INFLUENCE OF PHYSIOLOGICAL CONDITION ON THE BEHAVIORAL RESPONSE OF *ANOPHELES DARLINGI* TO DDT[1]

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ABSTRACT. Wild-caught *Anopheles darlingi* females were tested with one control and one DDT treated excito-repellency test box in March of 1980 and 1981 at Amazonas, Brazil. Three tests each were conducted with unengorged (not blood fed), recently (recent fed) engorged and late (late fed) engorged specimens. The initial tests were conducted during 1980. Following this the boxes were left undisturbed at the field site for 1 year, at which time 3 additional tests with recently engorged specimens were conducted.

The escape rate of *An. darlingi* females from the DDT treated box was uniformly greater than from the control box in all tests, regardless of physiological condition of the mosquitoes. Significant differences in escape rates occurred during the first 5–10 min observation period(s) in most tests and continued to be significant for the remainder of each 60 min test period. "Recent fed" females were less prone to escape from both the DDT and control boxes than were "late fed" and unfed females. Escape rates for "recent fed" females from the DDT treated box in April 1981 were essentially identical to those obtained with the same box and DDT treatment in March 1980. Included is a discussion of the test results plus comments on the test method and options for analyses of excito-repellency test data.

INTRODUCTION

The irritability of DDT for anopheline vectors of malaria has been the subject of study and controversy for over 35 years (Trapido 1946, Kennedy 1947).[a] Avoidance of DDT treated surfaces by various species has been documented during field studies and by tests in the laboratory. Unfortunately neither sufficient data nor a standardized method for collecting such data exists which permits both a quantitative comparison of DDT irritability between species and an objective inference on the meaning of avoidance behavior for the continued use of DDT in a malaria control program. The latter continues to be a subject of great importance as DDT is still employed in most malarious areas of the world.

Attempts to demonstrate avoidance of DDT as an acquired behavior have been inconclusive, primarily because insufficient information exists on vector behavior prior to the introduction of DDT. This dearth of reliable data has resulted in considerable debate as to which descriptive terms are appropriate for avoidance behavior. Since there is no consensus, terms used in this report will be according to the definitions proposed by Georghiou (1972) who proposed that behavioristic avoidance could be either stimulus-dependent or stimulus-independent. Georghiou further classified stimulus-dependent resistance as resulting from increased irritability or increased repellency. According to his definitions irritability resulted from physical contact with insecticidal residues whereas repellency entails the detection of an insecticide residue without physical contact.

Although various methods have been proposed to study DDT irritability in anophelines (World Health Organization 1975)[b], no test seems to be widely accepted. One of the most promising irritability tests involves use of the excito-repellency test box designed by Rachou et al. (1973) for studies on the response of *Anopheles albimanus* Wiedemann to DDT residues in El Salvador. Similar boxes were employed by Charlwood and Paraluppi (1978) to test populations of *Anopheles darlingi* Root for excito-repellency to DDT. As we were studying the effect of DDT sprayed housewalls on the endophagic behavior of *An. darlingi* populations, we too used the excito-repellency test box to obtain data comparable to observations of Charlwood and Paraluppi (1978). Included herein are results from testing sample populations of *An. darlingi* in different physiological

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[a] Unpublished WHO report by Elliott, R. and J. de Zulueta. 1975. "Ethiological resistance in malaria vectors. Behavioral response to intradomestic residual insecticides." WHO/VBC/75.569.

[b] Unpublished WHO report by T. G. Gheorghiou, E. M. Ungureanu and C. Garrett-Jones. 1971. "An apparatus for the study of the behavior pattern of mosquitoes under the influence of insecticides." WHO/VBC/71.278.

conditions, as well as our observations on the test method and options for analysis of excito-repellency data.

MATERIALS AND METHODS

Specimens of *An. darlingi* released into an assembled excito-repellency box (0.5 m^3) could escape into one of two small cages (21.5 × 31 cm) via funnels attached to the side and top of each box (Fig. 1). To facilitate transportation to the field study site, the excito-repellency boxes were modified to be collapsible. Sheets of absorbent poster paper were sprayed with a wettable powder formulation of DDT at a rate of 2 gm DDT (active)/m^2. The DDT was applied with a 10 gallon (37.8 liter) manual sprayer by personnel of the National Malaria Control Program (SUCAM) in Labrea, Amazonas, Brazil. Upon arrival at our study site, the boxes were assembled and the top and sides of one box were lined with DDT treated paper, with the box bottom left bare to duplicate the test condition described by Charlwood and Paraluppi (1978). The second box was lined with untreated poster paper.

