DEVELOPMENT TIME, OVIPOSITION ACTIVITY AND ONSET OF DIAPAUSE IN *CULEX TARSALIS, CULEX RESTUANS* AND *CULISETA INORNATA* IN SOUTHERN MANITOBA

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ABSTRACT. Development times and survival of immatures and reproductive diapause of adult females of *Culex tarsalis, Cx. restuans* and *Culiseta inornata* were investigated from hatching to adult emergence at 15, 20 and 25°C in the laboratory and at natural temperatures and photoperiods in southern Manitoba. Based on patterns of oviposition in artificial pools operated from mid-April to the end of September and development time of the immature stages, 3 generations of *Cx. tarsalis, Cx. restuans* and *Cs. inornata* were possible in 1980 and 1981. In 1981, 70% of field-reared *Cx. tarsalis* females emerging in mid-August were in diapause. Field-reared *Cx. restuans* and *Cs. inornata* entered reproductive diapause 2–3 weeks later than *Cx. tarsalis*.

INTRODUCTION

Culex tarsalis Coq., *Culex restuans* Theobald and *Culiseta inornata* (Williston) overwinter as inseminated adult females in the northern United States and southern Canada. Females of *Cx.tarsalis* and *Cs. inornata* emerge from hibernation when the soil surface temperature exceeds the subsurface temperature. They immediately seek blood meals and begin oviposition before the end of May in southern Alberta (Shemanchuk 1965). Western equine encephalitis (WEE) virus has been isolated from *Cx. tarsalis* and *Cs. inornata* females during 4 recent outbreaks of the disease in Manitoba (Sekla 1976, 1982; Sekla et al. 1980, Artsob 1983). The virus of WEE was isolated earlier from *Cx. restuans* (Norris 1946) and from *Cx. tarsalis* (McLintock 1947) in Manitoba. *Culex tarsalis* is the main vector in Manitoba (Sekla et al. 1980) and throughout western North America (Reeves and Hammon 1962).

In spite of the importance of *Cx. tarsalis* as a vector of WEE virus and *Cx. restuans* and *Cs. inornata* as potential vectors, the population dynamics of the 3 species in Manitoba and the northcentral United States are poorly known. As a result, our research focused on the following objectives: 1) determine the development time of immatures and their survival at different densities in the field and under environmentally controlled laboratory conditions, 2) estimate the number of generations by monitoring seasonal oviposition patterns in artificial pools and 3) determine the calendar date for the onset of reproductive diapause in each species over one field season in southern Manitoba. The results of the latter objective are of particular importance in planning an arbovirus control program against vector species that overwinter as adults.

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MATERIALS AND METHODS

Oviposition activity: Seasonal patterns in oviposition activity were monitored from mid-April to the end of September during the summers of 1980 and 1981, by collecting egg rafts from 3 oviposition pools set up adjacent to a wooded area in Winnipeg. The pools consisted of wooden boxes (1 × 1 × 0.3 m) lined with black polyethylene and lawn sod and filled with 200 liters of water. All egg rafts from the oviposition pools were collected daily. The number of eggs per raft was determined from the mean of 2 counts of each raft. All egg rafts were allowed to hatch in 500 ml polystyrene containers kept at room temperature. The first instar larvae were identified using Dodge's (1966) key. Significant differences in mean egg raft size per month were tested using ANOVA and Duncan's New Multiple Range Test (DNMRT).

Developmental rate and survival of immatures: The effects of larval density, rearing temperature and photoperiod on the development time of *Cx. tarsalis, Cx. restuans* and *Cs. inornata* immatures were investigated in the laboratory. *Culex tarsalis* and *Cs. inornata* populations were obtained from laboratory colonies. Laboratory colonies of *Cx. tarsalis* from Manitoba are difficult to establish, and it can take many months to develop a large viable colony. Therefore, a *Cx. tarsalis* colony which originated from Glenlea, MB, in 1973 was used for these experiments. The *Cs. inornata* colony originated from females collected in 1980 from Winnipeg, MB. *Culex restuans* was obtained from artificial pools in Winnipeg, MB.

Larvae, <12 h old, were placed in 21 × 32 × 7 cm polypropylene pans with 1 liter of dechlorinated water. They were maintained in environmentally controlled chambers at 15, 20, and 25 ± 1°C, and fed daily with enough liver powder (ICN Biochemicals, Inc.) to feed the larvae without fouling the rearing water. The photoperiods were 16L:8D and 8L:16D. The larval densities were 50 and 500 per pan. There were 5 replicates of 50 larvae per pan and 3 replicates of 500

larvae per pan. Development was monitored daily to record time to pupation and time to adult eclosion.

In 1981 development of immatures was investigated in the field using the same colonies and rearing methods described above. Pans containing 50 or 500 larvae were covered and maintained outdoors in a shaded area near oviposition pools. Air temperature was monitored adjacent to the pools (see Table 5). Different cohorts were begun at 2-week intervals to track development times under natural conditions.

