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SCIENTIFIC NOTE

EFFICACY OF THREE ATTRACTANT BLENDS TESTED IN COMBINATION WITH CARBON DIOXIDE AGAINST NATURAL POPULATIONS OF MOSQUITOES AND BITING FLIES AT THE LOWER SUWANNEE WILDLIFE REFUGE

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ABSTRACT. Synthetic blends of chemicals identified previously from human skin emanations were evaluated against mosquito and biting fly populations at the Lower Suwannee Wildlife Refuge near Cedar Key, FL. Mosquito Magnet™-Experimental traps were baited with the Red (400 ml acetone:10 ml 1-hexen-3-ol:10 ml 1-octen-3-ol), Blue (400 ml acetone:1 g/liter lactic acid:20 ml glycolic acid), or Green blend (400 ml acetone:1.5 g/liter lactic acid:20 ml dimethyl disulfide) plus CO_2 or with CO_2 alone (control). A relative index of efficacy was determined by dividing each mean blend trap catch by the mean control trap catch. Five mosquitoes (*Aedes infirmatus*, *Ae. taeniorhynchus*, *Ae. triseriatus*, *Anopheles crucians*, and *Culex nigripalpus*), 2 ceratopogonid (*Culicoides floridensis* and *C. furens*), and 1 tabanid (*Diachlorus ferrugatus*) and phlebotomine (*Lutzomyia shannoni*) species were trapped. The Red blend + CO_2 treatment significantly increased collections of *Ae. taeniorhynchus* (3.4×), *An. crucians* (2.8×), total mosquitoes (2.7×), *C. furens* (17.6×), and *L. shannoni* (10.8×) compared with control traps. Traps baited with either the Blue or Green blends generally captured fewer insects than traps baited with the other 2 treatments. However, traps baited with the Green blend caught 7× as many *C. furens* as the control traps. Responses clearly varied according to species; therefore, "one size does not fit all" when it comes to attractant blends.

KEY WORDS *Aedes taeniorhynchus*, *Culex nigripalpus*, *Culicoides*, *Diachlorus ferrugatus*, *Lutzomyia shannoni*

Synthetic blends of chemicals have been evaluated against mosquitoes and other biting insects under laboratory and field conditions. From observations made in a dual-choice olfactometer, Acree et al. (1968) determined that L-lactic acid attracted female *Aedes aegypti* (L.), but the attraction was not great. In subsequent trials, Schreck and James (1968) demonstrated that CO_2 activated the flight of *Ae. aegypti* females toward an odor source. Smith et al. (1970) produced a strong synergistic attraction response by using L-lactic acid in combination with CO_2. Schreck et al. (1981, 1990) showed that unidentified human skin emanations play a role in the attraction of female *Ae. aegypti* to humans, which could explain the differential attraction among individuals. Using purge and trap gas chromatography/mass spectrometry, >300 compounds from human skin were identified (Bernier et al. 2000, 2002). Twenty-six compounds were of background origin, leaving 277 as candidate *Ae. aegypti* attractants. Carboxylic and lactic acids were present in the highest amounts in the moderately volatile emanations (Bernier et al. 2000). Additional studies with more volatile components revealed that acetone was the most abundant component emanating from the human hand (Bernier et al. 2007b).

In triple-cage, dual-port, dual-choice olfactometer studies, acetone, 1-hexen-3-ol (hexenol), glycolic acid, acetone, dimethyl disulfide (DMDS), and lactic acid produced the best attractant responses in female *Ae. aegypti* (Posey et al. 1998, Bernier et al. 2001). Bernier et al. (2003) found that binary combinations of these chemicals were very attractive to mosquitoes, and that several binary and tertiary blends (e.g., acetone + DMDS + lactic acid) were highly attractive, even without CO_2 (Bernier et al. 2007a).

Earlier studies have also shown 1-octen-3-ol (octenol) as a promising attractant for mosquitoes in a variety of habitats (Takken and Kline 1989; Takken 1991; Kline et al. 1990a, 1990b, 1991a, 1991b), biting midges (Kline et al. 1994), and tabanids (French and Kline 1989); and that an octenol/CO_2 combination attracted some species more efficiently than others. In olfactometer trials, hexenol and octenol combined produced good (ca. 50%) attractant responses in female *Ae. aegypti* and *Anopheles albimanus* Wiedemann, but very poor (<10%) in *Culex quinquefasciatus* Say. Responses by *Ae. aegypti* were greater to the combination than to either compound alone. Very good responses (>80%) were also observed in to blends consisting of either glycolic acid + acetone, or glycolic acid +

