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Attraction of the Mosquitoes *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) to a 3-Part Phytochemical Blend in a Mesocosm

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Abstract

Aedes aegypti (L.) and *Aedes albopictus* (Skuse) mosquitoes of both sexes were attracted to a 3-part volatile synthetic phytochemical blend but differed according to their component ratios, 7:3:2 or 1:1:1, and their initial concentrations. These arbovirus vectors were presented with the blends as baits in paired baited and blank CFG traps in a large greenhouse mesocosm. *Ae. aegypti* attraction was highest at a 7:3:2 blend ratio, but at a concentration half that found most effective for an anopheline mosquito species in outdoor screenhouses. Both lower and higher concentrations yielded substantially lower attraction scores for *Ae. aegypti*. By contrast, the few tests conducted on *Ae. albopictus* showed that it was not as sensitive to concentration, but again it was more responsive to the 7:3:2 ratio of components than to the 1:1:1 ratio. The two sexes of both species were represented equally in the trap catches, indicating the potential value of this and similar attractive blends for population surveillance and control of *Aedes* mosquitoes.

Key words: *Aedes aegypti*, *Aedes albopictus*, mosquito, phytochemicals, attractants

Plant sugar is an important part of the adult diet of both sexes of nearly all species of mosquitoes. To locate the plants producing accessible sugar, mosquitoes appear to rely heavily on their odor (Foster 1995, Nyasembe and Torto 2014, Peach et al. 2019, Wooding et al. 2020). These odors have been proposed as a possible means of luring them into traps or toxic bait stations without the need for electric lights, fans, human and animal kairomones, or CO_2 (Foster and Hancock 1993). Several studies with wind-tunnel olfactometers have demonstrated the attractiveness of synthetic phytochemicals to mosquitoes (e.g., Jhumur et al. 2007, Nyasembe et al. 2012, Otienoburu et al. 2012, Yu et al. 2015, 2019, Peach et al. 2019, Meza et al. 2020). So far, successful attraction in the field with natural plant materials has been achieved multiple times with several genera and species of mosquitoes, mainly by deploying concoctions of natural plant substances such as fruit (e.g., Qualls et al. 2015, Junnila et al. 2015, Scott-Fiorenzano et al. 2017, Bilgo et al. 2018, Jacob et al. 2018, Furnival-Adams et al. 2020, Traore et al. 2020). Synthetic phytochemicals, presented as attractants in semi-field enclosures and in the field, have received little attention so far. Nyasembe et al. (2014) used a blend of synthetic phytochemicals to attract *Anopheles gambiae* Giles to field traps, and they reported that a single compound from that blend, linalool oxide, was attractive to *Aedes aegypti* (L.) (Nyasembe et al. 2015). Its effects on *An. gambiae* and other anopheline mosquitoes, as well as on *Ae. aegypti*, gave a mix of positive and negative results by itself and in combination with other phytochemicals and with zoochemicals either in an olfactometer or in the field (Jacob et al. 2018, Omondi et al. 2019, Peach et al. 2019). An enclosure study of *Ae. aegypti* (Fikrig et al. 2017) failed to detect attractive qualities of synthetic phytochemicals previously reported to be effective for other mosquitoes, including a blend containing linalool oxide.

With these conflicting results in mind, we conducted preliminary tests of attraction of *Ae. aegypti* and *Ae. albopictus* (Skuse) to a three-part blend of volatile organic compounds (VOCs) generated by plants that previously had been found to be attractive to the African malaria vector *An. gambiae* s.s. in traps baited with that blend (B. Ebrahimi, B. N. Njiru, and W.A. Foster, unpublished data). These three volatiles, linalool, 1-hexanol, and phenylacetaldehyde, are among a long list of common phytochemical components of the headspace of flowers attractive to mosquitoes and other insects (e.g., Knudsen et al. 2006, Yu et al. 2015). But their combined effect on these two important *Aedes* vectors of the arboviruses causing yellow fever, dengue, Chikungunya, and Zika, is unknown. The degree of attraction in a greenhouse mesocosm was evaluated by simultaneously comparing the numbers of mosquitoes caught in two traps, one baited with the blend, the other an unbaited control. The blend was presented in several concentrations and at two different ratios of the VOC components: 7:3:2 or 1:1:1. Most trials used *Ae. aegypti*. As a separate test, we measured the release rate of the components at the concentrations used in one of the 7:3:2 trials.

