2006

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Journal of the American Mosquito Control Association, 22(4):641–647, 2006

LABORATORY AND FIELD ASSESSMENT OF SOME KAIROMONE BLENDS FOR HOST-SEEKING *AEDES AEGYPTI*[1]

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ABSTRACT. Using laboratory Y-tube olfactometers, the attractiveness of lactic acid and 2 kairomone blends from the United States Department of Agriculture (USDA) and BioGents GmbH (BG) was assessed for attractiveness to *Aedes aegypti*. Four geographically disparate populations were assessed: North Queensland Australia (NQA), Florida USA, Minas Gerais Brazil (MGB), and Singapore. In descending order, populations were attracted to USDA, BG blends, and lactic acid. MGB was poorly attracted to lactic acid alone. The blends were less attractive than human odor. Proprietary blends were modified, and their attractiveness was assessed to find the optimum attractive mixture for NQA. Adding acetone to BG, and ammonia and caproic acid to USDA, improved attractiveness in the laboratory. Field attractiveness was assessed by coupling the blends with a newly developed BG-Sentinel *Ae. aegypti* trap. Trials were carried out using the BG blend, BG blend plus acetone, USDA blend, USDA blend plus ammonia and caproic acid, and a control trap with no kairomones. The traps were highly effective, with mean 24-h collections up to 11.15 *Ae. aegypti* per trap, and this species made up 91.7% of collections. However, the effectiveness of the unbaited control trap indicated that the BG-Sentinel has visual attractive properties for *Ae. aegypti* and that the kairomone lures added little to trap performance in NQA.

KEY WORDS *Aedes aegypti*, BG-Sentinel traps, dengue, kairomones, host seeking, Y-tube olfactometer

INTRODUCTION

Aedes aegypti (L.) is a prominent vector of dengue viruses throughout tropical regions, and the resurgence of dengue worldwide is necessitating novel approaches to improve surveillance and control of this species (Gubler 1998). Human-biting catch and aspiration samples pose a risk of arbovirus infection and are labor-intensive (Focks 2003). Hence, there is a need to develop new more efficient sampling methods that can be readily related to dengue epidemiology. Traps with kairomone lures for host-seeking *Ae. aegypti* would provide more efficient and epidemiologically relevant alternatives for surveillance. "Lure and kill" strategies for host-seeking *Ae. aegypti* may ultimately augment or even replace current population reduction methods. These strategies have been implemented in north Queensland, where regular dengue epidemics occur. Sticky ovitraps are used in surveillance (Ritchie et al. 2003), and lethal ovitraps that kill ovipositing females (Zeichner and Perich 1999) are used for population reduction (Queensland Health 2005).

Several *Ae. aegypti* attractants have been identified from human skin. Lactic acid is one such compound whose attractancy can be enhanced in the laboratory by combining it with fatty acids (Acree et al. 1968, Eiras and Jepson 1991, Geier and Boeckh 1999, Bosch et al. 2000), ammonia (Geier et al. 1999a), dimethyl disulfide (Bernier et al. 2003), carbon dioxide (Eiras and Jepson 1991, Geier et al. 1996), or acetone (Bernier et al. 2003). Many of these are components of proprietary kairomone blends for *Ae. aegypti*. The BioGents (BG) blend (Geier and Eiras 2003) is composed of lactic acid, caproic acid, and ammonia, whereas the United States Department of Agriculture (USDA) blend is a mixture of acetone, lactic acid, and dimethyl disulfide (Bernier et al. 2001).

Laboratory studies of kairomones have traditionally been conducted using long-established *Ae. aegypti* colonies. However, the attractiveness of any proprietary blends should be assessed against *Ae. aegypti* strains of disparate origins to assess conserved or divergent responses to kairomones. Although methods for assessing attraction of *Ae. aegypti* in the laboratory are well established (e.g., Y-tube olfactometers; Geier and Boeckh 1999a), field methods are less developed. Carbon dioxide-baited mosquito traps are not very effective in *Ae. aegypti* collection, and human bait and aspiration remain the most effective field sampling methods (Canyon and Hii 1997, Jones et al. 2003, Schoeler et al. 2004). The BG-Sentinel trap (Kröckel et al. 2006)

[1] Mention of a commercial product does not constitute endorsement by James Cook University, Queensland Health, or University of Sydney in Australia, or by USDA in the USA.

