FACTORS AFFECTING OVIPOSITION SITE PREFERENCE BY *TOXORHYNCHITES SPLENDENS* IN THE LABORATORY[1]

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ABSTRACT. In a series of laboratory oviposition assays, gravid *Toxorhynchites splendens* exhibited a preference for cups containing *Aedes aegypti* larval rearing water, but not for cups containing liquid cultures of bacteria, live *Ae. aegypti* in distilled water, *Ae. aegypti* larval holding water with reduced bacterial contamination, or methyl propionate at 0.1, 0.2 and 0.3% in distilled water. Preoviposition flight behavior was elicited by dark-colored containers, but few eggs were deposited if they contained no water. An invisible source of humidity placed in cups enhanced oviposition, but a reflective surface placed in dry cups did not. It is concluded that this species is strongly influenced by humidity and visual stimuli in the acceptance of a site for oviposition.

INTRODUCTION

Larvae of mosquitoes in the genus *Toxorhynchites* are predacious on the larvae of *Aedes aegypti* (Linn.) and other container-breeding mosquitoes. The degree of overlap between larval habitats of predator and prey will strongly influence the overall impact of a predator on a given prey population. Therefore, oviposition site preference by *Toxorhynchites* is an important aspect of their biocontrol potential. Habitat overlap or lack thereof has been clearly implicated in successes or failures of control efforts involving several species of *Toxorhynchites* (Focks 1985).

The ability of a female *Toxorhynchites* to discriminate between sites actually containing prey larvae and those without prey, may be critical to the success of a biocontrol program. *Toxorhynchites amboinensis* (Doleschall), which is particularly adept at locating and ovipositing in artificial containers in urban environments, appears to oviposit in response to unidentified cues correlated with the presence or absence of prey larvae in the field (Focks et al. 1983). *Toxorhynchites rutilus septentrionalis* (Dyar and Knab) (Trimble 1978) and *Tx. r. rutilus* (Coquillett) (Focks and Hall 1977) exhibited an oviposition preference for *Ae. aegypti* larval rearing medium over distilled water in the laboratory, though for the former species this behavior may have been due to a visual response to differences in color rather than to an olfactory response to volatile compounds (Trimble 1978). *Toxorhynchites r. septentrionalis* was shown by Slaff et al. (1975) to prefer black to white oviposition containers. *Toxorhynchites splendens* (Wiedemann) rarely oviposited in white containers (G. L. Benzon, unpublished data).

As an adjunct to our work with oviposition preferences of *Ae. aegypti*, we conducted laboratory experiments to determine if certain physical and chemical factors affect the oviposition preference of *Tx. splendens*. The influence of larval rearing water, larval holding water with reduced bacteria and bacterial cultures were of particular interest.

METHODS AND MATERIALS

A colony of *Tx. splendens* was established from eggs obtained from the New Orleans Mosquito Control Board. Eighty to 100 eggs were hatched in 26 × 40 × 8 cm clear plastic pans containing 2 liters of tap water aged at least 24 hours. Upon hatching, larvae were provided 200–300 first instar *Ae. aegypti*, and were thereafter provided daily with an excess of *Ae. aegypti* of approximately the same stadium as themselves. Pupae were transferred to a 15 cm diam. × 6 cm height white emergence dish containing distilled water which was placed in a 45 × 45 × 45 cm screened cage. Adults were provided with three 10 cm long cotton dental wicks protruding from 30 ml reservoirs of 10% sucrose solution. About 60 to 120 adults of mixed ages were held in each of 2 cages in a male to female ratio of 2:1. The colony was maintained at 26.6 ± 0.5°C and 70 ± 2% RH under a fluorescent lighting regime of 14 h light:10 h dark.

All assays were conducted in a room where conditions were similar to the rearing room but where no mosquitoes were reared. Four 500 ml black plastic cups with a top diam. of 9 cm (Louisiana Plastics, St. Louis, MO) were used; one cup was placed 10 cm from each corner of a colony cage. Two cups receiving the same treatment were placed diagonally opposite one another. Cups were removed after 45 min. and the eggs immediately removed and counted, after

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which cups were returned to the cage with the positions of treated and control cups reversed. The total number of eggs deposited in the two treatment and two control cups for each 45 min. period were recorded. Pairs of trials were repeated 2–6 times.