Tests were conducted in March of 1980 and 1981 at Floresta on the Ituxi River, Amazonas, Brazil, with *An. darlingi* females that were caught in human bait collections. Unfed and "recent fed" specimens were collected during the interval 0600–0700 on the day that each test was performed, whereas "late fed" specimens were allowed to engorge on humans and were collected from 1830–2000 of the day preceding the test. Water-soaked cotton pads were placed in cages during all holding periods prior to testing. Tests were conducted with approximately 50 specimens/box/test. A single test consisted of releasing test populations in each of the treated and control boxes. The standardized test procedures were as follows:

1) Approximately 100 specimens of a specified physiological condition were separated into 2 cages of 50 specimens per cage.

2) Tests were performed by a 2 man team, or 1 man/box.

3) The excito-repellency boxes and escape cages were cleared of living or dead insects before each test.

4) A waxed paper cup filled with wet cotton was placed in each box. Escape cages were put in place and each funnel was closed with disposable towels.

5) Ambient temperature and humidity readings were recorded at the beginning and end of each test.

6) Test specimens were gently aspirated from holding cages and blown into each box simultaneously.

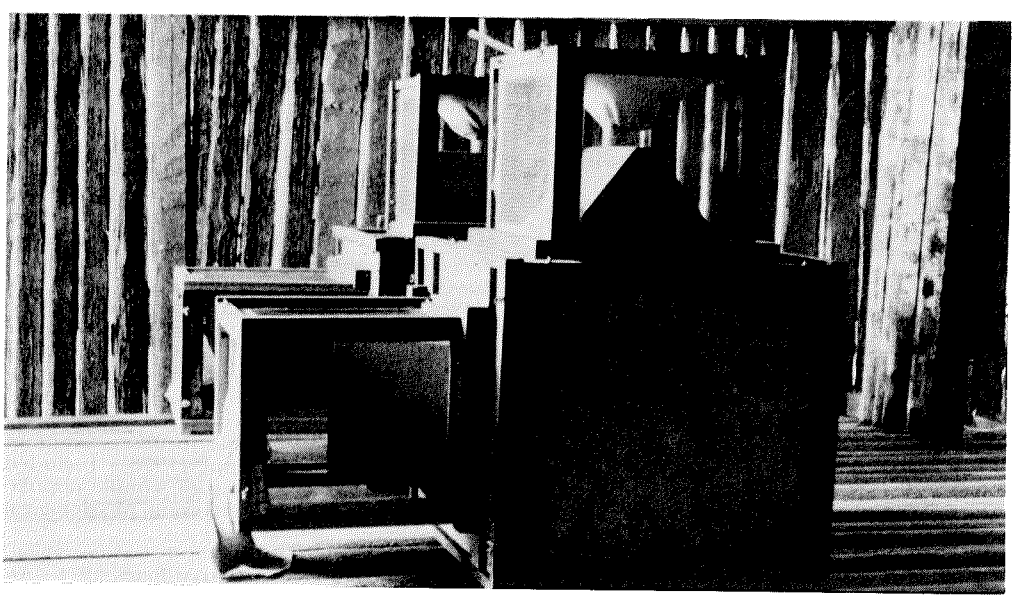

Fig. 1. Excito-repellency test boxes (with escape cages) inside an experimental house along the Ituxi River, Amazonas, Brazil.

7) Timing began with the transferral of specimens into the boxes (which required about 1–2 min). After 5 min towels were removed from the funnel openings. This 5 min interval during which the funnels were closed served as an adjustment time for the transferred specimens.

8) At the end of each subsequent 5 min interval after the funnels were opened, all specimens were collected from the escape cages with mechanical aspirators.

9) The number of escaped specimens was recorded for each 5 min interval.

10) The last observation was made 60 min after the funnels were opened. Live specimens left in the boxes were encouraged to enter the escape cages by gently rocking the boxes. The top escape cage was then partially removed and all live and dead specimens remaining inside the boxes were collected.

