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EVALUATION OF 1-OCTEN-3-OL, CARBON DIOXIDE, AND LIGHT AS ATTRACTANTS FOR MOSQUITOES ASSOCIATED WITH TWO DISTINCT HABITATS IN NORTH CAROLINA

LEOPOLDO M. RUEDA,[1] BRUCE A. HARRISON,[1] JEFFREY S. BROWN,[1] PARKER B. WHITT,[1]
RYAN L. HARRISON[2] AND ROBERT C. GARDNER[3]

ABSTRACT. Field studies were conducted in North Carolina to determine the responses of mosquitoes found in salt marsh and inland creek flood plain areas to 1-octen-3-ol (octenol), carbon dioxide (CO_2), and light in various combinations with Centers for Disease Control (CDC) light traps. Over 56,000 adult mosquito specimens of 12 species in 4 genera were collected in the salt marsh. They exhibited a general response pattern of octenol + CO_2 + light > CO_2 + light = octenol + CO_2 > octenol + light > octenol alone. Significantly, more *Aedes sollicitans, Ae. taeniorhynchus, Anopheles bradleyi,* and *Culex salinarius* were attracted to octenol + CO_2 + light than to CO_2 + light. Over 19,000 specimens of 24 species in 7 genera were collected in the inland creek flood plain. Although the response patterns to the attractants were similar to those in the salt marsh area, there was no significant difference between octenol + CO_2 + light and CO_2 + light. *Aedes vexans, An. crucians,* and *An. punctipennis* were attracted nearly equally to these two attractant combinations. These studies demonstrate that responses to combinations of these attractants are species specific. However, different combinations of attractants can significantly increase the collection of targeted species important in arbovirus transmission. The use of these combinations would be very beneficial in mosquito-borne virus surveillance studies. The use of octenol by itself or in conjunction with light was found the least useful for collecting mosquitoes in both habitats.

KEY WORDS Attractants, mosquitoes, octenol, carbon dioxide, light, North Carolina

INTRODUCTION

Many mosquito control techniques and programs rely heavily on both ground and aerial applications of adulticides. These pesticides, plus application-related costs, constitute a significant expense. In addition to high costs, the traditional reliance on adulticides has other drawbacks, including increasing resistance of mosquitoes to pesticides, perpetuating detrimental effects on nontarget biological control organisms, increasing temporary chemical pollution, and delaying the implementation of sound integrated pest management (IPM) approaches that use alternate control methods. Invariably, successful IPM approaches are based on sound natural history data accrued through mapping, surveillance, and monitoring. Costs and problems generated by the use of adulticides in the absence of sound natural history data has renewed interest in alternative control methods, particularly the use of baited traps that collect mosquitoes in sufficient numbers to function as a control mechanism by eliminating large numbers of the targeted species (Day and Sjorgen 1994).

Carbon dioxide (CO_2) and 1-octen-3-ol (octenol) baited traps have been evaluated against mosquitoes and other biting flies associated with a variety of habitats including salt marshes, swamps, rice fields, and animal holding areas (Jaenson et al. 1991, Torr 1994, Kline and Mann 1998). Responses of North Carolina mosquitoes associated with creek flood plains and salt marshes to octenol attractants have not been reported. These mosquitoes are important in the epidemiology of eastern equine encephalomyelitis (EEE), LaCrosse encephalitis (LAC) and other arboviruses in North Carolina. Thus, baited adult traps could have real value in accruing more target species during mosquito-borne virus surveys. Effective surveillance and alternative control programs for these mosquitoes are needed because of increases in residential housing developments, business establishments, and recreational areas near these salt marshes and flood plains. In this paper, we present an evaluation of incorporating attractants into surveillance and monitoring for mosquitoes in these habitats in eastern and central North Carolina.

MATERIALS AND METHODS

Study sites

From October 6 through October 11, 1998, trials were conducted in a salt marsh area with predominant plant species consisting of smooth cordgrass (*Spartina alterniflora* Loisel.), salt grass (*Distichlis spicata* L.), and black needle grass (*Juncus roemerianus* Scheele) in the northern part of Topsail Island, Onslow County, North Carolina (NC) (about 1 km from NC Highway 210). Margins of the salt marsh support dominant species such as sea myrtle (*Baccharis frutescens* L.), wax myrtle (*Myrica cerifera* L.), marsh elder (*Iva imbricata* Walt.), and

[1] Public Health Pest Management Section, Division of Environmental Health, North Carolina Department of Environment and Natural Resources, PO Box 29593, Raleigh, NC 27626-0593.

