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TECHNICAL
REPORT
DPGR 759

UNANNOUNCED

DECEMBER 1960

AD596046

OUTDOOR MOSQUITO BITING ACTIVITY STUDIES, PROJECT BELLWETHER-I, BW 459 (U)

SHORT TITLE: BELLWETHER-I

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SHORT TITLE: BELLWETHER-I

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FOREWORD

(S) The U. S. Army Chemical Corps has been assigned the task of providing the Department of Defense with adequate CBR weaponry. Certain entomological vector-agent systems have, after a period of laboratory demonstrations, qualitative field experience, and theoretical evaluations, reached the quantitative field test stage, and Dugway Proving Ground has been assigned the field testing responsibility. The present volume reports on Dugway Proving Ground's first independently designed and conducted mosquito field trials.

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DIGEST

(C) The primary objective of this test was to ascertain the effects of major meteorological parameters on the biting rates of starved, virgin female Aedes aegypti mosquitoes upon troops in the open. Other objectives were (1) to see whether this mosquito, a domesticated, house-loving tropic l mosquito, could be effectively tested in hot, open, temperate desert terrain and (2) to ascertain whether traps could be used to replace human samplers.

(C) A total of 52 field trials were conducted between 1 September and 9 October 1959. The basic trial design consisted of three 15-foot radius circles, located 1/8-mile apart on a crosswind line. Ten men were located equidistantly around the perimeter of one circle, and either ten guinea pig baited traps or ten nonbaited traps were placed around the perimeters of the other two circles. The volunteers all remained seated in these trials. One hundred vectors were released in the center of each circle, and sampling was conducted for 30 minutes. A mobile meteorological station was located 1/4-mile upwind of the center circle.

(S) Using uninfected, virgin female A. aegypti mosquitoes in Phase A of BW 459, the results obtained within the ranges of conditions encompasse in these trials indicate that:

1. It is feasible to test this mosquito under hot, dry, desert conditions, at least for the initial primary time period, and to assess the effects of various meteorological variables upon biting activity.

2. Although many of these trials produced erratic and unpredictable results, it would appear from the analysis of these data that each of the meteorological variables studied--wind speed, temperature, relative humidity, and solar radiation--exert a significant influence on the biting activity of the A. aegypti mosquito, and all would have to be considered as important parameters in any model designed to predict biting activity. However, the effects of the latter three factors were manifested only in terms of interaction with wind speed and with each other; wind speed alone had a direct effect upon biting activity. Moreover, within the ranges of conditions encompassed in these trials, it appears that wind speed was the most important factor affecting biting activity.

3. An increase of 1 mile per hour in the ambient wind speed was associated with a decrease of approximately six bites in a 15-foot radius circle with 10 volunteers over a 30-minute time interval.

4. The data suggest that the previously determined lower temperature limit of 59°F for vector biting activity of the non-cold resistant strain is placed too high; however, at these lower temperatures some other factor(s), at present unknown, produce erratic results.

6. Guinea pig-baited traps captured one and one-half times as many mosquitoes as did nonbaited traps.

7. Whereas, on the over-all average, a single mosquito in a baited trap was equivalent to two bites on a human, excessive intertrial variation precludes replacing human samplers with traps to determine biting rate activity.

8. With a vector/host ratio of 10:1, in only a very few trials did 100 per cent of the volunteers report bites. The mean percentage of test subjects bitten lay between 60 and 70 per cent.

9. The over-all average outdoor biting rate for this vector was 40 bites per 100 mosquitoes in the time period studied.

10. No evidence of crepuscular-period biting preference was obtained in those trials.

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INTRODUCTION

BACKGROUND

(S) Arthropod-borne diseases of man and his domestic animals, both fatal and debilitating, have exacted an enormous toll of life and have caused untold misery throughout recorded history. In general, these diseases are characterized by epidemic potentiality and endemic persistency. The causative disease organisms, ranging in size from nemotodes to viruses, produce such diseases as bubonic and sylvatic plague, typhus, malaria, yellow fever, filariases, various encephalitides, sleeping sickness, dengue, spotted fever, Texas tick fever, and many others. The arthropod vectors involved include ticks, lice, fleas, flies, mosquitoes, and bugs.

(S) The biological effectiveness of laboratory-reared and laboratory-infected arthropods in transmitting disease to human beings is well documented in the literature. One hundred and thirty human volunteers have been infected with yellow fever and over two hundred with dengue by laboratory-reared and laboratory-infected yellow fever mosquitoes. Ticks reared and infected in the laboratory with Rocky Mountain spotted fever, tularemia, and relapsing fever have transmitted these diseases to volunteer test subjects (1).[1] The literature dealing with accidental and intentional laboratory infection indicates that the deliberate employment of infected arthropod vectors against enemy targets holds great strategic potential (2) and limited, feasibility field tests with several vectors have borne out this possibility (1, 3 through 7). Concurrent with these feasibility-demonstration field trials, mass production techniques of rearing selected vectors, producing agent, and infecting vectors with agent have been developed (see 1).

(S) There are other requirements, however, that ordinarily must be satisfied in accepting and standardizing a potential weapon system for use. Some requirements of obvious importance would be realistic predictions of target effectiveness and casualty rates upon which munition expenditure calculations ultimately would be based. These requirements are, of course, formidable for any of the more conventional CW or BW weapons, and the numerous factors that must be considered and quantitatively appraised to satisfy these requirements need not be mentioned here. Although many of these problems would be common to any weapon system, there are others attendant to the evaluation of an entomological weapon that are inherently unique. A cursory review of the state of the art of assessing the field performance of conventional CW and BW weapon systems will serve to emphasize the uniqueness and complexity of the problems associated with the assessment of EW weapons systems.

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[1]Figures within parentheses denote references in the LITERATURE CITED section, see page 51.

target compact by a given weapon system. Excluding the effects of chemical or biological decay on the quantitative distribution of effective particulates, these dosage fields arise entirely as a result of physical forces (i.e., mode of dissemination, ballistic effects on droplets, and meteorological diffusion parameters) acting upon the airborne agents; these dosage fields are usually determined by the routine laboratory assay of regulated mechanical sampling devices that have been exposed systematically throughout the area of interest. The collection efficiency of the sampling devices being either known or assumed, dosage-field data can then be used, in conjunction with the appropriate dose-response curves that may be available, to arrive at direct or indirect estimates of the effects on exposed man. Although far from perfect, satisfactory sampling devices and techniques for the above assessments have been developed and have been more or less standardized.

(C) For EW weapons, comparable field testing techniques have not been developed. The agent is carried to the human target by the target-seeking entomological vector and consequently is rather independent of the meteorological diffusion processes. Therefore, the dissemination of arthropod vectors from an EW munition results in behavior quite unique from other CB weapons. This uniqueness is basically associated with three broad but mutually dependent vector characteristics, all of which are related to the potential transmission of the disease agent to a target population. These characteristics are (1) movement and distribution, (2) biting habits, and (3) survival or persistency of effect.

(C) Whereas aerosols, once released, are subject solely to the effects of the various meteorological parameters, arthropod behavior is quite different. The vectors move actively in all directions, and this movement is erratic both in time and space. Temperature, seldom limiting in aerosol work, have a sharp lower cutoff in entomological applications and the biting rate curves sharply downward as this cutoff is approached. Variation in the relative attractiveness of different human individuals to mosquitoes is a recognized but little studied problem. Further, a diurnal biting cycle is reported for Aedes aegypti, biting peaks occuring in early morning or evening. Although they may bite at anytime, especially if they have gained entry into lighted, inhabited buildings, biting has been reported to approach zero during the night with those mosquitoes that remain outside of buildings. A female mosquito may bite and probe many times on one or more individuals in order to gain a full blood meal; however, once she has accomplished this meal, she is lost to the primary target effect until she has deposited her eggs. This involves an average of 3 to 4 days, but after oviposition is completed, she is again ready to continue the primary target effects.

cated mechanical sampler requirement. Although certain mechanical devices have been used in earlier tests, they have not proven to be satisfactory for general purposes with the present vector species. Aside from the relative nonattractiveness of traps to A. aegypti, there is the further basic problem of translating trap capture data to human biting rates.

(C) Project BELLWETHER-I, BW 459, is a preliminary step in evaluating some of these problems with the A. aegypti mosquito, and it is an approach to the over-all problem of developing field assessment techniques; i.e. techniques whereby valid data for the development of Munition Expenditure Tables can eventually be obtained. The results of these trials will also be used in designing subsequent tests under BELLWETHER-II where some of the above cited problems, as well as others, will be further investigated and elucidated.

