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EVALUATION OF CARBON DIOXIDE, 1-OCTEN-3-OL, AND LACTIC ACID AS BAITS IN MOSQUITO MAGNET™ PRO TRAPS FOR *AEDES ALBOPICTUS* IN NORTH CENTRAL FLORIDA[1]

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ABSTRACT. The impact of the attractants 1-octen-3-ol (octenol) and L-lactic acid (Lurex™) on collection of *Aedes albopictus* in suburban backyards was assessed in Mosquito Magnet™ Pro traps. These carbon dioxide-producing traps were additionally baited with commercial formulated lures with octenol, lactic acid, octenol + lactic acid, or no attractant (control) and evaluated in 4 residential sites. Three repetitions of the study resulted in the total collection of 1,321 *Ae. albopictus*. Significantly more *Ae. albopictus* were captured in traps baited with octenol + lactic acid than in traps baited only with octenol. Lactic acid-baited and control trap captures were not significantly different from octenol + lactic acid- or octenol-baited trap totals. Octenol- + lactic acid-baited traps collected 36.2% and 52.0% more *Ae. albopictus* than lactic acid-baited and control traps, respectively. Male *Ae. albopictus* accounted for 26.7% of the total capture. Other mosquito species collected in sufficient numbers for analysis included *Cx. nigripalpus*, *Ochlerotatus infirmatus*, *Psorophora ferox*, and *Cx. erraticus*. Larger numbers of these species were collected in traps that were unbaited or baited with only octenol than in traps baited with lactic acid or octenol + lactic acid.

KEY WORDS *Aedes albopictus*, attractants, Mosquito Magnet™ Pro trap, carbon dioxide, octenol, lactic acid

INTRODUCTION

The introduced mosquito *Aedes albopictus* (Skuse) is one of the most prevalent nuisance mosquitoes associated with suburban environments in the southern and midwestern United States. In a recent Centers for Disease Control and Prevention (CDC)-sponsored national mosquito survey of vector control and public health personnel, *Ae. albopictus* was ranked 2nd only to *Ae. vexans* as the most troublesome mosquito in America (McKnight, personal communication). The 1st established North American population of *Ae. albopictus* was discovered in Houston, TX, in 1985 (Sprenger and Wuithiranyagool 1986). Since its introduction, *Ae. albopictus* has increased its range in the southeastern and central portions of the United States from 911 counties in 25 states in 1999 (Moore 1999) to 1,035 counties in 32 states as of December 2004 (McAllister, personal communication), including California (Linthicum et al. 2003).

Aedes albopictus is well adapted for breeding in artificial containers in and around households; quickly establishes large populations; effectively colonizes rural, forested, and unpopulated areas; and is very difficult to control. This species thrives in temperate and tropical climates similar to those in Asia where it originated (Hawley 1988) and may further expand its range within the United States. This expansion is of concern because *Ae. albopictus* is a competent vector of disease agents such as dengue viruses (Calisher et al. 1981) and been reported with infections of eastern equine encephalitis virus (Mitchell et al. 1992), West Nile virus (Holick et al. 2002), and La Crosse encephalitis virus (Gerhardt et al. 2001) within the United States. Distribution and population assessments are difficult because this species is not readily attracted to standard mosquito surveillance light traps (Thurman and Thurman 1955, Service 1993).

Several models of residential mosquito traps have within the last 10 years become available to the public as an aid for control of biting insects. Some traps have manufacturer's claims that use of the traps will keep mosquito populations below nuisance levels with protection of as much as 1.25 acres per trap. Homeowner mosquito trapping offers advantages over traditional chemical control methods, including reduction of biting insects (i.e., reduction of insect bites) on their immediate premises, control without pesticide use, continuous as opposed to periodic control, and the potential for reduced exposure to infected vectors. In addition, many of these traps are easy to operate and work independently for 2–3 wk. Some models offer mosquito attractants in gel- or matrix-formulated cartridges.