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incorporates visual attractants and includes the BG blend. Although the field efficacy of the BG-Sentinel for capturing *Ae. aegypti* has been demonstrated (Kröckel et al. 2006), the contribution of the BG blend to trap efficacy remains untested.

We present 3 phases of research. First, using laboratory Y-tube olfactometers, we assess the attractiveness of 2 proprietary kairomone blends to disparate collections of *Ae. aegypti* to assess responses to kairomones. Second, in Y-tube olfactometers, we optimize the kairomone blend for attraction of north Queensland *Ae. aegypti* (NQA). Finally, field trials were conducted in north Queensland, by using the optimal blends from laboratory studies, in the BG-Sentinel *Ae. aegypti* trap.

MATERIALS AND METHODS

Laboratory assays

F_1 generations were reared from field-collected eggs from NQA and Minas Gerais, Brazil (MGB). Eggs also were hatched from long-standing laboratory colonies maintained in Singapore and Florida, USA (USDA–ARS–CMAVE). Although probably unrepresentative of field *Ae. aegypti*, these laboratory colonies provide a geographic diversity of strains. Larvae were fed with Tetramin® fish food. Adults were maintained in plastic containers (50 × 40 × 25 cm) at 26–28 C, 60–70% relative humidity, a photoperiod of 12:12 (L:D) h, and with continuous access to a 10% glucose solution.

Olfactometers

Four identical Plexiglas® Y-tube olfactometers were used to measure the attractiveness of volatile compounds as described previously (Geier and Boeckh 1999). Mosquitoes were released from a chamber at the end of the stem, and through upwind anemotaxis they were exposed to odors released at 1 of 2 parallel stimulus chambers with the other chamber maintained as a blank control. Twenty milliliters of a compound or blend was placed into a 200-ml Erlenmeyer flask. Charcoal-filtered air was then passed through the flask, carrying by Teflon tubing headspace volatiles to ports immediately proximal to the treatment arms of the Y-tube. The dosage of kairomone was determined by the airflow rate through the flask as in previous work with the same apparatus (Geier and Boeckh 1999, Steib et al. 2001). Human odor was introduced into the stimulus chambers by inserting the right index finger through a snug-fitting hole (C.R.W.). A balanced, randomized block design was used so that *Ae. aegypti* strains would be uniformly exposed to human odors (Geier and Boeckh 1999, Geier et al. 1999b).

Bioassay and statistical analyses

Bioassay procedures were published previously (Geier and Boeckh 1999, Geier et al. 1999, Bosch et al. 2000, Williams et al. 2006). Groups of 17–25 nonblood-fed nulliparous females (10–20 days postemergence) were lured from their holding cages by human odor (Williams et al. 2006). This process identified host-seeking mosquitoes as described by Posey and Schreck (1981). The release cage containing mosquitoes was fitted to the stem of the Y-tube, the cage was flushed with clean air, and mosquitoes were acclimatized for 20 min. When odor stimuli were introduced, the mosquitoes were released from the chamber and allowed 30 sec to respond, after which the rotating screens were closed, trapping mosquitoes in the treatment arm, control arm, and in the release cage. The order in which each strain was exposed to each volatile stimulus was assigned randomly, with equal numbers of replicate tests (n = 12) for each strain–stimulus combination. Each experiment involved the use of all 4 *Ae. aegypti* strains assigned randomly to each of the 4 Y-tubes. Attracted mosquitoes were those that left the release cage, flew upwind, and were captured in the treatment arm of the olfactometer. The number captured in the control arm also was recorded. The number of mosquitoes in the test arm as a percentage of those in the release chamber was arcsine-transformed before analysis of variance (ANOVA). Least significant difference post hoc tests were performed using SPSS statistical software release 11.0.1 (SPSS Inc. Chicago, IL).

