENDIX VIII

ATTEMPTED ISOLATION TO WESTERN ENCEPHALITIS IN MANITOBA, 1966

Date	Genus	Number	Locality	W.E.E. Virus Isol'n.
July 22	Culiseta	3	Delta	Negative
25	Aedes	1	Delta	"
25	Aedes	25	Park	"
25	Aedes	20	Brandon	"
25	Aedes	15	Delta	"
25	Culex	13	Brandon	"
25	Culex	2	Lagoon	"
25	Culex	15	Park	"
25	Culex	6	Brandon	"
25	Culiseta	13	Park	"
26	Aedes	20	Delta	"
27	Aedes	50	Brandon	"
27	Aedes	27	Delta	"
27	Anopheles	1	Brandon	"
27	Culex	10	Park	"
27	Culex	40	Lagoon	"
27	Culex	11	Delta	"
27	Culiseta	15	Delta	"
27	Culiseta	20	Lagoon	"
27	Culiseta	30	Brandon	"
28	Aedes	10	Park	"
28	Aedes	10	Delta	"
28	Aedes	1	Brandon	"
28	Anopheles	4	Brandon	"
28	Culex	15	Brandon	"
28	Culex	9	Delta	"
28	Culiseta	1		
28	Culiseta	2	Park	"
28	Culiseta	30	Park	"
29	Aedes	15	Delta	"
29	Aedes	40	Lagoon	"
29	Aedes	40	Lagoon	"
29	Culex	10	Brandon	"
29	Culex	10	Delta	"
29	Culiseta	3	Brandon	"
29	Culiseta	10	Lagoon	"
29	Culiseta	9	Park	"
29	Culiseta	16	Lagoon	"
August 2	Aedes	?	Delta	"
3	Aedes	30	Lagoon	"
3	Aedes	3	Brandon	"
3	Anopheles	?	Delta omit	"

APPENDIX VIII

ATTEMPTED ISOLATION OF WESTERN ENCEPHALITIS IN MANITOBA, 1966

Date	Genus	Locality	Number	W.E. E. Virus Isol'n Negs
August 3	Culex	Brandon	3	Negative
3	Culex	Lagoon	46	"
3	Culiseta	Brandon	2	"
3	Culiseta	Lagoon	50	"
3	Culiseta	Delta	5	"
3	Culiseta	Park	3	"
4	Aedes	Brandon	4	"
4	Culex	Brandon	4	"
5	Aedes	Lagoon	?	"
5	Aedes	Park	15	"
5	Aedes	Delta	?	"
5	Culex	Delta	?	"
5	Culex	Lagoon	?	"
5	Culiseta	Lagoon	?	"
5	Culiseta	Delta	?	"
8	Aedes	Park	?	"
8	Aedes	Brandon	7	"
8	Culex	Delta	1	"
8	Culiseta	Delta	6	"
9	Aedes	Delta	13	"
9	Aedes	Brandon	5	"
9	Aedes	Park	30	"
9	Anopheles	Delta	40	"
9	Culex	Brandon	5	"
9	Culiseta	Lagoon	5	"
9	Culiseta	Delta	3	"
10	Aedes	Brandon	6	"
10	Aedes	Delta	3	"
10	Anopheles	Brandon	7	"
10	Culex	Delta	2	"
10	Culex	Lagoon	20	"
10	Culex	Brandon	15	"
10	Culiseta	Brandon	4	"
11	Aedes	Lagoon	8	"
11	Culex	Lagoon	?	"
11	Culiseta	Brandon	2	"
11	Culiseta	Lagoon	1	"
12	Aedes	Delta	8	"
12	Aedes	Park	3	"
12	Anopheles	Delta	11	"
			7	