Under both laboratory and field conditions, development time to pupation and to adult eclosion were calculated, based on the time it took 50% of fourth instar larvae to molt to pupae, and the time it took 50% of the pupae to reach the adult stage. Mortality of larvae and pupae was analyzed using an arcsin transformation, with factorial ANOVA and DNMRT. Development times of the different stages were analyzed by ANOVA and DNMRT.

Onset of reproductive diapause: Experiments on the effects of larval density, rearing temperature, and photoperiod on the ovarian development of *Cx. tarsalis*, *Cx. restuans*, and *Cs. inornata* were conducted in environmentally controlled chambers. Pupae from the experiments on larval development times, as described above, were removed from the pans and placed in Plexiglas® cages at the same temperature and photoperiod as the larvae. Adults were provided with moistened wicks of 10% sucrose and water. After 2 weeks, 20 females were either dissected immediately or stored at −20°C and dissected later. The females were dissected in a physiological saline solution. Five of the largest primary ovarian follicles per female were measured, together with the germarium (secondary follicle), under phase microscopy at 400× and 500× magnification (Watts and Smith 1978). Adult mortality during the 2 weeks following eclosion often resulted in less than 20 females per replicate available for dissection. As a result, the females from the replicates were combined; and 20 from each photoperiod and temperature were dissected. Statistical analysis could not be conducted due to the pooling of females. The criteria used for determining if a female was in diapause are discussed below.

Ovarian development of populations reared outdoors at Winnipeg in 1981 was investigated in the same manner as laboratory-reared populations.

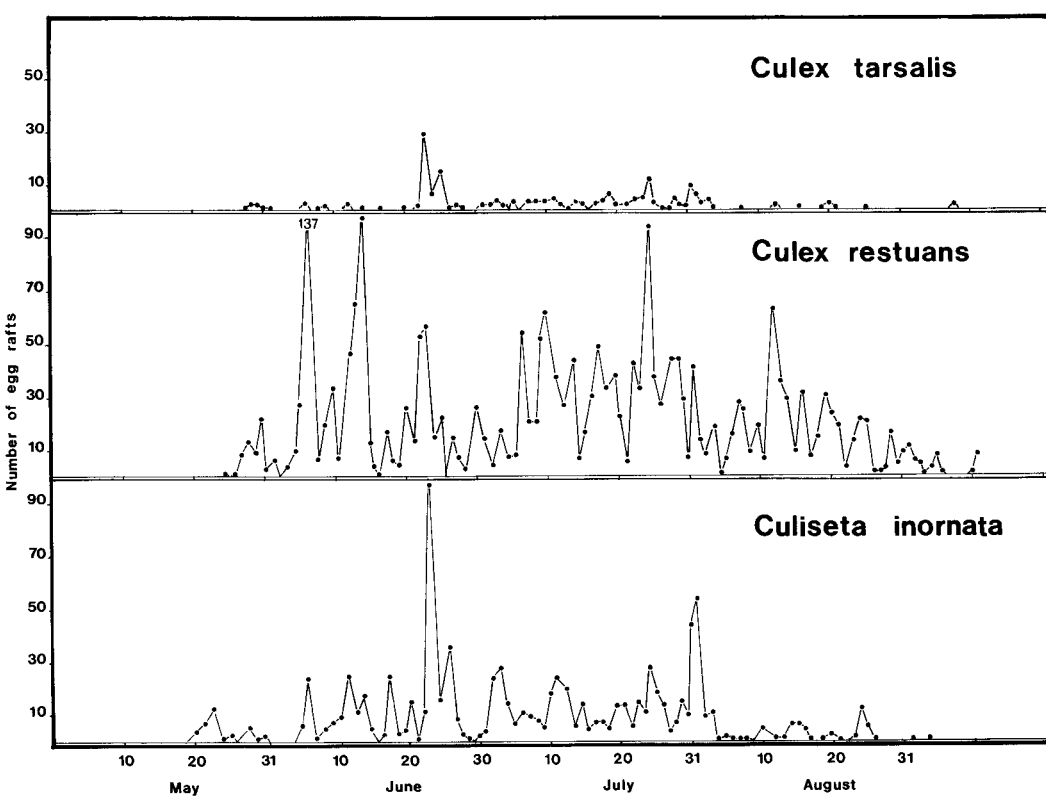

Fig. 1. Number of egg rafts laid daily by *Culex tarsalis, Cx. restuans* and *Culiseta inornata* in 3 pools, Winnipeg, Manitoba, 1980.

RESULTS

Oviposition activity and egg raft size: The first and last *Cx. tarsalis* egg rafts were collected on May 28 and August 27, 1980, and May 7 and August 27, 1981, respectively (Figs. 1 and 2). The total number of egg rafts was greater in 1981 (Table 1), and the peak oviposition occurred 3 weeks later than in 1980 (Figs. 1 and 2). The first and last *Cx. restuans* egg rafts were collected on May 24 and September 12, 1980, and May 21 and September 13, 1981, respectively. Oviposition peaks occurred periodically throughout the season in 1980. However, in 1981 the increase in rafts was limited to July (Figs. 1 and 2). The first and last *Cs. inornata* egg rafts were collected May 22 and September 3, 1980, and May 4 and September 13, 1981, respectively. Oviposition peaks occurred in mid-June and late July 1980, and in mid-August 1981 (Figs. 1 and 2).

