

**Response of Mosquitoes to 1-Octen-3-ol
at the University of Notre Dame
Ecology Research Center**

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Introduction

Recent studies have suggested that 1-octen-3-ol (octenol) acts as a chemical attractant for mosquitoes. Octenol is a volatile compound extracted from many natural sources such as the breath of oxen, the anal sac of wolves, clover, alfalfa and mushrooms. The chemical structure of octenol consists of an 8-carbon mono-unsaturated alcohol, two optical isomers and a terminal double bond. First isolated in 1984 from the breath of oxen, octenol was found to act as a powerful attractant among certain species of tsetse flies (Hall et. Al 1984 in Kline 1994). Since then an interest in octenol's effectiveness as a potential attractant among species of mosquitoes has invoked research studies.

Specifically, many studies have focused on whether octenol works by itself or whether there exists a synergistic effect between CO₂ and octenol. CO₂ has been established as a standard attractant among all species of mosquitoes (Service 1976). CO₂ elicits two different responses. First, it acts as an attractant independently, and, second, the effectiveness of CO₂ as an attractant on mosquitoes is enhanced at a distance by odor substances (Gullies 1980). This leads to the question whether octenol can act synergistically as a volatile odor substance with CO₂ or possibly by itself.

In fact, studies performed by Takken and Kline (1989) and Kline et. Al (1990a, 1991b) suggest that octenol with CO₂ increase collections of aedine mosquitoes. Unfortunately, only certain species act synergistically with CO₂. Kline (1994) found a

positive response in *Aedes taeniorhynchus*, a Neotropical salt marsh, and Kemme (1993) found a similar response among *Ae. vigilax* and *Ae. funereus*. Kemme (1993) also found that octenol alone enhances the attraction of *Ae. vigilax*, but that collections of *Culex sitiens* and *Cx. annulirostris* in the presence of octenol were stymied. These studies show that unlike CO₂, octenol is not a universal attractant among all species. Limited research has been performed on determining why it attracts certain species and whether there exists a geographical basis for these results.

Though the Collier Mosquito Control District, located in southwest Florida routinely surveys mosquito population with 25 New Jersey (NJ) light traps and octenol (Kline 1994), studies performed in other geographical locations have not found the combination of CO₂ and octenol to be effective. Dr. Craig, biology professor at the University of Notre Dame, evaluated octenol in the summer of 1994 in Upper Peninsula region of Michigan on the UNDERC property. Traps with and without octenol were run at three different highly-populated mosquito populations: Morris Lake, Tender Bog and Cranberry Lake. The data showed no variance among traps with octenol (distributed at low concentration by American Biophysics Corp, 18 Southwest Ave. Jamestown, RI 02835) and traps without octenol. More intense studies need to be performed before evaluating the effectiveness of the supposed mosquito attractant, octenol.

Therefore, the objective of this study, which was performed on the UNDERC property during the summer of 1995, was to test the hypothesis that octenol works synergistically with CO₂ as a mosquito attractant.

It is important to note that the octenol used during this

experiment was distributed by the Fluka Chemical Company at a much higher concentration (99% octenol) than the octenol packets used by Dr. Craig the previous summer at UNDERC.

Method

Study area: All experiments were run on the UNDERC property in the Upper Peninsula of Michigan. The complete study was run at four different locations: Tenderbog, Morris Lake, the Tire Dump and Cranberry Lake. For each location two positions were established 40 meters apart from one another. Treatments 1) ABC trap + CO₂ and 2) ABC trap + CO₂ + Octenol were run nightly for twelve hours. The ABC Dipteran Collection Trap is distributed by the *American Biophysics Corp.* (18 Southwest Ave. Jamestown, RI 02835) and the concentrated 99% octenol is distributed by *Fluka Chemical Company* (S.2nd St. Ronkonkoma, NY 11779-7238). Each of the two treatments were routinely switched from position 1 to position 2 at the beginning of each trapping night to factor out a possible position effect and to assure a more precise analysis. This process reduces experimental error by removing the differences among the days and the trap locations. A total of 16 trapping nights were used in the data analysis.

