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ATTRACTION OF *AEDES ALBOPICTUS* ADULTS TO SOD INFUSION

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ABSTRACT. *Aedes albopictus* adults were recovered from sod-baited gravid traps at 7 sites in 5 counties of southern and southwestern Illinois in areas ranging from suburban to rural. The attraction of this species to a lure usually associated with members of the *Culex pipiens* complex was investigated under laboratory and field conditions. Olfactometer and oviposition preference tests in the laboratory showed that *Ae. albopictus* is attracted to sod infusion and females readily oviposit on substrates in contact with the infusion. Both males and females responded to the sod infusion in the olfactometer. The attraction of multiple mosquito species to sod-baited gravid traps was demonstrated at a waste tire site in Jasper County, Illinois. Females of *Ae. albopictus, Aedes triseriatus,* and *Culex* species were collected from gravid traps placed along the edge of woods at distances ranging from 100 to 200 m from the tire site. Only a small percentage of the *Ae. albopictus* females appeared to be gravid (less than 5%), unlike the majority of *Culex* and *Ae. tristeriatus* females.

INTRODUCTION

The rapid dispersal and establishment of *Aedes albopictus* (Skuse) throughout much of the southern and eastern United States, presumably through the transport and storage of used tires, has serious public health implications because this species is a potential vector of numerous exotic and indigenous pathogens (Shroyer 1986, Hawley 1988, Rai 1991, Mitchell et al. 1992). Although its characteristic larval habitat in North America is tire casings, *Ae. albopictus* has also become a major nuisance mosquito in urban areas, where it is frequently found in domestic containers (O'Meara et al. 1992, Nasci et al. 1994). In Illinois, *Ae. albopictus* has been collected from waste tires along the southern (Pulaski County), southwestern (Madison, St. Clair, and Macoupin counties), and northern (Cook County) borders of the state (Webb 1993). Despite tire modification (shredding and splitting of tires), tire removal, and treatment with insecticides, *Ae. albopictus* continues to be collected from many of these areas, as well as several newly discovered sites in Illinois (Hanson et al. 1993 and this report).

The development of efficient collection methods for adult *Ae. albopictus* would facilitate the study of their dispersal from waste tire sites, their impact on other mosquito species, and their role in the transmission of pathogens (Freier and Francy 1991, Jensen et al. 1994). In 1993, new county and city distribution records for *Ae. albopictus* were made in southern Illinois based on the capture of adults in sod-baited gravid traps. The response of *Ae. albopictus* to sod infusion, an oviposition lure usually associated with members of the *Culex pipiens* complex (Madder et al. 1980), was investigated in the laboratory using 2-choice oviposition preference tests and an olfactometer. Additional field tests demonstrated the utility of sod-baited gravid traps for the collection of multiple mosquito species.

MATERIALS AND METHODS

Field studies: From mid-June to late September in 1993, mosquitoes were periodically collected from standard CDC gravid traps (Reiter 1983) baited with sod infusion in southern and southwestern Illinois. The infusions were prepared by adding 30 × 30-cm sections of Kentucky bluegrass sod (*Poa pratensis* Linn.) to 10–15 liters of water. Each mixture was aged for 2–5 days in closed, 18.9-liter plastic buckets. Each gravid trap was baited by adding 5 or 6 liters of the liquid infusion and ½ of the sod (a 15 × 30-cm section). The test sites included Hoffman, East St. Louis, Red Bud, Steeleville, Coulterville, Mounds, Ullin, Dongola, Goreville, Vienna, and Campbell Hill. Pairs of gravid traps were positioned 10–20 m apart at the edge of tree lines near sewage lagoons in these cities except in East St. Louis and Campbell Hill, where the traps were placed near waste tires. Seven of the test sites (Centralia, Red Bud, Steeleville, Coulterville, Dongola, Goreville, and Vienna) did not have an apparent source of used tires in the immediate area. The Campbell Hill site was discovered late in the season (September 22, 1993), and mosquitoes were collected from this site on one date only. The style of gravid trap, type of infusion lure, and most of the test sites were originally chosen for the surveillance of *Culex* species.

To confirm the attraction of *Ae. albopictus* and other mosquito species to sod-baited gravid traps, 4 gravid traps were placed along the edge of woods at distances ranging from 100 to 200 m from a waste tire site in Jasper County on September 14, 1995. One trap was placed at each distance, 100, 150, 175, and 200 m, approximately 2 m inside the woods.

