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FIELD AND LABORATORY EVALUATIONS OF POTENTIAL OVIPOSITION ATTRACTANTS FOR *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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ABSTRACT. We tested five volatile synthetic chemicals (dimethyl disulfide, indole, 4-methylphenol, 3-methylindole, and trimethylamine) as potential oviposition attractants of *Aedes albopictus* in field and laboratory experiments. The 5 synthetic compounds were loaded into controlled-release packets, which consisted of a cellulose material sealed within a permeable plastic membrane, that were used to bait water-filled ovitraps at 5 field sites. *Aedes albopictus* exhibited no oviposition preference for any of the baited traps versus adjacent traps containing only water. In addition, there was no difference in the mean number of eggs laid per trap-day by *Ae. albopictus* among ovitraps treated with the five compounds. We conducted behavioral bioassays to determine if the lack of response to the putative oviposition chemicals in the field was due to a concentration effect. A binary sticky-screen bioassay was used to measure attraction of gravid females to olfactory stimuli. Compounds were evaluated over a range of concentrations that spanned 3–5 logs (0.0083 to 8.3 or 83 mg/liter). Three concentrations of 4-methylphenol (0.083 mg/liter, 0.83 mg/liter, and 8.3 mg/liter) and 1 concentration of 3-methylindole (8.3 mg/liter) were significantly repellent. All other concentrations of the 5 chemicals tested did not attract more females than did a water control. Electoantennography indicated that *Ae. albopictus* did not exhibit a physiological response to 0.25 ng of any of the five chemicals tested. Because *Ae. albopictus* did not exhibit attraction, greater oviposition, or an electrophysiological response to any of the compounds tested, these compounds do not appear to be effective lures for baiting ovitraps for surveillance or control of this mosquito.

KEY WORDS *Aedes albopictus*, oviposition, attractant, electroantennogram

INTRODUCTION

Mosquitoes choose oviposition sites on the basis of physical characteristics, such as color, substrate texture, and odorants and other chemicals (Bentley and Day 1989). In the aqueous mixture of an oviposition site, microbial degradation of organic material can produce volatile attractants or repellents as well as nonvolatile arrestants and oviposition stimulants or deterrents. Of the biologically active compounds that have been isolated and the chemical structures identified, only a few have been tested both in the laboratory and in the field.

Millar et al. (1992) identified 5 compounds from hay infusions (3-methylindole, 4-methylphenol, 4-ethylphenol, indole, and phenol) that increased oviposition by *Culex quinquefasciatus* Say. One compound, 3-methylindole (skatole), was active at concentrations that spanned 5 orders of magnitude (0.01–100 μg/liter). Experimental ponds treated with skatole received significantly more *Cx. quinquefasciatus* egg rafts than did adjacent untreated ponds (Beehler et al. 1994). In addition, 4-methylphenol, indole, and 3-methylindole elicited significant antennal responses from *Cx. quinquefasciatus* and *Culex tarsalis* Coquillett in electroantennogram (EAG) studies (Du and Millar 1999).

Allan and Kline (1995) evaluated the 5 *Culex* oviposition chemicals against *Aedes albopictus* (Skuse) and *Aedes aegypti* L. in the laboratory and concluded that the compounds were only weakly active. A dose–response relationship could not be established for any of the *Culex* oviposition chemicals. Only only 1 concentration of 3-methylindole and 1 concentration of 4-methylphenol elicited a slightly greater oviposition response by *Ae. albopictus* relative to the well water control. In addition, gravid *Ae. aegypti* did not discriminate when laying eggs between well water and water treated with 3-methylindole. However, in field cages, 3-methylindole elicited a moderate oviposition response relative to well water.

Bentley et al. (1979) identified 4-methylphenol from birch bark infusions as an oviposition attractant of *Ochlerotatus triseriatus* (Say). Under laboratory conditions, they determined that the compound acted as a contact chemical stimulant, but it also attracted gravid *Oc. triseriatus* from a distance.

The objective of our investigation was to determine whether gravid *Ae. albopictus* were attracted to synthetic chemicals that are putative oviposition attractants on the basis of results from other mosquito oviposition research. Some compounds were tested because they occur in organic infusions that were reported to increase the numbers of mosquito eggs laid in oviposition traps. Specifically, we tested whether 3-methylindole, indole, 4-methylphenol, trimethylamine, and dimethyl disulfide increased oviposition by *Ae. albopictus* under field conditions

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and attracted gravid females in laboratory bioassays. In addition, we evaluated the EAG responses of *Ae. albopictus* to these test compounds.

