Western Equine Encephalomyelitis Virus Infection Affects the Life Table Characteristics of *Culex tarsalis* (Diptera: Culicidae)

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**ABSTRACT** The life table attributes of *Culex tarsalis* Coquilletti females infected experimentally by feeding on 4 and 6 log$_{10}$ plaque-forming units (PFU) of western equine encephalomyelitis virus (WEEV) per milliliter of heparinized chicken blood were compared with an uninfected control group. Females continually were offered 10% sucrose and an oviposition substrate and daily a blood meal through a biomembrane feeder. Mortality (dead females) and fecundity (female eggs per female) were monitored daily until all females died. Overall, 94% of 198 females in the two virus-infected groups were positive for WEEV at death when tested by plaque assay; the average body virus titer at death did not differ between groups. WEEV infection significantly altered the life table characteristics of *Cx. tarsalis*. Life expectancy at infection in days ($e_i$), reproductive effort in female eggs per female per generation ($R_0$), and generation time ($T$) in days for the infected cohorts were significantly lower than for the uninfected controls, whereas the reproductive rate ($r_e$) in female eggs per female per day was higher for infected than uninfected cohorts. In agreement with the WEEV infection data that showed similar body titers, there were few differences between the life table parameters for the 4 and 6 log$_{10}$ PFU treatment groups. Greatest differences were observed for survivorship between days 17–40 when virus titers in infected dying females were greatest. Our data extend recent studies that indicate mosquito infection with encephalitis viruses has a cost of reduced life expectancy and fitness.

**KEY WORDS** *Culex tarsalis*, western equine encephalomyelitis virus, life table, survivorship, fitness

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Although encephalitis virus epidemics can markedly alter the mortality schedules of vertebrate populations, far less is known about the impact of infection on vector mosquito population dynamics. Cohorts experimentally infected with western or eastern equine encephalomyelitis viruses (WEEV or EEEV, *Alphavirus, Togaviridae*) have been shown to have significantly a shorter life span and reduced fitness than uninfected controls (Scott and Lorenz 1998; Lee et al. 2000). These effects might have been caused by the multiplication of virus within infected mosquito midgut cells and salivary glands resulting in pathological changes that impact blood digestion and the frequency of blood feeding (Mims et al. 1966; Weaver et al. 1988, 1992; Weaver and Scott 1990).

Crowding or food stress during the immature stages (Reisen et al. 1984) and temperature during both immature and adult stages (Reisen 1995) significantly altered the life table attributes of *Culex tarsalis* Coquilletti, the primary vector of WEEV in western North America. The current research extends these laboratory life table studies and describes the impact of WEEV infection on the life table attributes of *Cx. tarsalis*.

**Materials and Methods**

**Mosquitoes.** The high virus producer (HVP) strain (Hardy et al. 1983) was derived from the original WEEV-susceptible (WS) strain of *Cx. tarsalis* (Hardy et al. 1978). This strain has been maintained in our insectary for the past 20 yr and was reselected for three generations to ensure a consistent response to WEEV infection. Mosquitoes were raised by standard methods (Mahmood 1999) at a density of three rafts per pan at 26°C. Adults were offered 10% sucrose for 3–6 d before experimentation.

**Virus.** The BFS1703 strain of WEEV originally isolated from *Cx. tarsalis* in Kern County, California, in 1953 was at suckling mouse passage 1 and Vero passage 3 when used in the current experiment. For infection, WEEV was diluted in heparinized chicken blood to produce infectious doses of $\approx$6 and 4 log$_{10}$ plaque-forming units (PFU) / ml. To determine titers of virus present in freshly fed and dead mosquitoes, whole mosquitoes were triturated in 1 ml of diluent and then tested by standard plaque assay on Vero cells (Kramer et al. 2002).
Infection. Two groups of 150 females each were offered a mixture of 6 or 4 log$_{10}$ PFU/ml of WEEV in heparinized chicken blood presented in a water-jacketed biomembrane feeder maintained at 37°C (Mahmood 1999). A control group was fed the same way but without virus. Twenty-five females that fully engorged within 15–30 min were allocated to each screen-topped, 3.8-liter carton cage, with four replicate cages per virus concentration. Three bloodfed females per group were frozen at −80°C immediately after feeding and then tested to determine the quantity of virus imbibed per female.

Life Table Methods. Females in each carton had constant access to 10% sucrose on cotton pads and a cup of tap water as an oviposition substrate. The entire carton was placed within a plastic bag to maintain humidity and held at 25°C in an environmental chamber. Cages were examined daily. Dead females were removed and placed into individual cryovials, labeled by cage and date, and frozen immediately at −80°C for later virus assay. Egg rafts were removed and placed into individually numbered vials with water. After 3 d, rafts were removed and eggs counted to estimate fecundity. Females were offered uninfected heparinized chicken blood through a biomembrane each day as a blood meal source for egg production.

