ECOLOGY AND OVERWINTERING BIOLOGY OF POTENTIAL MOSQUITO VECTORS OF WESTERN EQUINE ENCEPHALITIS IN MANITOBA

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William John Gallaway

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ABSTRACT

Outbreaks of western equine encephalitis (WEE) virus in man and horses are a reoccurring problem in southern Manitoba. In Manitoba this virus has been isolated from Anopheles earlei Vargas, Culex restuans Theobald, Culex tarsalis Coquillett, and Culiseta inornata (Williston). These species all overwinter as adult females.

The population dynamics of Cx. restuans, Cx. tarsalis and Cs. inornata were studied in southern Manitoba by sampling the population of adults attracted to carbon dioxide baited light traps and by sampling the population of gravid females attracted to oviposition pools.

Monitored oviposition activity and light trap catches showed that Cx. restuans and Cx. tarsalis may have 3 generations/year and that Cs. inornata has 2 generations/year. The peaks in oviposition activity and CDC light trap catches of Cx. tarsalis best fit the seasonal activity of WEE, which lends support to the idea that it is the primary vector in Manitoba. Culex restuans may be an important epizootic vector, while Cs. inornata is probably not an important vector of WEE. The oviposition pools attracted fair numbers of gravid Cx. restuans and Cs. inornata, and the relative numbers of egg rafts followed a similar trend each season. The oviposition pools did not attract gravid females of Cx. tarsalis in numbers representative of the adult population taken in CO_2 baited light traps. Diel monitoring of oviposition activity showed 2 peaks by Cx. restuans; a major one 2 to 4 hours after sunset, and a minor one at sunrise. Culex restuans egg rafts laid in early summer had significantly more eggs per raft than those laid in the spring and late summer.

Diapausing An. earlei, Cx. tarsalis and Cs. inornata were collected and offered a blood meal to see if they would exhibit gonotrophic dissociation and therefore be a possible overwintering mechanism for WEE. Overwintering An. earlei were collected in December, 1980. Forty-two were offered a human blood meal, and of 25 that fed 24 exhibited gonotrophic dissociation. Two Cx. tarsalis collected in December, 1980, were offered human blood meals. Both fed, but neither of them developed eggs. Culiseta inornata females collected in late August, readily took blood (19/20). Eighteen of the 19 blood-fed females developed eggs. Some ejected their first blood meal but accepted a second.

Anopheles earlei and Cx. tarsalis may be important in the overwintering of WEE virus. It is possible that diapausing females may take an infected blood meal, exhibit gonotrophic dissociation and then harbour the virus over the winter.

FOREWORD

The format followed for this Thesis is the manuscript style. This Thesis contains four manuscripts. The manuscript entitled "Some population parameters of Culex restuans, Culex tarsalis and Culiseta inornata (Culicidae:Diptera) from southern Manitoba" will be submitted for publication at a later date. The paper entitled "Blood feeding and gonotrophic dissociation in overwintering Anopheles earlei (Diptera:Culicidae) from southern Manitoba" by W.J. Gallaway and R.A. Brust has been published in The Canadian Entomologist 114:1105-1107. The paper entitled "Blood feeding in overwintering Culex tarsalis (Diptera:Culicidae) from Manitoba" by P.W. Arntfield, W.J. Gallaway and R.A. Brust has been published in The Canadian Entomologist 114:85-86. The final manuscript "Blood feeding in diapausing Culiseta inornata (Diptera:Culicidae) from southern Manitoba" is preliminary, and will require additional work to complete.

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INTRODUCTION

In Manitoba, the first known outbreak of western equine encephalitis (WEE) occurred in horses in 1935 (Medovy 1976). Since then, outbreaks have occurred periodically in both horses and man (Medovy 1976). Western equine encephalitis virus has been isolated during July and August from several species of mosquitoes found in Manitoba (Norris 1946, Sekla et al. 1980). Four of these species, Anopheles earlei Vargas, Culex restuans Theobald, Culex tarsalis Coquillett, and Culiseta inornata (Williston) overwinter as adult females (Wood et al. 1979). Culex tarsalis is probably the primary vector of this virus in Manitoba (Henderson et al. 1979, Sekla et al. 1980); the other 3 species may be secondary vectors.

Aerial spraying of propoxur (Baygon) was carried out to control vector populations of mosquitoes during the 3 most recent outbreaks of WEE in Manitoba (Ellis, R. pers. comm.). This method of vector control is costly, its effectiveness is disputed, and it may damage the environment. Increased knowledge of vector biology can increase the efficiency of surveillance techniques for both vector populations and arbovirus activity and increase the effectiveness of control methods by enabling insecticides to be applied when vector populations are most vulnerable. With these goals in mind a study was carried out to investigate the seasonal population dynamics of Cx. restuans, Cx. tarsalis and Cs. inornata. An oviposition monitoring technique and CDC light traps were used in this study.

The overwintering mechanism of WEE is unknown, although there are many hypotheses (Reeves 1974). A possible way for WEE to overwinter is

in overwintering adult female mosquitoes that have taken an infected blood meal and then used it as an energy source, instead of a protein source for vitellogenesis. The process of not developing eggs after taking a blood meal is called gonotrophic dissociation. It is known to occur in several species of Anopheles and Culex (Washino 1977). The possibility of gonotrophic dissociation occurring in diapausing An. earlei, Cx. tarsalis and Cs. inornata from Manitoba was investigated in this study.

LITERATURE REVIEW

New Jersey and CDC Light Traps

Sampling techniques for adult female mosquitoes have been reviewed by Service (1976). The New Jersey light trap (NJLT) is used throughout North America for monitoring mosquito populations. The CDC light trap is most commonly used for collecting live mosquitoes for arbovirus isolations. The history and uses of these 2 traps will be briefly reviewed.

The NJLT was originally designed by Headlee (1932) and then improved by Mulhern (1934) (see Mulhern 1942, Huffaker and Back 1943). Mulhern (1942) described, illustrated and discussed the uses of the present NJLT.

Huffaker and Back (1943) compared NJLT catches from traps with light as the attractant, light and CO_2, CO_2, and no attractant. The trap with light and CO_2 collected 3 times more mosquitoes than the others combined. They found that the trap with no attractant collected the most representative sample, although it did not capture many mosquitoes. It was concluded that attractants affected both the numbers and types of mosquitoes collected.

New Jersey light traps are used in mosquito control and in arbovirus surveillance programs. Both the City of Winnipeg and City of Brandon Mosquito Control Districts use the NJLT to monitor mosquito populations (Ellis, R.A. pers. comm.; pers. obser.). These types of traps were used in arbovirus surveillance by the Manitoba government (Brust and Ellis 1976). Olson *et al.* (1979) used the NJLT in California from 1953 to 1973. They correlated *Cx. tarsalis* population indices with the incidences of St. Louis encephalitis (SLE) and WEE.

The CDC miniature light trap is a portable, battery-powered light trap that was developed by Sudia and Chamberlain (1962). The use of dry ice (CO_2) bait with this trap was found to increase the catch by at least four-fold and increase the number of species trapped by 20 to 25% (Newhouse et al. 1966).

CDC miniature light traps baited with dry ice have been used to collect mosquitoes for arbovirus surveillance and for population studies. Taylor et al. (1971) used a CDC light trap to obtain large numbers of mosquitoes for California group arbovirus surveillance in Florida. In Iowa, Rowley et al. (1973) used these traps to collect 69,464 mosquitoes in 1971 for arbovirus surveillance. Mitchell et al. (1979) used CDC light traps with dry ice to collect mosquitoes and Culicoides during an eastern equine encephalitis outbreak in the Dominican Republic. In New York, Srihongse et al. (1980) used dry ice baited traps to collect 918,047 mosquitoes of 5 genera from 1972 to 1977. They obtained 228 viral isolations from 20,616 pools tested. Helson et al. (1980) used these traps to collect mosquitoes for a SLE surveillance program in southwestern Ontario where Cx. pipiens Linnaeus is probably the main vector of SLE. In 1982, the Manitoba Arbovirus Surveillance Program began using CDC light traps baited with dry ice in place of the NJLT. The CDC traps are used to collect live mosquitoes for virus tests and for population studies (Brust, R.A. pers. comm.). These studies indicate that the CDC light trap baited with dry ice is a suitable trap for collecting mosquitoes.

There appears to be a trend towards the use of the CDC light traps

in recent years. It is hoped that this trend will result in a standardization of trapping methods. In this way comparisons of mosquito population levels can be made over large areas and base line data can be established so that, in the future, a better prediction of arbovirus outbreaks can be made.

Oviposition Monitoring Techniques for *Culex* and *Culiseta* spp.

Oviposition activity is often monitored in studies on mosquito population dynamics. Most of the *Culex* and *Culiseta* species are well suited to oviposition monitoring because they lay their eggs in visible rafts on the water surface. This technique may represent an inexpensive alternative to light traps for the monitoring of *Culex* and *Culiseta* species in arbovirus and pest surveillance programs. Pools of various sizes have been used to study the oviposition biology of a variety of species. A few of these studies will be reviewed.

De Meillon *et al*. (1967) used a 90x60x120 cm tray filled with septic tank water to study the oviposition behavior of *Cx. quinquefasciatus* Say in Rangoon, Burma. They studied the daily oviposition cycle and the relationship between time of feeding and time of oviposition. It was found that an evening and morning peak in oviposition activity occurred. These peaks were not endogenous, but depended upon the time and the date of feeding. Oviposition by gravid females was found to be triggered by a change in light levels. They felt that their monitoring techniques could be used to assess mosquito populations and the effects of control programs.

Oda (1967) used earthenware jars filled with a rice straw infusion

to study the hourly and seasonal distribution of egg rafts laid by Cx. pipiens. He found these jars to be a suitable monitoring technique for his study.

Smith and Jones (1972) used 76x46x8 cm pools lined with black plastic to determine the presence, abundance and oviposition preference of Cx. nigripalpus Theobald in Florida. These pools were a quick and inexpensive method for demonstrating the presence and abundance of Cx. nigripalpus in isolated areas.

Maw and Bracken (1971) used $1m^2$ and $0.09m^2$ pools to assess Cx. restuans populations in Ontario. Pools treated with capric acid were found to be more attractive to ovipositing females than untreated pools. They observed that low damp areas along streams were the best locations for pools when monitoring Cx. restuans. The pools were more effective than light traps for monitoring overwintering females. The larger pools ($1m^2$) collected more egg rafts per unit surface area and were more efficient than the small pools when adult population densities were low. The small pools ($0.09m^2$) were easier to install, generally effective and easier to monitor.

Lowe et al. (1974) used egg raft collections to obtain information on the relative abundance throughout the year of Cx. quinquefasciatus, Cx. nigripalpus, Cx. restuans and Cx. salinarius Coquillett in Florida. They destroyed all known breeding sites and then set up tubs filled with an infusion medium. Egg raft collections and subsequent identification of the larvae was a better monitoring technique than trapping for these species, because of the difficulty of identifying adults.

Hayes (1975) and Hayes and Hsi (1975) used a water trough with a

surface area of 15, 235 cm^2 for oviposition by an isolated population of Cx. quinquefasciatus.

Surgeoner and Helson (1978) combined a CDC light trap and an ovipool to develop a technique which would capture Cx. pipiens and Cx. restuans females during oviposition and therefore ensure parous females for viral tests. They used an 84 cm diameter inflatable pool lined with sod. A CDC light trap was suspended in the middle. The pool acted as an attractant for gravid females, which were in turn captured in the CDC light trap. The parity rate of captured females greatly increased with this method. It was felt that this method would reduce the cost of arbovirus surveillance, through decreased time for sorting and identification, and an increased probablity of recovering virus. The only problem they found was that the apparatus had to be serviced daily to empty the CDC light trap.

Madder et al. (1980) used inflatable swimming pools 84 cm in diameter, lined with sod, to monitor Cx. pipiens and Cx. restuans in Ontario. These 2 species are believed to be the vectors of SLE in Ontario. The oviposition data was compared with numbers of adult females from CDC light trap catches. Egg raft collections were comparable to adult catches. They found the pools to be an efficient, inexpensive and sensitive technique for monitoring the populations of these 2 species. This technique enabled them to avoid the expense of sorting light trap catches and the need for expertise in identification of female adults.

Madder et al. (1980) compared the effects of 3 different treatments on the attractiveness of 84 cm diameter pools to ovipositing Cx. pipiens

and Cx. restuans. The treatments were: pool lined with sod, with capric acid added to the water; pool lined with sod; pool lined with dark green plastic. Capric acid was not necessary as an attractant all that was needed was organic material. Ikeshoji et al. (1975) found that Pseudomonas bacteria produced oviposition attractants for Cx. pipiens and that these attractants were intermediate metabolites of capric acid, not the acid itself.

It has been reported that the brightness of a pool's lining affects the oviposition behavior of Cx. restuans (Belton 1967). In a woodland environment, 3 times more rafts were laid in pools with dark linings compared to pools with translucent linings. Maw and Bracken (1971) found that Cx. restuans preferred to oviposit in pools located in damp low areas along streams.

The monitoring of oviposition activity through the use of man made pools is a reliable technique in studying the population dynamics of populations of Culex species. This method has not been evaluated on any Culiseta species. It is evident that many factors such as metabolites of bacteria, brightness of the pool lining, pool location and possibly other factors are important in the attractiveness of a pool to gravid females.

Life History of Culex restuans

Culex restuans Theobald is found from central Alberta south to Mexico, east to Nova Scotia and Florida (Wood et al. 1979). This species is a possible vector of 3 arboviruses. The first isolation of WEE from Manitoba was from this species (Norris 1946). Sekla et al. (1980) isolated WEE from a combined pool of Cx. restuans (3%) and Cx. tarsalis

(97%) collected in Manitoba. Hayes et al. (1960) isolated eastern equine encephalitis from Cx. restuans female adults collected in New Jersey. This species is a suspected vector of SLE in southern Ontario (Madder et al. 1980).

Adult females of Cx. restuans overwinter in a state of gonotrophic diapause. Diapause in this species is characterized by reduced blood feeding (Wallis 1959, Eldridge et al. 1972), fat body development (Wallis 1959), gonotrophic dissociation (Eldridge et al. 1972) and failure of the ovarian follicles to develop from stage N to the resting stage Ib (Eldridge et al. 1976). Eldridge et al. (1972, 1976) found that the temperature and photoperiod that a female is subjected to from the pupal stage until a 6 to 8 day old adult influences the diapause response. A low temperature (15^{o}C) and short photoperiod (8L:16D) induce gonotrophic diapause (Eldridge et al. 1972).