MATERIALS AND METHODS

Field experiments: Dimethyl disulfide (250 mg), trimethylamine (27% water solution), indole (25 mg in 75% ethanol), 3-methylindole (25 mg in 75% ethanol), and 4-methylphenol (25 mg in 75% ethanol) were each loaded separately in 1-ml volumes onto cellulose pads in controlled-release packets (BioLure™, Suterra LLC [formerly Consep, Inc.], Bend, OR). The packets (8 × 8 cm) were heat sealed plastic pouches (sachets) that each contained a permeable plastic membrane sealed under a 3.5-cm-diam hole in an impermeable plastic backing material. Removal of an overseal exposed the permeable surface. BioLure is used to formulate pheromone lures for monitoring some insect pests of fruit.

Field experiments were conducted at 5 residences in the Raleigh, NC, area where mosquito populations were known to be active (Trexler et al. 1997, 1998) from June to July 1998. Five ovitrap pairs were placed on the ground around the perimeter of each residence in shaded locations. The ovitrap pairs were spaced at least 25 m apart, and the ovitraps within each pair were 1 m apart. Oviposition traps were black polypropylene cups (ca. 250 ml) with a drain hole drilled near the lip of each cup. Cups were filled with 100 ml of tap water. Red velour papers (2.5 cm × 11 cm) were clipped to the inside of the ovitraps as oviposition substrates. A controlled-release packet containing a putative oviposition attractant was taped to the top inside the lip of 1 ovitrap of each pair. The other ovitrap contained only water. A controlled-release packet filled only with solvent was not taped to the control cup, which in retrospect was an oversight. A 6th pair of ovitraps containing only water was placed as an additional control at each site. One ovitrap in this pair was designated permanently as the test ovitrap so that egg densities in these traps could be included in statistical analyses of oviposition data collected for the other traps at each site.

At each site, the location of the initial placement of each compound was selected randomly. Ovitraps were serviced every 2 days. When the traps were serviced, ovistrips were collected, controlled-release packets were carefully removed, and cups were emptied and lightly scrubbed and rinsed. New ovistrips and water were placed in the ovitraps, and the controlled-release packets were reattached to the test ovitraps. Each ovitrap pair was systematically rotated to the next location at each residence so that each compound was evaluated at each location within a site. In addition, the position of each cup within the ovitrap pair was randomized for each new location. Eight 2-day trapping periods were completed over the 4-wk duration of the study. After collection, the ovistrips were taken back to the laboratory where the eggs on each strip were identified to species (Linley 1989a, 1989b) and counted.

Mosquito colony origin and maintenance: Aedes albopictus was colonized with eggs collected in oviposition traps in Raleigh, NC, in 1997. The colony was maintained at ca. 26°C and a relative humidity of ca. 75% under a photo regime of 14 h: 10 h (L:D). Included in the light phase were 2 30-min crepuscular periods simulated by a 40-watt incandescent bulb. Larvae were fed a 2:1 (wt.:wt.) mixture of liver powder:baker's yeast on a standardized schedule (Gerberg et al. 1994). Adults were kept in 30 × 30 × 30-cm Plexiglas® cages fitted with cotton surgical stocking tops and were provided a 10% sucrose solution ad libitum. Females that had fed on porcine blood via a membrane feeder (Benzon and Apperson 1987) were allowed to oviposit on seed germination paper (Steinley et al. 1994) so we could obtain eggs.

Laboratory experiments: After the field trials, the 5 synthetic compounds (dimethyl disulfide, indole, 4-methylphenol, 3-methylindole, trimethylamine) (Sigma Chemical Co., St. Louis, MO) were evaluated by the sticky screen bioassay method of Isoe et al. (1995) as modified by Trexler et al. (1998) to determine if the chemicals were attractive to gravid *Ae. albopictus.* The sticky screen bioassay differentiates between oviposition responses due to attraction to odorants and those due to contact chemical stimulation. In the bioassay, gravid female mosquitoes were presented a choice between a test and a control cup. Each cup was covered with a sticky screen that mosquitoes had to 1st land upon before they could enter the cup and contact the oviposition substrate. The proportion of mosquitoes adhering to screens on the test and control cups is used as a measure of the attraction or repulsion of gravid females to odorants in the test cup.