OBJECTIVES

(C) The specific objectives of Project BELLWETHER-I, using uninfected, virgin female Aedes aegypti mosquitoes, were:

1. To determine the feasibility of testing this mosquito under hot, dry, desert conditions;

2. To determine the relative significance of temperature, relative humidity, wind speed, and exposure duration upon vector outdoor biting activity; and

3. To determine the relative value of guinea pig-baited mosquito traps compared with human test subjects as assessment techniques.

SCOPE

(C) Phase A consisted of 52 field trials conducted between 1 September and 9 October 1959. The first six trials were exploratory, variant trials used to obtain data to establish a basic design; several other trials later in the test program also varied from the basic design. All variant trials, together with their deviations, are listed in Table 1. A total of 44 trials were conducted on the basic design of either 10 men or 10 traps located equidistantly along the perimeter of a 15-foot radius circle, with 100 test mosquitoes released in the center of each circle. The traps were either nonbaited or were baited with live guinea pigs. Sampling was conducted for 30 minutes, with the traps recording total captures and the volunteers recording bites for the 1-, 3-, 5-, 7-, 10-, 15-, and 30-minute time intervals.

(U) A rather wide range of meteorological conditions were experienced in these trials, viz: temperatures of 53.9 to 93.7°F; average wind speeds of 1.6 to 13.5 miles per hour; and relative humidities of 16.2 to 69.5 per cent.

SAMPLING PERSONNEL

(U) A request was initiated in August, 1959, through BW Operations Division, for 30 screened, volunteer military personnel to be furnished for a 6-week period. These men were supplied by the 45th and 46th Chemical Companies and the 1503.00 Headquarters Detachment, all attached to Dugway Proving Ground, Utah (DPG). The men were thoroughly briefed on what was expected of them and, on the whole, the individual interest and cooperation in the conduct of these trials was remarkably high. The men were clothed in the standard Army work uniform, with trousers bloused into combat boots, and fatigue caps. On cold and/or windy days, field jackets were worn.

TEST VECTOR

(U) The mosquitoes used in these trials were reared at Baker Laboratory, DPG, from egg papers furnished by the Entomology Division, U. S. Army Chemical Corps Biological Laboratories (BioLabs), Fort Detrick, Frederick, Maryland. New batches were seeded every 7 days throughout the test period. When the larvae reached the pupal stage, the females were separated from the males by means of a Fort Detrick pupal separator and 100 female pupae each were placed in 1-quart ice cream cartons in which the cardboard tops were replaced by squares of nylon bobbinette. Each carton was marked with the date of pupation, and the adults were fed a 1 molar solution of sucrose until 16 to 24 hours prior to use. All cartons of vectors to be used on a test day were picked up in the morning and carried about the field in the cabs of the vehicles until used. The mosquitoes were hardened for 3 or more days before being used, except those used in Trials A-3 through A-6 when this factor was overlooked;[2] in these four trials, pretrial field mortality was encountered, probably because of excessive dehydration through the soft exoskeleton.

TEST FIXTURE

(U) The test fixtures used in each trial of Project BELLWETHER-I were round, 1-quart cardboard, ice cream cartons suspended approximately 5 feet above ground level between two driven, wooden stakes. Prior to filling, the tops of each carton was pushed out and replaced by a square of nylon bobbinette; four-string harnesses were affixed to the top and to the bottom of each carton to serve as hanging points. Just before each trial, the carton was hung with the top, previously loosened, affixed to one stake; the carton body was suspended by a 50-foot lanyard running through a hole in the second stake. At function time, the operator pulled

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[2]"Hardening" is that term used to denote the drying of the wings and the general hardening of the chitoncus exoskeleton after emergence from the pupal case.

TABLE 1: List of Trials Varying from the Basic Design in Project [redacted]-I, [redacted] 459 (CONFIDENTIAL)

TRIAL NUMBER	VOLUNTEER CIRCLE			GUINEA PIG-BAITED TRAPS			NONBAITED TRAPS		
	Number of Vectors	Radius (feet)	Time of Exposure (minutes)	Number of Vectors	Radius (feet)	Time of Exposure (minutes)	Number of Vectors	Radius (feet)	Time of Exposure (minutes)
A- 1	100	15	60	100	15	60	100	15	60
A- 2	100	15	60	100	15	60	100	15	60
A- 3	100	15	60	350	15	60	1000	15	60
A- 4	100	15	-*	1000	45	60	1000	45	60
A- 5	1000	45	10	1000	45	60	1000	15	60
A- 6	1000	45	10	1000	45	60	1000	15	60
A-32	10 men and 10 traps (alternating baited-nonbaited) were used in one 15-foot radius circle with a 100-vector release; 4 men and 5 baited traps were placed along a 90-degree arc 100 feet downwind.								
A-42	All 30 subjects (10 men, 10 baited traps, 10 nonbaited traps) were placed on one 20-foot radius circle and 90 ± 3 vectors were released.								
A-43 to 50	100	15	30	Guinea pig-baited and nonbaited traps were used alternately on both circles: baited on even numbered positions and nonbaited on odd numbered positions.					
	(Basic Design)								
A-51 and A-52	100	15	30	100	15	30	100	15	30
	(Swat trials - see text)			(Basic Design)			(Basic Design)		

*Munition malfunctioned.

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all of the mosquitoes from the carton. At the volunteer circle, the fixture operator was one of the numbered volunteers seated in the circle. At the trap circles, the operator, after releasing the mosquitoes, quickly left the area by means of vehicle transport. A typical test fixture is shown in Figure 1. Except for two or three cool weather trials, no difficulty was encountered in getting the vectors out of the fixture.

(UNCLASSIFIED)

Fig. 1.- A typical test fixture set-up, BW 459.

MOSQUITO TRAPS

(U) The traps used in these trials were fabricated from 26-gauge galvanized steel sheeting. A 5-foot length of sheeting, 31 inches wide, was spot welded to form a tube 19 inches in diameter and 31 inches long. Screen funnels, 9 inches deep with a ¼-inch hole at the apex, were soldered to removable rings that fit over each end of the basic tube. In the

accommodated the guinea pig and the addition of a sliding gate between two metal guides completed the trap. The basic design of the traps was furnished by BioLabs. The size of the test vector called for the use of 20-mesh screening in the funnels, but the nonavailability of this material precluded its use on all but four of the traps. These 16 remaining traps were fitted with 12 by 18-mesh screen funnels. While the test mosquito can sometimes escape through such a mesh, it was felt that escapes in the time periods considered would be insignificant, and a laboratory-type experiment supported this belief.

TEST SITES AND TEST PROCEDURE

(U) Possible persistence of the test species used in this test precluded conducting all of the trials at a single test site; therefore, several general areas were utilized. These areas were the Clay Flats Grid north of Dog Area, the area south and east of GPI-1, the area south of the West Gate, and, especially after rainstorms, various laterals of the Downwind Grid. These general areas are depicted in Figure 2. Usually all of the tests accomplished on a single day were conducted in the same general area--the troops merely moving several miles upwind from the last test site.