Females overwinter in basements, caves, hollow trees and crevices and cracks in the ground (Hayles et al. 1979, Wood et al. 1979). In Connecticut the females emerge from overwintering sites in late March or early April (Wallis 1959). Oviposition by overwintered females occurs from mid-May to mid-June in Ontario (Maw and Bracken 1971, Madder et al. 1980). In Canada this species probably begins to emerge from their hibernacula from early to late May, depending on the area.

Madder et al. (1980) reported that in Ontario this species has 3 generations per year. Eggs of the first generation are laid by the overwintered females between mid-May and mid-June. The first generation lays the eggs giving rise to the second generation, during early June to mid-July. The second generation is the largest. Females of the

second generation lay the eggs giving rise to the third generation during mid-July and early August. The third generation is reduced because a high proportion of the females in the second generation are in gonotrophic diapause and do not lay eggs until the next year.

Gravid females prefer to oviposit in pools located in low damp areas along streams (Maw and Bracken 1971). Pools with a dark background are more attractive to gravid females than pools with a light coloured background (Belton 1967).

Maw and Bracken (1971) found capric acid to be an oviposition attractant for this species. They attributed its attractive properties to a fertilizer effect on bacteria of the family Pseudomonadaceae. Madder et al. (1980) found that capric acid had no attractive effect on this species.

Egg rafts laid by the overwintered generation average 185 eggs per raft; the summer generations average 220 eggs (Madder 1981). In southern Ontario, maximum oviposition activity occurs within 2 hours of sunset and a minor peak of activity occurs at sunrise (MacDonald et al. 1981).

This species is probably not a serious pest of man or his domestic animals. The preferred hosts of this species are birds (Tempelis 1975). Blood meals are fully digested in 6 days at 10°and 15°C and 3 to 4 days at 20°and 25°C (Eldridge et al. 1976).

Development of the immature stages is relatively rapid. At 12°C, 25 days are required for development from first instar larvae to adults, at 26°and 29°C only 6 days are required (Shelton 1973). Maximum larval survival occurs between 12° and 26°C; 32°C is lethal (Shelton 1973). The optimal temperature for development and survival of larvae would appear

to be 26°C.

Life History of Culex tarsalis

Culex tarsalis Coquillett is distributed throughout western North America, from the central MacKenzie Valley south to Mexico and occurs eastward to southwestern Ontario and Florida (Wood et al. 1979). Western equine encephalitis has been isolated from this species in Manitoba (Sekla et al. 1980). Henderson et al (1979) reported that a Manitoba strain of this species was an efficient vector of a Manitoba strain of WEE. This species is believed to be the main vector of WEE and SLE throughout western North America.

Adult females of Cx. tarsalis overwinter in a state of gonotrophic diapause. Females utilize plant sugars in the fall to develop a fat body which is used as an energy source during the diapause period (Schaefer and Miura 1972). The majority of females that attempt to overwinter are nulliparous (Blackmore and Dow 1962, Mitchell 1979). Gonotrophic dissociation has been shown to occur in diapausing females, but it is not thought to be a common phenomenon (Mitchell 1981, Arntfield et al. 1982).

Photoperiod affects fat body and ovarian development in this species. Harwood and Halfhill (1964) showed that females responded to short daily photoperiod (8 hr) by increased size and compactness of fat body and decreased ovary length. Lower temperature reinforced the fat body response. Photoperiod also had an affect on the larvae. These experiments were conducted on a California strain which was probably not as responsive as a northerly strain would be, because of the milder southern climate.

Diapausing females have been taken from piles of rock (Rush et al.

1958, Rush 1962), mammal burrows (Shemanchuk 1965), crevices in the ground (Hayles et al. 1979), and caves, subterranean tunnels, and mines (Price et al. 1960, Blackmore and Dow 1962, Arntfield et al. 1981, Mitchell 1981). Females emerge in the spring when soil surface temperatures become warmer than the subsurface (Shemanchuk 1965).

Mitchell (1981) found that diapause termination was a function of increased day length and not just an increase in temperature. He also found that the topical application of methoprene terminated diapause. Therefore diapause termination may involve the secretion of juvenile hormone by a reactivated corpus allatum.

In nature, females feed on birds and mammals. Reeves et al. (1963) and Tempelis et al. (1965) have collected and analysed large numbers of blood fed females and found them to prefer birds but also to feed on mammals.

Downe and Archer (1975) showed that females that fed on chicken blood produced more eggs than those that fed on the blood of snakes or guinea pigs. One colony that had fed on chickens developed an average of 247.8 eggs/female, while another colony produced 314.8 eggs/female. When the same colonies were allowed to feed on the blood of snakes they produced 205.2 eggs/female and 221.1 eggs/female respectively. Hence the number of eggs developed depends in part at least on the blood meal source.

Females appear to prefer to oviposit in pools exposed to sunlight. Walters and Smith (1980) inspected a variety of larval habitats and found larvae most often in eutrophic habitats that were fully exposed or at least partially exposed to the sun. Oviposition is cued by changes

in light levels and occurs at dusk or dawn (Logen and Harwood 1965).

Hagstrum and Workman (1971) found that at high feeding rates, larval development from first instar to pupa required 11.7 days at 30°C and 14.5 days at 20°C. Larval mortality was evenly distributed throughout the instars.

Major flight activity occurs within 2 hours after sunset and between temperatures of 13° to 33°C (Bailey et al. 1965). Temperatures below 18°C usually caused a reduction in activity (Bailey et al. 1965).

Mitchell (1981) described the seasonal dynamics of Cx. tarsalis in northern Colorado. Inseminated nulliparous females overwinter. These females begin to emerge from their hibernacula in late March and by early May blood fed females can be found, and oviposition commences. The population peaks near the end of July, and at the same time blood feeding activity decreases, probably due to a decrease in photoperiod. In mid-August, females begin to develop fat bodies from nectar carbohydrates. From early September until October, and even early November, females seek out hibernacula. In Manitoba, the life cycle probably follows much the same pattern, except there are undoubtedly differences in the dates for the events listed above due to a shorter season.

Life History of Culiseta inornata

Culiseta inornata (Williston) is found from the Yukon Territory and Northern Mexico east to New Hampshire and Florida (Wood et al. 1979). In Manitoba, Sekla et al. (1980) isolated WEE and an unidentified California encephalitis virus from females of this species. Isolations

of WEE have occurred from this species in various areas of the U.S.A. and Canada (McLintock and Iversen 1975).

In the northern parts of its range this species overwinters as nulliparous females (Hudson 1979). Hudson (1979) examined females captured in Edmonton during September and October. All were nulliparous, all had small ovaries and none had blood in their gut. In Colorado, Dow et al. (1976) collected post-hibernating female Cs. inornata underneath bridges from late March onward. All of the mosquitoes they examined from late March until the end of April were gravid or parous. They concluded that only parous or gravid females overwintered. This may be possible but not probable. It is more likely that these females had taken a blood meal when they left their hibernacula and had flown to resting and oviposition sites to develop and lay their eggs and were then collected.

In Canada and the northern U.S.A. females have been found during the winter months in rock piles, mammal burrows, and crevices and cracks in the ground (Rush et al. 1958, Shemanchuk 1965, Hayles et al. 1979).

Hudson (1977) demonstrated that in lab reared Cs. inornata a decrease in photoperiod resulted in adult females with follicles the size and stage characteristic of diapausing wild females. He transferred females that had just moulted to fourth instar larvae or pupae from 16L:8D, 20°C to 12L:12D, 10°C. These females had small ovaries with follicles in stage N-I. Females reared at a constant 16L:8D, 20°C or 12L:12D, 10°C developed large ovaries and follicles characteristic of gonoactive females. Diapausing females were reluctant to feed, and those that fed ejected the blood meal prematurely (Hudson 1979).

In the southern parts of its range this species aestivates in the summer months and is active during the winter months (Barnard and Mulla 1977c, 1978). Barnard and Mulla (1978) found that the aestivating population consists of gravid and parous females.

Barnard and Mulla (1977b) reared Cs. inornata from southern California at 16L:8D and 8L:16D at 15°, 20° and 25°C. Females raised at long days showed reduced blood feeding and developed fat bodies. The reduction in blood feeding decreased further as temperature increased. There was no difference in fat body development between nulliparous and parous females.

In Manitoba, this species has 2 generations a year (Hanec and Brust 1967). I have observed that females leave their hibernacula during April and May.

The optimal survival temperature for larvae of this species is 21°C. At this temperature, an average of 15 days is required for development from first instar to pupa (Brust 1967). Brust (1967) found the lethal high temperature to be 29°C, and the lethal low temperature to be 6°C. Fanara and Mulla (1974) reported that in California breeding by this species was restricted to the winter months because during the summer pool temperatures exceeded 29°C.

Diel periodicity of pupation and emergence has been demonstrated in this species. Barnard and Mulla (1977a) found that two peaks in pupation occurred. A small peak was recorded 1.5 hours before sunrise and a large peak at sunset. A large peak in emergence occurred at dusk, and this peak in emergence was correlated with water temperature (Barnard and Mulla 1977a). They reported that the pupation peaks did not correlate with any of the weather variables measured.

Gonotrophic Dissociation and Potential Overwintering of Arboviruses

In theory, some arboviruses may overwinter in hibernating female mosquitoes. This would be possible if a mosquito in a state of gonotrophic diapause took a blood meal and used it as an energy source instead of a protein source for egg development. The cessation of egg production despite the continued taking of blood meals is called gonotrophic dissociation. In North American mosquitoes, this phenomena occurs in An. freeborni Aitken (Washino 1970, Washino et al. 1971), An. punctipennis (Say) (Magnarelli 1979), Cx. pipiens (Eldridge and Bailey 1979), Cx. restuans (Eldridge et al. 1972) and Cx. tarsalis (Mitchell 1981). In all of these species, gonotrophic dissociation occurred in at least a small proportion of the test population, when the females were kept at low temperatures and short photoperiods. Diapausing female mosquitoes that exhibit gonotrophic dissociation are potential overwintering hosts for some arboviruses (Reeves 1974). The mosquito may feed on an animal infected with an arbovirus and the virus would then overwinter in the mosquito. In the spring the infected mosquito would transmit the virus while blood feeding.

Some Population Parameters of *Culex restuans*, *Culex tarsalis* and *Culiseta inornata* (Diptera: Culicidae) from Southern Manitoba

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ABSTRACT

An oviposition monitoring technique and CO_2 baited, CDC light traps were used to study the population dynamics of Culex restuans, Cx. tarsalis and Culiseta inornata.

Culex restuans may have 3 generations/year. During 1980-1982, eggs of the first generation were laid by overwintered females during late May to early June. Eggs of the second generation were laid from late June to late July and this generation was the largest. Eggs of the third generation were deposited between mid-July and late August. This generation was small, probably because many of the females from the second generation were in a state of gonotrophic diapause. Females were not readily attracted to CO_2 baited CDC light traps.

Oviposition by Cx. restuans showed a diel periodicity with a peak in activity 2 to 4 hours after dusk and a second peak at dawn.

Significant differences were found in the number of eggs per egg raft laid by spring, early summer and later summer generations of Cx. restuans. Egg rafts laid in the early summer had the greatest number of eggs per egg raft.

Culex tarsalis may also have 3 generations/year; an early spring generation and 2 summer generations. Oviposition activity and CDC light trap catches were greatest in July and early August. This species appears to prefer to oviposit in pools exposed to the sun.

Culiseta inornata appears to have 2 generations/year. Overwintered females lay eggs, which will give rise to the spring generation, between mid-May and early June. Eggs of the summer generation are laid during June to late July.

The population dynamics of Cx. tarsalis best fits the seasonal occurrence of western equine encephalitis (WEE) virus activity in Manitoba. This is further evidence that this species is the main vector of WEE virus in Manitoba. Culex restuans may be an important epizootic vector. Culiseta inornata probably is not an important vector of this virus because spring populations and feeding activity peak before WEE virus is found in mosquitoes, and well before equine or human cases are reported. The fall population peak occurs after a WEE epidemic subsides, and WEE virus is not found in mosquitoes during this time.

The use of pools, of the type used in this study, to monitor oviposition activity are suitable for Cx. restuans and Cs. inornata but not Cx. tarsalis. Oviposition activity levels may be an accurate reflection of adult female activity and population levels in Cx. restuans and Cs. inornata. Oviposition monitoring would be a suitable and inexpensive addition to an arbovirus surveillance program involving these two species.

INTRODUCTION

Western equine encephalitis (WEE) is the most common arbovirus isolated from Manitoba mosquitoes (Sekla et al. 1980). In Manitoba, recent outbreaks of WEE infection in horses and man occurred in 1975, 1977 and 1981. Culex tarsalis Coquillett is the primary vector of WEE in Manitoba (Henderson et al. 1979, Sekla et al. 1980). Western equine encephalitis has also been isolated from Cx. restuans Theobald (Norris 1946, Sekla et al. 1980) and Culiseta inornata (Williston) (Sekla et al. 1980), these species may be secondary vectors of WEE.

In Ontario, oviposition monitoring techniques have been used to study and monitor populations of Cx. restuans (Maw and Bracken 1971, Madder et al. 1980) and Cx. pipiens Linnaeus (Madder et al. 1980), the vectors of St. Louis encephalitis in that province (Helson et al. 1980). Madder et al. (1980) compared adult CDC light trap catches of Cx. restuans and Cx. pipiens with monitored oviposition activity data and concluded that oviposition activity followed the same trends as adult numbers. They feel that oviposition pools would be useful for monitoring populations of Cx. spp. Leiser and Beier (1982) obtained a positive correlation between total weekly egg raft numbers of Cx. restuans and Cx. pipiens collected from oviposition traps and total weekly adult numbers collected by New Jersey light traps. They found that both sampling methods demonstrated a similar pattern of Culex activity.

In the present investigation, an oviposition monitoring technique and CO_2 baited CDC light traps were used in the study of the population dynamics and reproductive biology of Cx. restuans, Cx. tarsalis and

Cs. inornata. The monitoring of oviposition activity in these species should be a good indicator of the population levels of reproductively active females and therefore potential WEE vectors. Other parameters that may be measured include the earliest date of oviposition after emergence from their winter hibernacula, the time of gonotrophic diapause in the autumn and increases or decreases in the number of gravid females attracted to the oviposition pools. When used in conjunction with CO_2 baited CDC light traps, oviposition monitoring could be a valuable aid to arbovirus surveillance programs.