Life table attributes for four replicate cohorts of 25 females each per treatment were calculated using methods applied previously to Cx. tarsalis (Reisen et al. 1984). Daily survivorship ($l_x$, the proportion living on day x) was used to calculate age-specific life expectancy ($e_x$). Age-specific fecundity ($m_x$) expressed in female eggs per living female per day was calculated by dividing the number of eggs laid by two, assuming a 1:1 sex ratio. Because immature survival is extremely variable and dependent upon environmental conditions (Reisen et al. 1984), we used the production of female eggs as an indication of reproductive effort. Age-specific reproductive effort ($l_x m_x$) was used to estimate generation time T and then summed to estimate reproductive effort per generation ($R_e$). The approximate rate of population change per female per day was calculated as $r_e = \ln(R_e)/T$.

Results

Infection. The titer of virus imbibed per female from the biomembrane feeder ranged from 2.9 to 3.2 and 1.7 to 1.8 log$_{10}$ PFU per blood meal for the 6 and 4 log$_{10}$ PFU/ml groups, respectively. Presuming a blood meal volume of 3 µl per female, the concentration of virus in the membrane feeders was 5.4–5.7 and 4.2–4.3 log$_{10}$ PFU/ml, respectively. Overall, WEEV was detected in 187 of the 198 (94.4%) females in the 6 and 4 log$_{10}$ PFU groups at death. Two females drowned in oviposition dishes and were not suitable for testing. Overall, the titer of WEEV per female at death averaged 4.9 log$_{10}$ PFU per female and did not differ between the 4 (mean = 4.9 log$_{10}$ PFU per female, $n = 100$) and the 6 log$_{10}$ (mean = 4.9, $n = 98$) treatment groups ($F < 0.01, df = 1, 195; P > 0.1$) when tested by an analysis of covariance (Hintze 1998). The covariate, age at death, was significant ($F = 7.65; df = 1, 195; P = 0.006$), indicating that virus titers at death changed as function of age. Data then were pooled over the 4 and 6 log$_{10}$ PFU treatment groups. A linear regression model provided a poor fit for the data ($R^2 = 0.04$) and therefore we fit a piece-meal linear polynomial model (Fig. 1) that improved the fit ($R^2 = 0.21$). Initially, virus titers increased as virus replicated after infection (slope b1 = 0.296), remained relatively constant during the period of greatest death rate (slope b2 = −0.036), but then decreased again as the oldest females died (slope b3 = −0.161).

Life Table Attributes. WEEV infection significantly altered the life table characteristics of Cx. tarsalis (Table 1). When tested by one-way analysis of variance (Hintze 1998), life expectancy at infection ($e_x$) ($F = 55.3; df = 2, 9; P < 0.001$), reproductive effort in female eggs per female per generation ($R_e$) ($F = 6.7; df = 2, 9; P = 0.016$), and generation time (T) in days ($F = 50.9; df = 2, 9; P < 0.001$) for the infected cohorts...
were significantly lower than for the uninfected controls, whereas the reproductive rate ($r_c$) in female eggs per female per day ($F/H_1<0.05$) was higher for infected than uninfected cohorts. In agreement with the similarity in WEEV titers at death, there were few differences between mean life table parameters for the 4 and 6 log$_{10}$ PFU treatment groups. Greatest differences in mean survivorship among treatments were observed between days 17 and 40 (Fig. 2A), when virus titers in infected dying females were greatest (Fig. 1).

Egg development was initiated among cohorts by the initial bloodmeal, and oviposition occurred synchronously at ~4-d intervals thereafter (Fig. 2B). Control and virus infected groups produced comparable numbers of eggs during gonotrophic cycles one and 2, but controls produced markedly more eggs during cycles 3–5. Differences in fecundity were related to the number of ovipositions per cohort and not egg raft size. When tested by an ANCOVA with age as the covariate, there were no differences in the mean numbers of eggs per raft among treatment groups [$F = 2.59; df = 2, 814; P = 0.07$; means ($n$) for control = 121 ($n = 374$), 4 log$_{10}$ = 113 ($n = 221$), 6 log$_{10}$ = 119 ($n = 223$)]. The number of eggs per raft pooled over treatment groups decreased significantly as a function of age in days ($x$) for high (6 log$_{10}$ PFU/ml) and low (4 log$_{10}$ PFU/ml) WEEV treatment and control (uninfected) groups.