The traps were hung underneath tree branches at a height of 5 ft. CO₂ tanks released CO₂ above the fan on the ABC trap with a rigid foam insulation allowing a 500ml/min sublimation rate. The liquid octenol was placed in a small glass vial. A pipe cleaner was inserted to soak up the octenol and release it into the environment. Approximately 1ml/12 hr of Octenol at a

concentration of 99% was used. The octenol was released at the same height and area as the CO₂. Once again, it is important to note that a stronger concentration of octenol was released in this experiment than was used in the previous year under Dr. Craig.

Mosquito collections: The collected mosquitoes were placed in the freezer for one hour in order to kill them. Then the mosquitoes were counted and identified. All the trap counts were statistically analyzed by transforming the counts to $\log(n+1)$. Multifactor Analysis of Variance was used to determine the significance of the attractant, position and location factors. (*Statgraphics, Statistical Graphics Corporation, 1989*).

Results

Graph 1 shows the results of the two treatments 1) CO₂ and Octenol and 2) CO₂ for the four trapping locations Tenderbog, Tire Dump, Morris Lake and Cranberry Lake. The average mosquito count/night was determined for each treatment at all four locations. The standard errors were also included on all the data points. Multifactor analysis of variance was run, and out of the three factor effects: attractant, position and location, only the attractant factor had a p value of <.05. Position and location p values were >.05 (Table 2). Summing all the mosquito counts for the two treatments at all locations gave a total of 26,486 mosquitoes for the CO₂ & Octenol treatment and a total of 6,561 mosquitoes for the CO₂ treatment. The average count/night for the CO₂ & Octenol treatments at all locations was 1,655.4 mosquitoes with a standard error of 415.0, and the average count/night for

the CO₂ treatment was 410.0 with a standard error of 80.1 (Graph 2). The total counts for each night of trapping are shown on TABLE 1.

In addition to taking a total count of mosquitoes for each trap, the mosquitoes were identified to the species level. A pie graph was drawn for each of the two treatments showing the percentages of all the main species present (Graph 3 and Graph 4). *Ma.perturbans* had the highest percentage followed by *Ae. punctor*. This relation was true for both treatments 1) CO₂ & Octenol and 2) CO₂. The following mosquito species were also found during the experiment on the UNDERC property but were all under 1% of the total count for treatment 1 and treatment 2: *Ae. canadensis*, *Ae. cinereus*, *Ae. excrucians*, *Ae. provocans*, *An. punctipennis*, *An. walkeri*, *Cl. morsitans*, *Cx. restuans* and *Cx. territans*.

ANALYSES

Based on the results of this experiment, it was found that using a 99% treatment of octenol synergistically with CO₂ significantly increased the number of mosquitoes captured in the ABC Dipteran traps. The multifactor analyses run on all the possible factors influencing mosquito counts assures the validity of the results. Out of the three possible factors; attractant, position and location influencing the mosquito counts, only the attractant factor had a P value less than .05. Thus, the octenol is responsible for the established results. Conversely, position and location of the traps, having p values far above .05, did not influence the results significantly. Spreadsheet information of the multifactor analyses can be found in Table 2.

Graph 1 gives the results for the average mosquito counts per night at the four different locations. The effectiveness of using octenol with CO₂ rather than only using CO₂ is especially evident at locations Tenderbog, Morris Lake and Cranberry. The smaller difference at the Tire Dump location may be due to the lack of mosquitoes present at the location. Whereas the other three sites were located in low, wet areas near a lake or a bog, the Tire Dump site was located on a small hill farther away from any water source. Since mosquito larvae live in water, the Tire Dump site may have been an inadequate source for mosquitoes.