Methods and Materials

Mosquito Rearing

Mosquitoes were reared and maintained according to conditions and methods described by Haramis and Foster (1983): a 16-h d including crepuscular periods, constant 27 °C and 70–80% RH, larvae reared in shallow pans at low density and high-quantity powdered Tetramin diet. The *Ae. aegypti* colony was established in 2014 from collections taken by L.P. Lounibos in Fort Myers, FL. The *Ae. albopictus* colony was established in 2012 from a collection made by W.A. Foster in Columbus, OH. The female adults of the colonies were fed human blood from the hand and arm of W.A.Foster according to human-subjects and biosafety protocols, IRB permit 2004H0193, and IBC permit 2005R0020, respectively.

Testing Environment

The experiment was conducted in a netting mesocosm (4.87 m width, 5.66 m length, 3.0 m height) described by Jackson et al. (2015) within a large greenhouse room in the Biological Sciences Greenhouse at The Ohio State University. The temperature range in the mesocosm was 25–31°C (mean = 27.4 °C), and the relative humidity range was 63–76% (mean = 68%), except one day at 90%. As resting sites, terracotta pots were placed on their sides at each of the four corners of the mesocosm. Two CFG traps (counter-flow geometry traps) (= MM-X traps), American Biophysics Corp. (Woodstream Corp.), North Kingstown, RI, were suspended from the ceiling. This type of trap has a black cap, a large clear plastic collection chamber, and two white PVC plastic openings at its lower end (see Kline 1999). That end consists of a strongly-sucking circular intake, to pull mosquitoes into the collection chamber, surrounding a central weakly-blowing exhaust tube situated over the dispenser wicks. Inside the collection chamber was some loose deltamethrin-treated netting to immobilize and kill the trapped mosquitoes for easy removal. Both the trap's intake and outflow fans were powered by a 110v AC electrical source connected to the 12v DC converter. The traps were positioned so that the exhaust tube was 20–25 cm above the floor, near the center of each half of the mesocosm. The two traps were 3.6 m apart. The baited trap held the three VOC-impregnated wicks, each with its own VOC, and the other identical trap had no wicks. One tray of aged tap water, 10 cm deep, was placed centrally between the two traps.

Preparation of Chemicals Tested

The three-part blend consisted of the phytochemicals phenylacetaldehyde (CAS: 122-78-1), 1-hexanol (CAS: 111-27-3), and linalool (CAS: 78-70-6), abbreviated P-H-L hereafter. All were obtained from Sigma-Aldrich. These VOCs were prepared separately at several concentrations, first applied to wicks at a ratio of 7:3:2, respectively (the ratio found most effective for *An. gambiae*) and later applied at a 1:1:1 ratio, as noted below. They were presented on separate cotton wicks. To create a particular concentration of each compound, mineral oil was used as the diluent as well as a slow-release medium. Assuming equal density for all chemicals (ρ = 1), the desired amounts of mineral oil (in mg) and of the compound (in µl) were added to a 1.5-ml vial to make a total volume of 1 ml (≈ 1 g), the amount applied to each release wick. For example, when making a concentration consisting of 70% phenylacetaldehyde, we first weighed 300 mg of mineral oil, then added 700 µl (700 mg) of phenylacetaldehyde to the vial. For a concentration of 7% phenylacetaldehyde, 930 mg of mineral oil was added to a 1.5-ml vial, followed by 70 µl of phenylacetaldehyde. The proportion of mineral oil-to-compound changed, depending on the concentrations of each compound desired, but the total mass was always 1 g. This was done for all three of the VOCs individually. The contents of each vial were then blended thoroughly in a vortex mixer until a homogeneous mix was obtained. Each oil-volatile mixture was then pipetted over the length of its respective 38 x 10 mm cylindrical cotton dental wick (Econo Cotton Roll 216206, Richmond Dental Co., Charlotte, NC) until the wick had absorbed all of it.