Sticky traps were constructed with 125-ml polypropylene cups painted flat black on the outside and galvanized hardware cloth. Painted cups were aged for 2 wk prior to use and were not repellent to gravid mosquitoes. The hardware cloth screen (6-mm mesh, Gilbert and Bennet, Toccoa, GA) was cut into disks and dipped into an adhesive solution made by dissolving a glue (Tanglefoot, Grand Rapids, MI) in hexane. Immediately prior to their use, the disks were placed in a fume hood for 2 h to evaporate the hexane. Each compound was tested over a range of concentrations that spanned 5 orders of magnitude (0.0083 to 83 mg/liter). To achieve a desired final concentration, each experimental cup was filled with 29 ml of distilled water and 1 ml of the appropriate ethanolic stock solution of the test compound. Control cups contained 29 ml of distilled water and 1 ml of 75% ethanol. Test and control cups were each covered with a glue-coated screen and then randomly placed in opposite diagonal corners of each bioassay cage (Trexler et

al. 1998). Bioassay cages consisted of the same Plexiglas cages that were used for mosquito colony maintenance. The cages were juxtaposed on metal racks with overhead lighting as described by Trexler et al. (2003). The sleeves of the cages oriented upward so that odorants from 1 cage would not influence the response of mosquitoes in adjacent cages. Conditions under which bioassays were conducted were the same as for colony maintenance except for relative humidity, which fluctuated from 30% to 50% during experimentation.

Mosquitoes from the F_4–F_7 generations were used in our experiments. Four days prior to the initiation of a trial, *Ae. albopictus* were allowed to feed to repletion on a human hand. The protocol for bloodfeeding virus-free mosquitoes on a human was approved by the Institutional Review Board at North Carolina State University (Human Use Protocol IRB 1388). Ten gravid females were placed in each bioassay cage, and after a 24-h exposure period, we counted the females trapped on screens covering the test and control cups.

We used the oviposition activity index (OAI) (Kramer and Mulla 1979) to evaluate the responses of the females to each compound. We calculated the OAI for each experimental replicate as $OAI = (N_t - N_c)/(N_t + N_c)$, in which N_t is the number of females trapped on the screen over the test cup and N_c is the number of females trapped on the screen over the control cup. The OAI is a measure of the proportion of females trapped on the screen over the test cup after correcting for the proportion of females trapped on the screen over the control cup. The OAI varies from −1 to 1, with 0 indicating no response.

Electrophysiology: The physiological response of gravid females to the 5 compounds was determined by electroantennography. Electroantennogram recordings were made on excised heads of gravid female mosquitoes (Blackwell et al. 1993, Du and Millar 1999). Ag-AgCl wires, 0.5 mm in diameter, were inserted into glass capillary tubes that were filled with physiological saline (Kurtti and Brooks 1976). The end of 1 antenna was severed just below the penultimate segment and inserted into a glass capillary tube that contained the recording electrode. The base of the head was placed into the glass capillary tube that contained the reference electrode. The antenna experienced a constant flow of humidified air (1.5 liter/min), which adapted the mechanoreceptors on the antenna. Each test solution (10 µl) was applied to a filter paper strip, and the solvent was allowed to evaporate. The filter paper was then inserted into a Pasteur pipette attached to a glass syringe. A single, rapid 2-ml puff of test odorant was then introduced into the airstream.

The signal was amplified by a variable DC amplifier (Grass P16, Astro-Med, West Warwick, RI). It was acquired through an A/D board installed in an HP5890 GC and recorded and analyzed with ChemStation software (Agilent Technologies, Palo Alto, CA). To ensure that the equipment was functioning properly and antennal preparations were responsive, we used a 100% concentration of ethyl acetate (Fisher Scientific, Pittsburgh, PA), three concentrations (1%, 10%, and 100%) of the insect repellent OFF® (S. C. Johnson, Racine, WI), and three concentrations (1%, 10%, and 100%) of isoamyl alcohol (Fisher Scientific) as standard positive controls. Hexane was used as a negative control. All electroantennography experiments were completed with 0.25 ng of the 5 putative oviposition attractants.

