Laboratory and Field Evaluations of Oviposition Responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to Oak Leaf Infusions

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ABSTRACT Organic infusions created by fermenting white oak (*Quercus alba* L.) leaves in water were evaluated as sources of attractant odorants and contact oviposition stimulants for gravid *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say). Infusions were bioassayed in the laboratory by giving single females a choice of ovipositing in 1 container with infusion and 7 containers with water. *Ae. albopictus* laid significantly more eggs in containers with infusion, regardless of concentration (dilutions ranging from 10 to 100%) or age (fermentation periods of 7, 28, 60 d), than in containers holding water. The largest proportion of eggs (76.8%) was deposited in response to a 60% concentration of 7-d-old infusion. In contrast, *Ae. triseriatus* exhibited variable oviposition responses but generally deposited the largest number of eggs in only a few concentrations of older age infusions. In binary "sticky screen" bioassays, there was no difference between the numbers of females attracted to infusion or water, indicating that oviposition responses to infusion were mediated by contact chemostimulants rather than by attraction to odorants. Oviposition responses to infusions by field populations of *Ae. albopictus* and *Ae. triseriatus* in Raleigh, NC, were evaluated with pairs of oviposition traps, one containing infusion and the other containing water. Generally, *Ae. albopictus* laid significantly more eggs in ovitraps containing infusion regardless of its age (7, 28, and 60 d old) or the mass of leaves fermented (126 g = 1× or 504 g = 4×) than in water. In contrast, *Ae. triseriatus* deposited an equivalent number of eggs in traps containing water or 1×, 80% infusion regardless of its age; however, the oviposition response to ovitraps containing 4×, 7-d-old, 50% infusion was significant. Placement of an automobile tire behind an ovitrap did not increase the number of *Ae. albopictus* eggs laid in ovitraps containing 4×, 7-d-old, 50% infusion or water relative to ovitraps without a tire. Our research indicates that baiting ovitraps with oak leaf infusion would increase the sensitivity of surveillance efforts for *Ae. albopictus* and *Ae. triseriatus*.

KEY WORDS *Aedes albopictus, Aedes triseriatus*, oviposition, infusion, oviposition trap

Aedes albopictus (SKUSE) and *Aedes triseriatus* (Say) are container-inhabiting mosquitoes that commonly inhabit wooded suburban areas in the southeastern United States. Both species are day-active, pestiferous, and potential or proven vectors of La Crosse virus (Grimstad et al. 1989, Szumlas et al. 1996b), eastern equine encephalomyelitis virus (Scott et al. 1990), and dengue virus (Mitchell et al. 1987).

Container-inhabiting mosquitoes use both physical and chemical cues in oviposition site selection (Bentley and Day 1989). These cues include the color and optical density of the water, oviposition substrate texture and moisture, and temperature and reflectance as well as olfactory cues and nonvolatile chemical cues received by contact chemoreception (Bentley and Day 1989). In addition, some container-breeding *Aedes* mosquitoes exhibit a behavior known as "skip oviposition" (Mogi and Mokry 1980), which occurs when females lay their eggs in several containers as opposed to laying their entire clutch in 1 container (Fay and Perry 1965, Rozeboom et al. 1973, Chadee and Corbet 1987, Apostol et al. 1994). This behavior increases the distribution of eggs in an area and may be increased by the tendency of gravid females to avoid ovipositing in sites where eggs of conspecific females have been laid (Kitron et al. 1989, Chadee et al. 1990, Apostol et al. 1994).

Because container-inhabiting *Aedes* are not highly responsive to light traps supplemented with dry ice (Loor and DeFoliart 1969, Chan 1985), these mosquitoes commonly are monitored with oviposition traps (ovitraps). Ovitraps typically consist of dark-colored, water-filled containers supplemented with balsa paddles or velour papers as oviposition substrates. Ovitraps originally were developed and used during the U.S. *Aedes aegypti* (L.) Eradication Program (Schliessmann 1964). This sampling procedure also has been used to monitor the prevalence, distribution, and oviposition periodicity of *Ae. triseriatus* (Loor and DeFoliart 1969, Hanson et al. 1988, Trexler et al. 1997) and *Ae. albopictus* (Holck et al. 1988, Hobbs et al. 1991, Trexler et al. 1997).

Ovitraps have been baited with organic infusions to increase trap use by gravid mosquitoes. Many different organic materials, such as leaves, grass and sod, have been fermented to create infusions reported to be active for gravid *Aedes* mosquitoes (Loor and DeFoliart 1969, Gubler 1971, Holck et al. 1988, Kitron et al. 1989, Allan and Kline 1995, Lampman and Novak 1996). Although organic infusions are easy to make, procedures for producing optimally active infusions for use in ovitraps have not been researched carefully. Loor and DeFoliart (1969) used an average of 5.2 g of oak leaves in 12-oz beer cans. In northern Indiana, ≈2 g of dry maple leaf litter were added to 500 ml of water in 3.1-liter ovitraps in a study of the geographic distribution of *Ae. triseriatus* (Hanson et al. 1988). Szumlas et al. (1996a) added 3–5 cm of dry oak leaf litter to ovitraps used to monitor *Ae. triseriatus* in western North Carolina. The mass of leaves added to ovitraps used in these studies appears to have been selected arbitrarily. Similarly, few attempts have been made to evaluate effects of fermentation time on the activity of the resultant infusion or to determine the optimal concentration of infusion to use for monitoring natural mosquito populations. A 6-d-old hay infusion mixture (approximating Reiter 1986) was used in ovitraps to detect *Ae. albopictus* in Louisiana (Holck et al. 1988). Undiluted grass infusions were used to sample *Ae. aegypti* populations in Florida (Frank and Lynn 1982). Reiter et al. (1991) used a 7-d-old Bermuda hay infusion in monitoring *Ae. aegypti* in Puerto Rico and found that an ovitrap containing a 10% solution paired with an ovitrap containing a 100% infusion collected more eggs than single ovitraps containing tap water. However, Chadee et al. (1993) determined that ovitraps containing 9 different concentrations of 7-d-old Bermuda hay infusion were equivalently attractive to gravid *Ae. aegypti* as water.