Using the above listed procedure three tests each were conducted for: 1) unengorged specimens (not blood fed), 2) recently ("recent fed") engorged specimens and 3) late engorged ("late fed") specimens. The standardized tests were conducted under conditions of incidental light inside an experimental house that had not been sprayed with DDT. The tests were conducted from 0900 to 1230, March 15–27, 1980. Range limits for measurements of ambient temperature and humidity at times when the excito-repellency tests were conducted were 25.5–30° C and 76–95% RH.

The excito-repellency test boxes were left undisturbed indoors at the field site for one year, at which time 3 additional tests with "recent fed" specimens were conducted 0955 to 1430, March 2–4, 1981. The test procedure was repeated as described above.

Results of the excito-repellency tests were analyzed graphically and graphs are presented for the proportion of the test populations remaining in test boxes at the end of each 5 min interval. In the narrative, however, these results are discussed in terms of escape rates, which are the inverse of the graphical representations. In addition to graphical analyses, the Mantel-Haenszel Chi-Square (Lee 1980), was calculated on each set of data from each test. Calculations were based on the number remaining in the test box at the end of each 5 min interval. A p value of < 0.01 was accepted as the level of significance between the DDT treated and control test chambers.

RESULTS

We found no correlation in extremes of temperature or humidity with variations in test results.

The escape rate of *An. darlingi* females from the DDT treated box was markedly greater than from the control box in all tests, regardless of physiological condition of the test populations (Fig. 2). Significant differences in escape rates occurred during the first 5–10 min observation period(s) in most tests and continued to be significant for the remainder of each test period. The only exception was a single test with "late fed" specimens in which significant differences in escape rates did not occur until the 20–25 min observation period. The "recent fed" females were less prone to escape from both the DDT and control boxes than were late and unfed females. The escape responses for late and unfed populations were similar and a uniformly greater percentage of these females escaped from the DDT treated box earlier in each test than did "recent fed" females.

The escape rates from control boxes were more constant throughout each test period than were escape rates from the DDT treated boxes. Most females which escaped from the DDT treated boxes did so within the first 20 min of the test period, and females that did not escape generally died within the 60 min test period. Although there was a peak of escape activity for late and unfed females from the control box in the first 5 minutes, the overall pattern of escaping females was more uniform throughout the 60 min observation periods, particularly for "recent fed" females.

The pattern of escape rates for "recent fed" females from the DDT treated box in April 1981 was essentially identical to the pattern documented with tests using the same box and DDT treatment in March 1980 (Fig. 3). In contrast, fewer "recent fed" females escaped from the untreated box during tests conducted in April 1981.

DISCUSSION

The excito-repellency test results reported by Charlwood and Paraluppi (1978) for unfed populations of *An. darlingi* collected north of Manaus, Amazonas, Brazil were quite similar to the general escape patterns documented in this report, except a greater proportion of our test populations escaped from both treated and untreated boxes. However the relatively greater proportion that escaped from our untreated box precludes any speculation that the Ituxi River populations were more sensitive to the DDT residues. In fact the differences in test results may be due to dissimilar pretest holding times, as Charlwood and Paraluppi (1978) held their specimens overnight before each test, whereas we collected unfed specimens at sunrise on the day of each test.