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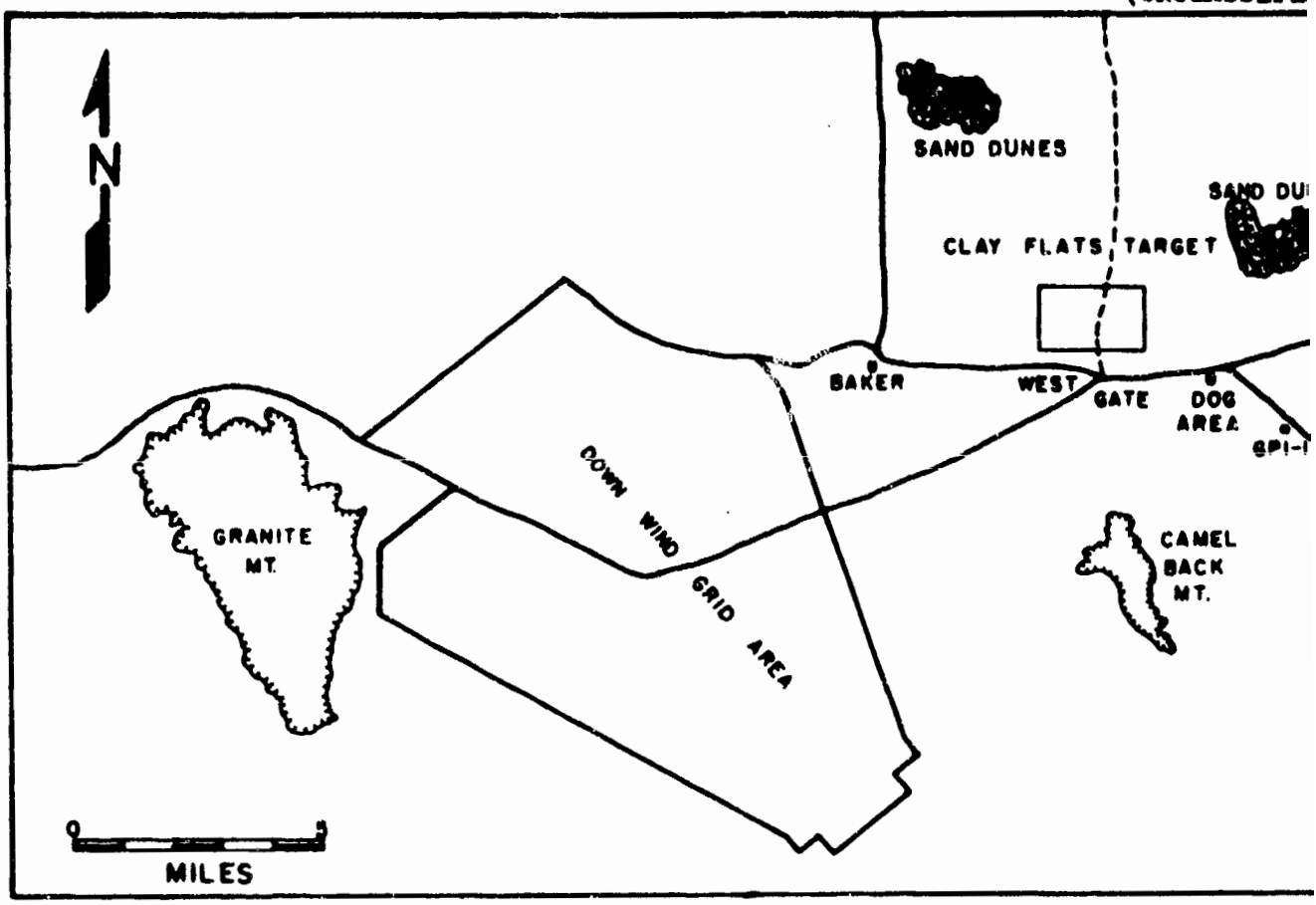

Fig. 2.- Map showing the general areas used in BELLWETHER-I, BW 459.

stake would be driven in the center of the open area and a 15-foot radius circle would be scribed, compass-like, with a measured rope or wire. The test fixture was then installed, and the traps were positioned equidistantly along the perimeter of the circle with their long axes at right angle to the perimeter. The number 1 position in all three circles was orientated to the north in the first trials, but from Trial A-32 on it was oriented into the wind prevailing at test time. Similar procedures were followed in the volunteer circle.

(C) The first six trials were largely exploratory in nature: Trials A-1 through A-4, with 100-vector releases, were conducted for 60 minutes and Trials A-5 and A-6, with 1000-vector releases, were conducted for 10 minutes with the volunteers and for 60 minutes with the traps. In all but two of the remaining trials (A-32 and A-42), the basic design of 100 vectors, 15-foot radius, and 30-minute sampling period was used, and the men in the volunteer circles were seated and remained as motionless as possible during the trial period. In Trial A-32, relatively high wind speeds dictated that a limited downwind study be conducted. A man and a trap were located at each of the 10 ring positions and 4 men and 5 traps were located along an arc 100 feet downwind. In Trial A-42, the loss, by excessive heat, of 200 of the 300 available vectors resulted in all three circles being combined for a single 100-vector release. In summary, 44 trials were conducted on the basic design and 8 were variants. Table 1, supra, lists these variant trials.

(U) Systematic randomization of the test personnel to be used in the volunteer circle was attempted, but their other military obligations prevented this system from working. As a consequence, the Test Officer randomly changed the relative positions of the men from trial to trial.

(U) A typical volunteer circle is shown in Figure 3 and a representative guinea pig-baited trap circle being set up is shown in Figure 4.

TRIAL ASSESSMENT

(U) The volunteers kept a written record of all bites received during each trial on a record form furnished them. The number of bites received were recorded for each of the following time periods: 0-1, 1-3, 3-5, 5-7, 7-10, 10-15, and 15-30 minutes. A medical entomologist frequently checked the reported bites against overt physical evidences of bites at the end of the trial.

(U) At the conclusion of each trap trial, the guinea pigs were removed to a holding box, and the traps were brought to a central position for assessment. The number of entrapped mosquitoes were counted by looking through the trap toward a clear area of sky (see Fig. 5). Mosquitoes

Fig. 3.- A typical volunteer circle, BW 459.

(UNCLASSIFIED)

Fig. 4.- A typical guinea pig-baited trap circle being set up, BW 459.

as captures. At no time were the number of captures high enough [illegible] rant use of the chloroform and the plastic covers available for such an event. Wherever the count was 10 or more, it was verified by one or more of the other test participants. Any off color or native mosquitoes were referred to the medical entomologist for identification. The dark A. aegypti were quite distinct from the few, light colored natives encountered. When the assessment was complete, one of the funnel ends was removed from each positive trap and the occupants were released, or killed if possible.

(UNCLASSIFIED)

Fig. 5.- Determining the number of entrapped mosquitoes in a trap, BW 459.

ECOLOGY AND EPIDEMIOLOGY BRANCH PARTICIPATION

(U) The Ecology and Epidemiology Branch, BW Operations Division, DPG, furnished one medical entomologist who participated as an observer in every trial. This man helped assess the number of bites on the volunteer personnel and assisted in trap assessment and native mosquito identification. In addition, his judgment was followed in determining the travel distance required before setting up new trial sites in the same general area of a previous trial.

(U) Meteorological coverage was furnished by a single mobile meteorological station installed in a jeep-pulled trailer. After arriving at the selected site position, the men would set up an aiming circle and mount the anemometer and wind speed heads. These were connected to Esterline-Angus chart roll recorders mounted in the trailer. At a distance of approximately 100 feet, a steel post was driven into the ground and a battery aspirated psychrometer was mounted thereon. Wind speed and direction data were recorded continuously, and temperature and relative humidity readings were taken every 10 minutes throughout each trial. In addition, since the Dog Area pyrheliometer was inoperative throughout the test period, incoming solar radiation value readings were taken every 10 minutes in the field with a General Electric radiation meter (Model 8DW40Y16). Light value readings were obtained with a standard photographic light meter. A typical meteorological station is shown in Figure 6.

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Fig. 6.- A typical meteorological station used in BW 459.

METEOROLOGICAL DATA

(U) The upwind location of the single meteorological station resulted in the recording of average values applicable to the three test sites, but specific values applicable only to the meteorological location. Therefore, rather than publish the specific meteorological data, as is the usual procedure in DPG technical reports, a summary of the average conditions existing during each trial is presented as Table 2. Complete meteorological data are on file at BW Branch, Test Design and Analysis Office, DPG. The 5-minute minimum and maximum wind speeds and wind directions were further averaged (average range) to smooth out the extremes and to give a better picture of the over-all meteorological conditions extant during the trials.

SAMPLING RESULTS

(U) A summary of the biting and trap capture data from these trials is presented in Table 3. Complete trial data are on file at the BW Branch, Test Design and Analysis Office, DPG. Relative to the biting data, several reasons contributed to equating a probe with a bite in these trials. One reason was the difficulty for inexperienced troops to distinguish between the two, and another was the fact that in disease transmission studies one probe was equivalent to one bite (Dr. Dale Jenkins, BioLabs, Fort Detrick, Maryland, in conversation).

SWAT TRIALS

(C) As the test progressed, it became apparent that second or third biting by the vectors would preclude any direct comparison between human biting rates and trap captures, since a trap capture effectively removed the mosquito from the test whereas a vector having bitten once could return or go elsewhere for additional bites. Hence, two "swat" trials, Trials A-51 and A-52, were conducted. In these trials, the men in the volunteer rings attempted to swat and kill every mosquito that actually probed or bit them, and their reported swatting effectiveness ranged from about 30 to 100 per cent. The attempt failed because it developed that the men could not effect a 100 per cent kill of the biting vectors. The approximate efficiency of killing appeared to be in the neighborhood of 60 per cent.

