SCIENTIFIC NOTE

# Effect of Oviposition Site Deprivation on Oviposition Performance and Egg Hatch Rate of Naturally Blood–fed Gravid *Culex quinquefasciatus* (Diptera: Culicidae)

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**Abstract:** The effect of oviposition site deprivation on oviposition performance and egg hatch rate of naturally blood–fed gravid *Culex quinquefasciatus* was examined in the laboratory. The rate of gravid females ovipositing within 24 hours was not significantly affected by the period of oviposition site deprivation that ranged from 1 day to 9 weeks. However, some gravid females failed to form egg rafts, and significant differences were found among some treatments. The egg hatch rate decreased significantly with the period of oviposition site deprivation. The implications of these findings on the introduction of mosquito borne viruses are discussed.

**Key words:** Gravid *Culex quinquefasciatus*, oviposition site deprivation

The Southern House Mosquito, *Culex quinquefasciatus* (Say), an important vector of St. Louis encephalitis in the U.S. mainland, causes major mosquito nuisance complaints in Hawaii (Department of Health, Vector Control Branch Manual, 1991). Since the introduction of the West Nile virus (WNV) in North America, *Cx. quinquefasciatus* has caused more concern because of its status as primary vector of WNV (Turrell et al. 2001). To prevent WNV from being introduced and established in Hawaii, we need to thoroughly understand the behavior and population dynamics of *Cx. quinquefasciatus* in the field.

It has been found that some gravid female mosquitoes are forced to retain mature eggs if there is lack of a suitable oviposition site (Bentley and Day 1989). The forced egg-retention can affect oviposition patterns in *Aedes aegypti* (L.) (Chadee 1997), and affect adult survival and reproduction in *Aedes albopictus* Skuse (Xue et al. 2005). Little is known about this aspect on *Cx. quinquefasciatus*, especially in wild gravid females.

Mosquitoes frequently board aircraft and hitch-hike considerable distances (Joyce 1961), and obviously they can reach Hawaii within a day by aircraft from any major city in the U.S. Mainland. Mosquitoes may also get aboard ships bound for Hawaii (Joyce 1961, Kilpatrick et al. 2004), and they have to survive without a suitable oviposition site for an extended period of time. Furthermore, *Culex* mosquitoes can carry WNV virus through winter (Nasci et al. 2001), which indicates that WNV can survive at least several months in a mosquito body. If the oviposition performance and egg hatch rate of mosquitoes are not affected by oviposition site deprivation, WNV infected mosquitoes in cargo ships from North America and other WNV endemic regions of the Old World may introduce WNV into Hawaii or other WNV-free regions. This study was carried out to investigate the effect of oviposition site deprivation on oviposition performance and egg hatch rate of naturally blood–fed gravid *C. quinquefasciatus*.

## Materials and Methods

**Mosquitoes.** Gravid traps (CDC type) baited with 2.5 gallons of infused water (tap water, horse manure and dry hay fermented for 4 days) were set up at different locations in the town of Lihue, on the island of Kauai, Hawaii. The traps were set up between 08:00 to 09:30 h and gravid female mosquitoes were collected between 08:00 to 08:30 h next morning. All naturally blood–fed gravid female mosquitoes were collected from February to July 2005.

**Experimental design.** Since the numbers of naturally blood–fed gravid females obtained from each trap varied, thirty five to forty gravid females in good condition (no injuries) trapped on the same day but at different locations were randomly chosen and transferred with a respirator into a cubical screen cage (30 x 30 x 30 cm) (BioQuip Products Co. Gardena, CA). Seven colonies were established within 2 weeks and each one received a different treatment. These colonies were provided with infused water on different days after trapping as follows: day 1 (control treatment), and 2, 4, 6, 7, 8 and 9 weeks after trapping. The infused water was kept in a plastic container (10 cm high, 11 cm in diameter). Vials with a cotton wick soaking in a 10% sucrose solution were provided in each cage as adult food. Solution and wicks were changed twice a week. The six treatments and the control were replicated a total of three times. All tests were conducted in a room maintained at 25–28°C, 50–60 % RH, and a photoperiod of ca. 12L:12D.

**Oviposition.** Once the infusion water was provided to the colony, the number of egg rafts was recorded after 24h along with the number of females that failed to lay eggs or failed to form egg rafts (laid a group of separated eggs).

