December 1988

Bacteria as Ovipositional Attractants for *Aedes Aegypti* (Diptera: Culicidae)

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BACTERIA AS OVIPOSITIONAL ATTRACTANTS FOR *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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ABSTRACT

The effectiveness of selected bacterial species as ovipositional attractants for *Aedes aegypti* was compared. Bacterial washes, in glass containers, were utilized as ovipositional substrates and subsequent egg counts determined the degree of ovipositional attractiveness. Among others, *Bacillus cereus* and *Pseudomonas aeruginosa* were noted as being effective attractants. It was concluded that *A. aegypti* displays discriminatory behavior in selecting individual bacterial species for oviposition.

Studies concerning ovipositional attractants in mosquitoes have been limited in scope and number. The current idea is that ovipositional activities in mosquitoes involve the selection of an environment which provides the necessary physical, chemical, and biotic factors required for larval development. In researching biotic factors, investigators such as Suleman and Shirin (1981) have noted that the presence of organic matter in association with bacteria cause an aquatic environment to be more attractive for ovipositing mosquitoes. Hazard et al. (1967) concluded that bacterial attraction for ovipositing mosquitoes was of adaptive value for the mosquitoes since bacteria are reported to stimulate the growth of mosquito larvae. While the general consensus among researchers in oviposition biology is that bacteria may serve as ovipositional attractants, few individuals have attempted to identify the bacterial species which serve as the actual attractants. In working with *Culex pipiens quinquefasciatus* Say, Steelman and Colmer (1970) were unique in that they did attempt to identify specific coliform bacteria as attractants. Rockett (1987) further investigated the specific role of bacteria as ovipositional attractants for *C. p. pipiens* L. (northern house mosquito). He concluded that *C. pipiens* displays discriminatory behavior in selecting individual bacterial species commonly encountered in the mosquito's natural breeding waters. The purpose of this work was to compare the effectiveness of selected bacterial species as ovipositional attractants for *Aedes aegypti* (L.).

Probably more is known about the biology of *A. aegypti* than any other species of mosquitoes. The literature dealing with this species is voluminous (Silverly 1972). However, information on the role of bacteria as ovipositional attractants is extremely limited. Maw (1970) determined that n-capric acid made artificial field water attractive for oviposition by a variety of mosquitoes, including *A. aegypti*. He suggested that pseudomonad bacteria were responsible for the production of the ovipositional attractants. Ikeshoji et al. (1979) isolated a specific ovipositional attractant for *A. aegypti* which was produced by *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

A colony of *A. aegypti* was maintained in the laboratory. Eggs were collected and larvae subsequently reared on a diet of powdered dog chow in 15 × 15 × 30 cm plastic

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containers. Adults were kept in screen cages (80 × 40 × 40 cm) at 27 ± 3°C, 80% RH, and a photoperiod of L:D 16:8 hrs. Cotton pads soaked with 10% sucrose solution were utilized as an interim food source prior to blood feeding. Approximately 5 days after adult emergence, the mosquitoes were fed overnight on a laboratory mouse.

Oviposition substrates were prepared by using 125 ml wide-mouthed glass containers filled with single-species bacterial washes. The bacterial washes were prepared by scraping individual-species cultures from petri dishes with a bent glass rod, and rinsing with 100 ml of water. When noted, percent transmittance relative to the number of bacterial cells was measured with a spectrophotometer. This measurement was taken in an attempt to eliminate possible variables related to dilution or concentration of bacterial cells in separate washes. A paper towel (15 × 4 cm), previously soaked for five minutes in a specific bacterial wash, was subsequently placed in a wash container. To insure a constant moisture level in the towel, half of the towel remained immersed in the wash, while the other half of the towel was draped over the edge of the glass container. The "set-ups" to be compared for oviposition attractiveness were placed in a cage containing ± 100–200 mosquitoes which had been fed 48 hrs beforehand and were normally ready for oviposition. The washes were left in the cage overnight. To minimize any effect that the individual container's location may have had on its selection by ovipositing mosquitoes, the containers holding the different washes were repositioned between replicates, within individual experiments. Eggs deposited on the towels were removed and counted. The number of eggs on each towel was used as the indicator for oviposition preference displayed by *A. aegypti*.