MATERIALS AND METHODS

One meter square pools were used to collect egg rafts of Cx. restuans, Cx. tarsalis and Cs. inornata during the spring and summer of 1980, 1981 and 1982. CDC light traps baited with dry ice were used for the collection of adult mosquitoes in 1980 and 1981.

The pools consisted of $1m^2$, 20 cm deep, wooden frames lined with clear polyethylene sheeting, the bottoms of which were lined with grass sod. They were filled with water from a local source or from the City of Winnipeg water supply. Water was added to the pools as required.

Pools were set up at the University of Manitoba, Fort Garry campus (U of M) and the Glenlea Research Station (GRS). The U of M site is located within the City of Winnipeg limits; the GRS is 15 km south of Winnipeg. The pool at the U of M was set up along the bank of the Red River where it was shaded by trees. In 1980 the GRS pool was set up near a small group of trees, where it was exposed to the sun and wind. In 1981 and 1982 the GRS pool was set up in a ravine where the pool was shaded by trees. The pools were set up in late April and dismantled in mid-September.

Egg rafts were collected 3 days/week during the daytime, and occasionally 5 days/week. An effort was made to collect all rafts. The egg rafts were placed in individual cups, allowed to hatch, and the larvae identified. Weekly totals are comprised of egg rafts laid during each 7 day interval.

CDC light traps baited with dry ice, as described by (Sudia and Chamberlain 1962, Newhouse et al. 1966) were used to collect female mosquitoes. These traps were used in 1980 and 1981. Traps were set up Tuesday and Thursday, in the late afternoon and the catches collected

the next morning. Due to equipment failures, the number of operational traps was reduced to 1 trap-day/week.

The seasonal distribution of egg raft numbers and light trap data were used to determine the approximate times when new generations may have occurred.

The numbers of eggs per egg raft were determined for randomly selected rafts. The number of eggs was counted the day the egg rafts were collected. The number of eggs per raft was compared between the 3 generations of Cx. restuans at the U of M in 1980.

Diel oviposition activity in Cx. restuans was monitored at the GRS on 29-30 June 1981, 7-8 July 1982 and 20-21 July 1982. Four pools were set up in a square grid pattern with the pools approximately 50 cm apart. In 1981 each pool was covered with a screen and then a randomly chosen pool was uncovered for a 2 hr period. It was then covered again and another pool was uncovered. Egg rafts were collected 0 to 4 hr after the test.

In 1982, 2 sets of 4 pools were used. One set of 4 was placed in a shaded area that had been used in 1981. The other set of 4 was placed at the edge of a field, where the pools were exposed to the sun and wind. All of the pools were left exposed to ovipositing females. The pools were inspected for egg rafts every 2 hr.

RESULTS

In 1980, oviposition by Cx. restuans females began in late May at the U of M (Fig. 1) and the GRS (Fig. 2). At the U of M, peaks in oviposition activity occurred during late May, the last half of July and during mid-August (Fig. 1). Peaks in oviposition by Cx. restuans at the GRS occurred in early June and mid-July (Fig. 2).

During 1981, oviposition by Cx. restuans began in late May at both sites (Fig. 3 and 4). Peaks in oviposition activity occurred in early June, early July and late August (Fig. 3 and 4). An additional peak in activity occurred in early August at the GRS (Fig. 4).

In 1982 oviposition by Cx. restuans began in early June, peaked in late June early July and then declined (Fig. 5 and 6).

Very few adult Cx. restuans were collected in the light traps. The greatest numbers were taken at the U of M in 1980 where two peaks in activity occurred, one in July and another in mid-August (Fig. 7).

In 1980 Cx. restuans females produced an average of 198.4±52.3 eggs/raft, n = 196 egg rafts. In 1981, 19 egg rafts averaged 171.3±49.7 eggs/raft.

A comparison of the number of eggs produced by females of 3 different generations at the U of M in 1980, showed that there were significant differences in the number of eggs produced per female (Table 1). The overwintered females laid 178.3 eggs/raft; the spring generation laid 230.6 eggs/raft; the early summer generation laid 209.8 eggs/raft.

Culex tarsalis egg rafts were not numerous; the greatest numbers were collected at the GRS in 1980. At this site oviposition began in late May, with peaks in late June and mid-July (Fig. 8).

At the GRS in 1980, Cx. tarsalis females were abundant in the light trap catches during mid to late July (Fig. 9). During 1981, one peak occurred at the end of June and one at the end of July (Fig. 10).

The average number of eggs produced by Cx. tarsalis females in 1980 was 237.7±74.7 eggs/raft, n = 9.

During 1980, oviposition by Cs. inornata females began in mid-May and peaks in activity occurred in early June at both sites (Fig. 11 and 12). An additional peak in activity occurred in early July at the U of M (Fig. 11).

In 1981 oviposition by Cs. inornata females began in mid-May. Oviposition activity peaked in early June at both sites (Fig. 13 and 14). At the U of M, oviposition activity continued at a relatively steady level until mid-August, when it began to decline (Fig. 13).

In 1982, at the U of M and the GRS, oviposition activity by Cs. inornata began in late May, peaked in late June and then declined to a low level (Fig. 15 and 16).

In 1980 the first Cs. inornata females were collected in mid-May (Fig. 17 and 18). Peaks in the numbers of females collected at the U of M and the GRS occurred during May, June and July (Fig. 17 and 18).

During 1981 fewer females were collected than in 1980. The largest collections were obtained at the GRS where the first female was collected in late May, and a peak in numbers collected occurred in mid-August (Fig. 19).

In 1980, the average number of eggs produced by 132 Cs. inornata females was 216.6±61.3 eggs/raft. In 1981, 31 females laid an average of 224.3±52 eggs/raft.

The monitoring of oviposition activity during a 24 hr period was done on 3 occasions. Culex restuans egg rafts were the only ones laid in large numbers. Oviposition activity of Cx. restuans was greatest just after sunset (Fig. 20, 21, 22 and 23) and at sunrise (Fig. 21, 22 and 23). Very little activity occurred during the daylight hours.

The pool water temperature on 29-30 July, 1981, ranged from 16°to 20°C. On 7-8 July, 1982, pool water temperatures ranged from 14°to 20°C. Pool water temperature on 20-21 July, 1982, ranged from 19°to 26°C for the bush pool and 18°to 32°C for the open pool.

DISCUSSION

From three years of egg raft collections in Manitoba, Cx. restuans probably has 3 generations a year. The overwintered females begin oviposition in late May to early June. These females would have to emerge from their hibernacula at least 1 week prior to oviposition, allowing enough time to find a blood meal and develop eggs. Oviposition by these females followed a similar pattern as those in Ontario. Madder et al. (1980) reported that overwintered females oviposited between mid-May to mid-June. In Manitoba, females of the spring generation laid their eggs between late June to late July, depending on the year. The second generation was the largest one each year. Eggs of the third generation were laid from mid-July to late August. Madder et al. (1980) found that in Ontario, the third generation was smaller than the second. They postulated that this may be due to many of the females from the second generation being in gonotrophic diapause. This may have been the case in the Manitoba population, resulting in a considerable decline in the population.

In 1980, at the U of M site (Fig. 1) and, in 1981, at both sites (Fig. 3 and 4), oviposition activity by Cx. restuans was high in August and is an indication of extensive blood-feeding activity by the females of the second generation. The oviposition activity in August of 1981 may have been significant in the epidemiology of WEE, since 1981 was an epidemic year. Blood-feeding by the second generation females may have aided in the spread of the virus. In 1980, at the GRS and in 1982 at both sites, there was very little oviposition activity in August. Perhaps many of the females in the second generation were in

gonotrophic diapause and presumably would not take blood meals.

The CO_2 baited CDC light trap at the U of M in 1980 collected the most Cx. restuans females (Fig. 7). When the number of egg rafts collected at the same site (Fig. 1) is compared with the number of adult females collected, it is evident that Cx. restuans is not readily attracted to this type of trap.

Oviposition activity by Cx. restuans showed a definite diel periodicity (Fig. 20, 21, 22 and 23). Oviposition activity was greatest during the 2 to 4 hour period following sunset and again at dawn. MacDonald et al. (1981) found a diel periodicity of oviposition in Cx. restuans and Cx. pipiens at Guelph, Ontario. A major peak in activity occurred 1 to 3 hours after dusk and a minor peak occurred at dawn. Similar biphasic cycles have been demonstrated in Cx. pipiens in Japan (Oda 1967) and Cx. quinquefasciatus (Say) from Burma (DeMeillon et al. 1967). The diel oviposition cycle in Cx. restuans is probably cued by a change in light levels as in Cx. quinquefasciatus (DeMeillon et al. (1967) and Cx. tarsalis (Logen and Harwood 1965).

In 1980 at the U of M, 3 generations of Cx. restuans showed significant differences in the number of eggs per egg raft (Table 1). Females laying eggs in the early summer lay significantly more eggs than females in the spring or late summer. Madder (1981) found that summer generations of Cx. restuans in Ontario lay significantly more eggs per egg raft than females of the overwintered generation.

The differences in egg production of females from these 3 generations may be due to a change in blood feeding habits, a change in the efficiency of digestion or changes in the age of the reproductive segments

of the population. It has been demonstrated that in Cx. tarsalis, the source of the blood meal effects the number of eggs produced. Downe and Archer (1975) found that Cx. tarsalis females that had fed on chicken blood produced more eggs than females that had fed on snake blood. They postulated that this may be due to Cx. tarsalis evolving a specialized digestive process enabling them to obtain more nutritive value from bird blood meals, their preferred hosts. The preferred hosts of Cx. restuans are birds (Tempelis 1975). If Cx. restuans has a specialized digestive process suited to the blood of birds, a shift in feeding habits could cause a difference in the numbers of eggs produced. The feeding habits, and digestive enzyme activity in Cx. restuans have not been studied sufficiently to speculate further.

In Manitoba, overwintered females are at least 9 months old. Their age and the effects of overwintering on their physiology may prevent them from producing as many eggs as the younger females of the summer generation. Females laying eggs in the late summer may be older, and in their second or third reproductive cycle. They may not be able to produce the optimal number of eggs for the species.

Culex tarsalis egg rafts were collected in adequate numbers from the GRS 1980 site only (Fig. 8). Overwintered females must have been actively seeking blood meals by mid-May, as the first egg rafts in our collections were taken at the end of May. There would appear to have been 3 generations; one spring and 2 summer generations. The light trap data does not show the May activity peak. The population reached its highest level in July (Fig. 9 and 10). The lack of activity after early August was probably due to many of the females being in gonotrophic diapause and

therefore not seeking blood meals. Mitchell (1981) states that in northern Colorado females begin entering diapause in mid-August. Therefore in Manitoba, early to mid-August would probably be the time when females enter diapause.

During the recent WEE outbreaksin Manitoba, the peak time of WEE activity has been from mid-July to early August. This is the time female Cx. tarsalis are most active (Fig. 8, 9 and 10). This may also be the time when females would be entering their second gonotrophic cycle, enabling them to spread the arbovirus. Nelson and Milby (1982) found that females usually did not feed again until at least the second night after oviposition. However they were unable to determine the length of time of a gonotrophic cycle.

The only pool from which Cx. tarsalis egg rafts were collected in relatively large numbers was from the GRS 1980 site. This site was exposed to the sun and wind. Walters and Smith (1980) found Cx. tarsalis larvae to be most common in eutrophic sunlit pools and least common in shaded pools. The apparent attraction of gravid females to pools exposed to the sun may account for the lack of egg rafts on the shaded pools.

Egg raft collections over the 3 year period show that Cs. inornata has 2 generations/year in Manitoba; a spring and a summer generation. This agrees with Hanec and Brust (1967), who examined light trap data from the U of M and concluded that this species has 2 generations/year. Overwintered females appeared in light trap catches in early to mid-May (Figures 17, 18 and 19), and egg rafts were collected in mid-May. McLintock and Iversen (1975) proposed that this species may be an early season vector of WEE, spreading the virus throughout the mammal and

bird populations before the other vectors are active. This may be possible, as overwintered females are on the wing by early May. However the vector potential of this species has never been proven in Canada.

Oviposition activity by the first generation was usually greatest in June to mid-July (Fig. 11, 12, 14, 15 and 16). The optimal temperature for larval growth in this species is 21°C, and higher temperatures are detrimental (Brust 1967). This may limit this mosquito species to the spring and early summer, when temperatures are low. The lack of oviposition activity in late-July and August is also undoubtedly due to a large proportion of the population entering gonotrophic diapause. The lack of oviposition activity in mid-summer indicates a lack of blood feeding in the population, and therefore this species is probably not an important vector of WEE.

Duck week (Lemna minor L.) growth was excessive and hard to control in the GRS pool in 1980. It is known that Cs. inornata and Cx. pipiens lay fewer egg rafts on ponds with duck weed than in plantless ponds (Angerilli and Beirne 1980). The duck weed growth on the GRS pool in 1980 probably affected the number of egg rafts obtained.

In conclusion, the use of pools to monitor oviposition activity is a suitable technique to monitor populations of Cx. restuans and Cs. inornata, but not Cx. tarsalis. Oviposition activity data and CO_2 baited CDC light trap catches showed that in Manitoba Cx. restuans and Cx. tarsalis have 3 generations a year and Cs. inornata has 2 generations a year. Overwintered females of all these species are rarely taken in light traps. The first indication of the size of the overwintered population is the number of egg rafts laid during mid to late May.

A decrease in oviposition activity during August is probably due to many of the females being in a state of gonotrophic diapause. Oviposition data suggests that Cx. restuans is more common in the Winnipeg area than light trap data indicates. This species may therefore be an important epizootic vector of WEE. Oviposition activity and light trap collection peaks of Cx. tarsalis occur at the same time as peaks in WEE activity. Culex tarsalis is probably the main epizootic and epidemic vector of WEE in Manitoba. Culiseta inornata is probably not important in the transmission of WEE.