(C) The total number of reported vector kills in Trial A-51 exceeded the number released; this can only be explained by the men believing they were killing more than they actually were by a factor of at least 40 per cent. This appears to be most probable at Positions 5, 6, 7, and 8 in Trial A-51 where 91, 60, 22, and 19 bites (and kills), respectively, were recorded in the 30-minute period. Although these trials failed in

bites and kills, were still vulnerable to this vector. The penetration efficiency would be greatly increased if this vector was used against unsuspecting troops.

PERCENTAGE OF MEN BITTEN

(S) In every trial where 1000 vectors were released, all ten men were bitten. In the regular trials wherein 100 vectors were released, a varying number of the men were bitten. The percentage of men bitten increased with an increase in the total number of bites, but the trial with the highest number of bites (Swat Trial A-51, 206 reported bites) saw only 80 per cent of the men bitten. Higher wind speeds tended to reduce the percentage but a 13 mph wind was associated with an 80 per cent biting occurrence in Trial A-16. Apparently, the interaction of wind speed, wind direction (variable or steady), and temperature is contributory to the percentage bitten. The median percentage of men bitten in all non-zero bite trials was about 70 per cent. In actual operations, however, this percentage would be greater because those mosquitoes blown from the ring would still be effective at other sites downwind. The percentage of traps recording captures in a trial closely paralleled the percentage of humans reporting bites.

MISCELLANEOUS NOTES

(C) In Trial A-42, in which all 10 test subjects and 20 traps were placed on a single 20-foot radius circle, the men proved to be much more attractive than the traps, with 27 bites and only 1 trap capture being reported.

(U) In a final briefing with the troops at the end of the test series, several interesting observations were reported. Although these are strictly qualitative in nature, they are included here both for the sake of completeness and for their intrinsic interest.

(U) It was brought out that a differential swelling reaction was encountered by at least one-third of the test personnel. One day they would suffer a pronounced swelling under every bite and on other days they would experience no reaction whatsoever. No overt correlation of this inflammatory reaction was noted with any obvious parameter.

(U) On hot, sunny days, it was observed that most of the vectors moved onto the shady side of the person to hover, land, or bite. On windy days, they moved to the lee side and on cooler days, they were observed to move up inside of the sleeves before biting.

TABLE 2: Summary of Meteorological Data, BW 459 (UNCLASSIFIED)

TRIAL NUMBER	WIND DIRECTION (°)					WIND SPEED (mph)					TEMPERATURE (°F)	RELATIVE HUMIDITY (%)	HEAT VALUE (g-cal/cm²/min)
	Avg	Range		Average Range		Avg	Range		Average Range				
		min	max	min	max		min	max	min	max			
A- 1	254	180	026	205	307	6.3	0.5	12.8	2.2	10.9	73.4	28.7	-*
A- 2	299	204	354	257	337	8.9	1.2	15.0	4.6	13.0	77.2	25.5	-
A- 3	289	192	050	231	340	5.2	0.5	10.2	1.8	8.4	73.9	25.7	-
A- 4	312	204	012	255	360	5.5	0.8	10.2	2.6	9.0	82.4	19.8	-
A- 5	165	027	032	110	228	4.7	0.5	12.2	1.5	8.2	93.2	17.6	1.3
A- 6	179	114	270	149	205	7.6	1.3	14.7	3.5	11.8	93.7	16.2	1.2
A- 7	285	180	350	234	323	6.1	0.5	14.5	2.2	10.2	85.5	24.0	1.1
A- 8	283	227	315	232	306	7.2	0.9	12.1	1.9	11.3	86.6	20.5	1.3
A- 9	329	297	009	309	001	12.5	3.6	20.0	6.5	16.8	90.5	17.6	1.1
A-10	268	190	019	214	323	1.7	0.5	3.9	0.8	2.6	67.3	20.0	1.1
A-11	230	196	270	208	255	3.2	1.5	4.9	2.0	4.5	70.0	34.7	1.3
A-12	281	180	319	231	305	3.3	0.8	5.9	1.5	5.0	78.9	20.5	1.3
A-13	310	189	007	225	346	2.2	0.7	4.1	1.1	3.2	76.4	20.0	1.2
A-14	320	180	072	244	031	1.6	0.5	4.4	0.5	3.3	84.8	16.2	1.2
A-15	074	031	150	066	088	2.0	1.4	2.4	1.7	2.3	78.8	27.6	0.05
A-16	183	162	198	172	194	6.5	3.6	8.9	4.3	8.6	80.1	29.7	0.8
A-17	246	180	336	203	287	6.8	0.5	13.1	2.5	11.5	86.9	24.0	0.9
A-18	238	190	284	196	265	11.4	5.6	15.0	7.0	14.5	84.9	23.7	0.4
A-19	184	169	200	173	194	13.5	6.8	20.4	8.6	18.2	73.2	49.0	0.6
A-20	345	294	025	312	009	2.7	0.0	6.1	1.0	4.8	67.2	58.3	0.8
A-21	252	187	323	196	301	4.7	1.9	8.3	2.5	7.1	56.7	69.5	0.9
A-22	234	189	342	201	288	1.7	1.0	10.4	1.7	9.0	62.1	61.1	1.2
A-23	245	179	327	194	294	5.2	0.5	12.9	1.9	9.0	64.8	57.0	0.9
A-24	206	103	270	176	231	2.4	0.5	5.0	0.7	4.3	55.8	68.0	0.7
A-25	155	102	180	121	177	6.1	1.6	11.0	2.6	9.8	56.5	61.5	1.1
A-26	167	103	223	130	205	8.5	4.1	14.8	4.5	13.1	66.5	45.0	1.4

*No data.

SECRET

TABLE 2: Summary of Meteorological Data, BW 459 (Concluded)

TRIAL NUMBER	WIND DIRECTION (°)					WIND SPEED (mph)					TEMPER-ATURE (°F)	RELATIVE HUMIDITY (%)	HEAT VALUE (gm-cal/ cm^2/min)	LI VA (0 t on s
	Avg	Range		Average Range		Avg	Range		Average Range					
		min	max	min	max		min	max	min	max				
A-27	156	079	225	122	193	8.2	0.8	15.0	3.6	13.3	68.1	41.5	1.1	1
A-28	148	106	172	127	165	7.6	4.0	11.8	4.2	10.8	61.5	59.0	0.5	1
A-29	169	150	189	155	184	7.5	4.0	11.0	4.6	10.9	64.2	57.0	1.1	1
A-30	278	243	332	259	304	6.4	1.8	8.6	3.4	8.9	58.1	48.0	1.2	1
A-31	341	280	059	306	017	5.8	2.5	7.6	3.2	8.4	60.6	45.0	1.3	1
A-32	332	271	014	301	356	5.2	2.3	8.6	3.4	7.6	61.1	45.5	0.7	1
A-33	056	306	156	062	126	3.2	0.5	7.3	0.9	5.8	57.7	49.0	1.2	1
A-34	284	180	035	194	357	5.0	0.5	10.6	1.0	8.3	64.1	37.0	1.0	1
A-35	235	180	050	193	326	3.0	0.5	8.6	0.6	6.4	64.9	36.5	1.0	1
A-36	232	197	285	208	266	6.8	0.5	13.0	2.1	10.1	56.5	57.0	0.5	1
A-37	355	206	039	276	030	9.6	0.5	20.4	3.3	17.2	60.3	48.5	0.4	1
A-38	053	346	104	013	084	6.6	3.0	13.1	4.0	10.2	64.1	39.5	1.0	1
A-39	229	178	250	191	261	5.2	1.0	9.5	2.0	8.4	58.5	55.0	1.1	1
A-40	232	176	342	196	271	4.6	0.5	8.5	1.4	7.3	59.7	50.0	1.2	1
A-41	351	186	145	204	045	3.2	0.5	8.6	1.1	6.8	63.9	37.0	1.2	1
A-42	243	174	306	201	275	3.9	0.5	10.2	0.75	8.0	69.5	30.0	1.1	1
A-43	285	213	009	232	335	5.1	0.5	11.6	1.8	9.7	54.3	42.5	1.0	1
A-44	343	281	013	318	004	9.4	4.3	15.0	5.0	14.4	54.3	42.5	1.1	1
A-45	320	237	017	264	005	5.0**	- - -	- - -	- - -	- - -	53.9	39.0	1.0	1
A-46	022	213	129	312	071	4.0**	- - -	- - -	- - -	- - -	53.1	38.5	0.7	1
A-47	003	234	135	292	076	3.1	0.5	9.4	0.8	7.2	56.9	30.0	0.4	1
A-48	345	288	068	302	031	7.0	1.0	13.9	2.8	10.5	57.2	28.5	1.1	1
A-49	353	304	023	325	015	11.2	6.6	16.6	6.8	15.1	57.8	29.5	1.0	1
A-50	316	275	358	288	347	9.5	5.5	15.6	7.2	11.4	57.3	34.0	1.2	1
A-51	183	150	268	143	220	3.4	0.5	7.6	0.6	6.9	65.2	27.0	1.1	1
A-52	309	243	353	281	332	10.2	8.7	>15.0	6.1	14.8	69.0	37.0	0.3	1

