THE BIONOMICS OF THREE POTENTIAL VECTORS OF WESTERN EQUINE ENCEPHALITIS IN MANITOBA

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Jo Anne Leona Buth

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ABSTRACT

The oviposition activity and egg production of C. tarsalis, C. restuans and C. inornata in Manitoba were monitored during the summers of 1980 and 1981 using artificial pools.

From examination of oviposition activity and development times of the immature stages, C. tarsalis and C. restuans had 3 generations per year. Culiseta inornata had 2 generations per year. Peak C. tarsalis oviposition activity coincided with the highest WEE transmission rate.

In 1980, female C. tarsalis and C. inornata produced fewer eggs per raft in the early spring and summer as compared to later in the summer. Female C. restuans produced fewer eggs per raft in the early spring and summer as compared to later in the summer in 1980 and 1981.

Development times and survival of C. tarsalis, C. restuans and C. inornata were studied in the laboratory at temperatures of 15°, 20° and 25°C, photoperiods of 16L:8D and 8L:16D and densitites of 50 and 500 larvae per pan. Larval and pupal development times of C. tarsalis, C. restuans and C. inornata in the laboratory were not affected by photoperiod. Development times of the three species were longest at 15° and shortest at 25°C. C. restuans and C. inornata were more tolerant of cool temperatures than C. tarsalis.

Development time increased at the higher density. There was a statisically significant interaction between temperature and density on the development times of the three species. At 15°C and the high density, development was longer, while at 20° and 25°C the differences between development times at the high and low density were not as great.

Mortality of C. tarsalis larvae and pupae was affected by temperature when reared at 50 larvae per pan. The highest mortality occurred at 15°C. Mortality of C. restuans and C. inornata was highest at 25°C.

Under field conditions, C. tarsalis required 20 to 25 days to complete development to adults, while C. restuans required 16 to 23 days. C. inornata completed development in 20 to 21.5 days. Mortality in the field for C. tarsalis was dependent on larval density. Mortality of C. restuans and C. inornata was dependent on larval density and the time period studied.

In laboratory studies, the follicle:germarium ratio and the stage of follicular development were chosen as the criteria for differentiating between diapausing and non-diapausing females. Females of C. tarsalis, C. restuans and C. inornata with pre-resting stage follicles and a follicle:germarium ratio less than 2.5:1 were considered to be in diapause.

Photoperiod was the main factor affecting the number of females of the three species entering diapause when studied in the laboratory.

In 1981, field reared C. tarsalis started entering diapause by 27 July, when 20-30% were in diapause. Female C. restuans started entering diapause about 10 August, when 15% were in diapause. By 26 August, 40-60% of C. restuans were in diapause. Female C. inornata started entering diapause by 28 August, when 30-40% were in diapause.

FOREWARD

The format followed for this thesis is that of the manuscript or paper style thesis. None of the three manuscripts have been published, but they will be submitted for publication at a later date.

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INTRODUCTION

Western Equine Encephalitis (WEE) virus is an alphavirus which is transmitted by mosquitoes and attacks the central nervous system of humans and horses. The disease has a widespread distribution throughout the United States and western Canada. However, human and equine cases occur most frequently in the western United States and Canada (Monath 1979, Anon 1978). In the United States, between 1955 and 1978, 947 human cases of WEE were reported with a fatality rate of 3 to 4% (Monath 1979).

Historically, WEE virus has been the most important arbovirus in Canada with respect to human disease. Since 1935, when the disease was first isolated in Saskatchewan (Fulton 1938), there have been 13 separate epidemics of WEE in humans extending from British Columbia west to Ontario (Artsob and Spence 1979; Sekla *et al.* 1980; Brust 1982). Three epidemics have occurred in Manitoba in the last eight years: i.e., 1975, 1977 and 1981 (Medovy 1976; Sekla 1976; Sekla 1982).

Western equine encephalitis is maintained in nature through a bird-mosquito-bird cycle (Hess and Hayes 1967). Humans and horses are considered to be "dead-end" hosts. *Culex tarsalis* Coquillett is considered the primary vector of WEE virus in the western United States and Canada (Chamberlain and Sudia 1957; McLintock *et al.* 1970; Henderson *et al.* 1979). *Culiseta inornata* (Williston) may be a significant vector in horse epizootics (McLintock *et al.* 1970) and in

northern areas where C. tarsalis is not abundant. The virus has also been isolated from Culex restuans Theobald (Norris 1946; Sekla 1982). This latter species may be important in the maintenance and amplification of the virus in the bird populations.

Understanding the biology of the mosquito vectors is essential in understanding the disease cycle and controlling outbreaks. This research was initiated to obtain more detailed information on the biology of three potential vectors of WEE in Manitoba.

Three studies were undertaken. Seasonal oviposition and egg production of the three species was examined in an attempt to determine the number of generations per year, the onset of reproductive diapause in the populations and the changes in egg production throughout the season.

The second study examined the development and survival of the immature stages of these mosquitoes under different temperatures, photoperiods and densities in the laboratory. Studies of the development and survival of the immature stages under field conditions and at different densities were also done.

Finally, the ovarian development of the adult female mosquitoes when reared under different temperatures, photoperiods and densities in the laboratory was monitored. Ovarian development in the field was also examined.

LITERATURE REVIEW

DISTRIBUTION

Culex tarsalis is widely distributed in the western, central and southern United States (Carpenter and LaCasse 1955). Although common west of the Mississippi, it is uncommon or rare in the eastern United States (Jenkins 1950). In Canada, C. tarsalis has been recorded from British Columbia, Alberta, Saskatchewan and Manitoba (Wood et al. 1979). Specimens have also been collected in southern Ontario (Wood et al. 1979; Helson et al. 1980).

Culex restuans is widely distributed in North America from the Gulf of Mexico to southern Canada (Carpenter and LaCasse 1955; Darsie and Ward 1981). In the United States, west of the Rockies, this species is not common and appears to be largely replaced by Culex erythrothorax Dyar (Bohart and Washino 1978). In Canada, C. restuans has been recorded from central Alberta, Saskatchewan, Manitoba, Ontario, Quebec, New Brunswick and Nova Scotia (Wood et al. 1979).

Culiseta inornata is distributed throughout the United States and northern Mexico (Carpenter and LaCasse 1955). It is common in northern and western Canada from the Yukon, British Columbia, Saskatchewan and Manitoba. In eastern Canada it is found only in southern Ontario (Wood et al. 1979).

ABUNDANCE

Studies on the abundance of these species have been conducted using light traps without dry ice, light traps baited with dry ice and flock traps baited with chickens. Because of the different kinds of mosquito traps used in collections, comparison between areas was difficult. However, some statements can be made about the abundance of these species in relation to other species within an area. The reports below refer to the percentage of a certain species within the entire trap collection. For an accurate indication of the mosquito population the actual trap numbers should also be examined.

Although C. tarsalis is widely distributed in western Canada, it is most common in the prairie provinces. On the prairies, it is uncommon in the aspen parkland belt (Graham 1966), but common in the agricultural areas.

In Manitoba, during 1947 and 1948, C. tarsalis comprised 9.5% (McLintock 1948) and 8.0% (Wardle 1949) of the total light trap catches. Brust and Ellis (1976) summarized the studies on abundance of C. tarsalis collected in New Jersey style light traps in Manitoba for the years 1947 to 1975. The percentage of C. tarsalis collected ranged from less than 1% to over 8%, depending on the year. In studies by McLintock and Rempel (1963), the percent C. tarsalis captured in light traps in Manitoba ranged from less than 1% to over 10% of the total mosquitoes collected. In Winnipeg during the 1975

epidemic of WEE, C. tarsalis comprised over 45% of light trap catches on two days in August. In 1975, C. tarsalis comprised 60% of trap collections from southern Manitoba (Brust and Ellis 1976).

In Saskatchewan, C. tarsalis comprised over 30% of light trap contents, making it the most abundant species at some locations (McLintock and Rempel 1963; McLintock et al. 1966). McLintock and co-workers (1970) found C. tarsalis to be the third and fourth most abundant species of mosquito collected in light traps in 1963 and 1965. In Saskatoon, C. tarsalis composed 4.0% of adults collected in Center for Disease Control-style (CDC) traps, making it the sixth most abundant species (Stewart 1973).

In Alberta, C. tarsalis was the most abundant species collected in flock traps (Shemanchuk 1969).

C. restuans composed less than 1% of the mosquitoes collected in light traps on the prairies in 1947 lnd 1948 (McLintock 1948; Wardle 1949). McLintock and Rempel (1963) showed that C. restuans comprised less than 1% of collections from 1944 to 1948. Numbers ranged from 3 to 465 per trap. In 1962 also, C. restuans was low in numbers.

Culiseta inornata comprised up to 54.2% of total light trap collections in Manitoba between the years 1942 and 1948 (McLintock and Rempel 1963). Several authors have reported that C. inornata is the most abundant species collected in light traps in Saskatchewan (McLintock et al. 1966 and 1970; Spalatin et al. 1963), comprising over 62% of the mosquitoes collected in some years. In Saskatoon, C.

inornata composed 80.6% of CDC light trap collections (Stewart 1973).

In Alberta, Shemanchuk (1969) found that C. inornata was the second or third most abundant species in both light traps and flock traps.

BIOLOGY

These three species have similar life cycles in that they overwinter as inseminated adult females. The females emerge in the spring, seek a blood meal and commence egg laying. As populations decrease in size in the northern portions of their range, they exhibit reduced blood-feeding, fat body accumulation and ovarian diapause. In the southern limits of its range, C. inornata is considered a winter mosquito and spends the summer in a state of aestivation.

Larval Habitats

Larval habitats differ, but all three species are tolerant of a wide range of environmental conditions and have been found in almost every freshwater source available.

Culex tarsalis larvae have been reported frequently from roadside ditches (Beck 1961; Breeland et al. 1961; Edmunds 1955; Harmston et al. 1956; Lungstrom 1955; Rempel 1950; Rueger et al. 1964; Snow and Pickard 1956) and irrigation ditches or seepages (Beck 1961; Breeland

et al. 1961; Dyar 1921; Edmunds 1955; Harmston et al. 1956; Owen and Gebhardt 1957; Washino and Bellamy 1963).

Many other C. tarsalis habitats have been recorded in the literature, including a variety of permanent, semi-permanent and temporary pools and artificial containers (Breeland et al. 1961; McLintock 1944; Howard 1916; Barton 1978; Harmston et al. 1956).

Culex restuans is generally considered to be a container breeding mosquito, but reports in the literature indicate that its habitats are actually diverse. Permanent freshwater swamps, ponds, streams, marshes and ditches have been reported frequently as habitats for C. restuans (Niebanck 1957; Breeland et al. 1961; Lewis and Bennett 1979; James et al. 1969; McLintock 1944; Berlin 1977). Tree holes seem to be a common habitat in some areas (Breeland et al. 1961; Shipp and Wright 1978; Shipp et al. 1978; Barton 1978; Porter et al. 1961).

C. restuans has been reported from a variety of containers such as rain barrels (Niebanck 1957; Breeland et al. 1961; James et al. 1969; Howard 1916; Judd 1954), tin cans (Barton 1978) and tires (Rupp 1977; Barton 1978).

Culiseta inornata larvae were found in a wide variety of temporary, semi-permanent and permanent habitats (McLintock 1944). Ditches were the most frequently reported habitats (Bohart and Washino 1978; Breeland et al. 1961; Carpenter and LaCasse 1955; Rempel 1950; Washino et al. 1962). Several authors have collected C. inornata in

pools covered with thin sheets of ice (Breeland et al. 1961; Beck 1961; Washino et al. 1962). Owen (1937) found C. inornata larvae in early spring, snow-melt pools in Minnesota.

Larval Development

Larval development of mosquitoes depends on temperature, food availability and density (Hanec and Brust 1967; Trpis 1972; Tauthong and Brust 1977; Hagstrum and Workman 1971; Wada 1965).

Culex tarsalis

Edmunds (1955) found that larval development of C. tarsalis in the field lasted 14 days at an average air temperature of 25.8°C and 31 days at an average air temperature of 17.4°C. In laboratory experiments, Brennan and Harwood (1953) found the duration of larval stages averaged 10 days (range 13 to 18 days) at 24° to 26°C.

Hagstrum and Workman (1971) conducted laboratory experiments on the relationship between the duration of the larval instars and the feeding rates at 20° and 30°C. Development time from first to fourth instar ranged from 11.7 days at 30°C to 25.3 days at 20°C.

Culex tarsalis larvae tolerate high temperatures better than C. inornata in California. First instar larvae of C. tarsalis were still present in field pools when temperatures reached 37°C (Fanara and Mulla 1974).

Culex restuans

In laboratory studies, Shelton (1973) reported that C. restuans larvae show a broad tolerance to water temperatures. Larvae developed to adults at 12°, 15°, 20° 23°, 26° and 29°C. Larvae did not complete development at 32° and 35°C. Development times ranged from 25.3 days at 12°C to 6.3 days at 29°C, but survival was fairly consistent at 85% for all temperatures up to 29°C. At this temperature, survival decreased to about 50%. The pupation period at 16L:8D photoperiod ranged from 6 days at 10°C to one day at 20° and 25°C (Eldridge et al. 1976).

Culiseta inornata

Several authors noted that this species is tolerant of low temperatures (McLintock 1964; Brust 1967; Hanec and Brust 1967). McLintock (1964) found the optimum temperature for rearing was 20.5°C. At 26.6°C, only about 2% of larvae survived to emergence and the resulting adults were weak. The average time from the egg of one generation to the egg of the next was 21 to 22 days at 20° to 21°C.

Larval development took at least 15 days at 15.6° to 21°C (Owen 1942). C. inornata required 30 days to complete its life cycle at this temperature.

Brust (1967) found developmental temperatures for obtaining healthy adults was considerably lower for C. inornata than for Aedes vexans (Meigen) or Aedes nigromaculis (Ludlow). At 10°C, 36% of C. inornata emerged as healthy adults. The optimal survival temperature was calculated to be 21° ± 1°C. Development time decreased markedly with an increase in temperature from 21°C to 26.5°C. A rearing temperature of 32°C was lethal, as no larvae survived beyond the second instar. At 21°C it took 312 ± 6 hours for males to develop from first instar to pupae whereas females required 402 ± 4 hours. At 26°C, it took 210 ± 5.5 hours for males and 274.5 ± 5 hours for females.

Hanec and Brust (1967) also found 21°C to be the optimum temperature for rearing C. inornata. Temperatures below 21°C caused an extended period of development whereas at temperatures above 21°C, high mortality occurred among fourth instar larvae and pupae. Although larvae required up to 239 days to reach the pupal stage at 5°C, no adults emerged at this temperature.

Culiseta inornata did not complete development at temperatures of 12°C or below and at 29°C and above (Shelton 1973). A temperature increase from 15° to 20°C reduced the length of the immature period by 5 days (19 days to 14 days) but survival to adults and average body weight of the adult females remained the same. As the temperature was increased from 20° to 23°C, adult survival decreased from 60% to 35% and average body weight decreased 0.6 mg. Time from hatching to adults at 20° and 23°C differed by less than 5 hours. Survival was not

affected as the temperature was increased from 23° to 26°C, but a 56% loss in body weight occurred. Percent survival was optimum (60%) at 15° and 20°C.

Oviposition

Culex tarsalis

Culex tarsalis females oviposit rafts of eggs directly onto the surface of water. In laboratory experiments, Hudson and McLintock (1967) found that females selected water containing pupae, exuviae and emerging adults of their own species for oviposition in preference to distilled water and to emergence water of C. inornata, Aedes aegypti (Linnaeus) and Culex pipiens Linnaeus. This preference still existed after removing all the stages and boiling the water. In another study, oviposition attractants were extracted from distillate of water in which C. tarsalis had been breeding (Ikeshoji and Mulla 1970) and this is the first record of oviposition attractants being found from C. tarsalis in natural breeding waters.

