Laboratory and Field Evaluations of Potential Human Host Odors for *Aedes albopictus* Skuse (Diptera: Culicidae)

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**ABSTRACT** Behavioral responses of female *Aedes albopictus* Skuse (Diptera: Culicidae) to ethyl butyrate, L-lactic acid, dimethyl disulfide, and hexanoic acid over a range of concentrations (0.01–100 mg/L) were investigated in a Y-tube olfactometer. The results indicated that 55–81% of female mosquitoes were activated to fly in all these tests. Ethyl butyrate, hexanoic acid and dimethyl disulfide had significantly greater levels of activation than L-lactic acid (*P* < 0.05), but activation was not significantly different between concentrations (0.01, 0.1, 1, 10, 100 mg/L; *P* = 0.80). Treatment of 0.01 mg/L ethyl butyrate, 0.1 mg/L L-lactic acid, and 0.01 mg/L dimethyl disulfide were significantly more attractive than control (*P* < 0.05). Field evaluation of ethyl butyrate, or L-lactic acid, 1-octen-3-ol, ammonia, 6-methyl-5-hepten-2-one, hexanoic acid, dimethyl disulfide, and their blends over a range of concentrations (0.1–1000 mg/L) for mosquito traps showed that the odors tested attracted many fewer mosquitoes (*Culex* and *Aedes*) compared with the control (a live mouse).

**KEY WORDS** *Aedes albopictus*, human skin volatile, activation, attraction, trap

Native to Asia, *Aedes albopictus* Skuse (Diptera: Culicidae) has been reported in more than 25 countries on the five continents outside its natural region since the end of the 1970s. It is an important vector of several arboviruses, including dengue, yellow fever, and diverse types of encephalitis. This mosquito is widely distributed in China and causes prevalent occurrence of dengue in some local areas, such as the cities of Guangzhou and Fuzhou.

Common approaches to control mosquitoes are the use of chemical insecticides for area-wide mosquito abatement and repellents for personal protection. However, because of insecticide resistance in mosquitoes and social concerns about environmental pollution, there has been an increasing emphasis on the development of alternative mosquito control technologies, such as the use of attractant-baited traps for mass trapping. Odors that attract mosquitoes could be useful in the development of new trapping methods (Meijerink & van Loon 1999, Silva et al. 2005).

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Many compounds that emanate from human skin and breath play an important role in the attraction of female mosquitoes to the host at a relatively close range (Gillies 1980, Schreck et al. 1990, Takken 1991). L-lactic acid is only slightly effective by itself but acts synergistically with other compounds from human skin for Anopheles gambiae Giles sensu stricto (Dekker et al. 2002). Ammonia is attractive to Aedes aegypti L. when presented together with lactic acid (Geier et al. 1999a). Hexanoic acid elicits responses of the carboxylic acid excited cells of An. gambiae in an electrophysiological study (Meijerink & van Loon 1999). Dimethyl disulfide is detected in human skin emanations (Bernier et al. 2000), and it synergistically attracts female Ae. aegypti with L-lactic acid, but the role of this disulfide in mosquito attraction process is not thoroughly understood (Bernier et al. 2003). Bernier et al. (2002) found that 6-methyl-5-hepten-2-one is more abundant on the host that attracts a greater percentage of Ae. aegypti. Certain combinations of chemicals synergistically attract some species more than others, although each attractant is capable of attracting mosquitoes on its own. Recent studies on mosquito behaviors to human odors have focused mostly on Ae. aegypti and An. gambiae. The work reported here comprises laboratory and field studies, and the purpose is to evaluate the response of female Ae. albopictus to seven synthetic chemicals that have been reported as attractants of other mosquitoes, individually or as blends over a wide range of concentrations.

Materials and Methods

Mosquitoes. Ae. albopictus mosquitoes were obtained from the colony maintained at Shanghai Institute of Entomology, Chinese Academy of Sciences. The insects were maintained at 25°C and a L:D 14:10 h photoperiod without simulated dusk. Female and male adults were kept together in a gauze cage (35 × 35 × 35 cm) at 70% relative humidity and provided with diet of 5% sucrose solution on cotton. Females were routinely offered a mouse as bloodmeal from the fifth day after emergence. Eggs were laid on wet tissue paper, hatched in water trays, and the larvae were fed with rat food. Pupae were collected into cups and placed in cages for adults to emerge. For olfactometer tests, we used nonblood-fed, 5- to 7-d-old female Ae. albopictus.