Oak leaf litter has been used to enhance ovitraps in studies with *Ae. triseriatus* (Loor and DeFoliart 1969, Szumlas et al. 1996a). In addition, laboratory studies of the oviposition responses of *Ae. albopictus* to natural and artificial oak leaf infusions have been conducted (Allan and Kline 1995, Lampman and Novak 1996). These studies demonstrated that oak leaf infusions contain substances that are active as mosquito attractants or oviposition stimulants. However, with the exception of the research of Allan and Kline (1995) with *Ae. albopictus*, methodical laboratory evaluations of oviposition attractants/stimulants in oak leaf infusions active against gravid *Ae. albopictus* and *Ae. triseriatus* have not been conducted. Accordingly, the 3 objectives of our current research were the following: (1) to determine effects of age and concentration of an infusion of white oak leaves on the oviposition responses of *Ae. albopictus* and *Ae. triseriatus* in the laboratory; (2) to ascertain whether the increased oviposition response by either species was the result of volatile chemicals or contact chemostimulants; and (3) to determine if optimally active infusion age and concentration for each species could eliminate or diminish skip oviposition behavior. In addition, we verified the laboratory findings of the activity of these oak leaf infusions against field populations of mosquitoes. Specifically, we determined if concentrations of various oak leaf infusions that were optimally active under laboratory conditions would increase oviposition by *Ae. albopictus* and *Ae. triseriatus* in infusion-baited oviposition traps under field conditions. Additionally, we evaluated effects of increasing the amount of leaves fermented on infusion activity, and ascertained if the attractiveness of infusion-baited ovitraps could be enhanced visually by propinquity to an automobile tire.

Materials and Methods

Mosquito Colony Origin and Maintenance. *Aedes albopictus* and *Ae. triseriatus* were collected as larvae and pupae in New Hanover County, NC, in the summer of 1994. The colonies were maintained at ≈26°C, ≈75% RH, and a photoperiod of 14:10 (L:D) h. Included in the photophase were two 30-min crepuscular periods corresponding to dawn and dusk that were provided by a single 40-W incandescent bulb. Larvae were fed a 2:1 mixture (wt:wt) of liver powder (ICN Biochemicals, Cleveland, OH) and brewer's yeast (ICN Biochemicals) on a standardized schedule (Gerberg et al. 1994). Adults were kept in Plexiglas cages (30 by 30 by 30 cm) fitted with cotton surgical stocking tops and were continuously provided a 10% sucrose solution. The F_1–F_7 generations were used in the experiments.

Preparation of Infusions. Infusions were prepared by fermenting 126 g of white oak (*Quercus alba* L.) leaves in 15 liters of tap water, approximating the methods of Reiter et al. (1991). White oak leaves were used because previous studies have established that oak leaf infusions elicit oviposition responses from container-inhabiting *Aedes* mosquitoes (Beehler et al. 1992, Allan and Kline 1995). Also, *Q. alba* is distributed widely in the eastern and southeastern United States (Christensen 1988, Greller 1988), and therefore leaves of this species would be readily available for preparation of infusions by other researchers or vector control agencies for surveillance of container-inhabiting mosquitoes. In our current study, fallen leaves were collected at a single site. Leaf material and water were contained within an 18-liter black plastic bucket double-lined with polypropylene trash bags and were fermented for periods of 7, 28, and 60 d at ≈26°C. Infusions were strained through a screen and frozen until used. Plant infusions can be stored at −18°C without loss of activity (Millar et al. 1992). Because small variations in ingredients or conditions can cause marked variations in infusions (Beehler et al. 1994), attempts were made to limit the variability in infusion preparation. White oak leaf litter was used from a single site, tap water was used from 1 source, infusions were prepared under a relatively constant temperature, and in the laboratory, infusions were evaluated from the same batch.

Laboratory Bioassays of Infusions. Infusions were bioassayed according to the methods of Corbet and Chadee (1993). Briefly, 8 polypropylene cups (120 ml each) (Fisher no. 09-800), spray-painted black, were placed in a circle within a Plexiglas cage so that each cup was an equal distance from its nearest neighbor. One experimental cup contained 30 ml of infusion, and each of the 7 remaining cups contained 30 ml of water. Each cup was lined with filter paper as an oviposition substrate. The oviposition responses to 7, 28- and 60-d-old leaf infusions, ranging in concentration from 10 to 100%, were measured at increments of 10%. Preliminary experiments using 8 cups of tap water indicated that cup position affected oviposition response. Therefore, the position of the cup with the infusion was randomized for each replicate. Oviposition by both species peaked 4 d after a blood meal (Trexler et al. 1997). Consequently, 4- to 7-d-old females were blood-fed on a human arm and placed in oviposition cages 4 d later. Laboratory colonies of both mosquito species are virus-free, and our protocol that involved blood feeding mosquitoes on a human was approved by the Institutional Review Board at North Carolina State University (Human Use Protocol IRB# 1388). Single females were used in each replicate, because the presence of conspecific eggs affects the numbers of eggs that subsequently are laid in a container (Chadee et al. 1990, Beehler et al. 1992). After a 3-d exposure period, filter papers were removed from the cups and the number of eggs counted under a dissection microscope. Eggs remaining in or floating on the infusion or tap water were removed, counted, and added to the count of eggs on the ovistrip. Females were dissected and examined for retained eggs as well as the presence or absence of sperm in the spermathecae. A replicate was discarded if a female retained any eggs or was not inseminated.