In field studies, Fanara and Mulla (1974) found females were more attracted to freshly flooded ponds than ponds which had been flooded earlier.

C. tarsalis showed a regular, temperature-independent oviposition rhythm at photoperiods of 8, 12 or 16 hours of light with peaks occurring in the first hour of light and the first hour of dark (Logen

and Harwood 1965). There was usually a corresponding change in oviposition rhythm when photoperiod was changed.

Egg rafts, collected from the field in Nebraska, contained an average of 244 eggs per raft, with a range of 143 - 331 eggs per raft (Edmunds 1955). Brennan and Harwood (1953) found that egg rafts from a laboratory colony of C. tarsalis contained an average of 230 eggs per raft.

Culex restuans

In southern Ontario, maximum oviposition occurred within one to three hours of dusk with a second, smaller peak at dawn. Ovipositional activity was complete by 1000 h (MacDonald et al. 1981).

Belton (1967) found artificial pools lined with black polyethylene were three times more attractive to ovipositing C. restuans females than pools lined with translucent polyethylene. Illumination at night prevented oviposition.

Artificial pools containing water-borne bacteria of the family Psuedomonadaceae and treated with capric acid and NH_4NO_3 were attractive oviposition sites for C. restuans and C. pipiens females (Maw and Bracken 1971). However, Madder and co-workers (1980) found that neither species showed greater attraction to pools containing decanoic acid.

Girault (1908), as cited by Barr (1958), found that female C. restuans laid an average of 267 eggs. Madder (1981) found that females laid an average of 189 to 303 eggs per raft over a three year period.

Culiseta inornata

Owen (1942) found that C. inornata females laid an average of 140 eggs per raft, whereas Brust (1967) found each egg raft consisted of approximately 200 eggs per raft.

Adult Feeding Preferences

Culex tarsalis

Many authors report an obvious feeding preference of females for avian hosts (Bellamy and Reeves 1963; Tempelis et al. 1967; Tempelis and Washino 1967), but females are attracted to and will feed readily on mammals (Edman and Downe 1964; Shemanchuk et al. 1963; Gunstream et al. 1971). Culex tarsalis also feeds on reptiles and amphibians (Gunstream et al 1971; Henderson and Senior 1961; McIver 1968).

An apparent bi-phasic feeding pattern has been reported by several authors (Gunstream et al. 1971; Reeves et al. 1963; Tempelis

1975; Tempelis et al. 1965; Tempelis et al. 1967). Culex tarsalis preferentially feeds on birds in spring and early summer, with a shift to feeding on significant numbers of mammals in mid- to late summer periods. The scope of its feeding on birds depends on the physiography of the area, to some extent on the fauna present in the area, and on the time of year.

Culex restuans

Females of C. restuans are generally considered to prefer avian hosts (Barr 1958) and this is substantiated by studies with baited traps and precipitin tests of blood-fed females (Means 1968; Murphey et al. 1967; Meyer and Bennett 1976; Magnarelli 1977).

Culiseta inornata

Female C. inornata feed almost exclusively on large mammals (Anderson et al. 1967; Edman et al. 1972; Tempelis 1975; Tempelis et al. 1967). Although in some of these studies C. inornata fed on birds, the percentages were low.

SEASONAL DEVELOPMENT of POPULATIONS

Culex tarsalis

Female *C. tarsalis* leave overwintering sites in the spring. The cue for emergence is related to the onset of warm weather (Keener 1952) and soil temperature inversion (Bennington *et al.* 1958a; Shemanchuk 1965).

From reports of light trap collections in western Canada, *C. tarsalis* is most abundant in late July and early August (Brust 1982; Brust and Ellis 1976; McLintock 1944; McLintock 1948; Spalatin *et al.* 1963).

Increases in the *C. tarsalis* population are related to certain weather factors. In Utah, Graham and Bradley (1969) found that changes in *C. tarsalis* populations were the result of the interaction of man's water management, patterns of precipitation, temperature and relationships between common species of mosquitoes in the area. The magnitude of Winnipeg's *C. tarsalis* population peak which normally occurs in late July, depends on the precipitation and temperature regimes of the latter part of May (Raddatz 1982). A large July peak occurred when late May had normal to above normal precipitation with normal to above normal temperatures. Late May conditions must be both wet and warm. Wet but cold or warm and dry conditions produce significantly smaller peaks.

Culex restuans

Female C. restuans also leave hibernation sites with the onset of warm weather in the spring. In southern Ontario, several authors report collecting C. restuans in late May and early June (Belton and Galloway 1966; Helson et al 1980; James et al. 1969; Judd 1954).

C. restuans egg rafts were first collected in oviposition traps in southern Ontario on 17 May and were most abundant in spring and early summer. Oviposition continued at low levels until 15 October (Madder et al. 1980).

A peak in numbers also depends on geographic location and weather. In Kentucky, C. restuans populations are highest in August and September (Covell 1968). In New York, numbers are highest during August (Niebanck 1957). Siverly and DeFoliart (1968) found C. restuans was most common in July in Wisconsin. In southern Ontario, populations were highest between mid-July and mid-August (Helson et al. 1980).

In Canada and the northern United States, populations decrease towards the end of summer. In southerly locations, where C. restuans is not as abundant in the hot summer, populations may increase again in the fall (Covell and Resh 1971). In New York, Niebanck (1957) collected C. restuans in September. In southern Ontario, C. restuans was collected as late as October (Belton and Galloway 1966).

Culiseta inornata

The seasonal development of C. inornata populations varies considerably depending on its location within its north-south distribution. In the southern United States, C. inornata is essentially a fall, winter and spring mosquito which spends the summer as an aestivating adult. However, in more northern locations, it is a summer mosquito which overwinters in sheltered locations in a state of facultative ovarian diapause.

In Manitoba, C. inornata overwinters as an adult in sheltered locations and becomes active in the early spring (Hanec and Brust 1967). Females have been captured as early as 15 April and are quite numerous by 1 May. Two population peaks occur during the summer. One peak occurs at the end of June and the second one during the second and third week of August. There are probably two generations per year.

Rempel (1950) noted that C. inornata did not appear in large numbers on the prairies until July or early August and speculated that there are several generations per year.

In Belleville, Ontario, C. inornata males were collected in NJLTs in the last week of August, mid-September and mid-October (Belton and Galloway 1966). In southwestern Ontario, C. inornata females were most common in June (Helson et al. 1980).

OVERWINTERING AND DIAPAUSE

Culex tarsalis

Overwintering is characterized by reduced blood-feeding, the accumulation of fat body and ovarian diapause (Bellamy and Reeves 1963). *C. tarsalis* females begin to enter overwintering sites in late summer and fall, depending upon geographic location and weather conditions.

The overwintering success of female mosquitoes depends on sufficient energy reserves of the mosquito and the temperature and relative humidity of the overwintering site (Anderson and Harwood 1966).

Schaefer and co-workers (1971) found that *C. tarsalis* females synthesize lipids as a source of energy during overwintering. In California, the field population reaches a maximum lipid content in November, after which there is a steady decline during the overwintering period.

In northern California, female *C. tarsalis* move into the foothills area adjacent to the San Joaquin Valley in the fall to overwinter. (Kliewer *et al*. 1969). During this period, the females are gonotrophically inactive and have well developed fat bodies. Females fed on blood and became gonotrophically active in January, after which and there was a general movement out of the foothill area.

All of the overwintering females collected were inseminated but very few were parous.

Female C. tarsalis entering overwintering sites are considered to be in reproductive diapause and probably enter overwintering sites without taking a blood meal. Parity, as measured by ovarian dilitations, indicates that a female has taken a blood meal (if the female is not autogenous) and has produced and oviposited some eggs. Burdick and Kardos (1963) found that the ovaries of overwintering C. tarsalis were nulliparous. Mitchell (1979) found that 89.9% of C. tarsalis collected from abandoned mines in Colorado were nulliparous. Only 2.8% were parous. The remainder could not be identified.

Bennington and co-workers (1958b) collected C. tarsalis from resting sites in Colorado throughout the summer and fall. The percent of engorged females decreased steadily from May until about 15 August when it reached 10%. Females with fat bodies first appeared about 15 August. They noted three distinct phases in the population: i.e., first, the refusal of fall broods to take blood; second, fat body development without taking blood and third, hibernation.

In California, levels of parity in the C. tarsalis population were high throughout the fall among blood engorged females from shelters and females with empty midguts that were attracted to CO_2 bait (Nelson 1964). Parity levels became progressively lower in the fall among females without blood in the midgut taken from shelters. All but a few females collected from shelters throughout the mid-

winter were nulliparous. Many blood-engorged, parous females were found in February and March prior to the first emergence of adults from pupae in the spring.

Burdick and Kardos (1963) found that 30% to 50% of females collected in the summer in California were parous. The percent nulliparous increased in the autumn. Between early November and late January, only a small percentage were parous. A large proportion of overwintering C. tarsalis became blood-engorged in late January and February. Fat body development began in early October and peaked in late December. By mid-January, when blood-feeding began, only 64% had well developed fat body. A marked depletion of fat body coincided with a rapid increase in blood-feeding.

The overwintering population of C. tarsalis studied during April and May in Oregon showed a similar trend (Rush and Tempelis 1967). Collections from resting sites consistently included recently blood-engorged females. As spring progressed, a decreasing proportion of the population contained visible fat. Parity increased throughout the spring and by mid-May reached 79% of the non-engorged population.

When overwintering females were studied in the laboratory, Mitchell (1981) found that exposure to a long photopheriod (15L:9D) resulted in diapause termination in essentially all mosquitoes by day 7 at 25°C. Warming alone did not terminate diapause in a significant proportion of females maintained at typical autumn day lengths.

A California strain of C. tarsalis responded to a short photoperiod (8L:16D) at 22°C by increased size and compactness of fat body and by minimum ovary length. Lower temperature reinforces the response of the fat body (Harwood and Halfhill 1964). No differences were noted in the ability of various photoperiod groups to blood-feed.

Culex restuans

After leaving hibernation sites in the spring, C. restuans females seek a blood meal. In Connecticut, during April, May and June, increasing numbers of blood-fed females appeared in diurnal resting sites (Wallis 1959). By July, 75% of the females were blood-fed. Later in the summer, the incidence of blood-fed females declined and by October the majority of females were engorged with clear fluid or had developed fat body (Wallis 1959).

Wallis (1959) encountered increasing difficulty towards the fall in inducing females to blood-feed when C. restuans was reared outside. Most females preferred a sucrose solution and would develop fat body when placed in experimental hibernation conditions. Generally, this group had a higher winter survival rate than those which had taken a blood meal. Wallis concluded that blood-feeding was not necessary for fat body formation and did not generally occur in nature.

In studies of the effect of photoperiod and temperature on diapause development and gonotrophic dissociation, Eldridge and co-workers (1976) found that, at 25°C, follicles in C. restuans developed equally at both long (16L:8D) and short (8L:16D) photoperiods. Follicles also developed fully at 15°C and a long photoperiod but at 15°C and a short photoperiod, they remained small. Gonotrophic dissociation was expressed in C. restuans under conditions of low temperature and short photoperiod (Eldridge et al. 1972).

Culiseta inornata

Near Edmonton, Alberta, some female C. inornata continue to fly until late October. However, blood-fed, gravid and parous individuals are rarely taken after the end of August (Hudson 1977). Hudson induced diapause in females by transferring them from 16 hour to 12 hour daylengths. Diapause did not occur when females were reared at a constant daylength of 12 hours. Kalpage (1970) reared C. inornata females at a series of constant temperatures from 10° to 30°C and constant daylengths from 8 to 16 hours. He found that both short days and low temperatures reduced the blood-feeding rate. The females which blood-fed did not exhibit gonotrophic dissociation and their eggs matured.

Diapause termination occurred when overwintering females were kept for 7 days at 16 hour daylength and 20°C (Hudson 1979). After termination, follicle growth and yolk deposition occurred. Females which blood-fed then matured their eggs.

Seasonal Oviposition and Egg Production of *Culex tarsalis* Coquillett, *Culex restuans* Theobald and *Culiseta inornata* (Williston) (Diptera: Culicidae) in Manitoba

INTRODUCTION

Historically, Western equine encephalitis (WEE) has been the most important arbovirus in Manitoba. In the last eight years, three outbreaks of the disease involving humans and horses have occurred: i.e., 1975, 1977 and 1981 (Medovy 1976; Sekla 1976; Sekla 1982). Culex tarsalis Coquillett is considered the primary vector of WEE in Manitoba (Henderson et al. 1979; Sekla et al. 1980). The virus has also been isolated from Culex restuans Theobald (Norris 1946; Sekla et al. 1980) and Culiseta inornata (Williston) (Sekla et al. 1980) and these species may also be involved in the transmission of the disease.

Oviposition monitoring has been used to study populations of various Culex and Culiseta species in many parts of the world (De Meillon et al 1967; Lowe et al 1974; Maw and Bracken 1971; Smith and Jones 1972). Oviposition activity of C. restuans and Culex pipiens Linnaeus has been studied in southern Ontario (Madder et al. 1980) and in New Jersey (Leiser and Beir 1982). In both studies, the oviposition activity closely followed the trends in adult populations as measured by light traps.

The oviposition of C. tarsalis, C. restuans and C. inornata in Manitoba is an indicator of the population of reproductively active females and therefore an indicator of potential WEE vectors. These species overwinter as inseminated females in a state of reproductive

diapause. The downward trend in oviposition in the late summer is the result of onset of diapause and results in decreased transmission of the disease during epidemics.

Population increases are dependent upon a variety of factors, including the survival of overwintering females, the availability of breeding sites, the number of eggs produced by a female and the weather. Further, the number of eggs which a female produces is related to the age of the female (Clements 1963; Walter and Hacker 1967), the size of the female and/or blood meal (Shannon and Hadjinicalao 1941) and the blood source (Spielman 1967).

In this study, the oviposition activity of the three potential vectors of WEE in Manitoba was monitored to study the reproductive activity and onset of diapause of these mosquitoes. Differences in egg production throughout the summer were also investigated.

MATERIALS AND METHODS

Seasonal changes in oviposition activity were monitored from May to October during the summers of 1980 and 1981 by collecting egg rafts from three artificial pools set up adjacent to a wooded area in west Winnipeg. The pools consisted of one meter square boxes, lined with black polyethylene and sod and filled with tap water. Tap water was added to the pools when required to maintain a water depth of 20 cm. The pools were set up in mid-April in both collection years.

All egg rafts were collected daily from the pools and placed in polystyrene cups with 150 ml tap water to allow hatching and identification. After hatching the first instar larvae were identified using Dodge's (1966) keys.

The seasonal distribution of egg rafts was used to determine the number of generations per year and to estimate the time when females would be entering ovarian diapause.

Step-wise linear regression was used to correlate the daily fluctuations in oviposition activity with weather parameters during the oviposition period. The following parameters were used: temperature, windspeed, relative humidity, dewpoint, total cloud opacity and the percentage of time with precipitation. The values for each parameter were averaged for two four hour periods: i.e., 1) the hour before sunset, the hour in which sunset occurred and the two

hours after sunset; 2) the two hours before sunrise, the hour of sunrise and one hour after sunrise.

Differences in egg production were investigated by counting the number of eggs per raft before hatching. Two counts were made of each raft and the average of the two counts was used for analysis of variance and Duncan's New multiple range test.

RESULTS

Seasonal Oviposition

The oviposition data are presented graphically on a daily basis (Figs. 1 and 3). The data are also summarized on a weekly basis to smooth the daily fluctuations and make population trends more apparent (Figs. 2 and 4).

In 1980, the first and last *C. tarsalis* egg rafts were collected on 28 May and 27 August, respectively (Fig. 1). Compared to *C. restuans* and *C. inornata*, the number of egg rafts collected remained relatively low throughout the summer (Figs. 1 and 2). Two increases were noted, i.e., one in the third week of June and the other in late July. The highest number of rafts collected on one day, 30, was collected on 23 June.

