OVIPOSITION ATTRACTANTS OF THE WESTERN TREEHOLE MOSQUITO, *AEDES SIERRENSIS*

ABBAS AHMADI AND G. A. H. McCLELLAND
Department of Entomology, University of California, Davis, CA 95616

ABSTRACT. Experiments were conducted to determine the role of mosquito-produced attractants and chemicals of environmental origin in the selection of oviposition sites by the western treehole mosquito. Under laboratory conditions, treehole water and laboratory rearing water were attractive, but emergence water, larval holding water and water exposed to newly laid eggs were not attractive to ovipositing mosquitoes. The results indicate that decayed organic matter in natural breeding water acts as an oviposition attractant for this species.

The western treehole mosquito, *Aedes sierrensis* (Ludlow), is one of the most annoying biting pests in recreational and wooded residential areas of California (Bohart and Washino 1978). It is also a vector of canine heartworm, *Dirofilaria immitis*, in northern California (Walters and Lavoipierre 1982). Larvae have been found in treeholes of at least 21 different species of trees with a wide range of pH (3.9–10.1) and total soluble salts (3,950–8,102 ppm) (Baerg unpublished[1], Chapman 1965). Adult oviposition behavior is a key determinant of larval habitats, and may depend on a variety of long- and short-range stimuli. Previous studies on other *Aedes* species have identified 4 categories of chemicals in the breeding water that may play a role in oviposition site selection: (1) egg-associated oviposition attractants (2) larval-produced attractants (3) pupal-produced attractants and (4) chemicals of environmental origin (such as produced by decaying organic matter). A summary of the literature dealing with species of *Aedes* is presented in Table 1. Although *Ae. sierrensis* is the only treehole mosquito of economic importance in California, no investigation has focussed on its oviposition attractants. The present study was to determine if mosquito-produced attractants and chemicals of environmental origin influence the selection of oviposition sites by *Ae. sierrensis*.

MATERIALS AND METHODS

The mosquitoes used were obtained from a 5-year old stock colony derived from a wild population near Vacaville, California. Sugar water was available to adults at all times and blood was offered, at intervals, by placing restrained guinea pigs in adult mosquito cages. Mosquitoes were reared from egg to pupa in white enameled pans 40 × 25 × 7 cm filled with 300 ml of tap water. Two hundred larvae were kept in each pan and fed liver powder. Unless otherwise specified all work was conducted at 25 ± 2°C, 35 ± 5% RH, and 12L:12D photoperiod.

Lots of 100 pupae were removed from the stock colony, placed in cages 60 × 30 × 30 cm and allowed to emerge. Seven days after emergence adults were fed on restrained guinea pigs. Bloodfed females were aspirated and transferred to 3.8-liter (1-gallon) ice cream cartons and held for 10 days, with access to sugar water. Females were tested once only, during the first gonotrophic cycle.

Oviposition bioassays were conducted in 3.8-liter ice cream cartons, with screen tops, placed in a 432-liter environmental chamber. Air within the chamber was exhausted at a rate of 5 liters per min to prevent saturation of the air space with any chemicals. Each carton held 2 oviposition sites 7.5 cm apart, consisting of small plastic cups (43 × 43 mm) lined with white filter paper (Whatman no. 1) and containing either the standard or treatment water. To prepare each unit for oviposition bioassay, 10 ml of a test medium was placed in one plastic cup and 10 ml of a standard (distilled water) in the other. The positions of treatment and standard plastic cups within each unit were randomly assigned. Five gravid female mosquitoes were placed in each bioassay unit and kept for 16 hours in the dark. Darkness was necessary to eliminate the effects of visual stimuli. Filter papers and plastic cups were replaced after each test to prevent possible contamination by test chemicals.

Solutions were prepared as follows: (1) egg water; 1000 freshly laid eggs were placed in 100 ml distilled water and held for 24 hr; (2) larval holding water; 100 4th instar larvae were washed 3 times with distilled water and kept without food in 100 ml distilled water for 24 hr; (3) emergence water; 100 pupae were washed 3 times with distilled water and held in 100 ml distilled water until emergence was completed; (4) treehole water; 20 liters of treehole water (5°C, pH 6.5) containing larvae of *Ae. sierrensis* were collected from a walnut orchard near Vacaville, California in January and kept for 5

[1] Baerg, D. C. 1965. A study of the biology and ecology of the treehole mosquitoes of California. M. S. thesis. Univ. Calif. Library, Davis. 66 pp.