[2] Forsyth County Health Department, Division of Environmental Health, PO Box 686, Winston-Salem, NC 27102-0686.

[3] Mosquito Control Section, Onslow County Public Works, 1222 Onslow Pines Road, Jacksonville, NC 28540.

yellow jasmine (*Gelsemium sempervirens* L.). A dirt road runs across the middle of the marsh, and the traps were positioned along the edges of this road and marsh area.

From June 6 through June 11, 1999, trials were conducted in Davidson County, about 3.5 km southeast of Lexington in the Homestead Acres Development off Becks Church Road. The homes back up to both Abbotts Creek and Pounder Fork Creek. Our study site was in the flood plain of Abbotts Creek, adjacent to Pounder Fork Creek. This site floods annually and is considered a protected wetland on Alcoa property. Abbotts Creek and Pounder Fork Creek are major headwaters for the High Rock Reservoir. The dominant tree species included red maple (*Acer rubrum* L.), yellow poplar (*Liriodendron tulipifera* L.), green ash (*Fraxinus pennsylvanica* Marsh), river birch (*Betula nigra* L.), sweet gum (*Liquidambar styraciflua* L.), and American sycamore (*Platanus occidentales* L.).

Attractants

The three attractants evaluated in these field trials consisted of octenol, CO_2, and light. The CO_2 was from a 2-liter plastic thermos containing about 1 kg of dry ice. The average CO_2 release rate was calculated to be 57 g/h. Octenol (Biosensory, Inc., Willimantic, CT) was in a patented wax-like medium that releases 1.5 mg/h at 27°C. It was packaged in a crush-resistant plastic housing containing 3 mg octenol. The CO_2 container and octenol package were suspended from the same piece of wood beside the trap near the air intake.

Trap sites and study design

Each site had one Model 512, Centers for Disease Control (CDC)-type, 6-V, battery-powered trap (John Hock Co., Gainesville, FL) suspended from a piece of wood (with each end tied on top of 2 standing parallel metal poles) about 1.3 m above ground level. Each trap was equipped with an attractant combination (see above). Mosquitoes near the intake were sucked into the trap and blown into a 1,000-ml plastic container. Traps were set each afternoon between 1630 and 1700 h, and the collections were picked up the following morning between 0800 and 0830 h. The responses of mosquitoes to CDC traps baited with octenol + CO_2 + light, octenol + CO_2, octenol + light, and octenol alone were compared with traps baited with CO_2 + light. Using a 5 × 5 Latin square design, we utilized 5 trap sites separated from each other by about 20 m. Trials were conducted on 5 consecutive nights to minimize variation due to population change. Two replicates (5 traps each) were run in 1999 in the flood plain site at Davidson County. Attractant, day, and trap position effects were evaluated using 3-way ANOVA for total number and

Table 1. Mean females per trap night (SE) of collected mosquito species in CDC light trap baited with octenol + carbon dioxide (CO_2) + light, CO_2 + light, octenol + CO_2, octenol + light, and octenol in salt marsh, Topsail, NC.

Species	Treatment (n = 25)				
	Octenol + CO_2 + light	CO_2 + light	Octenol + CO_2	Octenol + light	Octenol
Aedes infirmatus	0.00	0.00	0.20 (0.20)	0.00	0.00
Aedes sollicitans	143.40 (79.14)	80.80 (33.76)	71.20 (39.93)	1.20 (0.73)	0.20 (0.20)
Aedes taeniorhynchus	273.60 (56.66)	136.20 (68.41)	123.40 (29.84)	6.80 (3.22)	3.20 (2.06)
Aedes vexans	1.60 (1.03)	1.00 (0.63)	0.40 (0.24)	0.40 (0.40)	0.00
Anopheles atropos	1,787.80 (617.43)	474.40 (214.65)	1,518.00 (454.28)	115.00 (59.04)	28.20 (9.55)
Anopheles bradleyi	1,472.00 (522.75)	702.60 (246.54)	407.60 (151.02)	254.80 (40.20)	7.60 (3.79)
Anopheles crucians	0.00	0.60 (0.60)	0.00	0.00	0.00
Anopheles punctipennis	0.80 (0.31)	0.00	0.00	0.00	0.00
Culex pipiens	4.00 (0.24)	0.00	0.00	0.00	0.00
Culex restuans	1.00 (0.74)	1.60 (1.60)	0.40 (0.24)	0.00	0.00
Culex salinarius	2,282.20 (1,079.91)	707.60 (255.34)	579.20 (191.48)	155.40 (75.73)	22.80 (10.86)
Uranotaenia sapphirina	0.20 (0.20)	0.00	0.40 (0.24)	0.00	0.00