Gravid traps were typically left in the field for 18–24 h. Adult mosquitoes were returned to the laboratory where they were identified and sexed. The females were visually inspected and graded as gravid or nongravid. Nongravid females were not dissected; therefore, the insemination status, ovarian development, and parity of these specimens are unknown.

Two-choice preference tests: Oviposition preference was determined by comparing the number of eggs oviposited on a substrate in contact with sod infusion and either oak infusion or water. Two hundred milliliters of each treatment were added to 237-ml waxed paper cups (James River Co., Norwalk, CT) that were lined with seed germination paper, 305 × 98 mm (Steinly et al. 1991). In each test, 2 treatment cups were placed inside the same screened cage (0.3 × 0.3 × 0.3 m) containing 100–250 gravid *Ae. albopictus* for 18–24 h. For the comparison of sod infusion with oak infusion, 2 replicates were conducted without India ink and 3 replicates with 3–4 drops of India ink per cup. In the comparison of sod infusion with water, 4 replicates were conducted without India ink and 3 replicates with India ink. The ink was added to both cups in order to determine whether visual differences in the treatments affected oviposition preference. The preference tests used whatever strains of *Ae. albopictus* were available at the time. This included *Ae. albopictus* OAHU (from Oahu, HI), *Ae. albopictus* TODAI (from Tokyo, Japan), *Ae. albopictus* COMP (a composite strain of several temperate strains), and *Ae. albopictus* MOBOT (from Missouri Botanical Garden, St. Louis, MO).

Oak leaf infusion was included in the preference tests because *Ae. albopictus* has been shown to prefer leaf infusions for oviposition (Gubler 1971). The oak leaf infusion was prepared by adding 400 g of locally collected oak leaves (a mixture of *Quercus* species) to 10 liters of tap water and aging the mixture for 6–8 months at 22 ± 4°C. The sod infusion was prepared by adding a 30 × 30-cm section of Kentucky bluegrass sod to 15 liters of water and aging the solution for 2–4 days at 20 ± 5°C. The sod infusion was held at −20°C until thawed for the preference tests. All infusions were stirred, strained through cheesecloth, and filtered through No. 1 Whatman filter paper before each test. Distilled water was used for the water treatment.

Olfactometer tests: The olfactometer consisted of a central release chamber (a 3.8-liter cylindrical cardboard container) and 2 treatment chambers of equal size, 180° apart. The side chambers were connected to the central chamber by cardboard tubes about 8 cm in diameter and 20 cm long that were fit inside cotton stockinette attached to each chamber. Male and female pupae of *Ae. albopictus* were separated into waxed paper cups 1 or 2 days before eclosion. The adults were held in separate cardboard containers for 1–5 days until they were transferred to the olfactometer. Although adults were originally retested several times, only the results from their first exposure are reported.

Several olfactometer tests were conducted with 50–80 *Ae. albopictus* ESL (from East St. Louis, IL) adults to provide baseline data on the movement of mosquitoes in the test device. To determine the movement of adults after contacting a treatment, mosquitoes of the appropriate sex were released into a side chamber containing a waxed paper cup with either 200 ml of water or sod infusion. The other chambers of the olfactometer were empty. This experiment was conducted once with males and females for water and sod infusion. In the next test, adults were released into the central chamber and each side chamber was empty in order to determine whether there was a bias in the choice of one treatment chamber over the other. This was tested once with males and females. The final challenge of the olfactometer consisted of waxed paper cups with 200 ml of water and 3 or 4 drops of India ink. Identical treatments were placed in both side chambers, and mosquitoes were released in the central chamber. This was performed once with males and females to determine whether there was any bias in mosquito movement when the cups were placed in the olfactometer. The side chambers were covered with clear plexiglass at the onset of each test. The distribution of mosquitoes in the chambers was recorded after 16–18 h.

After the initial olfactometer tests, one waxed paper cup containing 200 ml of either tap water or sod infusion was placed in each of the side chambers. India ink (3 or 4 drops) was added to each treatment. As many naive adults of the appropriate sex as were available were released into the central chamber for each test. The experiment was conducted twice for males and females using 2 cohorts of *Ae. albopictus* ESL and once using males and females from the *Ae. albopictus* MOBOT strain. The side chambers were covered with clear plexiglass to promote the diffusion of volatiles into the central release

chamber. The number of mosquitoes in each chamber was recorded approximately 18 h after their release. The distribution of mosquitoes was checked periodically during the first 2–4 h of the test.