Test odors. Dimethyl disulfide (98%), hexanoic acid (98%), 1-octen-3-ol (98%), L-lactic acid (85–90% w/w aqueous solution), and 6-methyl-5-hepten-2-one (96%) were purchased from Fluka Company (Buchs, Switzerland); ammonia (25–28% w/w aqueous solution) from Changzheng Chemical Product Company (Hangzhou, China); ethyl butyrate (98%) from Medicine Group Company of Shanghai (Shanghai, China).

Ethyl butyrate (0.117 mL), dimethyl disulfide (0.096 mL), hexanoic acid (0.110 mL), 1-octen-3-ol (0.122 mL), and 6-methyl-5-hepten-2-one (0.122 mL) were dissolved in 2 mL of absolute alcohol and then diluted with distilled water to a series of concentrations (0.01, 0.1, 1, 10, 100, and 1000 mg/L). Ammonia and L-lactic acid were directly diluted with distilled water to the same concentrations.

Experimental set-up and testing procedures. We used a modified dual-choice olfactometer of Plexiglas® (Geier & Boeckh 1999). A constant air stream was purified with activated charcoal filter and adjusted to 70 ± 5% relative humidity and 26 ± 1°C. The wind speed was 0.2 m/sec in the arms and 0.4 m/sec in the downwind arm. A 40-W light bulb was provided as overhead illumination. An
hour before each experiment, 10–25 female mosquitoes were lured from the holding cage into a release cage and left in the olfactometer air stream for 20 min to acclimatize. A piece of filter paper (6.2 cm diameter) was dipped into odor solutions for 5 sec for bioassay. Filter paper treated with distilled water served as control. The treated filter paper was placed in one of the arms, and the control paper was placed in the other arm for 60 sec, then the test was started by slowly opening the release cage. During the 5-min test period, mosquitoes could enter either upwind arm. After each test the upwind arms and the release cage were closed and the mosquitoes in each arm of the olfactometer were counted. Female mosquitoes were exposed to ethyl butyrate, dimethyl disulfide, hexanoic acid, and L-lactic acid in concentrations ranging from 0.01–100 mg/L. Each treatment test was replicated eight times. To eliminate environmental influence, test and control arm were exchanged every four replicates. The olfactometer was cleaned with absolute alcohol and dried with a hair drier before each test. To avoid possible side effects such as learning or habituation, each group of mosquitoes was tested only once. Tests were conducted during 17.00–22.00 h everyday.

Finally, we examined the field trapping effect of seven chemicals over concentrations ranging from 0.1 to 1000 mg/L or blends that were mixed with equal volumes of its components. A vial (8 mL) containing 5 mL of solution was suspended in the middle of a gauze cage (35 × 35 × 35 cm). To trap and prevent mosquitoes from escaping out of the cage once trapped, four paper funnels, tapering from 9 cm to 3 cm, were inserted in the middle of four walls of the cages, respectively. The traps were placed 10 m apart in the field. Each treatment was replicated three times. A live mouse wrapped in gauze served as control bait. The trial was implemented in a grove between 17.00 h and 7.00 h the next morning from April to July 2006.

Data analysis. The olfactometer results were expressed in terms of three parameters (Bosch et al. 2000): (i) the percentage of mosquitoes found outside of the release cage after 5 min was taken as a measure of activation; (ii) the percentage of mosquitoes trapped in the treated arm was a measure for attraction to the treatment; and (iii) the percentage of mosquitoes in the control arm measured the response to pure air. All these data were arcsin transformed for statistical analysis. Data of activation were analyzed with a two-way analysis of variance (ANOVA) and least significant difference (LSD) to sort out differences between treatments. The relative attractiveness of the treatment and the control was analyzed with a one-sample t-test (Dekker et al. 2002). Data of attraction of the same compound were analyzed by a one-way ANOVA and LSD to sort out differences between five concentrations. The field results were analyzed by a one-way ANOVA and LSD for comparison of treatment. All data were analyzed by means of DPS© software (Tang & Feng 2002).