Step-wise linear regression was used to correlate the daily fluctuations in oviposition activity with the following weather parameters during the oviposition period: temperature, wind speed, relative humidity, dew point, total cloud opacity and the percentage of time with precipitation. There was no significant correlation between daily oviposition and the weather parameters.

The number of eggs per raft of *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata* in 1980 and of *Cx. restuans* in 1981 were significantly lower in May and June than in later months (Table 1). In 1981, there was no difference between the mean monthly numbers of *Cx. tarsalis* eggs or *Cs. inornata* eggs per raft (Table 1).

Development rate and survival of immature stages: All 3 species successfully developed to the adult stage at each of the temperatures used in the laboratory (Table 2). For each species, there were no significant differences in development times between photoperiods of 16L:8D and 8L:16D. Thus, the data were pooled for each temperature.

The shortest mean development time for *Cx. tarsalis* was 12.8 days at 25°C and at low density compared with 45.9 days at 15°C and at high density. Mortality was significantly different between temperatures at the low density. At 25°C, mortality was 7.4% whereas, at 15°C, it was 63.8%. At high larval density, mortality exceeded 75% at all temperatures (Table 2).

For *Cx. restuans* the shortest development

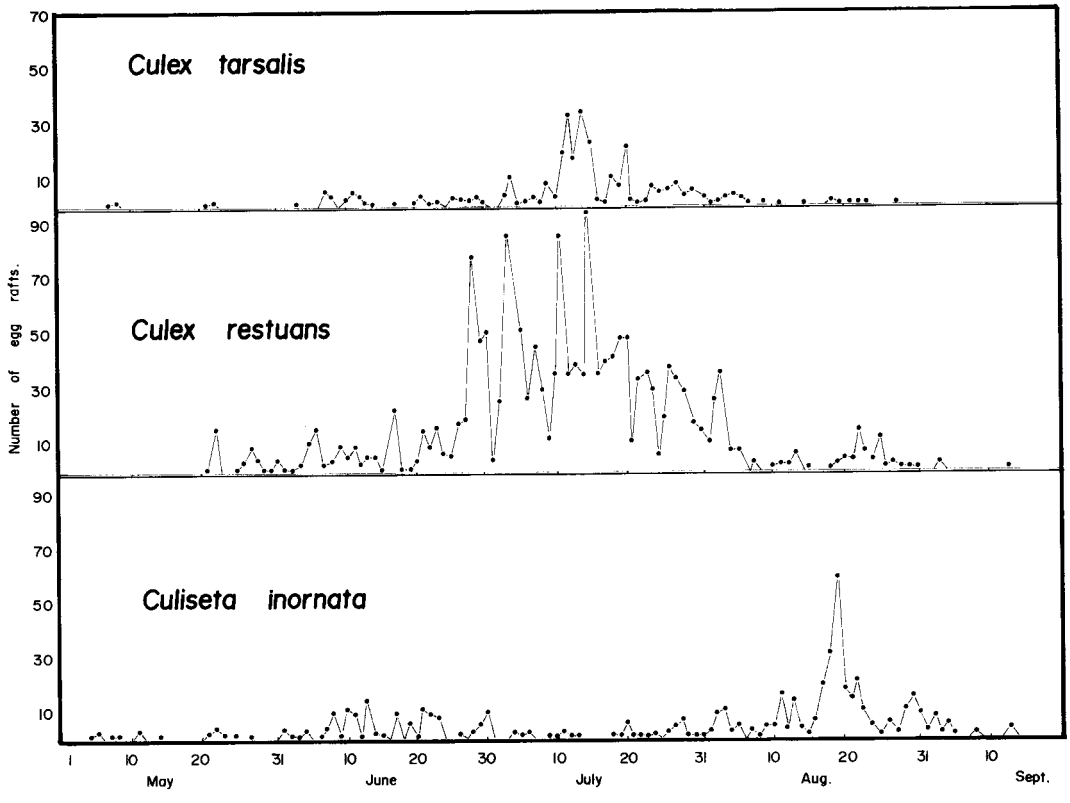

Fig. 2. Number of egg rafts laid daily by *Culex tarsalis, Cx. restuans* and *Culiseta inornata* in 3 pools, Winnipeg, Manitoba, 1981.

time was a mean of 8.1 days at 25°C and the low density. Mortality was 2–8% at low density, but was greater than 47% at high density (Table 2).

Development times to adults for *Cs. inornata* varied from 13.6 days at 25°C and the low density to 40.9 days at 15°C and the high density (Table 2). Mortality was 13.8 and 8.4% at 15 and 20°C at low density but was 51% at 25°C. Mortality at high density exceeded 60% (Table 2).