Graph 2 combines the average mosquito counts for all locations and clearly gives evidence that the 99% octenol greatly enhances the CO₂ attractant ability. The error bars on Graph 1 and Graph 2 refer to the standard internal errors calculated through the *Statgraphics Multifactor Analysis*.

Females were the dominant gender captured in the traps for both treatments. The total percentage of males caught was less than 1%. As well as taking a total count of the captured mosquitoes, they were identified to the specie level. Traps consisting of both treatments (CO₂ with and without octenol) contained primarily *Ae. punctor* during the first few weeks and *Ma. perturbans* for the remainder of the summer. All other mosquito species identified in these traps were insignificant in comparison to the dominant species *Ae. punctor* and *Ma. perturbans*. The actual percentages of species found for both treatments are shown and displayed in pie charts (Graph 3 and Graph 4). *Ae. cinereus*, *Ae. canadensis*, *An punctipennis*, *Cl. morsitans*, *Cx. restuans* and *Cx. territans* are grouped together due to their small numbers.

Conclusion

Hence, the conclusion that the 99% octenol significantly enhances the effectiveness of CO₂ as an attractant for mosquitoes may be drawn from this experiment. Further experiments however are needed to determine the effectiveness of CO₂ with octenol on all species. It was not determined in this experiment whether *Ae. punctor* and *Ma. perturbans* were more attracted to the octenol than all the other species or whether the high percentage of these two species captured in the traps merely reflect their high population on the property.

In addition, it must be noted that a comparison between the octenol packets distributed by *American Biophysics Corp.* and the 99% octenol distributed by *Fluka Chemical Co.* needs attention. Whereas the experiment run by Dr. Craig using the octenol packets does not support the effectiveness of octenol, this experiment using 99% liquid octenol does support the effectiveness of octenol.

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TABLE 1

	A	B	C	D
1	Date	Location	No Octenol	Octenol
2				
3				
4	17-Jun	Tenderbog	263	951
5	19-Jun	Morris	259	2727
6	21-Jun	Morris	783	833
7	28-Jun	Cranberry	438	2388
8	29-Jun	Cranberry	816	3008
9	1-Jul	Cranberry	96	207
10	7-Jul	Cranberry	167	269
11	9-Jul	Tenderbog	631	2112
12	10-Jul	Tenderbog	514	2840
13	13-Jul	Tenderbog	282	4032
14	14-Jul	Tenderbog	1223	5648
15	20-Jul	Tenderbog	68	110
16	21-Jul	Tire Dump	52	219
17	22-Jul	Tire Dump	369	198
18	24-Jul	Tire Dump	415	632
19	25-Jul	Tire Dump	185	312