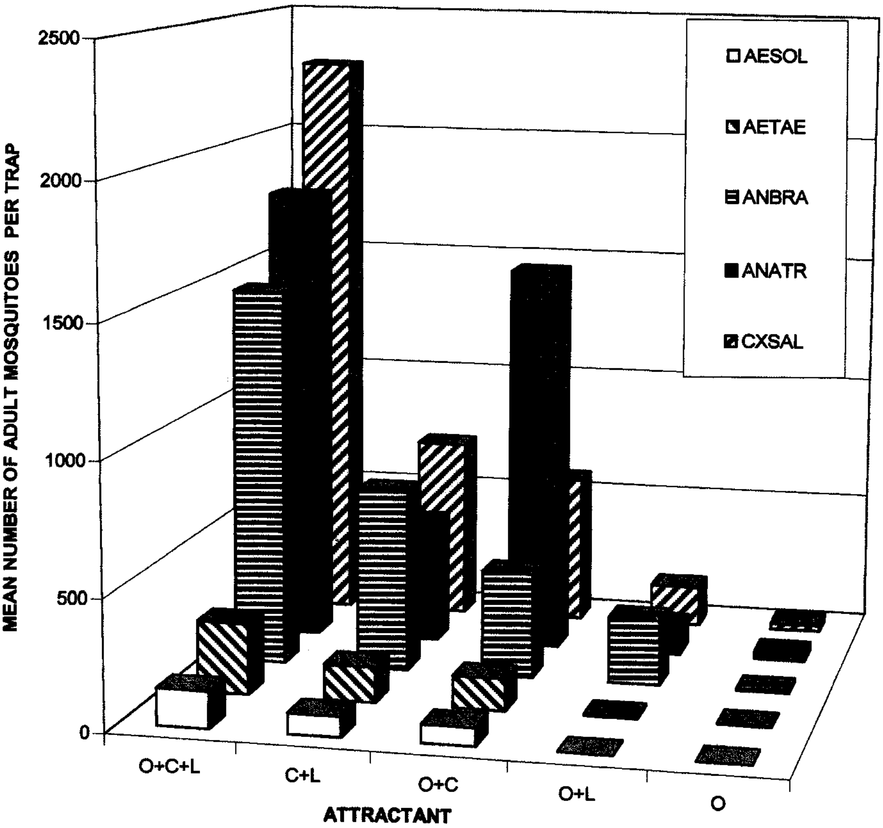

Fig. 1. Mean (females/trap night) response of 5 commonly collected mosquito species in Model 512 Centers for Disease Control (CDC)-type trap baited with octenol + carbon dioxide (CO_2) + light, CO_2 + light (control), octenol + CO_2, octenol + light, or octenol alone in salt marsh area, North Topsail, NC. (AESOL = *Aedes sollicitans*; AETAE = *Ae. taeniorhynchus*; ANBRA = *Anopheles bradleyi*; ANATR = *An. atropos*; CXSAL = *Culex salinarius*; O = octenol; C = carbon dioxide; L = light).

common species. Statistical Analysis System (SAS Institute 1985) was used for all statistical analysis.

Processing specimens

Trap collection containers were removed each morning of the test period. Specimens were removed, taken to the laboratory, sorted and identified using Slaff and Apperson (1989), and totaled. The females of *Aedes atlanticus* Dyar and Knab and *Ae. tormentor* Dyar and Knab cannot be differentiated. Thus, *Ae. atlanticus/tormentor* is used hereafter.