Results

Effects of odors on female mosquitoes in olfactometer. When any compound was tested, 55–81% of the mosquitoes left the release chamber (Fig. 1). Activation was significantly different between four compounds (ANOVA: $F = 4.354$, df = 3,140, $P = 0.006$), and ethyl butyrate, hexanoic acid and dimethyl disulfide had significantly higher activation than L-lactic acid ($P < 0.001$, $P =$
Activation was not significantly different between concentrations (0.01, 0.1, 1, 10, 100 mg/L; ANOVA: $F = 0.411$, $df = 4, 140$, $P = 0.800$).

Compared with the control, 0.01 mg/L ethyl butyrate ($t$-test: $t = 2.731$, $df = 7$, $P = 0.029$), 0.1 mg/L L-lactic acid ($t$-test: $t = 6.902$, $df = 7$, $P < 0.001$), and 0.01 mg/L dimethyl disulfide ($t$-test: $t = 2.648$, $df = 7$, $P = 0.033$) solution had significantly greater attractiveness to mosquitoes than distilled water and 100 mg/L dimethyl disulfide solution also had greater attraction than distilled water, although not significantly ($t$-test: $t = 2.315$, $df = 7$, $P = 0.054$; Fig. 2). These results suggest that they could attract *Ae. albopictus* females at the level of the aforementioned concentration. Attractiveness to mosquitoes was significantly different between five concentrations of ethyl butyrate (ANOVA: $F = 3.952$, $df = 4, 35$, $P = 0.009$) but was not significantly different between five concentrations of dimethyl disulfide (ANOVA: $F = 0.813$, $df = 4, 35$, $P = 0.525$), hexanoic acid (ANOVA: $F = 2.564$, $df = 4, 35$, $P = 0.055$), or L-lactic acid (ANOVA: $F = 0.104$, $df = 4, 35$, $P = 0.980$).

**Attraction of odors on mosquitoes in field.** Compared with a positive control (a live mouse), ethyl butyrate, L-lactic acid, 1-octen-3-ol, ammonia, 6-methyl-5-hepten-2-one, dimethyl disulfide, and hexanoic acid at the concentrations from 0.1 to 1000 mg/L attracted many fewer mosquitoes (*Culex* and *Aedes*) into the field traps. Blending L-lactic acid or ammonia with any other compound did not increase the attraction of mosquitoes over that of the single compound tested at the respective concentration (Table 1). These data indicated the synergist was not observed for blends of L-lactic acid or ammonia with the tested compounds.

**Discussion**

Similar to methyl butyrate in chemical structure, which is a tentatively identified component from human emanations (Bernier et al. 2000), ethyl butyrate
was tested in this study. Bernier et al. (2003) found that the blend of L-lactic acid with dimethyl disulfide was synergistic to attract female *Ae. aegypti*. In our olfactometer, the 0.01 mg/L ethyl butyrate solution, 0.01 and 100 mg/L dimethyl disulfide solutions produced greater attractiveness for *Ae. albopictus* than control. It also could be concluded that dimethyl disulfide exhibits some attractiveness for mosquitoes over a wide range of concentration. However, field results showed that the combination of ethyl butyrate or dimethyl disulfide with L-lactic acid was no more attractive to mosquitoes than either compound without L-lactic acid, at the respective concentrations. However, it is possible that different ratios of some compounds or all of them could still be promising attractants for *Ae. albopictus* and must be useful in the development of new trapping methods for control of mosquitoes.

L-lactic acid is a well-known attractant for *Ae. aegypti* (Acree et al. 1968). It also plays a significant role in host-finding by female *An. gambiae* (Dekker et al. 2002). Geier et al. (1999b) found the most effective lactic acid concentration was 1.7 nmol/L (153 ng/L), resulting in 20% upwind-flying mosquitoes in the homogeneous plume. In contrast to these reports suggesting when L-lactic acid attracts mosquitoes, Shirai et al. (2001) found that, compared with water-treated control skin, L-lactic acid repelled *Ae. albopictus* using human and mouse skin and the number of mosquito alightments decreased at increasing concentrations of L-lactic acid. Attraction of lactic acid to mosquitoes varied between 16% and 27% in our study, which was consistent with earlier findings of Bosch et al. (2000), and 0.1 mg/L L-lactic acid showed significant attractiveness when compared with the negative control in an olfactometer test. These findings imply that the attractiveness of L-lactic acid to mosquitoes is related to its concentration.