Skip Oviposition Studies. Effects of the oak leaf infusions on the expression of "skip oviposition" behavior by both species was determined by averaging the number of containers used by a species in response to the presence or absence of infusion. The bioassay method of Corbet and Chadee (1993) was again used to investigate the oviposition response of both mosquito species. The mean number of cups in which eggs were deposited by *Ae. albopictus* in response to 8 cups of water was compared with the number of cups used for oviposition when 1 of the 8 cups contained a 60% concentration of 7-d-old infusion. Likewise, the number of cups used by *Ae. triseriatus* in response to 8 cups of water was compared with the number of cups used when 1 of the 8 cups contained a 30% concentration of 7-d-old infusion.

Attraction Versus Contact Stimulation. The "sticky-screen" bioassay of Isoe et al. (1995b) was used to differentiate oviposition responses caused by volatile and contact chemical stimulants. A 5-cm-diameter disk cut from galvanized hardware cloth screen (6-mm mesh, Gilbert and Bennet, Toccoa, GA) and coated with a sticky material (Tanglefoot, Grand Rapids, MI) was placed on top of 1 test (infusion) and 1 control (water) cup in each bioassay cage. The response of *Ae. albopictus* was evaluated using a 60% concentration of a 7-d-old infusion and the response of *Ae. triseriatus* was evaluated using a 60% concentration of a 60-d-old infusion. The test cup was assigned randomly to 1 corner, and the control cup was placed in the opposite corner, in each of 12 cages. Ten gravid mosquitoes were placed in each cage. A 10% sucrose solution was provided continuously in each cage during the experiment. After a 2-d exposure period, the number of females adhering to the screen on each cup in each cage was counted. For these experiments, the oviposition activity index (OAI) described by Kramer and Mulla (1979) was used to evaluate the response of both mosquito species to the infusion. The oviposition activity index was calculated for each experimental replicate using the following formula:

$$OAI = \frac{N_t - N_c}{N_t + N_c}$$

in which N_t was the number of adults trapped on the test cup and N_c was the number of adults trapped on the control cup. The oviposition activity index represents the proportion of adults trapped on a specific test (infusion) cup after correcting for the number adults trapped on the control (water) cup. The oviposition activity index ranges from -1 to 1, with zero indicating no response.

Field Evaluations. Oviposition by *Ae. albopictus* and *Ae. triseriatus* was monitored in the field at 6 residences in Raleigh, Wake County, NC, during the summer of 1996. Three of these home sites were used again in 1997. Each of the 6 residences was located in suburban subdivisions. The plant overstory on these lots was dominated by a mixture of mature oak and pine. Various shrubs and knee-high herbaceous plants were distributed across each site. Although *Ae. triseriatus* was present at all sites, at only 1 of these sites was *Ae. triseriatus* sufficiently active to be included in our investigation. The residences were at least 1.6 km from each other. At each of the sites, ovitraps were placed in pairs, side-by-side on the ground in the shade, at 6 locations with a distance of ≥25 m between each ovitrap pair. Generally, traps were placed along the border of each lot where natural plant growth was the most dense. Ovitraps consisted of no. 10 tin cans spray-painted flat black inside and out with rust resistant paint. Red velour strips (2.5 by 17.8 cm) were paper-clipped to the inside of the ovitraps as oviposition substrates (ovistrips). For each ovitrap pair, the ovistrips were clipped to the outer edges of the ovitraps so that the 2 oviposition substrates were a maximum distance apart. Each ovitrap was numbered, and the placement of either 500 ml of tap water or 500 ml infusion in the ovitrap initially was chosen randomly. At the beginning of a new sampling period, the media (water or infusion) placed in each ovitrap was switched so that each ovitrap did not receive the same treatment for 2 consecutive sampling periods. Infusions were assayed at 3 residences, and the remaining 3 residences were used as controls to obtain a relative measure of the activity of the gravid mosquito population. With this design, we could determine if declines in egg densities in infusion-baited ovitraps resulted from declines in mosquito population levels.

Ovitraps were set in the field in the morning before 1200 hours EDT, and ovistrips were collected in the morning 2 d later. Ovistrips were numbered and stapled to white photocopy paper, placed in self-sealing plastic bags and transported to the laboratory. When ovistrips were collected, each trap was rinsed with tap water and either refilled with water or infusion or turned upside down until the beginning of the next ovitrapping period. In the laboratory, eggs were identified to species (Linley 1989a, b) and counted under a dissecting microscope.

Because *Ae. albopictus* deposited significantly more eggs in every concentration of every age of infusion tested in the laboratory than in water, only the most active concentrations of infusions found for *Ae. triseriatus* in the laboratory were tested under field conditions. In preliminary field trials in June and July 1996, no significant differences ($P > 0.05$) were observed between the number of eggs deposited in the 28- or 60-d-old 80% infusion and water-baited ovitraps. Consequently, a stronger infusion was prepared by increasing the weight of leaves fermented by 4-fold from 126 to 504 g. From 15 July to 2 August 1996 a 50% concentration of this 4×, 7-d-old infusion was tested. To clarify effects of fermenting an increased amount of leaves on infusion activity, a 50% concentration of 1×, 7-d-old infusion was tested in the late summer from 11 to 20 September 1996 and again from 2 to 25 July 1997.

Because the 28- and 60-d-old 80%, 1× infusions were tested in midsummer when air temperatures were elevated, these same infusions were reevaluated during cooler weather in the fall from 23 to 29 September and 30 September to 4 October 1996, respectively, to determine if air temperature affected mosquito response or infusion activity.