In 1981, the first and last *C. tarsalis* rafts were collected on 7 May and 27 August, respectively (Fig. 3). The number of rafts remained low until mid-July when oviposition activity increased for about two weeks (Figs. 3 and 4). On 27 July the Province of Manitoba declared a health emergency due to an outbreak of WEE. On 4 August, the oviposition study area was treated with an aerial application of a mosquito adulticide containing 12 % propoxur at a rate of 52.5 g active ingredient /ha. Few rafts were collected in August after the treatment.

In 1980, the first and last C. restuans egg rafts were collected on 24 May and 12 September, respectively (Fig. 1). The number of rafts began to increase at the beginning of June. On 6 June, 137 rafts were collected, the highest number for the summer. Numbers decreased slightly at the end of June, but increased the second week of July. There was a slight decrease at the end of July, followed by a brief increase at the beginning of August and then a steady decline into September.

In 1981, the first and last C. restuans egg rafts were collected on 21 May and 13 September (Fig. 3). Daily numbers remained low, i.e., less than 20, until the last week of June. Numbers increased and remained fairly high throughout July (Figs. 3 and 4). The highest number of rafts, 99, was collected on 15 July. Numbers declined in August after the aerial spraying and remained low until the last raft was collected.

In 1980, the first and last C. inornata egg rafts were collected on 22 May and 3 September (Fig. 1). The number of C. inornata egg rafts remained at low levels throughout the summer except for an increase towards the end of June and another smaller increase at the end of July (Figs. 1 and 2).

In 1981, the first and last C. inornata egg raft was collected on 4 May and 13 September (Fig. 3). Numbers remained at low levels throughout the summer except for a small increase in mid-June and a larger increase in mid-August (Figs. 3 and 4).

The correlation co-efficients of daily oviposition activity with weather parameters are listed in Table 1.

Egg production

The number of eggs per raft produced by C. tarsalis in 1980 ranged from 188 ± 67.6 in May to 303.2 ± 119.3 in August. Statistical differences were found between the mean number of eggs produced in May/June and July/August (Table 2). In 1981 there were no significant differences in the number of eggs per raft throughout the summer.

The number of eggs per raft produced by C. restuans in 1980 ranged from 155.0 ± 61.4 in May to 200 ± 55.8 in August. The number of eggs produced in May/June was significantly different than July/August/September (Table 3). Significant differences were also noted between July and August, and August and September. In 1981 the number of eggs per raft ranged from 146.9 ± 40.4 in May to 203.1 ± 79.8 in August. Significant differences were found between all months except July and August.

The number of eggs per raft produced by C. inornata in 1980 ranged from 161.4 ± 55.9 in May to 228.6 ± 86.4 in July. Significant differences were found between May and June (Table 4). No differences were found between July and August, however May/June was different from July/August. In 1981, no differences were found in the numbers of eggs per raft throughout the summer months.

DISCUSSION

Seasonal Oviposition

Distinct generations are not easily discernible with these three species because they breed continuously throughout the summer and an overlap of generations occurs. However, based on the weekly fluctuations in the numbers of egg rafts, the numbers of generations will be estimated.

Female *C. tarsalis* began laying eggs in late May, 1980. In 1981, females began laying eggs in early May, probably due to the warmer spring weather as compared to 1980. Females would have to leave overwintering sites as early as late April in order to seek a blood meal, mature eggs and oviposit in mid-May.

Based on increases in the number of egg rafts, *C. tarsalis* probably has three generations per year with population peaks occurring in mid-June, mid-July and mid-August. The second generation was the largest with a peak in oviposition in mid-to late-July. Gallaway (1983) found a similar pattern of oviposition and also reported three generations at the Glenlea Research Station in Manitoba.

The numbers of egg rafts collected in 1981, an epidemic year, was higher than in 1980, a non-epidemic year. Many factors contribute to a larger population. A larger number of *C. tarsalis* females may have

entered overwintering sites the previous fall. Overwintering survival may have been greater. Females left overwintering sites earlier in the spring and oviposition started in early May. The weather in 1981 was probably a major factor contributing to the higher population in 1981.

During recent outbreaks of WEE in Manitoba, the virus transmission rate has been highest in late July and early August (Sekla 1982). In this study, C. tarsalis oviposition in 1981 was highest in mid-July. Females from this second generation would be seeking a blood meal in late July and, if infected, may be capable of transmitting the virus.

The number of C. tarsalis egg rafts started to decline in late July in both 1980 and 1981. The number of egg rafts remained fairly low throughout August in 1980. In 1981, the number of egg rafts collected did not increase after the aerial spraying conducted on 4 August. This indicates that the females emerging after the aerial spray program were probably in ovarian diapause and not seeking a blood meal for egg production.

Female C. restuans emerged from overwintering sites and began egg laying in late May in both 1980 and 1981. This species probably has three generations per year with population peaks occurring in early June, mid-July and early August. The second generation was the largest. Gallaway (1983) reported a similar pattern in Manitoba and Madder (1981) found that C. restuans had three generations in southern

Ontario, with the second generation being the largest.

Culex restuans is not as common as C. tarsalis or C. inornata in light trap collections in Winnipeg (Gallaway 1983; R.A. Ellis pers. comm.). However, C. restuans may be much more common than C. tarsalis and C. inornata based on data from oviposition pools. Gallaway (1983) reported similar findings in oviposition pools in the Winnipeg area.

Culex restuans feeds mainly on birds (Barr 1958; Means 1968; Murphey et al. 1967; Magnarelli 1977) and has been found naturally infected with WEE virus in Manitoba (Norris 1946; Sekla et al 1980). Numbers of C. restuans egg rafts peaked in July, when WEE transmission was highest in 1981. Although C. restuans is probably not important as an epidemic vector because of its feeding preferences, it may be an important enzootic vector involved in the amplification of the virus in bird populations.

Culex restuans oviposition began to decrease in mid-August in 1980. In 1981, after the aerial spraying conducted on 4 August, the number of egg rafts increased slightly. However, the numbers did not reach the same levels as in August, 1980. A large proportion of the female C. restuans emerging after 4 August were probably entering diapause.

Female C. inornata females left overwintering sites and began egg laying in early May in 1981. In 1980, the first raft was collected later in May. In southern Manitoba, C. inornata probably has only two

generations per year with peak numbers occurring in June and August. Research in Manitoba on oviposition (Gallaway 1983) and light trap collections (Hanec and Brust 1967) also suggested that C. inornata has two generations per year.

In 1980, the number of C. inornata egg rafts declined in early August. However, in 1981, numbers did not start to decline until late August. In the southern United States, C. inornata is considered a winter mosquito and is generally considered to be tolerant of cool temperatures throughout its range (McLintock 1964; Brust 1967; Hanec and Brust 1967). C. inornata is active later in the summer and females may not enter diapause until later in the season.

There was no significant correlation between daily oviposition activity and the weather parameters examined (Table 1). The highest coefficient of 0.48390 was between C. restuans oviposition activity and sunrise dewpoint, sunset windspeed and sunset temperature. The weather parameters were measured at the Winnipeg International Airport and may not be an accurate measurement of the weather at the study site. The site was sheltered by a building on one side and a row of trees on the other. As a result, temperature and wind direction and windspeed were probably affected.

Egg Production

The number of eggs per raft produced by C. tarsalis and C. inornata in 1980 and C. restuans in 1980 and 1981 was significantly lower in the early spring and summer in comparison to later in the summer. The lower numbers in May and June may be due to a variety of factors including the poorer physiological condition of the overwintering females which is dependent on the weather conditions of the fall and winter, the temperature and humidity within the overwintering hibernacula and the amount of lipid the females accumulated before entering overwintering sites. The size and source of the blood meal in the spring may also affect the number of eggs a female produces. Weather conditions in the spring after the females have left overwintering sites may also be important.

In 1981, there was no significant difference between the mean number of C. tarsalis eggs per raft over the course of the summer. This constant number of eggs per raft and the slighlty higher production in the spring probably contributed to a higher population in 1981.

In conclusion, C. tarsalis and C. restuans probably have three generations per year in Manitoba. The second generation, which occurred in July, was the largest and correlated with peak WEE transmission rates during the epidemic in 1981. This supports the literature which considers C. tarsalis as the primary vector of WEE in

Manitoba.

C. restuans prefers to feed on birds and therefore is probably not an epidemic vector. However, this species may be important as an enzootic vector involved in the amplification of the virus in the bird population.

Culiseta inornata probably has only two generations per year. It may be important in the transmission of the virus in the early summer and early fall when C. tarsalis is not common.

The egg production of these three species was shown to differ throughout the summer in some years. Many factors determine the number of eggs a female produces and the reasons for the differences in this study are unclear. The number of eggs per raft may influence the size of the population.

Fig. 1. Total number of egg rafts laid each day by *Culex tarsalis*, *Culex restuans* and *Culiseta inornata* in three pools in 1980.

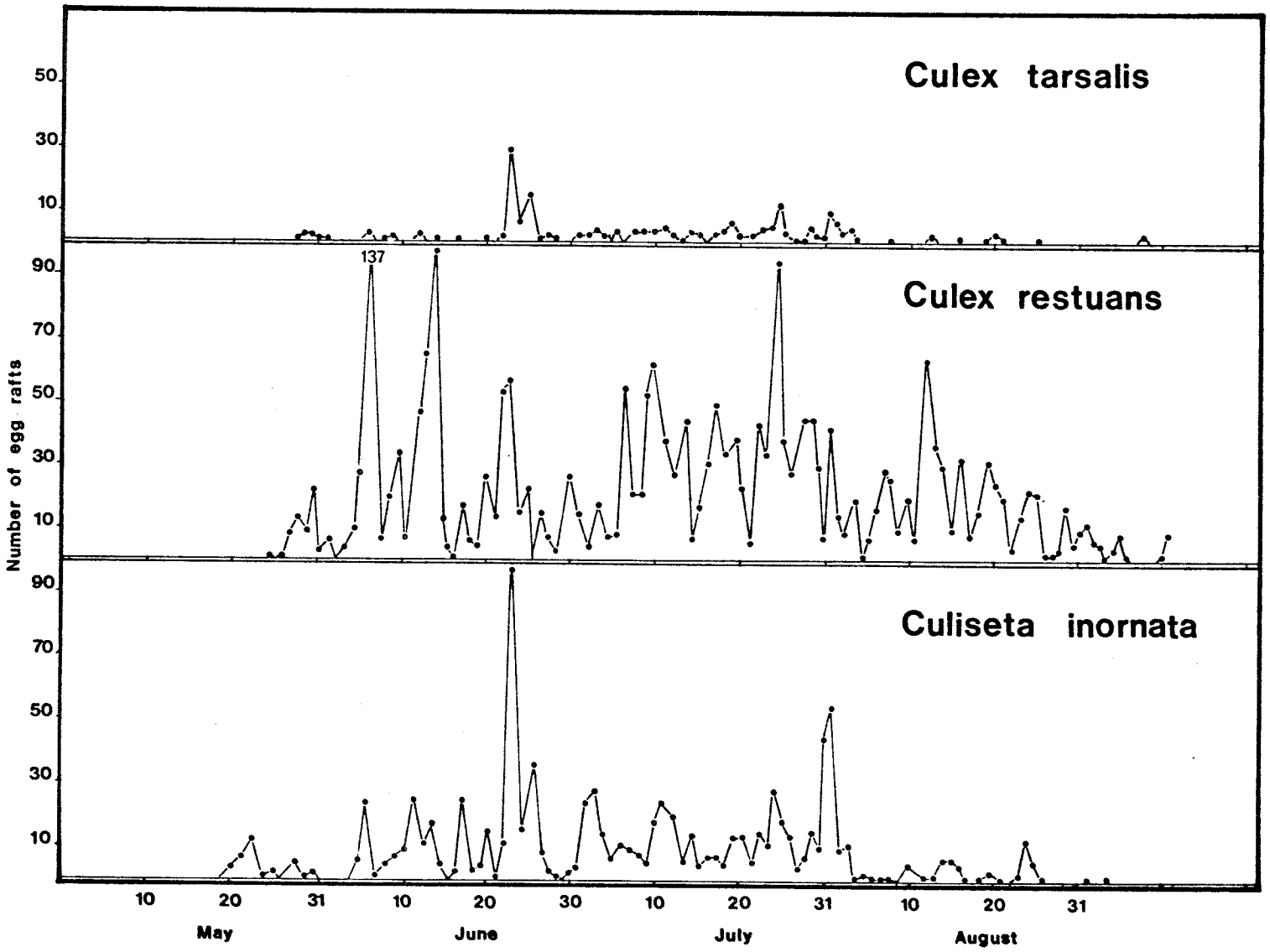

Culex tarsalis
Culex restuans
Culiseta inornata
137
Number of egg rafts
50
30
10
90
70
50
30
10
90
70
50
30
10
10
20
31
10
20
30
10
20
31
10
20
31
May
June
July
August

Fig. 2 Total number of egg rafts laid each week by *Culex tarsalis*, *Culex restuans* and *Culiseta inornata* in three pools in 1980.

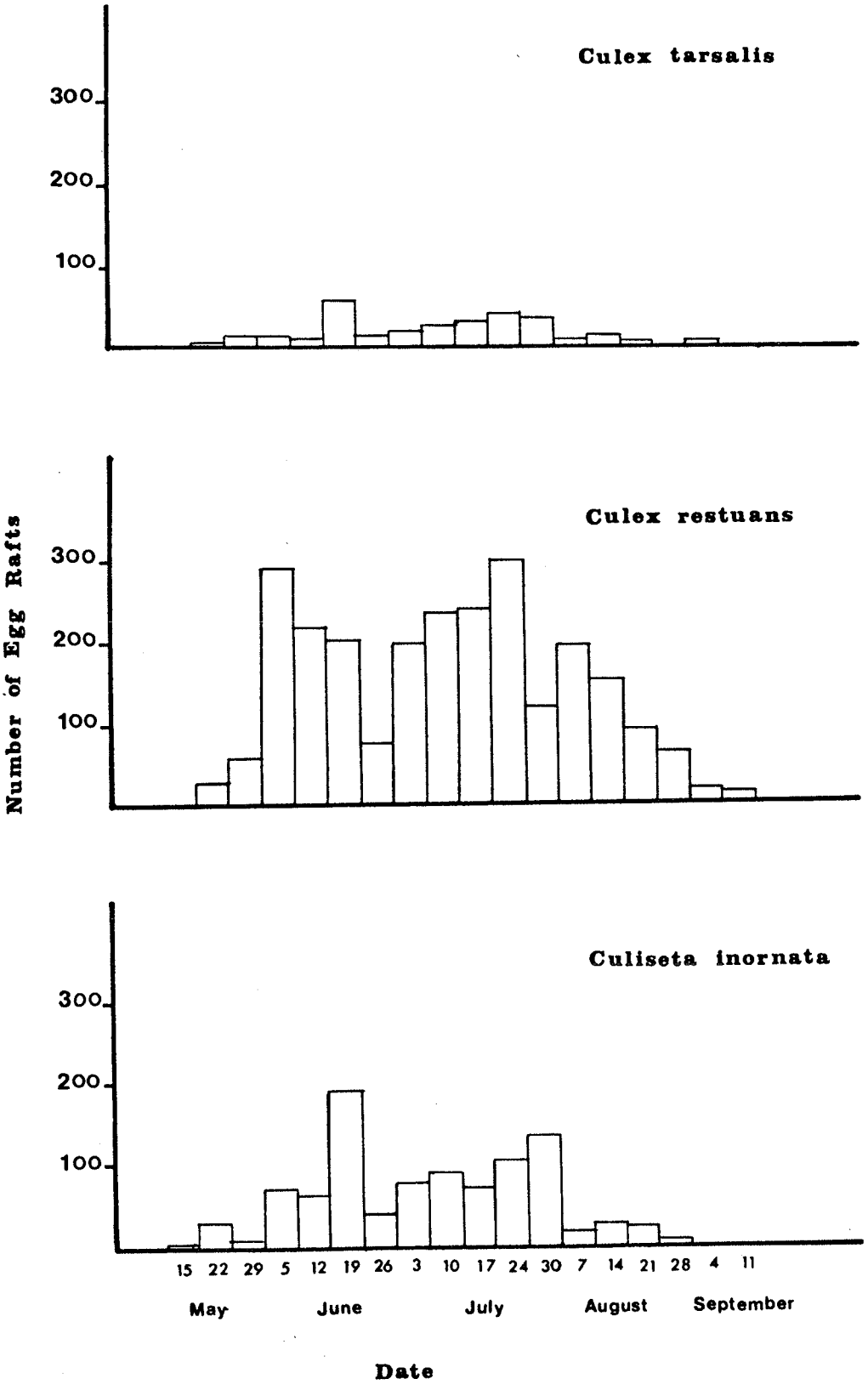

Culex tarsalis
300
200
100
Culex restuans
300
200
100
Number of Egg Rafts
Culiseta inornata
300
200
100
15 22 29 5 12 19 26 3 10 17 24 30 7 14 21 28 4 11
May
June
July
August
September
Date

Fig. 3. Total number of egg rafts laid each day by Culex tarsalis, Culex restuans and Culiseta inornata in three pools in 1981.

