ENHANCEMENT OF THE CDC OVITRAP WITH HAY INFUSIONS FOR DAILY MONITORING OF *AEDES AEGYPTI* POPULATIONS

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ABSTRACT. An ovitrap containing hay infusion and a second ovitrap adjacent to it containing a 10% dilution of the infusion in tap water together yielded 8 times more *Aedes aegypti* eggs than single CDC ovitraps containing tap water. These "enhanced pairs" were significantly more attractive than pairs with other combinations of infusion, water or methyl propionate, and have proven useful for daily monitoring of *Ae. aegypti* populations. Our results shed light on the oviposition behavior of *Ae. aegypti* in the field.

INTRODUCTION

Artificial oviposition sites, or "ovitraps," have been extensively used to detect the presence of *Aedes aegypti* (Linn.) (Service 1976). During the *Ae. aegypti* eradication program (Schliessmann 1964), the "CDC ovitrap" (Fay and Eliason 1966) was adopted as the standard in the United States. This consists of a glass jar painted glossy black on the outside, 12.7 cm high and 7.6 cm in diameter at the top, with slightly tapered sides. A paddle of water-absorbent fiberboard ("Masonite" or hardboard), 12.7 cm long and 1.9 cm wide, is clipped to the inside of the jar with the rough side exposed. The jar contains tap water. *Aedes aegypti* mosquitoes attracted to the jar oviposit on the wet fiberboard, close to the interface with the water.

Generally, CDC ovitraps are left in the field for 7 days. Some workers have used them for day-to-day surveillance, but the number of traps receiving eggs and the number of eggs per positive trap are usually low (Chadee and Corbet 1987). Buxton and Hopkins (1927) observed that infusions of vegetable matter gave increased egg yields, and a number of subsequent workers have used such infusions as "attractants" (Beattie 1932, Frank and Lynn 1982, Kitron et al. 1989). In groups of ovijars with various dilutions of hay infusion in tap water, we found that the largest number of eggs appeared in jars containing 10% infusion. However, single jars with 10% infusion gave much lower yields, suggesting that strong infusions can contribute to long-range attraction, but mosquitoes prefer to oviposit in weaker solutions. In this paper we present egg collection data for paired ovijars using various combinations of water and hay infusion. We also include data for methyl propionate solution, as this has been reported to enhance oviposition in the laboratory (Fay and Perry 1965, Klowden and Blackmer 1987).

METHODS

Hay infusion was made by steeping 1 kg of dry grass hay in 120 liters of tap water for 7 days in a tightly closed plastic garbage can in a shaded outdoor place. The product had a strong, foul smell. A new infusion was started in advance of every collection day. Hay from the same bale was used throughout the study.

Four attractants were compared: undiluted infusion, 10% infusion in tap water, 0.5% methyl propionate in tap water, and tap water. Ten combinations of these attractants were tested, including 4 in which an attractant was paired with an empty ("blank") jar. Forty ovijar pairs were used per day, with 4 replicates of the 10 combinations. Assignment of the combinations to the 40 sites was randomized each morning. Ovijars in polyethylene trays (restaurant "tote boxes," 10 pairs of ovijars per tray), were allotted 220 ml of the appropriate attractant in the order they were to be set in the field. Trays were protected from direct sunlight during transport to the field.

Collections were made in a residential zone (Puerto Nuevo) in the center of San Juan, Puerto Rico. Plots in the area were mostly 15 × 27 m, with little or no garden. The majority of houses were single story, constructed of cement blocks. They were well maintained, with white or pastel colored exterior walls. Trap sites were selected at every fifth house, on alternate sides of the road. If the fifth house was unavailable, the next was chosen. If this too was unavailable, the house across the road was used.

Ovijar pairs were set under the eaves of the house, where they contrasted with the color of the wall. To maintain consistency of day-to-day position, they were arranged with the paddles nearest to the wall and parallel to it. They were set between 0900–1200 h, the time of lowest oviposition activity (Haddow and Gillett 1957, Chadee and Corbet 1987) and collected 24 h later. On collection, attractants were discarded from the side of the jar opposite to the paddle, to prevent eggs from being washed from the fiberboard. If an ovijar had been moved, emptied or interfered with in any way, the collection was eliminated from the record.

A rain gauge was operated in the center of the collection area. Data from rainy days were eliminated because rainfall was considered a complicating factor.

At the laboratory, paddles were stacked with their egg-bearing surfaces well separated, and allowed to dry for 3 days (fiberboard lightens as it dries, making the black eggs more visible). Eggs were counted under a binocular microscope. Most counts could be made to within a 2% error, but accuracy was lower when several hundred eggs were present. Jars were meticulously scrubbed in clean water before reuse to prevent accumulation of deposits that would encourage mosquitoes to oviposit away from the paddles.

RESULTS

A total of 54,196 eggs were collected in 28 days of trapping. Yields per positive paddle ranged from a single egg to 734.

The enhancing effect of the infusion was well marked (Table 1). The 100%/10% pair gave the highest yield (92.2 eggs per collection). This was 8.1 times more than the water/blank pair (11.4 eggs per collection, $P < 0.001$, Mann-Whitney U test), the combination most similar to a standard CDC ovitrap.

The 10%/blank and 100%/blank combinations had 3.0 and 5.6 times more eggs than water/blank pairs ($P < 0.01$ and $P < 0.001$, respectively). The same was true for the 10%/10% and 100%/100% pairs, compared with water/water pairs, although the ratios were less marked (2.1:1, $P < 0.05$, and 4.4:1, $P < 0.001$, respectively). The methyl propionate/blank pair had only 1.6 times more eggs than the water/blank, and the difference was not significant ($P > 0.05$).