RESULTS

During 25 trap-nights in the salt marsh area, 56,855 mosquitoes (12 species in 4 genera) were collected (Table 1). The general species response pattern was that total collection size for octenol + CO_2 + light > octenol + CO_2 = CO_2 + light > octenol + light > octenol alone. Ten species were collected with the octenol + CO_2 + light treatment, 8 species responded to CO_2 + light, 9 responded to octenol + CO_2, 6 responded to octenol + light, and 5 responded to octenol alone. *Aedes sollicitans* (Walker), *Ae. taeniorhynchus* (Wiedemann), *Anopheles bradleyi* King, and *Culex salinarius* Coquillett were most attracted by octenol + CO_2 + light (Table 1). The responses of *Ae. sollicitans, Ae. taeniorhynchus,* and *Cx. salinarius* to octenol + CO_2 did not differ significantly from their responses to CO_2 + light ($P \leq 0.05$, Tukey's studentized range test). *Aedes sollicitans* and *Ae. taeniorhynchus* were least attracted by octenol + light and octenol alone ($P \leq 0.05$). The response of *An. atropos* Dyar and Knab to octenol + CO_2 + light did not differ significantly from its response to octenol + CO_2 ($P \leq 0.05$). Octenol alone attracted fewer mosquitoes than any other treatment in most cases. *Anopheles punctipennis* (Say) and *Cx. pipiens* L. were collected only from octenol + CO_2 + light. *Aedes infirmatus* Dyar and Knab and *An. crucians* Wiedemann were attracted only by octenol + CO_2 and CO_2 + light, respectively. Figure 1 shows the mean response of abundant mosquito species as-

Table 2. Mean females per trap night (SE) of collected mosquito species in CDC light trap baited with octenol + carbon dioxide (CO_2) + light, CO_2 + light, octenol + CO_2, octenol + light, or octenol in creek flood plain woods near Lexington, NC.

Species	Treatment (n = 50)				
	Octenol + CO_2 + light	CO_2 + light	Octenol + CO_2	Octenol + light	Octenol
Aedes albopictus	0.10 (0.10)	0.00	0.10 (0.10)	0.10 (0.10)	0.00
Aedes atlanticus/tormentor	0.40 (0.22)	0.50 (0.27)	0.40 (0.22)	0.10 (0.10)	0.30 (0.21)
Aedes canadensis canadensis	0.60 (0.31)	0.20 (0.13)	0.30 (0.15)	0.00	0.00
Aedes cinereus	0.70 (0.15)	0.20 (0.13)	0.50 (0.26)	0.00	0.00
Aedes dupreei	0.40 (0.22)	0.20 (0.10)	0.50 (0.22)	0.10 (0.10)	0.10 (0.10)
Aedes fulvus pallens	0.20 (0.13)	0.00	0.30 (0.15)	0.10 (0.10)	0.00
Aedes infirmatus	0.00	0.10 (0.10)	0.00	0.00	0.00
Aedes sticticus	118.80 (27.15)	85.50 (12.48)	90.70 (30.26)	9.80 (2.80)	3.90 (1.17)
Aedes triseriatus	0.70 (0.33)	2.50 (0.97)	0.80 (0.37)	0.40 (0.40)	0.00
Aedes trivittatus	6.40 (1.19)	4.60 (0.91)	6.30 (1.49)	0.40 (0.22)	0.50 (0.22)
Aedes vexans	525.20 (81.49)	511.70 (57.22)	125.90 (35.67)	107.70 (13.97)	2.60 (1.20)
Anopheles crucians	16.20 (4.61)	14.50 (3.62)	2.80 (0.74)	8.60 (2.77)	0.30 (0.21)
Anopheles punctipennis	25.00 (4.89)	14.70 (3.35)	4.30 (1.00)	8.40 (2.73)	0.20 (0.20)
Anopheles quadrimaculatus	0.50 (0.30)	0.70 (0.21)	0.50 (0.16)	0.40 (0.31)	0.00
Coquillettidia perturbans	0.00	0.00	0.10 (0.10)	0.00	0.00
Culex erraticus	0.10 (0.10)	1.50 (0.42)	0.10 (0.10)	0.20 (0.20)	0.10 (0.10)
Culex restuans	0.10 (0.10)	0.50 (0.30)	0.00	0.20 (0.20)	0.00
Culex salinarius	1.10 (1.10)	0.10 (0.10)	0.20 (0.13)	0.20 (0.13)	0.00
Culex territans	1.00 (0.78)	2.90 (0.92)	0.00	1.50 (0.97)	0.00
Culiseta melanura	0.10 (0.10)	0.00	0.00	0.00	0.00
Psorophora ferox	48.40 (9.14)	39.70 (7.26)	49.80 (7.87)	2.90 (0.97)	0.50 (0.22)
Psorophora horrida	1.30 (0.39)	0.70 (0.33)	2.10 (0.65)	0.00	0.20 (0.13)
Psorophora mathesoni	2.70 (1.44)	3.20 (1.37)	6.20 (1.60)	0.30 (0.21)	0.70 (0.05)
Uranotaenia sapphirina	0.00	0.50 (0.34)	0.00	0.10 (0.10)	0.00