Effects of a Visual Attractant. We attempted to increase the attraction of infusion-baited ovitraps to gravid mosquitoes by placing a discarded automobile tire behind an ovitrap containing a 50% concentration of 4×, 7-d-old infusion or tap water. Effects of the tire on the responses of gravid females to ovitraps were determined from 5 to 23 August 1996. Before use, automobile tires were cleaned thoroughly and the wheel openings covered with black plastic trash bags taped to the sides of the tires. These evaluations were carried out concurrently at each of the 6 ovitrap pair locations at 5 study sites. At each location, a tire was set so that it was standing on edge, and 1 ovitrap was set in front of it. The other ovitrap was placed to one side, 1 m from the tire ovitrap. Each tire–ovitrap pair was assigned randomly to 1 of 3 of the following conditions: (1) tire–ovitrap with 500 ml infusion and 2nd ovitrap with 500 ml water; (2) tire–ovitrap with 500 ml water and 2nd ovitrap with 500 ml infusion; and (3) both tire–ovitrap and 2nd ovitrap with 500 ml water. The condition of each tire–ovitrap pair was assigned randomly and then switched to the alternative condition during subsequent sampling periods. Infusions were set out in the morning before 1200 hours EST and ovistrips were collected in the morning 2 d later.

Temperature and Rainfall Data. Mean daily temperatures and daily rainfall totals for the field trials were obtained from the National Climatic Data Center of the National Oceanic and Atmospheric Administration recorded at Raleigh-Durham International Airport, which is located ≈15 km from the closest of our study sites.

Statistical Procedures. Results of experiments with infusions were analyzed by analysis of variance (ANOVA) on square-root transformed counts of the numbers of eggs deposited in test and control cups using a general linear models (GLM) procedure (SAS Institute 1989). A contrast procedure (SAS Institute 1989) was used to test for differences in the mean number of eggs deposited in the 1 test cup versus the 7 remaining.

Effects of optimally active concentrations of oak leaf infusion on skip oviposition behavior of both mosquitoes was analyzed by ANOVA on square-root transformed egg counts, and significant differences among the mean numbers of cups used by both mosquito species were separately analyzed by the Tukey studentized range honestly significant difference (HSD) test (SAS Institute 1989). A nonparametric signed rank test (PROC UNIVARIATE, SAS Institute 1989) was used to determine if the mean oviposition activity index for each species was significantly different from zero.

Results of the field experiments for *Ae. triseriatus* and *Ae. albopictus* were initially analyzed by 2- and 3-way ANOVAs, respectively, using a GLM procedure (SAS Institute 1989) to determine if oviposition activity at the various sampling sites and between sampling dates at each site was different. Before statistical analyses, the numbers of eggs collected in ovitraps was subjected to a $\sqrt{(y + 0.5)}$ transformation. Data for each mosquito species were analyzed separately. These preliminary analyses included site (*Ae. albopictus* only), sampling date, and media (infusion or tap water) as main effect variables. Site was not included as a variable for *Ae. triseriatus* because sufficient numbers only were collected at a single residence. To determine if differences in the oviposition responses to infusion and water were significant, separate paired comparisons for each infusion that was field-tested were made between the numbers of eggs deposited in the infusion-baited and water-filled ovitraps over each sampling period. A data set containing the differences in egg densities between each paired ovitrap was used to generate a t statistic (PROC MEANS, SAS Institute 1989). Effects of placing an automobile tire behind an ovitrap on the numbers of eggs deposited in the ovitrap were evaluated using these same procedures. Differences in egg densities between the paired ovitraps were tested to determine if the mean difference was significantly different from zero.

Results

Oviposition Responses to Oak Leaf Infusion. Significantly ($P < 0.01$) more eggs of *Ae. albopictus* were deposited in cups containing all concentrations and ages of oak leaf infusion than in cups containing water (Table 1). The highest percentage of eggs was laid in cups containing a 60% concentration of 7-d-old infusion. Responses to 7-d-old infusion followed a "bell-shaped" pattern, in which the 40–90% concentrations

Table 1. Oviposition responses of *Ae. albopictus* to oak leaf infusion in laboratory bioassays

Infusion conc, (%)	*n*	7-d-old infusion			*n*	28-d-old infusion			*n*	60-d-old infusion		
		Mean no. eggs/female	Mean % of eggs deposited in infusion cup[a]	$P > F^b$		Mean no. eggs/female	Mean % of eggs deposited in infusion cup[a]	$P > F^b$		Mean no. eggs/female	Mean % of eggs deposited in infusion cup[a]	$P > F^b$
10	11	61.3	28.5 (5.4)	0.0095	11	67.4	35.8 (8.2)	0.0001	14	68.6	42.4 (5.3)	0.0001
20	10	50.0	36.8 (9.1)	0.0012	13	55.4	49.3 (8.9)	0.0001	10	66.7	46.9 (12.7)	0.0001
30	10	57.8	44.2 (8.6)	0.0002	12	60.4	36.3 (8.1)	0.0010	14	70.0	56.1 (9.1)	0.0001
40	10	62.1	63.4 (9.3)	0.0001	7	62.6	43.6 (9.5)	0.0001	12	68.5	46.7 (8.3)	0.0001
50	11	61.2	48.6 (10.9)	0.0003	8	55.8	59.9 (4.6)	0.0001	18	64.2	60.9 (6.7)	0.0001
60	12	65.9	76.8 (6.7)	0.0001	7	54.6	49.5 (9.8)	0.0001	10	63.9	55.5 (8.4)	0.0001
70	9	71.1	47.2 (14.2)	0.0050	7	58.9	51.3 (13.2)	0.0005	15	64.7	59.4 (11.0)	0.0001
80	10	67.7	70.7 (10.8)	0.0001	8	68.0	43.4 (10.8)	0.0020	14	67.0	56.2 (8.2)	0.0001
90	9	62.1	52.5 (10.1)	0.0001	8	63.2	47.6 (12.1)	0.0001	16	68.9	61.6 (6.9)	0.0001
100	10	70.9	37.6 (9.0)	0.0005	8	70.8	53.7 (10.2)	0.0001	14	66.1	63.1 (9.8)	0.0001

[a] Values are mean percent of eggs (±SE) laid in the cup containing oak leaf infusion.

