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EVALUATION OF 1-OCTEN-3-OL AND CARBON DIOXIDE AS ATTRACTANTS FOR MOSQUITOES ASSOCIATED WITH IRRIGATED RICE FIELDS IN ARKANSAS[1]

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ABSTRACT. Field studies were conducted to determine the responses of mosquitoes associated with irrigated riceland in Arkansas to 1-octen-3-ol (octenol), carbon dioxide (CO_2) and combinations of both. Octenol was released at 4.1 (wick in) and 41.1 (wick out) mg/h and CO_2 at 200, 500 and 1,000 ml/min. Octenol alone did not result in a significant ($P > 0.05$) increased response for any species relative to unbaited traps. An increase in CO_2 release rate generally resulted in an increase in collection size. All octenol + CO_2 combinations increased the collections of *Coquillettidia perturbans*, *Culex salinarius* and *Psorophora columbiae* relative to equivalent CO_2 release rates alone. Mixed responses for these same treatment combinations were obtained for *Anopheles crucians*, *An. quadrimaculatus* and *Cx. erraticus*.

INTRODUCTION

Recent studies by Takken and Kline (1989) and Kline et al. (1990a, 1990b, 1991) have shown that both carbon dioxide (CO_2) and 1-octen-3-ol (octenol) are mosquito attractants. Carbon dioxide was first reported as a mosquito attractant by Rudolfs (1922) and has since been widely used for mosquito surveillance. Octenol was first reported as a mosquito attractant by Takken and Kline (1989), and its role as a mosquito attractant is still under investigation.

When used alone, octenol has been shown to be a good attractant for only a few species, such as *Aedes taeniorhynchus* (Wied.) (Takken and Kline 1989), *Culex salinarius* Coq., *Coquillettidia perturbans* Walker and *Mansonia titillans* (Walker) (Kline et al. 1990a). However, there appears to be a synergistic response of these species and some populations of *Anopheles* spp. (Kline et al. 1990b) to the combination of octenol and CO_2.

The major objective of this study was to determine the responses of mosquito species associated with irrigated riceland in Arkansas to various combinations of octenol and CO_2.

MATERIALS AND METHODS

Field studies were conducted in 1987 and 1988 in the Grand Prairie region near Stuttgart, AR. This predominantly agricultural region is characterized by a flat open landscape that is occasionally interrupted by hardwood swamps and bayous. Fields are often bordered by ditches lined with hardwoods and woody and herbaceous brush. The primary crops are rice and soybeans, both of which are frequently irrigated during the growing season. The vast acreage in rice culture, which consists of fields with shallow surface waters and adjacent irrigation ditches that are intermittently flooded with irrigation and/or rain water, provides excellent breeding conditions for *Psorophora columbiae* (Dyar and Knab) and *Anopheles quadrimaculatus* Say, the 2 predominant mosquito species found in this region (Schwardt 1939, Williams and Meisch 1983). The specific study area was a wooded site that was located adjacent to a large (>32 ha) rice field located on the property of the University of Arkansas Agricultural Rice Research and Extension Center. A trap line was established through the woods parallel to the rice field. Each trap location was established as a discrete site located ca. 170 m apart. The Model 512 CDC trap (John Hock Co., Gainesville, FL) without a light source but baited with different combinations of chemical attractants was used to trap the mosquitoes. Collections were made into pint Mason jars containing a small piece of Vapona (Shell No-Pest Strip®) as the killing agent.

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the United States Department of Agriculture.

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A Latin square experimental design was used both years. A requirement of this design was that each trap location be provided with a different treatment combination daily without repeating any treatment combination at that location during the test period. This design enabled removal of the differences among rows (days) and columns (trap locations) from the experimental error and allowed for a more precise analysis of the effects of treatment combinations on mosquito collections. The randomization scheme for each experiment was selected from Cochran and Cox (1957).

The treatments used in these studies consisted of baiting the traps with octenol or CO_2 alone, in various combinations with each other, or no attractant (unbaited). Octenol was released from 5-ml microreaction vials (Supelco Co., Bellefonte, PA) via a wick (Dills® 15-cm pipe cleaner) either in contact with or protruding through a neoprene septum (Fig. 1). The octenol release system with the wick in contact with the rubber septum will be referred to as the "wick in" method, and the release system with the pipe cleaner wick protruding through the rubber septum as the "wick out" method throughout this manuscript. In the wick out arrangement, the pipe cleaner was doubled over and arranged so that 2 cm protruded above the septum. At the beginning of each trapping session the vial was inverted for several seconds to assure complete wick saturation. The microreaction vial was attached ca. 5 cm from the top trap entrance, and when used in combination with CO_2, the vial was located adjacent to the CO_2 release point. The actual release rates of octenol were not calculated during this study, but based on previous studies the wick in and wick out release rates would be ca. 4 and 41 mg/h, respectively (Kline et al. 1990a, 1991). The CO_2 was metered at the desired rates from a 9 kg (20 lb) compressed gas cylinder fitted with a single stage regulator and Gilmont compact #12 flowmeter (Gilmont Instruments, Inc., Great Neck, NY). The gas was delivered to its release point ca. 5 cm from the trap entrance via polyethylene tubing. A no bait control was used, which corresponded to the zero level of both octenol and CO_2.