Statistical procedures: Results of the field experiments were analyzed by analysis of variance (ANOVA) on square root $(y_i + 0.5)$ transformed counts of eggs (y_i) deposited in each trap (PROC GLM, SAS 1999b). Because our model contained both fixed and random effects, we used a mixed model. Experiments were replicated over time, and experimental sites were considered random effects, whereas trap treatment was a fixed effect. For the hypothesis of no site main effect, the F-test was computed with the site mean square (MS) as the numerator and the week (site) MS as the denominator. For the treatment main effect, an F-test was computed with the treatment MS in the numerator, and the treatment × site interaction MS as the denominator. The F tests for the treatment × trap condition and treatment × site interactions used the treatment × condition × site interaction MS as the denominator. Trap condition indicates treatment versus control for the individual trap. Significantly different means were differentiated by the LSMEANS statement in PROC GLM (SAS 1999b) under the hypothesis $LSM_i = LSM_j$.

Laboratory sticky screen experiments were analyzed by a nonparametric signed-rank test (PROC UNIVARIATE, SAS 1999a) to determine if the mean OAI for each treatment was significantly different from zero.

EAG responses were determined by measuring the amplitude of the peak of the action potential produced by antennae. Peak heights produced by antennal responses to the negative control substance were subtracted from peak heights produced in response to the positive control and test solutions to form a data set of differences (PROC MEANS, SAS 1999a). A t-statistic generated from the data set was used to determine if mean differences were significantly different from zero.

RESULTS

Field trials

Aedes albopictus was the predominant mosquito collected in ovitraps. Occasionally at some sites, eggs of *Oc. triseriatus* were identified on ovistrips. However, in general, numbers of eggs of this mosquito species were insufficient at any site to allow

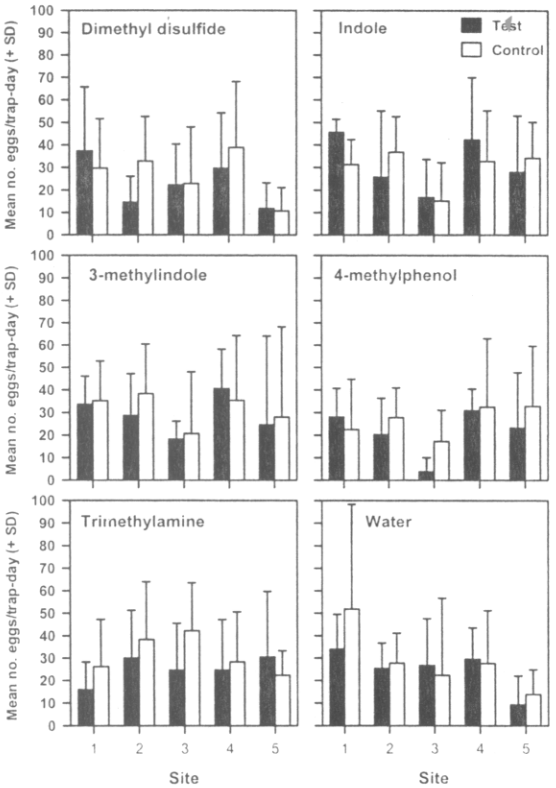

Fig. 1. Oviposition responses of *Aedes albopictus* to pairs of water-filled ovitraps that were placed at 5 different field sites. One trap in each pair was baited with a candidate attractant (test) and the other trap contained water (control). Mean (±SD) egg counts per ovitrap were averaged over 8 2-day ovitrapping periods from June to July 1998.

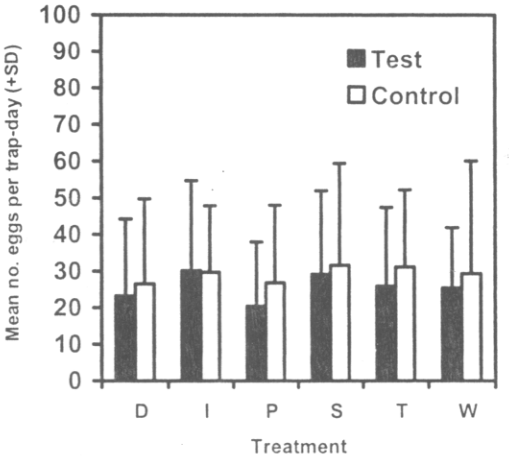

Fig. 2. Mean (±SD) number of eggs laid by *Aedes albopictus* in test ovitraps baited with 5 synthetic chemicals (D = dimethyl disulfide, I = indole, P = 4-methylphenol, S = 3-methylindole, T = trimethylamine) or in adjacent control ovitraps that contained water. A 6th pair of ovitraps was included at each site. Both of these ovitraps contained only water (W), but 1 was designated as the test and the other the control. Egg numbers were averaged by chemical over all field sites over the 4-wk ovitrapping period from June to July 1998.