The following treatments were evaluated:

1. Water in which 300–400 *Ae. aegypti* had been reared to fourth instars according to a standard feeding schedule[3] and used unfiltered but with larvae removed ("larval rearing water"), vs. distilled water.
2. Water prepared by holding 4th instar *Ae. aegypti* at a density of 1 larva/ml of sterile distilled water for 24 hours, using a screen partitioning and membrane filtration method[3] that reduced bacterial contamination ("larval holding water"), vs. sterile distilled water.
3. Distilled water to which 100 live, rinsed, 4th instar *Ae. aegypti* were added and left for the course of each trial, vs. distilled water without larvae.
4. Cultures of the bacterium *Acinitobacter calcoaceticus* prepared by inoculating 7.5×10^8 cells into 200 ml of ⅛th strength sterile Difco nutrient broth incubated at 26.6°C for 24 h. The dilute broth allowed bacteria to multiply at approximately the same rate as in larval rearing medium[3], reaching a maximum density of approximately 1×10^8 colony forming units/ml. Uninoculated nutrient broth from the same batch was used in control cups.
5. Distilled water vs. no water.
6. Transparent acrylic plastic disks (7 cm diam. × 1.5 mm thick) placed in the treatment cups to simulate the reflective surface of water (no water added), vs. cups without disks or water.
7. Six water-saturated layers of filter paper placed in the bottom of treatment cups covered by a 7.5 cm diam. disk of 20-mesh steel screen, vs. cups with dry filter paper and screen.
8. Distilled water containing methyl propionate at one of three concentrations (0.1, 0.2 and 0.4%), vs. distilled water alone. Methyl propionate in this range of concentrations was found to strongly enhance *Ae. aegypti* oviposition in the laboratory (Perry and Fay 1967).

The contributions of treatment, cup positions and time period to the experimental variance were analyzed for each treatment type using the SAS ANOVA procedure (SAS Institute 1985). Unless indicated otherwise, statistical analyses were carried out at the $\alpha = 0.05$ level of significance.

RESULTS AND DISCUSSION

Results of all assays are given in Table 1. In Experiment 1, *Tx. splendens* deposited significantly more eggs in cups containing *Ae. aegypti* larval rearing water (63.1%) than cups containing distilled water. Trimble (1978) reported a strong preference for similar larval rearing water by *Tx. rutilus septentrionalis* (80.4%). As with gravid *Aedes aegypti* in previous

[3] Benzon, G. L. 1987. Oviposition attraction and stimulation in *Aedes aegypti* (L.) and in a predator of mosquito larvae, *Toxorhynchites splendens* (Weidemann). Ph.D. Dissertation. Department of Entomology, North Carolina State Univ., Raleigh, NC.

Table 1. Results of *Toxorhynchites splendens* oviposition assays of selected physical and chemical factors. F statistic is for treatment effect. See text for explanation of treatment types.

Treatment vs. control	No. of trials	Mean no. eggs per trial		F	Prob > F
		Treatment	Control		
1. Larval rearing water vs. distilled water	8	139.0	81.1	6.78	0.02
2. Larval holding water vs. distilled water	8	172.6	166.0	0.10	0.76
3. Live larvae in distilled water vs. distilled water	4	155.0	163.8	0.17	0.70
4. Bacterial culture vs. uninoculated broth	14	82.4	68.4	0.85	0.37
5. Distilled water vs. dry	4	189.5	9.0	18.12	0.01
6. Wet filter paper vs. dry filter paper	4	59.5	6.3	47.21	0.002
7. Plastic disk vs. no disk	4	4.8	7.3	0.39	0.56
8. Methyl propionate in distilled water vs. distilled water					
0.1%	4	106.5	104.5	0.00	0.95
0.2%	4	125.5	122.5	0.03	0.86
0.4%	4	66.3	71.3	0.05	0.84

experiments[3], *Tx. splendens* showed no preference for *Ae. aegypti* larval holding water prepared in a manner to reduce bacterial contamination (Experiment 2). Also, the presence of live *Ae. aegypti* larvae had no significant effect on oviposition (Experiment 3). These results led us to assay the *Acinitobacter* cultures (Experiment 4). Though bacterial cultures were shown in previous experiments[3] to influence gravid *Ae. aegypti*, they had no significant effect upon oviposition by *Tx. splendens*. Albeit, we cannot conclude that either bacteria or prey larvae do not produce some volatile substances that enhance oviposition. Larval holding water produced over a 24 hr period (to exclude the effects of added food and resultant fecal contamination) probably does not contain the entire complement of endogenous products that might accumulate in a small breeding site over the entire course of larval development. The larval rearing water used in our experiment was a complex and concentrated mixture of several different bacteria, decomposing food, and feces from a high density of larvae. It was likely to be considerably more concentrated than that which may be found in the field.