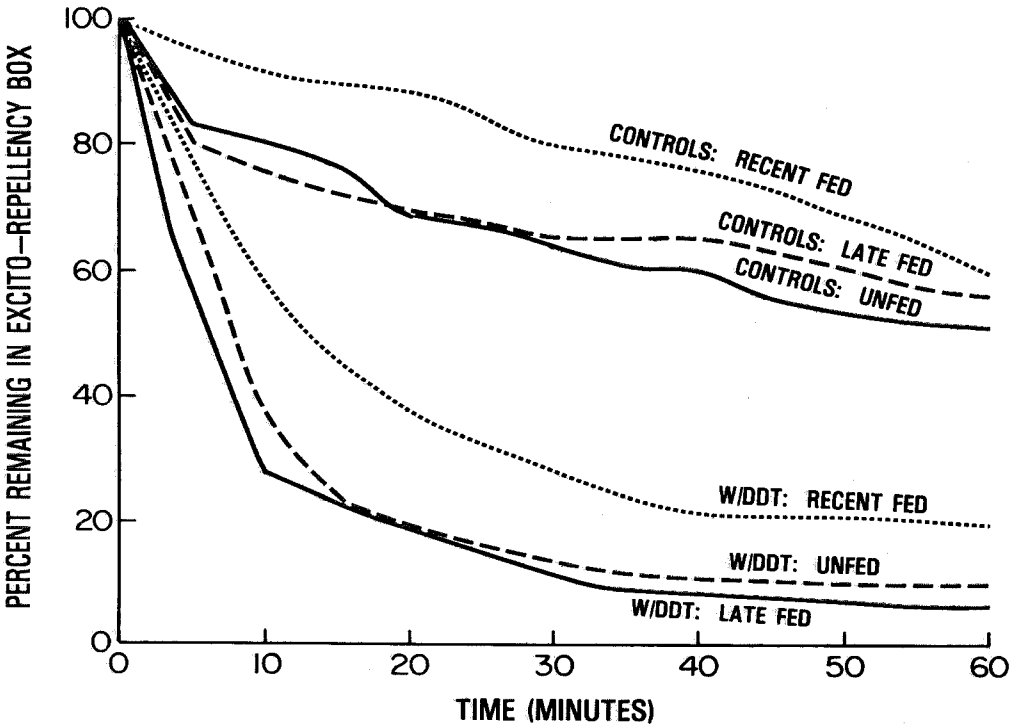

Fig. 2. Percent of *Anopheles darlingi* females remaining in DDT treated and untreated (control) excito-repellency boxes by 5 min intervals during 60 min test periods. Averages calculated from 3 replicates of each test with recent fed, late fed and unfed populations. All tests were conducted at a study site (Floresta) along the Ituxi River in Amazonas state, Brazil in March 1980.

A more gradual pattern of escape activity was documented for "recent fed" females than for "late or unfed" specimens. This diminished response of "recent fed" females to the DDT residues may be attributable to reduced flight because of the weight of large blood meals. Escape rates for "recent fed" females in tests conducted 12 months after the DDT treatments (in March 1981) were essentially identical to rates documented with tests immediately following treatment (in March 1980). The degree of similarity in persistence of excito-repellency versus toxic actions of DDT is unknown, as is the conformation of DDT that produces the behavioral responses. We have no explanation for the reduced rate of escaping females from the untreated box during tests conducted in March 1981. However, we can speculate that aging of the box for 12 months under conditions of high temperature and humidity may have produced an environment within the untreated box that was more acceptable to the test populations.

Regardless of differences and similarities in response patterns by physiological condition, the dominant influence on escape patterns for all populations in the treated chambers was the presence of DDT. Although we confirmed the pattern of escape behavior from the DDT treated box as reported by Charlwood and Paraluppi (1978), our respective interpretations differ. Based on preliminary observations from field studies north of Manaus, Hayes and Charlwood (1977) concluded that *An. darlingi* females were entering DDT sprayed houses, taking blood meals and subsequently resting on vegetation outside the houses. Charlwood and Paraluppi subsequently interpreted their excito-repellency data as further evidence for behavioral resistance to DDT. In studies of experimental houses along the Ituxi River, however, we found that the level of DDT avoidance

