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 x 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 ovitraps.

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>
<thead>
<tr>
<th>Ovijar contents</th>
<th>Ovijar 1</th>
<th>Ovijar 2</th>
<th>Total eggs (per pair)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Blank</td>
<td></td>
<td>11.4</td>
</tr>
<tr>
<td>10% infusion</td>
<td>Blank</td>
<td></td>
<td>33.6</td>
</tr>
<tr>
<td>100% infusion</td>
<td>Blank</td>
<td></td>
<td>63.4</td>
</tr>
<tr>
<td>Water</td>
<td>Water</td>
<td></td>
<td>9.7</td>
</tr>
<tr>
<td>10% infusion</td>
<td>10% infusion</td>
<td>22.2</td>
<td>40.6</td>
</tr>
<tr>
<td>100% infusion</td>
<td>100% infusion</td>
<td>42.7</td>
<td>85.5</td>
</tr>
<tr>
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<td>24.0</td>
</tr>
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<td>100% infusion</td>
<td>Water</td>
<td></td>
<td>36.6</td>
</tr>
<tr>
<td>100% infusion</td>
<td>10% infusion</td>
<td>37.5</td>
<td>92.2</td>
</tr>
<tr>
<td>Methyl propionate</td>
<td>Blank</td>
<td></td>
<td>18.0</td>
</tr>
</tbody>
</table>
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 (Riviere 1985).
In many cases such larvae may actually be short
of food (Southwood et al. 1972, Subra and
Mouchet 1984). Indeed, there is laboratory evi-
dence 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 feed-
ing: 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 Klodwen 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 ovitraps
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 contain-
ing 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 inter-
action 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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