

*rimaculatus* and *Cx. erraticus*. Thirty-seven and 41% of the *Ps. columbiae* and *Cx. erraticus* were respectively blood-fed. Although only 5 *Ps. ciliata* were collected, 60% were blood-fed.

Resting stations were constructed according to Edman et al. (1968). Four resting stations were placed at interfaces of wooded and open field or pasture areas. *Anopheles quadrimaculatus* and *Cx. erraticus* made up 95.0 and 4.9% respectively of the mosquitoes captured representing 48 trap collections. Only 4 other species were recorded, but in low numbers. These were *Cx. nigripalpus*, *An. crucians*, *An. punctipennis* and *Ae. vexans*. Forty percent of the *An. quadrimaculatus* and 13.0% of the *Cx. erraticus* captured by this method were blood-fed.

Hand-held aspirator collections from barn walls yielded predominantly *An. quadrimaculatus* followed by *Ps. columbiae* and *Cx. erraticus*. All mosquitoes collected by this method were blood-fed. An obvious bias for blood-fed mosquitoes collected in this manner to have fed on bovine animals contained in the barn is recognized. However, many different domestic animals were present in and around the barns and blood host tests conducted on these mosquitoes might have demonstrated some host preference.

Lancaster et al. (1968) listed 6 genera and 25 species present in Arkansas County. Only 14 species were collected and identified in this study, but 3 of these were new county records. These were *Ps. howardii*, *Cx. nigripalpus*, and *Cq. perturbans*. No *Ps. discolor* (Coquillett) were collected although Schwardt (1939) and Horsfall (1942) indicated that in light trap collections *Ps. discolor* was second in abundance only to *Ps. columbiae* and was occasionally more abundant.

The greatest diversity of species was obtained with the truck trap; however, this technique provided the lowest percentage of blood-fed mosquitoes. The hand aspirator collected the highest percentage of blood-fed mosquitoes, but these collections were strongly biased toward bovines. Neither of these techniques was as applicable for riceland mosquito collections as the resting station and backpack aspirator. The resting station was not effective for obtaining unbiased blood-fed *An. quadrimaculatus* and *Cx. erraticus* while the backpack aspirator functioned best for *Ps. columbiae*. The 3 most commonly collected species, *Ps. columbiae*, *An. quadrimaculatus* and *Cx. erraticus*, require different collecting techniques for blood-feeding investigations.

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## EFFECTS OF FLOTATION METHODS AND OVERNIGHT HOLDING ON THE TOXICITY OF CHLORPYRIFOS TO LARVAE OF *CULICOIDES VARIIPENNIS* (CERATOPOGONIDAE)<sup>1, 2</sup>

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Research on *Culicoides variipennis* (Coquillett) as a vector of bluetongue (BT) virus in ruminants is conducted at the USDA, Agriculture Research Service Arthropod-borne Animal Diseases Research Laboratory in Denver, CO. Several of the research projects require a rather large number of field-collected larvae, some of which are shipped from distant parts of the United States. The common method used in our lab for separating these larvae from mud samples is direct flotation with dechlorinated tap water.

Kline et al. (1975) showed that direct flotation by using saturated solutions of magnesium sulfate was satisfactory to recover *Culicoides* larvae from salt marshes or intertidal zones. The following evaluations were undertaken to determine if such flotation would affect the toxicity of chlorpyrifos to the larvae of *C. variipennis*

<sup>1</sup> This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended. Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA.

<sup>2</sup> This research was conducted in facilities of the U.S. Army Environmental Hygiene Agency, Denver, CO. under a Memorandum of Understanding entitled "Research on the control of insect vectors of mammalian diseases."

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from our colony and from a field site northeast of Denver, CO.

A solution of 100 ppm (w/v) technical chlorpyrifos was prepared in reagent grade acetone with a small amount of Toximul (Stepan Chemical Company) as an emulsifier and held at 0°C. On each test day an aliquot was used to prepare 30 ml of a 1 ppm stock solution in deionized water. Then 400 ml samples of 12.5, 6.25, 3.125, 1.563 and 0.781 ppb test dilutions were prepared in deionized water and stirred for 30 min. Three replicates of each dilution and a deionized water control were prepared in disposable petri dishes<sup>4</sup> (15 × 100 mm) with 50 ml of solution per dish. Fifty to 100 *C. variipennis* larvae were placed in each of the dishes using the hanging droplet technique (Holbrook 1982). The dishes were covered and held at 22±1°C for 24 hr for mortality counts.