Development times to adults in the field and mortality of immatures for all 3 species during the summer of 1981 is shown in Tables 3 and 4, respectively. Development time and mortality were greater at high larval densities. The mean development time to adults at low larval densities was 19.8, 16.3 and 20 days for *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata*, respectively (Table 3). The mean mortality of larvae and pupae at low larval densities was 48.6, 26.1 and 59.1% for *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata*, respectively (Table 4).

Onset of reproductive diapause: The development stage of ovarian follicles in females at 2 weeks of age and the ratio of the length of the primary follicle:length of the germarium were the criteria used to determine the presence or absence of reproductive diapause. The non-diapause resting stage of the ovarian follicles was determined to be Watts and Smith's (1978) stage II. The follicle:germarium length was 2.5:1 or greater for *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata*.

Based on the above criteria, a high percentage of females of all 3 species reared under a short photoperiod of 8L:16D in the laboratory entered reproductive diapause (Figs. 3–5). Photoperiod proved to have a greater influence than temperature on the induction of reproductive diapause. The different rearing temperatures and densities for immatures and the different maintenance temperatures for adults had little affect on the rate at which females entered the diapause state. The maximum diapause of each species, under laboratory conditions, was 75%, 95% and 85% in *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata*, respectively (Figs. 3–5).

In the laboratory, a high percentage of all 3

Table 1. Mean number of eggs per raft for *Culex tarsalis*, *Cx. restuans* and *Culiseta inornata*, 1980 and 1981.

Collection period	*Cx. tarsalis*		*Cx. restuans*		*Cs. inornata*	
	Number of eggs/ raft ($\bar{x} \pm SE$)	No. rafts	Number of eggs/ raft ($\bar{x} \pm SE$)	No. rafts	Number of eggs/ raft ($\bar{x} \pm SE$)	No. rafts
1980						
May	188.4 ± 2.4 a[1]	12	155.0 ± 5.7 a	32	161.4 ± 0.5 a	210
June	202.8 ± 1.3 a	41	161.8 ± 0.5 a	237	186.5 ± 0.7 b	151
July	275.2 ± 1.2 b	92	182.4 ± 0.6 b	219	228.6 ± 0.6 c	222
Aug.	303.2 ± 3.6 b	24	198.9 ± 0.5 c	242	220.5 ± 0.9 c	103
Sept.			200.1 ± 1.0 bc	54		
Total		169		784		686
1981						
May	209.6 ± 3.2 a	6	146.9 ± 1.1 a	36	197.6 ± 1.7 a	25
June	222.1 ± 1.6 a	52	173.5 ± 0.6 b	188	201.8 ± 0.7 a	131
July	258.8 ± 0.6 a	225	199.8 ± 0.5 c	298	216.7 ± 1.4 a	40
Aug.	261.2 ± 1.7 a	32	203.1 ± 0.8 c	127	197.1 ± 0.6 a	190
Total		315		649		386

[1] Means within each year and species followed by different letters are significantly different at the 5% level.

Table 2. Mean development time and mortality of *Culex tarsalis*, *Cx. restuans* and *Culiseta inornata* reared at 3 temperatures and 2 densities in the laboratory.

Temperature (°C)	Development time – days ($\bar{x} \pm SE$)			% mortality ($\bar{x} \pm SE$)		
	Cx. tarsalis	*Cx. restuans*	*Cs. inornata*	*Cx. tarsalis*	*Cx. restuans*	*Cx. inornata*
50 larvae/pan						
15	34.6 ± 0.6 a[1]	22.0 ± 0.4 a	23.8 ± 0.5 a	63.8 ± 1.6 a	1.9 ± 0.8 a	13.8 ± 0.9 a
20	18.2 ± 0.5 b	11.6 ± 0.2 b	20.6 ± 0.6 b	21.8 ± 1.9 b	5.0 ± 0.9 ab	8.4 ± 0.8 a
25	12.8 ± 0.4 c	8.1 ± 0.5 c	13.6 ± 0.5 c	7.4 ± 1.3 c	8.4 ± 1.1 b	51.2 ± 2.4 b
500 larvae/pan						
15	45.9 ± 0.6 d	31.8 ± 0.6 d	40.9 ± 0.5 d	78.3 ± 2.0 d	46.7 ± 1.3 c	65.3 ± 1.5 c
20	21.1 ± 0.6 e	13.2 ± 0.6 e	24.6 ± 0.6 a	76.4 ± 1.9 ad	72.8 ± 1.5 d	60.4 ± 1.9 bc
25	18.5 ± 0.3 b	12.8 ± 0.5 e	14.8 ± 0.5 e	79.2 ± 0.9 d	76.2 ± 1.3 d	51.2 ± 2.9 b

[1] Means within each species followed by different letters are significantly different at the 5% level.

species was in a non-diapause state when immatures and adults were maintained at a long photoperiod (16L:8D). The ovarian follicles reached the resting stage II (Watts and Smith 1978) within 2 weeks. The ratio of the primary follicle to the germarium in a non-diapausing female was greater than 2.5:1. At a photoperiod of 8L:16D, some females of all 3 species failed to reach the resting stage II by 2 weeks of age; and the 5 largest primary follicles of these females were less than 2.5× the size of the germarium. These females were judged to be in reproductive diapause. Using these criteria, most of the females maintained at short photoperiod were in reproductive diapause (Figs. 3–5).

