AN ANALYSIS OF THE OVIPOSITIONAL RESPONSE OF *AEDES ATROPALPUS* TO EXPERIMENTAL OVIPOSITION WATERS

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ABSTRACT. A set of seven experiments was conducted under laboratory conditions to determine the respective influence of larval-produced attractants or stimulants, microbial activity and chemicals of environmental origin on the oviposition site selection by *Aedes atropalpus*, a rock-pool mosquito. The more definitive responses were obtained with the larval holding waters. Mosquito larvae of the same species act as oviposition attractants or stimulants; this larval effect is persistent at room temperature and *Ae. atropalpus* females do not distinguish between *Ae. atropalpus* and *Ae. communis* larval holding waters. Statistical analysis (ANOVA) indicates no interaction between the larval factor and the tested environmental factors.

INTRODUCTION

Most entomologists agree that gravid females of many mosquito species have a marked oviposition site preference for aquatic habitats containing immature stages. Nevertheless, the study of factors and mechanisms inducing this oviposition behavior is still a relatively new area for research (Maire 1982, 1983).

For both historical reasons and convenience, almost all laboratory experiments have been conducted with mosquito species that have a tropical-temperate distribution (particularly species of the genus *Culex* and also *Aedes aegypti* (Linn.)). Few boreal-temperate species have been tested as most of them are difficult to rear under laboratory conditions. Most are univoltine species with an obligatory egg diapause of several months (Kardatzke 1981). They are also eurygamous and their mass rearing necessitates the use of forced copulation (McDaniel and Horsfall 1957, Brust 1971).

For these reasons, two boreal-temperate species have been more frequently used in laboratory experiments: *Ae. atropalpus* (Coquillett), an autogenous rock-pool mosquito (Kalpage and Brust 1974) and *Ae. triseriatus* (Say), a tree-hole mosquito, the main vector of Lacrosse encephalitis virus (Bentley et al. 1976, 1979, 1981, 1982; McDaniel et al. 1976, 1979). These two species are multivoltine, stenogamous and can be easily reared at room temperature.

This laboratory study compared the respective roles of environmental and biological factors in the oviposition site preference of *Ae. atropalpus*. The experiments were designed to answer four questions: (1) Is there an ovipositional attractive effect due to the chemical composition of natural breeding waters? (2) Is there an effect of conspecific larvae or of larvae of another species (*Ae. communis* (deGeer)), never found together with *Ae. atropalpus*? (3) Is there an effect of the microbial activity in breeding waters? (4) Is there an interaction among these factors?

MATERIALS AND METHODS

COLONY MAINTENANCE. The *Ae. atropalpus* colony, originally from La Gabelle, near Trois-Rivières, Quebec (46°18'N, 72°37'W), has been maintained in our laboratory for several years under environmental conditions described by Kalpage and Brust (1974): 21°C temperature, 16L:8D photoperiod and 75% RH. Mosquitoes were reared from eggs to pupae in pans filled with tap water. They were fed a mixture of yeast extract (10%) and Purina dog food. Pupae were removed daily and placed in cages 30 × 30 × 50 cm at a density of approximately 300 per cage. Only aqueous honey solution (1:1 volume) was provided for food since *Ae. atropalpus* is autogenous. Six days after the first adult emergence, four oviposition dishes (Petri dish glass covers) containing test solutions were introduced into the cage. They were randomly placed. Every day fresh test solutions were placed in each cage. Eggs laid overnight were removed daily and counted. Each experiment was conducted over 5 days (first gonotrophic cycle) and was started the second day after the first eggs were laid.

The *Ae. communis* larvae were collected in a shallow pool near Trois-Rivières in a deciduous woodland dominated by *Acer saccharum* Marsh., *A. rubrum* L. and *Fagus grandifolia* Ehrh.

LEAF LITTER SOLUTIONS. During the fall of 1982, autumn-shed leaves of *Acer saccharum* and *A. rubrum* were collected at the bottom of a dried shallow woodland pool. Leaves were placed in distilled water (8 grams of wet leaves per liter), macerated and stored at room temperature (approximately 23°C) for 7 days. Then the litter solution was filtered through Whatman #2 filter paper and the filtrate was used as the test solution. One sample of the leaf litter solution was made from previously autoclaved leaves macerated under sterile conditions; the second sample was made from non-autoclaved leaves under open non-sterile conditions. Filtrates were kept refrigerated until used.

LARVAL HOLDING MEDIUM. Fourth-instar larvae of *Ae. atropalpus*, previously washed several times with distilled water, were placed for 48 hr in 1000 ml of ten-fold diluted leaf litter solution, at a density of 1 larva per ml. The larval holding solution was filtered and stored as described above.

BREEDING WATERS. Two enameled pans were filled with distilled water at room temperature (23°C). As in routine mass rearing, food was added to each pan 24 hr later. Three samples were successively taken from each pan: one the first day, just before addition of food; a second 48 hr after the food addition and a last 10 days later, once all pupae had been removed. Each sample was filtered and stored as described above. Filtrates were test solutions.