Tests were conducted by comparing *C. variipennis* larvae of the Sonora (Texas) strain colonized in 1957 (Jones et al. 1969) with larvae collected from a site adjacent to a beef cattle feedlot northeast of Denver, CO. Field collections were made with a garden trowel by removing a ca 2 cm thick layer of mud and larvae for transportation to the lab in plastic bags on wet ice. The field sample of mud and larvae and a sample of mature colony larvae on dacron<sup>®</sup> fiber mats (Jones et al., 1969) were each divided in half and placed in 4 containers. One container each of the field and colony samples was filled with dechlorinated tap water to a depth of ca 30 cm and stirred. More water was slowly added and the larvae swimming on the surface

were collected in a fine mesh screen held under an overflow spout. The remaining 2 containers were similarly processed except that the flooding agent was a 40% solution (w/v) of magnesium sulfate. Each of these 4 larval samples were then divided into 2 equal subsamples, 4 tested immediately and 4 held at 26±1°C for testing 24 hr later. As an additional control fresh colony larvae floated with water were tested at the same times as the larvae that had been held for 24 hr. Data were analyzed by probit analyses (Finney 1971), using programs modified from Daum (1970).

The uniformity of the slopes (Table 1) calculated from the dosage-response data indicates that all *C. variipennis* larvae tested reacted to the chlorpyrifos in a similar manner. Flotation with 40% magnesium sulfate solution or dechlorinated tap water resulted in no significant differences in the toxicity of chlorpyrifos to either colonized or field-collected larvae, but the former increased the number of larvae recovered. Holding field-collected larvae for 24 hr after flotations did not significantly affect test results. However, the LC<sub>50</sub> of the colonized larvae on the day of flotation averaged ca twice that of the field-collected larvae, but at 24 hr no significant differences were present. Fresh colony larvae tested with those held 24 hr resulted in an LC<sub>50</sub> similar to that of the colony larvae tested on Day 0.

The differential effects of chlorpyrifos to Sonora Strain and field-collected (Colorado) larvae was reported by Holbrook (1982), with LC<sub>50</sub> values of 0.0043 and 0.0023 ppm, respec-

Table 1. Toxicity of chlorpyrifos to *Culicoides variipennis* larvae recovered by flotation with dechlorinated water or 40% magnesium sulfate solution.

| Day            | Larval source | Flotation         | LC <sub>50</sub> (ppb) <sup>1</sup> | Fiducial limits (5% level) | Slope <sup>2</sup> |
|----------------|---------------|-------------------|-------------------------------------|----------------------------|--------------------|
| 0              | Colony        | H <sub>2</sub> O  | 4.394a                              | 3.950-4.888                | 3.405              |
|                |               | MgSO <sub>4</sub> | 3.596ab                             | 2.996-4.316                | 3.156              |
|                | Field         | H <sub>2</sub> O  | 2.171c                              | 1.956-2.411                | 3.595              |
|                |               | MgSO <sub>4</sub> | 2.042c                              | 1.788-2.332                | 2.999              |
| 1              | Colony        | H <sub>2</sub> O  | 2.960bc                             | 2.611-3.356                | 2.973              |
|                |               | MgSO <sub>4</sub> | 2.702c                              | 2.396-3.141                | 3.132              |
|                | Field         | H <sub>2</sub> O  | 2.565c                              | 2.305-2.855                | 3.241              |
|                |               | MgSO <sub>4</sub> | 2.352c                              | 2.044-2.706                | 3.352              |
| 0 <sup>3</sup> | Colony        | H <sub>2</sub> O  | 4.714a                              | 4.238-5.243                | 3.476              |

<sup>1</sup> Data from 4 tests combined; numbers followed by the same letter do not differ at the 5% level, by Duncan's multiple range test.