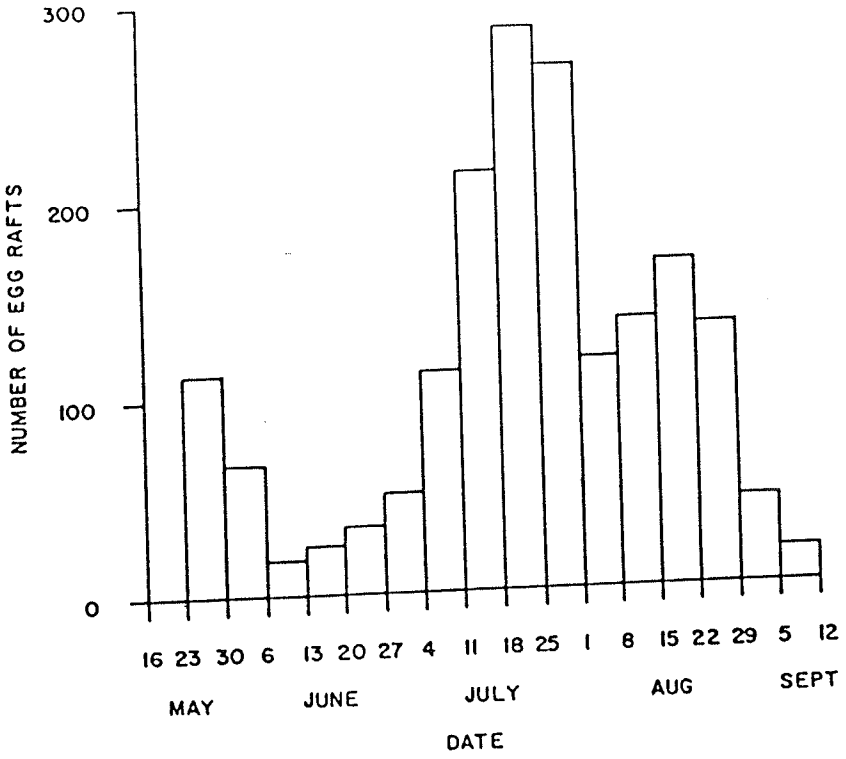
Table 1. Number of eggs per egg raft for 3 generations of Cx. restuans collected from the U of M pool, 1980.

Date of collection	Number of eggs/ egg raft ($\bar{x}$±S.D.)	Range	n
26 May - 4 July	178.3±42.5a[1]	92-253	30
16 July - 1 Aug.	230.6±51.8b	112-332	39
15 Aug. - 29 Aug.	209.8±45.6c	91-294	42

[1]From paired comparison test different letters indicate a difference at $P<0.05$.

Fig. 1. Seasonal distribution of the number of egg rafts laid by Culex restuans in the pool at the U of M in 1980.

Fig. 2. Seasonal distribution of the number of egg rafts laid by Culex restuans in the pool at the GRS in 1980.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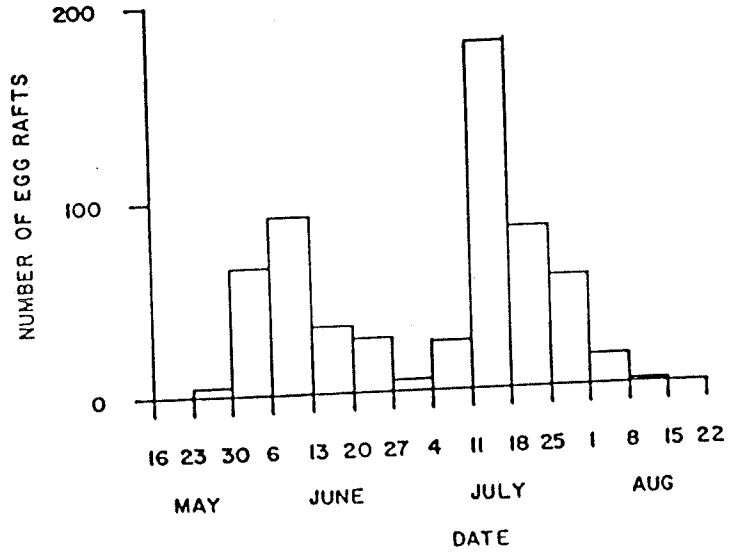
MAY
JUNE
JULY
AUG
SEPT
DATE

Fig. 1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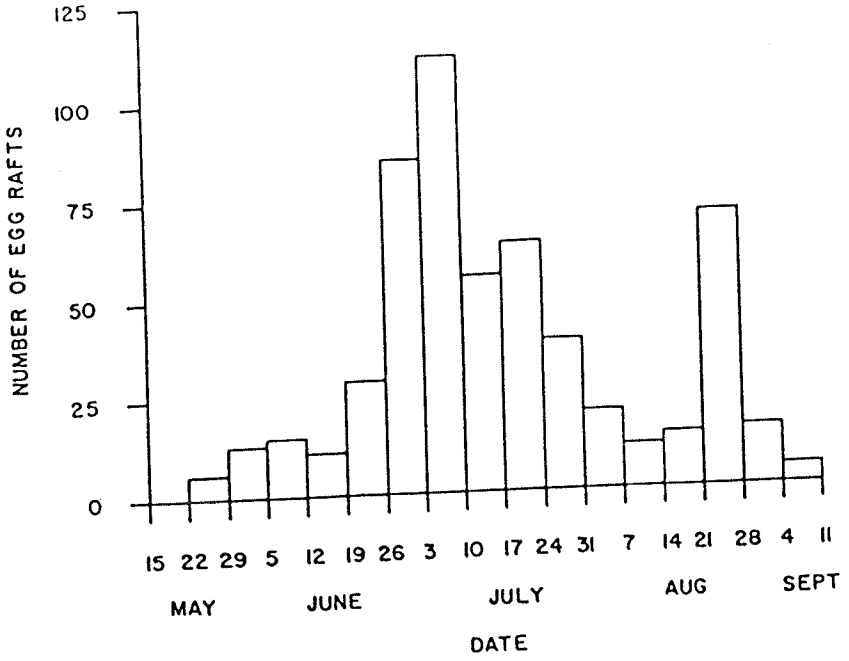
MAY
JUNE
JULY
AUG
DATE

Fig. 2

Fig. 3. Seasonal distribution of the number of egg rafts laid by Culex restuans in the pool at the U of M in 1981.

Fig. 4. Seasonal distribution of the number of egg rafts laid by Culex restuans in the pool at the GRS in 1981.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75
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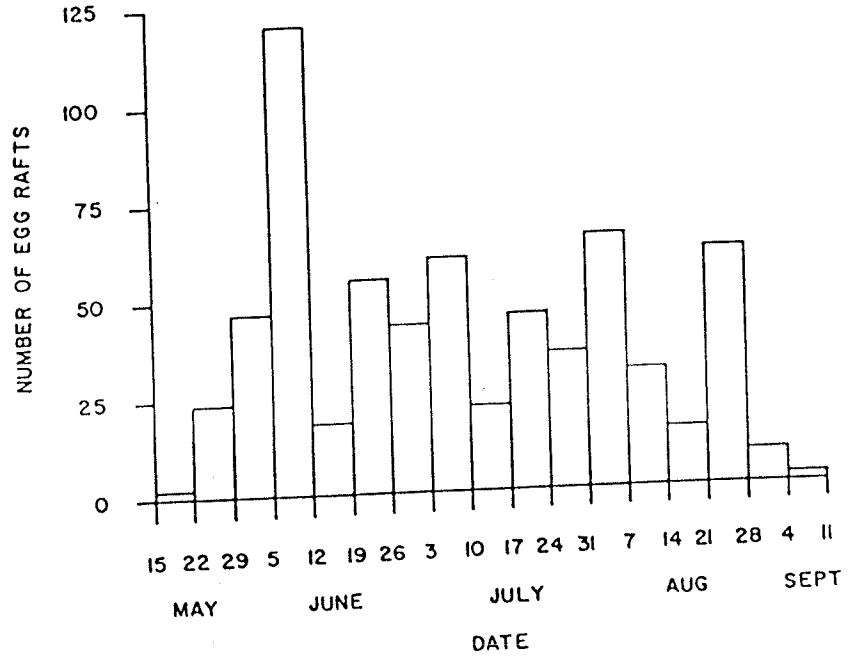
MAY
JUNE
JULY
AUG
SEPT
DATE

Fig. 3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MAY
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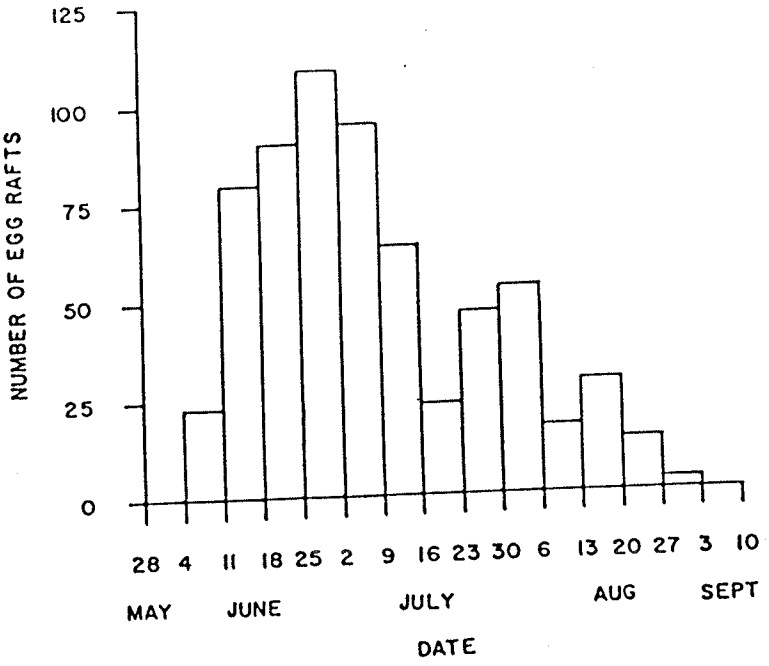
JULY
AUG
SEPT
DATE

Fig. 4

Fig. 5. Seasonal distribution of the number of egg rafts laid by Culex restuans in the pool at the U of M in 1982.

Fig. 6. Seasonal distribution of the number of egg rafts laid by Culex restuans in the pool at the GRS in 1982.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MAY
JUNE
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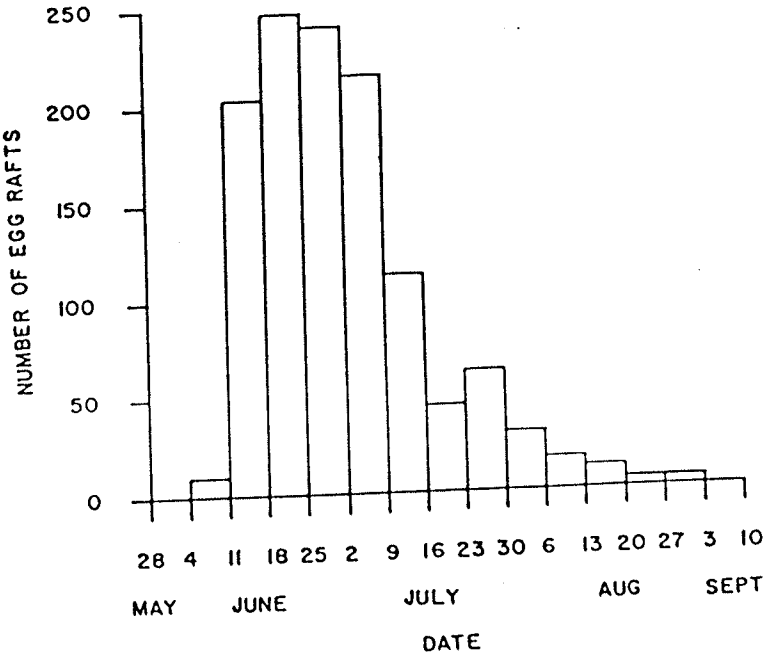
SEPT
DATE

Fig. 5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MAY
JUNE
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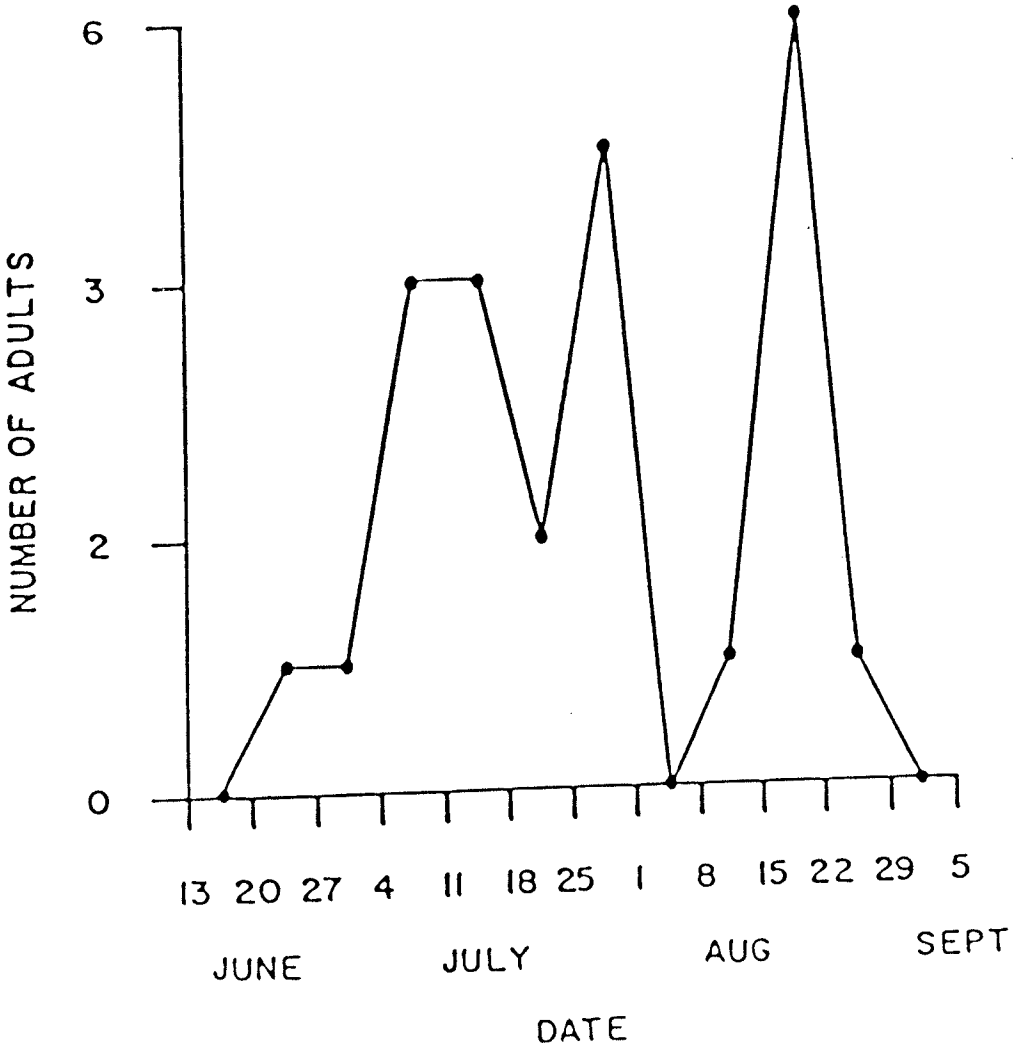
SEPT
DATE