**Field instrument inoperative; general data taken from Dog Area information.

SECRET

TABLE 3: Summary of Recorded Bite and Trap Capture Data, BW 459 (CONFIDENTIAL)

TRIAL NUMBER	DATE (Sep 1959)	FUNCTION TIME (MST)	BAITED TRAP CAPTURES		NONBAITED TRAP CAPTURES		BITES ON HUMAN VOLUNTEERS	
			Total	Number of Positive Traps	Total	Number of Positive Traps	Total	Number of Men Bitten
A- 1	1	1050	30	10	6	ND*	128	10
A- 2	1	1445	37	8	13	6	74	8
A- 3	2	0945	20	8	53	10	36	7
A- 4	2	1445	83	8	4	3	ND	ND
A- 5	3	1405	50	10	180	10	120	10
A- 6	3	1605	40	9	72	7	135	10
A- 7	8	1030	27	9	26	6	50	8
A- 8	8	1302	24	7	6	5	7	4
A- 9	8	1445	4	3	6	2	9	2
A-10	9	0934	20	7	10	6	59	5
A-11	9	1100	19	7	25	6	99	9
A-12	9	1340	12	6	19	6	47	10
A-13	10	1022	24	9	2	2	104	8
A-14	10	1312	13	8	4	3	138	9
A-15	10	1837	4	2	2	2	53	10
A-16	11	0930	28	7	10	3	70	8
A-17	11	1222	23	6	29	7	76	10
A-18	11	1335	34	5	44	8	44	6
A-19	14	0908	0	0	0	0	0	0
A-20	15	1500	7	6	24	9	23	4
A-21	16	1005	8	3	7	7	14	3
A-22	16	1250	22	10	16	5	90	9
A-23	16	1400	10	6	18	8	23	5
A-24	17	0922	7	5	9	4	0	0
A-25	17	1025	4	3	4	4	12	3
A-26	17	1309	15	7	3	3	28	7

*No data

Continued

TABLE 3: Summary of Recorded Bite and Trap Capture Data, BW 459 (Concluded)

TRIAL NUMBER	DATE (Sep 1959)	FUNCTION TIME (MST)	BAITED TRAP CAPTURES		NONBAITED TRAP CAPTURES		BITES ON HUMAN VOLUNTE	
			Total	Number of Positive Traps	Total	Number of Positive Traps	Total	Numb of M Bitt
A-27	17	1408	9	4	14	7	15	6
A-28	18	0903	0	0	3	3	2	2
A-29	18	1006	6	6	0	0	3	3
A-30	21	1106	3	5	3	3	1	1
A-31	21	1343	0	0	4	2	36	8
A-32	21	1542	0	0	1	1	0	0
A-33	22	1038	21	9	2	2	103	9
A-34	22	1320	38	10	10	7	56	8
A-35	22	1435	25	6	0	0	86	7
A-36	23	1120	3	1	5	3	13	5
A-37	23	1325	6	4	0	0	40	8
A-38	23	1430	7	4	3	2	32	5
A-39	24	0945	5	4	0	0	0	0
A-40	24	1043	8	7	0	0	52	8
A-41	24	1312	16	6	9	3	47	8
A-42	24	1511	0	0	1	1	27	5
A-43	28	1330	5	2	1	1	25	7
A-44	28	1445	1	1	5	3	2	2
A-45	29	1340	8	3	2	1	14	6
A-46	29	1500	15	7	3	2	45	8
A-47	30 (Oct 1959)	1450	19	7	3	2	21	5
A-48	1	1343	1	1	0	0	1	1
A-49	1	1500	0	0	3	1	ND	NI
A-50	2	Traps: 1235 Humans: 1310	0	0	0	0	0	0
A-51	9	1105	16	6	10	4	206	[illegible]
A-52	9	1320	15	7	5	3	31	[illegible]

leased vectors would fly towards them and then pass by and fly to another person without stopping.

(U) The volunteers that had functioned the test fixtures observed that the vectors were attracted by movement--the movement involved in releasing the vectors resulted in many mosquitoes moving to that man. Other personnel observed that the vectors appeared to be attracted by sound--the more garrulous men getting more bites.

(U) It was observed that many of the mosquitoes could not be felt while they were probing or taking blood meals. It is not known what percentage the reported bites represent of the total number, but it is likely that it is less. For a single example, early in the trial series, one man reported 15 bites at the test site. Later that night he counted the swellings and found 31. Other similar disparities between reported and actual bites undoubtedly occurred. However, the reported over-kill of Swat Trial A-51 leaves this point somewhat in doubt.

(U) Several of the men noticed that some of the vectors would become so engorged with blood that they apparently could not fly, but instead dropped to the ground and crawled away.

(U) One further incident appears noteworthy. Each man in the volunteer circle was provided with a clipboard, and all of the clipboards were transported to the field in a wooden box. This box, with open slanted sides, was used at times in the first few trials by different men to sit on (see Figure 3), but they never did it twice. It appeared as if the mosquitoes, in numbers, went inside the box, venturing forth to bite the sitter, and then returning to the box.

PERSISTENCY AND SPREAD

(C) When this test was designed, it was felt that moving the test sites at least 1 mile upwind would alleviate any effects of vectors persisting to affect a second trial in the same general area. Apparently, this was true, since no test vectors were reported prior to release in any trial. One otherwise observed persistency of a single A. aegypti was noted in the Clay Flats area on 8 September 1959. The last previous trial conducted in this general area had been on 4 September; therefore, this one vector had persisted for 4 days.

(C) In several of the first few trials the vehicles used to transport the volunteers were left approximately 150 to 200 yards away from the test circle. It was soon found, however, that this distance was too short, and that some of the mosquitoes had immediately moved to the trucks. In the ensuing trials, all vehicles were driven to the meteorological station. It was noticed, moreover, that during the time that the men or traps were being picked up after a trial several mosquitoes often entered the trucks; attempts were made to destroy all of these vectors before moving to a new test site.

INTRODUCTION

(U) Outlines of the methods used and the results of the statistical analyses performed on the BW 459 data are given below; more detailed presentation of several major aspects of this analysis can be found in the Appendix. In the large, these analyses were performed by Messrs. R. F. White and S. J. Amster of the General Analysis Corporation. General Analysis Corporation is under contract with Dugway Proving Ground to assist in the statistical design and evaluation of CBR field tests and laboratory research.

ACCUMULATION OF BITES IN TIME (MODIFIED RAO METHOD)

(U) For each trial, the biting data at the human circle consisted of the accumulated number of probes and bites received by individuals up to 1, 3, 5, 7, 10, 15, and 30 minutes following the release of the mosquitoes.

(U) If the data from a trial are plotted as accumulated bites versus time, a nondecreasing function is observed. An analysis of these data depends initially on some description of this function. There are several ways of describing it; however, the one chosen was the following modification of a method given by Rao (8):

$$b_i = \frac{1}{m} \sum_{j=1}^{m} \frac{(\Delta y)_{ij}}{(\Delta x)_j}$$

where,

b_i = average rate of increase of the function in the i-th trial;

$(\Delta y)_{ij}$ = total number of mosquito bites obtained in the i-th trial during the j-th time interval

where,

$i = 1, 2, \ldots n$ (39 trials in this case);

$j = 1, 2, \ldots m$ (7 intervals in all trials);

$(\Delta x)_j = \frac{1}{n} \sum_{i=1}^{n} (\Delta y)_{ij}$ = the average number of mosquito bites obtained in all trials during the j-th time interval.

ological conditions present during the trial on the rate of increase of total bites in time and is greater or less than unity accordingly as a trial had a greater or lesser increase than "average". Therefore, in order to ascertain the extent of the effect of each measured meteorological variable, the trials were compared on the basis of these values (b_i's) by means of an analysis of variance.