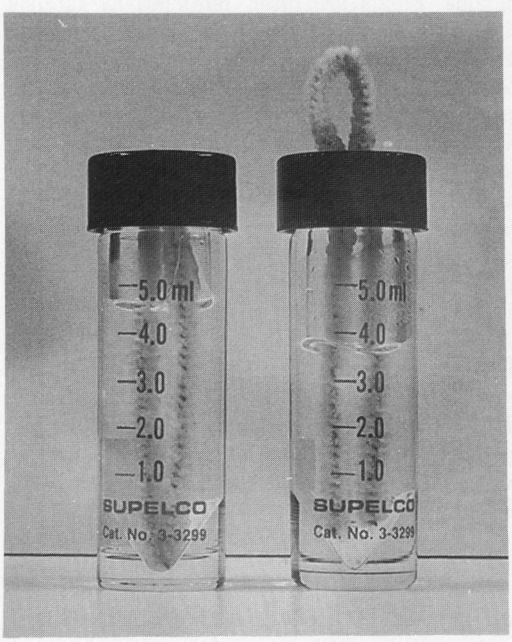

Fig. 1. Octenol is released from vials either through a "wick in" (left vial) arrangement that has a pipe cleaner in contact with a neoprene septum or as a "wick out" arrangement (right vial) that has a pipe cleaner protruding through a neoprene septum.

The first field tests were conducted during July 1987. The first test period consisted of 5 days (July 8–12) during which a 5 × 5 Latin square design was used to test the following treatment combinations: 1) no attractant, 2) octenol (wick in) only, 3) 500 ml/min CO_2 only, 4) octenol (wick in) + 500 ml/min CO_2, and 5) 1,000 ml/min CO_2. The trapping interval was 24 h, and each trap was serviced daily between 1700 and 1800 h Central Standard Time (CST). This same experiment was repeated during another 5-day period (July 14–18).

Field trials were also conducted in July and August 1988. A 6 × 6 Latin square experimental design was used for each test period. The treatments for the first test period (July 18–23) included: 1) 200 ml/min CO_2 only, 2) 500 ml/min CO_2 only, 3) 200 ml/min CO_2 + octenol (wick in), 4) 500 ml/min CO_2 + octenol (wick in), 5) octenol (wick in), and 6) no bait. Treatments for the second test period (July 28–August 2) were: 1) 200 ml/min CO_2 only, 2) 200 ml/min CO_2 + octenol (wick out), 3) 200 ml/min CO_2 + octenol (wick in), 4) octenol (wick out), 5) octenol (wick in), and 6) no bait. The traps were operated from 1400 to 0800 CST each day.

All specimens collected were counted and identified to species. Catches for each test period were transformed to log (n + 1) for analysis of variance (Sokal and Rohlf 1969). The transformed data were analyzed with Statistical Analysis System (SAS) programs PROC GLM and Means/DUNCAN for analysis of variance and mean comparisons (SAS Institute 1985). Because different attractant combinations were

Table 1. Mean catch ± SE per trap day[1] for different treatments of odor baited unlighted CDC traps operated near rice fields at Stuttgart, AR, July 8–18, 1987.

	Control	Attractant			
Species	No bait	Octenol	500 ml/min CO_2	Octenol + 500 ml/min CO_2	1,000 ml/min CO_2
Anopheles crucians	0.0 ± 0.0 B	0.0 ± 0.0 B	10.9 ± 5.8 A	8.7 ± 2.9 A	7.6 ± 2.2 A
An. quadrimaculatus	0.7 ± 0.3 B	1.9 ± 0.8 B	355.8 ± 91.1 A	823.7 ± 282.0 A	574.2 ± 92.9 A
Coquillettidia perturbans	0.0 ± 0.0 B	0.0 ± 0.0 B	0.9 ± 0.4 AB	3.1 ± 2.1 A	1.3 ± 0.7 AB
Culex erraticus	6.5 ± 4.5 B	10.9 ± 7.6 B	62.3 ± 25.2 A	92.1 ± 29.1 A	162.3 ± 69.6 A
Cx. salinarius	0.1 ± 0.1 B	0.2 ± 0.1 B	10.5 ± 2.6 A	18.2 ± 4.3 A	23.8 ± 9.2 A
Psorophora ciliata	0.0 ± 0.0 C	0.0 ± 0.0 C	1.3 ± 0.5 B	3.4 ± 1.1 A	1.6 ± 0.8 B
Ps. columbiae	9.8 ± 6.7 B	12.8 ± 5.7 B	718.8 ± 352.7 A	2,314.8 ± 899.6 A	2,786.3 ± 872.6 A
Ps. mathesoni	0.3 ± 0.2 BC	0.0 ± 0.0 C	4.7 ± 2.7 A	4.2 ± 2.6 A	3.3 ± 2.0 AB