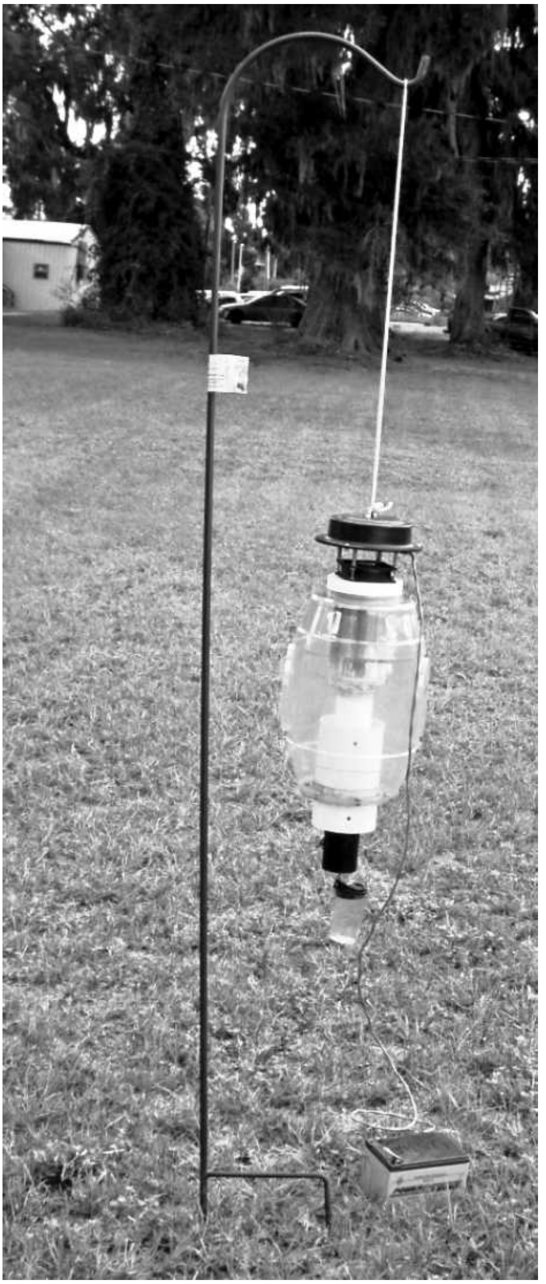

Fig. 1. Mosquito Magnet™-Experimental trap with jar of blend suspended near the carbon dioxide outlet port.

acetone + lactic acid (Kline, unpublished data). Responses to compounds/blends in laboratory olfactometer studies may differ from those of field populations of mosquitoes or biting flies. The present field study was carried out to determine if traps baited with blends of selected compounds from human skin emanations in combination with CO_2 would capture greater numbers of mosquitoes and/or biting flies than traps baited with CO_2 alone.

Experiments were conducted at the Lower Suwannee Wildlife Refuge, near Cedar Key, FL, with natural populations of salt-marsh and woodland mosquitoes and biting flies. This ca. 21,448-ha (53,000-acre) refuge contains a diversity of habitats that provide numerous developmental sites for a large variety of target species. Four Mosquito Magnet-Experimental (MM-X; American Biophysics Corp., North Kingston, RI) counterflow geometry traps, which are not sold commercially, were used to evaluate our candidate blends. Counterflow technology utilizes an outgoing airflow to disseminate attractive lures and an incoming airflow to suck insects into a collecting chamber (Kline 1999). Trap fans were powered by rechargeable 12-V batteries. The CO_2 was supplied by 9-kg compressed-gas cylinders equipped with FLOWSET1 pressure regulators (Clarke, Roselle, IL) with a fixed output of 15 psi, a 10-µm line filter, and a 0.007 in-line orifice, which maintained the flow rate at 500 ml/min. Polythene tubing (outer diam: 8 mm) equipped with quick-connect lure fittings at both ends connected the regulator assembly with the MM-X trap.

Three candidate blends were designated as Red, Blue, or Green to provide a blind test. The Red blend consisted of 400 ml acetone, 10 ml 1-hexen-3-ol, and 10 ml 1-octen-3-ol; the Blue blend consisted of 400 ml acetone, 1 g/liter lactic acid, and 20 ml glycolic acid; the Green blend consisted of 400 ml acetone, 1.5 g/liter lactic acid, and 20 ml DMDS. The L-lactic acid (99% purity) was purchased from Fluka (Milwaukee, WI). Remaining reagent-grade chemicals (≥97% purity) were purchased from Sigma-Aldrich (Milwaukee, WI). Blends were released from Quorpak® (Bridgeville, PA) 120-ml clear glass jars (40-mm mouth opening, lids removed) suspended below the traps near the CO_2 outlet (Fig. 1). Blends in Quorpak jars were brought up to the 120-ml level after daily insect removal.

