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EFFICACY OF CARBON DIOXIDE, 1-OCTEN-3-OL, AND LACTIC ACID IN MODIFIED FAY-PRINCE TRAPS AS COMPARED TO MAN-LANDING CATCH OF *AEDES AEGYPTI*

D. V. CANYON AND J. L. K. HII

Vector Research Laboratory, Department of Public Health and Tropical Medicine, James Cook University, Townsville, OLD 4811, Australia

ABSTRACT. The attractants 1-octen-3-ol and lactic acid significantly decreased catches of *Aedes aegypti* in Townsville, Australia, by 50% in a controlled laboratory environment and by 100% in the field when compared to carbon dioxide baited bidirectional Fay-Prince trap catches. Evaluation of an omnidirectional alteration on a bidirectional Fay-Prince trap revealed no significant improvement in catch size when compared to both the bidirectional trap and man-landing catch (MLC). Cumulative evening MLC (1730–2000 h) was twice that of the morning MLC (0600–0830 h), which has implications on the precise estimation of the man-biting rate. The MLC sampling method is shown to be a quick, simple, effective, and cheap alternative to expensive traps in areas not currently experiencing arbovirus transmission.

INTRODUCTION

Aedes aegypti (Linn.) is widely distributed in northern Queensland (Sinclair 1992) and has been incriminated as a primary dengue vector (Gubler 1988). Entomological surveillance of *Ae. aegypti* for detecting changes in adult or immature populations or for viral isolations is a crucial component of active dengue surveillance. Ecological and behavioral information such as population densities, distribution, survivorship, and seasonal abundance are urgently required for an understanding of epidemic potential, and for the formulation of control strategies. Periodic surveys designed to detect changes in key adult indices are also important for an epidemiological early-warning system.

Host-seeking behavior in mosquitoes is influenced by visual cues (Bidlingmayer 1994) and chemical attractants emanating from the human body (Eiras and Jepson 1994). The development of attractants has thus tended to focus on those substances emanating from vertebrates. Carbon dioxide, one such substance, is well documented as being an attractant for *Ae. aegypti* (Christophers 1960). Eiras and Jepson (1991) found a positive correlation between mosquito probing and landing and the presence of CO_2 and lactic acid, which indicated the presence of a synergistic relationship. Lactic acid has been reported to attract at a distance, but to repel at short range. A synergistic relationship between CO_2 and octenol has also been demonstrated (Takken and Kline 1989). Kline et al. (1990a, 1990b, 1991a, 1991b), who have produced a large proportion of the literature on octenol, state that octenol produces variable results for some mosquito species, which are repelled and then attracted on different occasions. Response to octenol has been shown by many researchers to be variable and species specific. Kline (1994) also states that response to octenol and CO_2 varies geographically, seasonally, and according to the physiological state of the mosquito. "Unlike CO_2," Kline (1994) states, ". . . octenol appears to be an attractant for only a few mosquito species." Additionally, Vythilingam et al. (1992) collected 9 different mosquito species and showed no significant difference between CO_2-baited catch sizes with or without octenol. In light of Takken's (1991) statement, "it should not be ruled out that olfactory cues are involved at short distance as well," and the overwhelming number of research papers that used octenol and did not list *Ae. aegypti* as being caught, this study tests the attractant abilities of octenol, CO_2, and lactic acid alone and in combination on *Ae. aegypti* in a controlled environment with a bidirectional Fay-Prince trap (Jensen et al. 1994).

This study also compares the bidirectionally modified trap with the most effective attractant with an omnidirectionally modified Fay-Prince trap (Fay and Prince 1970) to determine the more effective modification. The omnidirectional trap was first developed by Jensen et al. (1994) and improved by the John W. Hock Company (Gainesville, FL), who replaced the color scheme and tactile surface with the glossy black and white contrasting colors and slick surfaces of the Fay-Prince trap.

In areas where traps are not economical to use and in areas of high *Ae. aegypti* density, the man-landing catch (MLC), which involves a person acting both as bait and trap, is very effective for indoor and outdoor bait collections (Trpis et al. 1995). Our laboratory and field trials also compare MLC and trap results in order to determine the trap/attractant combination that is more representative of a human host.

