HAY INFUSION AND ISOPROPYL ALCOHOL-BAITED CDC LIGHT TRAP: A SIMPLE, EFFECTIVE TRAP FOR GRAVID CULEX MOSQUITOES

SCOTT A. RITCHIE
Collier Mosquito Control District, P.O. Box 7069, Naples, FL 33941

ABSTRACT. A CDC light trap baited with oviposition attractants effectively collected gravid Culex mosquitoes. Addition of isopropyl alcohol enhanced the effectiveness of hay infusion to attract gravid Culex. Paired tests indicated that the hay infusion + alcohol-baited CDC light trap collected fewer Culex but significantly more gravid Culex than the CO₂-baited CDC light trap. The hay infusion + alcohol-baited trap also collected a significantly higher percentage of parous Culex. The hay infusion + alcohol mixture appears to be a valuable supplement to CDC light traps and has potential as an arbovirus surveillance tool.

INTRODUCTION

New Jersey light traps and CO₂-baited CDC light traps have been popular methods of monitoring Culex populations for arbovirus surveillance (Service 1976). However, the propensity of these traps to collect nuliparous mosquitoes has necessitated the development of methods to sample gravid (presumed to have taken a blood meal) Culex (Leiser and Beier 1982). This is especially relevant for collecting live mosquitoes for arbovirus assay (Reiter 1983).

Two approaches have been used to monitor and collect gravid Culex. Oviposition traps sample egg rafts deposited into an oviposition attractant by gravid females. These traps provide population indices and positive identification of morphologically similar adults (Leiser and Beier 1982). However, gravid Culex traps provide a live mosquito collection which can be assayed for arboviruses. While seemingly advantageous to New Jersey and CDC light traps (Reiter 1983), gravid Culex traps have received little application; most designs seem inconvenient and relatively ineffective. Traps designed by Lewis et al. (1974) required construction and aspiration of the collection while only collecting an average of 12.1 Culex quinquefasciatus Say per trap night. Surgeoner and Helson's (1978) trap involved the costly and laborious use of a child's wading pool, sod and water to fill the pool.

Recently, Reiter (1983) developed a more effective gravid Culex trap. A modified CDC trap was baited with hay infusion; collections averaged 141.3 Culex per trap night, over 90% of which were gravid. Concurrent collections from resting sites and with New Jersey light traps were considerably smaller. Unfortunately, no comparison was made with CO₂-baited CDC light traps.

Therefore, a need exists to develop a simple, effective trap for collecting gravid Culex and compare this trap relative with CO₂-baited CDC light trap.

MATERIALS AND METHODS

Testing of oviposition attractants involved a CDC miniature light trap (Fig. 1) hung above a dark brown dishpan (29 × 34 × 12 cm) containing 6.0 liters (1.3 gal) of oviposition attractant. Two oviposition attractants were used. The standard hay infusion (modified from Reiter 1983) was produced by adding 0.9 kg (2 lb) hay, 10 gm (0.35 oz) brewer's yeast and 114 liters water (25 gal) to a 136 liter (30 gal) plastic trash can. The solution was mixed and the can covered with black plastic, securing the lid to prevent mosquito access. The can was kept out-

Fig. 1. An infusion-baited CDC light trap.
doors and the hay infusion was ready after 1 week. Every week thereafter, 0.25 kg (0.5 lb) hay, 10 gm (0.35 oz) Brewer's yeast and water were added to fill the can to the 114 liter (25 gal) mark. Old hay was removed biweekly. The other oviposition attractant consisted of a 2:1 mix of hay infusion and industrial-grade isopropl alcohol.

Paired trappings were conducted to determine which oviposition attractant more effectively collected gravid Culex. Subsequently, the more effective attractant was paired with CO2 (Addison et al. 1979) to target-engorged specimens which appeared translucent. Parity was determined by the state of ovarian tracheoles dissected in 30 nongravid Culex selected randomly from each collection (10 paired trappings). The ovariolar dilatation method (Detinova 1962) was rejected since Nayar and Knight (1981) found aberrant dilations in nulliparous Cx. nigripennis. The total number of Culex (excluding blood-engorged specimens) which had taken a blood meal sufficient to develop eggs was calculated by adding the number gravid to the number parous. The number parous was estimated by multiplying the number nongravid by the proportion parous as determined by dissection. Data from all tests were compared using Student's t-test (Snedecor and Cochran 1987); data from malfunctioning traps were eliminated.