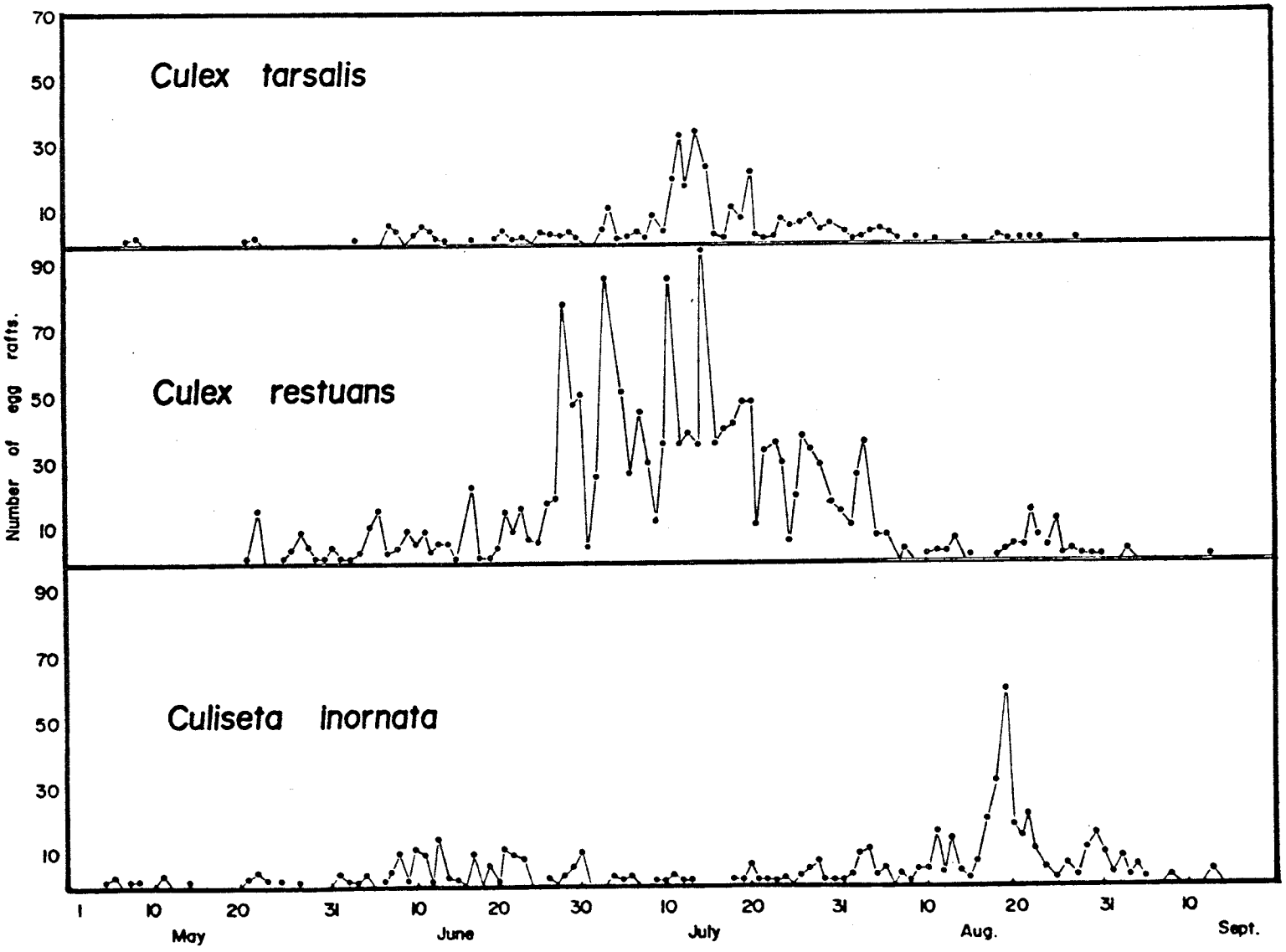

Culex tarsalis
Culex restuans
Culiseta inornata
Number of egg rafts.
70
50
30
10
90
70
50
30
10
90
70
50
30
10
1
10
20
31
10
20
30
10
20
31
10
20
31
10
May
June
July
Aug.
Sept.

Fig. 4. Total number of egg rafts laid each week by *Culex tarsalis*, *culex restuans* and *Culiseta inornata* in three pools in 1981.

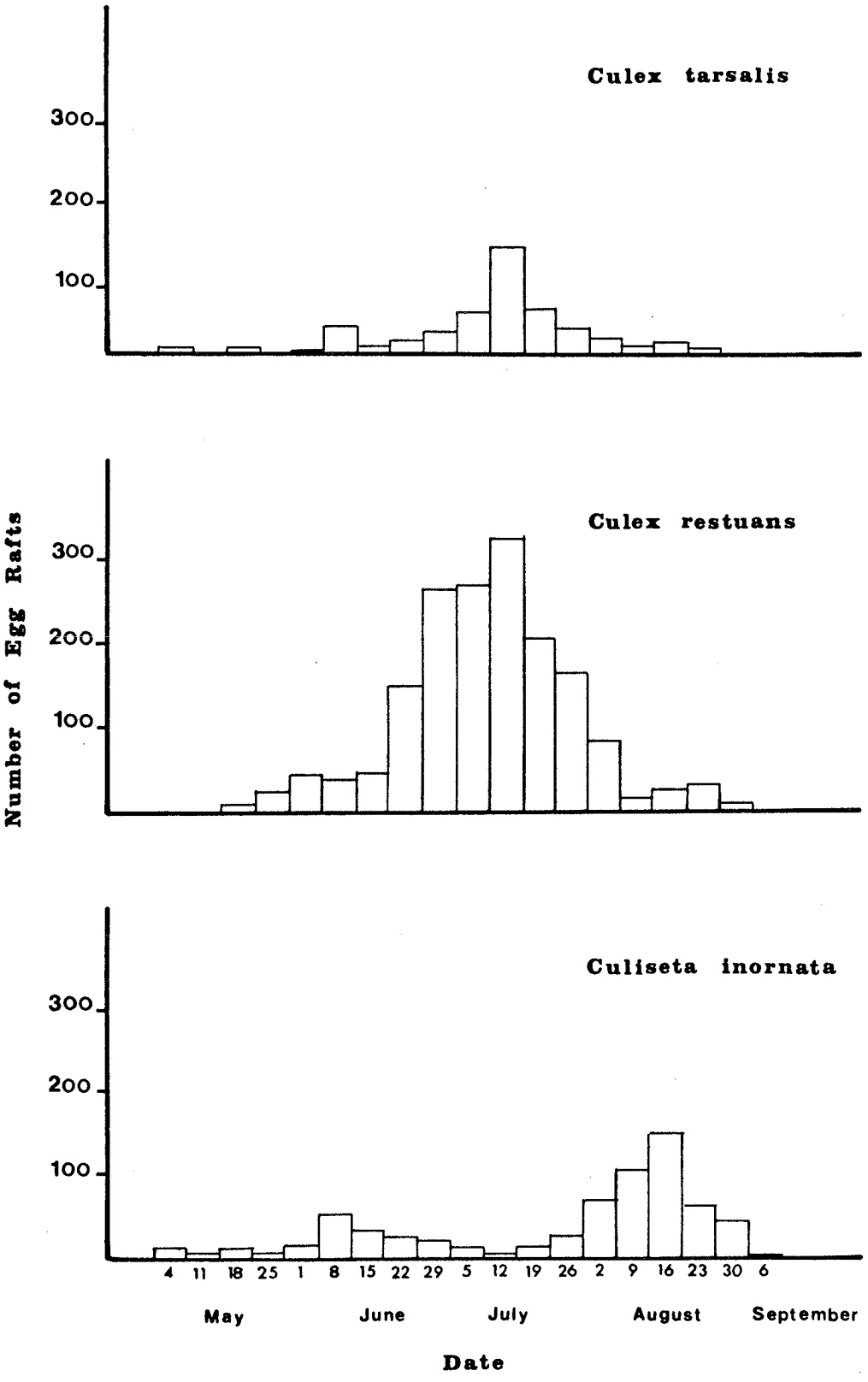

Culex tarsalis
300
200
100
Culex restuans
300
200
100
Number of Egg Rafts
Culiseta inornata
300
200
100
4 11 18 25 1 8 15 22 29 5 12 19 26 2 9 16 23 30 6
May
June
July
August
September
Date

Table 1. Correlation coefficients of daily ovipositon activity of *Culex tarsalis*, *Culex restuans* and *Culiseta inornata* with weather parameters.

Species	Correlation Coefficient	Weather Parameters
C. tarsalis	0.35907	Sunset temperature Sunrise windspeed
C. restuans	0.48390	Sunrise dewpoint Sunset windspeed Sunset temperature
C. inornata	0.36930	Sunset temperature Percentage of time with precipitation around sunrise.

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Table 2. Number of Culex tarsalis eggs per raft in 1980 and 1981.

Collection Period	Number of eggs/ raft ($\bar{x} \pm$ S.D.)	Range	Sample Size (n)
1980			
May	188.4 ± 67.6 a[1]	88-342	12
June	202.8 ± 69.0 a	101-367	41
July	275.2 ± 125.7 b	71-605	92
August	303.2 ± 119.3 b	61-485	24
1981			
May	209.6 ± 62.0 a	116-277	6
June	222.1 ± 69.3 a	96-356	52
July	258.8 ± 90.0 a	48-476	225
August	261.2 ± 94.8 a	77-479	32

1/ Means followed by different letters are significantly different at the 5% level by one-way analysis of variance and Duncan's New multiple range test.

Table 3. Number of *Culex restuans* eggs per raft in 1980 and 1981.

Collection Period	Number of eggs/ raft ($\bar{X} \pm$ S.D.)	Range	Sample Size (n)
1980			
May	155.0 ± 61.4 a[1]	45-231	32
June	161.8 ± 65.6 a	43-349	237
July	182.4 ± 70.7 b	15-384	219
August	198.9 ± 68.2 c	62-459	242
September	200.1 ± 55.8 bc	105-283	54
1981			
May	146.9 ± 40.4 a	66-225	36
June	173.5 ± 65.5 b	42-333	188
July	199.8 ± 67.6 c	33-386	298
August	203.1 ± 79.8 c	48-449	127

1/ Means followed by different letters are significantly different at the 5% level by one-way analysis of variance and Duncan's New multiple range test.

Table 4. Number of Culiseta inornata eggs per raft in 1980 and 1981.

Collection Period	Number of eggs/ raft($\bar{x}\pm$S.D.)	Range	Sample Size (n)
1980			
May	161.4 ± 55.9 a[1]	43-300	210
June	186.5 ± 68.9 b	42-300	151
July	228.6 ± 86.4 c	19-624	222
August	220.5 ± 90.7 c	20-408	103
1981			
May	197.6 ± 69.1 a	64-319	25
June	201.8 ± 71.7 a	14-414	131
July	216.7 ± 75.2 a	68-367	40
August	197.1 ± 70.3 a	42-359	190

1/ Means followed by different letters are significantly different at the 1% level by one-way analysis of variance and Duncan's New mulitple range test. Means for 1981 data are not significant at the 5% level.

The Effect of Temperature, Photoperiod and Density on the Immature Stages of *Culex tarsalis* Coquillett, *Culex restuans* Theobald and *Culiseta inornata* (Williston) (Diptera:Culicidae) in Manitoba

INTRODUCTION

Culex tarsalis Coquillett is the primary vector of Western equine encephalitis (WEE) virus in Manitoba (Henderson et al 1979; Sekla et al. 1980). The virus has also been isolated from Culex restuans Theobald and Culiseta inornata in Manitoba and these mosquitoes may be involved in the disease cycle (Norris 1946; Sekla et al. 1980).

All three species overwinter as inseminated adult females. They emerge in the spring and, in Manitoba, begin oviposition in May. The number of generations per year and the size of the population is dependent upon many factors, including the overwintering success of the females, the availability of hosts, breeding sites, competition, predation and weather. One important factor is the rate of larval and pupal development and survival of the larvae to adults. The developmental rate and survival of the larvae depends on temperature, food availability and density (Hagstrum and Workman 1971; Hanec and Brust 1967; Tauthong and Brust 1977; Trpis 1972; Wada 1963).

Development of C. tarsalis has been studied both in the laboratory (Brennan and Harwood 1953) and the field (Edmunds 1955). Development time to pupae in the laboratory has ranged from 25.3 to 11.7 days at 20°C and 30°C, respectively, depending on food availability (Hagstrum and Workman 1971).

Culex restuans showed a broad tolerance of temperatures when studied in the laboratory (Shelton 1973) as development times ranged

from 25.3 to 6.3 days at 12°C and 29°C, respectively.

Several authors report that C. inornata larvae are tolerant of low temperatures (McLintock 1964; Brust 1967; Hanec and Brust 1967). The optimum temperature for rearing this species is about 20°C.

In this study, the effect of three different temperatures, two photoperiods and two densities on the development time and survival of the immature stages of three potential vectors of WEE in Manitoba was examined in the laboratory. Development times and survival under field conditions and at two different densities were also studied.

MATERIALS AND METHODS

Laboratory Experiments

The effects of larval density, rearing temperature and photoperiod on the development time of *C. tarsalis*, *C. restuans* and *C. inornata* from first instar larvae to adults were studied in the laboratory. Larvae of *C. tarsalis* and *C. inornata* were obtained from laboratory colonies. The *C. tarsalis* colony originated from Glenlea, Manitoba and the *C. inornata* colony originated from Winnipeg, Manitoba. *Culex restuans* larvae were obtained from field-collected egg rafts.

Larvae less than 12 hours old were placed in plastic pans (21x32x7 cm) in 1 litre of dechlorinated tap water and reared in incubators at temperatures of 15°, 20° and 25°C (± 1.0°C), at photoperiods of 16L:8D and 8L:16D and at densities of 50 and 500 larvae per pan. The pans were covered with plastic lids. Five replicates were used for the lower density and 3 replicates were used for the higher density.

The pans were checked daily and the number of individuals passing from one instar or stage to the next was recorded in the low density replicates. In the high density replicates the number of fourth instar larvae which moulted into pupae and the number of emerging adults were recorded. Larvae were fed daily with an excess of liver powder/water suspension.