[b] *P* values were generated using a contrast procedure to compare the mean number of eggs oviposited in 1 test cup containing oak leaf infusion versus the mean number of eggs oviposited in 7 remaining control cups that contained tap water. The experiment-wise error mean square for the numbers of eggs deposited in the test and control cups was used as the denominator in *F* tests.

received more eggs than the higher or lower concentrations. However, in general, for the 28- and 60-d-old infusions the mean percentage of eggs deposited in the infusion cup increased as the concentration increased.

Gravid *Ae. triseriatus* were comparatively less responsive to the 7-d-old oak leaf infusion than were *Ae. albopictus*. Concentrations of 10 and 30% infusion received significantly more eggs than water (Table 2). Numbers of eggs deposited in the other infusion concentrations (20%, 40–100%) were not significantly different from water.

In response to the 28-d-old infusion, *Ae. triseriatus* laid significantly more ($P < 0.01$) eggs only in bioassay cups containing an 80% infusion. The remaining infusion concentrations elicited no greater oviposition than water (Table 2). Oviposition responses to the 60-d-old infusion were also variable. Significantly more ($P < 0.01$) eggs were deposited only in cups containing 60 and 90% concentrations of infusion. The remaining concentrations elicited oviposition responses that varied from repellence (10%, 20%) to no effect (30–50%, 70–80%, 100%) (Table 2).

Skip Oviposition. In response to 8 cups of water, individual *Ae. albopictus* laid eggs in a mean of 5.7 ± 0.2 cups ($n = 31$). In contrast, *Ae. triseriatus* females averaged only 2.3 ± 0.3 cups ($n = 27$), with 70% of females ovipositing in 1 or 2 cups. The number of cups used by *Ae. albopictus* in response to a 60% concentration of 7-d-old infusion (mean = 3.5 ± 0.3 cups, $n = 26$) was significantly lower ($P < 0.05$) than the number of cups used in response to water alone (Table 3). *Ae. triseriatus* laid eggs in an average of 2.6 cups (±0.4) in the presence of a 30% concentration of the 7-d-old infusion (Table 3). This number of cups was not significantly different ($P > 0.05$) than the number of cups used in response to water alone.

Odor-Mediated Attraction Versus Contact Chemoreception. In the "sticky-screen" bioassay for *Ae. albopictus*, 108 out of 120 females from 12 cages were trapped, with 59 females trapped on the screens covering the infusion cups and 49 females trapped on screens covering the water cups. The mean oviposition activity index for a 60% concentration of 7-d-old infusion was 0.079 (±0.11). A total of 90 of 100 *Ae.*

Table 2. Oviposition responses of *Ae. triseriatus* to oak leaf infusion in laboratory bioassays

Infusion conc, (%)	*n*	7-d-old infusion			*n*	28-d-old infusion			*n*	60-d-old infusion		
		Mean no. eggs/female	Mean % of eggs deposited in infusion cup[a]	$P > F^b$		Mean no. eggs/female	Mean % of eggs deposited in infusion cup[a]	$P > F^b$		Mean no. eggs/female	Mean % of eggs deposited in infusion cup[a]	$P > F^b$
10	16	74.1	28.4 (10.6)	0.0001	10	53.2	18.2 (11.6)	0.1996	11	62.0	0.0 (−)	0.2329
20	21	67.1	31.6 (9.7)	0.0712	10	51.2	19.9 (11.3)	0.3270	12	76.0	0.2 (0.2)	0.1562
30	17	78.6	31.8 (10.3)	0.0020	10	43.6	5.0 (3.9)	0.7922	12	69.4	16.7 (11.9)	0.9749
40	8	72.1	22.2 (10.3)	0.4527	9	58.9	29.1 (15.5)	0.0482	13	77.7	7.6 (4.5)	0.5979
50	8	61.5	28.4 (14.9)	0.4562	9	47.1	11.8 (9.8)	0.7221	16	60.9	26.9 (11.7)	0.0298
60	8	72.0	18.2 (12.0)	0.6008	8	49.9	26.1 (13.9)	0.2472	16	71.2	43.8 (12.5)	0.0006
70	8	62.8	28.3 (16.9)	0.2522	11	50.8	18.1 (12.1)	0.0332	16	68.2	19.8 (9.5)	0.0783
80	8	67.6	15.5 (13.3)	0.5436	8	48.8	44.1 (23.2)	0.0049	15	81.5	37.1 (12.4)	0.0228
90	8	66.5	0.0 (−)	0.2480	9	53.6	26.4 (17.6)	0.3627	16	72.7	30.3 (11.2)	0.0049
100	8	71.4	21.4 (17.5)	0.2561	9	61.3	22.5 (12.4)	0.6295	13	72.4	17.3 (8.5)	0.2653

[a] Values are mean percent of eggs (±SE) oviposited in the cup containing oak leaf infusion.

[b] *P* values were generated using a contrast procedure to compare the mean number of eggs oviposited in 1 test cup containing oak leaf infusion versus the mean number of eggs oviposited in 7 remaining control cups that contained water. The experiment-wise error mean square for the numbers of eggs deposited in the test and control cups was used as the denominator in *F* tests.

Table 3. Effects of oak leaf infusion on skip oviposition behavior of *Ae. albopictus* and *Ae. triseriatus*

Media	n	Mean no. cups used[a]		
		Ae. albopictus[b]	n	*Ae. triseriatus*[b]
Water	31	5.7 (0.2)a	27	2.3 (0.3)a
Water + infusion	26	3.5 (0.3)b	17	2.6 (0.4)a

[a] Mean number of cups (±SE) oviposited in by *Ae. albopictus* in response to water and water + 60% concentration of 7-d-old infusion, and by *Ae. triseriatus* in response to water and water + 30% concentration of 7-d-old infusion. In each replicate assay, only one female was used per cage.