(U) To accomplish this, the 39 trials (A-7 and A-10 through A-47) were first classified on a "high" or "low" basis for the four meteorological criteria: wind speed, temperature, relative humidity, and solar radiation. For each of these criteria, the 39 trials were ordered from low to high. The 20-th value in such an ordering is the median and the trials which were below the median were called the "low" trials (for that meteorological criterion) and those above were called the "high" trials. For wind speed, temperature, and relative humidity, the median (20th) trial was arbitrarily placed in the high group, but for solar radiation the 17th, 18th, 19th, and 20th trials were "tied" with solar radiation equal to 1.05 gram-calories/cm^2/minute so that it was necessary to place the median (20th) trial in the low group. The results of these classifications are given in Table 4.

TABLE 4: Summary of General Group Classification Data for 39 Human Circle Trials (UNCLASSIFIED)

CRITERION	MEDIAN OF ALL TRIALS	LOW GROUP			HIGH GROUP		
		Mean	Range*	Number of Trials	Mean	Range*	Number of Trials
Wind speed (mph)	5.1	3.18	3.4	19	7.38	8.4	20
Temperature (°F)	64.1	58.1	10.8	19	73.0	22.8	20
Relative humidity (%)	42.5	30.0	25.3	19	53.4	27.0	20
Solar radiation (gm-cal/cm^2/min)	1.05	0.73	1.00	20	1.19	0.30	19

*The range in this analysis is the difference between the highest and the lowest value in the group.

(U) Although it would be possible, albeit tedious, to perform an analysis of variance of these 39 trials based on a four-way classification created by these four partitions, not all of the questions answered by such an analysis would be of major interest. Therefore, several analyses of variance were performed, based on the following three-way classifications:

2. By wind speed, temperature, and solar radiation; and

3. By wind speed, relative humidity, and solar radiation.

Since it was clear from a visual examination of the data that wind speed had a major effect on biting activity, wind speed was included in all analyses.

(U) A three-way table of means (of b_i values) was computed for each of the three analyses. These are given in Tables 5, 6, and 7.

TABLE 5: Three-way Table of Means for Wind Speed, Temperature, and Relative Humidity Analyses (CONFIDENTIAL)

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Relative Humidity		High Relative Humidity		Low Relative Humidity		High Relative Humidity	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low Temperature	0.7562	4	1.2770	5	-*	0	0.3654	10
High Temperature	1.8848	9	0.5190	1	1.1780	6	0.3443	4

*Missing combination (high wind speed, low temperature, and low relative humidity).

TABLE 6: Three-way Table of Means for Wind Speed, Temperature, and Solar Radiation Analyses (CONFIDENTIAL)

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Solar Radiation		High Solar Radiation		Low Solar Radiation		High Solar Radiation	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low Temperature	0.4488	5	1.7915	4	0.4792	5	0.2516	5
High Temperature	1.3407	4	2.0200	6	1.0033	6	0.6062	4

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Solar Radiation		High Solar Radiation		Low Solar Radiation		High Solar Radiation	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low Relative Humidity	1.1268	6	1.8897	7	1.3655	4	0.8030	2
High Relative Humidity	0.2820	3	2.0193	3	0.4220	7	0.2967	7

(U) Analysis of variance of three-way classifications with unequal numbers in the cells is not a simple matter. It does not arise frequently in experimental data where the control of the factors enables the experimenter to keep the cell numbers equal, but is quite likely to arise in observational data where the factors, such as meteorological conditions, are relatively uncontrolled. The least complex and perhaps most common case is the one here, where each of the three factors is at two levels. Furthermore there were no trials at high wind speed, low temperature, and low relative humidity as can be seen by noting the missing cell in the first analysis (Table 5). This is not serious, although it means that the three-factor interaction of wind speed, temperature, and relative humidity cannot be estimated. A full exposition of the analyses performed on these data is given in the Appendix and the results of these analyses are presented in Tables 8, 9, and 10.

(C) If the three analyses are considered together it is clear that high wind speed was associated with significantly lower biting activity. The effect of temperature is somewhat obscured by the temperature-relative humidity (T x H) interaction (Table 8) which is significantly negative. This may be a result of the fact that H (relative humidity) is not an independent variable but is intrinsically related to T (temperature). High temperature was associated with higher biting activity than was low temperature (Tables 8 and 9), especially when solar radiation was relatively constant and humidity was variable (Table 9). But the significantly negative T x H interaction in the first analysis (Table 8) indicates that high temperature was associated with a smaller increase in biting activity at high humidity than at low. Data in the first of the three tables of means (Tables 5, 6, and 7), show that high

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT	STANDARD ERROR OF EFFECT
Wind Speed, W	1	4.2340	8.569	< 5	-0.7401	0.2528
Temperature, T	1	0.9172	1.856	< 20	0.3768	0.2765
Relative Humidity, H	1	0.7771	1.573	NS*	-0.3752	0.2992
W x T	1	0.3503	<1	NS	positive	
W x H	1	0.1853	<1	NS	positive	
T x H	1	2.2801	4.615	< 5	negative	
Error	32	0.4941				

*Not significant.

TABLE 9: Summary of the Results of the Analysis by Wind Speed, Temperature, and Solar Radiation (CONFIDENTIAL)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT	STANDARD ERROR OF EFFECT
Wind speed, W	1	5.9816	13.115	< 1	-0.7027	0.2189
Temperature, T	1	2.3543	5.162	< 5	0.4961	0.2183
Solar Radiation, V	1	1.0041	2.201	< 20	0.5248	0.2189
W x T	1	0.0391	<1	NS*	negative	
W x V	1	4.1463	9.091	<1	negative	
T x V	1	0.3980	<1	NS	negative	
W x T x V	1	0.1449	<1	NS	positive	
Error	31	0.4561				

*Not significant

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT	STANDARD ERROR OF EFFECT
Wind Speed, W	1	2.4202	5.801	<5	-0.5448	0.2262
Relative Humidity, H	1	2.5065	6.200	<5	-0.5616	0.2256
Solar Radiation, V	1	1.5764	3.779	<10	0.4061	0.2089
W x H	1	0.2288	<1	NS*	negative	
W x V	1	5.1580	12.364	<1	negative	
H x V	1	1.0133	2.429	<10	positive	
W x H x V	1	0.1435	<1	NS	negative	
Error	31	0.4172				

*Not significant

temperature actually was associated with a decrease in biting activity at high humidity.[3] It appears that biting activity is highest on warm, dry days; however, since test conditions never included coincident high temperatures and high humidity, this last finding should not be construed to mean that biting rates would be less on warm, moist days than on warm, dry days. Although investigators have consistently listed relative humidity as an important factor in the survival of A. aegypti, little mention has been made of its effect on vector activity for the first day following release.

(C) Aside from the decrease in biting activity associated with high wind speed, the most statistically significant result was the negative interaction of wind speed and solar radiation (W x V in the second and third analyses, Tables 9 and 10). Since wind speed is certainly negative

[3]This point perhaps needs further clarification. Whereas absolute humidity is an independent meteorological variable, relative humidity is dependent to a large extent upon temperature. Since Dugway Proving Ground lies in a continental, high latitude desert, the combination of high temperature and high humidity is rare and was not encountered in this test. As a result, the grouping of "high" temperature trials (Table 4) in order of increasing temperature is associated, in general, with decreasing "high" relative humidities. Thus, the statement made above that "high temperature actually was associated with a decrease in biting activity at high humidity (over that encountered at low humidity)" really means that lower biting activity was recorded at high humidity because the temperature was lower and not because the humidity was higher.

at high rather that at low solar radiation. Thus the idea of high solar radiation acting as a counteractant to a windchill factor (9), such that high solar radiation would tend to nullify the cooling effect of the wind, does not appear tenable. Without further interpretation, all that can be said here is that biting activity is highest on days of low wind speed and high solar radiation.

ACCUMULATION OF BITES IN TIME (WHITE'S METHOD[4])

(U) Rao's analysis does not give a complete description of the functional relationship between time and total bites. Therefore, the following model was derived:[5]

$$x_{ij} = \frac{n_i \gamma_i}{2} \left[1 + \sin(\alpha_i + \beta_i \log t_{ij}) \right], \quad -\frac{\pi}{2} < \alpha_i + \beta_i \log t_{ij} < +\frac{\pi}{2}$$

or

$$y_{ij} = \alpha_i + \beta_i \log t_{ij}$$

where,

x_{ij} = the total accumulated number of bites obtained in the i-th trial by the j-th time, where i = 1, 2, ...n and j - 1, 3, ...m;

n_i = the number of mosquitoes released in the i-th trial;

t_{ij} = the j-th time in the i-th trial;

$\alpha_i, \beta_i, \gamma_i$ = parameters to be estimated; and

$$y_{ij} = \arcsin \frac{2x_{ij}}{n_i \gamma_i} - 1.$$

(U) The human circle data from 28 trials (A-10 through 18, 20 through 23, 25 through 27, 29, 31, 34 through 37, 40 through 41, 43, and 45 through 47) were fitted to this model and values of α_i, β_i, and γ_i were determined by the method of minimum chi-square.