MATERIALS AND METHODS

Laboratory trials: All laboratory trials were conducted with F_1 to F_4 generations of the Townsville COUNCIL strain of *Ae. aegypti* mosquitoes, which were obtained from the Townsville City Council.

The colony was maintained according to the guidelines set by Foster (1980).

Attractant trials were conducted using a bidirectional Fay-Prince trap suspended 1 m above the floor. The most effective attractant obtained from this trial was then used to evaluate omnidirectional alterations to the bidirectional trap. These alterations constituted a reduction in suction fans from 2 to 1 and an increase in entry vents from 2 to 4. Apart from these changes, which may have canceled each other out, the traps were so similar in appearance that a full trial with all attractant combinations on the omnidirectional trap was not warranted.

Randomly designed laboratory attractant trials were conducted from August through September 1995 in a small experimental room (18 m^3). The MLC and attractant trials lasted 1 and 3 h, respectively, and were repeated 4 times in a random pattern. A 3-h period for the attractant trials was selected because *Ae. aegypti* host-seeking behavior typically reveals two peaks, dawn and dusk, each lasting approximately 3 h (MacDonald 1956). Since this study aimed to look at subtle differences in response to attractants, the trials were held midway between those two peaks, at which time host-seeking behavior in *Ae. aegypti* is still evident although much reduced.

Carbon dioxide was provided by placing 0.3 kg of dry ice pellets in the standard black insulated container suspended above the trap. Two milliliters of 1-octen-3-ol was provided at release rates ranging between 4.4 and 5.6 mg/h in a microreaction vial (Van Essen et al. 1994). Lactic acid (85%) was provided by soaking a cotton pad in a small Petri dish with 10 ml of the acid. The calculation of a release rate for lactic acid was not possible since its hydroscopic nature resulted in a moisture absorbance rate of 0.31 mg/h. Both 1-octen-3-ol and lactic acid containers were placed in a central position on the top of the trap so that their vapors would be drawn down and dispersed by the suction fans.

Ceiling lighting was provided via 4 white fluorescent tubes. Temperature was kept at 27 ± 2°C and relative humidity varied between 68 and 74%. Since male mosquitoes are present in natural circumstances, they were included in each trial. Fifty female and 50 male *Ae. aegypti* were released in each trial and those not caught by the sampling methods were aspirated at the end of the trials to calculate recovery rates.

Field trials: A suburban residence with 5 key breeding containers situated under 1.5- to 2.5-m-high garden shrubbery, accommodating a naturally occurring adult *Ae. aegypti* mosquito population, was used for all trials. The only mosquito species present over the duration of the trials was *Ae. aegypti*. In the first part of this trial, the morning (0600–0830 h) and evening (1730–2000 h) MLCs, which were taken on the same day, were alternated with CO_2-baited bidirectional Fay-Prince traps (0600–2000 h) every 3 days from October through November. This was done to allow the local mosquito population time to recover and because the gonotrophic cycle of *Ae. aegypti* is 3 days (Pant and Yasuno 1973). In December 1995, another trial tested the bidirectional trap without attractants, with lactic acid and with 1-octen-3-ol alternately on consecutive days. Ten replicates were made for each test and the field trials were limited to a single attractant, because laboratory trials revealed no benefit in combining attractants. Since *Ae. aegypti* were breeding on-site and in neighboring premises, from October through December, only a slight decrease in the natural adult *Ae. aegypti* population over the duration of the 2 field trials was expected.

All catch data are presented in raw form, but were transformed to $\log(n + 1)$ to allow more realistic means to be calculated and for the application of parametric tests (Service 1993). Transformed laboratory data were analyzed in the SPSS (Windows version 6.1, 1994) program MEANS/DUNCAN for mean comparisons. Transformed field data were analyzed using the SPSS Student's *t*-test for variance analysis and means comparisons.

RESULTS

Laboratory trials

Attractant trials: The bidirectional trap alone (B) with no attractants sampled an average of 26.8 ± 5.72 (SE) *Ae. aegypti* females in a 3-h period (Fig. 1). The mean catch was significantly higher ($P <$ 0.05) than mean catches with 1-octen-3-ol (B-O: 10.0 ± 2.55) and lactic acid (B-L: 10.3 ± 2.53) either separately or in combination (B-OL: 13.3 ± 4.33). The addition of carbon dioxide (CO_2) to lactic acid (B-CL: 18.8 ± 2.66) and 1-octen-3-ol (B-CO: 22.3 ± 1.65) and a combination of the two (B-CLO: 26.5 ± 2.99) significantly increased trap catch to the point where it was no different from a trap baited with CO_2 (B-C: 31.3 ± 3.57) or an unbaited trap (B). The mean recovery rate for these laboratory trials was 97.3 ± 0.67% (range: 95–100%), which indicates that the experimental room was sufficiently secure.