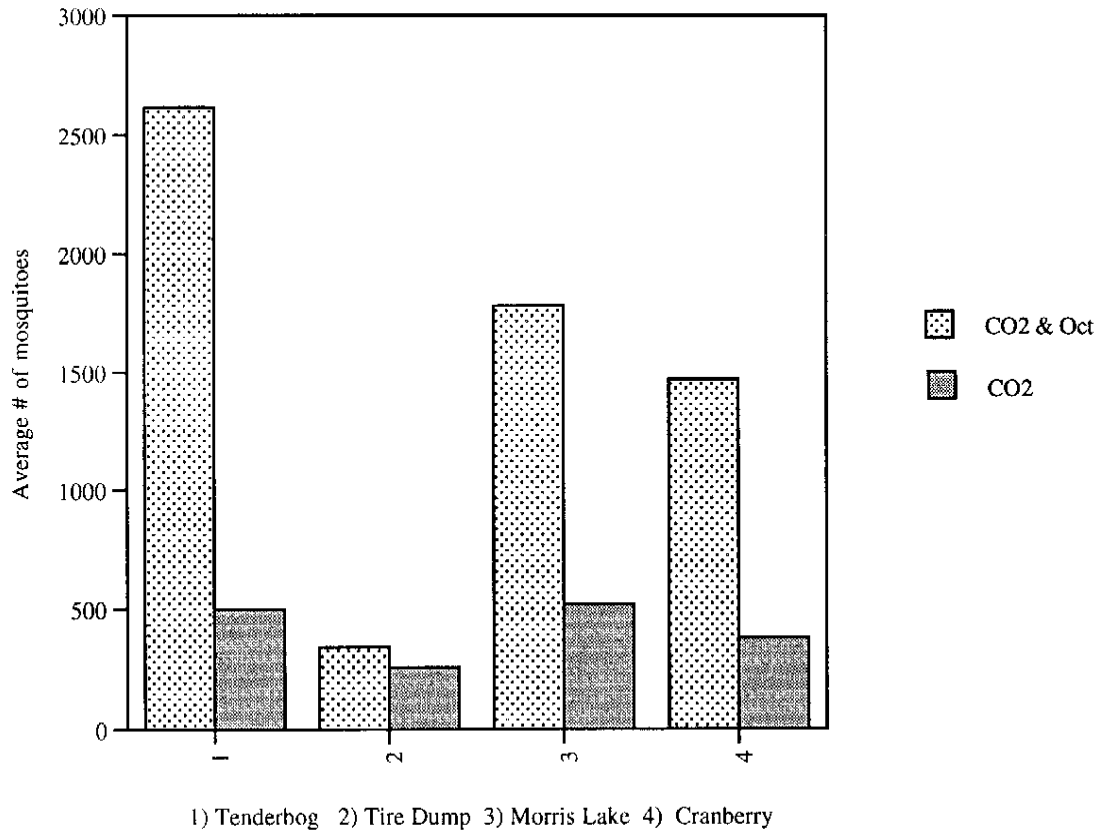
ANALYSIS OF VARIANCE FOR ERG.LUG1012

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	3.3107561	5	.6621512	2.513	.0659
ATTRACT	1.7531281	1	1.7531281	6.653	.0184
LOCATN	1.3202675	3	.4400892	1.670	.2071
POSIT	.2373605	1	.2373605	.901	.3645
2-FACTOR INTERACTIONS	.3908459	7	.0558351	.212	.9783
ATTRACT LOCATN	.2484621	3	.0828207	.314	.8148
ATTRACT POSIT	.0005780	1	.0005780	.002	.9636
LOCATN POSIT	.1418058	3	.0472686	.179	.9091
RESIDUAL	5.0069789	19	.2635252		
TOTAL (CORR.)	8.7085809	31			

0 missing values have been excluded.