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[4]For a complete description of this model, see the Appendix.

[5]This model, as used, is a method of estimating the initial primary effects following the release of starved vectors in the near vicinity of human subjects. As stated earlier, the full primary effects must take into consideration the entire life span of the released vector and, therefore, are beyond the scope of this model.

criteria: wind speed, temperature, relative humidity, and solar radiation. The 28 trials were ordered from "low" to "high" for the first three of these criteria; the 14 lower trials were called the low trials and the 14 higher trials were called the high trials. The 12th, 13th, 14th, and 15th trials had equal solar radiation so the lower 15 trials were called low solar radiation and the upper 13, high. The results of these classifications are given in Table 11.

TABLE 11: Summary of General Group Classification Data for 28 Trials, BW 459 (CONFIDENTIAL)

CRITERION	LOW GROUP		HIGH GROUP	
	Mean	Range*	Mean	Range*
Wind Speed (mph)	2.93	3.1	6.96	6.4
Temperature (°F)	58.9	11.1	74.3	22.1
Relative Humidity (%)	28.2	22.3	52.3	30.5
Solar Radiation (gm-cal/cm^2/min)	0.72	0.95	1.21	0.30

*The range in this analysis is the difference between the highest and the lowest value in the group.

(U) Two analyses of variance, similar to those discussed in the analysis of the b_i values (modified Rao method) were performed on the γ_i values. The two analyses were by wind speed, temperature, and relative humidity; and by wind speed, temperature, and solar radiation. The tables of means for the γ_i's are presented in Tables 12 and 13.

TABLE 12: Table of Means for Wind Speed, Temperature, and Relative Humidity, BW 459 (CONFIDENTIAL)

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Relative Humidity		High Relative Humidity		Low Relative Humidity		High Relative Humidity	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low Temperature	0.4138	4	1.3539	3	-*	0	0.3253	5
High Temperature	1.0090	8	0.2427	1	0.7468	4	0.2240	3

*Missing combination (high wind speed, low temperature, and low relative humidity).

TABLE 13: Table of Means for Wind Speed, Temperature, and Solar Radiation, BW 459 (CONFIDENTIAL)

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Solar Radiation Value		High Solar Radiation Value		Low Solar Radiation Value		High Solar Radiation Val	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	numb of tria
Low Temperature	0.3281	4	1.4731	3	0.3245	3	0.3266	2
High Temperature	0.6397	4	1.1512	5	0.7575	4	0.2098	3

(U) The reader should interpret these mean values as being estimates of total bites per mosquito if the time of exposure were extended long enough. The results of the analyses are given in Tables 14 and 15.

TABLE 14: Summary of Results of Analysis by Wind Speed, Temperature, and Relative Humidity, BW 459 (CONFIDENTIAL)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT
Wind Speed, W	1	1.3292	3.068	<10	negative
Temperature, T	1	0.0592	<1	NS*	positive
Relative Humidity, H	1	0.0004	<1	NS	positive
W x T	1	0.5520	1.274	NS	positive
W x H	1	0.0347	<1	NS	positive
T x H	1	1.7146	3.957	<10	negative
Error	21	0.4333			

*Not significant.

TABLE 15: Summary of Results of Analysis by Wind Speed, Temperature, and Solar Radiation (CONFIDENTIAL)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT
Wind Speed, W	1	1.2495	3.019	<10	negative
Temperature, T	1	0.0569	<1	NS*	positive
Solar Radiation, V	1	0.6831	1.650	NS	positive
W x T	1	0.0415	<1	NS	positive
W x V	1	2.0050	4.844	<5	negative
T x V	1	0.5989	1.447	NS	negative
W x T x V	1	0.0029	<1	NS	positive
Error	20	0.4139			

*Not significant.

(C) As in the analysis of the b_i values, it is clear that higher wind speed is associated with a lower biting activity, and that higher temperature is associated with a greater increase in biting activity when the relative humidity is low than when the humidity is high (see the negative T x H effect in Table 14 and the preceding discussion on page 32). Again, the W x V interaction (Table 15) is significantly negative, indicating that the effects of wind speed and solar radiation are opposed in the sense that higher wind speed is associated with a greater decrease in biting activity when solar radiation is high than it is when solar radiation is low. This apparent paradox was unexpected and, as of this time, remains unexplained.

(U) The same two analyses of variance were performed on the values of log t_i^{**} (the time at which the biting process would end), but the results were inconclusive. This is further complicated by the fact that a large t_i^{**} may be interpreted as representing a long biting period with moderate or low biting activity. A study of the t_i^{**} values would have intrinsic fundamental interest, but the inherent variability of these quantities is such as to require more data than are presently available for meaningful conclusions to be reached. A possibility for future investigation is to examine the quantity γ_i/t_i^{**}.

(U) No analysis of the values of log t_i^* (the time at which the biting process would begin) was performed.

have been obtained had the sampling period been extended to completion (in the initial primary biting period), it was found that the total number of bites observed in the 30-minute sampling period averaged 81 per cent with a range extending from 44 to 97 per cent. (One trial, A-22, which obviously did not fit, was excluded from this aspect.) Therefore, since 81 per cent of the expected bites were received in the 30-minute sampling interval, it appears that test periods of this length should provide adequate data in future field testing. This is important since participant interest can be maintained at a rather high rate for 30 minutes (as observed on these trials) while their interest and accuracy would tend to decline in longer periods.

BAITED VERSUS NONBAITED TRAPS

(U) The traps in the trap circles were quite shiny and therefore bypassed the known attraction of A. aegypti to dark objects. However, the traps did afford several other attraction factors. These included:

1. Bulk. The traps constituted relatively large bodies on the flat desert floor, especially in the sparsely vegetated areas picked for these trials.

2. Shelter. The traps afforded protection both from direct sunlight and from wind. This factor can be further separated into shade and windbreak.

3. Bait. Guinea pigs were supported in the center of each baited trap. They furnished moisture and carbon dioxide gradients, olfactory stimuli, and visual stimuli (they could be seen and they moved somewhat, although their range of movement was limited).

(U) A total of 35 trials (A-7 through A-13, A-15 through A-31, A-33 through A-41, and A-51 through A-52) were used in this analysis. Each trial consisted of observations of two rings--one with baited traps and the other with unbaited traps. One hundred mosquitoes were released from the center of a given ring of 10 traps and after 30 minutes the number of entrapped mosquitoes were counted. If r_i is the observed total number of trapped mosquitoes in the $_i$th trial and n_i is the number released, then p_i, the proportion entrapped in the $_i$th trial, is:

$$p_i = \frac{r_i}{n_i}$$

This p_i was then transformed to

$$y_i = 2 \arcsin (\sqrt{p_i}) \quad ,$$

which has constant variance. However, there would be bias in this y_i

value; therefore, to correct for this bias, p_i was first modified to

$$p_i = \frac{r_i + \frac{1}{2}}{n_i + \frac{1}{2}} = \frac{4r_i + 1}{4n_i + 2} \quad .$$

The resulting y_i values were then subjected to an analysis of variance.

(C) The 35 trials (each consisting of a pair of trap rings) were classified on the basis of two meteorological criteria: wind speed and solar radiation. These were selected since the analysis of the biting rate on humans had indicated that these two criteria would probably affect trapping recoveries the most. The 35 trials were ranked from "low" to "high," and the trials below the median were called low and those above were called high, for the given criterion. For wind speed, the median trial was arbitrarily placed in the low group, but for solar radiation a "tie" made it necessary to place the median trial in the high group. The results of these classifications are given in Table 16.

TABLE 16: Classification Criteria for Baited- Versus Nonbaited-Trap Data (UNCLASSIFIED)

CRITERION	MEDIAN OF ALL TRIALS	LOW GROUP		HIGH GROUP	
		Mean	Range*	Mean	Range*
Wind Speed (mph)	6.1	3.47	4.1	8.32	7.4
Solar Radiation (gm-cal/cm^2/min)	1.1	0.67	0.85	1.19	0.30

*Range as used in this analysis is the difference between the highest and the lowest value in the group.

(U) For each trial, both the sum of and the difference between the two y_i-values (baited-nonbaited) were computed. The means of these values, classified by meteorological conditions, are presented in Table 17.