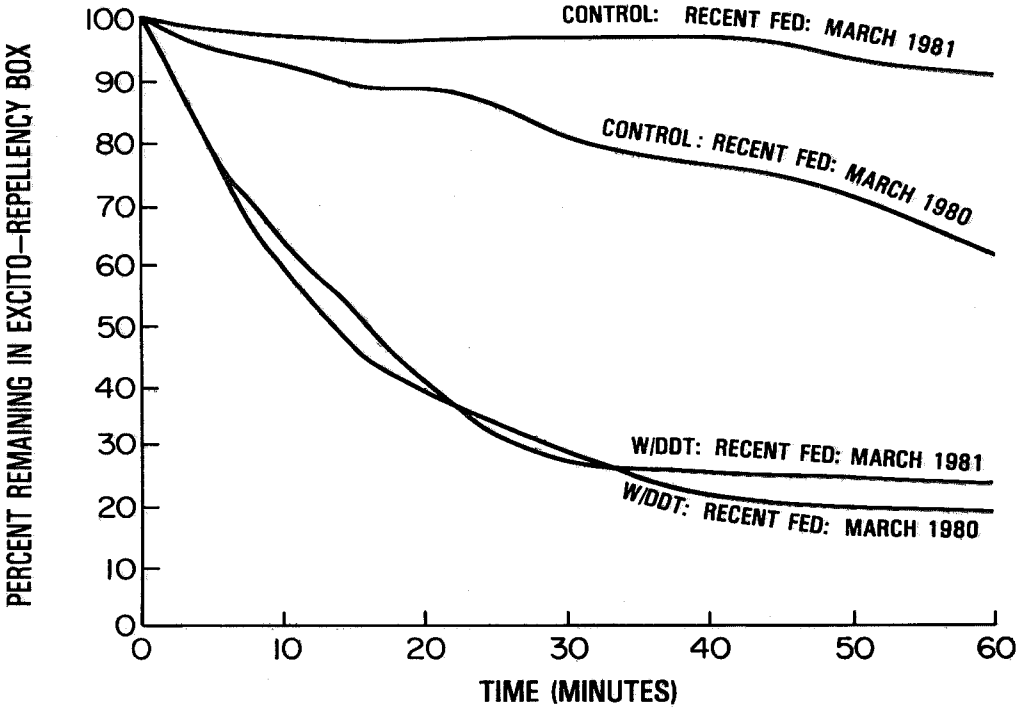

Fig. 3. Percent of *Anopheles darlingi* females remaining in DDT treated and untreated (control) excito-repellency boxes by 5 min intervals during 60 min test periods. Averages calculated from 3 replicates of each test with recent fed females in March 1980 and with recent fed females in March 1981. All tests were conducted at a study site (Floresta) along the Ituxi River in Amazonas state, Brazil in March 1980.

by *An. darlingi* was so great that females rarely entered a house that had been sprayed with the insecticide (Roberts, unpublished data). Consequently, we merely interpreted the excito-repellency test data as confirmation that *An. darlingi* females were strongly and immediately repelled by DDT residues.

This difference in the interpretation of similar data reveals a basic problem with the excito-repellency test, viz., that tests for behavioral responses of anophelines to DDT excitation, irritation, repellency or a combination thereof, provide limited insight into the meaning of such behavior in the field. Thus we invariably make interpretations that are consistent with what is perceived to occur in nature. This problem will be overcome only with more basic research on the underlying biological mechanisms, as listed above, responsible for the behavioral responses and the means by which the insecticidal vapors/residues are detected by mosquitoes. A better understanding of these basic considerations, particularly the latter, will greatly improve the usefulness of excito-repellency test data.

Although reproducible results were obtained, a number of deficiencies were found with use of the excito-repellency test boxes. Even though the boxes used in these studies were collapsible, they were still difficult to transport and assemble, as well as large and cumbersome. It was difficult to introduce the specimens into the boxes and remove live specimens at the end of the test period. Boxes and escape cages had to be handled very carefully during the test period because any disturbance caused an increase in the numbers that escaped. The test dose of insecticide is of concern since the amount applied varies with the spray operator or technician treating the paper, i.e., there is no provision for a standardized dose of DDT. Each test required 2 man-hours of labor, excluding the time re-

quired to collect and prepare the test populations. The test data were collected in discrete 5 min intervals and most specimens that were going to escape from the DDT treated box did so within the first 1–2 test intervals. Consequently, a detailed pattern of escape behavior could not be documented and subjected to statistical analysis.

There is no accepted method for the analysis of excito-repellency data and probably none will be developed until more is known about the behavioral responses of mosquitoes to insecticides. Although graphical analysis is valid for visual comparisons of such data, much information is lost with this method and descriptive summary statistics are not easily derived from the graphs. The Mantel-Haenszel Chi-Square test is useful for detecting when and for how long significant differences in escape rates from treated and untreated boxes occur, but unfortunately this information is of limited value in making a biological interpretation of test results or in comparing test results between species or populations. In summation, there is a need for more information on the effect of insecticides on mosquito behavior as well as improved and simplified test and analytical methods for studying these behavioral responses.

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