[1] $n = 10$ days; means in the same row followed by the same letter are not significantly different ($P > 0.05$); Duncan's multiple range test (SAS Institute 1985) applied to log (n + 1) transformed data.

used during different sampling periods, each experiment was analyzed separately.

RESULTS

In 1987, a total of 80,370 mosquitoes, all females, consisting of 8 species were collected (Table 1). Three species (*Ps. columbiae* (72.7%), *An. quadrimaculatus* (21.9%) and *Cx. erraticus* (Dyar and Knab) (4.2%)) were predominant in the collections. Traps baited with octenol (wick in) only did not result in a significantly ($P > 0.05$) increased response of any species relative to unbaited traps (Table 1). When the CO_2 rate was doubled from 500 ml/min to 1,000 ml/min, trap collections of all species except *An. crucians* Wied. and *Ps. mathesoni* Belkin and Heinemann were increased, but none of these increases was statistically significant. Although the combination of 500 ml/min CO_2 + octenol (wick in) generally resulted in increased trap collections for most species, this increase was statistically significant ($P < 0.05$) only for *Ps. ciliata* (Fabricius).

In 1988, six mosquito species (*An. crucians, An. quadrimaculatus, Cq. perturbans, Cx. erraticus, Cx. salinarius* and *Ps. columbiae*) were collected (Tables 2 and 3). During the first sampling period (July 18–23), 103,907 mosquitoes were collected. *Psorophora columbiae* (58.7%), *An. quadrimaculatus* (28.5%) and *Cx. erraticus* (12.3%) were again the predominant species. During the second sampling period (July 28–August 2), 47,758 mosquitoes were collected. In addition to *Ps. columbiae* (34.3%), *An. quadrimaculatus* (29.9%) and *Cx. erraticus* (29.7%), *Cx. salinarius* (5.3%) was abundant in the collections. No males were collected during either sampling period.

During the first sampling period (Table 2), no mosquitoes were collected in either the no bait or octenol (wick in) baited traps. Increasing the CO_2 release rate from 200 ml/min to 500 ml/min increased, though not significantly ($P > 0.05$), collections of all species except *Cx. erraticus* and *Cx. salinarius*. The various combinations of octenol (wick in) and CO_2, with few exceptions, did increase the size of the trap collections (Table 2) when compared with the same rate of CO_2 alone, but in only one case was the increase statistically significant. *Psorophora columbiae* collections were significantly ($P < 0.05$) increased by the combination of octenol (wick in) and 500 ml/min CO_2 as compared with 500 ml/min CO_2 alone.

During the second sampling period in 1988, two rates of octenol (wick in and wick out) were compared alone and in combination with 200 ml/min CO_2. Neither rate of octenol alone resulted in any statistically significant change in trap collection size when compared with the no bait treatments. The basic trend was that octenol supplemented CO_2 traps tended to increase collections of most species (Table 3), but the only trap catch comparisons that were statistically significant were the 200 ml/min CO_2 with and without octenol (wick in and wick out).

DISCUSSION

These data generally support trends observed in our previous field studies conducted with octenol and CO_2 (Takken and Kline 1989, Kline et al. 1990a, 1990b, 1991). In our previous studies we observed that: 1) very few mosquito species respond in large numbers to octenol alone, 2) the combination CO_2 + octenol causes a large increase (2× or greater) in the collections of many mosquito species, especially species of *Aedes, Anopheles, Psorophora, Coquillettidia* and *Mansonia* (the increase appears to be due to a synergistic effect), and 3) *Culex* spp. show little response to octenol alone or in combination with CO_2 (any increase tends to be an additive effect).

Table 2. Mean catch ± SE per trap day[1] for different treatments of odor-baited unlighted CDC traps operated near rice fields at Stuttgart, AR, July 18–23, 1988.