The experimental design was a 4 × 4 Latin square and 4 treatments were evaluated. Three traps were baited with 500 ml/min CO_2 plus 1 of 3 blends; 1 trap served as a control and was baited only with 500 ml/min CO_2. The MM-X traps were placed ca. 160 m apart along a dirt road that transected a mixture of bottomland hardwood swamp and salt-marsh habitats. Traps were suspended from shepherd's hooks so that the lowest point of each trap was ca. 15–20 cm above the ground. Traps were operated continuously for ca. 23 h each trapping day, beginning at 2 h before sunset. On the 1st night of testing, the treatments were randomly assigned to trap stations. On subsequent nights, the treatments were rotated to the next station until all treatments were tested at each station.

Table 1. Mean[1] (± SE) and index[2] of insect species captured with blend treatments 1–4 ($n = 4$).

Insects	Control treatment 1 (CO_2 alone)	Red Blend treatment 2 (+ CO_2)	Red Blend treatment 2 Index	Blue Blend treatment 3 (+ CO_2)	Blue Blend treatment 3 Index	Green Blend treatment 4 (+ CO_2)	Green Blend treatment 4 Index
Aedes infirmatus	32.3 ± 1.5ab	66.1 ± 1.3a	2.04	24.7 ± 1.8b	0.76	20.9 ± 1.7b	0.64
Ae. taeniorhynchus	249.2 ± 1.3b	838.1 ± 1.1a	3.36	139.5 ± 1.5b	0.56	207.1 ± 1.1b	0.84
Ae. triseriatus	2.2 ± 1.6a	4.3 ± 1.9a	2.93	2.5 ± 1.1a	1.11	2.0 ± 1.3a	0.90
Anopheles crucians	55.0 ± 1.6b	155.4 ± 1.6a	2.82	30.5 ± 2.0b	0.55	48.2 ± 2.1b	0.88
Culex nigripalpus	55.2 ± 1.6a	16.9 ± 1.4b	0.30	11.0 ± 1.7b	0.20	10.0 ± 1.9b	0.18
Total mosquitoes	431.4 ± 1.3b	1177.9 ± 1.1a	2.73	222.8 ± 1.6c	0.52	330.0 ± 1.3bc	0.74
Culicoides floridensis	468.1 ± 1.7a	234.0 ± 2.0a	0.49	363.9 ± 2.5a	0.78	293.5 ± 1.8a	0.63
C. furens	16.2 ± 2.3b	285.8 ± 2.6a	17.64	17.0 ± 1.8b	1.05	114.2 ± 1.5ab	7.05
Diachlorus ferrugatus	14.0 ± 1.4a	37.1 ± 1.5a	2.66	10.4 ± 1.3a	0.75	11.5 ± 2.4a	0.82
Lutzomyia shannoni	16.4 ± 2.0b	176.9 ± 1.5a	10.81	10.1 ± 2.3b	0.62	8.2 ± 1.7b	0.50

[1] Means in rows followed by the same letter are not significantly different ($P < 0.05$; Ryan–Einot–Gabriel–Welsch multiple range test [SAS/STAT user's guide, version 9.2; SAS Institute, Cary, NC]).
[2] Index = Blend treatment mean/Control treatment mean.

Data were subjected to the General Linear Models procedure to determine the effects of treatment (blend), trap location, and trap day on numbers of insects captured. Means were separated with the Ryan–Einot–Gabriel–Welsh multiple range test (SAS/STAT user's guide, version 9.2.; SAS Institute, Cary, NC) and, unless otherwise stated, $P = 0.05$. Insect capture data were transformed with $\log_{10}(n + 1)$ prior to analysis but back-transformed numbers are shown in text and tables. A relative index of efficacy was calculated by dividing the mean trap catch for each blend by the mean control trap catch.

Five mosquito species (*Ae. infirmatus* Dyar and Knab, *Ae. taeniorhynchus* (Wiedemann), *Ae. triseriatus* (Say), *An. crucians* Wiedemann, and *Cx. nigripalpus* Theobald), 2 ceratopogonid species (*Culicoides floridensis* Beck and *C. furens* (Poey)), and 1 tabanid (*Diachlorus ferrugatus* (Fabricius)) and 1 phlebotomine species (*Lutzomyia shannoni* Dyar) were caught (Table 1). The mean numbers of *Ae. taeniorhynchus*, *An. crucians*, total mosquitoes, *C. furens*, and *L. shannoni* captured with the Red blend + CO_2 were significantly greater than those captured with CO_2 alone. The Red blend + CO_2 increased mean captures of *Ae. infirmatus* (2.04×), *Ae. triseriatus* (2.04×), and *D. ferrugatus* (2.66×) compared with CO_2 alone, but results were not significant. Mean numbers of *Cx. nigripalpus* captured with the Red blend + CO_2 were significantly smaller than those captured with CO_2 alone, but the collection size of *C. floridensis* was numerically but not significantly smaller. Traps baited with the Red blend + CO_2 caught statistically more of all individual species and total mosquitoes, except *Ae. triseriatus*, *Cx. nigripalpus*, *C. floridensis,* and *D. ferrugatus* when compared with traps baited with the Blue blend + CO_2. Only *C. floridensis* was caught in numerically but not significantly greater numbers with the Blue (363.9) compared with the Red (234.0) blend + CO_2. When traps baited with the Red blend + CO_2 and the Green blend + CO_2 were compared, trends were similar to those observed with the Red versus Blue blend comparison. However, mean numbers of *C. furens* captured with the Green blend + CO_2 and the Red blend + CO_2 were not significantly different. Mean numbers of insects captured with the Blue and Green blends + CO_2 were not significantly different. Baiting the traps with any of the 3 blends statistically reduced collections of *Cx. nigripalpus* when compared with traps baited CO_2 alone. Also, traps baited with the Blue blend + CO_2 captured statistically fewer total mosquitoes than traps baited with the Red blend + CO_2 or CO_2 alone.