OVIPOSITION BIOASSAYS. A series of seven experiments was designated:

1. Distilled water and larval holding distilled water. For subsequent statistical analysis, 3 cages (replicates) were tested.
2. Same test solutions, but dried at room temperature by evaporation and then refilled with distilled water. Three cages were tested.
3. Ten-fold diluted leaf litter solution with *Ae. atropalpus* larvae and same solution with *Ae. communis* larvae. Three cages were replicates.
4. Distilled water, the 48 hr breeding water, the 10 day breeding water. One series contained larvae of *Ae. atropalpus*, the other did not. Six cages were replicates.
5. Distilled water and ten-fold diluted litter solution, sterile and non-sterile. Five cages were replicates.
6. Distilled water and ten-fold diluted leaf litter solution, with and without larvae. Three cages were replicates.
7. Distilled water, pure leaf litter solution, leaf litter solution diluted tenfold and leaf litter solution diluted 100-fold. Five cages were tested.

STATISTICAL ANALYSIS. The total number of eggs laid per dish and per cage for each experiment was expressed in percentage and then arcsine transformed before statistical analysis (Carpenter 1983). When only two types of oviposition sites were tested, results were submitted to a paired *t*-test analysis (experiments 1, 2 and 3). When more oviposition sites were tested, data were analyzed with a multi-way analysis of variance (ANOVA). In the last experiment (7), ANOVA was completed with a Newman-Keuls analysis.

RESULTS AND DISCUSSION

The results of the experimentation are shown in Table 1 (1, 2 and 3), Table 2 (4, 5 and 6) and Table 3 (experiment 7). There is evidence of an attractive or stimulatory ovipositional effect of the tested larval holding waters (experiments 1, 2, 4 and 5). Such a larval effect was previously demonstrated for *Culex* (Hudson and McLintock 1967, Dadd and Kleinjan 1974, Rotraut et al. 1973, Suleman and Shirin 1981), for *Anopheles* (Reisen and Siddiqui 1978) and for several species of *Aedes:* Kalpage and Brust (1974) for *Ae. atropalpus;* Soman and Reuben (1970), Roberts and Hsi (1977) for *Ae. aegypti;* Trimble and Wellington (1980) for *Ae. togoi* (Theobald); Bentley et al. (1976) for *Ae. triseriatus*. More recently, Ahmadi and McClelland (1983) failed to show such an attraction by larvae or pupae of *Ae. sierrensis* (Ludlow), a western American tree-hole mosquito closely related to *Ae. triseriatus* (Wood et al. 1979).

Experiment 2 shows, as previously indicated by Kalpage and Brust (1974) for the same species, that this larval effect is stable even after a complete drying of the oviposition site. From a general point of view, ecological implications of such semiochemical (pheromones) stability of the larval effect are of interest. These attractants could influence the ecological behavior of the snow-melt *Aedes;* as the massive emergence

Table 1. Results of offering *Aedes atropalpus* females a choice between two types of oviposition sites.

Experiment	No. of cages (replicates)	Contents of oviposition dishes	Mean of no. of eggs	Mean arcsine value	*t* value	*P*
1	3	Distilled water without larvae	1054 2363	33.97 56.04	6.175	$0.05 > P > 0.02$
2	3	Same as in 1 but after evaporation	530 2011	26.23 63.77	9.968	$0.01 > P > 0.005$
3	3	Leaf litter with *Aedes atropalpus* larvae	963	45.34	0.760	NS
		Leaf litter with *Aedes communis* larvae	929	44.65		

of the adults takes place, a simultaneous fast drying of the shallow pools occurs. It may be advantageous for gravid females of this homogeneous group to detect persistent semiochemical attractants or stimulants released several weeks earlier by the immatures. For these reasons, it would be of interest to detect the presence of stable oviposition attractants in other species of *Aedes* of the subgenus *Ochlerotatus*.

Females of *Ae. atropalpus* did not differentiate between *Ae. atropalpus* and *Ae. communis* larval holding waters for their oviposition site selection (experiment 3). The larval effect thus does not seem to be strictly species specific. This lack of specificity was not expected, for these two species colonize separate habitats and, as a rock pool and a shallow pool species, they never coexist in the field. Bentley et al. (1976) however demonstrated that females of *Ae. triseriatus* were also attracted by *Ae. atropalpus* larval holding waters. This aspect of the oviposition site selection necessitates further studies.

When the choice was offered to *Ae. atropalpus* females among distilled water and several concentrations of leaf litter solutions (experiment 7, Table 3), a significant difference was shown in the respective number of eggs laid ($F=5.09$; $0.025 > P > 0.01$). A complementary statistical analysis using the Newman-Keuls test indicated that this difference was due to the pure and ten-fold diluted leaf litter solutions which were more attractive than other offered water. Nevertheless, results of experiment 6 indicated no significant difference between distilled water and the ten-fold diluted leaf litter solution. The results of this last experiment are not very conclusive since no larval effect could be demonstrated. This is not consistent with the responses obtained in experiments 1, 2, 4 and 5.