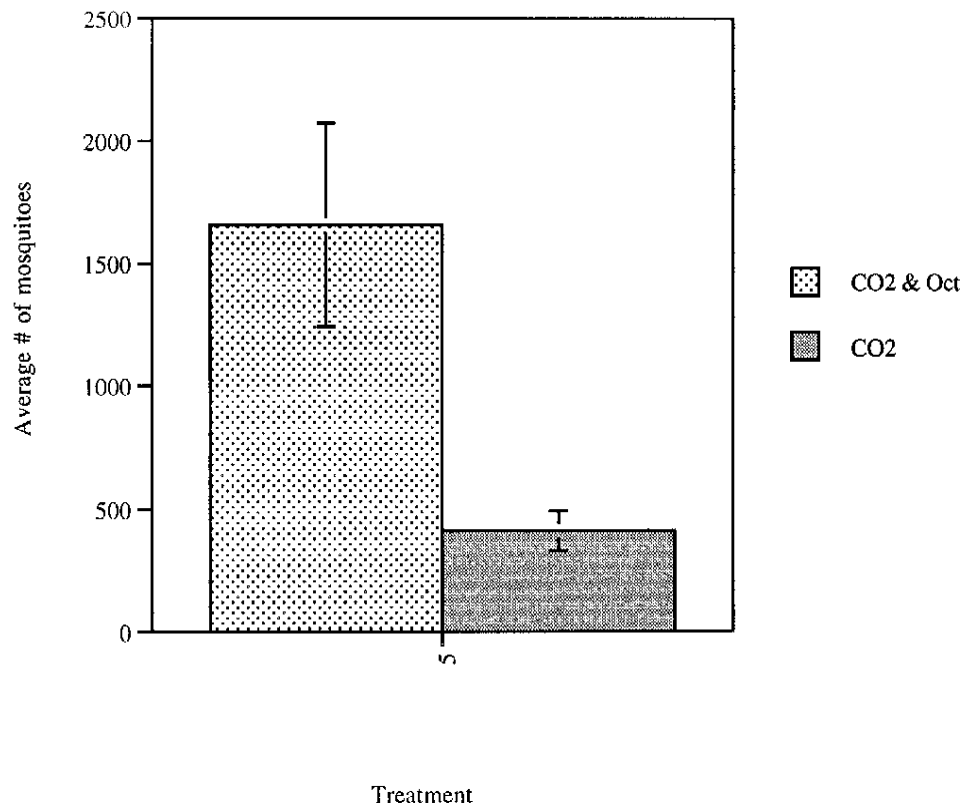
GRAPH 1

Average nightly mosquito counts for treatments
1) CO2 and Octenol and 2) CO2
for four location sites