Culex were collected separately to assay for St. Louis encephalitis virus. Mosquitoes were frozen then separated into pools of 50 on a cold table. Pools were packed in dry ice and shipped to the Florida Epidemiological Research Center in Tampa, FL; pools were stored at -70°C until processed (Hammon and Sather 1969). Individual pools were triturated in sterile chilled mortars using alundum and 2 ml of 20% fetal bovine serum-buffered saline. The resulting suspensions were clarified by centrifugation at 4°C for 30 min at 1,200 x g. Supernatant fluids were immediately inoculated into 24 4-day-old Swiss white mice; 0.02 ml intracerebrally and 0.03 intraperitoneally. Mice were observed daily for 14 days post-inoculation for clinical signs of illness. Brains were removed from any dead or morbid mice and 10% suspensions used for successive passage into litters of 2-4-day-old mice.

RESULTS

The addition of isopropl alcohol to hay infusion increased the collection of Culex mosquitoes with CDC light traps (Table 1). The hay infusion + alcohol-baited trap collected significantly more Culex (P < 0.001) and gravid Culex (P < 0.01) than hay infusion-baited traps. The control, isopropl alcohol-baited CDC light trap, collected fewer Culex (P < 0.10) and significantly fewer gravid Culex (P < 0.01) than hay infusion + alcohol-baited CDC light traps. Carbon dioxide-baited CDC light traps collected more specimens of different species (12) than the hay infusion + alcohol-baited traps (9). Uranotaenia lousa Theobald was the only species collected in larger numbers (14.2 vs 4.6) by the hay infusion + alcohol-baited trap; many of these appeared gravid. The percent Culex, 87.7% and 76.3% respectively, was comparable for hay infusion + alcohol and CO2-baited CDC light traps.

Results of paired trappings with CDC traps baited with CO2 and hay infusion + alcohol are summarized in Table 2. While CO2-baited traps collected nearly 5 times the number of Culex, hay infusion + alcohol-baited traps collected nearly 50 times the number of gravid Culex. The estimated number of Culex which had taken a blood meal sufficient to develop eggs

---

Table 1. Comparison of Culex collected from CDC light traps baited with different oviposition attractants.

<table>
<thead>
<tr>
<th>Attraction</th>
<th>Hay infusion + Isopropl alcohol* (X ± S.D.)</th>
<th>Hay infusion* (X ± S.D.)</th>
<th>Isopropl alcohol† (X ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Culex</td>
<td>405.2 ± 288.2</td>
<td>227.7 ± 294.7</td>
<td>168.8 ± 146.4</td>
</tr>
<tr>
<td>Gravid Culex</td>
<td>231.3 ± 250.5</td>
<td>69.1 ± 78.1</td>
<td>10.4 ± 18.5</td>
</tr>
</tbody>
</table>

* Traps set in western Collier County, FL.; 16 replicates.
† Traps set in similar area; 12 replicates.
‡ Culex includes predominately Cx. nigripennis and possibly a few Cx. quinquiesquisitum and Cx. salinarvus.
Table 2. Summary of paired trapping with CDC light traps: Hay infusion + alcohol vs CO₂ bait.

<table>
<thead>
<tr>
<th>Description</th>
<th>Hay infusion + alcohol (X ± S.D.)</th>
<th>Carbon dioxide (X ± S.D.)</th>
<th>Probability of greater t (replications)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total Culex*</td>
<td>335.1 ± 263.2</td>
<td>1,562.7 ± 1,689.5</td>
<td>P&lt;0.005 (20)</td>
</tr>
<tr>
<td>2. Gravid Culex*</td>
<td>147.6 ± 98.3</td>
<td>3.0 ± 3.96</td>
<td>P&lt;0.001 (20)</td>
</tr>
<tr>
<td>3. Parity (%) of non gravid Culex*</td>
<td>42.7 ± 18.4</td>
<td>29.7 ± 16.4</td>
<td>P&lt;0.01 (10)</td>
</tr>
<tr>
<td>4. Combined gravid + parous Culex*</td>
<td>190.1 ± 110.0</td>
<td>232.6 ± 167.8</td>
<td>P&lt;0.50 (10)</td>
</tr>
<tr>
<td>5. % gravid + parous Culex*</td>
<td>65.2 ± 18.2</td>
<td>26.1 ± 13.1</td>
<td>P&lt;0.001 (10)</td>
</tr>
</tbody>
</table>

*a Culex includes predominately *C. nigrripalpus* and possibly a few *C. quinquefasciatus* and *C. salinarius*.
*b Represents an estimate of Culex which have taken a previous blood meal.

was not significantly different for either bait. However, the percentage of said Culex was significantly greater using the oviposition attractant.