[b] Means followed by the same letter within the same column are not significantly different ($P > 0.05$) by Tukey studentized range (HSD) test.

triseriatus was trapped, with 41 mosquitoes trapped on cups containing infusion and 49 females trapped on cups containing water. The mean oviposition activity index for *Ae. triseriatus* in response to a 30% concentration of 7-d-old infusion was -0.075 (±0.11). Neither the oviposition activity index value for *Ae. albopictus* ($n = 12$, signed rank = 5, $P = 0.60$) or *Ae. triseriatus* ($n = 10$, signed rank = −7, $P = 0.38$) was significantly different from zero, indicating that both mosquitoes found cups containing oak leaf infusion or water to be equally attractive.

Field Evaluations. The oviposition responses to infusion or water for both species did not differ between the various sampling sites and dates. In the preliminary ANOVAs of data for *Ae. albopictus*, no significant interaction effects ($P > 0.05$) were found between sites and sampling dates, sites and oviposition media, or sites, sampling dates, and oviposition media. Similarly, no significant interaction effects were found for *Ae. triseriatus* between oviposition media and sampling date ($P > 0.05$).

Aedes albopictus exhibited a strong oviposition response to ovitraps containing 4×, 7-d-old infusion (Table 4). Similarly, *Ae. albopictus* was highly responsive to the 1×, 7-d-old infusions in September 1996 and in July 1997, when significantly more eggs were laid in ovitraps containing infusion than in ovitraps with water. In fall 1996, substantially more eggs were collected in ovitraps with 1×, 28-d-old or 1×, 60-d-old infusion than in traps containing water (Table 4).

In the preliminary field trials in which *Ae. albopictus* did not exhibit a significant oviposition response to oak leaf infusion, ≈50% of the eggs were deposited in both the test and control ovitraps. When mosquitoes exhibited a significant oviposition response to infusion-baited traps, an average of 64–69% of the total number of eggs were laid in ovitraps containing infusion. Over the course of our field study, the numbers of eggs deposited in infusion-baited ovitraps ranged from a mean of ≈22–178 eggs per ovitrap per 2-d period. Egg densities at the control sites followed the same seasonal profile and ranged from an average of ≈11–59 eggs per ovitrap per 2-d period.

In paired comparisons, the number of eggs deposited by *Ae. triseriatus* females in traps baited with 1× infusion were not significantly different ($P > 0.05$) than traps containing water regardless of infusion age (Table 5). *Ae. triseriatus* exhibited a significant oviposition response to infusion-baited traps only when they contained 4×, 7-d-old infusion produced by fermenting a 4-fold amount of leaf material (Table 5). For all trials, the proportion of eggs deposited in infusion-baited traps ranged from ≈12 to 81% of the total eggs collected in both test and control ovitraps. The densities of eggs in ovitraps were highly variable, ranging from an arithmetic mean of 0.2–23.9 eggs per trap per 2-d period.

Effects of Visual Attractant. Placing a tire behind infusion-baited ovitraps did not significantly increase the number of *Ae. albopictus* eggs collected. More eggs were collected in infusion-baited ovitraps than in traps containing water regardless of which ovitrap the tire was placed behind (Table 6). When both ovitraps contained water, approximately equal numbers of eggs were deposited in the tire and control ovitraps. The response of *Ae. triseriatus* was not evaluated because oviposition activity of this species was too low to obtain meaningful results when experiments with *Ae. albopictus* were conducted.

Table 4. Paired comparisons of densities of eggs laid in ovitraps containing oak leaf infusion and water by populations of *Ae. albopictus* at 3 residential sites in Raleigh, NC, in 1996 and 1997

Field trial	No. ovitrap pairs	t	$P > t$	Oak leaf infusion		Water	
				Mean no. eggs/trap (95% CL)[a,b]	Mean % of total eggs (±SE)[c]	Mean no. eggs/trap (95% CL)[a,b]	Mean % of total eggs (±SE)[c]
1×, 7-d-old, 50% infusion							
11-20 Sept.	35	3.94	0.0004	33.8 (19.2–52.6)	69.2 (11.4)	14.7 (8.0–23.2)	30.8 (5.9)
2–25 July	78	4.82	0.0001	33.4 (25.1–42.8)	64.0 (6.9)	19.3 (14.8–24.5)	36.0 (3.3)
4×, 7-d-old, 50% infusion							
15 July–2 Aug.	107	7.31	0.0001	55.6 (46.4–65.7)	64.8 (5.0)	29.7 (24.5–35.4)	35.2 (2.7)
1×, 28-d-old, 80% infusion							
23–29 Sept.	23	4.20	0.0004	82.7 (45.9–130.2)	66.6 (12.7)	38.4 (18.9–64.5)	33.4 (7.7)
1×, 60-d-old, 80% infusion							
30 Sept.-4 Oct.	24	4.17	0.0004	85.9 (54.0–125.2)	65.5 (9.5)	41.7 (23.2–65.4)	34.4 (5.9)

Egg densities were subjected to $\sqrt{(y + 0.5)}$ before statistical analyses were performed.

[a] Means and confidence limits are back-transformed.

[b] Per 2-d period.

[c] Mean percentage of the arithmetic mean number of eggs.

