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THE RELATIVE ATTRACTIVENESS OF CARBON DIOXIDE TO PAROUS AND NULLIPAROUS MOSQUITOES[1]

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Abstract: Standard New Jersey light traps were compared with and without a dry-ice supplement in a coastal area of New Jersey. The tests were conducted to determine the relative attractiveness of CO_2 to parous and nulliparous specimens and to determine if the addition of CO_2 as an attractant affected the age composition of the mosquitoes in the sample. More than 15,000 female mosquitoes were collected during the study; *Culex salinarius*, *Aedes canadensis*, *Ae. cantator*, *Ae. vollucians* and *Ae. vexans* were the predominant species. Ovarian dissections revealed that a significantly greater percentage of parous mosquitoes was collected in the trap where light was the only attractant. The addition of CO_2 consistently attracted a greater proportion of the nulliparous specimens. The light trap baited with dry ice, however, caught more than 17 times the number of mosquitoes and increased the incidence of the major species by 8- to 45-fold. Data suggested that the addition of CO_2 in the form of dry ice attracted a greater percentage of nulliparous mosquitoes but increased the total catch sufficiently to warrant its continued use for arbovirus sampling in most situations.

The benefits of using carbon dioxide as a mosquito attractant have been known for many years. Headlee (1941) and Reeves & Hammon (1942), as well as Hufflaker & Back (1943), reported increases in light-trap catches when CO_2 was used as a supplement, in the form of dry ice. The system has also been employed to collect specimens for arbovirus surveillance. Most public health agencies have used dry ice in conjunction with light since Newhouse et al. (1966) reported that light traps baited with dry ice collect greater numbers of mosquitoes for virus testing as well as increased number of species.

Although the use of dry ice as an adjuvant increases the number of mosquitoes trapped, relatively little information is available on the physiological age composition of the sample. In the absence of transovarial transmission, only parous mosquitoes are able to function as biological vectors of disease. As a result, public health personnel would ideally prefer to sample and test only the parous portion of the population. Morris & DeFoliart (1971) concluded that the efficiency of virus assays would be increased if the periods of high parous rates in a population could be predicted. Limiting virus isolation attempts to periods when parity is high would eliminate the costly tests during those periods when virus recovery is unlikely.

Dissection and examination of numerous mosquitoes are time-consuming, however, and in most cases economically unfeasible. A trapping method that was selective for parous individuals would represent the ideal alternative. Since dry ice is often employed to collect specimens for arbovirus surveillance, a study was designed to field-test the relative attractiveness of CO_2 to parous and nulliparous mosquitoes. The results of this study are presented in this paper.

MATERIALS AND METHODS

Trapping was conducted near Adamston, a coastal community in Ocean County, New Jersey. The majority of land at the site was composed of heavy undergrowth and 2nd-growth woodland bordering tidal salt marsh. Larval sampling revealed an abundance of diverse mosquito habitats in the immediate area ranging from fresh floodwater to tidal pools.

Two standard New Jersey light traps placed approximately 100 m apart were used to make the comparison. The traps were located so that competition was minimal; heavy undergrowth, shrubs and trees obscured light from one source to another. One trap was operated with light as the only attractant. The other was baited with 2–3 kg of dry ice, which was wrapped in aluminum foil to retard sublimation and suspended in orthopedic stockinet near the trap. Traps were activated at dusk and operated until the collections were picked up at 0700 h the following morning. The traps were alternately baited at weekly intervals from 6 June to 15 September 1973. The procedure yielded 16 live collections on which the comparisons were based.

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TABLE 1. A comparison of standard New Jersey light-trap collections at Adamston, New Jersey with and without a CO_2 supplement, based on the 5 predominant species collected.

Mosquito species	No. ♀ collected		Fold increase with CO_2 supplement
	Without CO_2	With CO_2	
Culex salinarius	473	7055	14.9
Aedes canadensis	76	3420	45.0
Ae. cantator	61	1872	30.7
Ae. vexans	116	1118	9.6
Ae. sollicitans	93	763	8.3
Totals	819	14,228	17.4

TABLE 2. Percentages of parous mosquitoes captured by standard New Jersey light traps at Adamston, New Jersey with and without a CO_2 supplement, based on the 5 predominant species collected.

Mosquito species	No. dissected	% parous†	
		Without CO_2	With CO_2
Culex salinarius	1736	35.4***	24.1
Aedes canadensis	1009	56.2***	35.1
Ae. cantator	604	60.7***	33.3
Ae. vexans	571	38.6*	40.7
Ae. sollicitans	501	45.3**	30.1
Weighted average		44.8***	31.4

† Differences between trapping methods significant at the following probability levels. ***0.005, **0.01, *0.05, according to 2 × 2 chi-square test (1 df) based on actual number of individuals

In the laboratory, the mosquitoes were lightly anesthetized with chloroform and identified to species. The females were sorted into 10-cm plastic petri dishes, which were lined with moist filter paper. After the mosquitoes revived, the petri dishes were refrigerated at 5 C until the specimens were dissected. Mosquitoes were dissected according to the method of Meadows (1968) and parity was determined by Detinova's method of ovarian tracheation (Detinova 1962).