Experiment 1: laboratory comparison of proprietary blends

The attractiveness of the BG and USDA blends was assessed, along with distilled water (a negative control) and human odor (positive control) (Table 1). Lactic acid (analytical reagent [AR] grade) also was included to determine the contribution of other compounds to the attractiveness of proprietary blends.

Experiment 2: laboratory testing modified proprietary blends for NQA

Attempted enhancements of proprietary blends were made by the addition of compounds known to provide enhanced attraction with lactic acid. These blends were then assessed in Y-tube olfactometers (Table 1). Acetone (AR grade) also was tested to determine its role in the USDA blend and to verify previous attractancy findings (Bernier et al. 2003).

Table 1. Composition and flow rate of treatments and controls used in laboratory experiments with Y-tube olfactometers.

Experiment 1		
Treatment	Composition	Flow rate
Water (negative control)	Distilled water	10 ml/min
Human odor (positive control)	Finger[1]	
Lactic acid	Lactic acid (Merck, Darmstadt, Germany)	50 ml/min
BG blend	Lactic acid	50 ml/min
	Caproic acid (Fluka Chemika, Neu-ulm, Germany)	5 ml/min
	Ammonia (Merck)	1 ml/min
USDA-blend (Bernier et al. 2001)	Acetone + lactic acid + dimethyl disulfide (mixture)	50 ml/min
Experiment 2		
USDA-blend	Composition	Flow rate
USDA-blend + ammonia + caproic acid	USDA-blend	50 ml/min
	Ammonia	1 ml/min
	Caproic acid	0.3 ml/min
BG blend	As in experiment 1	As in experiment 1
BG blend + acetone	BG blend Acetone (Fluka Chemika Neu-ulm, Germany)	As in experiment 1 50 ml/min
Acetone	Acetone	50 ml/min

[1] Finger directly inserted into treatment arm of Y-tube olfactometer.

Experiment 3: field studies of kairomone blends with traps in NQA

BG-Sentinels (BioGents GmbH, Regensburg, Germany) were used as an *Ae. aegypti* trap. Kairomone blends were placed inside these traps to assess their relative attractiveness to *Ae. aegypti* in NQA. BG-Sentinels are collapsible, white, cylindrical "buckets" (40 cm in height and 20 cm in diameter), with white gauze tops. In the top center of the trap is a black 10-cm-diameter opening to a catch bag. A 12-V DC fan draws air through the black opening and exhausts it through the perimeter of white gauze at the top of the trap. Kairomones placed in the bottom of the trap are exhausted by the fan.

Field testing was conducted in suburban Cairns, NQA, from January to March 2005. Traps were deployed in the bottom level of 2-story "Queenslander"-style houses. Such houses are mostly timber, with the well-ventilated lower story typically comprising a laundry, a secondary bedroom, and sometimes garaging. No houses used in this study were fully enclosed by walls and doors on the lower level, because they would impede the egress of mosquitoes. Such house design is conducive to *Ae. aegypti* harborage, because fly-screened windows and doors are rare, and there are ample refugia and human hosts for mosquitoes in the lower level.

Lures evaluated in the BG-Sentinel were the BG blend, the BG blend plus acetone, the USDA blend, and the USDA blend plus ammonia and caproic acid. The quantities of kairomones in BG-Sentinels were chosen on the basis of commercial availability and feasibility for field deployment, taking the volatility and flammability of acetone into account. The BG blend and its respective components were dispensed in amounts as supplied by the manufacturer. The BG blend dispenser consisted of 2 m of coiled 3/16-in. internal diameter (i.d.) silicon tubing (containing 15 ml of lactic acid), 50 cm of 0.4-mm-i.d. high-density polyethylene tubing (2 ml of caproic acid), and a slow release ammonia acrylic fibrous tablet (BioGents GmbH, Regensburg, Germany), all bound together with plastic cableties. There was no standard dispensing mechanism for acetone or the USDA blend, so these blends were dispensed in 50-ml volumes in a 300-ml (7-cm-diameter) glass jar with a perforated stainless steel lid. A trap without any lure served as a control.