sociated with the salt marsh area to traps baited with combinations of attractants.

In the creek flood plain site near Lexington (50 trap-nights), 19,498 mosquitoes (24 species in 7 genera) were collected (Table 2). The general species response pattern was that total collection size for octenol + CO_2 + light = CO_2 + light > octenol + CO_2 > octenol + light > octenol alone. Twenty-one species were collected with the octenol + CO_2 + light treatment, 20 species responded to CO_2 + light, 19 responded to octenol + CO_2, 18 responded to octenol + light, and 11 responded to octenol alone. *Aedes vexans* (Meigen), *An. crucians,* and *An. punctipennis* were most attracted by CO_2 + light with or without octenol. The responses of *Ae. sticticus* (Meigen) and *Psorophora ferox* (von Humboldt) to octenol + CO_2 + light were not significantly different from octenol + CO_2 or CO_2 + light ($P \leq 0.05$). Figure 2 shows the mean response of the 5 most common mosquito species associated with the flood plain area to traps baited with combinations of attractants.

DISCUSSION

Huffaker and Back (1943), Miller et al. (1969), and Herbert et al. (1972) showed that, for most mosquito species, light traps baited with CO_2 caught up to 30 times as many mosquitoes as light-only traps. In North Carolina, there were 6 times more mosquitoes collected from CO_2-baited light traps than from unbaited light traps (Newhouse et al. 1966). In our study, we used CO_2 + light as our control treatment for comparison with different combinations such as octenol + CO_2 + light, octenol + CO_2, octenol + light, and octenol only.

Since Takken and Kline (1989) initially reported the potential of octenol as a mosquito attractant, several studies (Kline et al. 1990, Kemme et al. 1993, Kline and Mann 1998) have been conducted that included various types of habitats and mosquito species with CDC light traps baited with various combinations of attractants (octenol, butanone, lactic acid, honey, phenols, CO_2). Generally, our data show that very few species were attracted to octenol alone, but when octenol + CO_2, either with or without light, was used, at least twice as many specimens of *Aedes, Anopheles, Culex,* and *Psorophora* were collected. This pattern of response also was observed in Florida (Kline and Mann 1998) and Australia (Kemme et al. 1993).

In the salt marsh, nearly twice or more *Ae. sollicitans, Ae. taeniorhynchus, An. bradleyi,* and *Cx. salinarius* were attracted to octenol + CO_2 + light than to CO_2 + light. These 4 species showed no significant attraction to either octenol alone or octenol in combination with light. However, *An. atropos,* a vicious day-time biter, demonstrated a highly significant response to octenol + CO_2, with or without light ($P \leq 0.001$). Currently, *Ae. sollicitans, Ae.*