statistical analyses to be carried out. In contrast, *Ae. albopictus* oviposition activity was comparable at all study sites. Statistical analyses of *Ae. albopictus* egg densities in ovitraps baited with putative attractants indicated that there were no differences in oviposition activity between sites (site main effect; df = 4, 15; $F = 1.00$; $P = 0.44$). Although the mean number of eggs laid in traps in response to each chemical lure varied among the sites (Fig. 1), the overall oviposition activity was not significantly different between sites when egg densities in the test ovitraps were averaged over the 4-wk ovitrapping period (site × treatment effect; df = 20, 20; $F = 1.20$; $P = 0.26$). Egg densities in ovitrap pairs that contained only water were similarly variable but not significantly different ($P > 0.05$) within or between sites.

In general, ovitraps baited with dimethyl disulfide, 3-methylindole, trimethylamine, or 4-methylphenol received fewer eggs than matching control ovitraps that contained water (Fig. 2). Only ovitraps baited with indole stimulated more oviposition than untreated traps within an ovitrap pair. However, the differences between treated and control ovitraps within ovitrap pairs were not significant (condition × treatment effect; df = 5, 20; $F = 0.52$; $P = 0.75$). In addition, there were no significant differences between treated and control ovitraps when egg densities were averaged over all sites and over all treatments (condition main effect; df = 1, 4; $F = 1.23$; $P = 0.30$).

Laboratory bioassays

No concentration of the test compounds elicited a significantly positive OAI in laboratory bioassays. *Aedes albopictus* females were either significantly repelled or exhibited no preference for all concentrations of each compound (Table 1).

Aedes albopictus females were significantly repelled by 3 concentrations of 4-methylphenol. The greatest overall response to any concentration of any compound was to 4-methylphenol at 8.3 mg/liter. The repellent effect at this concentration was highly significant (OAI = −0.64; $P < 0.0001$). Repellent effects of 4-methylphenol were also noted at 0.83 mg/liter ($P < 0.05$) and at 0.083 mg/liter ($P < 0.005$). The highest concentration of 3-methylindole (83 mg/liter) was significantly repellent ($P < 0.05$). The remaining concentrations of the rest of the chemicals did not have significant effects ($P > 0.05$) on the oviposition responses of gravid *Ae. albopictus*.

Table 1. Results of sticky screen bioassays to determine oviposition responses of *Aedes albopictus* to synthetic chemicals in the laboratory.

Compound	Concentration (mg/liter)	*n*	Mean OAI (SE)[1]	SR[2]	$P > t$
Dimethyl disulfide	0.0083	15	0.04 (0.09)	3.5	0.65
	0.083	12	−0.03 (0.12)	−6.0	0.82
	0.83	12	0.10 (0.10)	8.5	0.36
	8.3	12	−0.04 (0.11)	−3.5	0.74
	83.0	12	−0.00 (0.13)	1.0	0.99
Indole	0.0083	18	0.12 (0.08)	18.0	0.15
	0.083	12	−0.17 (0.12)	−15.5	0.21
	0.83	12	−0.19 (0.14)	−16.5	0.21
	8.3	12	−0.08 (0.17)	−7.0	0.62
4-Methylphenol	0.0083	12	−0.07 (0.11)	−9.0	0.55
	0.083	12	−0.30 (0.09)	−21.5	0.005
	0.83	12	−0.21 (0.10)	−24.5	0.05
	8.3	12	−0.64 (0.07)	−33.0	0.0001
3-Methylindole	0.0083	18	0.11 (0.09)	25.5	0.25
	0.083	12	−0.10 (0.08)	−11.0	0.24
	0.83	12	0.14 (0.18)	9.5	0.46
	8.3	12	−0.14 (0.11)	−16.0	0.21
	83.0	12	−0.24 (0.11)	−25.0	0.05
Trimethylamine	0.0083	15	−0.10 (0.11)	−17.5	0.38
	0.083	12	−0.07 (0.11)	−4.0	0.52
	0.83	12	−0.14 (0.11)	−13.5	0.21
	8.3	12	−0.06 (0.09)	−6.5	0.52

[1] OAI, ovisposition activity index.
[2] SR, signed-rank statistic derived through PROC UNIVARIATE (SAS 1999a).