The evaporative release rates for each of the kairomone components from the lures in the BG-Sentinels were measured in outdoor trials conducted near our laboratory in Cairns. Each lure component was weighed before deployment in a BG-Sentinel. The trap was then operated for 24 h, after which time the lures were removed and weighed. Temperature and humidity measurements were made concurrently. It was not possible to measure the release rate of lactic acid, because this compound is hygroscopic and therefore gains weight as it binds atmospheric water.

Two lure comparison experiments were conducted. In the 1st experiment, a Latin square design (Cochran and Cox 1957) was used whereby the BG blend, BG blend plus acetone,

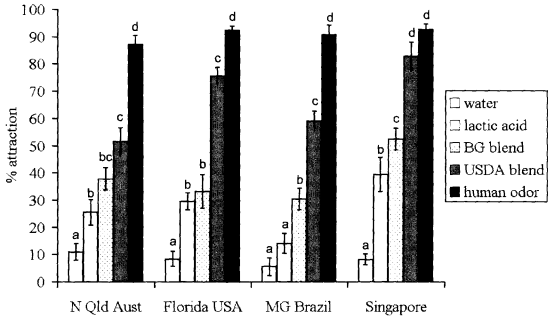

Fig. 1. Mean attraction (± SE) of *Aedes aegypti* to lactic acid and proprietary blends in laboratory Y-tube olfactometers (n = 12) (experiment 1).

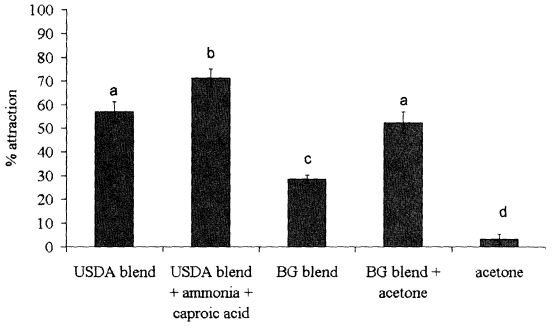

Fig. 2. Mean attraction (± SE) of *Aedes aegypti* from NQA to modified proprietary blends in laboratory Y-tube olfactometers (n = 12) (experiment 2).

the USDA blend, and a blank control were rotated amongst houses to account for positional effects. This experiment was repeated 5 times at different groups of houses, resulting in 20 replicates for each treatment. In the 2nd experiment, the USDA blend plus ammonia and caproic acid was compared with a blank control at 10 houses, with the treatment and control houses subsequently swapped to control for positional effects, giving 20 replicates for each treatment. Traps were deployed between 1100 and 1300 h and collected 24 h later. A minimum of 2 days was allowed between trap deployments at each house to prevent any substantial local population reduction. Data were log (x + 1) transformed before ANOVA (field trial 1) or t-tests (field trial 2) by using SPSS statistical software. The frequencies of males and females were analyzed using chi-square contingency tables to test for independence of sex and lure type.

RESULTS

Experiment 1: laboratory comparison of proprietary blends

Mosquitoes from all 4 populations exhibited upwind flight in response to volatiles in the Y-tube. There were significant differences in the attractiveness of the treatments compared with controls in all strains: NQA: $F = 50.07$ $P < 0.001$; Florida USA: $F = 94.39$, $P < 0.001$; Singapore: $F = 81.38$ $P < 0.001$; and MGB: $F = 58.58$ $P < 0.001$. The USDA blend was the most attractive in all strains, followed by the BG blend and lactic acid (Fig. 1). The Singapore strain was attracted equally to the USDA blend and human odor. Human odor was significantly more attractive than either kairomone blend in the other three strains. There was a slight response to the water control. The USDA blend was significantly more attractive than the BG blend for all populations except NQA. The BG blend was significantly more attractive than lactic acid alone for MGB and Singapore.

Experiment 2: laboratory attraction of NQA to modified blends

The modification of proprietary blends had a significant effect on attractiveness for NQA ($F = 32.44$, $P < 0.001$). The addition of ammonia and caproic acid to the USDA blend, and the addition of acetone to the BG blend, significantly improved the attractiveness of both blends (Fig. 2). There was no discernible attraction to acetone. In descending order of attractiveness, the best kairomone blends for NQA were the USDA blend plus caproic acid and ammonia, the BG blend plus acetone, the USDA blend, and last the BG blend.