Fig. 2. Proportion of female *Aedes albopictus* caught in the two upwind arms (test and control) of the olfactometers. Test arms are added with ethyl butyrate (EB), or L-lactic acid (LA), dimethyl disulfide (DD), and hexanoic acid (HA) at different concentrations. Significant difference at $P < 0.05$ indicated by *. Means of eight replicates ± SE.
<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration of Component I (mg/L)</th>
<th>Component II</th>
<th>Component III</th>
<th>Control$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>1</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>EB</td>
<td>0.3 ± 0.3</td>
<td>0.0</td>
<td>2.7 ± 1.7</td>
<td>0.3 ± 0.3</td>
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<tr>
<td>LA</td>
<td>0.0</td>
<td>2.0 ± 0.0</td>
<td>0.0</td>
<td>0.7 ± 0.7</td>
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<tr>
<td>Octenol</td>
<td>0.7 ± 0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>AM</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>MHO</td>
<td>0.7 ± 0.3</td>
<td>1.0 ± 0.0</td>
<td>0.0</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>HA</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7 ± 0.7</td>
<td>0.0</td>
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<tr>
<td>DD</td>
<td>0.3 ± 0.3</td>
<td>0.0</td>
<td>1.0 ± 1.0</td>
<td>0.7 ± 0.7</td>
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<tr>
<td>Octenol</td>
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<td>0.7 ± 0.3</td>
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<tr>
<td>HA</td>
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</tbody>
</table>

$^a$EB, ethyl butyrate; LA, L-lactic acid; Octenol, 1-octen-3-ol; AM, ammonia; MHO, 6-methyl-5-hepten-2-one; HA, hexanoic acid; DD, dimethyl disulfide.

$^b$Component II was 100 mg/L LA solution.

$^c$Component III was 100 mg/L AM solution.

$^d$Control was a live mouse.

$^*$_Significant difference existed at $P < 0.05$ level in the same line.
Geier et al. (1999a) found that the addition of 0.17 mg/L ammonia doubled the percentage of mosquitoes attracted to lactic acid alone, but greater concentrations did not further raise the attractiveness, and 0.017–1.7 mg/L ammonia alone was not attractive to mosquitoes compared with controls. Therefore, they concluded that attractive effects of certain compounds can be discovered in a bioassay only in combination with lactic acid. Bosch et al. (2000) showed a mixture of ammonia, lactic acid, propanoic acid, and valeric acid was the most attractive artificial odor blend for *Ae. aegypti* to date, with attraction levels that almost reached that of human skin washings. But L-lactic acid and ammonia showed no synergistic effect in trapping mosquitoes to other components in field test. Although Bosch et al. (2000) pointed out that the attractiveness of lactic acid was significantly augmented when combined with hexanoic acid, the hexanoic acid showed no significant attraction of mosquitoes in olfactometer and field tests even with the addition of L-lactic acid. Vale (1980) even found hexanoic acid was repellent for tsetse fly when used in an odor-baited trap. Our tests also indicated that when in combination with 1-octen-3-ol or lactic acid and hexanoic acid, ammonia was not attractive for *Ae. albopictus* in field.

Results from our field tests showed that all compounds attracted fewer mosquitoes than the control, but there was some minimum attractiveness of the chemicals tested. The concentration, ratio, and composition of chemical attractants in a blend can directly affect the efficacy of mosquitoes captured by a baited trap (Silva et al. 2005). It is conceivable that the composition, concentrations, and proportions of the synthetic compounds tested so far are not optimal. At the same time, host-seeking behavior of mosquitoes in the field also could be influenced by the mosquito physiological states such as sucrose-feeding, blood-feeding, oviposition, age, crowding and the environmental factors such as light, heat, windspeed, and moisture (van Dyk 2002). These factors also would undoubtedly influence the attractiveness of the test compounds for mosquitoes in field, especially single or simple blends, which only had very slight attraction of mosquitoes. In addition, the collection efficiency of mosquitoes can be strongly influenced by trap design. Silva et al. (2005) reported the attractive effects of a blend of acetone, L-lactic acid and dimethyl disulfide on *Ae. aegypti* varied among the trap designs under controlled laboratory conditions. Therefore, it may be important to study more factors in the design of mosquito traps to promote their efficiency of luring and trapping mosquitoes.

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