Electrophysiological responses to chemicals

The antennae of *Ae. albopictus* exhibited significant responses to compounds that were used as positive controls (Table 2). Significant ($P < 0.05$) EAG responses were obtained in response to 10% OFF and 100% isoamyl alcohol. In addition, the highest mean ratio value (2.59 ± 0.72) of any compound that we tested was in response to ethyl acetate, although this value was not significantly higher than controls ($P > 0.05$). None of the test compounds evaluated elicited a significant physiological response from *Ae. albopictus* antennae (Table 2). Each compound was evaluated with antennae from 6 or 7 gravid females. Indole, 4-methylphenol, and 3-methylindole elicited approximately the same response from antennae as the negative control. The EAG responses to dimethyl disulfide and trimethylamine were lower than responses to negative controls. However, there was no significant difference ($P > 0.05$) between the test compounds and the controls.

DISCUSSION

In both the field and the laboratory, *Ae. albopictus* exhibited either no preference or a negative ovi-

Table 2. Electroantennogram responses of *Aedes albopictus* to synthetic chemicals that were candidate oviposition attractants and to chemicals used as positive control substances.

Compound	*n*	Mean ratio (SE)[1]	*t*	$P > t$[2]
Dimethyl disulfide	7	0.78 (0.19)	−1.17	0.28
Indole	6	1.04 (0.07)	0.60	0.58
4-Methylphenol	7	1.06 (0.14)	0.41	0.69
3-Methylindole	6	0.98 (0.14)	−0.12	0.91
Trimethylamine	7	0.72 (0.12)	−2.30	0.06
1% OFF®	3	1.01 (0.10)	0.08	0.94
10% OFF	5	1.26 (0.08)	3.29	0.03
100% OFF	8	1.26 (0.14)	1.77	0.12
Ethyl acetate	6	2.59 (0.72)	2.21	0.08
1% Isoamyl alcohol	10	1.02 (0.08)	0.32	0.75
10% Isoamyl alcohol	12	1.35 (0.21)	1.67	0.12
100% Isoamyl alcohol	11	2.10 (0.28)	3.90	0.003

[1] Ratio of test substance and hexane (negative control).
[2] Tests the hypothesis that the mean differences of antennal responses to the chemicals versus hexane controls were equal to 0.

position response to all 5 compounds tested. In addition, none of the compounds elicited an antennal response from *Ae. albopictus* in EAG studies. The range of concentrations (0.0083–83 mg/liter) that we used in the laboratory produced positive oviposition responses in 2 independent studies targeting *Oc. triseriatus* and *Cx. quinquefasciatus*. First, Bentley et al. (1979) demonstrated that a concentration of 10 mg/liter of 4-methylphenol attracted significantly more *Oc. triseriatus* than did water controls. The highest concentrations we tested in the laboratory were 83 mg/liter (trimethylamine and dimethyl disulfide) and 8.3 mg/liter (indole, 4-methylphenol, 3-methylindole). Oviposition attractants should exhibit biological activity over a wide range of concentrations. Second, Millar et al. (1992) found that tubs baited with 0.01 and 100 μg/liter of 3-methylindole elicited significantly higher oviposition by *Cx. quinquefasciatus* than did water controls. The range of concentrations we evaluated is likely to include a potential response threshold concentration, on the basis of results of the aforementioned studies.

Allan and Kline (1995) evaluated 4-methylphenol, 3-methylindole, and indole as oviposition chemicals of *Ae. albopictus*. The concentrations we tested in the laboratory were generally higher than those used in their study, but the 2 lowest concentrations we used, 83 and 8.3 mg/liter, overlapped those used by Allan and Kline (1995). We obtained similar results in our tests of 3-methylindole and indole. Although only 1 concentration of 3-methylindole was significantly repellent in our study, negative OAI values were observed in our other experiments. However, whereas we found 4-methylphenol to be significantly repellent at the 3 highest concentrations, Allan and Kline (1995) showed that the oviposition response of *Ae. albopictus* to 4-methylphenol at concentrations similar to those evaluated in our study was not different from the response of this mosquito to the control. However, Allan and Kline did not report a repellent effect by this compound as we observed.