Experiment 3: field studies of kairomone blends with traps in NQA

The kairomone release rate from BG-Sentinels over 24 h of trap operation was determined at a mean minimum–maximum temperature of 21.1–32.2°C, and mean minimum–maximum relative humidity of 54.2–86.1%. These conditions are typical of the Cairns wet season. Dispensing rates for the BG blend components were caproic acid, 0.04 ± 0.01 g/24 h and ammonia, 0.03 ± 0.001 g/24 h. The USDA blend was dispensed at 30.3 ± 1.8 g/24 h and acetone at 30.8 ± 0.9 g/24 h. All postdeployment weights of lactic acid revealed an increase after field use.

In the first field experiment, 893 mosquitoes were captured in the BG-Sentinels. Of these mosquitoes, 811 (90.8%) were *Ae. aegypti*, with a 46 male:54 female ratio. There were 10 other species collected. A single BG-Sentinel captured a maximum of 62 *Ae. aegypti* in 24 h (USDA blend; 30 females, 32 males). The average total *Ae. aegypti* catch ranged from 9.0 (BG blend) to 11.15 (USDA blend). The numbers of males, females, or total *Ae. aegypti* caught per trap per 24 h were not significantly different among kairomone blends (Table 2). There was also no significant difference in the frequency of sexes.

Table 2. Twenty-four hour capture of *Aedes aegypti* by BG-Sentinel mosquito traps in Cairns, north Queensland, Australia (experiment 3) with different kairomone blends (n = 20).

Field experiment 1					
Treatment	Blank (control)	BG blend	BG blend + acetone	USDA blend	Statistical result
Mean ♀ (± SE)	5.7 ± 1.1	4.6 ± 1.2	5.9 ± 1.3	5.7 ± 1.4	$F = 0.532$, $P = 0.662$
Mean ♂ (± SE)	3.9 ± 1.4	4.4 ± 1.7	5.1 ± 1.4	5.5 ± 1.9	$F = 0.145$, $P = 0.933$
Mean total *Ae. aegypti* (± SE)	9.6 ± 2.4	9.0 ± 2.7	10.9 ± 2.3	11.1 ± 3.2	$F = 0.295$, $P = 0.829$
Total ♀:♂	114:78	92:88	118:101	114:109	$\chi^2 = 3.56$, $P = 0.312$

Field experiment 2			
Treatment	Blank (control)	USDA blend + ammonia + caproic acid	Statistical result
Mean ♀ (± SE)	2.4 ± 0.5	3.5 ± 0.9	$t = -0.743$, $P = 0.231$
Mean ♂ (± SE)	2.1 ± 0.6	3.3 ± 1.3	$t = -0.054$, $P = 0.479$
Mean total *Ae. aegypti* (± SE)	4.4 ± 1.0	6.7 ± 1.9	$t = -0.550$, $P = 0.293$
Total ♀:♂	47:41	69:65	$\chi^2 = 0.08$, $P = 0.780$

In the second experiment, 341 mosquitoes were captured. Of these mosquitoes, 222 (65.1%) were *Ae. aegypti*, with a sex ratio of 48 males:52 females. Four other species also were collected. As in the first field experiment, there was no significant difference in the number of *Ae. aegypti* caught per trap when using USDA blend plus ammonia and caproic acid or the blank control, and there was no significant difference in the frequency of sexes (Table 2).

DISCUSSION

Both kairomone blends were attractive to the 4 *Ae. aegypti* strains when presented in Y-tube olfactometers without the addition of carbon dioxide. Generally, these blends were not as attractive as human odor, with the exception of the USDA blend, which was equally attractive to the Singapore population as human odor. Although there were differences in the absolute attractiveness of the kairomone blends for different strains, the hierarchy of their attractiveness was equal in all strains. This suggests that the mechanisms making these blends attractive to *Ae. aegypti* are conserved among populations globally and augurs well for the applicability of a given kairomone blend across regions.