Fig. 6

Fig. 7. Number of adult female Culex restuans captured one night per week in a CO_2 baited CDC light trap at the U of M in 1980.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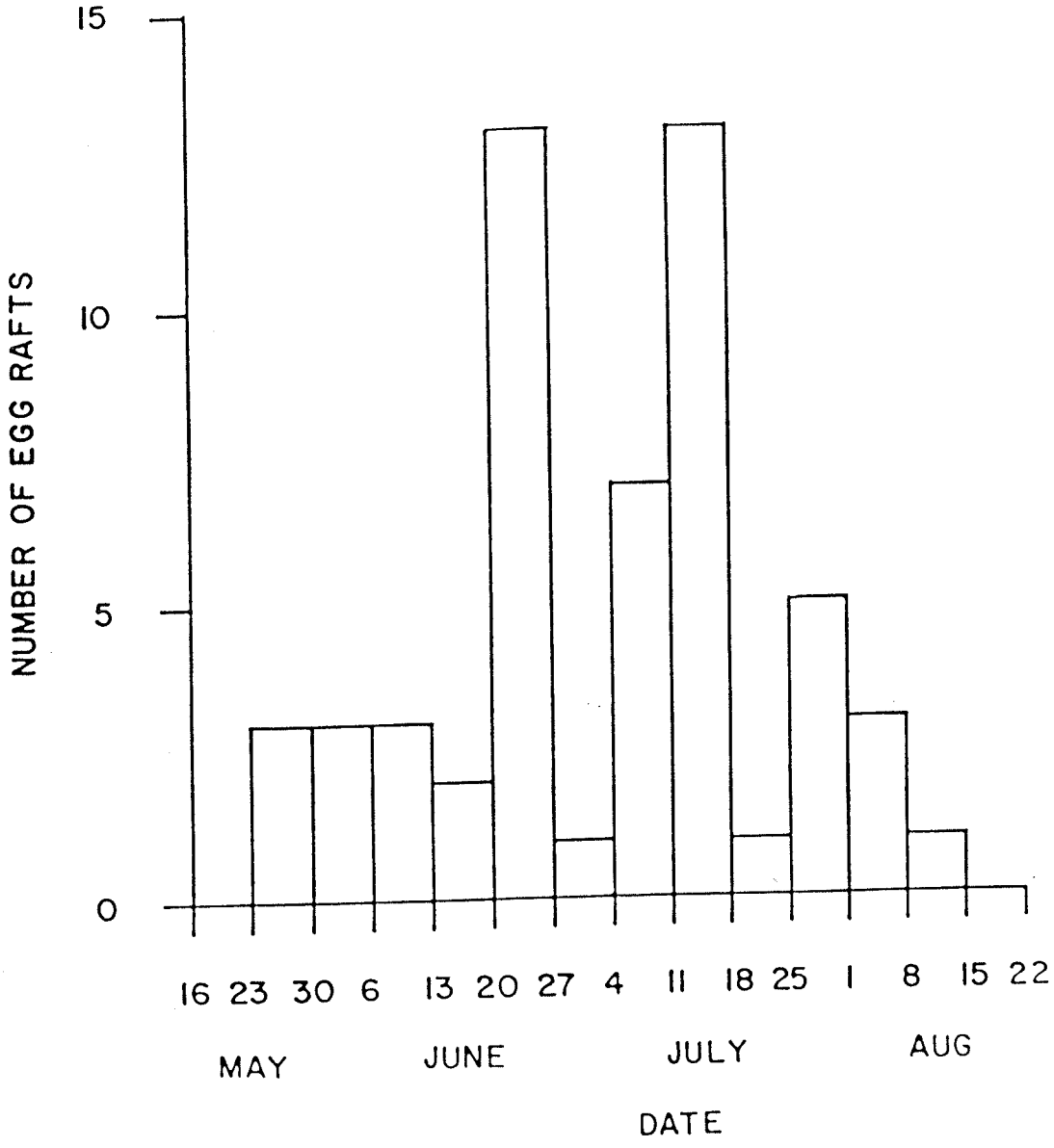
JUNE
JULY
AUG
SEPT
DATE

Fig. 7

Fig. 8. Seasonal distribution of the number of egg rafts laid by Culex tarsalis in the pool at the GRS in 1980.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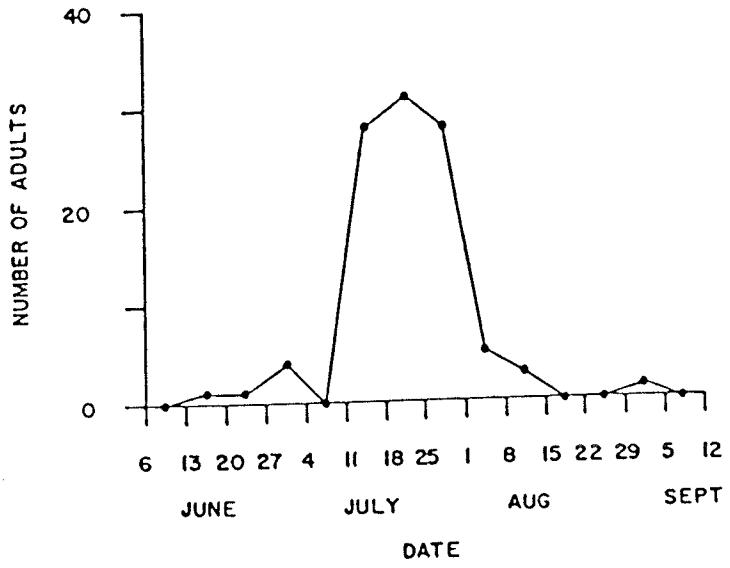
JUNE
JULY
AUG
DATE

Fig. 8

Fig. 9. Number of adult female Culex tarsalis captured one night per week in a CO_2 baited CDC light trap at the U of M in 1980.

Fig. 10. Number of adult female Culex tarsalis captured one night per week in a CO_2 baited CDC light trap at the GRS in 1981.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JUNE
JULY
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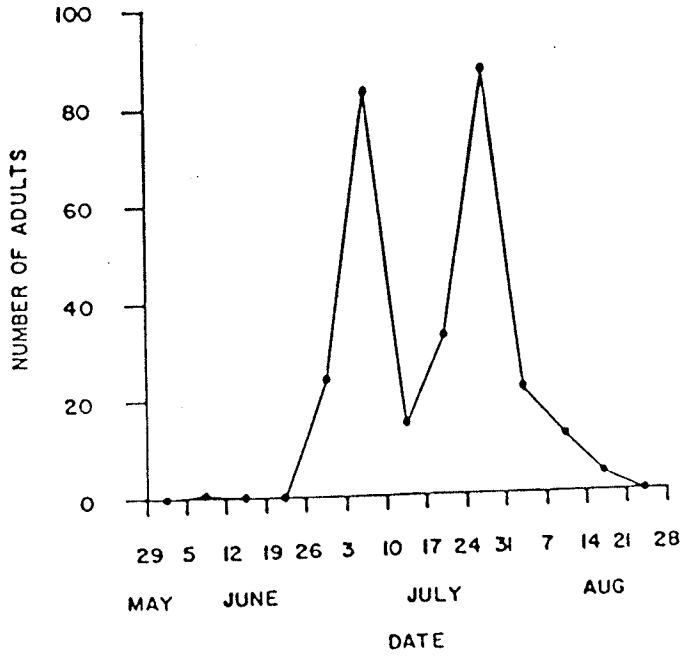
DATE

Fig. 9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MAY
JUNE
JULY
AUG
DATE

Fig. 10

Fig. 11. Seasonal distribution of the number of egg rafts laid by Culiseta inornata in the pool at the U of M in 1980.

Fig. 12. Seasonal distribution of the number of egg rafts laid by Culiseta inornata in the pool at the GRS in 1980.

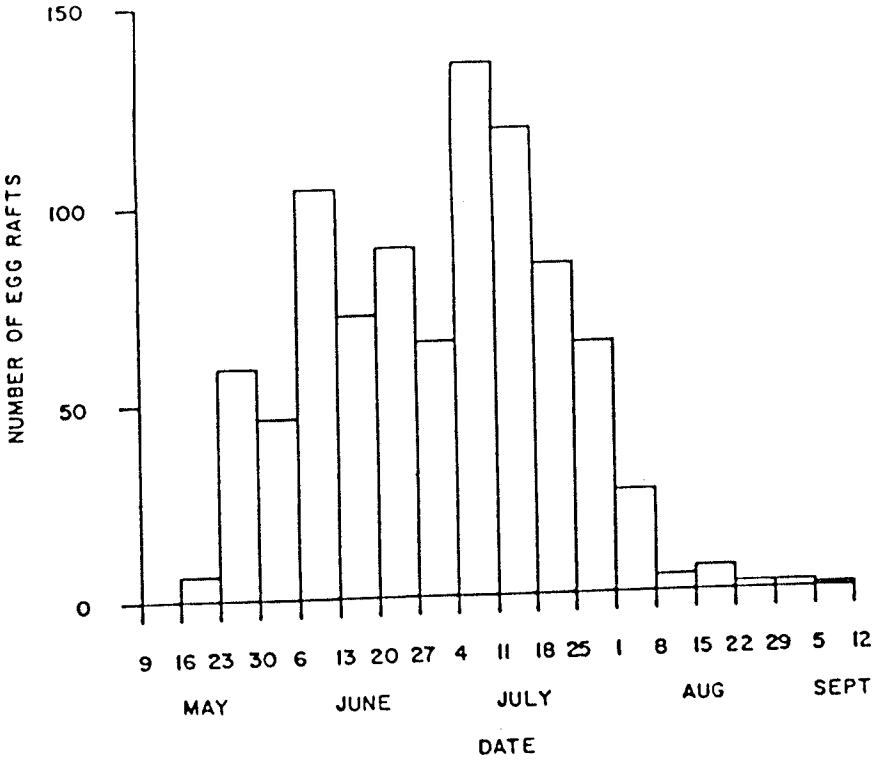
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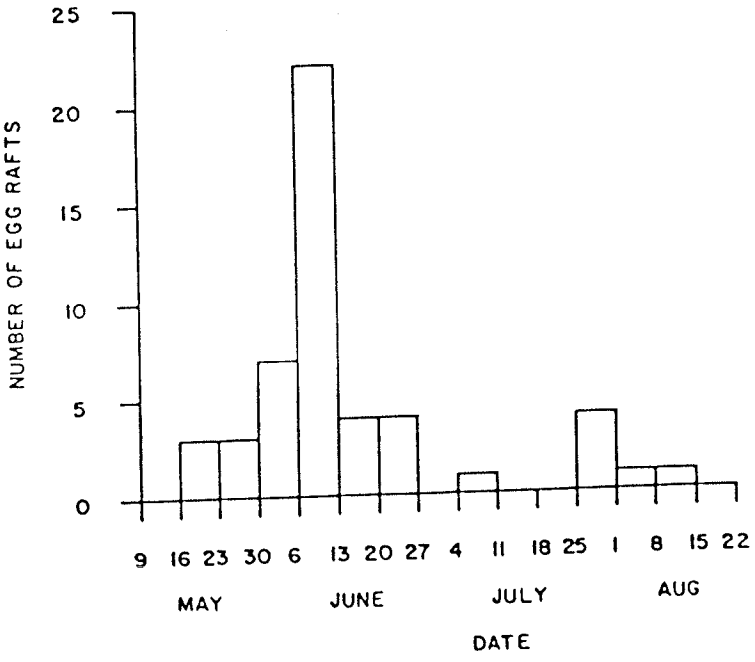
Fig. 11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JUNE
JULY
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Fig. 12

Fig. 13. Seasonal distribution of the number of egg rafts laid by Culiseta inornata in the pool at the U of M in 1981.

Fig. 14. Seasonal distribution of the number of egg rafts laid by Culiseta inornata in the pool at the GRS in 1981.