	Control	Attractant				
Species	No bait	Octenol (wick in)	200 ml/min CO_2	500 ml/min CO_2	Octenol (wick in) + 200 ml/min CO_2	Octenol (wick in) + 500 ml/min CO_2
Anopheles crucians	0.0 ± 0.0 B	0.0 ± 0.0 B	11.3 ± 5.9 A	14.7 ± 6.9 A	19.8 ± 8.3 A	13.3 ± 5.6 A
An. quadrimaculatus	0.0 ± 0.0 B	0.0 ± 0.0 B	1,078.5 ± 466.5 A	1,558.8 ± 517.2 A	1,103.5 ± 523.9 A	1,200.7 ± 414.6 A
Coquillettidia perturbans	0.0 ± 0.0 B	0.0 ± 0.0 B	1.5 ± 0.7 AB	1.7 ± 1.4 A	3.8 ± 5.8 A	3.5 ± 3.7 A
Culex erraticus	0.0 ± 0.0 B	0.0 ± 0.0 B	697.2 ± 235.9 A	536.2 ± 275.7 A	492.3 ± 94.7 A	398.7 ± 193.2 A
Cx. salinarius	0.0 ± 0.0 B	0.0 ± 0.0 B	39.3 ± 18.9 A	37.8 ± 9.9 A	123.0 ± 43.1 A	160.5 ± 77.6 A
Psorophora columbiae	0.0 ± 0.0 C	0.0 ± 0.0 C	1,001.3 ± 280.3 B	1,760.8 ± 1,398.2 B	3,446.5 ± 1,638.6 AB	3,960.5 ± 973.9 A

[1] $n = 6$ days; means in the same row followed by the same letter are not significantly different ($P > 0.05$); Duncan's multiple range test (SAS Institute 1985) applied to log (n + 1) transformed data.

Table 3. Mean catch ± SE per trap day[1] for different treatments of odor-baited unlighted CDC traps operated near rice fields at Stuttgart, AR, July 28–August 2, 1988.

	Control	Attractant				
Species	No bait	Octenol (wick in)	Octenol (wick out)	200 ml/min CO_2	Octenol (wick in) + 200 ml/min CO_2	Octenol (wick out) + 200 ml/min CO_2
Anopheles crucians	0.0 ± 0.0 B	0.0 ± 0.0 B	0.7 ± 0.5 B	7.7 ± 3.5 A	24.5 ± 12.2 A	18.5 ± 4.4 A
An. quadrimaculatus	0.3 ± 0.2 B	1.5 ± 0.9 B	3.3 ± 1.3 B	710.0 ± 173.5 A	1,100.2 ± 518.5 A	469.5 ± 191.7 A
Coquillettidia perturbans	0.0 ± 0.0 C	0.0 ± 0.0 C	0.0 ± 0.0 C	1.7 ± 0.2 B	4.2 ± 1.7 AB	9.3 ± 4.1 A
Culex erraticus	19.3 ± 11.7 B	40.8 ± 28.4 B	19.5 ± 12.1 B	560.7 ± 174.9 A	889.2 ± 517.3 A	832.2 ± 496.5 A
Cx. salinarius	0.3 ± 0.3 C	3.7 ± 1.7 C	1.7 ± 0.8 C	54.0 ± 37.3 B	139.5 ± 36.2 A	223.7 ± 98.2 A
Psorophora columbiae	3.2 ± 1.9 B	5.5 ± 3.3 B	26.0 ± 13.9 B	613.3 ± 329.1 A	1,013.8 ± 262.9 A	1,069.2 ± 360.5 A

[1] $n = 6$ days; means in the same row followed by the same letter are not significantly different ($P > 0.05$); Duncan's multiple range test (SAS Institute 1985) applied to log (n + 1) transformed data.

None of the species in the study reported herein showed any significant response to octenol alone. There was a twofold or greater increase in collection size of *Cq. perturbans*, *Cx. salinarius*, *Ps. ciliata* and *Ps. columbiae* with octenol-supplemented CO_2 compared with CO_2 alone treatments. Because of extreme variation in the daily catches, the means comparison procedure was unable to resolve many differences between them ($P > 0.05$). These increases were not statistically significant, but they may be biologically significant. Although the data for *Cx. erraticus* followed the generalized trend for *Culex* spp. in its response to octenol and CO_2, *Cx. salinarius* did not. But these data are consistent with the data obtained for *Cx. salinarius* trapped near phosphate mining operations in Florida (Kline et al. 1990a). In that study, trap collections of *Cx. salinarius* obtained with 500 ml/min CO_2 treatments supplemented with octenol were significantly greater than trap collections obtained with 500 ml/min CO_2 alone.

Of all the genera evaluated to date for their responses to octenol and CO_2, the responses of the anophelines, especially *An. crucians* and *An. quadrimaculatus* have been the most difficult to interpret. This remains true with the data obtained in the current study. At times octenol appears to enhance collections of these species, but other times it appears to repel these species. Data obtained from our studies with these species in other ecological and geographical zones show similar responses (Kline et al. 1990a, 1990b).

These results indicate that much remains to be learned about octenol's mode of action and its potential as an effective attractant in mosquito surveillance and possible removal trapping programs. Determination of why it attracts some species of mosquitoes, or some populations of the same species, and not others requires further investigation.

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