APPENDIX VIII

ATTEMPTED ISOLATION OF WESTERN ENCEPHALITIS IN MANITOBA, 1966

Date	Genus	Locality	Number	W.E.E. Virus Isol'n.
August 12	Culiseta	Brandon	4	Negative
25	Aedes	Brandon	4	"
25	Aedes	Delta	25	"
25	Anopheles	Brandon	9	"
25	Anopheles	Brandon	9	"
25	Culex	Delta	9	"
25	Culiseta	Brandon	18	"
25	Culiseta	Delta	40	"
26	Aedes	Brandon	15	"
26	Aedes	Delta	20	"
26	Aedes	Park	20	"
26	Anopheles	Brandon	6	"
26	Culiseta	Delta	25	"
26	Culiseta	Lagoon	6	"
September				
9	Aedes	Brandon	30	"
9	Aedes	Lagoon	30	"
9	Anopheles	Delta	1	"
9	Anopheles	Lagoon	1	"
9	Anopheles	Brandon	1	"
9	Culiseta	Lagoon	5	"
9	Culiseta	Park	1	"
9	Culiseta	Delta	1	"
?	?	?	300	"

Appendix IX

Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN ADULT MOSQUITOS

Record No.

Date September 22/66
Insecticide: DDT/dieldrin/other[1] D.D.T.
1. Investigator: R. Costello
2. Country: Canada
3. Province: Manitoba
4. Locality: Winnipeg
5. History of insecticide treatment (including agriculture): Spraying & fogging during the summer months.
Species: Culex restuans
6. Condition of mosquitos: blood-fed/gravid/unfed/sugar-fed/males[1]
7. Where collected: shelters sprayed/unsprayed/outdoors/biting/bred out[1]
8. Type of test on population: first time/routine check/complete retest[1]
9. Exposure period (minutes): 240

Tests	Preliminary	Replicate 1	Replicate 2	Replicate 3	Totals (for comparable tests only)
Date of test	Sept. 2	Sept. 2			
Temperature during exposure period	20°C	20°C			
Humidity during exposure period (%)	60	60			
Temperature during 24-hr holding period (°C)	Max. Min. 20°C	Max. Min. 20°C	Max. Min.	Max. Min.	

Insecticide concentration (%)	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]
2.0	15	15	100%	15	15	100									
1.0	13	15	85.8	14	15	92.4									
0.5	11	15	72.6	10	15	66.6									
0.25	11	15	72.6	13	15	85.8									
Control (oil alone)	0	15	0	0	15	0									

[1] Cross out what does not apply
[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

Remarks:
Interpretation of results (optional):

Signature of investigator: R. A. Costello

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.
A second copy to be sent on completion to the appropriate WHO Regional Office.

Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN ADULT MOSQUITOS

Record No.

Date June 10/66
Insecticide: DDT/dieldrin/other[1] D.D.T
1. Investigator: R. Costello
2. Country: Canada
3. Province: Manitoba
4. Locality: Standlands
5. History of insecticide treatment (including agriculture): Collected from area which is sprayed & fogged during spring & summer
Species: Aedes vexans
6. Condition of mosquitos: blood-fed/gravid/unfed/sugar-fed/males[1]
7. Where collected: shelters sprayed/unsprayed/outdoors/biting/bred out[1]
8. Type of test on population: first time/routine check/complete retest[1]
9. Exposure period (minutes): 60

Tests	Preliminary	Replicate 1	Replicate 2	Replicate 3	Totals (for comparable tests only)
Date of test	June 9	June 9			
Temperature during exposure period	20° C	20° C			
Humidity during exposure period (%)	50%	50%			
Temperature during 24-hr holding period (°C)	Max. Min. 20°C	Max. Min. 20°C	Max. Min.	Max. Min.	

Insecticide concentration (%)	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]
2.0	15	15	100	15	15	100									
1.0	14	15	92.4	15	15	100									
0.5	14	15	92.4	14	15	92.4									
0.25	12	15	89.2	11	15	72.6									
Control (oil alone)	0	15	0	0	15	0									

[1] Cross out what does not apply
[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

Remarks:
Interpretation of results (optional):

Signature of investigator: R. A. Costello

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.
A second copy to be sent on completion to the appropriate WHO Regional Office.