The high and low density cohorts for each species were combined for the field results to give the most realistic representation of diapause in natural populations. The field populations were not in diapause during June and July, but there were increasing numbers of females in reproductive diapause in August and September (Fig. 6). In 1981 *Cx. tarsalis* females entered diapause about 2 to 3 weeks earlier than *Cx. restuans* or *Cs. inornata* females. Most of the *Cx.*

Table 3. Mean development time of *Culex tarsalis*, *Cx. restuans* and *Culiseta inornata* at 2 densities under field conditions, Winnipeg, Manitoba, 1981.

	Development time (days)					
	Cx. tarsalis ($\bar{x}$ ± SE)		*Cx. restuans* ($\bar{x}$ ± SE)		*Cs. inornata* ($\bar{x}$ ± SE)	
Hatching date	50 larvae/pan	500 larvae/pan	50 larvae/pan	500 larvae/pan	50 larvae/pan	500 larvae/pan
May 14	25.8 ± 0.5[1] a	28.9 ± 0.6 a			23.5 ± 0.4 a	27.8 ± 0.7 a
June 2	25.0 ± 0.2 a	30.9 ± 0.4 bd	17.6 ± 0.5 a	30.4 ± 0.5 ac	23.0 ± 0.3 a	24.0 ± 0.5 b
June 15	18.2 ± 0.4 b	24.5 ± 0.6 b	16.9 ± 0.3 ab	25.7 ± 0.6 a	19.7 ± 0.4 b	21.6 ± 0.5 c
July 2	14.4 ± 0.5 c	19.8 ± 0.7 c	12.3 ± 0.6 b	18.9 ± 0.6 b	16.6 ± 0.4 c	16.8 ± 0.4 d
July 13	14.4 ± 0.5 c	20.5 ± 0.9 c	15.3 ± 0.3 ab	18.4 ± 0.5 b	17.2 ± 0.3 c	17.3 ± 0.6 d
July 27	13.2 ± 0.3 c	20.3 ± 0.6 c	14.2 ± 0.2 ab	17.8 ± 0.9 b	16.1 ± 0.4 c	16.6 ± 0.3 d
Aug. 12	19.6 ± 0.6 b	24.6 ± 0.5 b	13.9 ± 0.2 ab	16.3 ± 0.4 b	16.2 ± 0.3 c	16.7 ± 0.4 d
Aug. 26	27.6 ± 0.5 a	32.6 ± 1.0 d	24.1 ± 0.7 c	32.1 ± 0.6 c	28.1 ± 0.3 d	30.0 ± 0.8 a
Total	19.8 ± 1.2[2]	25.3 ± 0.5[2]	16.3 ± 0.3[2]	22.8 ± 0.5[2]	20.0 ± 0.3[2]	21.4 ± 0.5[2]

[1] Means within each species and densities followed by different letters are significantly different at the 5% level.
[2] Means are significantly different within each species at the 1% level by *t*-test.

Table 4. Percent mortality of *Culex tarsalis*, *Cx. restuans* and *Culiseta inornata* at 2 densities under field conditions, Winnipeg, Manitoba, 1981.

	Mortality to adult stage (%)					
	Cx. tarsalis ($\bar{x}$ ± SE)		*Cx. restuans* ($\bar{x}$ ± SE)		*Cs. inornata* ($\bar{x}$ ± SE)	
Hatching date	50 larvae/pan	500 larvae/pan	50 larvae/pan	500 larvae/pan	50 larvae/pan	500 larvae/pan
May 14	47.3 ± 1.5[1]	78.0 ± 1.0[1]			43.3 ± 1.6 a[2]	79.7 ± 1.4 a
June 2	44.0 ± 1.0	76.7 ± 1.1	14.0 ± 1.2 a[2]	69.9 ± 0.7[1]	59.3 ± 1.7 b	80.3 ± 1.3 a
June 15	64.7 ± 1.5	80.9 ± 1.1	14.0 ± 0.8 a	75.1 ± 1.3	49.3 ± 0.9c	77.4 ± 1.1 ab
July 2	48.0 ± 0.6	83.0 ± 1.1	28.7 ± 0.8 abc	71.0 ± 1.1	67.3 ± 1.6 b	87.7 ± 1.8 c
July 13	42.0 ± 1.3	74.7 ± 1.1	22.7 ± 0.9 ab	77.4 ± 1.2	76.6 ± 1.0 d	84.7 ± 1.5 d
July 27	35.3 ± 0.8	82.0 ± 1.1	34.0 ± 1.2 bc	78.0 ± 1.8	66.0 ± 1.2 b	75.3 ± 1.6 b
Aug. 12	46.3 ± 1.4	80.3 ± 1.5	40.0 ± 1.2 c	82.3 ± 1.0	57.3 ± 1.4 e	77.7 ± 1.6 be
Aug. 26	61.0 ± 1.5	86.7 ± 1.3	29.3 ± 0.9 abc	73.3 ± 1.8	53.3 ± 0.5 f	80.0 ± 1.0 b
Total	48.6 ± 0.5[3]	80.3 ± 0.5[3]	26.1 ± 0.6[3]	75.3 ± 0.6[3]	59.1 ± 0.6[3]	66.8 ± 1.0[3]