Although numerous chemical compounds have been screened as attractants for biting female

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mosquitoes, few are considered strong attractants based on both laboratory and field tests. The most attractant compounds include carbon dioxide (CO_2) (Gillies 1980, Mboera and Takken 1997), 1-octen-3-ol (octenol) (Kline et al. 1991a, 1991b; Kline 1994b; Kline and Mann 1998), lactic acid (Acree et al. 1968, Kline et al. 1990, Bernier et al. 2003), phenols and butanone (Kline et al. 1990), acetic acid (Vale and Hall 1985), and several amino acids (Brown and Carmichael 1961, Roessler and Brown 1964). These compounds are derived, in part, from the physiological processes of respiration, perspiration, and waste elimination in mammals, birds, and reptiles. Attractants commercially developed for use with mosquito traps include CO_2, octenol, and lactic acid.

Carbon dioxide is considered a universal attractant for hematophagous insects, especially mosquitoes (Kline 1994a) and has been used extensively to enhance capture rates in many field studies (Gillies 1980). Octenol was identified from ruminant breath (Hall et al. 1984) and is recognized as an attractant because it greatly increased trap capture of tsetse flies in East Africa (Vale and Hall 1985). Early field tests identified octenol as an attractant alone or in combination with CO_2 for certain mosquito species of *Ochlerotatus*, *Aedes*, *Anopheles*, *Psorophora*, *Coquillettidia*, *Mansonia*, and *Wyeomyia* (Takken and Kline 1989, Kline et al. 1991a, Kline 1994b). Lactic acid, a component of sweat, has increased trap captures of some *Ochlerotatus*, *Anopheles*, and *Culex* mosquitoes when combined with CO_2 (Stryker and Young 1970, Kline et al. 1990).

Octenol and L(+)-lactic acid are commercially available as mosquito trap supplements. The response of *Ae. albopictus* to these compounds in the field is not well documented, and there are no reports pertaining to lactic acid. Shone et al. (2003) used Fay-Prince traps supplemented with CO_2 and octenol to capture *Ae. albopictus* in Maryland. They found that traps baited with CO_2 alone or in combination with octenol attracted significantly more *Ae. albopictus* than traps lacking CO_2 or baited only with octenol. No significant difference in trap capture was seen between octenol + CO_2 and CO_2-only–baited traps. Dennett et al. (2004) also obtained higher collections of *Ae. albopictus* from an octenol-baited Mosquito Magnet™ Liberty trap as opposed to other unbaited traps from a tire repository in Houston, TX. Conversely, an octenol-baited Mosquito Deleto™ trap caught the smallest number of *Ae. albopictus* in that study. Similarly, observations from field trials in Hawaii indicate that octenol may depress capture rates of *Ae. albopictus* (Bedard, personal communication).

Little is known about the effect of lactic acid on attraction of *Ae. albopictus* because previous studies focused only on *Aedes aegypti* (Davis and Sokolove 1976, Bernier et al. 2003). Our objective was to determine the effect of baiting traps that produce carbon dioxide with commercial lures containing octenol, lactic acid, both, or neither on collection of *Ae. albopictus* in suburban settings and to monitor landing collections over the course of this trial for a possible reduction due to trapping. The effects of these treatments on other mosquito species trapped during our study are included.

MATERIALS AND METHODS

Field trials were conducted using the Mosquito Magnet Pro trap (American Biophysics Corporation, North Kingstown, RI) in 4 suburban neighborhoods in Gainesville, FL. Sites were selected based on homeowner complaints of nuisance populations of biting mosquitoes and initial surveys confirmed the presence of *Ae. albopictus*. All sites consisted of a mixture of pine and hardwood trees with sparse undergrowth. Of these sites, one was planted extensively with bromeliads, including *Neoregelra* (red finger nail), *Bilbergia pyramidalis*, and *Bilbergia* spp.; another site had lesser numbers of *Neoregelra* bromeliads. Tank bromeliads are excellent breeding sources for *Ae. albopictus* (O'Meara et al. 1995). Trapping was conducted in three trials from 13 to 21 August, 25 August to 2 September, and 9 to 17 September 2003. Traps were placed in shaded areas under trees or just inside a tree line next to open spaces.

The Mosquito Magnet Pro trap was chosen based on positive collection results with *Ae. albopictus* in previous Hawaiian field investigations (McKenzie, personal communication). This trap uses counterflow geometry technology and produces warm, moist air, and CO_2 at the rate of ~520 ml/min.