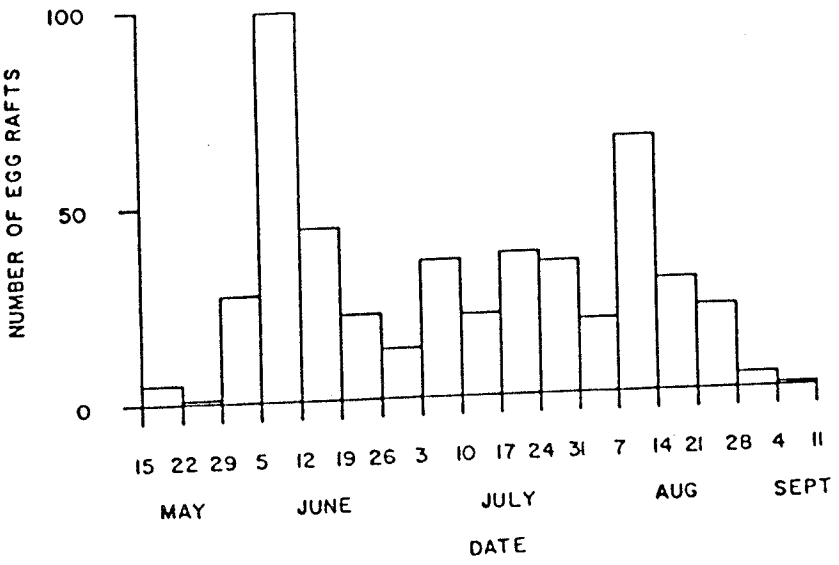


Fig. 13

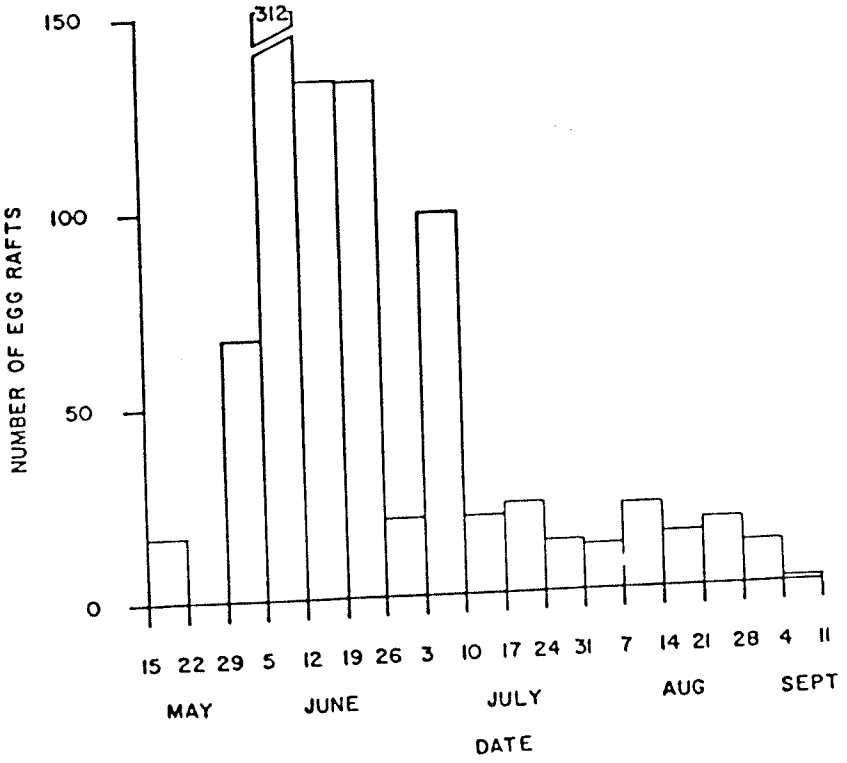
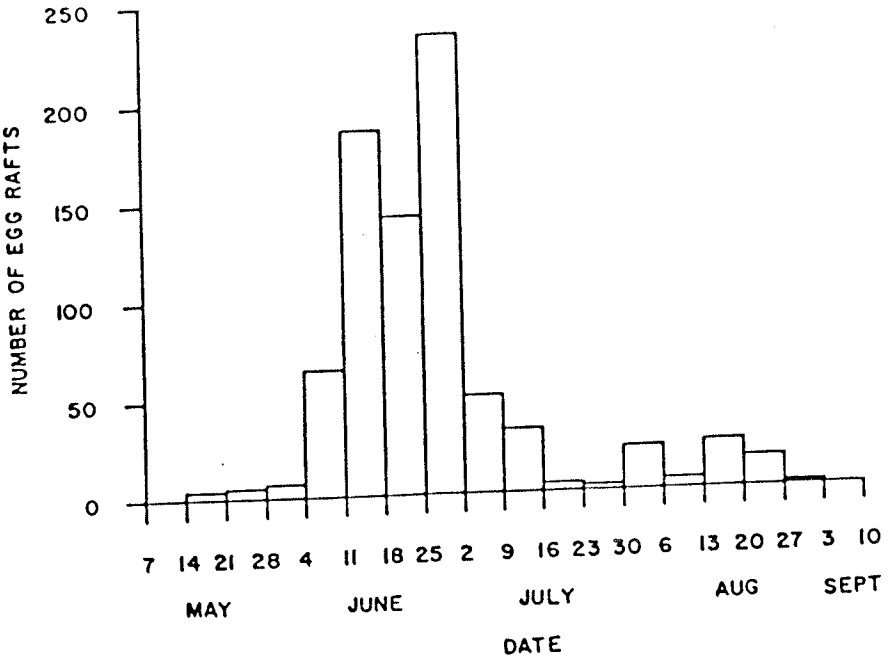


Fig. 14

Fig. 15 Seasonal distribution of the number of egg rafts laid by Culiseta inornata in the pool at the U of M in 1982

Fig. 16. Seasonal distribution of the number of egg rafts laid by Culiseta inornata in the pool at the GRS in 1982.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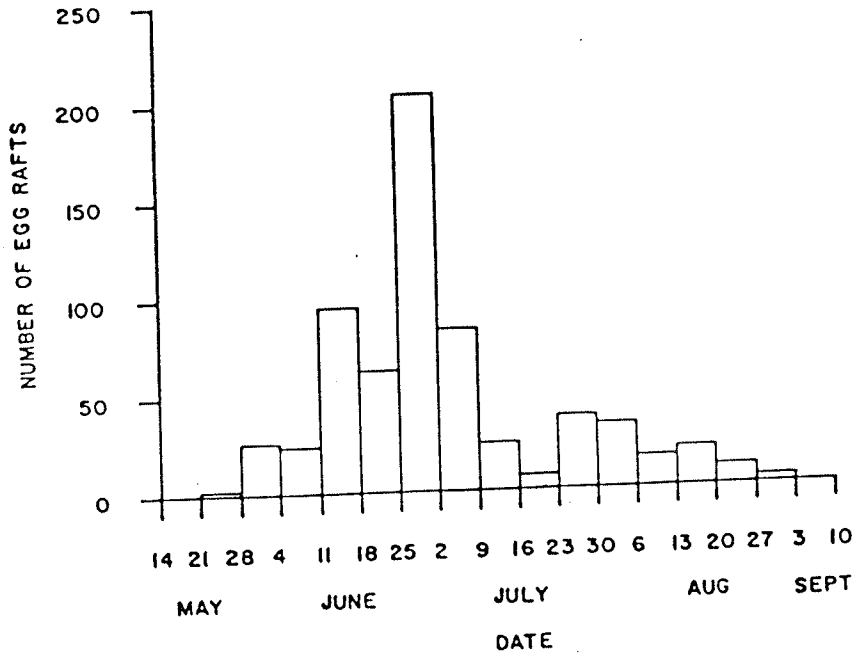
SEPT
DATE

Fig. 15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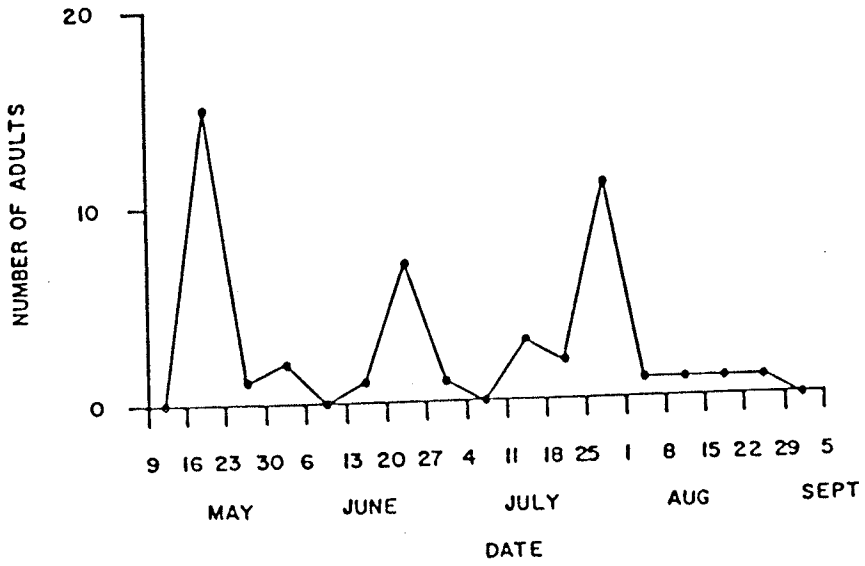
MAY
JUNE
JULY
AUG
SEPT
DATE

Fig. 16

Fig. 17. Number of adult female Culiseta inornata captured one night per week in a CO_2 baited CDC light trap at the U of M in 1980.

Fig. 18. Number of adult female Culiseta inornata captuted one night per week in a CO_2 baited CDC light trap at the GRS in 1980.


20
10
0
NUMBER OF ADULTS
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MAY
JUNE
JULY
AUG
SEPT
DATE

Fig. 17

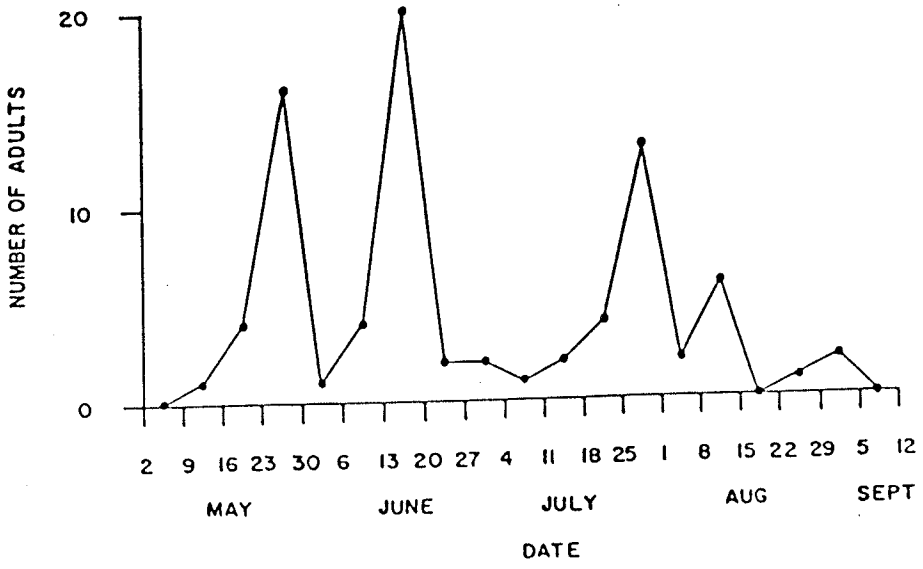
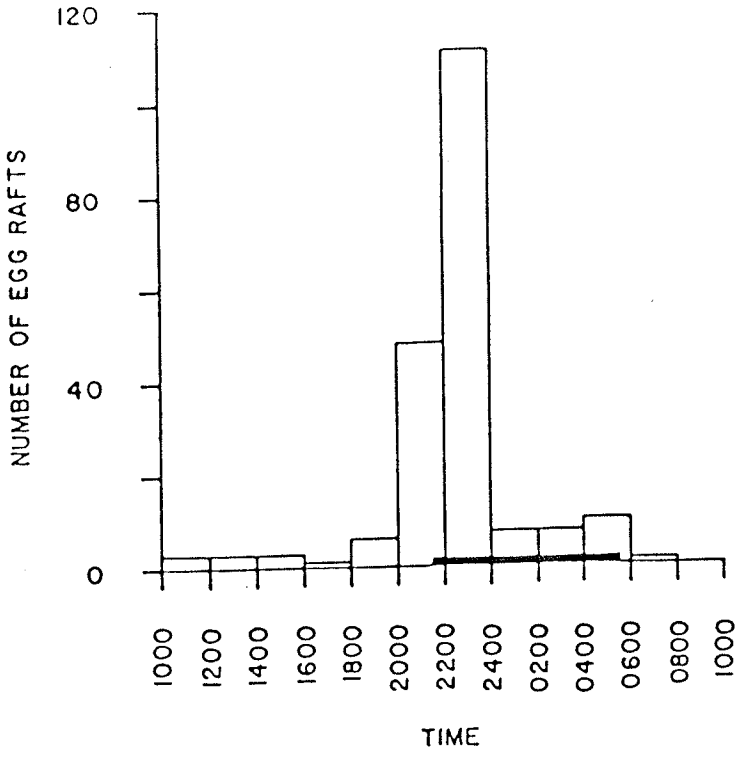

20
10
0
NUMBER OF ADULTS
2 9 16 23 30 6 13 20 27 4 11 18 25 1 8 15 22 29 5 12
MAY
JUNE
JULY
AUG
SEPT
DATE

Fig. 18

Fig. 19. Number of adult female Culiseta inornata captured one night per week in a CO_2 baited CDC light trap at the GRS in 1981.

Fig. 20. Diel periodicity of oviposition by Culex restuans at the GRS on the 29-30 June, 1981.

Fig. 21. Diel periodicity of oviposition by Culex restuans at the GRS on the 7-8 July, 1982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40
0
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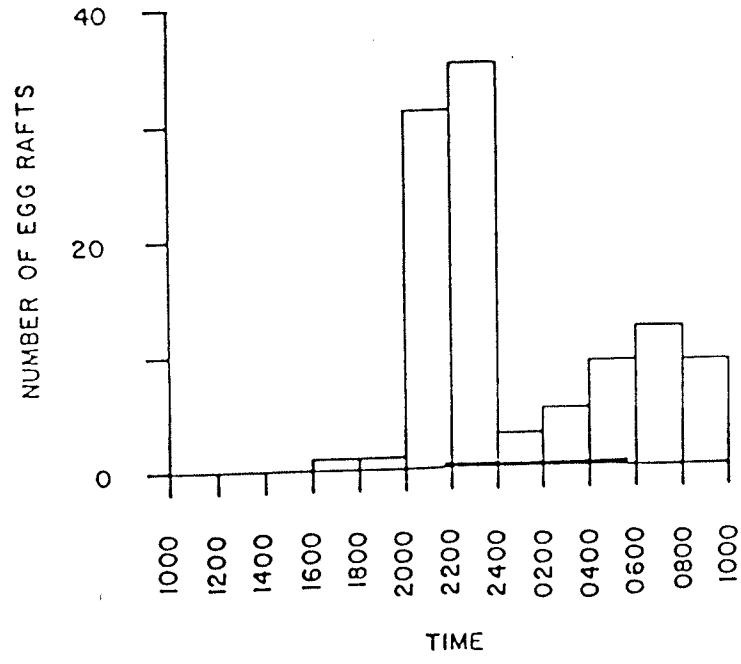
1200
1400
1600
1800
2000
2200
2400
0200
0400
0600
0800
1000
TIME

Fig. 20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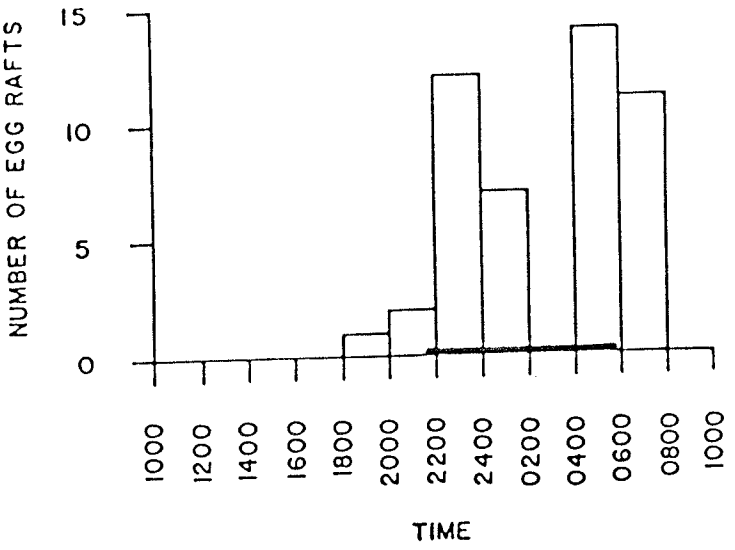
0600
0800
1000
TIME

Fig. 21

Fig. 22. Diel periodicity of oviposition by Culex restuans at the GRS in a treed area on the 20-21 July, 1982.