Table 1. Oviposition attractants of 7 species of *Aedes* mosquitoes.

Species	Oviposition attractants	Reference
Ae. aegypti (L.)	Horse manure infusion	O'Gower (1963)
	Hay infusion bacteria	Hazard et al. (1967)
	7,11-Dimethyloctadecane	Ikeshoji et al. (1979)
	Larval holding water	Soman and Reuben (1970)
	4th instar larval water	Roberts and Hsi (1977)
	Pupal exuviae	Ibid
Ae. albopictus Skuse	Grass infusion	Gubler (1971)
	Egg-associated pheromone	Ibid
Ae. atropalpus (Coq.)	Larval holding water	Kalpage and Brust (1973)
	Pupal holding water	Ibid
Ae. nigromaculis (Ludlow)	Natural breeding water	Ikeshoji and Mulla (1970)
Ae. polynesiensis Marks	Grass infusion	Gubler (1971)
	Egg-associated pheromone	Ibid
Ae. taeniorhynchus (Wied.)	Natural breeding water	Ikeshoji and Mulla (1970)
Ae. triseriatus (Say)	Decayed organic matter	Wilton (1968)
	Treehole water	Bentley et al. (1976)
	p-cresol	Bentley et al. (1979)
	p-ethylphenol	Bentley et al. (1981)
	Larval holding water	Bentley et al. (1976)
	Larval produced attractants	McDaniel et al. (1976)

days at room temperature and (5) laboratory rearing water; 3 liters of water collected from colony rearing pans. All solutions were filtered through Whatman no. 1 filter paper and the filtrate used for the bioassays. Solutions 4 and 5 were stored at −11°C prior to testing.

Paired *t*-tests were used to determine if the numbers of eggs laid in the test and control cups were significantly different at $P < 0.05$, following Bentley et al. (1981).

RESULTS AND DISCUSSION

The results of bioassay tests are shown in Table 2. Unlike *Ae. albopictus* and *Ae. polynesiensis* (Gubler 1971), an egg-associated attractant was not detected in *Ae. sierrensis*. Our assays failed to show the presence of attractants of larval or pupal origin for *Ae. sierrensis* although these have been reported for other species of *Aedes* (Table 1). For example, Kalpage and Brust (1973) reported that *Ae. atropalpus* preferred to oviposit on clean water which had held its larvae and pupae for 24 hr; we repeated the same test with *Ae. sierrensis*, but the results were negative. However, the treehole water and the laboratory breeding water were significantly more attractive than distilled water. *Aedes sierrensis* was similar to other container-breeding mosquitoes such as *Ae. aegypti* (Hazard et al. 1967) and *Ae. triseriatus* (Bentley et al. 1979) which respond positively to decayed organic matter. Bentley and coworkers (1979) found that *p*-cresol, a trace component of aqueous infusions of decayed organic matter, produced a highly selective oviposition response in *Ae. triseriatus*. Ikeshoji and coworkers (1979) isolated an oviposition attractant for *Ae. aegypti* from a bacterial culture medium, and identified it as 7,11-dimethyloctadecane. Since bacteria are important to the nutrition of *Ae. aegypti* larvae, Roberts and Hsi (1977) suggested that it is reasonable that microbal metabolites influence site selection for oviposition. In view of similarities between *Ae. sierrensis*, *Ae. aegypti* and *Ae. triseriatus*, it is possible that a similar mechanism exists for *Ae. sierrensis* as well.

The attractant chemical in treehole water was

Table 2. Attractiveness of 5 different solutions when tested against gravid female *Ae. sierrensis* mosquitoes using distilled water as standard.*

Test solution	No. tests	Mean no. eggs laid (± S.E.) Treatment	Standard	P (*t*-test)
Egg water	10	87 (22)	130 (37)	>0.05
Larval holding water	10	169 (40)	69 (39)	>0.05
Emergence water	9	93 (33)	85 (25)	>0.05
Treehole water	8	254 (43)	45 (21)	<0.01
Lab. rearing water	4	181 (28)	6 (5)	<0.01

* Five mosquitoes per bioassay unit. Mosquitoes were held 16 hr in darkness at 25 ± 2°C and 35 ± 5% RH.

stable (at least for 5 days). Since the mosquitoes were able to contact the liquid in the cups, both olfaction and gustation could be involved in chemical discrimination between the test solutions.

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