Oviposition was rare in cups lacking water. Of 794 eggs collected in Experiment 5, only 36 (4.5%) were found in dry cups. Apparently, a reflective surface does not, by itself, stimulate oviposition (Experiment 6). Over the four 45 min. periods, only 19 eggs were collected from cups containing clear plastic disks and 29 eggs from empty control cups. The importance of humidity was confirmed by the results of Experiment 7. Only 25 eggs were deposited in cups containing dry filter paper but 238 eggs were found in cups with moist filter paper. Because screen disks prevented females from coming in contact with the filter paper, we conclude that females were able to sense the increased humidity in the head space of the latter cups. Females often were observed to execute their looping pre-oviposition flight behavior (Furumizo and Rudnick 1978) over dry cups. This observation suggests that the looping flight facilitates the perception of humidity gradients that would indicate the presence of water even when the water is obscured from view. The extremely hydrophobic nature of the chorion of *Toxorhynchites* eggs allows them to bounce off the walls of oviposition sites to reach the water (Furumizo and Rudnick 1978) if more direct access is impossible.

Finally, none of the three concentrations of methyl propionate tested had a significant effect upon oviposition (Experiment 8). *Toxoryhnchites* may respond to other airborne odors but they do not respond to this compound found by Perry and Fay (1967) to strongly enhance *Ae. aegypti* oviposition in the laboratory. Based on other results presented above, the reflectance of the oviposition container and humidity seem to be more important factors relative to olfactory cues in the acceptance of sites for oviposition by *Tx. splendens* in the laboratory. The white pupal emergence cups were never observed to elicit pre-oviposition flight behavior and only a few eggs were found in them even after 4 days in the colony cages. Likewise, very few eggs were deposited in dry containers. Given the external stimuli and internal influences that may effect *Toxorhynchites* behavior under field conditions, we feel that investigations, such as Focks et al. (1983), that correlate *Toxorhynchites* oviposition site preference and natural occurrence of prey larvae would be especially valuable in evaluating the potential of this and other *Toxorhynchites* species as biological control agents of container-breeding mosquitoes.

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REFERENCES CITED

Focks, D. A. 1985. *Toxorhynchites*. pp. 42–45 *In:* H. C. Chapman (ed.), Biological control of mosquitoes. Bull. Am. Mosq. Control Assoc. 6.

Focks, D. A. and D. W. Hall. 1977. Laboratory colonization and biological observations of *Toxorhynchites rutilus rutilus* Mosq News. 37:751–755.

Focks, D. A., S. R. Sackett, D. A. Dame and D. L. Bailey. 1983. Ability of *Toxorhynchites amboinensis* (Doleschall) (Diptera: Culicidae) to locate and oviposit in artificial containers in an urban environment. Environ. Entomol. 12:1073–1077.

Furumizo, R. R. and A. Rudnick. 1978. Laboratory studies of *Toxorhynchites splendens* (Diptera: Culicidae): Biological observations. Ann. Entomol. Soc. Am. 71:670–673.

Perry, A. S. and R. W. Fay. 1967. Correlation of chemical constitution and physical properties of fatty acid esters with oviposition response of *Aedes aegypti*. Mosq. News 27:175–183.

SAS Institute Inc. 1985. SAS® user's guide: Statistics, Version 5th ed. Cary, NC.

Slaff, M. E., J. J. Reilly and W. J. Crans. 1975. Colonization of the predacious mosquito, *Toxorhynchites rutilus septentrionalis* (Dyer & Knab). Proc. N.J. Mosq. Exterm. Assoc. 62:146–148.

Trimble, R. M. 1978. Laboratory observations on oviposition by the predaceous tree-hole mosquito, *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae). Can. J. Zool. 57:1104–1108.