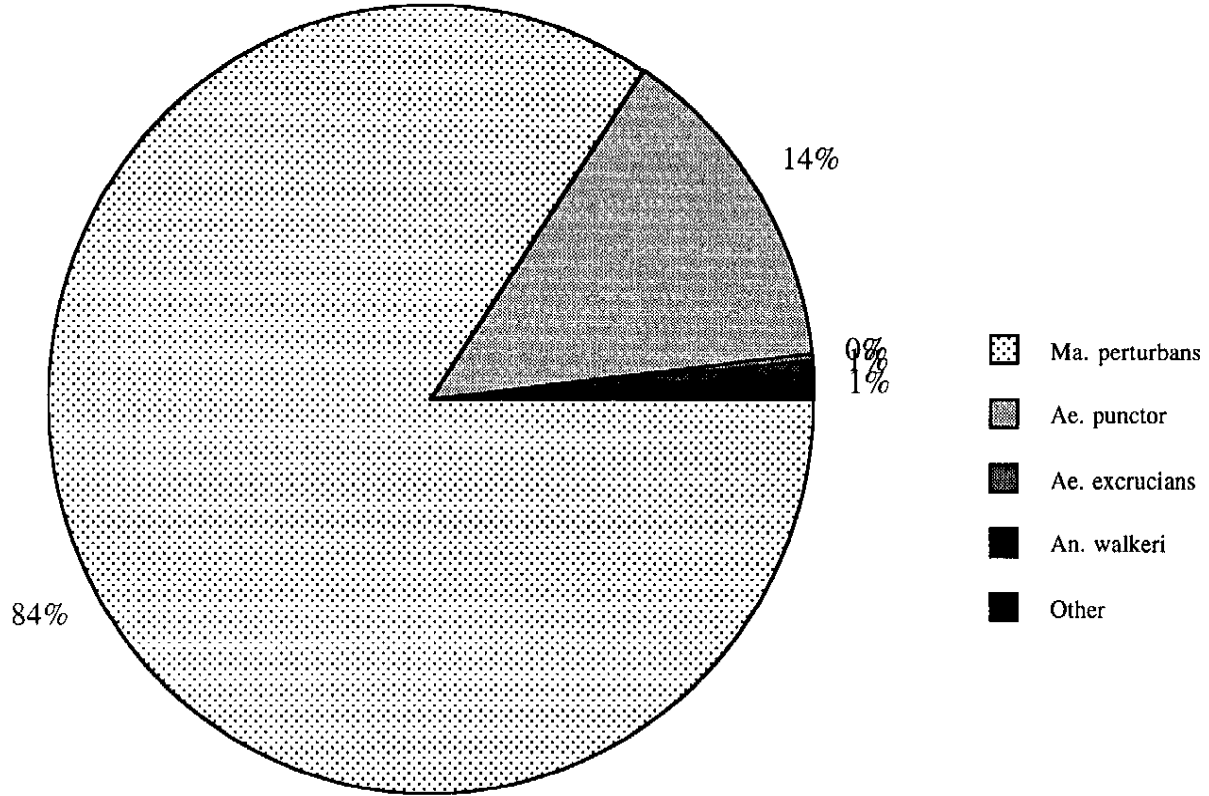


GRAPH 2

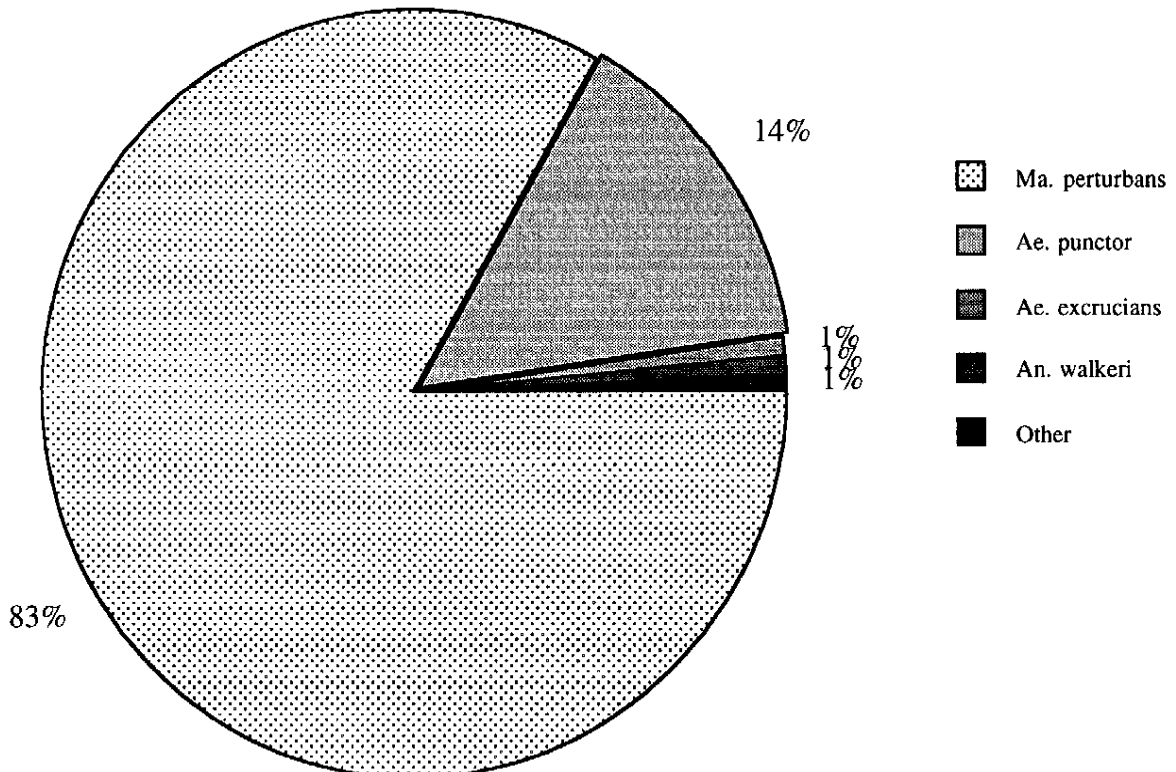
Average nightly mosquito counts for all locations



GRAPH3 Data #1
Specie percentage for
treatment CO₂



Data #1
GRAPH4
Specie percentage for
treatment CO₂ and Octenol



Materials

ABC Dipteran Trap

Silicon gasket

CO₂ tanks

External hanger and hook

Long life DC motor

PVC cylinder

Net bag

99% octenol

CO₂

Microscope

pipe cleaners

glass vials