Table 5 Paired comparisons of densities of eggs deposited in ovitraps containing oak leaf infusion and water by populations of *Ae. triseriatus* at a residential site in Raleigh, NC, in 1996 and 1997

Field trial	No. ovitrap pairs	$P > t$	Oak leaf infusion		Water	
			Mean no. eggs/trap (95% CL)[a,b]	Mean % of total eggs (±SE)[c]	Mean no. eggs/trap (95% CL)[a,b]	Mean % of total eggs (±SE)[c]
1×, 7-d-old, 50% infusion						
11–20 Sept.	18	0.41	0.6 (0.0–2.0)	11.8 (8.0)	0.1 (0.0–0.4)	88.2 (76.2)
2–25 July	30	0.45	12.9 (5.6–23.2)	52.2 (13.1)	8.6 (2.3–18.7)	47.8 (17.5)
4×, 7-d-old, 50% infusion						
15 July–2 Aug.	36	0.012	11.7 (5.1–21.0)	73.6 (18.3)	4.5 (2.0–7.9)	26.4 (7.0)
1×, 28-d-old, 80% infusion						
23–29 Sept.	11	0.63	1.4 (0.0–4.7)	36.8 (24.2)	2.3 (0.0–7.8)	63.2 (41.0)
1×, 60-d-old, 80% infusion						
30 Sept.–4 Oct.	12	0.35	3.0 (0.0–9.6)	81.0 (44.5)	0.9 (0.0–2.6)	19.0 (11.4)

Egg densities were subjected to $\sqrt{(y + 0.5)}$ before statistical analyses were performed.
[a] Means and confidence limits are back-transformed.
[b] Per 2-d period.
[c] Mean percentage of the arithmetic mean number of eggs.

Discussion

Laboratory Studies. The bioassay of Corbet and Chadee (1993) was used in our laboratory experiments because of the behavioral tendencies of container-inhabiting *Aedes* mosquitoes to use >1 container for oviposition. This method allowed us to use more than one index in evaluating oviposition responses to infusions—namely, the number or percentage of eggs laid in containers and the number of containers used for oviposition.

Aedes albopictus deposited significantly more eggs in cups containing oak leaf infusion than in cups containing water, regardless of infusion age or concentration. Among all concentrations and ages of infusion tested, the highest percentage of eggs was laid in response to a 60% concentration of the 7-d-old infusion. Because *Ae. albopictus* will oviposit readily in clean water (Gubler 1971), the greater response to the youngest infusion may reflect a propensity to oviposit in containers that recently have been flooded. In contrast, *Ae. triseriatus* deposited the highest percentage of eggs in response to an 80% concentration of 28-d-old infusion and a 60% concentration of the 60-d-old infusion. These results are similar to those of Isoe et al. (1995a) who studied the oviposition responses of *Culex tarsalis* Coquillett and *Culex quinquefasciatus* Say to Bermuda grass infusions. Every age of infusion tested (0–63 d) was significantly more stimulatory to *Cx. quinquefasciatus* than water, whereas only infusions aged 5–25 d were significantly more attractive to *Cx. tarsalis* than water. In our research, *Ae. albopictus* females deposited a significantly larger percentage of their eggs in cups containing infusion regardless of its concentration or age; however, *Ae. triseriatus* females laid significantly more eggs in only a few concentrations of each age of infusion. Although our results for *Ae. triseriatus* were variable, females laid the highest percentage of each clutch of eggs in cups containing older infusions. These findings indicate that for *Ae. triseriatus*, the attractiveness of oak leaf infusions increase with age. This species is collected frequently from tree holes (Loor and DeFoliart 1970) that hold water and organic debris for long periods. Therefore, responses to the 28- and 60-d-old infusion could reflect the adaptation of *Ae. triseriatus* to oviposit in rot cavities in trees.

Oak leaf infusions contained nonvolatile oviposition stimulants. Allan and Kline (1995) reported that under laboratory conditions, *Ae. albopictus* laid significantly more eggs in response to 25 and 75% concentrations of field-collected oak leaf infusion that had contained conspecific larvae and pupae than in well water. In related experiments, using a dual-port olfactometer, they demonstrated that the field water released olfactory attractants. Allan and Kline (1995) stated that because the field-water contained immature *Ae. albopictus*, the presence of immatures (and their associated bacteria), and naturally occurring microorganisms possibly created olfactory attractants or chemostimulants that mediated the oviposition re-

Table 6. Paired comparisons for effects of a visual attractant on the use of ovitraps by *Ae. albopictus* at 3 residential sites in Raleigh, NC, in 1997

Trap condition	n	$P > t$	Mean no. eggs/trap[a,b]	
			Tire (95% CL)	Control (95% CL)
Tire + infusion vs water	60	0.0001	74.5 (59.5–91.1)	37.1 (30.1–44.8)
Tire + water vs infusion	58	0.0001	37.3 (29.7–45.9)	66.0 (49.3–85.1)
Tire + water vs water	59	0.80	30.5 (23.6–38.3)	31.1 (23.8–39.5)

[a] Egg densities (per 2-d period) were subjected to $\sqrt{(y + 0.5)}$ transformations before statistical analyses were performed.
[b] Means and confidence limits are back-transformed.

sponses of gravid females. Benzon and Apperson (1988) showed that bacteria associated with *Ae. aegypti* larvae were attractive to gravid females, presumably through the production of volatile metabolic byproducts. In addition, Bentley et al. (1976) reported that the rearing water of 4th-instar *Ae. triseriatus* contained a volatile chemical that was highly attractive to gravid females. Our laboratory results indicated that if any volatile chemicals were produced during the fermentation of oak leaves, they occurred in insufficient quantities to attract mosquitoes from a distance.

The variable responses of *Ae. triseriatus* to oak leaf infusions may relate to the relatively low number of cups used by ovipositing females. Because our oak leaf infusion contained contact chemostimulants rather than volatile attractants, females probably deposited all or most of their eggs before encountering the infusion. In comparison, *Ae. albopictus* used a larger number of cups for oviposition; consequently, in our laboratory bioassays, there was a higher probability that when the infusion was encountered females had not completed oviposition.