Fig. 23. Diel periodicity of oviposition by Culex restuans at the GRS in an open area the 20-21 July, 1982.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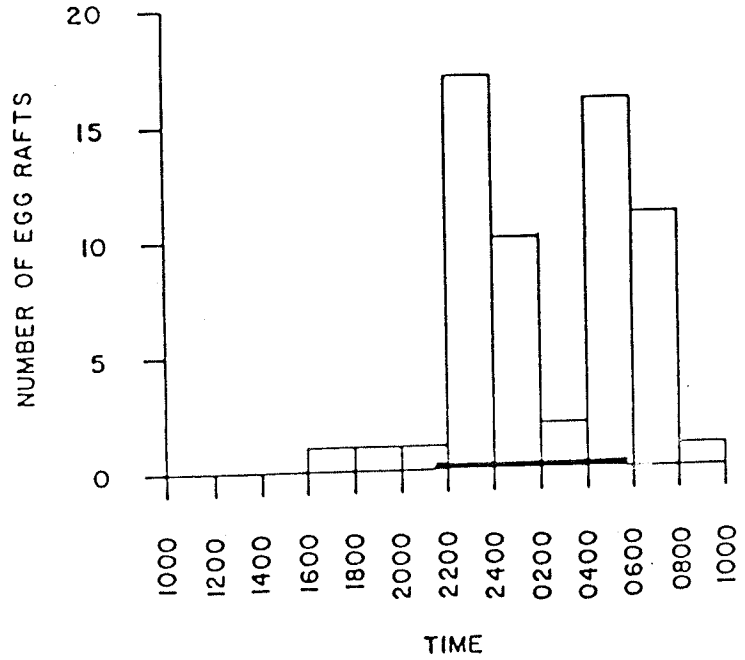
1600
1800
2000
2200
2400
0200
0400
0600
0800
1000
TIME

Fig. 22


NUMBER OF EGG RAFTS
20
15
10
5
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1000
1200
1400
1600
1800
2000
2200
2400
0200
0400
0600
0800
1000
TIME

Fig. 23

Blood-feeding and Gonotrophic Dissociation in Overwintering *Anopheles earlei* Vargas (Diptera:Culicidae) from Southern Manitoba

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ABSTRACT

A total of 72 overwintering Anopheles earlei Vargas was collected from southern Manitoba during December, in 1980 and 1981. These mosquitoes were maintained at 15° or 20°C, and a photoperiod of 8L:16D. Forty-two females were offered a blood meal and 25 of these fed. Extensive fat body development was seen in the blood-fed females collected in 1980; this was not seen in collections from 1981. Gonotrophic dissociation was observed in 24 of the blood-fed females.

INTRODUCTION

As far as is known, the mosquito Anopheles earlei Vargas overwinters as a non-blood-fed nulliparous female (Hudson 1978; Wood et al. 1979). It overwinters in buildings and other shelters (McLeod and McLintock 1947); in mammal burrows (Shemanchuk 1965; Hudson 1978; Hayles et al. 1979); in crevices and caverns (Hayles et al. 1979) and rock piles (Hudson 1978).

Sekla et al. (1980) reported that An. earlei may be an important vector of western equine encephalitis (WEE) virus, as it had the highest minimum infection ratio of any infected mosquito species in Manitoba. Hayles et al. (1979) considered An. earlei a possible overwintering reservoir for WEE in western Canada. Hudson (1978) thought that An. earlei was an unlikely overwintering reservoir of the virus, as there was no evidence that diapausing females would take a blood meal and exhibit gonotrophic dissociation.

In this paper we report blood feeding and gonotrophic dissociation in wild overwintering female An. earlei from southern Manitoba.

MATERIALS AND METHODS

Overwintering female An. earlei were collected during the month of December, in 1980 and 1981. These mosquitoes were used in blood feeding experiments to see if gonotrophic dissociation occurs. Blood-fed females were kept at 15° or 20°C and 8L:16D for 7 days.

On 22 December 1980, 16 An. earlei females were collected from a subterranean concrete tunnel located on the University of Manitoba Campus (Arntfield et al. 1982). These mosquitoes were maintained at 15°C, 8L:16D from 23 to 30 December 1980. During December 1981, An. earlei females were collected from highway culverts in southern Manitoba. On 10 December, 6 females were collected and maintained at 10°C, 8L:16D for 4 days. These were combined with 36 females collected on 14 December. The 42 females were placed at 15°C, 8L:16D from 15 to 22 December. Fourteen females collected on 17 December were maintained at 20°C, 8L:16D from 18 to 25 December. At the time of collection the temperature in the subterranean tunnel was 4°C (Arntfield et al. 1982); within the culverts it ranged from -7° to 0°C.

The females were separated into 4 groups; pre-treatment, blood-fed, sugar-fed, and non-fed. The pre-treatment group was dissected immediately (day 0). The blood-fed group was offered human blood (at ambient laboratory temperatures); the sugar-fed group was offered a 10% sucrose solution; the non-fed group consisted of those that were offered blood but did not feed. Some of the mosquitoes used in the pre-treatment and sugar-fed group were females that had been offered blood but did not feed. All groups were maintained on water. The treatment groups were dissected after 7 days.

Fat body and ovarian development were evaluated in all females. The quantity of fat body in the abdomen was rated on a scale of 0 to 3; 0 being no fat and 3 being fully distended with fat (Burdick and Kardos 1963). Ovarian follicular development was assessed using the classification of Christophers (1911).

RESULTS AND DISCUSSION

All of the mosquitoes in the pre-treatment groups had ovarian follicles at stage I (Table 1); this would indicate that they were in gonotrophic diapause (Hudson 1978). Out of 42 females offered blood, 25 (60%) took a full meal (Table 1). None of the blood-fed females kept at 15°C, 8L:16D for 7 days developed their ovarian follicles past stage II and 84% of these females retained their ovarian follicles at stage I (Table 1). One of the blood-fed females kept at 20°C, 8L:16D for 7 days developed 99 eggs (Table 1). Four of the other females appeared to have broken diapause with their ovarian follicles developing to stage II, and 1 female remained in diapause. Only 1 out of 25 blood-fed females developed eggs.

Washino (1977) has reviewed the physiological ecology of gonotrophic dissociation. He reported that the term was originally used to indicate a cessation in egg production despite the continued taking of blood meals. In my experiments, 19 (100%) blood-fed females kept at 15°C, 8L:16D and 5 (83%) blood-fed females kept at 20°C, 8L:16D underwent gonotrophic dissociation (Table 1). McLeod and McLintock (1947) found that 3 females of An. earlei collected near Portage la Prairie, Manitoba, on 21 January 1946 took repeated blood meals after 1 February 1946. This resulted in 2 eggs on 6 March 1946. Unfortunately they did not give enough information to indicate if gonotrophic dissociation occurred in these females.

Eleven out of 72 (15%) of the females terminated diapause, developing their ovarian follicles past stage I (Table 1). It is likely that the response was due to increased temperatures experienced in the

laboratory compared with field conditions.

The blood-fed females kept at 15°C, 8L:16D in 1980 used their blood meals to increase their fat bodies. Seven of the 9 blood-fed females developed a fat body rated at 3, compared to the 5 pre-treatment females that had a fat body rated at 1 (Table 1). This extensive fat body development was not seen in the blood-fed females kept at 15°C, 8L:16D in 1981 although their fat bodies were larger than in the water-fed females (Table 1). The difference in fat body development between the females collected in 1980 and in 1981, may have been due to differences in the temperatures the mosquitoes were subjected to prior to collection. The monthly averages of mean daily temperature for August, September, October, and November 1980 were 16.9°C, 11.2°C, 4.1°C and -2°C respectively, and for 1981 were 20.1°C, 12.4°C, 5.3°C and 1°C. The difference between the 2 years, was that during August to November, the monthly averages were 1.2-3.2°C lower in 1980 than in 1981.

The conditions under which these experiments were conducted do not parallel those in nature, however the results reveal some interesting characteristics concerning the overwintering biology of An. earlei. Blood-feeding by diapausing females may aid in survival by providing the mosquito with an energy source in the event sugars from flowering plants are not available in the fall (Washino 1977). As in Culex tarsalis Coquillett (Arntfield et al. 1982) blood-feeding and subsequent gonotrophic dissociation by diapausing adult female mosquitoes may provide a possible mechanism for the overwintering of WEE.

ACKNOWLEDGEMENTS

We are grateful to P.W. Arntfield for his help and suggestions in the preparation of this paper. Financial support provided by N.S.E.R.C. grant No. A2545.

Table 1. Number of overwintering females of An. earlei in each category, size of fat body and developmental stage of ovarian follicles.

Treatment & maintenance temperature (8L:16D)	Day	Fat body				Follicle stage		
		0	1	2	3	I	II	V
Pre-treatment								
23 Dec., 1980*	0		5			5		
15 Dec., 1981	0	4	7	1		12		
18 Dec., 1981	0	1	5	2		8		
Fed sucrose (10%)								
30 Dec., 1980, 15°C*	7			1	1	2		
22 Dec., 1981, 15°C	7		4	4	2	8	2	
Fed water								
22 Dec., 1981, 15°C*	7	7	3			9	1	
Fed blood								
30 Dec., 1980, 15°C	7			2	7	9		
22 Dec., 1981, 15°C	7	2	8			7	3	
25 Dec., 1981, 20°C	7	?	3			1	4	1

* Offered a blood meal but did not feed.

Blood-feeding in Overwintering *Culex tarsalis* (Diptera:Culicidae) from Manitoba

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Diapause development in Culex tarsalis Coquillett has been characterized by reduced blood-feeding to complete termination of ovariole development at or before stage IIa (Bennington et al. 1958, Schaefer and Washino 1970, Schaefer et al. 1971, Bellamy and Corbet 1973, Mitchell 1979). The role that diapausing Culex species have in overwintering arboviruses has been examined (Eldridge 1966, 1968, Eldridge et al. 1972, Reeves 1974, Eldridge and Bailey 1979, Mitchell 1979). Gonotrophic dissociation and its implications for survival of the vector and virus has been reviewed by Washino (1977). Eldridge (1966) indicated gonotrophic dissociation was possible in Cx. pipiens L. incubated at low temperature and short daylength and later reported failure of ovarioles of prehibernating Cx. pipiens to mature following a blood meal (Eldridge and Bailey 1979). Eldridge et al. (1972) reported that Cx. restuans Theobald exhibited gonotrophic dissociation in response to conditioning by short daylength and low temperature. Mitchell (1981) has reported blood-feeding and gonotrophic dissociation in a significant portion of diapausing Cx. tarsalis from Colorado, when females were kept at 15°C and at short daylength conditions.

At the University of Manitoba, three Cx. tarsalis were taken in a subterranean, concrete tunnel, 2 x 1.7 x 45m (Fig. 1). Ambient temperature in the tunnel was 3°C to 5°C. The females were half way along the tunnel and 1m above its floor.

A female collected 22 December 1980 was kept overnight in darkness at 5°C. The following day it was dissected and found to be nulliparous, mated, with ovarioles at Watts and Smith (1978) stage Ia (Fig. 2), with

a mean follicle-germarium ratio of 0.97:1.00±0.10 (n = 8) and with a fat body rated at 2 on a scale of 0 to 3 (Hudson 1978).

Two females collected on 6 January 1981 were kept for two days at 15°C and in a light:dark regime of 8:16. They were then offered human blood by placing a screened cage (2.5 x 2.5 x 15 cm) on the arm. Both females took full meals. After feeding, they were kept for seven days at 15°C and 8L:16D and then dissected. One female had retained the blood for only a few days. Ovarian follicle development of this female was at stage Ib and fat body was rated at 2. The other female had blood in its gut (Fig. 3) and ovaries with follicles of some proximal ovarioles developed to stage IIa (Fig. 4) and a fat body rated at 2.

It is of interest that diapausing Cx. tarsalis will take blood. The evidence for gonotrophic dissociation is that they did not develop eggs during the seven day period after taking blood. Non-diapausing Cx. tarsalis will mature eggs when kept at 15°C for seven days following a blood meal (Fig. 5).

Research was supported by the National Science and Engineering Research Council of Canada Grant A2545 to R.A. Brust.