Aedes albopictus mosquitoes are diurnally active with a bimodal feeding habit during daylight (Hawley 1988). A 48-h trapping interval was selected to allow a continuous 24- period with no interruption of diurnal activity. A trapping period occurred between 0800 and 1200 h and ended ~48 h afterward. Entire traps were rotated between sites because octenol can leave a residual odor on the trap that may bias trap performance across treatments. Preventive maintenance on traps was performed at the start of each trial by clearing the combustion chamber with compressed CO_2 gas following manufacturer's recommendations and by removing debris from inside of the trap collecting tube and net chamber with a moist sponge.

Treatments, all of which produced equivalent quantities of carbon dioxide, included a control trap (no lures), a trap baited with only octenol, a trap baited with only lactic acid, and a trap

Table 1. Numbers (mean ± SE)[1] of mosquitoes collected in a trap period (48 h) in Mosquito Magnet™ Pro traps producing carbon dioxide and baited with lures containing lactic acid or octenol.

Species	Lactic acid	Octenol	Lactic + octenol	Control (no lure)	P
Aedes albopictus[2]	27.8 ± 7.5ab	17.8 ± 4.4b	45.6 ± 10.8a	20.9 ± 4.0ab	0.0290
Anopheles crucians s.l.	0.0 ± 0.0a	0.6 ± 0.6a	0.1 ± 0.1a	0.3 ± 0.3a	0.6700
Coquillettidia perturbans	0.0 ± 0.0a	0.8 ± 0.3a	0.8 ± 0.6a	0.1 ± 0.1a	0.0400
Culex erraticus	0.5 ± 0.2a	5.6 ± 3.8a	1.9 ± 0.8a	2.8 ± 1.1a	0.0800
Cx. nigripalpus	14.3 ± 5.4b	106.8 ± 33.7a	29.5 ± 17.4b	173.3 ± 68.1a	0.0001
Ochlerotatus atlanticus	0.4 ± 0.2a	1.9 ± 0.1a	0.5 ± 0.2a	2.6 ± 1.1a	0.1100
Oc. infirmatus[3]	5.5 ± 2.1b	14.8 ± 3.0a	7.2 ± 2.6b	19.8 ± 5.2a	0.0003
Oc. triseriatus	0.4 ± 0.2a	1.1 ± 0.5a	0.9 ± 0.4a	1.3 ± 0.6a	0.4800
Psorophora columbiae	0.0 ± 0.0a	1.2 ± 0.7a	0.1 ± 0.1a	0.8 ± 0.7a	0.1200
Ps. ferox[2,4]	0.5 ± 0.3c	3.2 ± 1.3ab	1.1 ± 0.6bc	7.5 ± 3.3a	0.0001
Wyeomyia mitchellii	0.6 ± 0.4a	0.8 ± 0.5a	0.3 ± 0.2a	1.9 ± 1.4a	0.6300
Culex spp.	1.7 ± 0.1a	2.8 ± 1.5a	1.9 ± 1.3a	1.9 ± 0.6a	0.8100
Psorophora spp.	1.2 ± 0.7b	3.5 ± 1.3ab	1.8 ± 1.3b	9.3 ± 3.4a	0.0007

[1] Means within each row followed by the same letter are not significantly different (Ryan–Einot–Gabriel–Welsh multiple range test). $n = 12$ trap periods (48 h).
[2] Significant position effect ($P < 0.05$).
[3] Adults could not be distinguished from *Oc. tormenter*.
[4] Significant period effect ($P < 0.05$).

baited with both octenol + lactic acid. Commercial lures containing octenol and lactic acid were provided by American Biophysics Corporation. The lactic acid cartridges (Lurex™) contained 4.88 g of lactic acid embedded into a 13.8-g clear gelatin matrix sealed in a plastic package that releases lactic acid over 3 wk at ~230 mg/day. This lactic acid release rate is apparently below the repellency threshold of *Ae. albopictus* (Davis and Sokolove 1976). Octenol cartridges released 1.66 g of octenol from a microporous polyethylene block over 3 wk at 80°F (26.7°C) (79 mg/day). All cartridges were replaced at the beginning of each trial (after 8 days of use).