Kairomone blends were more attractive than lactic acid alone. Furthermore, we were able to improve the attractiveness of both blends by adding compounds that have been previously demonstrated to act synergistically with lactic acid (Geier et al. 1999, Bosch et al. 2000). The addition of ammonia and caproic acid to the USDA blend and acetone to the BG blend improved attraction. Bernier et al. (2003) suggested that acetone functions in a similar way to carbon dioxide, not as an attractant in the manner of lactic acid, but as an "activator." Our results (Fig. 2) also show no attractiveness to acetone alone but a synergistic attractiveness (a combination effect greater than the sum of the individual effects) with the BG blend. A similar synergistic effect involving acetone and lactic acid was reported in Bernier et al. (2003). However, attraction to acetone alone in that study was much greater than in the present trials (26.2% compared with 3.3%), and the attraction is probably due to behavioral differences between the Florida and NQA strains. In confirming this hypothesis, we found that the Florida strain showed a mean attraction to acetone of 25%, very similar to the 26.2% reported by Bernier et al. (2003), demonstrating a concordance between olfactometer studies in separate laboratories.

The field studies reported here showed no detectable effects of the kairomone blends when tested with the BG-Sentinel. This finding suggests that the visual properties of the BG-Sentinel are more important, at least in NQA, than olfactory cues from human skin in the absence of carbon dioxide.

This is inconsistent with laboratory results in which the kairomone blends were attractive. However, kairomones in human skin emanations only attract *Ae. aegypti* over short distances (Dekker et al. 2005). In Y-tubes, *Ae. aegypti* are exposed to concentrated kairomone plumes in confined spaces, less than 2 m from the odor source, thereby facilitating short-range attraction. Visual properties of the BG-Sentinel may be effective over larger distances. The collection size in the field will primarily be a function of the distance from which mosquitoes are attracted and how effectively they are collected when near the

trap. Provided that the addition of kairomone blends to BG-Sentinels did not effectively increase the range of attraction in the field, an increase of collection rates would not be expected. This explanation is well supported by a recent wind tunnel study where the behavioral responses of female *Ae. aegypti* to skin odors waned quickly with dilution (Dekker et al. 2005). In this way, the lack of effect of kairomones on BG-Sentinel efficacy may be related to the doses used here. In a laboratory cage study using the USDA blend coupled with 3 different mosquito traps (not including the BG-Sentinel), higher kairomone doses led to greater capture of MGB *Ae. aegypti* (Silva et al. 2005). The highest dispensing rate for the USDA blend in that trial (7.7 g/24 h) was much less than that here (30.3 g/24 h). The cage used in that study (2.0 × 2.5 × 2.8 m) may have permitted short-range attraction.

The kairomone dispensing method is critical in maintaining above a response threshold odorant concentration in the odor plumes. As odorants disperse in plumes, insects may respond to concentrated filaments even though the overall concentration of odorants in the plume may be below the response threshold (Murlis et al. 1992). In this study, the kairomone blends were placed in the bottom of the BG-Sentinels. Fans in the BG-Sentinel trap displace ~90 ft^3/m such that kairomones are pushed vigorously out of the trap and may be quickly diluted below the threshold detection for *Ae. aegypti*. Thus, a dispensing method that allows a filamentous plume of kairomones to disperse from the trap may enhance field collections.

Different kairomone doses, dispensing methods, or both are an obvious next step for experimentation. Carbon dioxide, an agent that presensitizes *Ae. aegypti* to human odors (Dekker et al. 2005), also may enhance field collections. Regardless, this work highlights the large gap between the observed behavioral responses of mosquitoes to olfactory signals in artificial laboratory bioassays and the function of these signals in the complex field environment.

ACKNOWLEDGMENTS

Sharron Long (Queensland Health) provided valuable field assistance in Cairns. Ulla Kröckel, Thomas Hörbrand, and Margit Lindner (all of BioGents GmbH) provided technical assistance at the University of Regensburg laboratories. Carl Baptista (Origin Exterminators PTE Ltd., Singapore) provided the Singapore mosquito colony. We thank the people of Cairns for access to personal properties. This work was funded by Australian National Health and Medical Research Council grant 279401 to S.A.R. and R.C.R.

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