Ovipositional wash media, containing pure cultures of individual bacterial species was varied according to the experiment being conducted. Excluding *Staphylococcus epidermidis, Pseudomonas aeruginosa, Bacillus subtilis,* and *Bacillus thuringiensis,* the bacterial species utilized were isolated from common breeding waters used by *Culex* species in Bowling Green, Ohio. *A. aegypti,* being a tropical mosquito, is not found in colder, temperate climates, such as Bowling Green. The bacterial species commonly isolated from the breeding waters included *Escherichia coli, Enterobacter aerogenes, Pseudomonas maltophilia,* and *Bacillus cereus.*

Eleven separate experiments were conducted (Table 1). Basic procedural methodology for all experiments were similar; any variation for an individual experiment is noted.

1. Three separate washes containing *Bacillus cereus, Enterobacter aerogenes,* and distilled water were all placed in a single cage. Two replicates were done for this experiment.

2. *Enterobacter aerogenes* and *Staphylococcus epidermidis* washes were compared. This combination was selected because *S. epidermidis* is not commonly found in mosquito breeding waters and *E. aerogenes* is. Three replicates were done in this experiment.

3. *Pseudomonas maltophilia* and *Pseudomonas aeruginosa* washes were compared. This comparison was done to test the ability of gravid mosquitoes to distinguish between two species of bacteria that are congeneric. Light transmittance values varied between replicates, but were standardized within replicates at 41, 55 and 55%. Three replicates were done for this experiment.

4. A comparison between *P. maltophilia, E. aerogenes,* and *S. epidermidis* was done. Transmittance values for replicates were 71, 19, and 50% respectively.

5. A comparison between *B. cereus* wash and pure *A. aegypti* egg homogenate suspended in 100 ml of water was conducted. This experiment was conducted to test the relative attractiveness of eggs versus bacteria. Egg homogenate was prepared by grinding *A. aegypti* eggs with a mortar and pestle and then rinsing with 100 ml of water. Three replicates were done; replicates were standardized at 95% transmittance.

6. To compare the ovipositional attractiveness of two commonly occurring bacteria in mosquito breeding waters, *Escherichia coli* and *B. cereus* bacterial washes were utilized. Three replicates were done for this experiment. Transmittance was standardized at 50% for all replicates.

7. *B. cereus* and *P. aeruginosa* washes were compared. This combination was selected because both bacterial washes were found to be very effective attractants when compared

Table 1. Comparisons of Selected Ovipositional Attractants for *Aedes aegypti* (L).

EXPERI-MENT	BACTERIAL WASHES OR OTHER MEDIA WITH RESULTANT EGG COUNT TOTALS		
1[a]	*Bacillus cereus* 719	*Enterobacter aerogenes* 444	Distilled water 307
2	*Staphylococcus epidermidis* 1457	*Enterobacter aerogenes* 1112	
3	*Pseudomonas aeruginosa* 1607	*Pseudomonas maltophilia* 933	
4	*Staphylococcus epidermidis* 218	*Enterobacter aerogenes* 93	*Pseudomonas maltophilia* 2
5	*Bacillus cereus* 772	*Aedes aegypti* Egg Homogenate 270	
6	*Bacillus cereus* 96	*Escherichia coli* 0	
7[b]	*Pseudomonas aeruginosa* 1126	*Bacillus cereus* 422	
8	*Bacillus cereus* 162	*Bacillus subtilis* 44	
9	*Bacillus thuringiensis* 399	Tap water 155	
10	*Bacillus cereus* 401	*Bacillus thuringiensis* 118	
11	*Pseudomonas aeruginosa* 1220	*Escherichia coli* 63	

[a,b]Indicates 2 and 4 replicates, respectively. Unmarked, indicates three replicates.

to other bacterial species separately. Four replicates were done for this experiment. Washes were standardized within replicates at 50, 50, 53, and 65% transmittance.

8. *B. cereus* and *subtilis* were compared to test for differences in the relative ovipositional attractiveness of two species of *Bacillus* bacteria. Three replicates were completed for this experiment. Transmittance varied between replicates, but were standardized within each replicate at 69, 75, and 88%, respectively.