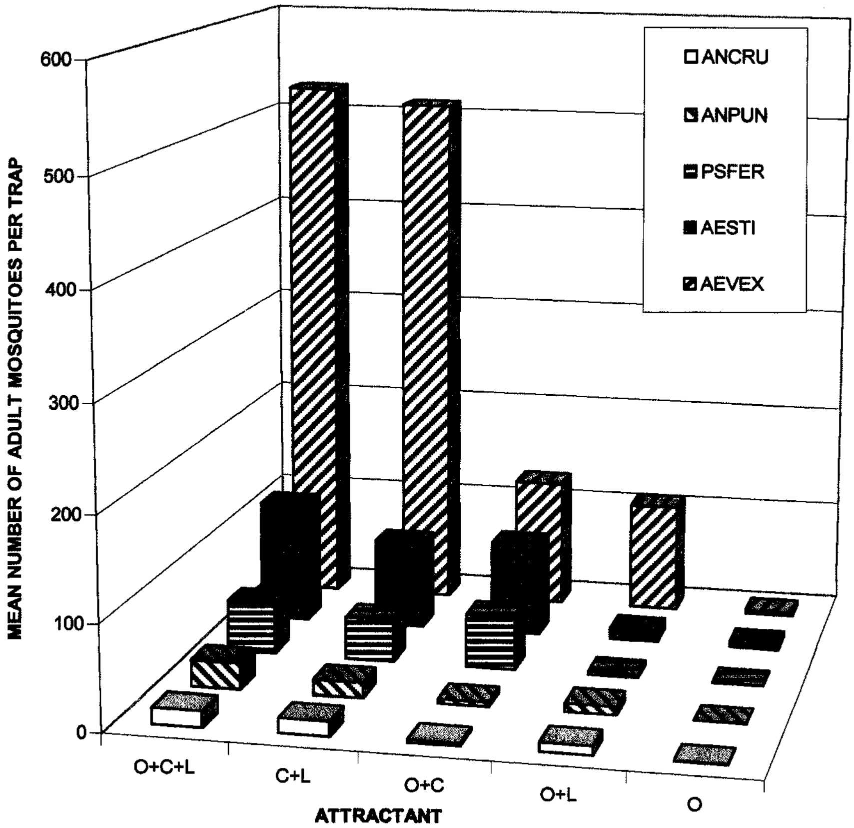

Fig. 2. Mean (females/trap night) response of 5 commonly collected mosquito species in Model 512 Centers for Disease Control (CDC)-type trap baited with octenol + carbon dioxide (CO_2) + light, CO_2 + light (control), octenol + CO_2, octenol + light, or octenol alone in creek flood plain near Lexington, NC. (ANCRU = *Anopheles crucians*; ANPUN = *An. punctipennis*; PSFER = *Psorophora ferox*; AESTI = *Ae. sticticus*; AEVEX = *Aedes vexans*; O = octenol; C = carbon dioxide; L = light).

taeniorhynchus, and *Cx. salinarius* are considered vectors of eastern equine encephalomyelitis (EEE) virus and are likely candidates to serve as bridge vectors for West Nile virus if it comes to North Carolina. Thus, the use of octenol in combination with CO_2 + light will provide a substantial increase in specimen numbers of these species in virus surveillance studies.

In the creek flood plain trials, none of the 24 species demonstrated a significant attraction to octenol alone. Furthermore, responses to octenol + CO_2 + light were also not significant when compared with CO_2 + light. However, other trends were noted. *Aedes vexans,* the dominant species, was attracted significantly to octenol with light or CO_2, and this attraction nearly quadrupled when CO_2 and light were combined, with or without octenol ($P \leq 0.0001$). This information is crucial in view of the isolation of West Nile virus from this species in Connecticut (Anderson et al. 1999). *Aedes sticticus* and *Ps. ferox,* however, were significantly attracted to CO_2, regardless of the presence of octenol or light. *Anopheles crucians* and *An. punctipennis* were significantly attracted to CO_2 + light ($P < 0.0001$) but not to octenol.

In the salt marsh area, total mosquito catch was significantly greater ($P \leq 0.001$) in CO_2-baited light traps with octenol than those without octenol. In the creek flood plain, however, there was no significant difference in mosquito catches between CO_2-baited light traps with or without octenol. Using traps with octenol alone, total mosquito catch was significantly lower compared with other treatments in the salt marsh ($P \leq 0.001$) and in the creek flood plain ($P \leq 0.0001$).

Culex pipiens, considered the enzootic vector of West Nile virus in New York (Centers for Disease Control and Prevention 1999), was not collected in the creek flood plain, and only very low numbers were collected in the salt marsh. *Culex restuans,* another potential enzootic vector of West Nile virus, was also collected at both sites along with *Cx. salinarius* at the creek flood plain site.

In summary, our data indicate that, for inland flood plain mosquitoes, octenol should be used only in conjunction with CO_2 + light. For the 5 common salt marsh mosquitoes that we encountered, the addition of octenol clearly resulted in higher catches. We do not recommend the use of octenol by itself or in conjunction with light for the species we studied. Our data also show that species within a given genus do not respond uniformly to the same combinations of attractants.

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