The mean development time for each stage was calculated using a weighted mean. The weighting factor was the number of individuals passing into the next instar or stage on day X_n, where X_n was the number of days from the start of the trial. The percent mortality was calculated based on the number of individuals passing into the next stage as compared to the number of first instar larvae originally introduced into the pan. The development data were analyzed by factorial analysis and Duncan's New multiple range test. The mortality data was analyzed using an arcsin transformation with factorial analysis and Duncan's New multiple range test.

Field Experiments

The development of these three species was also studied in the field during the summer of 1981. Plastic pans (21x32x7 cm) containing either 50 or 500 larvae were covered with plastic lids and placed in a wooded area in west Winnipeg. Three replicates were used for each density. Pans were placed in the area at two week intervals to study the changes in development time throughout the summer.

The pans were checked daily and the numbers of fourth instar larvae which molted into pupae and the numbers of emerging adults were recorded. The larvae were fed daily with an excess of liver powder/water suspension. The larvae were obtained in the same manner as the laboratory studies.

The development time from first instar larvae to pupae and to the adult stage was calculated using a weighted mean. The data were analyzed using factorial analysis and Duncan's New multiple range test. The percent mortality was also calculated as above, transformed into arcsin and analyzed using factorial analysis and Duncan's New muliple range test.

The mean air temperature for the period from the start of the trial until 50% of the adults emerged was calculated using the mean daily air temperature measured at the Winnipeg International Airport by Environment Canada.

RESULTS AND DISCUSSION

Laboratory Experiments

Development times for the immature stages of the three species are summarized (Tables 1,3,5). Comparisons between these results and those reported in the literature are difficult because different rearing techniques were used and the findings were often reported in a variety of forms.

All three species completed development to the adult stage at each of the temperatures. With all three species, factorial analysis showed no difference ($P<.05$) in development times between the two photoperiods studied. Therefore, the photoperiod data were pooled for each temperature.

The development times of *C. tarsalis* were significantly different ($P<.01$) among the three temperatures at the low density and the high density (Table 1). The shortest development time was 12.8 ± 0.6 days at 25°C under low density conditions. The longest time was 45.9 ± 1.0 days at 15°C under high density conditions. Hagstrum and Workman (1971) found that at 20°C and with an excess of food, *C. tarsalis* larvae developed to pupae in 14.6 days. In this study, development to pupae required 13.8 days at 20°C.

Development times for C. restuans were significantly different ($P<.01$) among the three temperatures at the low density (Table 3). At the high density there was no significant difference between the development times at 20° and 25°C. The shortest development time for C. restuans was 8.1±1.2 days at 25°C under low density conditions. The longest development time was 31.8±1.0 days at 15°C under high density conditions. Shelton (1973) found that, at 20° and 26°C, C. restuans required 6.5 and 10.0 days, respectively, to complete development. In this study C. restuans larvae developed in 8.1 and 11.6 days at 20° and 25°C, respectively.

There were significant differences ($P<.01$) among development times of C. inornata at the three temperatures and the low density, but not at the high density (Table 5). C. inornata larvae needed 13.6±1.0 days to develop to adults at 25°C under low density conditions. At 15°C, under high density conditions, immature development required 40.9±1.1 days. Hanec and Brust (1967) found that C. inornata required 22 days to reach the pupal stage at 15°C. In this study, development time to pupae was 23.8 days at 15°C. At 21° and 26°C, Hanec and Brust (1967) found the development time was 15 and 11.5 days, respectively. C. inornata required 17 and 10.9 days at 20° and 25°C, respectively in this study (Table 5). The differences between studies could be due to the type and availability of food.

Mortality of C. tarsalis was significantly different ($P<.01$) at the three temperatures and the low density (Table 2). The highest survival occurred at 25°C when mortality to the adult stage was only 7.4%. At 15°C, 63.8% mortality occurred. It appears that C. tarsalis larvae are not tolerant of cool temperatures. This may be partially due to the use of material from a laboratory colony rather than field collected larvae. The laboratory colony may have become less tolerant of cooler temperatures after many generations of rearing at the same temperature.

At the high density, mortality of C. tarsalis was over 75% at all three temperatures. There were no significant differences between the mortality at the three temperatures.

Mortality of C. restuans larvae was very low for all three temperatures at the low density (Table 4). At the high density, mortality was lowest at 15°C and significantly different ($P<.01$) from the mortality at 20° and 25°C.

Mortality of C. inornata at the low density was 6.4% and 8.4% at 15° and 20°C, respectively (Table 6). However, at 25°C, mortality was 51.2%. At the high density, mortality was 32.8% at 15°C and significantly different ($P<.01$) from 60.4% and 51.2% at 20° and 25°C, respectively.

With all three species, there was a significant interaction between temperature and density on development time (Fig. 1). At 15°C, development time for the three species under high density

conditions was substantially greater than development times under low density conditions. At 20° and 25°C, the differences were not as pronounced. However, the differences were still significant for C. tarsalis and C. restuans at these temperatures (Tables 1 and 3). At 25°C, there were no significant differences ($P<.05$) between C. inornata development times under high and low density conditions (Table 5). This interaction can be partially explained by examining the mortality of the immature stages (Tables 2,4 and 6).

At 15°C, under low density conditions, mortality of C. tarsalis occurred primarily in the fourth instar larval and pupal stages (Table 2). If this also occurred under high density conditions, then the larvae would be affected by the higher density in the earlier instars. At 25°C and the lower density, mortality was more evenly distributed throughout the immature stages. Under high density conditions and high temperatures, if most of the mortality occurred in the earlier instars, the effects of the high density would be reduced and development time would be shorter. When Hagstrum and Workman (1971) reared C. tarsalis at 20° and 30°C, they found that mortality was evenly distributed throughout all four larval instars.

Under high density conditions, C. restuans and C. inornata mortality at 15°C was significantly lower than at 20° and 25°C (Tables 4 and 6). The effects of high density would be more pronounced at the lower temperature, resulting in a longer development time. At 20° and 25°C, mortalities were higher, the effects of the high density not as pronounced and development times shorter.

To summarize, photoperiod has no effect on the development times of C. tarsalis, C. restuans and C. inornata. Rearing the larvae at high densities causes an increase in the development time from first instar to adult. There was a significant interaction between temperature and density on the development times of the three species. The immature stages of C. restuans and C. inornata are more tolerant of cool temperatures than C. tarsalis and develop faster than C. tarsalis at 15°C.

Mortality of C. tarsalis was affected by temperature when the larvae were reared at the low density. The highest mortality occurred at 15°C. Mortality of C. restuans was highest at 25°C at both low and high densities. Mortality of C. inornata was also highest at 25°C when the larvae were reared at the low density.

Field Experiments

Laboratory experiments provide a basic understanding of immature development and survival however, the larvae may respond differently to natural field conditions when temperature and photoperiod fluctuate daily.

Under field conditions, C. tarsalis development time ranged from

13.2 days for larvae started on 27 July and the low density to 32.6 days for larvae started on 26 August and the high density. There were significant differences ($P<.01$) among development times under low and high densities and among development times at different commencement dates. The varying temperatures during development time would be the primary cause of differences between dates. The higher density generally caused an increase in the development time, as was found in the laboratory experiments.

The average development times for the summer ranged from 19.8 days for the low density to 25.3 days for the high density. Assuming that ovarian development, blood feeding, egg maturation and embryonic development would require at least 10 days to two weeks, the minimum time for development from egg to egg in the field would be about 30 days under optimum temperatures, with available food and no predation. Field development is probably longer than this as conditions are not often optimum. Based on this estimate, only three generations would be likely for C. tarsalis which commences oviposition by the end of May (Gallaway 1983).

There were no significant differences between C. tarsalis mortalities at different commencement dates, indicating that temperature did not affect mortality in the field (Table 8). Density did affect mortality as there was a significant difference ($P<.01$) between the average percent mortality for the entire season at high and at low densities.

The development time of C. restuans was shorter than that of C. tarsalis (Table 9). This was expected, based on the laboratory results. Development ranged from 12.3 days for larvae started on 2 July and low density to 32.1 days for larvae started on 26 August and high density. The average development time for the summer ranged from 16.3 days at the low density to 22.8 days at the high density. Development time was affected by the date of commencement and by density. Madder (1980) found that C. restuans required 11.1 days to develop from first instar larvae to adults under field conditions in southern Ontario.

Assuming that C. restuans required an additional 10 days to two weeks for completion of the life cycle, a minimum of 26 to 33 days would be needed to complete development from egg to egg. Only three generations would be likely for C. restuans in Manitoba which commences oviposition by the end of May (Gallaway 1983).

Mortality of C. restuans changed throughout the summer (Table 10). Significant differences ($P<.01$) were found between the percent mortality of larvae reared at different densities and between the mortality at different commencement dates and the low density. At the high density no significant differences were found between the different dates. Mortality at the low density averaged 26.1%, while at the high density mortality averaged 75.3%.

The development of C. inornata in the field ranged from 16.1 days for larvae started on 27 July and low density to 30 days for larvae

started on 26 August and high density (Table 11). The average development time for the entire summer ranged from 20 days at the low density to 21.4 days at the high density. C. inornata would likely require at least 30 days to complete development from egg to egg under optimum conditions. Considering that C. inornata commences oviposition in May, three generations of C. inornata would be possible in Manitoba (Gallaway 1983).

Mortality of C. inornata was significantly different ($P<.01$) between dates of commencement and the high and low density. The average mortality at the low density was 59.1% as compared to 66.8% at the high density.

The development time and survival of the immature stages of all three species were affected by the date the trial commenced and the density of the larvae. Larval density under natural conditions will vary from breeding site to breeding site. Therefore, estimating development times in the field should consider both the temperature during the summer and the density of the larvae in the breeding site. These studies provide an average development time throughout the summer based on two different densities which could occur in the field. The results can be used to estimate development times in other studies in Manitoba.

Table 1. Development time of Culex tarsalis reared in the laboratory at three temperatures, i.e, 15°, 20° and 25°C and low (50 larvae/pan) and high (500 larvae/pan) density.

Temperature (°C)	Stage	Development Time (d)			
		Low Density (50 L/pan)		High Density (500 L/pan)	
		(x± S.D.)[1]	Range	(x± S.D.)[2]	Range
15	Instar I	-	-	-	-
	II	5.1 ± 0.4	4-8	-	-
	III	10.6 ± 0.5	8-14	-	-
	IV	16.4 ± 0.4	12-22	-	-
	Pupa	26.7 ± 0.7	22-32	31.3 ± 0.7	27-36
	Adult	34.6 ± 1.5 a[3]	31-45	45.9 ± 1.0 a[3]	40-51
20	Instar I	-	-	-	-
	II	3.0 ± 0.6	2-4	-	-
	III	6.0 ± 1.8	4-9	-	-
	IV	9.0 ± 2.4	6-13	-	-
	Pupa	13.8 ± 2.1	10-21	16.1 ± 1.8	13-24
	Adult	18.2 ± 1.2 b	12-21	21.1 ± 1.0 b	15-27
25	Instar I	-	-	-	-
	II	2.1 ± 0.1	2-3	-	-
	III	4.4 ± 0.3	4-6	-	-
	IV	6.6 ± 0.4	6-9	-	-
	Pupa	11.3 ± 0.6	9-18	17.0 ± 0.3	14-20
	Adult	12.8 ± 0.6 c	11-19	18.5 ± 0.3 c	16-23

1/ n=10
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by factorial analysis and Duncan's New multiple range test.

Table 2. Percent mortality of Culex tarsalis reared in the laboratory at three temperatures, i.e., 15°, 20° and 25°C, and low (50 larvae/pan) and high (500 larvae/pan) density.

Temperature °C	Stage	Mortality (%) Low density (50 L/pan) (x± S.D.) 1	Mortality (%) High Density (500 L/pan) (x± S.D.) 2
15	Instar I	6.0 ± 5.9	-
	II	12.4 ± 4.6	-
	III	19.0 ± 4.0	-
	IV	26.2 ± 4.3	57.8 ± 19.7
	Pupa	63.8 ± 12.4 a3	78.3 ± 12.2 a3
20	Instar I	0.0	-
	II	1.4 ± 2.1	-
	III	8.6 ± 9.5	-
	IV	18.0 ± 15.1	64.9 ± 15.0
	Pupa	21.8 ± 17.2 b	76.4 ± 10.8 a
25	Instar I	0.0	-
	II	0.6 ± 1.4	-
	III	3.0 ± 5.3	-
	IV	5.6 ± 7.5	72.7 ± 5.6
	Pupa	7.4 ± 8.2 c	79.2 ± 2.5 a

1/ n=10
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by anaylsis of variance and Duncan's New multiple range test.

Table 3. Development time of Culex restuans reared in the laboratory at three temperatures, i.e, 15°, 20° and 25°C and low (50 larvae/pan) and high (500 larvae/pan) density.

Temperature (°C)	Stage	Development Time (d)			
		Low Density (50 L/pan)		High Density (500 L/pan)	
		(x± S.D.)[1]	Range	(x± S.D.)[2]	Range
15	Instar I	-	-	-	-
	II	3.4 ± 0.1	2-5	-	-
	III	5.9 ± 0.7	5-9	-	-
	IV	9.0 ± 0.2	8-11	-	-
	Pupa	15.9 ± 0.3	15-21	25.8 ± 0.8	23-31
	Adult	22.0 ± 0.9 a[3]	19-27	31.8 ± 1.0 a[3]	30-40
20	Instar I	-	-	-	-
	II	1.4 ± 0.1	1-2	-	-
	III	3.1 ± 0.2	2-5	-	-
	IV	4.8 ± 0.2	4-6	-	-
	Pupa	7.9 ± 0.7	7-11	9.4 ± 0.6	8-12
	Adult	11.6 ± 0.3 b	9-17	13.2 ± 0.9 b	11-16
25	Instar I	-	-	-	-
	II	0.8 ± 0.1	0-1.5	-	-
	III	2.0 ± 0.1	1-2.5	-	-
	IV	4.2 ± 0.2	3-5	-	-
	Pupa	6.3 ± 1.3	5-8	7.6 ± 0.4	6-9
	Adult	8.1 ± 1.2 c	7-9	12.8 ± 0.8 b	9-15

1 n=10
2 n=6
3 Means followed by different letters are significantly different at the 1% level by factorial analysis and Duncan's New Multiple range test.

Table 4. Percent mortality of Culex restuans reared in the laboratory at three temperatures, i.e., 15°, 20° and 25°C, and low (50 larvae/pan) and high (500 larvae/pan) density.

Temperature °C	Stage	Mortality (%) Low density (50 L/pan) (x± S.D.) 1	Mortality (%) High Density (500 L/pan) (x± S.D.) 2
15	Instar I	0.0	-
	II	0.0	-
	III	0.2 ± 0.6	-
	IV	0.6 ± 1.3	40.0 ± 3.9
	Pupa	1.9 ± 2.8 a3	46.7 ± 4.7 a3
20	Instar I	0.2 ± 0.6	-
	II	0.8 ± 1.3	-
	III	1.8 ± 1.0	-
	IV	4.4 ± 3.2	59.6 ± 6.6
	Pupa	5.0 ± 3.8 b	72.8 ± 6.9 b
25	Instar I	0.0	-
	II	0.2 ± 0.6	-
	III	1.0 ± 1.0	-
	IV	2.4 ± 2.5	52.6 ± 4.0
	Pupa	6.4 ± 5.6 b	76.2 ± 4.7 b

1 n=10
2 n=6
3 Means followed by different letters are significantly different at the 1% level by anaylsis of variance and Duncan's New multiple range test.

Table 5. Development time of Culiseta inornata reared in the laboratory at three temperatures, i.e, 15°, 20° and 25°C and low (50 larvae/pan) and high (500 larvae/pan) density.