[1] No significant differences between means at the 5% level.
[2] Means within each species and densities followed by different letters are significantly different at the 5% level.
[3] Means are significantly different within each species at the 1% level by Student's *t*test.

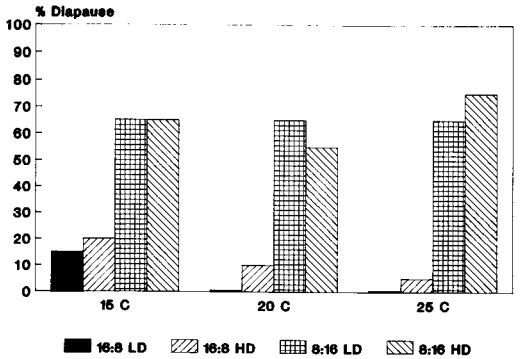

Fig. 3. Percent of *Culex tarsalis* females in diapause when reared under laboratory conditions. LD: 50 larvae/pan; HD: 500 larvae/pan.

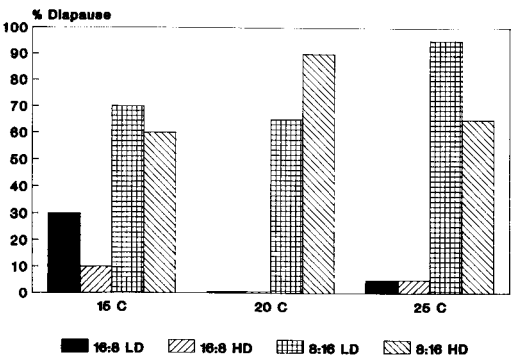

Fig. 4. Percent of *Culex restuans* females in diapause when reared under laboratory conditions. LD: 50 larvae/pan; HD: 500 larvae/pan.

tarsalis (70%) females were in reproductive diapause by the beginning of August.

DISCUSSION

Oviposition activity and egg raft size: The most abundant species, based on egg raft collections from the oviposition pools in Winnipeg in 1980 and 1981, was *Cx. restuans*, followed by *Cs. inornata* and *Cx. tarsalis* (Table 1). This is a different ratio from that of adult collections in New Jersey light traps in Winnipeg (Ellis 1980, 1981). In 1980, the light trap (19 locations throughout Winnipeg) collection ratio of *Cx. tarsalis*:*Cx. restuans*:*Cs. inornata* was 1:1:12. In 1981, the year of the largest WEE epidemic since 1941 in Manitoba, it was 7:1:21. The egg raft collection ratio for these 3 species in 1980 was 0.2:1:0.9. In 1981, it was 0.5:1:0.6 (Table 1). Oviposition pool data obviously provide very different population estimates for these 3 species in Winnipeg compared to light trap collections of adults. Although year to year variations occur, the trend of higher numbers of *Cx. restuans* in oviposition pools compared to light traps has been recorded for several years (Brust 1990).

Fewer eggs in the egg rafts of *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata* in the spring of 1980 (Table 1) may be related to the fate of the blood meal taken by overwintered adults. Females may use some components of the blood meal to replenish fat or to maintain metabolic processes which may require the same blood proteins that summer generations allocate to egg production. The reduced size of early spring egg rafts has also been reported in populations of *Cx. restuans* and in *Cx. pipiens* Linn. in Ontario (Madder et al. 1983a). In 1981 the number of eggs per raft of *Cx. tarsalis* and *Cs. inornata* was not lower in the spring. This may have been one of the factors leading to higher population levels of *Cx. tarsalis* as indicated by oviposition monitoring.

Development rate and survival of immatures: Studies have been published on the development rate of *Cx. tarsalis*, *Cx. restuans* and *Cs. inornata.* However, where data is available at the same temperature, the development period may not include the pupal stage. Hagstrum and Workman (1971) found that at 20°C, development to pupae in *Cx. tarsalis* was 14.6 days. This compares favorably with 18.2 days to the adult stage in our study, at low density. In a field study in California, *Cx. tarsalis* development to adult ranged from 8 days at 31°C to 16 days at 17°C (Milby and Meyer 1986). Laboratory studies conducted by Bailey and Gieke (1968) showed development time at 15.6, 21.1 and 26.7°C was 23.5, 13.2 and 9.1 days, respectively. In our study, *Cx. tarsalis* development required a longer time at slightly lower temperatures: e.g., 34.6, 18.2 and 12.8 days at 15, 20 and 25°C, respectively.