Pairs of jars containing the same liquid yielded more eggs than a single jar with that liquid coupled with a blank, but the differences were not significant ($P > 0.05$). However, the 10%/water and 100%/water pairs yielded 1.9 and 4.5 times more eggs than water/water pairs ($P < 0.05$ and $P < 0.001$), and 100%/10% pairs yielded 4.8 times more than water/water pairs ($P < 0.001$).

Within pairs, infusion received nearly twice as many eggs as water (ratio 1.9:1, $P < 0.01$) in the 10%/water combination, but in the 100%/water and 100%/10% combinations mosquitoes preferred water (ratio 1.4:1, $P < 0.01$) or 10% infusion (ratio 1.5:1, $P < 0.01$) to full strength infusion. The 100%/water and 100%/10% pairs collected more eggs than the 100%/blank pairs (1.4:1 and 1.5:1, respectively), although these differences were not significant at the 95% level.

DISCUSSION AND CONCLUSIONS

The foul smelling hay infusion clearly augmented the number of eggs collected. The microbial flora of such attractants is constantly changing, but the use of a strict routine for producing and using them should minimize the effect of such variations on collections. Filling the jars at the laboratory ensures that they reach the field in a uniform condition, with the paddles well wetted above the water line, thus further improving their "standardization."

We have used the 10%/100% combination in a long series of evaluations of the efficacy of adulticiding operations against *Ae. aegypti* (Centers for Disease Control, unpublished data). In some studies, oviposition preference for 10%

Table 1. Mean daily collections of *Aedes aegypti* eggs in paired ovitraps. Mean of 122 samples for each combination.

Ovitrap contents		No. of eggs		Total eggs
Ovijar 1	Ovijar 2	Ovijar 1	Ovijar 2	(per pair)
Water	Blank	11.4	—	11.4
10% infusion	Blank	33.6	—	33.6
100% infusion	Blank	63.4	—	63.4
Water	Water	9.7	9.8	19.3
10% infusion	10% infusion	22.2	18.7	40.6
100% infusion	100% infusion	42.7	44.3	85.5
10% infusion	Water	24.0	12.8	36.1
100% infusion	Water	36.6	50.4	87.0
100% infusion	10% infusion	37.5	56.5	92.2
Methyl propionate	Blank	18.0	—	18.0

over 100% has been more pronounced than in the data presented above, presumably due to differences in the infusion. We therefore prefer to use the combination, rather than single jars of 100% infusion, on the assumption that this gives the best overall result.

In an urban area, a pair of operators can service 80 ovitrap pairs in a morning without difficulty. Moreover, in contrast to aspirator collections of adults, which require diligence, skill, and consistency of effort, setting ovitraps requires no subjective effort and can be done with minimal training. In San Juan, 80 sites routinely yield 5,000–10,000 *Ae. aegypti* eggs per day. This high yield is useful in susceptibility testing and other studies for which maximum heterogeneity of field samples is required. We have also used the ovitrap pairs to monitor oviposition activity on a 2-hr basis.

Aedes aegypti is commonly said to prefer clean water for its breeding sites, so it may seem surprising that a foul hay infusion is favored for oviposition. However, clean water is a sterile environment for mosquito larvae, whereas the microbial fauna of the infusion is an excellent source of nutrition. Field surveys may give the impression that the species prefers clean water habitats because late instar larvae are often found in receptacles where they have cleansed the water of suspended matter (Rivière 1985). In many cases such larvae may actually be short of food (Southwood et al. 1972, Subra and Mouchet 1984). Indeed, there is laboratory evidence that microbial activity is an indicator to ovipositing females that the receptacle is not already crowded with competing larvae (Benzon and Apperson 1988). *Aedes aegypti* has even been found breeding in septic tanks and other foul water sites (Babu et al. 1983).

Pre-oviposition behavior in *Ae. aegypti* may be analogous to host selection before blood feeding: visual and olfactory stimuli for long-range attraction to a suitable site (host), give way to close-range stimuli that manage commitment to the site (host) and the initiation of oviposition (feeding). The enhancement of egg yield in the presence of a jar of undiluted infusion indicates long-range olfactory attraction (referred to as "pre-oviposition" by Klowden and Blackmer (1987)). The preference for diluted infusion where choice is available implies short range selection. It is also possible that the attractant could modulate the number of eggs deposited by a feedback mechanism similar to "desistance" during probing for a blood meal (Ribiero et al. 1985).

Kitron et al. (1989) found that the presence of *Aedes triseriatus* (Say) eggs on an ovitrap paddle was associated with a decrease in further oviposition by this species. In our study, single jars adjacent to a blank jar collected less eggs than the total for a pair of adjacent jars containing the same liquid, although the differences were not significant. Because mosquitoes had an equal chance of arriving at either jar of such a pair, these differences may indicate inhibition of a similar kind in *Ae. aegypti*. However, the difference could be also be due to simple interaction of females at the site. Certainly the wide range of numbers of eggs per positive paddle, from 1 to over 700, confirms that many females can contribute eggs to a single site during the short oviposition period.

A chemical substitute for infusion would be convenient, not only because preparation would be simpler, but because, as pointed out by Frank and Lynn (1982), it could be truly standardized. Methyl propionate did not meet this need, but a search for effective compounds would be worth pursuing.

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