Temperature (°C)	Stage	Development Time (d) Low Density (50 L/pan) (x± S.D.) [1]	Range	High Density (500 L/pan) (x± S.D.) [2]	Range
15	Instar I	-	-	-	-
	II	6.9 ± 0.6	3-9	-	-
	III	10.7 ± 0.6	7-12	-	-
	IV	14.3 ± 0.5	11-17	-	-
	Pupa	19.7 ± 1.3	16-23	32.8 ± 2.9	25-39
	Adult	23.8 ± 1.4 a3	19-27	40.9 ± 1.1 a3	35-45
20	Instar I	-	-	-	-
	II	3.5 ± 0.5	2-6	-	-
	III	7.8 ± 0.3	6-11	-	-
	IV	10.4 ± 0.5	8-12	-	-
	Pupa	17.1 ± 1.5	11-21	20.1 ± 1.3	16-26
	Adult	20.6 ± 1.5 b	13-25	24.6 ± 1.2 b	19-30
25	Instar I	-	-	-	-
	II	3.1 ± 0.3	2-5	-	-
	III	4.7 ± 0.3	3-6	-	-
	IV	6.7 ± 0.7	5-10	-	-
	Pupa	10.9 ± 1.0	8-14	11.9 ± 1.1	8-17
	Adult	13.6 ± 1.0 c	11-17	13.4 ± 0.7 c	11-20

1/ n=10
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by factorial analysis and Duncan's New Multiple range test.

Table 6. Percent mortality of Culiseta inornata reared in the laboratory at three temperatures, i.e., 15°, 20° and 25°C, and low (50 larvae/pan) and high (500 larvae/pan) density.

Temperature °C	Stage	Mortality (%) Low density (50 L/pan) (x± S.D.) 1	Mortality (%) High Density (500 L/pan) (x± S.D.) 2
15	Instar I	3.6 ± 2.1	-
	II	4.0 ± 2.6	-
	III	6.2 ± 3.0	-
	IV	6.2 ± 3.0	30.4 ± 6.2
	Pupa	6.4 ± 4.4 a3	32.8 ± 6.9 a3
20	Instar I	0.2 ± 0.6	-
	II	2.2 ± 2.6	-
	III	4.0 ± 3.7	-
	IV	6.2 ± 3.0	40.5 ± 5.3
	Pupa	8.4 ± 3.1 a	60.4 ± 10.9 b
25	Instar I	1.4 ± 1.4	-
	II	5.0 ± 4.6	-
	III	16.1 ± 7.2	-
	IV	41.6 ± 18.1	50.2 ± 15.0
	Pupa	51.2 ± 30.0 b	51.2 ± 24.9 b

1/ n=10
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.

Table 7. Development time of Culex tarsalis reared at low (50 larvae/pan) and high (500 larvae/pan) density under field conditions in 1981.

Date of Commencement	Development Time (d)					
	Low Density (50 L/pan)		High Density (500 L/pan)		Combined	
	x± S.D.[1]	Mean Air Temp.(°C)	x± S.D.[1]	Mean Air Temp.(°C)	x ± S.D.[2]	
May 14	25.8 ± 1.0 a[3]	15.2	28.9 ± 1.0 a[3]	15.3	27.3 ± 1.9 a[3]	
June 2	25.0 ± 0.3 a	16.4	30.9 ± 0.4 ad	16.4	28.0 ± 1.9 ab	
June 15	18.2 ± 0.6 b	19.7	24.5 ± 1.0 b	20.8	21.4 ± 3.5 c	
July 2	14.4 ± 1.4 c	23.6	19.8 ± 1.4 c	21.3	17.1 ± 3.2 d	
July 13	14.4 ± 1.0 c	19.5	20.5 ± 2.6 c	19.5	17.5 ± 3.8 d	
July 27	13.2 ± 0.4 c	19.4	20.3 ± 1.0 c	19.4	16.8 ± 4.0 d	
August 12	19.6 ± 2.0 b	21.1	24.6 ± 0.8 b	19.7	22.1 ± 3.0 c	
August 26	27.6 ± 1.1 a	15.1	32.6 ± 3.2 ad	14.2	30.1 ± 3.5 b	
Total	19.77 ± 5.5 [4]		25.3 ± 5.0 [4]			

1/ n=3
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.
4/ Means are significantly different at the 1% level by t-test.

Table 8. Percent mortality of Culex tarsalis reared at low (50 larvae/pan) and high (500 larvae/pan) density under field condition in 1981.

Date of Commencement	Mortality to Adult Stage (%)		
	Low Density (500 1/pan) x ± S.D.1	High Density (500 L/pan) x ± S.D.1	Combined x ± S.D.2
May 14	47.3 ± 11.0 [3]	78.0 ± 3.0 [3]	62.7 ± 18.3 [3]
June 2	44.0 ± 5.3	76.7 ± 3.5	60.4 ± 18.4
June 15	64.7 ± 11.0	80.9 ± 3.52	72.8 ± 11.5
July 2	48.0 ± 2.0	83.0 ± 3.5	65.5 ± 19.4
July 13	42.0 ± 8.7	74.7 ± 3.5	65.5 ± 18.8
July 27	35.3 ± 3.1	82.0 ± 3.6	50.5 ± 23.4
August 12	46.3 ± 10.4	80.3 ± 6.7	63.3 ± 20.2
August 26	61.0 ± 10.8	86.7 ± 4.7	73.8 ± 15.9
Total	48.6 ± 11.70 [4]	80.3 ± 5.0 [4]	

1/ n=3
2/ n=6
3/ No significant differences between means at the 5% level by analysis of variance.
4/ Significant difference between means at the 1% level by t-test.

Table 9. Development time of Culex restuans reared at low (50 larvae/pan) and high (500 larvae/pan) density under field conditions in 1981.

Date of Commencement	Development Time (d)					
	Low Density (50 L/pan)		High Density (500 L/pan)		Combined	
	x± S.D.[1]	Mean Air Temp.(°C)	x± S.D.[1]	Mean Air Temp.(°C)	x ± S.D.[2]	
June 2	17.6 ± 1.0 a[3]	16.2	30.4 ± 0.7 ac[3]	16.9	24.0 ± 7.0 ac[3]	
June 15	16.9 ± 0.4 ab	17.7	25.7 ± 1.0 a	19.5	21.3 ± 4.9 a	
July 2	12.3 ± 1.7 b	24.3	18.9 ± 1.2 b	22.4	15.6 ± 3.9 b	
July 13	15.3 ± 0.4 ab	19.7	18.4 ± 0.8 b	19.8	16.8 ± 1.8 b	
July 27	14.2 ± 0.3 ab	19.2	17.8 ± 2.2 b	19.8	16.0 ± 2.4 b	
August 12	13.9 ± 0.3 ab	22.1	16.3 ± 0.6 b	21.9	15.1 ± 1.4 b	
August 26	24.1 ± 2.2 c	16.1	32.1 ± 1.2 c	14.9	28.1 ± 4.7 c	
Total	16.3 ± 3.8[4]		22.8 ± 6.3[4]			

1/ n=3
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.
4/ Means are significantly different at the 1% level by t-test.

Table 10. Percent mortality of Culex restuans reared at low (50 larvae/pan) and high (500 larvae/pan) denisty under field conditions in 1981.

Date of Commencement	Mortality to Adult Stage (%)		
	Low Density (50 L/pan) x ± S.D.1	High Density (500 L/pan) x ± S.D.1	Combined x ± S.D.2
June 2	14.0 ± 7.2 a[3]	69.9 ± 1.5 a[3]	42.0 ± 31.0 a[3]
June 15	14.0 ± 3.5 a	75.1 ± 4.9 a	44.6 ± 33.7 a
July 2	28.7 ± 3.1 abc	71.0 ± 3.9 a	50.0 ± 23.4 ab
July 13	22.7 ± 4.2 ab	77.4 ± 4.0 a	50.0 ± 30.2 ab
July 27	34.0 ± 7.2 bc	78.0 ± 9.5 a	56.0 ± 25.3 bc
August 12	40.0 ± 6.9 c	82.3 ± 3.1 a	61.2 ± 23.7 bc
August 26	29.3 ± 4.2 abc	73.3 ± 9.6 a	51.3 ± 25.0 ab
Total	26.1 ± 10.4[4]	75.3 ± 6.5[4]	

1/ n=3
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New mulitple range test.
4/ Means are significantly different at the 1% level by t-test.

Table 11. Development time of Culiseta inornata reared at low (50 larvae/pan) and high (500 larvae/pan) density under field conditions in 1981.

Date of Commencement	Development Time (d)					
	Low Density (50 L/pan)		High Density (500 L/pan)		Combined	
	x± S.D.[1]	Mean Air Temp.(°C)	x± S.D.[1]	Mean Air Temp.(°C)	x ± S.D.[2]	
May 14	23.5 ± 0.7 a[3]	14.6	27.8 ± 1.4 a[3]	14.7	25.7 ± 2.6 a[3]	
June 2	23.0 ± 0.5 a	16.1	24.0 ± 0.8 b	16.1	23.5 ± 0.8 b	
June 15	19.7 ± 0.8 b	18.4	21.6 ± 0.8 c	19.1	20.7 ± 1.2 c	
July 2	16.6 ± 0.8 c	23.2	16.8 ± 0.4 d	23.2	16.7 ± 0.6 d	
July 13	17.2 ± 0.4 c	19.8	17.3 ± 1.0 d	19.8	17.2 ± 0.7 d	
July 27	16.1 ± 0.6 c	19.5	16.6 ± 0.3 d	19.5	16.4 ± 0.5 d	
August 12	16.2 ± 0.4 c	21.9	16.7 ± 0.6 d	21.8	16.4 ± 0.5 d	
August 26	28.1 ± 0.4 d	15.4	30.0 ± 2.1 a	15.1	29.0 ± 1.7 e	
Total	20.0 ± 4.2[4]		21.4 ± 5.2[4]			

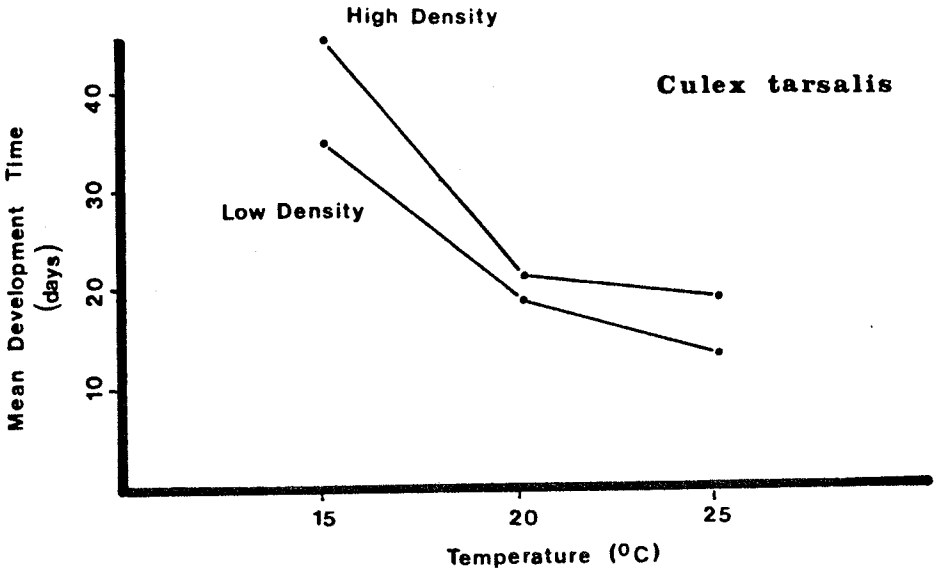
1/ n=3
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.
4/ Means are significantly different at the 1% level by t-test.

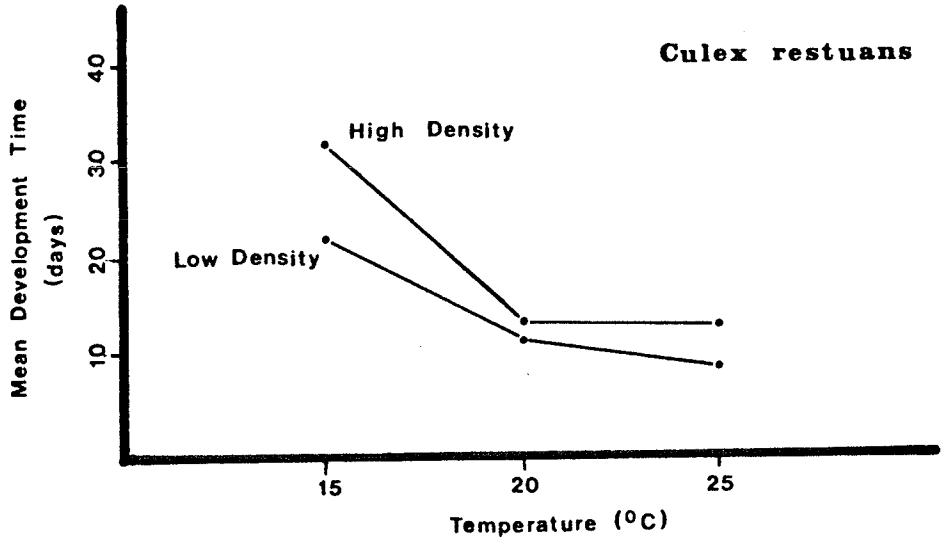
Table 12. Percent mortality of Culiseta inornata reared at low(50 larvae/pan) and high (500 larvae/pan) density under field conditions in 1981.

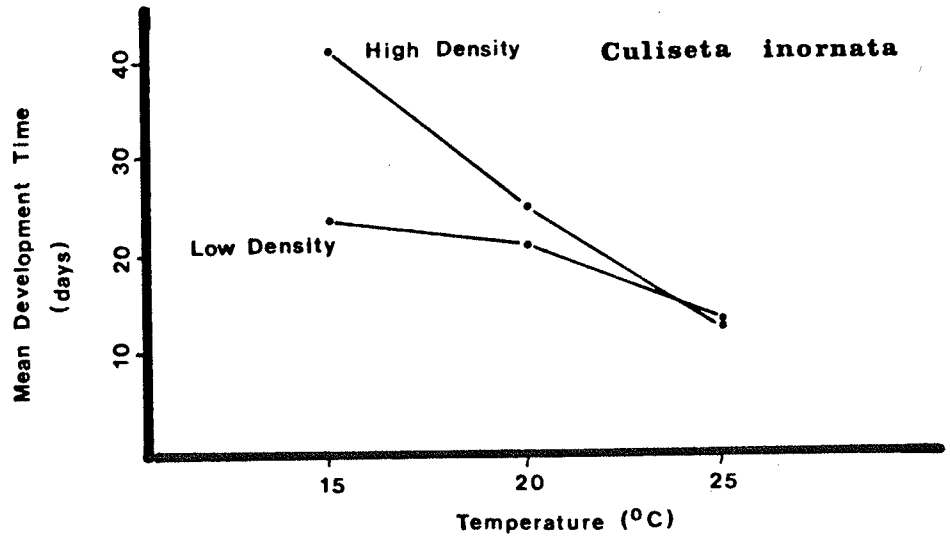
Date of Commencement	Mortality (%)		
	Low Density (50 L/pan) x ± S.D.1	High Density (500 L/pan) x ± S.D.1	Combined x ± S.D.2
May 14	43.3 ± 13.0 a[3]	79.7 ± 6.1 a[3]	61.5 ± 21.9 a[3]
June 2	59.3 ± 14.0 b	80.3 ± 5.0 a	69.8 ± 14.9 b
June 15	49.3 ± 4.2 c	77.4 ± 3.9 ab	72.8 ± 11.5 c
July 2	67.3 ± 12.9 b	87.7 ± 9.5 c	77.5 ± 15.0 d
July 13	76.6 ± 5.0 d	84.7 ± 7.0 d	80.7 ± 7.0 d
July 27	66.0 ± 7.2 b	75.3 ± 7.5 b	70.7 ± 8.3 b
August 12	57.3 ± 10.1 e	77.7 ± 7.4 be	67.5 ± 13.6 e
August 26	53.3 ± 1.2 f	80.0 ± 3.0 b	66.5 ± 14.6 e
Total	59.1 ± 13.0[4]	66.8 ± 24.9[4]	

1/ n=3
2/ n=6
3/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New mulitple range test.
4/ Means are significantly different at the 1% level by t-test.