Efficacy of traps on biting activity in the vicinity of the traps was assessed during the trials with landing counts of female *Ae. albopictus*. Collections were made before traps were rotated and included mosquitoes that landed on exposed legs during a 3-min period by using a hand-held flashlight aspirator (Hausherr's Machine Works, Toms River, NJ). All landing collections were made within a 1-acre radius of the trap (within 36 m of the trap). Mosquitoes were anesthetized with carbon dioxide and transferred to labeled paper cups (Solo Cup Company, Urbana, IL) for later identification to species by using Darsie and Morris (2000).

A 4 × 4 Latin square design was used for each of the 3 trials with trap location randomized between trials. Collections of *Ae. albopictus* were analyzed for treatment, site, and collection period (=48 h) effect by using a 3-way analysis of variance (ANOVA) (SAS Institute 2001). The Ryan–Einot–Gabriel–Welsh (REGW) multiple range test was used to identify significant differences between treatments ($P < 0.05$). Other mosquito species were then analyzed for treatment, site, and period effects by using a 3-way ANOVA and multiple comparisons with the REGW multiple range test ($P < 0.05$). All capture data were transformed with $\log_{10}(x + 1)$ before analysis.

RESULTS

Eleven species were collected in the traps (Table 1). In total, 6,787 mosquitoes were collected during the 12 trap periods with *Ae. albopictus* making up 19.5% of the catch (1,321). Collections of *Ae. albopictus* differed significantly between treatments ($F = 3.44$; df = 3, 30; $P = 0.029$) and collection sites ($F = 5.69$; df = 3, 30; $P = 0.003$). The collection site planted extensively in tank bromeliads yielded 40.2% (531) of all *Ae. albopictus* collected among the 4 sites. Collections of *Ae. albopictus* were largest from traps baited with lactic acid and octenol (45.6 ± 10.8), and the lowest collections were from traps baited with octenol only (17.8 ± 4.4). Moderate collections were obtained in traps baited with lactic acid alone (27.8 ± 7.5) and the control trap (20.9 ± 4.0), although these collections did not differ significantly from traps baited with octenol alone or octenol and lactic acid (Table 1).

Significantly more *Culex nigripalpus* Theobald ($F = 14.89$; df = 3, 30; $P = 0.0001$) were caught in octenol-baited and unbaited traps than in lactic acid-baited and octenol- + lactic acid-baited traps. A similar pattern was observed with *Ochlerotatus infirmatus* (Dyar and Knab) ($F = 8.41$; df = 3, 30; $P = 0.0003$). Collections of *Psorophora ferox* (von Humboldt) also differed among treatments with the largest collection in control traps ($F = 10.95$; df = 3, 30; $P = 0.001$).

The percentage of male and female *Ae. albopictus* collected in traps was similar for all

Table 2. Collection over the 3 trials of *Aedes albopictus* in Mosquito Magnet™ Pro traps producing carbon dioxide and baited with lures producing lactic acid or octenol.

	Mean (±SE)[1] collected/trap period			
Treatment	Trial 1	Trial 2	Trial 3	*P*
Lactic acid	49.7 (15.1)	23.2 (10.2)	10.5 (2.7)	0.06
Octenol	27.2 (8.9)	14.7 (9.1)	11.2 (3.6)	0.33
Octenol + lactic acid	80.0 (19.6)	21.5 (12.9)	29.2 (8.1)	0.03
Control	28.2 (9.4)	9.75 (3.3)	24.7 (2.6)	0.12

[1] Means compared by ANOVA ($P > 0.05$).

attractants. Traps baited with lactic acid were made up of 23.8% females and 29.4% males, whereas those baited with lactic acid + octenol contained 39.3% females and 39.6% males. Traps baited with octenol had 16.2% and 15.0% females and males, respectively, whereas control traps captured 20.8% females and 14.2% males.

The collection of *Ae. albopictus* over the 3 trials differed significantly for the octenol + lactic acid treatment. An ~5-fold reduction was seen with the lactic acid and control treatments (Table 2). No significant reduction was seen for the octenol and control treatments that captured the fewest *Ae. albopictus*. The landing activity of *Ae. albopictus* during this these trials remained stable. Three-minute landing collection totals of *Ae. albopictus* from trap sites on most collection days during these trials averaged 3.8 ± 0.7, 5.0 ± 0.4, and 3.5 ± 1.1 for trials 1, 2, and 3, respectively, and these differences were not significant ($F = 0.83$; $df = 2, 30$; $P = 0.49$).

