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## DEVELOPMENT OF A FLOATING BIOASSAY CONTAINER FOR MOSQUITO LARVAE<sup>1, 2</sup>

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Various containers have been developed to retain mosquito larvae or other target organisms in field experiments when evaluating biological control agents, commercial pesticides or environmental influences (Chapman et al. 1970, Case and Washino 1979, Hembree et al. 1980, Petersen 1982, Westerdall et al. 1982). We recently developed a new bioassay container in support of riceland mosquito research involving *Romanomermis culicivorax* Ross and Smith, a nematode parasite of mosquito larvae. The container is easily constructed, relatively inexpensive at ca. \$2.50 (U.S.)/unit, and reusable (except when contaminated with some pesticides). Barring strong, turbulent currents, fluctuations in water levels do not inhibit its ability to remain upright and in proper position for maximum exposure of the confined target organisms. In addition, the container is lightweight (260 g), easily transported to remote research sites and durable.

A diagram of the bioassay container is provided (Fig. 1) to assist in the following de-

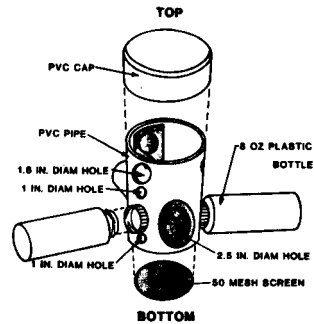


Fig. 1. Diagram of floating bioassay container for mosquito larvae.

scription of the materials and construction. A length of 4 in. (10.2 cm) diam schedule 20 polyvinylchloride (PVC) pipe is cut transversely into 7 in. (17.8 cm) sections. Each section requires a series of holes drilled along its sides to facilitate air and water interchange, and provide biocontrol agents and pesticides access to the confined target organisms. Three 2.5 in. (6.4 cm) diam holes are drilled equidistant around one end of the PVC section so that the edges of the resultant holes are 0.8 in. (1.9 cm) from the end of the PVC section. Three caps from 8 oz (237 ml) sample bottles<sup>4</sup> are individually affixed with hot melt glue<sup>5</sup> between the holes (centered horizontally and vertically). The positioning of these caps is the most important factor in construction, and must be placed in a manner to permit the horizontal diameters of the holes to be contiguous with the water surface to provide maximum surface exposure.

To supplement air and water exchange, another series of 1.0 in. (2.5 cm) and 1.6 in. (4.1 cm) holes are drilled in the PVC section. These holes are located in line and above and below each of the attached caps. The locations of these holes are not as important as the 2.5 in. holes previously mentioned; however, they should be drilled as near as possible to the pattern shown in Fig. 1. Inside the PVC containers, swatches of 50 mesh Saran (plastic) screen<sup>6</sup> are glued in place over all drilled holes and the container bottom. New Saran screen may have insecticidal properties so it should be thoroughly washed before use (Osgood 1974). After the glue has cured and the plastic bottles are tightly screwed

<sup>1</sup> Mention of a commercial or proprietary product does not constitute a recommendation or an endorsement of the product by Louisiana State University Agricultural Center and does not imply exclusion of other products that may also be compatible.

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<sup>4</sup> Model 6049-01, 8 oz sample bottle, Horizon Ecology Co., Chicago, IL 60648.

<sup>5</sup> Thermogrip®, Bostick Consumer Division, USM Corporation, 4408 Pottsville Pike, Reading, PA 19605.

<sup>6</sup> Lumite®, Chicopee Manufacturing, P. O. Box 2537, Gainesville, GA 30503.

into the caps, the bioassay container is ready for use. During field experiments, double-layered cheesecloth is placed over the top of the container and secured with rubber bands to prevent predators and debris from entering the container.

Another unique feature of this bioassay container is the ease with which intermittent counts of the target organisms (i.e., mosquito larvae) can be made in the field. This is accomplished by removing the cheesecloth from the top of the container, replacing the cloth with a schedule 20 PVC cap (Fig. 1) and inverting the entire unit. The mosquito larvae and approximately 250 ml of water are retained in the PVC cap after the excess water has drained from the container through the screen covered holes. The bioassay container is subsequently removed from the cap and the larvae are exposed in the cap to determine mortality/survival rates.

Prior to its inclusion in field tests, four laboratory trials were conducted to determine if the design of the bioassay container inhibited pre-parasitic juveniles of *R. culicivora* from gaining access to 1st instar mosquitoes located inside the containers. Each container was placed in a plastic pan filled with about 2.1 gal (8 liters) of well water maintained at 80°F (26.6°C). Fifty 1st instar *Culex quinquefasciatus* Say larvae were placed in the bioassay container and fifty larvae were placed in the pan. About 1000 newly hatched preparasites of *R. culicivora* were introduced into the pan giving a ratio of 10:1 (nematodes: mosquito larva). The containers were evaluated with and without float bottles attached to determine if the bottles provided a funneling effect as the preparasites approached the bioassay container. Larvae were fed daily

with 50 mg of ground rabbit chow. After three days, the surviving larval mosquitoes were removed from the pans and bioassay containers, and washed in a 100 mesh sieve to remove free swimming preparasites. The larvae were subsequently placed in pans of well water maintained at 80°F (26.6°C), reared to 4th instar and examined for parasitism. Data were subjected to analyses of variance using SAS general linear models procedure for testing the hypothesis that percent parasitism was equal (SAS 1982).