Shelton (1973) showed that *Cx. restuans* developed to the pupal stage in 10.2 days at 20°C. This is comparable to our results of 11.6 days to

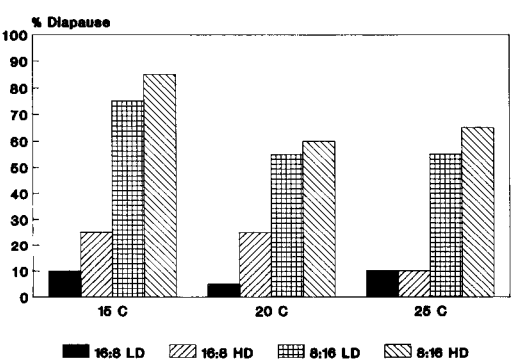

Fig. 5. Percent of *Cs. inornata* females in diapause when reared under laboratory conditions. LD: 50 larvae/pan; HD: 500 larvae/pan.

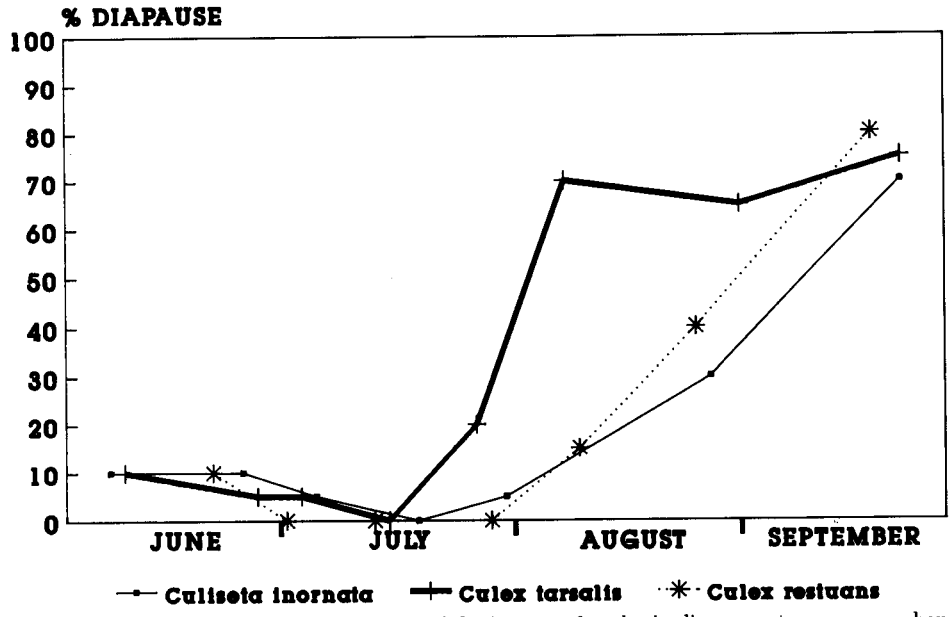

Fig. 6. Percent of *Culex tarsalis*, *Cx. restuans* and *Cs. inornata* females in diapause at emergence when reared under field conditions, Winnipeg, Manitoba, 1981.

Table 5. Mean air temperature for each cohort for development studies of *Culex tarsalis*, *Culex restuans* and *Culiseta inornata* at 2 densities under field conditions, Winnipeg, Manitoba, 1981.

	Mean air temperature (°C)					
	Cx. tarsalis		*Cx. restuans*		*Cs. inornata*	
Hatching date	50 larvae/ pan	500 larvae/ pan	50 larvae/ pan	500 larvae/ pan	50 larvae/ pan	500 larvae/ pan
May 14	15.2	15.3			14.6	14.7
June 2	16.4	16.4	16.2	16.9	16.1	16.1
June 15	19.7	20.8	17.7	19.5	18.4	19.1
July 2	23.6	21.3	24.3	22.4	23.2	23.2
July 13	19.5	19.5	19.7	19.8	19.8	19.8
July 27	19.4	19.4	19.2	19.8	19.5	19.5
Aug. 12	21.1	19.7	22.1	21.9	21.9	21.8
Aug. 26	15.1	14.2	16.1	14.9	15.4	15.1

the adult stage at 20°C. A comparison with Madder et al. (1983a) shows *Cx. restuans* required 26.5, 14.3 and 8.5 days at 15, 20 and 25°C. In our study *Cx. restuans* required 22, 11.6 and 8.1 days at the same respective temperatures. The Manitoba population developed faster at the lower temperatures.

Shelton's (1973) results on the development time of *Cs. inornata* at 20°C show development time to be about 20% more rapid than in our study at the same temperature. Our results are similar to Hanec and Brust (1967). In both Hanec and Brust (1967) and the present study, recent field-collected colony material (second or third laboratory generation) was used. The lower threshold of development of *Cs. inornata* from Manitoba (10°C of Hanec and Brust 1967), versus *Cs. inornata* from Louisiana (15°C of Shelton 1978), suggests that these populations are quite different.