Fig. 1 Mean development time for Culex tarsalis, Culex restuans and Culiseta inornata when reared at three different temperatures and two different densities in the laboratory.


High Density
Culex tarsalis
Low Density
Mean Development Time (days)
10
20
30
40
15
20
25
Temperature (°C)


Culex restuans
High Density
Low Density
Mean Development Time (days)
10
20
30
40
15
20
25
Temperature (°C)


High Density
Culiseta inornata
Low Density
Mean Development Time (days)
10
20
30
40
15
20
25
Temperature (°C)

The Effect of Temperature, Photoperiod and Density on Ovarian Development of *Culex tarsalis* Coquillett, *Culex restuans* Theobald and *Culiseta inornata* (Williston) (Diptera:Culicidae) in Manitoba

INTRODUCTION

In Manitoba, recent outbreaks of Western Equine Encephalitis (WEE) have occurred in 1975, 1977 and 1981 (Medovy 1976; Sekla 1976; Sekla 1982). Culex tarsalis Coquillett is considered the primary vector of the disease in Manitoba (Henderson et al. 1979; Sekla et al. 1980). The virus has also been isolated from Culex restuans Theobald (Norris 1946; Sekla et al 1980) and Culiseta inornata (Williston) (Sekla et al. 1980) and these species may also be involved in the disease cycle.

Culex tarsalis, C. restuans and C. inornata overwinter as inseminated females in ovarian diapause. Females in diapause do not seek a blood meal (Mitchell 1981) and ovarian development is arrested until females become active again in the spring (Clements 1963).

During recent outbreaks of WEE, the virus transmission rate has been highest in late July and early August (Sekla 1982). Widespread aerial mosquito adulticiding has been conducted over Manitoba communities in late July or early August to control the vectors. Females emerging late in the summer after adulticiding, have been assumed to enter ovarian diapause, would not be seeking a blood meal and therefore would not be transmitting the virus. However, the onset of diapause in these species has not been studied in Manitoba.

This study was designed to determine the criteria to distinguish between diapausing and non-diapausing females of C.tarsalis, C. restuans and C. inornata in Manitoba. The effect of temperature, photoperiod and larval density on induction of diapause were studied in the laboratory. The onset of diapause in the three species, under natural conditions during the summer of 1981, was also studied.

MATERIALS AND METHODS

Laboratory Experiments

The effects of larval density, rearing temperature and photoperiod on the ovarian development of C. tarsalis, C. restuans and C. inornata were studied in the laboratory. Larvae of C. tarsalis and C. inornata were obtained from a laboratory colony. The C. tarsalis colony originated from Glenlea, Manitoba. The C. inornata colony originated from Winnipeg, Manitoba. C. restuans larvae were obtained from field-collected egg rafts.

Larvae less than 12 hours old were placed in plastic pans (21x32x7 cm) containing 1 litre of tap water and reared in incubators at 15°, 20° and 25° C (± 1°C), photoperiods of 16L:8D and 8L:16D and densities of 50 and 500 larvae per pan. Five replicates were used for the lower density and three replicates were used for the higher density. The pans were checked daily and the larvae were fed with an excess of liver powder/water suspension.

Pupae were removed from the pans and the adults emerged in plexiglas cages (26x26x26 cm). Two moistened paper wicks were placed in the emergence cage, one with a sucrose water solution, and one with water.

Two weeks after emergence, females were either frozen and stored at -20°C or were dissected immediately and the ovaries examined in a physiological saline solution with a compound microscope at 400X or

500X magnification. The follicles were classified according to Christophers (1911), as modified by Watts and Smith (1978), and the follicle and germarium of the five largest follicles were measured.

The mean follicle length and the mean ratio of follicle:germarium length were examined to determine the best type of measurement to define diapause.

Field Experiments

The ovarian development of these three species was also studied in the field during the summer of 1981. Plastic pans (21x32x7 cm) containing either 50 or 500 larvae were placed in a wooded area in west Winnipeg. Pans were placed in the area at two week intervals to study the changes in ovarian development throughout the summer. Three replicates were used for each density.

The larvae were fed daily with a liver powder/water suspension. Pupae were removed daily and the adults emerged in plexiglas cages (26x26x26 cm) which were placed at the same site. Two moistened paper wicks were placed in the emergence cage, one with a sucrose water solution and one with water. The females were dissected, and ovaries were classified and measured as described above.

RESULTS AND DISCUSSION

The criteria for determining diapause in Culex and Culiseta species of mosquitoes has generally been the mean ratio of follicle:germarium length (Spielman and Wong 1973; Madder et al. 1983; Hudson 1979). Some authors use the length of the follicle to distinguish diapause (Eldridge and Bailey 1979; Oda and Kuhlow 1973).

The ratio chosen to distinguish between diapausing and non-diapausing females of a particular species has differed between authors (Spielman and Wong 1973; Madder et al 1983). Madder et al. (1983) used a higher ratio for C. pipiens than Spielman and Wong (1973). Madder et al. (1983) attribute this to a difference in measuring technique. However, this may also reflect a difference in the physiological response of the species from another geographic location. This emphasizes that caution should be used when trying to apply the results from one study to another study in a different area and with different techniques.

No significant differences were found between mean follicle lengths when females were reared at different temperatures, photoperiods and larval densities. This is probably due to the great variation in follicle lengths which occur within females and between females. Part of this variation is due to the dissection technique. Follicle length depends to a great extent on the amount of saline solution beneath the coverslip. As the saline evaporates from beneath

the coverslip the follicle flattens and lengthens.

Measuring the ratio of follicle:germarium length overcomes this variation. In this study differences were found between mean follicle:germarium ratios of females reared at different photoperiods. Therefore, the ratio was used to differentiate diapausing from non-diapausing females.

Culex tarsalis

Laboratory Experiments

When reared at 25°C and 16L:8D photoperiod, the ovaries of C. tarsalis mature to stage 2a-2b and remain at this stage until the females take a blood meal and mature their eggs. This stage is considered the resting stage and ovaries which have not reached the 2a-2b stage are considered to be in the pre-resting stage.

C. tarsalis females reared at a photoperiod of 8L:16D had a greater percentage of females with ovaries in the pre-resting stage (Table 1). At 15°, 20° and 25°C and at both the high and low density, at least 65% of the females dissected had pre-resting stage ovaries. At 16L:8D, 25% or less of the females examined had pre-resting stage ovaries.

Females with pre-resting stage ovaries had a mean ratio of

follicle:germarium length less than 2.5:1, while females with resting stage ovaries had a mean ratio of follicle:germarium length greater than 2.5:1. If one ignores the stage of the follicle and considers only the mean ratio of follicle:germarium length of all the females under each set of conditions, the differences between the two photoperiods are not as clear.

Therefore, both the stage of the follicle and the ratio of follicle:germarium length were chosen as the criteria to distinguish between diapausing and non-diapausing C. tarsalis females. A female was considered to be in diapause if her ovarian follicles were at the pre-resting stage and the mean ratio of follicle:germarium length was less than 2.5:1.

Photoperiod was the main factor affecting the number of females entering diapause. The mean ratios of follicle:germarium length were significantly lower in females reared at 8L:16D than at 16L:8D. Temperature and larval density had no significant effect on the mean ratio of follicle:germarium length.

Autogeny in C. tarsalis was first reported in laboratory colonies in 1958 (Chao 1958; Bellamy and Kardos 1958). In California, Spadoni et al. (1974) found that autogeny rates were highest in July and August. However, Moore (1963) found that autogeny decreased during the summer. Autogeny seems to be related to both photoperiod and temperature (Harwood 1966). At a photoperiod of 16L:8D, a higher proportion of females were autogenous. Moore (1963) found a moderate

correlation between autogenous egg development and water temperature during the larval stages.

In this study, autogeny was found only in females reared in the laboratory at 25°C (Table 1). The percentage of autogenous females was highest at 16L:8D and at the lower density.

Field Experiments

A significant difference between the mean ratio of follicle:germarium lengths of the total females at each rearing period was first noted in females which emerged on 9 August (Table 2). Of the females emerging on 27 July, 20-30% were in diapause. By 9 August 70-75% were in diapause and of the last females to emerge, on September 23, 75% were in diapause.

Autogeny in the field reared females occurred in July, mid-August and the beginning of September (Table 2). Autogeny was highest in females which emerged at the beginning of July. Autogenous females are probably found throughout the summer in Manitoba. The sample size in this study may not have been large enough to detect autogeny at other times.

The percentage of autogenous females in the field is important in the WEE disease cycle. Females which are autogenous will not seek their first blood meal until after the first batch of eggs are laid.

They will not become potential vectors of the disease until they seek their second blood meal which will be later than the autogenous females which emerged at the same time. If more autogenous females are emerging in mid-summer when temperatures are higher and photoperiods are longer, the peak transmission rate of WEE virus may occur later in the summer.

Culex restuans

Laboratory experiments

The resting stage of non-diapausing C. restuans females was also determined to be stage 2a-2b. Females with ovaries at an earlier stage of maturity were considered to have pre-resting stage ovaries.

When C. restuans larvae were reared at photoperiods of 8L:16D, at least 65% of the females had pre-resting stage ovaries and a mean ratio of follicle:germarium length less than 2.25:1 (Table 3). When reared at 16L:8D, 30% or fewer females had pre-resting stage ovaries and the mean follicle:germarium ratios were greater than 2.6:1.

The criteria used for determining diapause in C. restuans females were ovaries in the pre-resting stage and a mean follicle:germarium length less than 2.5:1. Madder et al. (1983) used a mean follicle:germarium ratio of 2.0:1 for C. restuans females in southern Ontario. If Madder's ratio had been used in this study, only two

groups of females would be considered in diapause, i.e., those reared at 20°C, 8L:16D and 500 larvae/pan and those females reared at 25'C, 8L:16D and 50 larvae/pan. No C. restuans females reared in the field would have entered diapause.

Photoperiod was the main factor determining diapause in C. restuans females. The mean ratios of follicle:germarium lengths were significantly lower at 8L:16D than at 16L:8D. Temperature also affected the mean follicle:germarium ratio. Females reared at 20°C had lower ratios than females reared at 15° and 25°C. Higher density rearing had no significant effect on the mean ratio of follicle:germarium length.

Field Experiments

C. restuans females emerging on or after 26 August had a significantly lower mean ratios of follicle:germarium length than females emerging earlier in the summer (Table 4). Females started entering diapause about 10 August. By 26 August, 40-60% of the females had pre-resting stage ovaries and a mean follicle:germarium ratio less than 2.5:1. By 19 September, 75-80% of the females were in diapause.

Madder et al. (1983) found that C. restuans females enter diapause in late July; 88% of C. restuans females emerging between 24 July and 28 July in southern Ontario were in diapause.

C. inornata

Laboratory Experiments

The resting stage of non-diapausing C. inornata ovarian follicles is 2a-2b. Females with less mature follicles are considered to be have pre-resting stage ovaries.

When C. inornata larvae were reared at a photoperiod of 8L:16D, over 55% of the females reared at 15°, 20° and 25°C had pre-resting stage ovaries (Table 5). At 16L:8D, 25% or fewer females had pre-resting stage ovaries. The mean follicle:germarium ratio for females with pre-resting stage ovaries was less than 2.3:1. The mean follicle:germarium ratio for females with resting stage ovaries was greater than 2.5:1 except in two cases.

The criteria chosen for determining diapause in C. inornata females were ovaries in the pre-resting stage and a mean ratio of follicle:germarium length of 2.5:1 or less.

Photoperiod was the main factor determining diapause. Females reared at 16L:8D had significantly a higher mean ratio of follicle:germarium lengths than females reared at 8L:16D. Temperature also affected the mean follicle:germarium length. Females reared at 25°C had a significantly higher mean follicle:germarium ratio than females reared at 15 and 25°C. Crowding had no significant effect on the ratios.

Hudson (1977) used a follicle:germarium ratio of 1.5:1 to distinguish diapausing females of C. inornata. If this ratio had been used here, no C. inornata females would be considered in diapause when reared in the laboratory. Hudson may have used a different technique to measure the follicles and germariums of C. inornata.

Hudson (1977) also found that diapause occurred when females were transferred from 16 hours daylength at 20°C to 12 hours, 10°C shortly after ecdysis to the fourth larval instar. Females reared at a constant 16 hr, at 20°C or at 12 hr, 10°C did not enter diapause. In this study, rearing the larvae at the constant photoperiod of 16L:8D and temperatures of 15°, 20° and 25°C did produce females in diapause.

Only one autogenous female was found when larvae were reared at 15°C, 8L:16D and 50 larvae/pan. Autogenous females were rarely observed in the laboratory colony of C. inornata and to my knowledge only one record of autogeny is noted in the literature (Owen 1942).

Field Experiments

Culiseta inornata females emerging on or after 28 August had a significantly lower mean ratio of follicle:germarium lengths than females emerging earlier (Table 6). The number of females in diapause reached 30-40% and 70-80% by 28 August and 23 September, respectively.

Hudson (1979) collected C. inornata in Edmonton September and October which had follicle:germarium ratios of less than 1.5:1. He considered females with ratios below 1.5:1 as diapausing. No C. inornata females in this study had follicle:germarium ratios less than 2.0:1 and therefore none of the females would be considered in diapause.

To summarize, photoperiod was the main factor determining diapause in the three species studied. Temperature affected the mean follicle:germarium ratios of C. restuans and C. inornata, but not C. tarsalis. Rearing the larvae at the high density did not affect the number of females entering diapause.

Culex tarsalis females began entering diapause in late July. By 9 August 70-75% of emerging females were in diapause. If an extensive aerial spray program was conducted over Winnipeg in late July or early August, 70-75% of the females emerging after the spraying would be in diapause. The transmission of WEE after an extensive aerial spray program depends on many factors besides the percentage of female C. tarsalis in diapause. The actual population of C. tarsalis and the effective control achieved are very important factors. The weather during the late summer may also be important, especially if conditions remain favourable for continued biting activity of mosquitoes. The migration of mosquitoes into the control area must also be considered.

Culex restuans females started entering diapause later than C. tarsalis around 10 August. By 26 August, 40-60% of the females emerging were in diapause. The majority of C. restuans females emerging after an extensive aerial spray program conducted at the beginning of August would not be in diapause. This species may be responsible for some of the late season transmissions of WEE virus to man and horses. However, considering C. restuans prefers to feed on birds this is probably unlikely.

Culiseta inornata females do not enter diapause until later in August. Only 30-40% of the females emerging on 28 August were in diapause. Considering the late season activity of C. inornata and its preference for feeding on large mammals, this species may be important in late season transmission of WEE virus to man and horses.

Table 1. Percent Culex tarsalis females (n=20) in resting stage and mean follicle:germarium (F:G) ratio under laboratory conditions.

Temperature (°C)	Photoperiod	Density (L/pan)	Diapause[1] (%)	Ovarian Stage of Females						Total
				Pre-resting		Resting		Autogenous		
				%	F:G	%	F:G	%		F:G
15	16L:8D	50	15	20	2.33	80	2.85	0		2.75 a[2]
		500	20	25	2.25	75	2.78	0		2.65 ab
	8L:16D	50	65	65	2.01	35	2.72	0		2.26 c
		500	65	65	1.93	35	2.71	0		2.21 cde
20	16L:8D	50	0	0	-	100	2.76	0		2.76 a
		500	10	10	2.34	90	2.83	0		2.77 a
	8L:16D	50	65	65	2.20	35	3.05	0		2.49 bde
		500	55	70	2.33	30	3.08	0		2.56 bd
25	16L:8D	50	0	0	-	75	2.79	25		2.79 a
		500	5	5	2.32	85	2.87	10		2.84 a
	8L:16D	50	65	65	1.68	25	2.59	10		2.00 c
		500	75	75	2.23	15	3.14	10		2.38 bce

1/ Females with pre-resting stage follicles and a mean F:G ratio < 2.5:1.
2/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.