9. A comparison between *Bacillus thuringiensis* and tap water was done. This comparison was conducted to test the relative ovipositional attractiveness of a potentially lethal bacterium, which is commonly used as a larvicide in mosquito control. Three replicates were completed. Transmittance values varied between replicates, but were standardized within replicates at 40, 55, and 60%, respectively. Tap water had a constant transmittance of approximately 100%.

10. *B. cereus* and *B. thuringiensis* washes were compared. Three replicates were done for this experiment. Transmittance values were standardized within replicates at 38, 60, and 59%.

11. *P. aeruginosa* and *E. coli* washes were compared. Three replicates were done; transmittance values, standardized within replicates were 65, 55, and 55%.

In all experiments, statistical analysis of total egg counts were done by using a Chi-square, goodness of fit (Zar 1984).

RESULTS

Compared replicates are listed in experimental sequence. Total egg counts for each comparison are listed in Table 1.

1. The comparison between *B. cereus* washes, *E. aerogenes* washes, and distilled water resulted in a highly significant difference ($P < .001$) in the total number of eggs deposited in each wash. Distilled water was significantly less attractive for oviposition to gravid female *A. aegypti* than either of the bacterial washes. Results regarding individual replicates were 421 and 298 for *B. cereus;* 271 and 173 for *E. aerogenes;* 182 and 125 for distilled water.

2. The comparison between *E. aerogenes* and *S. epidermidis* washes resulted in a highly significant difference ($P < .001$). Replicate counts for *S. epidermidis* were 816, 548, and 93; counts for *E. aerogenes* were 287, 714, and 111.

3. The comparison between *P. maltophilia* and *P. aeruginosa* washes resulted in a highly significant difference ($P < .001$). Replicate counts for *P. aeruginosa* were 129, 502, and 976; *P. maltophilia* egg counts were 92, 257, and 584.

4. In the comparison between *P. maltophilia, E. aerogenes,* and *S. epidermidis* washes, a highly significant difference ($P < .001$) in the degree of attractiveness to mosquitoes resulted. Individual replicates for *P. maltophilia* were 0, 1, and 1; 43, 1, and 49 for *E. aerogenes,* and 161, 51, and 6 for *S. epidermidis.*

5. In comparing *B. cereus* wash and egg homogenate suspended in water, a highly significant difference ($P < .001$) in the degree of attractiveness to *A. aegypti* resulted. Replicate egg counts for *B. cereus* were 286, 74, and 412; egg homogenate counts were 159, 52, and 59.

6. The comparison between *B. cereus* and *E. coli* washes resulted in a highly significant difference ($P < .001$) in the degree of attractiveness of the two washes. Replicate counts for *B. cereus* were 1, 93, and 2; replicate counts for *E. coli* were consistently 0.

7. In the comparison between *B. cereus* and *P. aeruginosa* washes, a highly significant difference ($P < .001$) resulted in the number of eggs deposited in each wash, Replicate egg counts for *B. cereus* were 89, 291, 27, and 15; *P. aeruginosa* counts were 364, 442, 187, and 133.

8. The comparison between *B. cereus* and *B. subtilis* washes resulted in a highly significant difference ($P < .001$) in the degree of attractiveness. Individual replicate counts for *B. cereus* were 104, 7, and 51; while counts for *B. subtilis* were 41, 0, and 3.

9. In comparing *B. thuringiensis* wash with tap water a highly significant difference ($P < .001$) resulted in the number of eggs deposited. Replicate counts for *B. thuringiensis* were 95, 267, and 37; counts for tap water were 68, 87, and 0.

10. In the comparison between *B. cereus* and *B. thuringiensis* washes a highly significant difference ($P < .001$) in the relative attractiveness of these two washes was noted. Individual replicate counts for *B. cereus* were 172, 128, and 101; counts for *B. thuringiensis* were 36, 82, and 0.

11. In comparing *P. aeruginosa* and *E. coli* washes a highly significant difference ($P < .001$) in the relative attractiveness of these two washes resulted. Replicate egg counts for *P. aeruginosa* were 358, 696, and 166; *E. coli* egg counts were 54, 9, and 0.