All of the tests were conducted under long-day photoperiod (18:6 h L:D) at 26 ± 2°C and 60 ± 5% relative humidity. Fresh sod infusion was prepared for the olfactometer tests by adding a 30 × 30-cm piece of Kentucky bluegrass sod to about 15 liters of water and aging the mixture for 2–4 days. Distilled water was used for the water treatment.

Statistical analysis: StatView, a statistical package for personal computers (Abacus Concepts 1992), was used for all analyses. For oviposition preference, the number of *Ae. albopictus* eggs was analyzed by 2-way ANOVA including treatment and India ink (presence or absence) as variables. For the olfactometer results, the pooled number of adults in each side chamber was subjected to chi-square analysis for each sex to show whether the observed number of mosquitoes in the 2 side chambers was significantly different at the 5% level from the expected number, assuming an equal distribution of those that moved out of the central chamber.

RESULTS

Field studies: In 1993, *Ae. albopictus* was recovered from sod-baited gravid traps at 6 of the 11 test sites in southern Illinois. Four of these test areas (East St. Louis, Mounds, Ullin, and Campbell Hill) were within 0.5–1.0 km of a waste tire site. The other areas that were positive for *Ae. albopictus* (Red Bud and Steeleville) did not have an obvious source of tires in the vicinity. Ullin, Campbell Hill, Red Bud, and Steeleville were new city records and the latter 3 sites represented 2 new county records (Jackson and Randolph counties) for Illinois. The gravid traps placed near sewage lagoons in 1993 caught primarily *Culex* species.

In East St. Louis, the sod-baited gravid traps attracted 46 female and 21 male *Ae. albopictus* on 2 collection dates. About 15% of the females appeared gravid (7 out of 46). The number of males caught in the traps appeared to increase with an increase in the number of females. Gravid traps with 4 and 6 females had one male in each trap (June 18, 1993) and traps with 16 and 20 females had 6 and 13 males (July 15), respectively. At all other sites, few males were captured in the gravid traps; however, the number of females captured at these sites was also less than in East St. Louis. In Mounds, 7 females were collected from 3 dates, and in Ullin, 3 females from 2 dates. In Red Bud, 11 females and one male were collected from 3 dates, and in Steeleville, 6 females from 2 dates. None of the 27 females captured outside of East St. Louis appeared gravid. Host-seeking adults were observed only in East St. Louis and Campbell Hill (see below).

The presence of *Ae. albopictus* in Red Bud and Steeleville, 2 areas lacking an obvious tire source of larvae, prompted a survey of nearby cities. A waste tire site was found in Campbell Hill (Jackson County) about 13 km from Steeleville in late September. Although the gravid traps at this site were rendered inoperable by heavy rain and flooding (September 23, 1993), one gravid trap caught 2 *Ae. albopictus* females. Ten host-seeking *Ae. albopictus* were counted during the 10-min trap set-up period at Campbell Hill.

In 1995, additional field tests were conducted with sod-baited gravid traps near a waste tire site in Jasper County. Larval collections at this site included a variety of *Aedes* and *Culex* species (Lampman, unpublished data). When traps were placed at 4 distances from the same tire pile on September 14, the gravid trap at 100 m had 184 female and 10 male *Ae. albopictus,* 7 female *Culex,* and 7 female *Aedes triseriatus* (Say). At 150 m, there were 90 female *Ae. albopictus,* 3 female *Culex,* and 20 female *Ae. triseriatus*; at 175 m, 67 female *Ae. albopictus,* 3 female *Culex,* and 6 female *Ae. triseriatus*; and at 200 m, 80 female *Ae. albopictus,* one female *Culex,* and 4 female *Ae. triseriatus.* A visual inspection of the females in the laboratory indicated that 5% of the *Ae. albopictus* were gravid (n = 421), 93% of the *Culex* (n = 14), and 68% of the *Ae. triseriatus* (n = 37). The *Culex* adults were identified as either *Culex pipiens* Linn. or *Culex restuans* Theobald. The most prevalent *Culex* species, based on larval identification, collected from the tire site in August 1995 was *Cx. pipiens* (Lampman, unpublished data).

Two-choice preference tests: In oviposition choice tests with sod and oak infusions, with and without India ink (Table 1), a 2-factor ANOVA showed that the number of eggs oviposited was not affected by presence or absence of ink, infusion type, or the interaction of these factors (P = 0.59, P = 0.27, and P = 0.70, respectively). The sod infusion had more eggs than oak infusion in each of the 5 tests; however, the difference between the 2 treatments was relatively small. In contrast, the sod infusion had significantly more eggs than the water treatment, whether or not it was treated with India ink (Table 1). A 2-factor ANOVA indicated that the presence of India ink did not influence the number of eggs (P = 0.86); however, treatment (sod infusion and water) was significant (P = 0.004).