The bioassay containers did not inhibit pre-parasitic *R. culicivora* from gaining access to *Cx. quinquefasciatus* larvae (Table 1). In fact, average infection rates (80.9 and 81.8%) for containers with float bottles were significantly greater ( $P < 0.01$ ) than infection rates within the pan (77.1 and 69.9%). The arrangement of the float bottles may have contributed to the higher incidence of parasitism in the container by providing a funneling effect for the surface dwelling preparasites. There was no significant difference ( $P > 0.05$ ) in percent parasitism between the pan and containers without float bottles.

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Table 1. Comparative parasitism of *Culex quinquefasciatus* by *Romanomermis culicivora* in pans and in bioassay containers placed in pans.<sup>1</sup>

Trial	Larval location	No. nematodes per larva					Total		
		0	1	2	3	4	5+	Mosquitoes examined	Percent parasitism
<i>With float bottles</i>									
Trial I <sup>2</sup>	Container	42	124	37	10	7	0	220	80.9
	Pan	52	131	32	10	1	1	227	77.1
Trial II <sup>2</sup>	Container	30	97	35	2	1	0	165	81.8
	Pan	74	129	40	2	1	0	246	69.9
<i>Without float bottles</i>									
Trial I <sup>2</sup>	Container	4	60	43	30	10	10	157	97.5
	Pan	13	57	44	18	9	7	148	91.2
Trial II <sup>3</sup>	Container	2	6	7	16	13	31	75	97.3
	Pan	4	31	26	20	6	5	92	95.7

<sup>1</sup> Nematode to host ratio of 10:1, respectively. Each replication consisted of 50 first instar mosquito larvae in the container and 50 in the pan. Nematode larvae were placed in the pan.

<sup>2</sup> Means for 5 replications.

<sup>3</sup> Means for 2 replications.

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### AEDES PURPUREIPES IN WESTERN ARIZONA

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Rapid melting of record snowfall and rains in the upper reaches of the Colorado River drainage in 1983 led to the release, from storage facilities, of huge quantities of water into the lower Colorado River basin. The resultant flooding increased areas for mosquito breeding, and arboviral activity was noted in vector species (D. B. Francy, unpublished data). In 1984, subsequent studies of virus activity in mosquitoes by the Centers for Disease Control (CDC) and routine surveillance by the U.S. Army Environmental Hygiene Agency revealed the presence of *Aedes (Kompsia) purpureipes* Aitken in two counties of western Arizona. All specimens collected were females.

*Aedes purpureipes* occurs in northwestern Mexico and southern Baja California; and until the time of this study, it was known in the U.S. from only Pima, Cochise and Santa Cruz counties of southeastern Arizona (Zavortink 1972, McDonald et al. 1973). It is a tree hole breeder and is associated with cottonwood (*Populus*), hackberry (*Celtis*), and palo verde (*Cercidium*) in the hotter and drier regions of its range. It is

also found in riparian woodland and oak-pine forest at elevations up to 1700 m. The integument of the thorax and abdomen of this striking, medium-sized mosquito is yellowish to golden in color and marked with silvery scale patches.

The first specimen was collected on August 2, 1984, at Blue Water Marina, Parker, La Paz Co., Arizona, in a CDC light trap supplemented with dry ice (coll.—Gordon C. Smith<sup>1</sup>). The specimen was identified as *Ae. purpureipes* by E. L. Peyton and Dr. R. E. Harbach, Walter Reed Biosystematics Unit (WRBU), National Museum of Natural History, Washington, DC. Traps were hung in a cluster of mesquite (*Prosopis*) and salt cedar (*Tamarix*) and also yielded *Culex tarsalis* Coquillett, *Psorophora signipennis* (Coquillett) and a few *Ps. columbiae* (Dyar and Knab).

Two additional specimens were collected on September 7, 1984, at the Pre-School, Yuma Proving Ground, Yuma County, Arizona, in a CDC light trap supplemented with dry ice (coll.—Paul Weimann<sup>3</sup>). The trap was hung in a mature palo verde next to an oleander (*Nerium oleander*) and a California fan palm (*Washingtonia filifera*); it also yielded *Cx. tarsalis* and *Ae. taeniorhynchus* (Wiedemann). The identification of these specimens as *Ae. purpureipes* by the junior authors was confirmed by Dr. Bruce A. Harrison, WRBU. These specimens are deposited in the National Museum of Natural History, Washington, DC.

Another *Ae. purpureipes* was collected on October 3, 1984, in a salt cedar windbreak near the main gate of the Yuma Proving Ground in a CDC light trap supplemented with dry ice (coll.—Paul Weimann<sup>3</sup>). The collection also included *Cx. tarsalis*, *Ps. columbiae*, and *Anopheles franciscanus* McCracken. The site was about 400 m from the Pre-School site.

Collection of this species in Yuma and La Paz counties, Arizona, is a considerable western and northward extension of its range. It seems likely that collections on the California side of the river will also yield *Ae. purpureipes* since ecological conditions there are similar to the Arizona side of the river.

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