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

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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 CO_2-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 CO_2-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 nulliparous 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 CO_2-baited CDC light traps.

Therefore, a need exists to develop a simple, effective trap for collecting gravid *Culex* and compare this trap relative with CO_2-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, 0.23 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 isopropyl alcohol.

Paired trappings were conducted to determine which oviposition attractant more effectively collected gravid *Culex*. Subsequently, the more effective attractant was paired with CO_2 (Addison et al. 1979) to compare the species composition, number of gravid *Culex* and parity of nongravid *Culex*. *Culex* refers primarily to *Culex nigripalpus* Theobald since *Cx. quinquefasciatus* and *Culex salinarius* Coq. are rare in south Florida in the late summer (O'Meara and Evans 1983). Traps were set in a variety of *Culex* habitats in western Collier County, FL in the late afternoon and picked up by 0900 hr. The trapping period was from August to November, 1983. Isopropyl alcohol-baited CDC light traps were used as controls.

Collections were killed by freezing and gravid *Culex* were separated on a light table (designed to edit photographic transparencies) from nectar-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 dilation method (Detinova 1962) was rejected since Nayar and Knight (1981) found aberrant dilations in nulliparous *Cx. nigripalpus*. 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 1967); 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, pH 7.2 as diluent. The resulting suspensions were clarified by centrifugation at 4°C for 20 min at 1,200 × 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 postinoculation 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 isopropyl 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 trap. The control, isopropyl 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 lowii* 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 CO_2-baited CDC light traps.

Results of paired trappings with CDC traps baited with CO_2 and hay infusion + alcohol are summarized in Table 2. While CO_2-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 attractions.

	Hay infusion + isopropyl alcohol[a] ($\bar{X}$ ± S.D.)	Hay infusion[a] ($\bar{X}$ ± S.D.)	Isopropyl alcohol[b] ($\bar{X}$ ± S.D.)
Total *Culex*[c]	405.2 ± 288.2	227.7 ± 204.7	168.8 ± 146.4
Gravid *Culex*[c]	231.3 ± 259.5	69.1 ± 78.1	10.4 ± 18.5

[a] Traps set in western Collier County, FL; 16 replicates.
[b] Traps set in similar area; 12 replicates.
[c] *Culex* includes predominately *Cx. nigripalpus* and possibly a few *Cx. quinquefasciatus* and *Cx. salinarius*.

Table 2. Summary of paired trapping with CDC light traps: Hay infusion + alcohol vs CO_2 bait.

	Hay infusion + alcohol ($\bar{X}$ ± S.D.)	Carbon dioxide ($\bar{X}$ ± S.D.)	Probability of greater t (replications)
1. Total *Culex*[a]	335.1 ± 263.2	1,562.7 ± 1,689.5	P<0.005 (20)
2. Gravid *Culex*[a]	147.6 ± 98.3	3.0 ± 3.96	P<0.001 (20)
3. Parity (%) of nongravid *Culex*[a]	42.7 ± 18.4	29.7 ± 16.4	P<0.01 (10)
4. Combined gravid + parous *Culex*[a, b]	190.1 ± 110.0	232.6 ± 167.8	P>0.50 (10)
5. % gravid + parous *Culex*[a, b]	65.2 ± 18.2	26.1 ± 13.1	P<0.001 (10)

[a] *Culex* includes predominately *Cx. nigripalpus* and possibly a few *Cx. quinquefasciatus* and *Cx. 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 CO_2-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 CO_2 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, Osgood 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 parous *Culex* than CO_2-baited traps. *Culex* which have recently oviposited may still be attracted to the bait; many of the parous 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 bait may be more practical than CO_2 for collecting *Culex* for arbovirus assay. However, this estimate assumes: (1) No autogeny which would belie 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 *Cx. nigripalpus* populations in Florida are functionally anautogenous. Regarding assumption 2, Edman and Kale (1971) found that host defensive behavior elicited partial blood-feeding in *Cx. nigripalpus*. Additionally, Mitchell et al. (1979) demonstrated that partial blood meals may serve to infect *Cx. pipiens* Linn. with SLE yet not 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-

els. Additionally, CO_2 supplemented with oviposition attractant might increase the utility of CDC light traps as an arbovirus surveillance tool.

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