A VOC's release rate from its mineral oil diluent depends on its volatility, its starting concentration, and its duration of exposure to air. Those characteristics, in turn, describe their changing concentrations in the air around the trap, as they dissipate. Therefore, we measured the release rates of the three volatiles, gravimetrically, at one set of the concentrations used in attraction experiments: 3.5, 1.5, and 1% of P-H-L, respectively. This gave us a sense of the diminishing strengths of each VOC emanating from the trap over the course of the experimental period. After adding the mixture to the cotton wicks, each of the three wicks was then attached by a curved pin beneath the centrally located exhaust tube while the CFG trap was in operation within a fume hood. Weight loss was measured by an analytical balance at intervals over time, compared to mineral-oil controls. Conditions of temperature and humidity were made to simulate those in the mesocosm. A detailed set of results for all concentrations at all times is not presented here.

Experimental Procedure

Prior to their release, experimental mosquitoes of both sexes were maintained on only water on wicks in 7-liter emergence cages for 4 d after emergence. The number of mosquitoes released from the cage into the mesocosm, 140-317 ($\bar{x}$= 198.5) per trial, was based on the number of pupae placed in a cup in the cage on the day after pupation, minus the number of pupae that had failed to emerge or had died as adults prior to release. The numbers of each sex were recorded when they were recovered at the completion of each trial.

At the onset of a trial, the emergence cage was positioned in the center of the mesocosm between the traps at 16:00–17:30 h (usually 16:00 h), which was several hours before sundown (~21:00 h). At that time, the VOC-oil mixtures were prepared and applied to their respective wicks. Each wick was then attached by a curved pin to the exhaust tube of the CFG trap to be baited. The wicks hung ~1–2 cm below the tube, equidistant from each other, in the weak exhalent airstream. The unbaited trap lacked the wicks. The traps and wicks were handled with gloves at all times, to prevent their contamination with human skin. After 15 min, the mosquitoes were released passively into the mesocosm, by removal of the cage's access sleeve, when the traps' fan motors were turned on. The experimenter immediately left the mesocosm and returned the next morning at 10:00–12:00 h (usually 11:00 h), about 19 h later. The test included extended periods of time at both ends of photophase, because both *Aedes* species are bimodally active diurnally (Yee and Foster 1992) and thus likely to respond to plant odors primarily in the late afternoon and early morning. Two or three replicate trials were performed for each species and each variation in the concentration and ratio of the volatiles. At the end of each test, the traps' fans were switched off, and untrapped but living mosquitoes were recaptured with a back-pack power aspirator (John W. Hock Co, Gainesville, FL.) and killed by freezing. Dead specimens on the mesocosm floor or in the release cage also were collected. All untrapped specimens, alive and dead, were sorted by sex and counted. The traps' collection chambers were then emptied, and the two trapped categories of mosquitoes, baited and blank, were likewise sorted and counted.

Data Analysis

Three numbers were used to create measures of mosquito attraction: n = sample size, the total number recovered in the morning, dead or alive; T = number caught in the baited (treated) trap; and C = the number caught in the blank (control) trap. T/n was the overall trapping *Efficiency*, i.e., the proportion of the population caught by the baited trap, and $T/(T+C)$ was the *Preference*, i.e., the discrimination between baited and blank traps, which discounted other features of a trap, independent of the wicks containing the chemical bait. The product of these two measures, (T/n) x $(T/[T+C])$ x 100, was an *Attraction Index*, based on the raw scores of each replicate, with a potential range of scores from 0 to 100.

After testing the data for normality, three analyses were conducted. In all of them, sex was considered as one of the independent variables. 1) The performance of five widely divergent concentrations was measured, using the 7:3:2 ratio of VOCs, to determine the effect of concentration on *Ae. aegypti*. The optimum attractive concentration was estimated with a Generalized Linear Model regression of the *Attraction Index*, using a stepwise method. 2) The effect of the VOC ratio was evaluated with *Ae. aegypti* by two-way ANOVA with the *Attractive Indices* of three experiments as dependent variables: the optimum concentration from the previous experiment and the two concentrations having a 1:1:1 ratio. 3) To test whether the VOC blends were species-specific, some of the blends attractive to *Ae. aegypti* were tested against *Ae. albopictus*. Comparisons were by two-way ANOVA and posthoc Tukey's tests. All tests employed SYSTAT Version 13.0 for Windows (Systat Software, San Jose, CA), and α= 0.05 was the criterion for statistical significance.