Appendix IX

Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN MOSQUITO LARVAE

Date August 26/66 Species: Aedes vexans

Insecticide: DDT/dieldrin/BHC/other [1] DDT

1. Investigator: R. Costello graduate student
2. Country: CANADA 3. Province: MANITOBA 4. Locality: WINNIPEG
5. History of insecticide treatment (including agriculture): during summer spraying & fogging regularly
6. Condition of larvae: instar 4th reared/collected/other [1] reared
7. Results of test (abbreviations: "M" — moribund; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
Date of test	Aug. 26			Aug. 28											
Temperature during test	20°C			20°C											
Insecticide concentration (p.p.m.)	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
0.1	15	15	100	15	15	100									
0.02	15	16	100	15	15	100									
0.004	14	16	93.3	15	15	100									
Control 1	0	15	0	0	15	0									
CONTROL 2	0	16	0	0	15	0									

[1] Cross out what does not apply

[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

Remarks: .....

Signature of investigator: R.A. Costello

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit Division of Environmental Health, Geneva, Switzerland.
A second copy to be sent on completion to the appropriate WHO Regional Office.

Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN MOSQUITO LARVAE

Date August 15/66 Species: Culisata inornata

Insecticide: DDT/dieldrin/BHC/other [1] DDT

1. Investigator: R.A. Costello graduate Student
2. Country: Canada 3. Province: Manitoba 4. Locality: Winnipeg
5. History of insecticide treatment (including agriculture): spraying & fogging throughout the summer months
6. Condition of larvae: instar 4th reared/collected/other [1] collected
7. Results of test (abbreviations: "M" — moribund; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
Date of test	Aug. 15			Aug. 15											
Temperature during test	20° C			20°C											
Insecticide concentration (p.p.m.)	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
0.1	15	15	100	15	15	100									
0.02	15	15	100	15	15	100									
0.004	15	15	100	15	15	100									
Control 1	1	15	6.6	0	15	0									
CONTROL 2	2	15	13.2	0	15	0									

[1] Cross out what does not apply

[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

Remarks: .....

Signature of investigator: R.A. Costello

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit Division of Environmental Health, Geneva, Switzerland.
A second copy to be sent on completion to the appropriate WHO Regional Office.

Appendix IX

Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN MOSQUITO LARVAE

Date July 23/66

Insecticide: DDT/dieldrin/BHC/other [1] DDT *Species:* Culex restuans

1. Investigator: R. A. Costello Graduate Student
2. Country: Canada 3. Province: Manitoba 4. Locality: Winnipeg
5. History of insecticide treatment (including agriculture): Area sprayed almost daily during summer
6. Condition of larvae: instar 3rds & 4ths reared/collected/other [1] Collected
7. Results of test (abbreviations: "M" — moribund; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
Date of test	July 23/66			July 28/66											
Temperature during test	20° C			20° C											
Insecticide concentration (p.p.m.)	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
2.5	20	20	100	20	20	100									
0.5	20	20	100	20	20	100									
0.1	20	20	100	20	20	100									
0.02	20	20	100	19	20	95									
0.004	12	20	60	18	20	90									
Control 1	0	20	0	0	20	0									
Control 2	0	20	0	0	20	0									

[1] Cross out what does not apply

[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

Remarks:

Signature of investigator: R. A. Costello

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit Division of Environmental Health, Geneva, Switzerland. A second copy to be sent on completion to the appropriate WHO Regional Office.

Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN MOSQUITO LARVAE

Date August 18/66

Insecticide: DDT/dieldrin/BHC/other [1] DDT *Species:* Culex tarsalis

1. Investigator: R. A. Costello
2. Country: Canada 3. Province: Manitoba 4. Locality: Winnipeg
5. History of insecticide treatment (including agriculture): Spraying & fogging throughout the summer months
6. Condition of larvae: instar 4th reared/collected/other [1] collected
7. Results of test (abbreviations: "M" — moribund; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
Date of test	Aug. 18/66			Aug. 18/66											
Temperature during test	20° C			20° C											
Insecticide concentration (p.p.m.)	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
0.1	15	15	100	15	15	100									
0.02	15	15	100	15	15	100									
0.004	14	15	93.3	14	15	93.3									
Control 1	1	15	6.6	2	15	13.2									
Control 2	0	15	0	1	15	6.6									

[1] Cross out what does not apply

[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

Remarks:

Signature of investigator: R. A. Costello

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit Division of Environmental Health, Geneva, Switzerland. A second copy to be sent on completion to the appropriate WHO Regional Office.

APPENDIX X

MOSQUITOES CAUGHT IN 40 FOOT TAYLOR TRAP, 1965

Date		Species	Mosquitoes Caught ♀	♂
May	13	Culiseta inornata	1	
	17	Culiseta inornata	1	
	25	Culiseta inornata	1	
	31	Culiseta inornata	2	
June	3	Culiseta inornata	1	
	10	Culiseta inornata	1	
	14	Aedes vexans		1
	16	Culiseta inornata	2	4
		Aedes vexans	1	
	17	Aedes vexans		5
	19	Aedes vexans	1	
	20	Culiseta inornata	2	
	21	Aedes vexans		1
		Culiseta inornata	2	
	22	Aedes flavescens	1	
		Aedes vexans		1
		Culiseta inornata	2	
	23	Aedes spencerii	1	
		Aedes vexans	1	
		Culiseta inornata	1	
	24	Aedes spencerii	1	
		Aedes vexans	4	3
		Culiseta inornata	5	3
	25	Aedes vexans	1	1
		Culiseta inornata	2	
	26	Culiseta inornata	1	
	30	Aedes vexans	16	9
		Culiseta inornata	1	
July	1	Aedes vexans	1	
	2	Aedes vexans	1	
		Culiseta inornata	1	
	3	Aedes vexans	3	3
		Culex tarsalis	1	
	5	Aedes spencerii	1	
		Aedes vexans	4	3
		Culex tarsalis	7	4
		Culiseta inornata	3	1

APPENDIX X

MOSQUITOES CAUGHT IN 40 FOOT TRAP, 1965

Date		Species	Mosquitoes Caught ♀	♂
July	6	Aedes spencerii	1	
		Aedes vexans		1
		Culiseta inornata	4	2
	7	Aedes spencerii	2	
		Aedes stimulans	1	
		Aedes vexans	1	
		Culex tarsalis	7	5
	8	Aedes vexans	2	1
		Culex tarsalis	2	4
		Culiseta inornata	2	
	9	Aedes dorsalis	3	1
		Aedes vexans	1	
	11	Aedes vexans		17
	12	Aedes vexans	1	
	14	Aedes vexans		2
		Culiseta inornata	2	
	15	Aedes vexans	8	
	16	Aedes stimulans	4	426
		Aedes vexans	3	1
		Culiseta inornata	2	1
	17	Aedes vexans	3	
		Culex tarsalis	4	
		Culiseta inornata	1	
	18	Aedes vexans	2	
	23	Culex tarsalis	1	
		Culiseta inornata	13	
	25	Aedes vexans	4	
	28	Aedes vexans	1	
	29	Aedes vexans		
		Culiseta inornata	3	20
August	2	Aedes vexans	1	1
		Culiseta inornata	7	
	5	Aedes vexans	3	
	7	Aedes vexans	1	
		Culex tarsalis	6	6
	9	Aedes vexans	5	2
		Culex tarsalis		130
	12	Aedes vexans	25	
		Culiseta inornata	7	
	13	Aedes vexans	1	
	16	Aedes vexans		75
	18	Aedes vexans		2800
	19	Aedes vexans	2	1
	22	Aedes vexans		900
	26	Aedes vexans	3	3
				540