DIEL PERIODICITY OF OVIPOSITION AND SOIL MOISTURE PREFERENCE OF *PSOROPHORA COLUMBIAE*[1,2]

C. L. Meek and D. C. Williams
Department of Entomology
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Louisiana State University
Baton Rouge, LA 70803
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ABSTRACT

The ovipositional cycle of *Psorophora columbiae* was bimodal when gravid females were exposed to natural climatic conditions over three separate 24 hr periods. Peak egg deposition (49.8 eggs/cage) occurred during the first 24 hr period after sunset. Other cages of *Ps. columbiae* females were exposed to aliquots of soil with various moisture levels by weight. Average numbers of eggs deposited per cage in soil at 0, 20, 40, 60 and 80% moisture by weight differed significantly ($P < 0.001$) with 2.2, 30.2, 89.8, 28.3 and 14.0 eggs, respectively. When the intervals between moisture levels were reduced to 10% and caged mosquitoes were exposed to soil at 30, 40, 50, 60, 70 and 80% moisture, significant differences ($P < 0.008$) occurred among the mean numbers of eggs per moisture level (125.8, 188.6, 176.9, 39.6, 46.3, and 31.5, respectively).

Key Words: Diel periodicity, mosquitoes, soil moisture, egg laying, oviposition, *Psorophora columbiae*.

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INTRODUCTION

Two factors important to oviposition of floodwater mosquitoes, particularly *Psorophora columbiae* (Dyar and Knab), are diel periodicity and soil moisture preference. Only a few species of mosquitoes have been studied to determine their ovipositional cycles. *Aedes aegypti* (L.) and *Aedes africanus* Theobald exhibit peak oviposition in late afternoon (Haddow and Gillett 1957; Gillett and Haddow 1957; McClelland 1968). *Coquillettidia fuscopennata* (Theobald) has a bimodal distribution of oviposition with the first occurring early at night and a major peak just before sunrise (Haddow and Gillett 1958). The oviposition cycle of *Ae. apicoargenteus* Theobald is diurnal and cyclical, broadly resembling the biting cycle (Haddow et al. 1960). Oviposition for some permanent water species, such as *Anopheles gambiae* Giles, occurs 1 hr after sunset (Haddow and Ssenkubug 1962), whereas *Culex pipiens quinquefasciatus* Say begins egg laying 3 to 5 hrs after sunset (Suleman and Shirin 1981). No information is available on the diel periodicity of oviposition for the floodwater species, *Ps. columbiae*.

[1] Diptera: Culicidae

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Data for soil moisture preferences of ovipositing floodwater mosquitoes also are limited. Knight and Baker (1962) determined moisture requirements for *Aedes sollicitans* (Walker) and *Aedes taeniorhynchus* (Wiedmann) in the laboratory (Russo 1977; Strickman 1980) and in the field (Horsfall et al. 1975; Cassani and Bland 1978; Strickman 1980; Novak 1981). In Texas, most *Ps. columbiae* eggs were deposited on riceland soil with moisture levels ranging from 75% of field capacity to just above field capacity (Olson and Meek 1977, 1980). Field capacity is defined by Box and Bennett (1959) as the maximum amount of water that a given soil type can hold against drainage by gravity.

In studying factors influencing the attractiveness of sites to ovipositing *Ps. columbiae,* it would be beneficial to more precisely determine preferred soil moisture as well as define the time of day for the greatest ovipositional activity. These data may be of particular importance to researchers attempting to develop a self-mating colonization technique for this species. For these reasons, laboratory studies were conducted to determine the diel periodicity of ovipositing *Ps. columbiae* and the preferred soil moisture gradient for egg deposition.

MATERIALS AND METHODS

Diel Periodicity

Adults of *Ps. columbiae* were collected in Jefferson Davis Parish, LA, with CDC light traps baited with dry ice. Mosquitoes were transported to the laboratory in screened cages (30 × 30 × 30 cm) covered with damp toweling. In the laboratory, 20 *Ps. columbiae* females were transferred to cages (20 × 8 × 8 cm) which were constructed of aluminum screening (16-mesh) on 2 opposing sides and clear Plexiglas® (3.2 mm thick) on the other two sides. Both ends of each cage were Plexiglas® with one end having a centered hole (4 cm diam) for the introduction of the mosquitoes. Water soaked raisins, covered with moistened paper toweling, were maintained on top of the cages at all times to provide mosquitoes with a carbohydrate source. Mosquitoes were offered blood 4 and 3 days prior to the start of the oviposition tests.

Inside open-topped plastic containers (34 × 26 × 9 cm) lined with cellucotton, strips (20 × 8 cm) of double layered cheesecloth were laid perpendicular to the lengths of the containers. Cages of mosquitoes were placed over the cheesecloth strips. The containers were placed outdoors to take advantage of ambient summertime temperatures, natural photoperiod, and other natural climatic factors. The containers were tilted lengthwise at a 20° angle so that free water along the entire lower length of each container provided the gravid mosquitoes with a moisture gradient along the cheesecloth strip.