DISCUSSION

The results of this work add additional credibility to the idea that complex biological cues are involved in the selection of oviposition sites by gravid mosquitoes. These preliminary experiments suggest that bacteria act as ovipositional attractants for *A. aegypti.* While these investigations indicate that *A. aegypti* displays an actual preference for various bacterial species, including congeneric species, the mosquitoes seldom exhibited an "all or nothing" response to the wash samples presented for oviposition. However, in the comparison between *E. coli* and *B. cereus* washes (3 replicates), sufficient attractancy was observed for *B. cereus* to ensure 100% placement of the eggs in that wash. In the experiment comparing *P. aeruginosa* and *E. coli,* a highly significant difference ($P < .001$) was noted. *P. aeruginosa* was a much better attractant than *E. coli* for gravid *A. aegypti.* An aspect of particular interest in this experiment were the results obtained in one replicate where 100% placement of eggs occurred in the *P. aeruginosa* wash. In work with *C. pipiens,* Rockett (1987) noted a highly significant difference ($P < 0.001$) in the degree of attractiveness for gravid mosquitoes in comparing *E. coli* and *P. maltophilia* washes (11 and 61 egg rafts, respectively). These findings were particularly interesting since *E. coli* is commonly encountered in natural breeding waters containing *C. pipiens.* From the preliminary investigations now done with both a *Culex* and *Aedes* species it is tempting to speculate that *E. coli* may be a poor ovipositional attractant for numerous mosquitoes.

A concern throughout the course of these investigations was whether the same or related species of bacteria would serve as a strong ovipositional attractant for more than one species of mosquito. *Bacillus cereus* appeared to be highly favored for oviposition by *A. aegypti* based on comparisons with distilled water, *E. aerogenes, E. coli, B. subtilis, B. thuringiensis,* and *A. aegypti* eggs. Rockett (1987) found *B. cereus* was also significantly more attractive to *C. pipiens* for oviposition, when it was compared with plain agar wash, and *Enterobacter agglomerans.* However, *B. cereus* was not significantly more attractive to *C. pipiens* when it was compared to *P. maltophilia* (both species being common bacteria in *C. pipiens* breeding water). *P. aeruginosa* was a more effective ovipositional attractant than even *B. cereus* for *A. aegypti.* Rockett (1987) did not examine the ovipositional attractiveness of *P. aeruginosa* for *C. pipiens.* Other researchers have implicated the family Pseudomonaceae as possible attractants for both *Aedes* and *Culex* ovipositing mosquitoes. Maw (1970) found a pseudomonad to be attractive when cultured on capric acid substrate. Ikeshoji et al. (1967) found *Pseudomonas reptilivora* to serve as an ovipositional attractant for *C. p. quinquefasciatus,* and in a later work he found *P. aeruginosa* to be a possible attractant for *A. aegypti* (Ikeshoji et al. 1979).

Various research has indicated that eggs and larvae of some species of mosquitoes may act as effective attractants. Osgood (1971) found that *Culex tarsalis* preferred to oviposit in water containing 200-2000 egg rafts of *C. tarsalis.* Dadd and Kleinjan (1974) and Nakamura (1978) both found that water on which egg rafts of *C. pipiens* were deposited was attractive to gravid females in the *C. pipiens* group. Bentley et al. (1976) found *Aedes triseriatus* eggs were not as attractive to *A. triseriatus* for oviposition as *A. triseriatus* larvae. Kalpage and Brust (1973) noted a similar result in *Aedes atropalpus.* In this current study, *A. aegypti* eggs did appear to induce oviposition, however, it is interesting to note that bacterial washes, when compared with eggs alone, resulted in a much greater number of eggs deposited in the applicable wash. To our knowledge, no other work has been done in comparing the relative attractiveness of eggs and bacterial washes.

Information on specific chemical isolates produced by bacteria, which appear to induce mosquito oviposition, is extremely limited. Ikeshoji et al. (1979) was unique in that he isolated 7, 11 dimethyloctodecane from *P. aeruginosa,* cultured on capric acid substrate, and found the bacterium or its metabolites to be highly attractive for gravid *A. aegypti.* As mentioned by Rockett (1987), continued investigations could result in the isolation of a "lure" for mosquito pest management.

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