Trap comparison trial: Since the B-C trap caught the most female mosquitoes, marginally although not significantly better than the B trap alone ($P >$ 0.05), CO_2 was added to the omnidirectional trap (OM-C) to compare it with the bidirectional design (B-C). Omnidirectional improvements made on the bidirectional design, which allowed mosquitoes to enter the trap from all directions, produced no significant increase in trap catch (B-C: 31.3 ± 3.57; OM-C: 30.3 ± 2.78).

Man-landing catch trial: Man-landing catches were compared with trap catches, since MLC has been reported as the standard and most useful method for collecting host-seeking and anthropophilic mosquitoes (Service 1976). MLC produced the

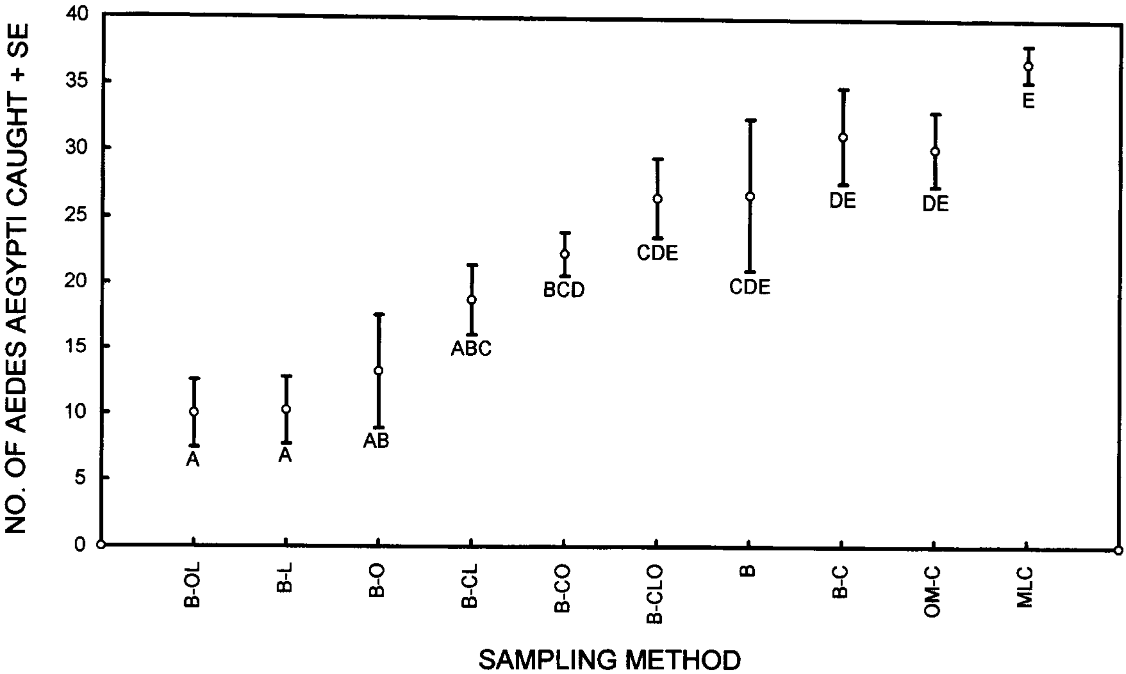

Fig. 1. Mean number (±SE) of *Aedes aegypti* females in various trap/attractant combinations compared to man-landing catch (MLC) in 10 laboratory trials. B—Bi-Fay trap; OM—Omni-Fay trap; C—CO_2; O—Octenol; L—Lactic acid. Means with the same letters are not significantly different at $P < 0.05$ by Duncan's multiple-range test.

highest catch over 1 h for female mosquitoes (36.8 ± 1.38) which was significantly higher ($P < 0.05$) than B-O, B-L, B-OL, B-CL, and B-CO catches (Fig. 1). Separation of the 1-h MLC into 10-min intervals and analysis of raw data revealed that significantly more (78%) mosquitoes were aspirated in the first 10 min. ($P < 0.01$). In view of this, field trail MLCs were limited to 10 min.