Data from the present study show that compounds used in blends may either synergize or antagonize each other's activity in the presence of CO_2 at the behavioral response level. The response can vary according to species. For all species except *Cx. nigripalpus*, trap collections were increased when the Red blend + CO_2 was used, compared with traps baited with CO_2 alone. For most species, collections were reduced when the Blue or Green blend was used. The Blue blend had a neutral effect on *Ae. triseriatus* and *C. furens*. Blend components alone and in various combinations produced high attractive responses by *Ae. aegypti* in olfactometer studies (Bernier et al. 2001, 2003). A combination of hexenol + octenol (without acetone or CO_2) was the only blend tested against *Cx. quinquefasciatus*, showing a very poor response (Bernier, unpublished data). This was supported by Mboera et al. (2000). Very good attractive responses (>80%) by *Ae. aegypti* to blends consisting of either glycolic acid + acetone, or glycolic acid + acetone + lactic acid (Bernier and Kline, unpublished data) were also documented with *Ae. albopictus* (Skuse) (Bernier et al. 2001).

Of particular interest is the performance of traps baited with the Red blend. As previously stated, the combination of hexenol + octenol resulted in greater responses of both *Ae. aegypti* and *An. albimanus* than the single components in olfactometer tests (Kline and Bernier, unpublished data). We are not aware of previous field studies conducted using hexenol as an attractant. Since we conducted our field studies, Mann et al. (2009) found that *L. shannoni* preferred MM-X traps baited with Red blend + CO_2 over traps baited with CO_2 alone. This supports our findings for this species. Many studies have been conducted with octenol alone and in combination with CO_2. Trends seen in our study resemble those reported previously. It is difficult to determine from our data whether hexenol contributed to the attractiveness of the Red blend. However, it appears that the effectiveness of octenol is not decreased by hexenol. Future studies should be conducted with blends consisting of octenol + acetone, hexenol + acetone, octenol + hexenol + acetone, and acetone alone with and without CO_2.

We are unaware of any published field studies with either glycolic acid or DMDS. There are an increasing number of published field studies in which L-lactic acid is a major component of blends (Smallengange et al. 2005). BioGents (BioGents GmbH, Regensburg, Germany) developed and commercialized the BG-Lure, which consists of lactic acid, ammonia, and caproic acid, all substances found on human skin (Krockel et al. 2006). This lure was specifically developed to attract host-seeking female *Ae. aegypti* without the use of CO_2. This lure has also been used successfully to attract other *Aedes* (*Stegomyia*) species, such as *Ae. polynesiensis* Marks (Schmaedick et al. 2008) and *Ae. albopictus* (Cilek et al. 2011, Meeraus et al. 2008), and also *Cx. quinquefasciatus* (Cilek et al. 2011). When the traps were baited with a combination of CO_2 + BG-Lure, *Ae. albopictus* collections increased 6×; this combination also increased the collection size of 13 of the other 17 mosquito species. In traps baited with CO_2 alone, the collections of *Ae. albopictus* decreased, but trap collections of all *Anopheles*, *Coquillettidia*, *Psorophora*, and most *Culex* and woodland floodwater species of *Aedes* increased 2–4× compared to the traps baited with a combination of CO_2 + BG-Lure. This implies that the BG-Lure contains at least one chemical compound that adversely affects the collection of most mosquito species. This trend is similar to the results we obtained with our Blue and Green blends.

These data demonstrate that "one size does not fit all" when it comes to attractant blends. Part of this may be related to host-preference differences in the various species collected. A major part of this may also come from the odors by the blend components being an imperfect approximation of the complex odor profile produced by a living host. More research is needed to develop the right blends for different species of mosquitoes and biting flies.

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