<sup>2</sup> Slopes do not differ at the 5% level, by potency probit analyses.

<sup>3</sup> Tested as fresh larvae on Day 2.

<sup>4</sup> Preliminary testing showed that the LC<sub>50</sub> and slope obtained using glass (4.286 ppb and 3.780) or disposable polystyrene (4.436 ppb and 4.069) petri dishes were similar.

tively. The transitory nature of this difference in the colonized larvae may involve the physical conditions under which the larvae are reared or the bacterial diet on which they feed. Pesticide

susceptibility may be used as a method for periodic assessment of quality control of colonized *C. variipennis*.

I express my appreciation to R. White and other U.S. Army Environmental Hygiene Agency personnel for their cooperation; also, to G. V. Richardson, Statistician, USDA-ARS, Ft. Collins, CO, and R. J. Bobian, Agric. Research Technician (Insects), USDA-ARS, Denver, CO, for assistance during these experiments.

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### A MULTI-PADDLE OVITRAP FOR COLLECTING *HAEMAGOGUS* AND *AEDES AEGYPTI* EGGS

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During studies on the natural history of yellow fever virus in Trinidad, it became necessary to collect large numbers of *Haemagogus* eggs for transovarial transmission experiments. Earlier, Tikasingh and Laurent (1981) had demonstrated the usefulness of the traditional ovitrap developed by Fay and Eliason (1966) for collecting *Hg. (Hag.) equinus* Theobald eggs in Tobago. These traps were also used to collect *Hg. (Hag.) janthinomys* Dyar and *Hg. (Con.) leucoclaelus* (Dyar and Shannon) in the forests of Trinidad. A multi-paddle ovitrap was also developed and used successfully in collecting *Haemagogus* eggs; its usefulness for collecting *Aedes aegypti* (Linn.) eggs was later demonstrated.

The modified ovitrap made from a 1.5 liter plastic ice cream container was approximately 165 mm in diameter and 100 mm deep, being

brick red in color. Later collections were also made from containers painted black (Fig. 1). This size container can hold 12 hard-board paddles each measuring approximately 25 × 130 mm and 4 mm thick<sup>1</sup>. In order to maintain the paddles in an erect position, wire loops were woven around the top of the container. The numbered paddles were placed with the rough side facing the center of the container to which was added 3 cm of water. Each ovitrap was set between 1 to 3 m above ground in the forest. Servicing was done at weekly intervals and consisted of replacing the exposed paddles with new ones and replenishing the water, if necessary. In the laboratory, the paddles were examined, under the low power of a dissecting microscope and eggs present were counted.

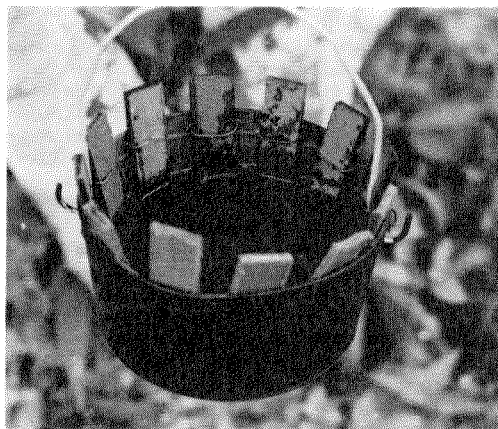


Fig. 1. A multi-paddle ovitrap *in situ*.

Four multi-paddle traps were operated in the Chaguaramas forest, Trinidad from 18th May 1981 through 12th February 1982. During this period 936 paddles were examined and 148 (15.8%) were positive. A total of 1013 eggs was collected giving a mean of 6.8 eggs per positive paddle. When a trap was positive, eggs were found on as many as 10/12 cohort paddles in any one week.

Since the containers were manufactured in a red color, one was painted (inside and outside) black and set approximately 4 m from the red one. The results obtained with these containers in which each held 12 paddles and which were

<sup>1</sup> Commercially available in the USA as Masonite™, a wood-fiber material, pressed in sheets and used for partitions, etc. The product used should have one surface with cross-hatched indentations about 1 mm apart (Editor).