Each test was conducted by removing the moistened cheesecloth strips from underneath the cages and replacing them with clean strips every 2 hrs throughout a 24 hr period. Once removed, egg ladened cheesecloth strips were maintained in a moistened state at 24°C until the number of eggs on each strip was counted, usually within 24 hrs.

Tests were started at various times of day, but scheduled in order that one 2 hr period would begin at civil sunset. Tests were conducted on July 22, July 28, and August 12, 1983. Sunset and sunrise on these days began at 7:40 pm and 6:40 am, 7:34 pm and 6:39 am, and 7:15 pm and 6:47 am (Central Daylight

Savings Time), respectively. Eleven cages were used in the July 22 test and 12 cages on each of the July 28 and August 12 tests.

Transformed data from the different time periods were subject to an analysis of variance (ANOVA SAS User's Guide: Statistics version 5th Edition, p. 114-30) to test the hypothesis that the mean numbers of eggs laid during the 2 hr time periods were equal. To equalize the variances, data were transformed by taking the square root of the number of eggs. A "t" test was performed to test the hypothesis that the mean number of eggs laid during darkness was equal to the mean number of eggs laid during the daylight hours.

Soil Moisture

The methodology for collecting, transporting, and maintaining caged adults of *Ps. columbiae* was similar to the procedures previously described. However, in this case, adult mosquitoes were maintained in screened alumninum cages (30 × 30 × 30 cm) prior to and during the soil moisture preference tests. The cages were kept in the laboratory at ca. 22°C and subjected to a 12:12 light:dark photoperiod. Mosquitoes were offered guinea pigs as a blood source 4 and 3 days prior to the beginning of the tests.

The substrate used in these tests was Jeanerette silt loam soil from a rice field in Vermilion Parish, LA. The soil was screened through a sieve (40-mesh) to remove vegetation and large pieces of debris and subsequently oven dried at 100°C until a constant soil weight was achieved. Strickman (1980) indicated that oven-drying may alter the chemical composition of the soil; however, results obtained using oven-dried soil are more relevant to field conditions than gauze, cheesecloth or sand as has been used by other authors in previous research. Aliquots of dry soil (50 g) were weighed in the bottom portions of petri dishes (9 cm diam).

Approximately 1 hr prior to beginning of a test, the appropriate amount of water was added to each dish to establish the desired percentage of moisture by weight. In each cage, mosquitoes were exposed to six moisture levels within the randomly spaced Petri dishes. The first series of trials involved soil at 0, 20, 40, 60, and 80% moisture by weight and a sixth petri dish containing only water. This provided an opportunity for gravid mosquitoes to oviposit over the entire gradient of soil moisture to include free standing water. Soil in petri dishes with lower percentages of soil moisture was stirred with a metal spatula to insure equal distribution of moisture throughout the sample.

Three trials each involving 3 cages of mosquitoes were conducted with the first series of moisture level experiments. A second series of tests were conducted to provide supportive data to the first tests and further define the percentage of soil moisture required for maximum egg deposition. These latter tests involved two trials of four cages each using soil aliquots of 30, 40, 50, 60, 70 and 80% soil moisture. The resultant data from these tests could be more appropriately compared to general field determinations of preferred soil moistures by *Ps. columbiae* as shown in ricelands (Olson and Meek 1977). Each test began approximately 1 hr before sunset and ended 2 hr after dawn. All petri dishes were reweighed at the conclusion of each test.

Eggs were recovered from soil samples by techniques described by Horsfall (1956). Collapsed or dessicated eggs were not counted but were assumed present in the soil prior to testing.

Transformed data from the tests were subjected to an analysis of variance to test the hypothesis that the mean numbers of eggs deposited at different soil moisture levels were equal. To equalize variances, data were transformed by adding 1 to each number and taking the natural logarithm of the resulting number. After rejection of the hypothesis, mean comparisons were made utilizing orthogonal contrasts (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Diel Periodicty

The results of the diel periodicity study are given in Figure 1. The "t" test indicated that significantly ($P < 0.05$) more total eggs were laid at night (mean 140.3 eggs/cage) than during the day (mean 49.2 eggs/cage). Analysis of variance revealed that oviposition among the time periods was significantly different ($P < 0.001$). More eggs (49.8) were deposited in the 2 hr period after sunset than in any of the other periods. Afterwards, the number of eggs per cage gradually decreased from 27.5 to 4.1 eggs/cage for the period that included sunrise. The mean number of eggs per cage dropped to its lowest point for the 24 hrs in the 2 hr period after sunrise with an average of 3.2 eggs/cage.