Table 1 lists the other mosquito species collected. In descending order, *Cx. nigripalpus*, *Ae. albopictus*, *Oc. infirmatus*, *Ps. ferox*, and *Cx. erraticus* were the 5 most abundant mosquitoes collected. Many *Psorophora* mosquitoes (188) were excluded from analysis because specimens were damaged and identification was impossible. It is likely that most of these specimens were *Ps. ferox*, but 24 *Ps. columbiae*, 17 *Ps. howardii* Coquillett, and a *Ps. ciliata* F. also were collected. Excluding *Ae. albopictus*, a small proportion of collections consisted of males (0.5% = 27 specimens).

DISCUSSION

Residential mosquito traps are promising for capture of *Ae. albopictus* (Smith et al. 2002, Smith and Walsh 2003, Dennett et al. 2004), especially with the addition of commercially formulated slow-release mosquito attractants. Two products contain either octenol or lactic acid. Our results indicated that the use of the lactic acid and octenol lures in the presence of CO_2 in Mosquito Magnet Pro traps resulted in higher collections than in traps with octenol lures only. Neither collection differed significantly from the control CO_2-baited Mosquito Magnet Pro traps. A similar trend was seen in field observations in Hawaii in which octenol-baited Mosquito Magnet Pro traps collected fewer *Ae. albopictus* than unbaited traps (Bedard, personal communication). In contrast, Dennett et al. (2004) trapped significantly more *Ae. albopictus* from an octenol-baited Mosquito Magnet Liberty trap than any of 6 other trap types; however, an unbaited Liberty trap was not used for comparison. Control traps that used only CO_2 as an attractant collected similar numbers of *Ae. albopictus* as well as other mosquito species as octenol + CO_2-baited traps in our study. Using CDC light traps, Vythilingam et al. (1992) collected twice as many *Ae. albopictus* in CO_2-baited traps than in octenol + CO_2-baited traps, although trap totals were small and trap mean differences were not significant. No octenol flow rate was provided.

Lactic acid is a known attractant for *Ae. aegypti* (Acree et al. 1968), which have lactic acid-sensitive receptors on their antennae (Davis and Sokolove 1976). In dual-port olfactometer studies, lactic acid + acetone blends were as attractive as lactic acid + CO_2 blends (Bernier et al. 2003). Few reports exist concerning the effects of lactic acid on attraction of *Ae. albopictus*. In a field study in Japan, lactic acid-baited traps were no more attractive to *Ae. albopictus* than unbaited traps (Kusakabe and Ikeshoji 1990); however, release rates were not provided. At high skin surface concentrations (>41.7 ppm), lactic acid was repellent to *Ae. albopictus* (Shirai et al. 2001).

The use of blends of 2 or 3 attractants often increases trap collections more than the use of 1 attractant alone (Gillies 1980, Kline et al. 1990, Lehane 1991, Bernier et al. 2003). In our study, the addition of lactic acid to octenol lures in the presence of CO_2 increased collections of *Ae. albopictus*. Based on these results, surveillance or population reduction efforts targeting *Ae. albopictus* with residential traps could be improved when using traps baited with octenol by adding lactic acid. The majority of all male and female *Ae. albopictus* were collected in octenol + lactic acid- or lactic acid-baited traps (64.9%). The use of octenol alone seemed to depress collections of *Ae. albopictus*, whereas the addition of lactic acid baits to octenol-baited traps had a synergistic effect on trap collections. Likewise, *Ae. aegypti* responds positively to low concentrations of lactic acid (10 µg) (Acree et al. 1968), and it seems that *Ae. albopictus* is lactic acid sensitive as well. A much higher percentage of male *Ae. albopictus* were collected (26.7% of total collection) compared with males of other species (<1.0%), possibly due to a limited flight range in this species (Bonnet and Worcester 1946), the attraction of males to hosts (Gubler and Bhattacharya 1972), or the wingbeat frequency of females (Kanda et al. 1987, Ikeshoji and Yap 1990).