Results

General Observations

In all trials of *Ae. aegypti* combined, the total number recovered, alive or dead, trapped or not, was 3,039 out of 3,110 released, a 97.7% recovery rate. For *Ae. albopictus*, the total number recovered was 1,398 out of 1,438 released, a 97.2% recovery rate. Among the untrapped morning collections, 6–17% of *Ae. aegypti* and 11–14% of *Ae. albopictus* were dead. Together, those not trapped accounted for 54% of all specimens recovered. Baited and blank traps together contained 46% of all mosquitoes recaptured.

Volatile Release Rate

The three compounds in the release-rate test (P-H-L at concentrations of 3.5, 1.5, and 1.0%, respectively) dissipated at a logarithmic rate, with >50% of each compound being released by 7 h. Phenylacetaldehyde was released at the highest rate from the outset and linalool the lowest, which was commensurate with their concentrations. This result is implicit for suspensions of volatiles with similar vapor pressures. As an example, the rate of dissipation of phenylacetaldehyde within 2.5 h after the test's set up was 8.4 µl/h. For hexanol it was 3.23 µl/h, and for linalool it was 1.05 µl/h. The release rates of all three volatiles slowed and stabilized at a similar rate after 7 h. By 27 h, about 96, 90, and 82% of each of the P-H-L components, respectively, was gone.

Sex Ratio

The male:female ratio of the total recovered was 1.000:0.996, essentially 1:1 (Tables 1 and 2). The numbers and proportions of each sex trapped indicate that there was no sex bias among the mosquitoes attracted, regardless of species. Percentages of each sex, both species combined, caught in the baited trap, compared to the total of all recovered mosquitoes of that sex (*Efficiency*), were nearly identical: males, 936 out of 2,228 (42%); females, 890 out of 2,199 (40%). Likewise, their *Preference* for the baited trap over the blank trap was identical: males, 963 out of 1,039 (90%); females, 890 out of 989 (90%). The *Attraction Index*, both species combined, was also nearly the same: 37.4 for males and 36.5 for females. These figures all indicate that females and males responded equally to the volatiles.

Table 1. *Aedes aegypti*: effect of concentration and ratio of P-H-L (phenylacetaldehyde,1-hexanol, and linalool) on attraction

Rank	Concentration (%) and ratio of P-H-L	Replicates[*]	Total recovered (Male + Female)	Baited-trap *Efficiency*[†] (*SE*)	Baited-trap *Preference*[††] (*SE*)	*Attraction index*[§] (*SE*)
1/5	1.6- 0.6-0.4	4	462 (228 + 234)	19.68 (1.41)	93.20 (2.73)	18.30 (1.35)
1/2	3.5-1.5-1.0	4	479 (244 + 235	34.83 (1.61)	96.58 (1.19)	33.63 (1.45)
1	7-3-2	4	438 (220 + 218)	43.83 (2.63)	99.50 (0.5)	43.60 (2.53)
5	35-15-10	6	598 (291 + 307)	58.70 (2.85)	96.65 (0.83)	56.75 (2.85)
10	70-30-20	6	482 (245 + 237)	10.08 (2.35)	81.50 (5.28)	8.48 (2.06)
-	30-30-30	4	329 (160 + 169)	32.22 (1.92)	98.15 (1.01)	41.55 (1.94)
-	5-5-5	4	462 (228 + 234)	24.89 (1.96)	93.50 (2.58)	32.18 (1.94)

[*] Replicates = no. of trials x 2 sex categories

[†] *Efficiency* = Mean no. in baited trap/total no. recovered (x 100).

[††] *Preference* = Mean no. in baited trap/no. in baited + blank traps (x 100).

[§] *Attraction Index* = Mean *Efficiency* x *Preference* (x 100), based on raw scores of each replicate.

SE = standard error of the mean.

Table 2. *Aedes albopictus*: effect of concentration and ratio of P-H-L (phenylacetaldehyde, 1-hexanol, and linalool) on attraction

Concentration (%) and ratio of P-H-L	Replicates*	Total recovered (Male + Female)	Baited-trap *Efficiency*† (*SE*)	Baited-trap *Preference*†† (*SE*)	*Attraction Index*§ (*SE*)
3.5-1.5-1.0	4	398 (202 + 196)	34.53 (1.41)	83.10 (2.08)	50.08 (2.45)
70-30-20	4	397 (228 + 169)	64.48 (4.94)	92.75 (3.02)	58.98 (3.47)
30-30-30	6	315 (281 + 315)	43.46 (2.52)	77.75 (1.58)	32.72 (2.43)

* Replicates = no. of trials x 2 sex categories

† *Efficiency* = Mean no. in baited trap/total no. recovered (x 100).