The attractive effect of bacteria in the

Table 2. ANOVA of the ovipositional responses of *Aedes atropalpus* females to oviposition factors (experiments 4 to 6).

Experiment 4 (6 cages were replicates)

Water type	Experimental design: Larvae −	Larvae +
DW	519[a]	730
48 hr breeding W	879	956
10 days breeding W	1193	1247

ANOVA

Source	SS	DL	MS	F
Water type	366.79	2	183.39	19.72[b]
Larvae	75.37	1	75.37	8.10[c]
Interaction	6.20	2	3.10	0.33 NS
Total	725.35	35		

Experiment 5 (5 cages)

Water type	Experimental design: Microbial activity −	Microbial activity +
DW	1021	980
Leaf litter	1106	981

ANOVA

Source	SS	DL	MS	F
Water type	17.30	1	17.30	0.69
Microbial activity	16.93	1	16.93	0.68 NS
Interaction	0.28	1	0.28	0.01 NS
Total	333.71	15		

Experiment 6 (3 cages)

Water type	Experimental design: Larvae −	Larvae +
DW	1194	1180
Leaf litter	878	1037

ANOVA

Source	SS	DL	MS	F
Water type	7.43	1	7.43	0.44 NS
Larvae	57.62	1	57.62	3.46 NS
Interaction	0.63	1	0.63	0.04 NS
Total	198.97	11		

[a] Mean values of the egg number per dish.
[b] Significant at the 0.001 level.
[c] At the 0.01 level.

Table 3. ANOVA and Newman-Keuls multiple range test of the ovipositional response of *Aedes atropalpus* to different concentrations of a maple leaf litter solution (LLS). Five cages were replicates (experiment 7).

ANOVA

Test solutions	Mean values (egg number)	Mean arcsine value
1 DW	611	24.24 (μ1)
2 LLS (pure)	803	29.77 (μ2)
3 LLS (1/10)	1004	35.46 (μ3)
4 LLS (1/100)	767	27.89 (μ4)

$F = 5.09$; $0.025 > P > 0.01$

	SS	df	MS
total	694.89	19	
group	328.79	3	109.60
error	366.10	17	21.53

Newman-Keuls multiple range test

Comparison	Difference of means	SE	q	p	$q^{0.05, 17, p}$	
3 vs 1	11.22	2.075	5.407	4	4.020	$\mu 3 \neq \mu 1$
3 vs 4	7.57	2.075	3.648	3	3.628	$\mu 3 \neq \mu 4$
3 vs 2	5.69	2.075	2.742	2	2.984	$\mu 3 \neq \mu 2$
2 vs 1	5.53	2.075	2.665	3	3.628	$\mu 3 \neq \mu 1$

overall conclusion: 1 4 2 3

oviposition site preference by females of *Culex* (several species) and *Ae. aegypti* has been directly demonstrated by Ikeshoji (1966) and Ikeshoji et al. (1967, 1975). The role of the microbial activity was also indirectly demonstrated with decayed organic matter and several types of infusions (Dadd and Kleinjan 1974, Suleman and Shirin 1981, Gjullin et al. 1965, Reisen and Siddiqui 1978, Roberts and Hsi 1977, Kramer and Mulla 1979). In experiment 5, responses to sterile and non-sterile waters were not significantly different. More nuances were, however, brought out in the results of experiment 4. Distilled water with food (yeast extract and dog food) is more attractive than distilled water alone. There is also a significant difference between responses to larval holding water and distilled water, but there is no statistical evidence of an interaction between the larval effect and the effect of different types of water. Furthermore, females were more attracted by the food solution after 48 hr than after 10 days of maceration. Water in the 48 hr sample was clear and the food granules could still be seen in the solution. Ten days later, a layer of microbial origin had spread out on the surface of the solution and the water was yellowish; also, mold had grown around some remaining food granules. There is a definite increase in microbial activity with time. The preference shown by females for the site with the 48 hr food solution rather for the 10 day-aged one possibly indicates that not only bacteria but also fungal mycelia could serve as environmental oviposition attractants or stimulants. Fish and Carpenter (1982), analyzing three kinds of leaf litter (sugar maple, beech and black oak) indicated, "... maple leaves decayed the most rapidly and supported the densest growth of microbes" and "biomass of fungal mycelia exceeds that of bacteria cells" (p. 287) of leaf litter in aquatic environments.

From this set of preliminary experiments one may conclude that: (a) mosquito larvae act as oviposition attractant or stimulants, (b) the larval attractive or stimulatory ovipositional effect is stable in the field under conditions of temperatures characteristic of the boreal and the temperate life-zones, and (c) the larval effect is less specific than expected. Statistical analysis indicated also that the larval factor and the tested environmental factors did not interact. These conclusions may in part help to explain the preference shown by the females of the *Aedes (Ochlerotatus)* subgenus for a common type of habitat: the wetlands. However, these data do not provide information about the more acute selectivity shown by different species which, as previously indicated for subarctic string bogs (Maire 1982), are able to make a distinction between closely related habitats. The mechanisms for this strong selectivity are yet unknown.

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