Field trials

MLC and B-C comparison: The morning (0600–0830 h) MLC, evening (1730–2000 h) MLC, and CO_2 baited bidirectional trap (14B-C) results for female mosquitoes (Table 1) were not significantly different from each other ($P > 0.05$).

Attractant trials: Comparison of baited and unbaited bidirectional trap catches (Table 1) revealed results that were somewhat contrary to the laboratory trial results in that the only trap that caught any *Ae. aegypti* mosquitoes was the CO_2-baited trap. The unbaited, lactic-acid-baited, and octenol-baited traps caught no mosquitoes.

Table 1. Mean catch ± SE of *Aedes aegypti* female and male mosquitoes caught by 6 trapping methods in a field site.

Method	Female
10-min man-landing catch—morning	1.70 ± 0.37a[1]
10-min man-landing catch—evening	3.60 ± 0.86a
14-h Bi-Fay + CO_2	1.80 ± 0.59a
14-h Bi-Fay (no attractants)	0
14-h Bi-Fay + octenol	0
14-h Bi-Fay + lactic acid	0

[1] Means with different letters are significantly different ($P < 0.05$).

DISCUSSION

Laboratory tests revealed that lactic acid and octenol repelled *Ae. aegypti* even when used in combination with carbon dioxide. The mean unbaited trap catch was higher than all but the CO_2-baited trap catch and the man-landing catch. The results confirm the negative influence of lactic acid on *Ae. aegypti* attraction in close proximity to a host, and show no evidence to support the existence of a synergistic relationship between lactic acid and CO_2. Our laboratory and field trial results for lactic acid are supported by the field trial results of Stryker and Young (1970), who also could not confirm this synergistic relationship. The negative effect of octenol on *Ae. aegypti* attraction is possibly due to octenol being a product of rumination (Takken 1991). It is reasonable to expect little or no attraction to the chemical by *Ae. aegypti,* which is a highly anthropophilic mosquito and, as such, probably has had little interest in bovines. Since octenol has been isolated from many plants and fungi (Hall et al. 1984), it might also be expected that any mosquito not usually attracted to omnivorous or semiomnivorous vertebrates will not be attracted to octenol.

As expected, the omnidirectional improvements we made to the Fay-Prince trap had little impact on catch size. Certainly the omnidirectional unit ($147) is far more cost-effective because it retails for almost half the price of a bidirectional trap ($274) (Gainesville, FL).

Since a trap sample ideally represents the number of mosquitoes attracted to a human host, MLC was compared to trap catches. That the MLC was not significantly different from CO_2-baited trap catches confirmed the suitability of CO_2 for use in the field trials.

Surprisingly, field and laboratory trial results were not compatible with regard to the use of attractants. Where the laboratory trials show a clear pattern of preference and repellency on the part of the adenines, the field experiments show clearly that carbon dioxide is the only one of the 3 attractants tested that actually works. Laboratory results do not represent what is actually happening in nature due to extraneous factors such as background illumination, which can interfere with the discrimination of light intensities perceived by mosquitoes (Barr et al. 1963). On the other hand, laboratory tests may provide insight into what could be expected in a far more extensive field trial.

In the MLC field trials, the cumulative evening catch was double that of the morning. This has implications in the precise estimation of the man-biting rate. Man-landing catch is one of the most basic parameters involved in the evaluation of any vector-borne disease transmission (Service 1976, 1993). Man-landing catch is also one of the first parameters that entomologists and malariologists have tried to measure, and is one that is probably the least accurately measured due to differences in human attractiveness (Khan 1977) and differences in the ability of individual collectors to capture mosquitoes. That MLC is shown in this study to vary with time of collection indicates a need to limit MLC collections to the evening peak of *Ae. aegypti* activity.

Ethical implications in using human catchers to collect *Ae. aegypti* in areas where vector-borne diseases occur may preclude MLC usage. However, where MLCs are still being practiced, dengue viruses have been isolated from *Ae. aegypti* mosquitoes collected by this method (Thavara et al. 1996). This risk may be hard to avoid in areas where the risk of importation or introduction of vector-borne diseases is high but unpredictive.

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