Table 1. Mean (standard deviation) life expectancy at infection in days ($e_x$), net reproduction per generation ($R_o$) in female eggs per female, generation time in days ($T$), and reproductive rate in female eggs per female ($r_c$) for four cohorts of 25 female Cx. tarsalis per treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$e_x$</th>
<th>$R_o$</th>
<th>$T$</th>
<th>$r_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 log$_{10}$</td>
<td>17.7(1.49)b</td>
<td>133(24.3)b</td>
<td>8.45(0.73)b</td>
<td>0.58(0.057)b</td>
</tr>
<tr>
<td>4 log$_{10}$</td>
<td>20.2(1.02)b</td>
<td>134(11.1)b</td>
<td>8.62(0.62)b</td>
<td>0.57(0.039)b</td>
</tr>
<tr>
<td>Control</td>
<td>29.1(3.71)a</td>
<td>213(26.9)a</td>
<td>12.34(0.46)a</td>
<td>0.44(0.024)a</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter were not different by a least significant range test ($P > 0.05$).
Discussion

WEEV infection in Cx. tarsalis females was associated with significant decreases in life expectancy ($e_x$) and fitness ($R_n$) compared with uninfected females from the same cohort. Unlike one previous study where survival of the low virus dose-infected group and uninfected controls was similar and differed from the high virus dose-infected group (Lee et al. 2000), the low and high dose groups in our study produced similar survivorship curves that differed significantly from uninfected controls. Infection in the previous study (Lee et al. 2000) for the high titer group was from uninfected controls. Infection in the previous study (Lee et al. 2000) for the high titer group was from uninfected controls. Infection in the previous study (Lee et al. 2000), and uninfected control was similar and differed from the low and high dose groups in our study produced where survival of the low virus dose-infected group and uninfected controls was similar and differed from the low and high dose groups in our study produced similar survivorship curves that differed significantly from uninfected controls. Infection in the previous study (Lee et al. 2000) for the high titer group was assumed to be 82% ($n = 72$ females from a concurrent experiment), but the mosquitoes in the survivorship experiments were not tested for infection. Possibly, the infection rate in the low titer group was <82%, and therefore the survivorship curve approximated that of the uninfected controls, because many of the females were not infected. We used the HVP laboratory colony reselected for a homogenous susceptible response to WEEV infection and practically all females (94%; $n = 198$) became infected regardless of the infectious dose. Our parallel experiment using the resistant WR colony to provide infection without possible pathology was not included, because most of the females were not infected when tested at the end of the experiment (data not shown).

The oldest females in our experiment had the lowest virus titers at death. This could be attributed to the females simply having lower titers infected or to the females modulating the virus titers in their infections. Previous studies described a decrease in WEEV body titers among old Cx. tarsalis females that was attributed to the ability of the mosquito immune system to lower body titers (Kramer et al. 1998).

Reproductive effort or fitness was lower in infected than uninfected Cx. tarsalis females, primarily because there were fewer ovipositions or egg rafts per cohort. Egg raft size did not differ significantly among treatments, differing from an EEEV infection experiment with Culiseta melanura Coquillett, where infection reduced both the number of ovipositions and egg raft size (Scott and Lorenz 1998). Because egg raft size in Cx. tarsalis was similar among infected and uninfected females, changes in gut pathology related to WEEV infection (Weaver et al. 1992) apparently did not prevent blood digestion and subsequent egg development. However, the overall outcome of our experiment agreed well with previous studies on EEEV infection in Cx. melanura where infection reduced both survival and fitness (Scott and Lorenz 1998).

The impact of WEEV infection on Cx. tarsalis adult life table attributes far exceeded stress induced by immature rearing temperature (18–31°C), food availability, or density (300–2,400 larvae per pan) that altered adult size and autogeny rate at emergence (Reisen et al. 1984). However, the combined effect of immature rearing and adult holding temperatures ranging from 14 to 32°C markedly reduced life expectancy at emergence from a mean of 26–9 d, respectively (Reisen 1995). The impact of temperature combined with infection stress has not been investigated.

The impact of virus infection on survival and reproduction was not readily discernible until >2 wk after the females imbibed the infectious blood meal and were 3–5 parous. In nature survivorship repeatedly was estimated vertically to be ≈85% per day and few tripars were detected during age grading studies (Reisen et al. 1995b). These estimates were slightly greater than survivorship estimated horizontally using mark–release-recapture methods (Reisen et al. 1992, Reisen and Lothrop 1995). Assuming a constant 85% value for survivorship, only ≈10% of the population would be expected to remain alive in the field after 14 d, and these few individuals would seem to be insufficient to alter vector population dynamics. In addition, vector infection rates in nature typically are low. Even during major epizootics, WEEV minimum infection rates (MIRs) typically remained <10 per 1000 (Reisen et al. 1995a), and most females remained uninfected. Therefore, even though infection may reduce fitness, low MIRs and inconsistent virus occurrence would seem to limit infection as a selective pressure on vector populations.

The impact of decreased survival among old infected females also may not markedly impact virus transmission dynamics. At extrinsic incubation temperatures of 25°C, 50% of Cx. tarsalis females were able to transmit WEEV by bite at 7 d postinfection (Reisen et al. 1993). These previous and ongoing experiments indicate that WEEV transmission rates declined after 14 d, the time during the current study when differences in mortality among infected and uninfected cohorts became distinct. Therefore, increased mortality among infected females >2 wk postinfection may not have a marked impact on WEEV transmission.
dynamics in nature, because these females contribute minimally to the force of transmission.

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