The most abundant species captured in this study was *Cx. nigripalpus*, and collections were significantly affected by the attractants. Control and octenol-baited traps collected significantly more *Cx. nigripalpus* than did traps baited with lactic acid. The 2 traps baited with lactic acid accounted for 13.5% of the total number collected, indicating that lactic acid may have negatively affected *Cx. nigripalpus* collections. Kline and Mann (1998) noted a similar reduction of *Cx. nigripalpus* captures in north Florida with octenol- + CO_2-baited CDC light traps. In the Florida Everglades, Kline et al. (1990) trapped similar numbers of *Cx. nigripalpus* in octenol- + CO_2-baited and CO_2-baited CDC light traps. These results indicate that octenol and lactic acid do not contribute to collection of *Cx. nigripalpus*. Smaller numbers of *Cx. erraticus* were collected in this study with no significant differences among attractants. Collections in octenol-baited traps were about 2-fold greater than to other traps. Similarly, Kline et al. (1991a) reported larger numbers of *Cx. erraticus* collected in octenol- + CO_2-baited traps than in CO_2-baited traps; however, differences were not significant.

Ochlerotatus infirmatus was an abundant species with significantly larger numbers in control (42%) and octenol-baited traps (31%) than in octenol- + lactic acid- or lactic acid-baited traps. The 2 traps baited with lactic acid captured <30% of collections of this species, indicating that lactic acid may be somewhat repellent. In a study on attraction of octenol and CO_2, Kline and Mann (1998) collected more *Oc. infirmatus* in CDC light traps baited with octenol + CO_2 than with CO_2 or octenol. Although it seems that the addition of octenol to CO_2-baited traps does not adversely affect *Oc. infirmatus* captures, lactic acid baits should be avoided if this species is targeted for capture.

Collections of *Ps. ferox* and other *Psorophora* species differed significantly among treatments, with the largest collection of *Ps. ferox* from the control traps. The addition of lures seemed to decrease collections of *Psorophora* species. Kline (1994b) reported that octenol + CO_2 had an increased synergistic effect on *Psorophora* spp. capture rates compared with octenol alone. Kline et al. (1991a) caught 3 times more *Ps. columbiae* in unlit CDC light traps baited with octenol + CO_2 than in CO_2-baited traps. Their results differ from ours with *Ps. ferox*, and this discrepancy may be related to differences in host preference between the species. Based on these data, control treatments (CO_2 alone) are best if targeting *Ps. ferox*.

There was a reduction of *Ae. albopictus* in yards with certain lures. Collections of *Ae. albopictus* from octenol- + lactic acid-baited traps decreased 4-fold; however, there were no significant reductions in yards with control traps or traps baited with lactic acid or octenol over the course of the 3 trials. During this same interval, *Ae. albopictus* collections in CDC light traps from 6 locations in Gainesville increased slightly, so that decreased catches in the octenol + lactic acid treatment could not be attributed to a declining source population. The decreases may indicate a reduction in backyard populations in those yards with the octenol + lactic acid baits. Landing rates of *Ae. albopictus* at these sites remained steady despite the presence of baited Mosquito Magnet Pro traps suggesting that humans are still preferred over baited traps. This preference may be due in part to the high attractancy of mosquitoes to moving targets (humans) compared to stationary targets (mechanical traps). Fieldworkers in an aedine mosquito-infested forest suspected that their movements initiated attack (Allan et al. 1987), and Sippel and Brown (1953) noted that transparent containers with anesthetized mice were 3.7 times less attractive to *Ae. aegypti* than containers with active, moving mice. The addition of movement in human collectors is an added attractancy factor that Mosquito Magnet Pro traps do not provide. However, Mosquito Magnet traps operate continuously and therefore constantly remove mosquitoes even when other competitive sources are not present.

Use of lactic acid + octenol with the CO_2-emitting Mosquito Magnet Pro traps resulted in more *Ae. albopictus* collected than with the octenol-baited traps. However, neither treatment differed significantly from traps without either attractant. Further efficacy studies using lactic acid-baited traps would be useful in determining its impact on suburban and woodland mosquito populations.

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