Table 1. Number of *Aedes albopictus* eggs in oviposition cups baited with either oak and sod infusion or water and sod infusion, with and without the addition of India ink to both treatments.

Strain[1]	India ink	Infusion type: Oak	Infusion type: Sod
OAHU	No	588 (42.2%)	803 (57.8%)
COMP	No	929 (34.7%)	1,748 (65.3%)
TODAI	Yes	420 (45.7%)	500 (54.3%)
COMP	Yes	601 (44.5%)	750 (55.5%)
COMP	Yes	1,100 (40.2%)	1,638 (59.8%)
Strain[1]	**India ink**	**Infusion type: Water**	**Infusion type: Sod**
TODAI	No	841 (24.9%)	2,541 (75.1%)
MOBOT	No	460 (20.7%)	1,767 (79.3%)
MOBOT	No	98 (9.9%)	890 (90.1%)
MOBOT	No	560 (37.8%)	920 (62.2%)
MOBOT	Yes	450 (18.7%)	1,900 (80.9%)
MOBOT	Yes	624 (36.7%)	1,078 (63.3%)
MOBOT	Yes	390 (22.8%)	1,320 (77.2%)

[1] OAHU is a strain originating from Oahu, HI; COMP, a composite strain of several temperate-zone strains from around the world; TODAI, a strain from Tokyo, Japan; and MOBOT, a strain from the Missouri Botanical Gardens, St. Louis, MO.

The interaction of the 2 factors was not significant ($P = 0.86$). The addition of India ink had little influence on the number of eggs oviposited on substrates in the sod infusion in either series of tests (56.3 and 61.5% of the eggs were in the sod infusion *vs.* the oak infusion, with and without India ink, and 73.8 and 76.7% of the eggs were in the sod infusion *vs.* water, with and without India ink, respectively).

Olfactometer tests: The preliminary tests with the olfactometer showed that male and female *Ae. albopictus* released into side chambers containing either water or sod infusion tended to remain in those chambers. In the tests with water, only 11% of males and 14% of females moved from the side chamber with water to either of the other 2 empty chambers. Similar results were obtained with sod infusion; only 14% of the males and 15% of the females moved from the chamber with the sod infusion to the empty chambers. When both side chambers were empty, males and females released into the central chamber showed no bias for moving to either side. After 18 h, 28% of the males were in the left chamber, 33% in the right, and 39% in the release chamber. For females, 21% were in the left chamber, 29% in the right, and 49% in the release chamber. The absence of chamber preference was also noted for males and females released into the central chamber when both side chambers contained colored water, except fewer mosquitoes remained in the release chamber. In the first trial, 48% of the males were in the right chamber, 43% in the left, and 9% in the release chamber. In the next trial, 45% of the females were in the left chamber, 45% in the right chamber, and 10% in the release chamber. This suggests that movement in the olfactometer is not biased by the test design and that both sexes may respond to humidity (hence the increased movement out of the release chamber).

After demonstrating the absence of any inherent bias in the olfactometer, males and females were released into the central chamber with one side chamber containing sod infusion and the other chamber containing water (Table 2). Both sexes consistently moved to the chamber containing the sod infusion. Chi-square analyses of the pooled data for each sex indicated a significant deviation from an equal distribution in the side chambers ($P = 0.001$ for both sexes). Means ($\pm$SD) of 63 $\pm$ 3.1% males and 62.0 $\pm$ 8.0% females moved from the release chamber to the chamber with the sod treatment ($n = 3$ for both sexes). Considering only the mosquitoes that moved out of the central release chamber into one of the side chambers, 77% of the males and 70% of the females moved to the sod-baited chambers. Periodic inspection of the distribution of mosquitoes indicated that the differential movement toward the sod was evident within the first 2–3 h of the test.

DISCUSSION

Aedes albopictus adults were recovered from sod-baited gravid traps at 7 sites in 5 counties of southern and southwestern Illinois in areas

Table 2. Number of male and female *Aedes albopictus* in olfactometer chambers (and percentage in each chamber) baited with sod infusion or water (both treated with India ink). The center treatment is the release chamber and represents the number of mosquitoes that made no choice after 18 h. The side chambers contained the treatments. The first 2 trials were with *Aedes albopictus* ESL and the last trial with *Aedes albopictus* MOBOT.