Table 2. Percent Culex tarsalis females (n=20) in resting stage and mean follicle:germarium (F:G) ratio under field conditions.

Date of Commencement	Date of 50% Emergence	Density (L/pan)	Diapause[1] (%)	Ovarian Stage of Females					
				Pre-resting		Resting		Autogenous	Total
				%	F:G	%	F:G	%	F:G
May 14	June 9	50	10	10	2.03	90	2.84	0	2.75 a[2]
	June 12	500	10	10	2.11	90	2.92	0	2.84 a
June 2	June 27	50	5	5	2.00	95	2.86	0	2.82 a
	July 3	500	0	0	-	95	2.91	5	2.91 a
June 15	July 3	50	5	5	2.14	55	2.93	40	2.86 a
	July 9	500	0	0	-	50	2.93	50	2.93 a
July 2	July 16	50	0	5	2.65	95	3.03	0	3.01 a
	July 22	500	0	0	-	100	3.02	0	3.02 a
July 13	July 27	50	20	30	2.29	70	2.98	0	2.77 a
	August 2	500	35	35	2.21	65	3.23	0	2.87 a
July 27	August 9	50	70	70	2.23	30	2.67	0	2.35 b
	August 16	500	75	80	2.31	5	2.76	15	2.33 b
Aug.12	Sept. 1	50	65	80	2.31	20	2.79	0	2.40 b
	Sept. 5	500	70	80	2.35	15	2.74	5	2.41 b
Aug. 26	Sept. 23	50	75	95	2.35	5	2.76	0	2.37 b
	Sept. 28	500	75	90	2.38	10	2.58	0	2.40 b

1/ Females with pre-resting stage follicles and a mean F:G ratio < 2.5:1.

2/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.

Table 3. Percent Culex restuans females (n=20) in resting stage and mean follicle:germarium (F:G) ratio under laboratory conditions.

Temperature (°C)	Photoperiod	Density (L/pan)	Diapause [1] (%)	Ovarian Stage of Females					
				Pre-resting		Resting		Autogenous	Total
				%	F:G	%	F:G	%	F:G
15	16L:8D	50	30	30	2.15	70	2.78	0	2.62 a [2]
		500	10	15	2.48	85	3.02	0	2.94 b
	8L:16D	50	70	70	2.19	30	2.66	0	2.33 cd
		500	60	70	2.08	30	2.82	0	2.30 cd
20	16L:8D	50	0	0	-	100	3.22	0	3.22 b
		500	0	5	2.40	95	3.13	0	3.09 b
	8L:16D	50	65	70	2.03	30	2.62	0	2.06 c
		500	90	90	1.98	10	2.82	0	2.06 c
25	16L:8D	50	5	5	2.48	95	3.21	0	3.17 b
		500	5	5	3.24	95	3.18	0	3.18 b
	8L:16D	50	95	95	1.99	5	3.18	0	2.27 cd
		500	65	65	2.14	35	2.94	0	2.42 da

1/ Females with pre-resting stage follicles and an F:G ratio < 2.5:1.
2/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New mulitple range test.

Table 4. Percent Culex restuans females (n=20) in resting stage and mean follicle:germarium (F:G) ratio under field conditions.

Date of Commencement	Date of 50% Emergence	Density (L/pan)	Diapause[1] (%)	Ovarian Stage of Females					
				Pre-resting		Resting		Autogenous	Total
				%	F:G	%	F:G	%	F:G
June 2	June 20	50	10	10	2.11	90	2.84	0	2.76 ab[2]
	July 22	500	5	10	2.47	90	2.96	0	2.91 ac
June 15	July 1	50	0	0	-	100	3.12	0	3.12 c
	July 10	500	0	10	2.60	90	2.99	0	2.95 ac
July 2	July 14	50	0	0	-	100	3.09	0	3.09 c
	July 21	500	5	5	2.46	95	3.11	0	3.08 c
July 13	July 28	50	0	10	2.60	90	2.93	0	2.90 ac
	July 31	500	10	15	2.34	85	3.11	0	2.99 ac
July 27	August 10	50	15	20	2.34	80	2.83	0	2.73 ab
	August 14	500	15	25	2.37	75	2.94	0	2.80 abc
Aug.12	August 26	50	40	55	2.29	45	2.92	0	2.57 bd
	August 28	500	60	60	2.06	40	2.58	0	2.27 d
Aug. 26	Sept. 19	50	80	95	2.25	5	3.22	0	2.30 d
	Sept. 27	500	75	95	2.22	5	2.88	0	2.26 d

1/ Females with pre-resting stage follicles and a mean F:G ratio < 2.5:1.
2/ Means followed by different letters are significantly different at the 1% by analysis of variance and Duncan's New mulitple range test.

Table 5. Percent Culiseta inornata females (n=20) in resting stage and mean follicle:germarium (F:G) ratio under laboratory conditions.

Temperature (°C)	Photoperiod	Density (L/pan)	Diapause [1] (%)	Ovarian Stage of Females					
				Pre-resting		Resting		Autogenous	Total
				%	F:G	%	F:G	%	F:G
15	16L:8D	50	10	10	2.21	90	2.59	0	2.56 ace [2]
		500	25	25	2.10	75	2.64	0	2.51 ac
	8L:16D	50	75	75	1.88	25	3.05	5	2.13 bd
		500	85	85	2.16	15	2.51	0	2.21 bd
20	16L:8D	50	5	5	2.33	95	2.78	0	2.76 ce
		500	25	25	2.30	75	2.87	0	2.73 ce
	8L:16D	50	55	55	1.95	45	2.47	0	2.18 bd
		500	60	60	1.78	40	2.34	0	2.00 d
25	16L:8D	50	10	10	2.19	90	2.91	0	2.84 ef
		500	10	15	2.47	85	2.95	0	2.88 fg
	8L:16D	50	55	55	2.28	45	2.93	0	2.57 ace
		500	65	65	2.20	35	2.73	0	2.39 ab

1/ Females with pre-resting stage follicles and a mean F:G ratio < 2.5:1.
2/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.

Table 6. Percent Culiseta inornata females (n=20) in resting stage and mean follicle:germarium (F:G) ratio under field conditions.

Date of Commencement	Date of 50% Emergence	Density (L/pan)	Diapause[1] (%)	Ovarian Stage of Females					
				Pre-resting		Resting		Autogenous	Total
				%	F:G	%	F:G	%	F:G
May 14	June 7	50	10	10	2.11	90	2.89	0	2.82 a[2]
	June 11	500	15	15	2.31	85	2.89	0	2.80 a
June 2	June 25	50	10	10	2.39	90	3.04	0	2.97 ab
	June 26	500	10	10	2.16	90	3.25	0	3.14 bc
June 15	July 5	50	5	10	2.44	90	3.25	0	3.17 bc
	July 7	500	0	10	2.66	90	3.33	0	3.26 bc
July 2	July 19	50	0	5	2.55	95	3.48	0	3.43 c
	July 19	500	10	10	2.38	90	3.45	0	3.34 c
July 13	July 30	50	5	5	2.23	95	3.27	0	3.21 bc
	July 30	500	0	5	2.54	95	3.43	0	3.39 c
July 27	August 12	50	0	0	-	100	3.26	0	3.26 bc
	August 13	500	5	5	2.34	95	3.02	0	2.99 ab
Aug. 12	August 28	50	30	40	2.37	60	2.99	0	2.74 a
	August 29	500	40	45	2.28	55	3.04	0	2.70 a
Aug. 26	Sept.23	50	70	80	2.28	5	2.88	0	2.40 d
	Sept. 25	500	80	90	2.28	10	2.77	0	2.33 d

1/ Females with pre-resting stage follicles and a mean F:G ratio < 2.5:1.
2/ Means followed by different letters are significantly different at the 1% level by analysis of variance and Duncan's New multiple range test.

GENERAL DISCUSSION

In Manitoba, C. tarsalis is an effective vector of WEE (Henderson et al. 1979). The virus has been isolated from field-collected C. tarsalis (Sekla et al. 1980). Brust (1982) found that high C. tarsalis populations, as measured by sentinel flock traps, are correlated with years when WEE epidemics occurred. C. tarsalis oviposition was higher in 1981, an epidemic year than in 1980, a non-epidemic year. The mean number of C. tarsalis eggs per raft was also higher in the spring of 1981.

Based on seasonal oviposition patterns and on an average larval plus pupal development time of 20 to 25 days in the field, C. tarsalis probably has three generations per year in southern Manitoba. The largest peak in oviposition occurred in the second generation and coincided with the period of the highest transmission rate of the WEE virus (Sekla 1982).

Female C. tarsalis began to enter diapause about the end of July in 1981. This explains the decrease in oviposition at the end of July and the smaller third generation. If mosquito adulticiding, e.g., aerial spraying of a large area such as Winnipeg and the surrounding area, was conducted about the the beginning of August, 70-80% of the C. tarsalis females emerging after the treatment would enter diapause. If these females do not seek a blood meal, as reported by Mitchell (1981), they would not be capable of transmitting the virus at that

time.

Larvae of C. tarsalis are not tolerant of cool temperatures. By entering diapause in late July and August, C. tarsalis avoids the cooler late summer temperatures.

Autogeny in C. tarsalis reach 40-50% in July of 1981. Autogeny must be taken into consideration when attempting to correlate the peak population of C. tarsalis with transmission of WEE. Autogenous females would not be vectors of the disease until their third gonotrophic cycle.

Culex restuans was more common in oviposition traps than C. tarsalis and C. inornata when compared to light trap collections where C. restuans was not as common (Gallaway 1983; R.A. Ellis pers. comm.). Based on oviposition activity and on larval plus pupal development times of 16 to 23 days in the field, C. restuans probably has three generations per year.

In Manitoba, WEE virus has been isolated from C. restuans females. The preferred hosts of C. restuans are birds (Barr 1958; Means 1968; Magnarelli 1977). This species may be important as an enzootic and epizootic vector involved in the amplification of the WEE virus in birds.

The immature stages of C. restuans are more tolerant of cool temperatures than C. tarsalis and development time is faster compared to C. tarsalis and C. inornata under both laboratory and field

conditions.

Culex restuans females begin to enter diapause later in the summer than C. tarsalis. Fifteen percent of C. restuans females emerging from 10 to 14 August were in diapause. By 26 August, 40-60% of the females were in diapause. In southern Ontario, 88% of C. restuans females were in diapause by the end of July (Madder et al. 1983). Madder et al. (1980) reported that C. restuans is primarily a spring and early summer mosquito in southern Ontario and that C. pipiens, which competes with C. restuans for breeding sites, occurs later in the summer. This earlier diapause in southern Ontario may be a survival mechanism for C. restuans. By entering diapause earlier, the overwintering female population is larger than it would be later in the summer when competing with C. pipiens.

Culiseta inornata is a winter mosquito which aestivates during the summer in the southern United States. In Manitoba, oviposition activity of C. inornata occurs mainly in the late spring and late summer when temperatures are cooler. The immature stages of C. inornata are tolerant of cool temperatures under both laboratory and field conditions.

Based on oviposition activity, C. inornata probably has two generations in Manitoba. However, based on the development time of the immature stages of 20 to 21.5 days, C. inornata could complete three generations. This mosquito may be seeking cool sheltered

locations during the hotter summer months. This would explain the decrease in oviposition in late June and July. Summer aestivation in the southern United States is characterized by an increase in lipid content (Barnard and Mulla 1978a) and decrease in parity rates (Barnard and Mulla 1978b). It would be interesting to examine these parameters to see if C. inornata responds similarly in Winnipeg.

C. inornata prefers to feed on large mammals, but will feed on birds (Anderson et al. 1967; Edman et al 1972; Tempelis 1975; Tempelis et al. 1967). The WEE virus has been isolated from field-collected C. inornata. This species may be important in the transmission of the disease in the early spring and late late summer and early fall during epidemic years.

Female C. inornata begin to enter diapause later in the summer than either C. tarsalis and C. restuans. Only 30-40% of females emerging around 28 August were in diapause. By 23 September, 70-80% were in diapause.

Although C. tarsalis is the primary vector of WEE in Manitoba, other mosquitoes such as C. restuans and C. inornata are probably involved in the disease cycle. Culex restuans is a common mosquito in the Winnipeg area which is well adapted to its environment. Its feeding habits and the WEE virus isolations from field-collected females indicate that this species is probably more important than previously suspected. More information on the population dynamics of

C. restuans, especially during epidemic years may provide a better understanding of the role this species plays in WEE transmission.

More research on the feeding habits of C. inornata, specifically in the spring and fall and on its population dynamics may elucidate the importance of C. inornata in the WEE cycle.

SUMMARY AND CONCLUSIONS

This thesis is comprised of three manuscripts, each describing an aspect of the bionomics of three potential vectors of Western Equine Encephalitis in Manitoba.

1. In Manitoba, C. tarsalis and C. restuans probably have three generations per year; C. inornata probably has two generations per year.

2. Overwintering females of C. tarsalis, C. restuans and C. inornata were active and laid eggs before the end of May. C. tarsalis and C. restuans oviposition peaked in mid-June to mid-July; C. inornata oviposition peaked in mid-June and late July to early August.

3. Female C. tarsalis and C. inornata produced fewer eggs per raft in early spring and summer as compared to later in the summer in 1980. Female C. restuans produced fewer eggs per raft in the early spring and summer as compared to later in the summer in 1980 and 1981.

4. Larval and pupal development times of C. tarsalis, C. restuans and C. inornata in the laboratory were not affected by photoperiod.

5. Development time of larvae and pupae of the three species was longest at 15° and shortest at 25°C when reared in the laboratory at 15°, 20° and 25°C. C. restuans and C. inornata are more

tolerant of cooler temperatures than C. tarsalis.

6. Rearing larvae at high densities caused an increase in development time from first instar larvae to adults. There was a significant interaction between temperature and density on development times of the three species. At low temperatures and the high density, the development time was lengthened, while at higher temperatures the differences between development times at the high and low density, although significantly different in some instances, was not as great.

7. Mortality of C. tarsalis larvae and pupae was affected by temperature when were reared at a density of 50 larvae per pan. The highest mortality occurred at 15°C. Mortality of C. restuans and C. inornata was highest at 25°C.

8. C. tarsalis required 20 to 25 days to complete development from first instar larvae to adults under field conditions, while C. restuans required 16 to 23 days. C. inornata completed development in 20 to 21.5 days.

9. Mortality in the field ranged from 48.6% to 80.3% for C. tarsalis throughout the summer and was dependent on the larval density. Mortality of C. restuans ranged from 26.1% to 75.3% and was dependent on the density of the larvae and the date the trial began. Mortality of C. inornata ranged from 59.1% to 66.8% and was also dependent on the density and the date the trial began.

10. The follicle:germarium ratio and the stage of follicular development were chosen as the best criteria for differentiating between diapausing and non-diapausing females. Females of C. tarsalis, C. restuans and C. inornata with pre-resting stage follicles and a follicle:germarium ratio less than 2.5:1.0 were considered to be in diapause.

11. Photoperiod was the main factor affecting the number of females of the three species entering diapause when studied in the laboratory.

12. Field reared C. tarsalis females began entering diapause by 27 July, when 20-35% were in diapause. Female C. restuans began entering diapause by 10 August when 15% were in diapause. By 26 August, 40-60% were in diapause. Female C. inornata started entering diapause by 28 August, when 30-40% were in diapause.

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