†† *Preference* = Mean no. in baited trap/no. in baited + blank traps (x 100).

§ *Attraction Index* = Mean *Efficiency* x *Preference* (x 100), based on raw scores of each replicate.

SE = standard error of the mean.

Effect of Concentration on *Ae. aegypti*

When the P-H-L blend was presented at a 7:3:2 ratio, but at five different concentrations, the proportion of *Ae. aegypti* caught in the baited trap varied widely with concentration (Table 1). The *Attraction Indices* of the 1.6-0.6-0.4 % P-H-L blend and the 70-30-20 % P-H-L blend, which were the lowest and highest concentrations, were 18.3 and 8.5, respectively. *Efficiencies* also were quite low at those two extremes: 19.7 and 10.1, respectively. By contrast, the most attractive concentration was a 35-15-10% P-H-L blend, which had an *Attraction Index* of 56.8 and an *Efficiency* score of 58.7.

Preference for the baited trap was high to very high at all concentrations, with an average of 96.0%. Out of a total of 450 trapped males, 432 (96%) were in the baited trap. Out of a total of 435 trapped females, 417 (96%) were in the baited trap. *Preference* for the baited-trap, for each of the first four concentrations were all in the 89.5–100 range for each sex. Only at the highest concentration (70-30-20%), which also had the lowest *Attraction Index*, the trap *Preference* was slightly lower. It was 81.5 for combined sexes. Separately, for males it was 82 (n = 27) and for females it was 80 (n = 31).

There was a positive quadratic response between *Attraction Index* and concentration that fits the data: r = 0.94, adjusted R^2 = 87.9%, and *Residual Mean Square* = 6.80 (F = 84.61; df = 2, 21; $P < 0.0001$), where *Attraction Index* = $-1.71\ (C^2) + 15.57\ (C) + 23.16$, and where C is the *Concentration rank* (that is, 0.2, 0.5, 1.0, 5.0, 10.0), which was based on the magnitude of the concentrations compared to the 7-3-2% P-H-L concentration (having a rank of 1.0) (see Table 1). In the model, the *Attraction Index* maximum was predicted to be these concentrations for P-H-L: 31.92-13.98-9.12%, respectively. These estimated optima were close to the actual concentrations of the most attractive blend: 35-15-10% of P-H-L, respectively.

Effect of Ratio on *Ae. aegypti*, Comparing 1:1:1 vs. 7:3:2 Blends

The *Attraction Index* of *Ae. aegypti* at the most attractive concentration of the 7:3:2 P-H-L blend (35-15-10% concentration) was 56.8, which was close to its predicted optimum set of concentrations. That was significantly higher than either of the two 1:1:1 P-H-L blends (30-30-30 % and 5-5-5%), which were 41.6 and 32.2, respectively (F = 25.24, df = 2, 11, $P < 0.0001$). The same was true for their trapping *Efficiencies*: 58.7 for the 7:3:2 ratio, vs. 32.2 and 24.9, respectively, for the two 1:1:1 ratios. Nonetheless, the *Preference* for the baited trap over the blank trap was very high at both concentrations of the 1:1:1 ratio: 98.2 and 93.5, respectively.

Comparison of *Ae. albopictus* and *Ae. aegypti* Attraction

Ae. albopictus was strongly attracted to a wide range of concentrations of the 7:3:2 ratio. The low and high concentrations (3.5-1.5-1.0% P-H-L and 70-30-20 % P-H-L) had *Attraction Indices* that were insignificantly different from each other: 50 and 59.0, respectively (despite one having 20 times the concentration of the other) (Table 2). They also were not significantly different than the *Attraction Index* of the most attractive *Ae. aegypti* concentration (35-15-10 %): 56.8 (see Table 1). However, when the ratio of the constituents was 1:1:1 (30-30-30% concentration), its *Attraction Index* for *Ae. albopictus* was 32.7, significantly lower than the 7:3:2 ratio (F = 19.618; $d.f.$ = 3, 16; $P < 0.0001$). This lower value was the result of both a low *Efficiency* score and a low *Preference* score: 43.5 and 77.8, respectively. Note that the unusually modest 77.8 *Preference* score of *Ae. albopictus* for the 30-30-30 % concentration was considerably lower than the 98.2 score of *Ae. aegypti* for the same 1:1:1 blend (Table 2)