Field Trials. In the preliminary trials, oviposition responses to 28- and 60-d-old oak leaf infusion by gravid *Ae. albopictus* increased markedly from summer to fall. The findings of Hazard et al. (1967) suggest that it is unlikely that high summer temperatures deleteriously affected infusion quality in our experiments, because they produced a heat-stable hay infusion that mediated oviposition by *Ae. aegypti* through contact chemoreception. The lack of an expressed oviposition response to oak leaf infusion in the preliminary field trials simply may have been an artifact of batch-to-batch variability in the fermentation process that resulted in production of infusions which lacked active ingredients. Unfortunately, we did not verify the activity of each batch of infusion through a laboratory bioassay. However, we have found little variation in the oviposition responses of *Ae. albopictus* to different batches of infusion in the laboratory (J.D.T., unpublished data). Seasonal changes in mosquito oviposition response provides another possible explanation for differences in egg densities in infusion-baited ovitraps in summer and early fall. In the fall, as the photoperiod shortens and diapausing eggs are laid (Focks et al. 1994), females may be more responsive to the oak leaf infusion.

Aedes albopictus laid a significantly larger number of eggs in ovitraps that contained all 3 ages of infusion (7, 28, and 60 d) than in water. The relatively greater mean number of eggs found in some infusion-baited traps was the result of an increase in the size of the population of gravid *Ae. albopictus* rather than to a greater oviposition response by females. For all infusions that elicited a significant response, the percent of total eggs deposited in the test ovitrap ranged from 64.0 to 69.2%. These results indicated that all 3 ages of oak leaf infusion were equally attractive to gravid *Ae. albopictus*.

Aedes triseriatus exhibited a highly variable oviposition response to ovitraps baited with infusion that made differences between the mean numbers of eggs deposited in test and control ovitraps statistically insignificant even when the mean percent of eggs deposited in infusion-baited traps was as high as 81%. The low percentage of eggs collected in some infusion-baited ovitraps indicated that *Ae. triseriatus* was repelled by some of the infusions tested. *Ae. triseriatus* also exhibited inconsistent oviposition responses to infusions tested under laboratory conditions. Results of other laboratory studies (Wilton 1968, McDaniel et al. 1976, Beehler et al. 1992) indicated that the color of the oviposition media is an important determinant of oviposition response for *Ae. triseriatus*. Perhaps the black colored oviposition traps that we used dampened the response of gravid *Ae. triseriatus* to the dark colored infusion. Wilton (1968) found that gravid *Ae. triseriatus* were attracted to natural treehole water. Our attempts to produce infusions that were equivalent to treehole water through the fermentation of white oak leaves, generally, were unsuccessful. Chemicals derived or associated with active mosquito breeding may be needed for infusions to elicit a significant oviposition response from *Ae. triseriatus*. Nevertheless, the significant response to 4× infusion in our investigation indicated that the concentration of chemical(s) that mediate oviposition by *Ae. triseriatus* is partially dependent on the amount of leaf material fermented.

Attempts to enhance visually the attractiveness of infusion-baited ovitraps to *Ae. albopictus* by placing a tire behind the ovitrap were not effective. This finding seems counter-intuitive because it has been observed that both *Ae. triseriatus* (Wilton 1968) and *Ae. albopictus* (Gubler 1971) are attracted to dark-colored backgrounds. Container- inhabiting *Aedes* mosquitoes readily will use discarded tires as oviposition sites (Beier et al. 1983, Reiter and Sprenger 1987, Pumpuni and Walker 1989). Attraction to tires as breeding sites probably results from the black coloration of the tires, water vapor emanating from flooded tires, and production of attractants from the breakdown of organic matter accumulated in the tires. Mosquitoes also prefer dark-colored areas as resting sites (Allan et al. 1987), and attraction to tires also may relate to this behavior. In our investigation, the ovitrap containing the infusion consistently received ≈⅔ of the eggs laid in each pair of ovitraps, regardless of whether a tire was placed behind the infusion-baited ovitrap or not.

Oak leaf infusions have been used to enhance the attractiveness of ovitraps to mosquitoes by other researchers. In these field experiments, infusions typically have been created in situ by adding leaf litter collected at the site to ovitraps (Loor and DeFoliart 1969, Holck et al. 1988, Szumlas et al. 1996a). Under these conditions leaf material would continue to ferment in the field or fermentation would be initiated after an unknown period following trap placement. Thus, production of attractants and stimulants would likely vary over time. In our experiments, we created infusions in the laboratory and transported them to the field. Infusions created in the laboratory may exhibit batch-to-batch variability in quality (Beehler et al. 1994). However, laboratory-prepared infusions are uniform within batches, and should elicit more consistent oviposition responses than infusions prepared by fermenting leaves in ovitraps in the field.

Oviposition attractants isolated and identified from Bermuda grass infusions (Millar et al. 1992) are promising mosquito pest management tools for *Culex* mosquitoes (Beehler et al. 1994). The use of specific chemicals in the monitoring of mosquito populations will eliminate the need to create infusions and allow the preparation of precise formulations, thereby eliminating problems related to variation in infusion quality. Consequently, the isolation and identification of specific chemical arrestants/stimulants in our white oak leaf infusions that influence the oviposition response of *Aedes* mosquitoes would appear to merit further research. Our field trials demonstrated that infusions that are active in the laboratory are not necessarily as active in the field, indicating that infusions should be tested experimentally against natural mosquito populations before being used operationally to monitor the activity of gravid mosquitoes. Our current and previous studies of *Ae. albopictus* and *Ae. triseriatus* (Trexler et al. 1997) indicate that these mosquitoes may exhibit markedly different ovipositional responses to environmental cues. Therefore, an understanding of the environmental determinants of oviposition must be obtained before the oviposition behavior of these mosquitoes can be exploited effectively for pest management purposes.

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