		Treatment and position of adults after 18 hours		
	Sex	Treatment Sod	Release	Treatment Water
Trial 1	Male	42 (63.6%)	4 (6.1%)	20 (30.3%)
	Female	53 (57.6%)	10 (10.9%)	29 (31.5%)
Trial 2	Male	47 (59.5%)	25 (31.6%)	7 (8.9%)
	Female	62 (55.3%)	18 (16.1%)	32 (28.6%)
Trial 3	Male	67 (67.0%)	14 (14.0%)	19 (19.0%)
	Female	52 (73.2%)	10 (14.1%)	9 (12.7%)

ranging from suburban (Red Bud) to rural (Jasper County) sites. Olfactometer and oviposition preference tests in the laboratory showed that *Ae. albopictus* is attracted to sod infusion and that females readily oviposit on substrates in contact with the infusion. Furthermore, sod-baited gravid traps near a waste tire site captured adult mosquitoes of 3 species, including *Ae. albopictus, Ae. triseriatus,* and a *Culex* species (probably *Cx. pipiens*). Sod and grass infusions were originally developed as lures for species in the *Culex pipiens* complex, presumably mimicking the highly eutrophic aquatic sites preferred by this group of mosquitoes (Madder et al. 1980, Reiter 1983). Conversely, ovitrap surveillance programs for *Aedes aegypti* (Linn.) and *Ae. albopictus* often use black jars or cans filled with water (McHugh 1993). These *Aedes* species are commonly found in containers with cleaner water than one associates with *Cx. pipiens* and *Cx. restuans* (Bentley and Day 1989). However, Reiter et al. (1991) demonstrated that *Ae. aegypti* is attracted to grass infusion despite its frequent collection from sites with less polluted water. Gubler (1971) found that gravid *Ae. albopictus* prefer to oviposit in grass infusion rather than colored or uncolored water, but they prefer aged leaf infusions (undescribed plant species) over grass infusions. Our study showed *Ae. albopictus* will lay more eggs in sod infusion than in colored or uncolored water but equally prefers oak leaf and sod infusions.

The response of *Ae. albopictus* to sod-baited gravid traps in the field may have occurred for a variety of reasons, such as orientation to visual stimuli (the black tub of the gravid trap), high relative humidity, or the presence of noncontact and contact semiochemicals associated with the infusion. In laboratory tests, the preference of this species for sod infusion over colored or uncolored water tends to rule out color and humidity as major factors; however, this type of test does not differentiate between contact and noncontact stimuli. The olfactometer tests suggest it is reasonable to assume that the differential movement toward the chambers with sod infusion was probably due to volatile (noncontact) semiochemicals. It should be noted that the factors determined to be important in laboratory tests do not necessarily reflect the significance of those factors in field tests. In fact, it is generally assumed that the relative importance and interaction of visual, tactile and chemosensory stimuli vary as a mosquito moves toward and finally contacts an oviposition site (Bentley and Day 1989). Our laboratory tests indicate that odor is a potentially important component of the oviposition selection process, and our field results demonstrate the utility of sod-baited gravid traps for the collection of *Ae. albopictus* and other potential vector species.

The sod-baited gravid traps in East St. Louis collected a relatively high percentage of males (about 30% of the total number of adults). Reports of male mosquitoes being attracted to stimuli usually associated with females are not uncommon (Kanda et al. 1987). Freier and Francy (1991) showed that a CDC gravid trap, baited with a rabbit pellet infusion, captured a significant number of *Ae. albopictus* males. The presence of males in field collections may reflect an attraction to females; however, our olfactometer data indicate that males are attracted to sod infusion independent of the presence of females. In our field tests, males were not always caught in the sod-baited gravid traps. Only one gravid trap out of 6 in 1995 caught male *Ae. albopictus* near a waste tire site, despite the abundance of

females in all traps. Unfortunately, the relative abundance of males and females in the area surrounding the gravid traps is unknown. One method of clarifying the response of males to these infusions would be to compare the electroantennographic response of the 2 sexes to various infusions and isolated volatile components of these infusions. Many of the volatiles generated by Bermuda sod infusion, which is used to attract *Culex quinquefasciatus* Say, have been identified in the Kentucky bluegrass sod infusion used in this study, particularly indole, skatole, and cresol (Millar et al. 1992; Lampman, unpublished data).

Gravid traps baited with sod infusion allowed us to detect the presence of *Ae. albopictus* in areas with and without an obvious source of tires as larval habitats and provided a means of capturing multiple mosquito species. The demonstration of the attraction of *Ae. albopictus* to sod infusion provides the basis for enhancing collection techniques and augments our understanding of the chemical ecology of mosquitoes.

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