Discussion

This preliminary study of phytochemicals that attract the most medically important two species of *Aedes* confirms that a 7:3:2 blend may have the potential for broad application in mosquito surveillance and control. Three prominent results of the tests were as follows: 1) Both sexes of both species of *Aedes* were attracted to the same 3-part ratio of volatiles that attracted *An. gambiae* (B. Ebrahimi B. N. Njiru, and W.A. Foster, unpublished data) indicating that its effects may be broadly applicable. The lack of a sex difference in the numbers caught in the baited traps accords with other behavioral studies on attraction of mosquitoes to sugar sources and sugar-related odors (Foster 1995). In a similar physiological state, i.e., with energy reserves either depleted or not yet accumulated and with the females neither digesting a blood meal nor holding a batch of eggs, both males and females will respond equally strongly to cues from plant-sugar sources. 2) The concentration eliciting a peak response from *Ae. aegypti* was 35-15-10% P-H-L. An optimum concentration is to be expected, because weak stimuli either push the lower limits of detectability or are interpreted as being very distant and thus likely to incur costs and risks. Very concentrated stimuli, on the other hand, may "overload the senses," so to speak, creating difficulty for the mosquito in orienting to the source of the stimuli or inducing repellency. In contrast, a high *Attraction Index* of *Ae. albopictus* covered a wide range of concentrations, from as high as 70-30-20% down to as low as 3.5-1.5-1.0% P-H-L. *Ae. aegypti* was only weakly attracted to those extremes. The reason for this species difference is only speculative but may be related to their differences in sugar-feeding and blood-feeding behavior in the field, such as *Ae. albopictus*'s less anthropophilic nature, i.e., greater dependency on plant sugar for energy when human blood meals are not readily available. 3) The 1:1:1 ratio of the compounds, at least at the concentrations tested, was attractive, but much less so than the 7:3:2 ratio, suggesting that these proportions are important. However, that result needs confirmation over a wider range of concentrations and different ratios. The omission of oiled wicks attached to the blank trap was an unfortunate oversight that we think had little or no effect on its extremely low *Preference* score, because the mineral oil itself and the small sterile wicks were very unlikely to add an important visual or chemical stimulus to either trap.

Aside from that technical caveat, the results demonstrate unequivocally that these *Aedes* species are attracted to a blend of sugar-related plant chemicals. However, both sexes of *Ae. aegypti* and *Ae. albopictus* are well known for their attraction to dark objects, especially in the presence of blood-host cues (e.g., van Breugel et al. 2015, Tang et al. 2021), which can serve either as sources of blood or as mating sites. So the black caps of the CFG traps may have been visually attractive to them. However, that effect must have been quite small, because the blank traps caught so few mosquitoes of either sex, as the *Preference* measures showed. Nonetheless, phytochemicals may play only a minor role among attractants salient to these two species. For example, females can be completely diverted from a sugar-odor cue to blood-host odor when both are present in the same airspace (Yee and Foster 1992). Blood is a high priority in these species, whose females derive large amounts of energy from human blood, owing to its isoleucine deficiency and thus a surplus of other amino acids that might otherwise be used in egg development (Briegel 1990, Braks et al. 2006). Yet, both sexes clearly are sugar-feeders in the field, depending on environment, season, and local circumstances (e.g., Martinez-Ibarra et al. 1997, Spencer et al. 2005, Sissoko et al. 2019, Fikrig et al. 2020).

The 3-part blend of VOCs appears to be a promising CO_2-free and human-kairomone-free attractant for both male and female *Ae. aegypti* and *Ae. albopictus*. Its use in sampling the male portion of populations may be particularly helpful. Further testing must confirm its usefulness in mosquitoes that vary in age and physiological state. More replicates of each type of test are needed, and perhaps modifications in the VOC component ratios should be explored. Field trials, where wind, rain, and fluctuating temperature are uncontrolled variables, must ultimately be conducted. But prior to that, its widespread use as a surveillance or control tool in the field must meet these three technical demands: a) a slow-release device that can maintain a steady, airborne, bioactive, plume of the blend, b) a light-weight portable trap or toxic bait station that is electricity-free, and c) a device and its placement that prevent or limit effects on nontarget organisms.

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