FIGURE CAPTIONS

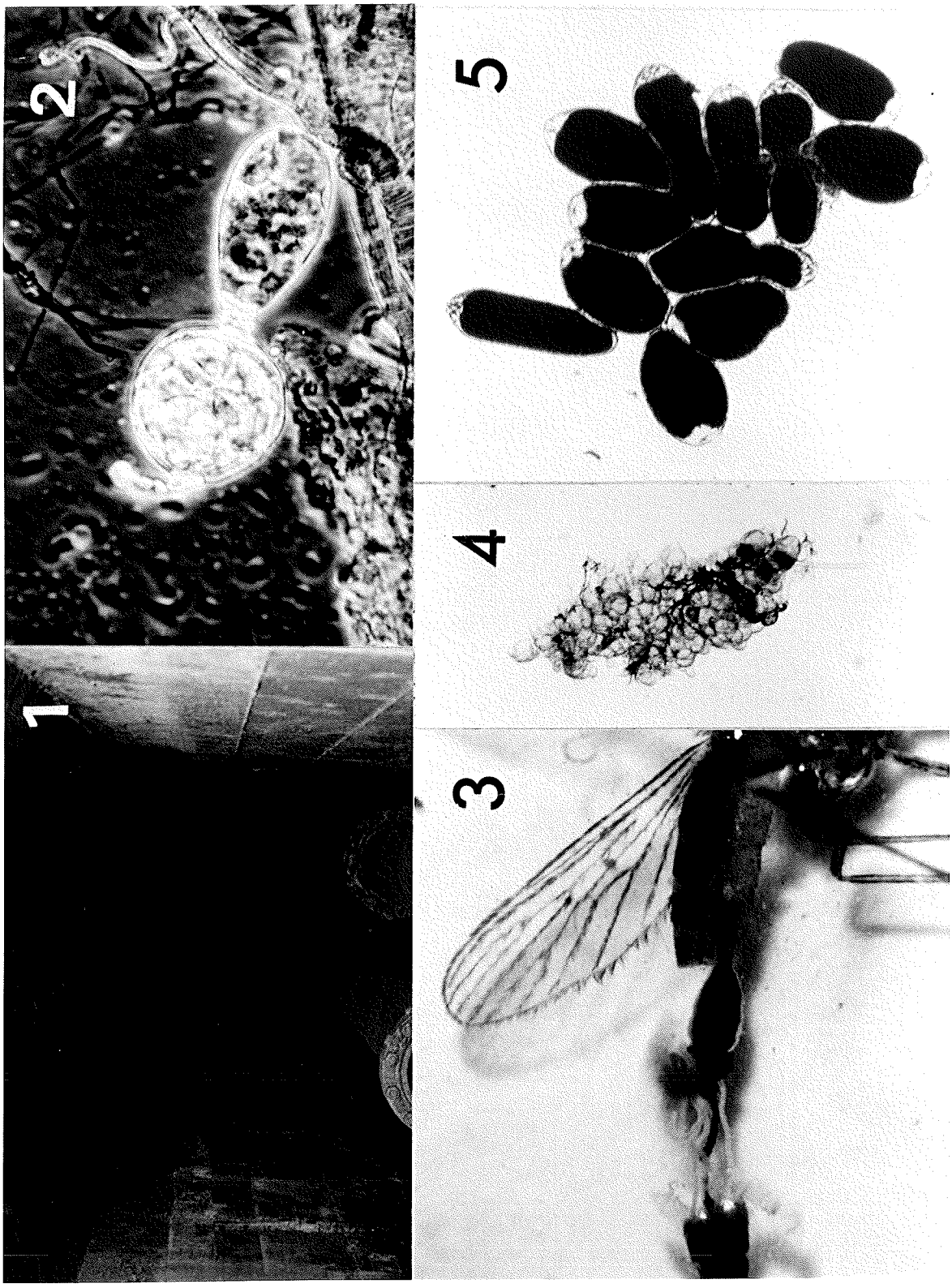
Fig. 1. Subterranean tunnel from which 3 overwintering Cx. tarsalis were removed in 1980-81.

Fig. 2. Ovarian follicle at stage Ia from an overwintering female Cx. tarsalis removed from the tunnel on 22 December 1980.

Fig. 3. Blood in the gut of an overwintering female Cx. tarsalis removed from the tunnel on 6 January 1981, blood-fed 2 days later and dissected 7 days after feeding.

Fig. 4. Ovary with proximal ovarioles developed to stage IIa of an overwintering Cx. tarsalis removed from the tunnel on 6 January 1981, blood-fed 2 days later and dissected 7 days after feeding.

Fig. 5. Stage IVa ovarioles from a blood-fed non-diapausing female Cx. tarsalis kept at 15°C and dissected 7 days after blood-feeding.


1
2
3
4
5

Blood-feeding in Diapausing *Culiseta inornata* (Diptera:Culicidae) from Southern Manitoba

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ABSTRACT

Diapausing adult female Culiseta inornata were collected in late August from an emergence trap set over an artificial pool. Twenty females were offered a blood meal and 19 of them fed. Some females expelled their first blood meal, but fed again the next day. Eighteen of 19 known blood-fed females developed eggs.

INTRODUCTION

Arboviruses may overwinter in diapausing adult female mosquitoes (Reeves 1974). The mosquito Culiseta inornata (Williston) probably overwinters as nulliparous non-blood-fed females (Hudson 1979), but there is the possibility that some parous blood-fed females may overwinter (Dow et al. 1976). Hudson (1979) observed that a small proportion of diapausing Cs. inornata females could be induced to take a blood meal, and which they often expelled before it was digested. The possibility exists that diapausing Cs. inornata may take an infected blood meal and acquire western equine encephalitis (WEE) virus. They may then expel the blood, overwinter successfully and transmit the virus to a new host in the spring.

MATERIALS AND METHODS

Culiseta inornata used to investigate the response of diapausing females to a blood meal were collected on 20 August 1981, from an emergence cage placed over an artificial pool. Females were kept at 17°C, 12L:12D and offered a 10% sucrose solution. On 27 August (day 0) 10 females were dissected. Twenty females were offered human blood by placing a cage (2.5 x 2.5 x 15 cm) on the arm. This was done twice, once on 27 August and again on 28 August. Ten blood-fed females were dissected at 7 days post-blood meal, and 10 at 20 days post-blood meal. Sucrose-fed females were dissected on the same dates.

The ovarian follicle stage was recorded (Mer 1936) and the fat body rated on a scale of 0 to 3, 0 being no fat in the abdomen and 3 being fully distended with fat (Burdick and Kardos 1963). Follicle:germarium (F:G) ratios (Hudson 1979) were computed for the females dissected on day 0.

RESULTS

Nine of 10 Cs. inornata dissected on day 0 had ovarian follicles at stage Ib (Table 1) and F:G ratios of $\leq$1.5; one female had ovarian follicles at stage IIa (Table 1) and a F:G ratio of 2.63.

Nineteen of 20 females took a blood meal (Table 1). Some of the blood fed females expelled the blood within 24 hours, but second blood meals were readily taken the next day. Two of the females in the blood-fed group, dissected on day 7, did not develop eggs (Table 1); one of these was probably the one female out of 20 that did not take blood. All of the blood-fed females dissected at 20 days developed eggs (Table 1). Two of the sucrose-fed females developed eggs (Table 1).

DISCUSSION

The majority (9/10) of the Cs. inornata females dissected on day 0 had follicles at the pre-resting stage Ib (Table 1) and a F:G ratio of $\leq$1.5. These are the conditions Hudson (1979) used to define gonotrophic diapause in this species. The females that were offered a blood meal fed readily. This is contrary to the restrained response observed by Hudson (1979). Almost all of the blood-fed females developed eggs (Table 1), so gonotrophic dissociation may not occur in this species.

An undetermined number of females expelled their first blood meal within 24 hours. This type of behavior was also reported by Hudson (1979). This means that if diapausing Cs. inornata females feed readily, but then expel the blood meal, they may be a possible overwintering reservoir for WEE virus. As exciting as this possibility may be, one must remember that Cs. inornata prefers to feed on large mammals (Tempelis 1975). The chances of females feeding on a virulent host in late summer are therefore quite low.

Table 1. Fat body size and stage of ovarian follicles of Cs. inornata females collected on 20 August 1981, and kept at 17°C, 12L:12D, and offered a blood meal on 27 and 28 August (Day 0) or maintained on sucrose solution (10%).

		Fat body				Follicle stage		
Treatment	Day	0	1	2	3	Ia-b	IIa	IIIa-V
Pre-treatment	0		3	6	1	9	1	
Blood-fed	7	3	7				2[1]	8
Sucrose-fed	7	3	6	1		3	6	1
Blood-fed	20	9						9
Sucrose-fed	20	9				5	3	1

[1]One may be the female that did not take a blood meal.

GENERAL DISCUSSION

Culex restuans may have up to 3 generations/year with the second being the largest. Madder et al. (1980) reported similar observations with Cx. restuans populations in southern Ontario. They postulated that the third generation was small because many of the females from the second generation were in a state of gonotrophic diapause. This is probably true for the Manitoba populations as well.

In this study many more Cx. restuans egg rafts were collected from ovipools than adults from CDC light traps. The large number of egg rafts indicates that this species is more abundant than CDC light trap catches indicate.

In Ontario, Cx. restuans is believed to be a vector of St. Louis encephalitis (Madder et al. 1980). In Manitoba, WEE has been isolated from this species (Norris 1946, Sekla et al. 1980). Blood meal identification studies have shown that Cx. restuans prefers bird hosts (Tempelis 1975). In Manitoba this species is probably an important epizootic vector of WEE.

Culex tarsalis egg rafts were not collected in large numbers. This is probably because all of the pools, except the pool at the GRS in 1980, were shaded. Walters and Smith (1980) found that pools exposed to the sun were more likely to contain Cx. tarsalis larvae, than were shaded pools. This species was attracted to the CDC light traps. Peaks in egg raft numbers and adult females occurred in July. This is when WEE activity is greatest during epidemic years (Sekla et al. 1980; Brust, R.A. pers. comm.). This species has been incriminated as the primary vector of WEE in Manitoba (Henderson et al. 1979, Sekla et al. 1980).

The abundance of Cx. tarsalis in July is further proof that this species is the primary vector of WEE in Manitoba.

Culex tarsalis may be an overwintering reservoir for WEE, although virus isolations from overwintering females are rare. Two females collected in December took blood-meals and did not develop eggs. Mitchell (1981) obtained the same results with overwintering Cx. tarsalis from Colorado. He also found that the females would only feed if placed next to the host. Therefore, blood feeding by overwintering females is not likely to occur unless they are next to a host. This may occur if the mosquitoes overwinter in a mammal burrow that contains a host. Diapausing Cx. tarsalis have been collected from mammal burrows (Shemanchuk 1965).

Culiseta inornata has 2 generations/year with much of the oviposition activity occurring in the spring and early summer, before the peak period of WEE activity. Sekla et al. (1980) isolated WEE from this species in Manitoba. McLintock and Iversen (1975) suggested that this species may be an important vector of WEE in the spring and in areas where Cx. tarsalis does not occur. Blood-meal identification studies have shown that Cs. inornata prefers large mammals (Tempelis 1975). It is believed that large mammals are dead-end hosts of WEE, and that mosquitoes cannot become infected when they feed on them. Therefore, Cs. inornata is probably not an important vector of WEE.

Diapausing Cs. inornata females took blood and developed eggs. Hudson (1979) obtained the same results with this species in Alberta. It is of interest that some females expelled their first blood meal before it was digested. This behavior was also reported by Hudson (1979). If this behavior occurs in nature, Cs. inornata may be an overwintering

reservoir for arboviruses.

Of all the mosquito species from Manitoba from which WEE was isolated An. earlei had the highest minimum infection ratio (Sekla et al. 1980). The ability of this species to vector WEE is unknown. In this study females collected in December and given a blood meal exhibited gonotrophic dissociation. Anopheles earlei may therefore be capable of overwintering WEE. It is unfortunate that the vector capability of this species is unknown, because it may be the key to winter maintenance of WEE in Manitoba.

SUMMARY AND CONCLUSIONS

1. In Manitoba, Cx. restuans and Cx. tarsalis may have 3 generations/year; Cs. inornata has 2 generations/year.
2. Overwintered females of Cx. restuans, Cx. tarsalis and Cs. inornata were active in May and laid eggs before the end of May. Oviposition activity in Cx. restuans and Cx. tarsalis generally peaked in July; in Cs. inornata it usually peaked in June.
3. Daily oviposition in Cx. restuans showed a diel periodicity, with peaks in activity 2 hours after dusk and again at dawn.
4. Female Cx. restuans of the spring generation lay significantly more eggs per egg raft than overwintered females or females of the summer generations.
5. Diapausing An. earlei and Cx. tarsalis exhibited gonotrophic dissociation, and may therefore be overwintering hosts for WEE.
6. The population dynamics of Cx. tarsalis best fits the seasonal occurrence of WEE. This species is likely the primary vector of this virus in Manitoba. Culex restuans may be an important epizootic vector of WEE.
7. The monitoring of oviposition activity, as carried out in this study is a suitable method for monitoring populations of Cx. restuans and Cs. inornata but not Cx. tarsalis.

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APPENDIX

Survival of *Anopheles earlei* and *Culiseta inornata* Released in a Natural Overwintering Site

The winter survival of mosquitoes in their natural overwintering sites is not well studied. Hudson (1978, Mosquito News 38:570-579) observed a 88.5% winter mortality in *Anopheles earlei* Vargas overwintering in a cellar, with a mean temperature of 6.4°C. Mitchell (1979, J. Med. Ent. 16:482-487) marked 379 hibernating *Culex tarsalis* Coquillett, his recovery of 8.7% of the marked population 3 to 5 months later gave a rough estimate of winter survival. The winter survival of wild diapausing *An. earlei* and *Culiseta inornata* (Williston) was studied at an overwintering site on the U of M campus (Arntfield, Gallaway and Brust, 1982, Can. Ent. 114:85-86). A screen door was fitted to the entrance of the tunnel to prevent mosquitoes from escaping.

Diapausing *An. earlei* females were collected from highway culverts in southern Manitoba during September and October 1981. These mosquitoes were kept at 15°C, 8L:16D until they were released into the tunnel. Fourth instar larvae and pupae of *Cs. inornata* were collected from road side ditches in mid-September 1981 and allowed to emerge into a cage kept outdoors. The *Cs. inornata* were kept outdoors in a cage and offered a 10% solution of sucrose until the females were released into the tunnel.

Table 1 shows the numbers of mosquitoes naturally occurring and released in the tunnel. None of the released mosquitoes could be found

in the tunnel at the end of January. At this time the temperature in the tunnel was 8°C. Diapausing females of these species have super cooling points below -10°C (Hudson 1978, Can. J. Zool. 56:1697-1709). The temperature in the tunnel was probably too high for successful overwintering of these species.

Table 1. Numbers of Anopheles earlei and Culiseta inornata naturally occurring and released in a subterranean tunnel on the U of M campus.

Date	Temperature (°C)	An. earlei	Cs. inornata
4 Sept. 1981	19	7	0
18 Sept.	15	15	0
2 Oct.	13	16	120*
19 Oct.	15	8	330*
6 Nov.	14	6	0
13 Nov.	13	124*	0
15 Jan. 1982	9	15	0
29 Jan.	8	0	0

* Released mosquitoes