Holck et al. (1988) reported evidence of increased oviposition by *Oc. triseriatus* in Louisiana to a 1% fish oil emulsion. In Wisconsin, however, Beehler and DeFoliart (1990) found that *Oc. triseriatus* was significantly repelled by 1% and 5% fish oil emulsion infusions in the laboratory and in the field. Trimethylamine is a chemical constituent of fish odors (Milo and Grosch 1995). In our field trials, fewer eggs were laid in ovitraps that were baited with trimethylamine. However, there was no significant difference between the baited and control ovitraps. Similarly, in our laboratory bioassays, *Ae. albopictus* did not respond to 4 concentrations of trimethylamine.

Dimethyl disulfide is a component in hog lagoon odors (Zahn et al. 2001). Hog waste lagoons and other animal waste sites are common areas in which *Culex* mosquitoes are produced (O'Meara and Evans 1983). Even though *Cx. quinquefasciatus* is stimulated to oviposit by manure infusions (Kramer and Mulla 1979), dimethyl disulfide apparently is not active against this species as an oviposition attractant (J. Millar, personal communication). Du and Millar (1999) isolated the related compound dimethyl trisulfide as a volatile component of hay infusions. They found that antennae of *Cx. quinquefasciatus* and *Cx. tarsalis* did not respond to the chemical, but the compound did stimulate oviposition at a single concentration. In our laboratory bioassays, dimethyl disulfide did not elicit an oviposition response from *Ae. albopictus*. Additionally, in our field trials, fewer eggs were laid in ovitraps baited with dimethyl disulfide than in control ovitraps. The lack of response to dimethyl disulfide may reflect the preference of *Ae. albopictus* to use container habitats for egg laying that are not highly organic or polluted.

Blackwell et al. (1993) conducted electrophysiological studies of the responses of female *Cx. quinquefasciatus* to a number of chemicals. In experiments with an EAG, females were presented with a range of concentrations of 3-methylindole that had previously elicited oviposition responses. They found that the threshold for the antennae (1 ng) was an order of magnitude greater than the behavioral threshold (0.1 ng). We conducted EAG experiments with *Ae. albopictus* with 0.25 ng of the 5 compounds. This concentration was 3× higher than the highest concentration of 3 of the compounds (indole, 4-methylphenol, and trimethylamine) and comparable with the highest concentrations of 2 of the compounds (dimethyl disulfide and 3-methylindole) we evaluated in laboratory oviposition experiments. Because 2 of the compounds exhibited significant repellent effects, it is not surprising that we did not obtain significant EAG responses.

The antennae of *Oc. triseriatus* and *Ae. aegypti* produce "strong" responses to 4-methylphenol in EAG experiments (Bentley et al. 1982). Bentley et al. (1979) previously demonstrated that 4-methylphenol was a significant attractant and oviposition stimulant of *Oc. triseriatus*. *Aedes aegypti* was significantly repelled by 0.01 and 1.0 μg/liter of 4-methylphenol (Allan and Kline 1995). Although we found that 4-methylphenol was significantly repellent to *Ae. albopictus* at concentrations that spanned 3 orders of magnitude, we did not obtain a significant EAG response. It is unlikely, but still possible, that we did not use a concentration that was high enough to elicit an antennal response.

Our field and laboratory experiments with known and potential mosquito oviposition attractants/stimulants failed to elicit a positive response from *Ae. albopictus*. The differential response exhibited to chemicals isolated from organic infusions reflects the adaptation of mosquito species to habitats that often vary substantially in physical and biological properties. Chemical attractants have the potential

to enhance the response to oviposition traps or to increase the number of mosquitoes trapped in gravid traps. The "cart before the horse" approach employed in our investigation illustrates the effort wasted by initiating field trials without first verifying the activity of putative oviposition attractants in behavioral bioassays. Also, the admonishment of Knight and Corbet (1991) about attractants exhibiting a dose-dependent reversal of effect makes completion of laboratory behavioral bioassays a first step in the screening process of putative oviposition chemicals prudent. In this regard, laboratory bioassays coupled with electrophysiological investigations provide a rigorous method for screening candidate attractants (Du and Millar 1999).

A controlled-release packet for delivery of oviposition attractants is an appealing concept. Relative to preparing organic infusions, a controlled-release packet would reduce the time needed to set up and maintain oviposition traps. The packets would provide a standardized delivery system for oviposition attractants, so that the variability in quality of active ingredients often seen in organic infusions (Beehler et al. 1994) can be avoided.

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