**Egg hatch rate.** The egg rafts or the group of separated eggs were collected and the numbers of eggs for each egg raft or group of separated eggs were counted. Then, they were individually (egg raft or group of separated eggs) placed into a glass bottle (10 cm in length, 1 cm in diameter) half-filled with infused water (same as above). Egg hatch rate was determined by counting the number of hatched eggs after 48 hours under a dissecting microscope.

**Data analyses.** Means of female oviposition performance (rate of ovipositing females within 24 hours of infusion water provision and egg raft forming rate) and egg hatch rate of each treatment were calculated. Comparisons of oviposition performance and egg hatch rate in gravid mosquito females among different treatments and control were examined using one-way ANOVA (Analytical Software 1996) and the Tukey HSD test. The data were transformed by arcsin square root before analysis; untransformed means are presented in Table 1.

## Results

Table 1 summarizes the rate (mean ± SE) of naturally blood–fed gravid females ovipositing within 24 hours of infusion water provision, egg raft formation rate, and egg hatch rate. For the rate of gravid females ovipositing within 24 hours no statistically significant differences were found among treatments and control ($F_{6,14} = 1.43$, $p>0.05$). For the egg raft formation rate, the rate of treatment with 8 weeks of oviposition site deprivation was significantly lower compared with the control, and the treatments of 2 and 6 weeks of oviposition site deprivation ($F_{6,14} = 5.75$, $p<0.05$) (Table 1). In general, the egg hatch rate significantly decreased with the increase in the length of oviposition site deprivation ($F_{6,14} = 25.02$; $p<0.001$). For example, the hatch rate in the control was 1, while the hatch rate was only 0.01 in the treatment deprived of infused water for 9 weeks (Table 1).

**Table 1. Oviposition performance and egg hatch rate for the naturally blood–fed gravid *Culex quinquefasciatus* under different treatments of oviposition site deprivation (mean ± SE).**

| | Rate of ovipositing females within 24 hours of infusion water provision | Egg raft formation rate | Egg hatch rate |
|---|---|---|---|
| Control | 1 (0) a | 1 (0) a | 0.98 (0.02) a |
| 2 weeks | 1 (0) a | 1 (0) a | 0.91 (0.09) ab |
| 4 weeks | 0.71 (0.15) a | 1 (0) a | 0.52 (0.19) b |
| 6 weeks | 0.88 (0.06) a | 0.58 (0.13) ab | 0.07 (0.07) c |
| 7 weeks | 0.89 (0.11) a | 0.67 (0.17) ab | 0.03 (0.02) c |
| 8 weeks | 0.61 (0.20) a | 0.44 (0.06) b | 0.01 (0.01) c |
| 9 weeks | 0.72 (0.15) a | 0.67 (0.17) ab | 0.01 (0.01) c |

*Means in the same column followed by the same letter are not significantly different (P>0.05, Tukey HSD test). Three replicates per treatment.

## Discussion

Because a few females exhibited an extended lifetime when confronted with oviposition site deprivation, we had a chance to determine their late oviposition performance and egg hatch rate. However, we do not know how long these naturally blood–fed females had already survived and how many times they had been gravid. Weidhaas et al. (1973) found that the first oviposition of *Cx. quinquefasciatus* usually occurred about 8 days after the females emerged. Thus, the gravid females used in this experiment must have been at least 8 days old when they were caught as all of them laid eggs within 24h of capture. Furthermore, because the colonies were formed by randomly chosen gravid females, the chance to have individuals with different ages and gravid phases for the colonies was equal.

**Egg hatch rate.** The results showed that late oviposition of mature eggs affected the egg hatch rate. However, from this trend we can not conclude that the egg hatch rate will be 0 if the period of oviposition site deprivation increases to more than 9 weeks. Perhaps, some females can lay live eggs as long as they live. This is based on the fact that a gravid female with 9 weeks of oviposition site deprivation laid an egg raft, from which the eggs hatched completely.

Since the oviposition ability remained high and egg hatch rate was considerably high when gravid *Cx. quinquefasciatus* were provided with oviposition sites 4 weeks after capture, the findings of this study have at least two implications: (1) A few gravid *Cx. quinquefasciatus* can survive long-distance transportation to reach Hawaii from other continents, and potentially deposit their mature eggs and establish a colony. (2) Since gravid females are more likely to contain the virus than unfed mosquitoes, the transmission of virus in the future may be from gravid females. For example, an arriving gravid *Cx. quinquefasciatus* with WNV may either transmit the virus transovarially (Goddard et al. 2002) or transmit the virus through feeding on the local birds.

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