A 2 × 2 chi-square contingency test was used to analyze the data, on both a trap-night and pooled-data basis.

RESULTS

Mosquito abundance More than 15,000 mosquitoes were collected and identified during the study. Although 14 species representing 6 genera were encountered, *Culex salinarius* Coquillett, *Aedes canadensis* (Theobald), *Ae. cantator* (Coquillett), *Ae. vexans* (Meigen) and *Ae. sollicitans* (Walker) were the predominant species, constituting more than 98% of the total. These 5 species showed that a variety of breeding habitats occurred in the area. *Ae. canadensis* is predominantly an early-season mosquito species that breeds in transient woodland pools. *Ae. vexans* is a floodwater species that utilizes transient rain pools somewhat later in the season. *Cx. salinarius* is a mosquito that requires fresh to brackish permanent water with considerable emergent vegetation. *Ae. cantator* and *Ae. sollicitans* are marine species that breed in tidal salt marshes.

Attractiveness of CO_2. In all cases the addition of CO_2 in the form of dry ice markedly increased the number of mosquitoes in the collection. TABLE 1 shows a comparison of the efficiency of the 2 trapping techniques for the predominant species. Over the entire season, the light trap without a CO_2 supplement collected 819 mosquitoes of these 5 species. The trap with the dry-ice supplement collected 14,228 individuals over the same period, an increase of 17.4-fold. The addition of dry ice had the greatest effect on *Ae. canadensis*, a mosquito that is not readily attracted to light. The trap with light alone collected only 76 specimens during the entire season. The trap with the dry-ice supplement collected 3420, an increase of 45-fold. *Ae. cantator* was also highly attracted to the CO_2 supplement, increasing by 30.7-fold in the dry ice-baited trap. Each of the remaining predominant species was collected in greater numbers by the CO_2-supplemented trap, but the differences were not as marked.

Physiological age. Ovarian dissections revealed significant differences in the physiological age of the mosquitoes collected by the 2 trapping methods. The results of 4421 dissections are presented in TABLE 2. In all cases, a greater percentage of parous mosquitoes was collected where light alone was the only attractant. The trap with the CO_2 supplement was consistently more attractive to the nulliparous portion of the population, for all mosquito species throughout the sampling period. The only exception was the last sampling date in the study, when few mosquitoes were captured and all of these were parous. The differences between trapping methods were significant for each of the predominant species when analyzed by 2 × 2 chi-square treatment using the actual number of mosquitoes for the computation. Significant differences were also obtained when individual sampling dates were analyzed (except where previously mentioned) and merely substantiated pooled-data analysis.

The attraction of nulliparous mosquitoes to CO_2 was most evident with *Ae. canadensis* and *Ae. cantator*. These were the species that showed the greatest increase when dry ice was used as a collection supplement. Although dry ice was an efficient means of increasing the numbers of mosquitoes in these studies, the addition of CO_2 depressed the overall parous rate by attracting a disproportionate number of nulliparous mosquitoes.

DISCUSSION

The results of these studies appear to be in general agreement with previous work. The overall increase of 17.4-fold by the addition of CO_2 is much greater than the 4-fold increase reported by Newhouse et al. (1966), but the traps used to make the comparisons were quite different. Newhouse et al. (1966) used CDC Miniature light traps which are small and easily portable. The standard New Jersey light trap has a larger motor with greater displacement and is capable of drawing in mosquitoes from a farther distance.

Magnarelli (1975) dissected mosquitoes for parity that were collected in CDC Miniature light traps with and without a dry-ice supplement. He reported that the highest percentage of parous mosquitoes (*Anopheles walkeri*, *Coquillettidia perturbans* and *Culiseta morsitans dyari*) was taken by the unsupplemented traps, but his sample size was not large enough to reach a firm conclusion on relative attractancy. Data from the present study confirm his observation. With all species, the highest percentage of parous mosquitoes was collected by the trap where light alone was the only attractant.

These results may question the broad use of CO_2 as an attractant to collect mosquitoes for arbovirus testing. Data indicated that dry ice resulted in collection of considerably more mosquitoes, but also increased the percentage of nulliparous specimens in the sample. This might adversely affect public health programs with small budgets allocated for virus testing. Where only limited numbers of mosquitoes can be screened, a greater number of unsupplemented traps placed over a wider geographic area may be preferable on economic grounds.

In most cases, however, the advantage of using unsupplemented light traps for the collection of physiologically "older" specimens is negated by the sheer numbers of mosquitoes attracted by the CO_2 supplement. Dry ice appears to depress the overall parous rate of the collection, but the sample size is increased to the point where considerably more parous specimens are captured when CO_2 is used. This is particularly true for mosquito species that are not readily attracted to light. It would be impractical to operate the number of unsupplemented traps required to collect adequate numbers of *Ae. canadensis* or *Ae. cantator* for virus testing. Mosquitoes that are highly attracted to light, however, might be more efficiently trapped without a CO_2 supplement. If adequate numbers can be obtained without the addition of CO_2, there is no reason to use the attractant. The selection of a trapping method should be based on the particular needs of the study.

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