Mortality at the high density in the laboratory and under natural conditions was similar and generally over 60% for all three species. However, at the low density, mortality in the laboratory was lower than in the field with all 3 species. The shorter the development time in the laboratory, the lower the mortality. In the field development times were longer, probably due to fluctuating temperatures. Hence, mortality was also higher.

Madder et al. (1983a) had mortality ranging from 6.7% at 15°C to 42% at 25°C. In our study,

Cx. restuans was much more tolerant of high temperatures. Mortality never exceeded 8.4%.

Because these 3 species breed continuously throughout the summer with overlapping generations, estimating the number of generations based only on the oviposition monitoring is difficult. Using the development times for each species under natural conditions, oviposition patterns and dates of first and last oviposition, we estimated the number of generations of each species. *Culex tarsalis* larval to pupal development time under natural conditions ranged from 19.8 to 25.3 days. Assuming an additional 15 days for embryonation, ovarian development, blood-feeding, egg development and oviposition, each generation would need 35–40 days. Oviposition commmenced as early as May 7 in 1981 and continued until late August in both years, resulting in a mean of 102 days of oviposition. Therefore, 2.6–2.9 generations could have occurred in 1980 and 1981. Similarly, with *Cx. restuans*, each generation would need 31–38 days. Oviposition continued over a mean of 113 days, and *Cx. restuans* could have had 3.0–3.6 generations. *Culiseta inornata* would require 35–36.4 days for each generation. In 1980 and 1981, the mean number of oviposition days was 118. Thus, 3.2–3.4 generations of *Cs. inornata* could have occurred.

In the southern United States, *Cs. inornata* is considered to be a winter mosquito. It is considered tolerant of cool temperatures throughout its range (McLintock 1964, Brust 1967, Hanec and Brust 1967). In this study, *Cs. inornata* was more tolerant of low temperatures than *Cx. tarsalis. Cx. restuans* is also more tolerant of low temperatures than *Cx. tarsalis.* Development time and survival is similar to the studies conducted in southern Ontario by Madder et al. (1983a).

Crowding affected all species similarly by increasing both development time and mortality. Mortality was generally greater than 60% at the high density. *Culiseta inornata* larvae were the most tolerant of crowding.

Onset of reproductive diapause: The criteria used to define reproductive diapause in females of *Culex* and *Culiseta* species have not been standardized. Some workers have used the size of the primary ovarian follicles at a specified age of the female (Oda and Kuhlow 1973, Barnard and Mulla 1978, Eldridge and Bailey 1979). Other authors have used the ratio of the mean length of several primary follicles: mean length of the corresponding secondary follicles per female (Spielman and Wong 1973, Hudson 1979, Madder et al. 1983b). Some authors have used the mean length of the primary follicle, in conjunction with the resting stage of the follicles, to determine if females were in reproductive diapause (Reisen 1986, Reisen et al.1986).

Madder et al. (1983b) showed that *Cx. restuans* in Ontario diapaused earlier in 1979 and 1980 than we found for *Cx. restuans* in Manitoba in 1981. Their study cannot be compared directly because they used a follicle:germarium ratio of 2.0:1. If we had used this ratio in our study all of the females, regardless of photoperiod, would have been in reproductive diapause.

As a result of the criteria we selected, some females (reared under 16L:8D photoperiod in the laboratory and under natural June and July photoperiods in the field) were in diapause. If we had chosen a ratio higher or lower than 2.5:1, the resulting percentage females in diapause would fluctuate accordingly.

The timing of reproductive diapause in *Cx. tarsalis* has implications in regard to vector control during a WEE outbreak in Manitoba and surrounding jurisdictions. The onset of most of the equine and human cases in Manitoba during 1975, 1977, 1981 and 1983 was during August (Sekla 1976, 1982; Artsob 1983). The supply of new vectors, particularly *Cx. tarsalis*, should diminish rapidly after July, as well as the need for larval control. This could be an important parameter in assessing the vector capacity of *Cx. tarsalis* populations during a WEE outbreak year. However, in addition to reproductive diapause, other basic information is needed to model the vector capacity of *Cx. tarsalis* populations in Canada and the northcentral United States. Local data are needed on longevity of natural populations of females in the field, the number of ovarian cycles in natural populations, the distance that females disperse and an expanded profile of habitat preferences. These data, together with the standard factors monitored in an arbovirus surveillance program (California Department of Health Services 1987), should provide the basic elements of a model which could be used to predict increasing or decreasing risk of a WEE outbreak.

ACKNOWLEDGMENTS

We wish to thank the City of Winnipeg's Parks and Recreation Department for its contribution to this research project and for their cooperation in the study. We acknowledge the support provided through the Natural Sciences and Engineering Research Council of Canada grant A2545 to R. A. Brust.

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