No virus was isolated from Culex collected with either bait (2,237 pooled from hay infusion + alcohol-baited trap collections; 2,750 pooled from CO₂-baited trap collections). Interestingly, 7/23 sentinel chickens located within 2 miles of mosquito-trapping areas seroconverted for SLE virus during the time mosquitoes were collected (Florida Department of Health and Rehabilitative Services, personal communication).

DISCUSSION

Isopropyl alcohol appears to enhance the efficacy of hay infusion to collect gravid Culex with CDC light traps. The rationale of the alcohol supplement was to increase the volatility of the hay infusion, thus exposing more gravid Culex to the attractant. However, isopropyl alcohol seemed to act as a short range oviposition repellent since egg rafts were never observed on the hay infusion + alcohol mixture. Indeed, the paucity of gravid Culex collected by the control traps suggests isopropyl alcohol in itself does not attract gravid Culex. The collection of many nongravid Culex in the controls is probably due to the attractiveness of light alone, traps with malfunctioning CO₂ tanks similarly collected numerous nongravid Culex. Reiter (1983) removed the lamp and minimized the collection of nongravid Culex. Further research with various concentrations of different infusions and volatile compounds might produce better results for different species of mosquitoes. Organic infusions (Surgeoner and Helson 1978, Lewis et al. 1974, Kramer and Mulla 1979, Leiser and Beier 1982), pheromones (Andreadis 1977, Kalpage and Brust 1973, Os- good 1971) and a variety of compounds (Maw 1970, Petersen and Willis 1970) have been shown to act as ovipositional attractants to species of Aedes, Culex and Psorophora.

Interestingly, the hay infusion + alcohol-baited CDC light trap collected a significantly greater percentage of gravid Culex than CO₂-baited traps. Culex which have recently oviposited may still be attracted to the bait; many of the gravid ovaries exhibited the dilated appearance characteristic of recent oviposition. Surgeoner and Helson (1978) made similar observations on Culex collected in an oviposition trap.

The significantly greater percentage of previously blood-fed Culex (gravid + estimated parous Culex/total Culex) collected with hay infusion + alcohol suggests this may be more practical than CO₂ for collecting Culex for arbovirus assay. However, this estimate assumes: (1) No autogeny which would believe blood as the source of egg production and, (2) All blood meals are sufficient to produce eggs as evidenced by parity determination. Regarding the first assumption, Nayar (1982) states that *C. nigrripalpus* populations in Florida are functionally anautogenous. Regarding assumption 2, Edman and Kale (1971) found that host defensive behavior elicited partial blood-feeding in *C. nigrripalpus*. Additionally, Mitchell et al. (1979) demonstrated that partial blood meals may serve to infect *C. pipiens* Linn. with SLE yet result in egg maturation. Therefore, it is impossible to accurately estimate the number of Culex which have previously blood-fed without knowledge of partial blood-feeding rates.

CONCLUSION

The addition of isopropyl alcohol enhanced the efficacy of hay infusion to collect gravid Culex with CDC light traps. No modification of CDC traps is necessary and the oviposition attractant is inexpensive (alcohol cost per trap was $1.29). This is an improvement over the inconvenience, expense and efficacy of earlier mod-
Additionally, CO₂ supplemented with oviposition attractant might increase the utility of CDC light traps as an arbovirus surveillance tool.

ACKNOWLEDGMENTS

My gratitude to F. M. Wellings and A. L. Lewis of the Florida Epidemiological Research Center for assaying mosquitoes for arbovirus and J. H. Frank of the Florida Medical Entomology Laboratory for his criticism of the manuscript. Judy Knight of the FMEI instructed me in determining mosquito parity and Kris Thoenike at Rookery Bay National Estuarine Sanctuary allowed use of a phase contrast microscope. I also wish to thank B. G. Watson for support during the research.

References Cited