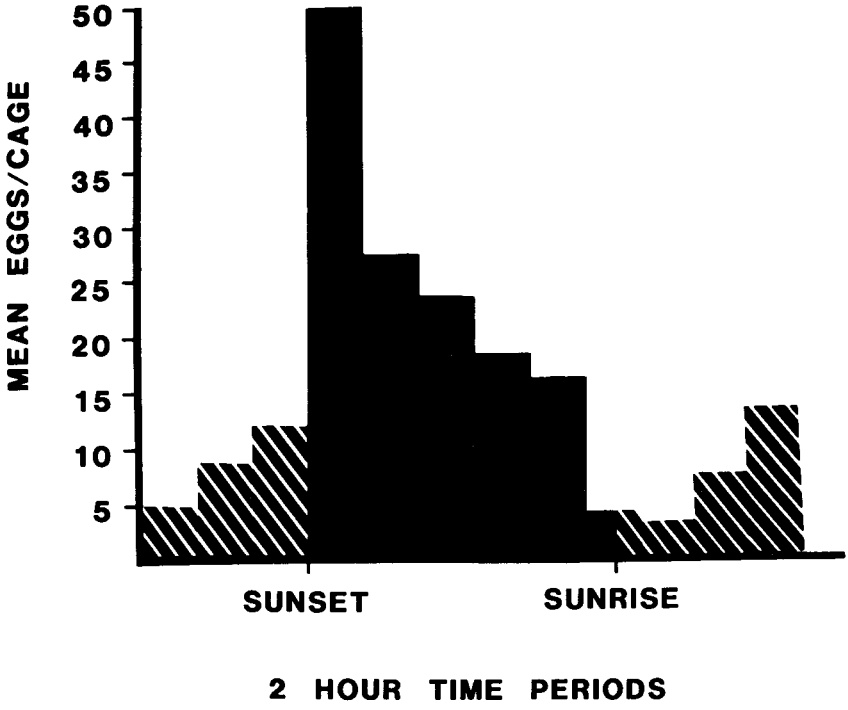

Fig. 1. Diel periodicity of oviposition by caged *Psorophora columbiae* exposed to natural climatic conditions for three 24 hr time periods in Louisiana (July - August 1983).

The diel periodicity of *Ps. columbiae* oviposition in this experiment was bimodal with the main activity occurring in the 2 hr period after sunset. However, a small peak in oviposition occurred near mid-day when 13.3 egss/cage were recorded. The former peak coincides with the greatest adult collections in light traps (Horsfall 1955; Al-Azawi and Chew 1959; Olson 1983). The timing of this peak in oviposition also coincides with observations by Steelman et al. (1972) concerning the number of blood-fed mosquitoes (including *Ps. columbiae*) resting on barn walls. Adult swarming of *Ps. columbiae* in Texas (Olson 1983) also occurred soon after sunset.

Soil Moisture

Females of *Ps. columbiae* deposited eggs on soil at all moisture levels. Analysis of variance on data from the first series of tests, in which 0, 20, 40, 60, 80, and 100% soil moisture levels were presented to mosquitoes, indicated that mean numbers of eggs laid at different levels were significantly different ($P < 0.004$). Orthogonal contrasts revealed that there was a significant quadratic effect ($P < 0.001$). Weight changes in the soil due to evaporation or absorption (taking up moisture from the air) averaged 3.3%. Soil moisture levels did not overlap from the beginning to the end of the tests. During the nine trials an average of 2.2 eggs were laid at zero moisture level. This was the moisture level at which the fewest eggs were deposited on the average whereas the 40% moisture level received the most eggs with a mean of 89.8 eggs/trial. At 20, 60 and 80% moisture levels averages of 30.2, 28.3, and 14.0 eggs/trial, respectively, were deposited. Surprisingly, an average of 43.2 eggs/trial were laid in petri dishes containing water. The authors recognize that oviposition on dry soil and in free-standing water is aberrant behavior for *Ps. columbiae*. Knight and Baker (1962) in laboratory tests with the floodwater species *Ae. sollicitans* and *Ae. taeniorhynchus* suggested that eggs deposited at moisture extremes may have been deposited by dying females and did not represent a preference selection.

Eggs also were deposited at all soil moisture levels in the second series of tests in which gravid females were exposed to petri dishes containing soil at 30, 40, 50, 60, 70, and 80% moisture levels. The mean numbers of eggs laid at different soil moistures were significantly different ($P < 0.01$). The orthogonal control revealed a significant ($P < 0.001$) linear effect due to soil moisture. Mean numbers of eggs per trial laid at 30, 40, and 50% moisture levels were 125.8, 188.6, and 176.9, respectively. Lower numbers of eggs were deposited at 60, 70, and 80% soil moistures with 39.6, 46.3, and 31.5 eggs/trial, respectively. The difference in the total number of eggs deposited between each test series was due to several factors. Tests were conducted on separate dates and therefore field collected adult mosquitoes used in the tests were not of similar age or reproductive potential. However, these dissimilarities did not appear to affect the eventual selection of the 40% moisture gradients for maximum egg deposition.

Data from the present study showed that silt loam soil containing 30% moisture by weight fit within the 75% field capacity to just above field capacity category as defined by Box and Bennett (1959) and demonstrated by Olson and Meek (1977) to be preferred by ovipositing *Ps. columbiae*. The majority of eggs were deposited in the 30 to 50% soil moisture range with the most eggs being deposited at 40% soil moisture in both series of tests.

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