TABLE 17: Means of Sums and of Differences in Baited- Versus Nonbaited-Trap Data (UNCLASSIFIED)

SOLAR RADIATION CATEGORY	MEANS OF SUMS (BAITED + NONBAITED)				MEANS OF DIFFERENCES (BAITED - NONBAITED)			
	Low Wind Speed		High Wind Speed		Low Wind Speed		High Wind Speed	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low	1.2996	7	1.2038	8	0.1269	7	0.0797	8
High	1.2894	11	1.1796	9	0.2192	11	0.1273	9

differences were positive, indicating that baited traps captured more mosquitoes than did nonbaited. The difference is more pronounced at high solar radiation than at low and is less pronounced at high wind speeds than at low. Detailed analyses of these and other factors were then performed to examine their significance.

(U) First, a straightforward analysis of variance of the 70 y_i values was performed. The results are given in Table 18.

TABLE 18: Standard Analysis of Variance of Baited- Versus Nonbaited-Trap Data (UNCLASSIFIED)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	SUM OF SQUARES	F-VALUE	APPROXIMATE PROBABILITY (%)
Meteorological groups, M	3	0.0153	0.0461	<1	NS*
Trials within meteorological groups, T	31	0.1838	5.7005	3.00	5
Baited versus nonbaited traps, B	1	0.3620	0.3620	5.92	5
B x M	3	0.0173	0.0519	<1	NS
B x T	31	0.0612	1.8984		
Total	69		8.0589		

*Not significant.

(U) The error term for M is T. The error term for the other sources is B x T. It is interesting to note the significant F-value for T (3.00); this indicates that day-to-day variability within meteorological groups is significantly greater than the ring-to-ring variability within days. Therefore it is important not to pool T and B x T into a single error term, but rather to use T as the error term for components of M and to use B x T as the error term for both B and the components of B x M. The F-value for B (5.92) indicates that the previously stated higher trapping rate for baited traps is significant.

(U) The analysis of variance shown in Table 18 was extended, by subdivisions of M and B x M components, to provide further information. Components of M give estimates of the association of wind speed and solar radiation with trapping rates for baited and nonbaited traps together. Components of B x M give estimates of the association of the increase of trapping rate of baited over nonbaited traps with these two meteorological conditions. The results of this extended analysis are given in Table 19.

TABLE 19: Extended Analysis of Variance of Baited- Versus Nonbaited-Trap Data (UNCLASSIFIED)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE
Wind Speed, W	1	0.0456	<1
Solar Radiation, V	1	0.0013	<1
W x V	1	0.0002	<1
Error (a)	31	0.1838	
Baited versus non-baited traps, B	1	0.3620	5.92
B x W	1	0.0218	<1
B x V	1	0.0208	<1
B x W x V	1	0.0021	<1
Error (b)	31	0.0612	

(C) Error (a) is the appropriate error term for W, V, and W x V; error (b) is the appropriate error term for B, B x W, B x V, and B x W x V. It is interesting to note that W and W x V are not significant, contrary to the findings on biting activity with human volunteers. Thus wind speed and its interaction with solar radiation are apparently not associated with trapping rates, although they are associated with biting activity. Again, although B is significant (trapping rates are higher with baited than with nonbaited traps), there is no evidence of interaction of B with wind speed and solar radiation.

(U) The baited traps captured significantly more mosquitoes than did the nonbaited. Moreover, it seems logical to ascribe the basic value of any trap to bulk and shelter, and the difference between nonbaited and baited to the specific attraction of the bait. Thus, the addition of bait does not seem to be of paramount importance, particularly since the effect of baiting was not found to be affected by wind speed and solar radiation. These results substantiate and enlarge the observation that A. aegypti will first seek out buildings, vehicles, and other bulky objects on their visual horizon.

EFFECT OF WIND DIRECTION

(C) Since higher wind speeds were consistently associated with lowered biting activity, the effect of wind direction with regard to specific ring locations was investigated. In Trials A-1 through A-31, the number one positions were the northernmost positions; from Trial A-32 on (to facilitate analysis) the number one positions were located at the farthest point upwind. It must be kept in mind that the wind

for the general area and could have been different at the three circles. Furthermore, the wind direction at the beginning of testing was not necessarily the same either during the intervening period or at the end of the test. However, these data should average out well enough to show the effect of wind direction on biting and trap captures. It should be further borne in mind that higher wind speeds would tend to be more constant in direction than the local vagaries of low velocity winds.

(U) The counts for the three upwind ring positions were segregated from the counts for the three downwind positions for each trial. That is, each ring of traps yielded two values: the number of mosquitoes entrapped upwind and the number entrapped downwind (each value based on 3 traps for 30 minutes). Each ring of humans also yielded two values: the number of bites upwind and the number of bites downwind (each value based on 3 human subjects in 30 minutes). The lateral ring positions were not considered.

Bites on Human Subjects

(U) In this analysis, the difference (downwind - upwind bites) was divided by the sum (downwind + upwind bites). This was done for 35 trials (the same trials used in the modified Rao method less four--Trials A-19, 24, 32, and 39; in these latter four trials, the sum, downwind + upwind bites, was zero and the ratio could not be computed.). The table of means of these ratios is presented as Table 20.

TABLE 20: Table of Means of Ratios of Differences/Sums of Upwind Versus Downwind Biting Activity (CONFIDENTIAL)

CATEGORY	LOW WIND SPEED		HIGH WIND SPEED	
	Mean	Number of Trials	Mean	Number of Trials
Low Solar Radiation	0.3673	8	0.4498	9
High Solar Radiation	0.4096	10	0.5058	8

(C) Since these means are positive, they indicate an apparent propensity of mosquitoes for downwind locations. Whether or not this propensity was significant or whether it was affected by wind speed and solar radiation was then considered by subjecting the data to an analysis of variance. The results are given in Table 21.

TABLE 21: Analysis of Variance of Ratios of Differences/Sums of Downwind Versus Upwind Biting Activity (CONFIDENTIAL)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	EFFECT
Direction, D	1	4.5050	12.495	<1	positive
D x W	1	0.0696	<1	NS**	positive
D x V	1	0.0208	<1	NS	positive
D x W x V	1	0.0004	<1	NS	positive
Error	31	0.3606			

*Not significant.

(C) The F-value of 12.495 is significant at the 0.01 level of probability and indicates that there were significantly more bites at the downwind then at the upwind locations and that this was not affected by either wind speed, solar radiation, or their interaction.

Captures in Baited Traps

(U) To investigate the association of wind direction with trap captures, the baited trap captures from 35 trials, Trials A-7 through A-13, A-15 through A-31, A-33 through A-41, A-51, and A-52 (the same trials as in the section entitled BAITED VERSUS NONBAITED TRAPS) were considered. For each ring of baited traps, the three 30-minute upwind trap captures were totaled, as were the three downwind trap captures. A square root transformation was then made of each sum, and for each trial the difference, d, was computed, viz.:

$$d = \sqrt{\text{Downwind total}} - \sqrt{\text{Upwind total}}$$

(U) These differences were analyzed in the same way as were the analogous quantities for the human-baited rings. It is not necessary to give the results in great detail, except to say that wind direction, D, was highly significant (at the 0.01 probability level) but D x W, D x V, and D x W x V were not; this indicates that mosquitoes prefer downwind baited trap targets (as in the case for human targets) and that this propensity is unaffected by either wind speed, solar radiation, or their interaction.

(U) It may be well to point out that the variables concerned here deal with the relative attractiveness of visual versus olfactory factors. All of the men and traps offered visual attraction (bulk, contrast, shadow), but to the mosquitoes released in the center of the ring, only the upwind positions offered olfactory stimuli. Therefore, it appears

the downwind segments--even overcoming the directory [illegible] the particular speed of the transporting wind is not important.

REGRESSION ANALYSIS OF TOTAL BITES

Regression on Meteorological Conditions

(C) The recorded biting data from 39 trials, Trials A-7 and A-10 through A-47, were analyzed by a multiple regression analysis. The model

$$y = a + bH + cT + dW + eV$$

was fitted where:

y = total number of bites,

H = relative humidity (%),

T = temperature (°F),

W = wind speed (mph),

V = solar radiation (gram-calories per cm^2 per minute), and

a, b, c, d, and e are partial regression coefficients determined by the method of least squares. The results were:

$a = -44.12$,

$b = -0.389$, $b/s_b = -0.08$,

$c = +1.612$, $c/s_c = +2.25$,

$d = -5.387$, $d/s_d = -2.77$, and

$e = +22.98$, $e/s_e = +1.39$.

The right-hand column immediately above gives the results of dividing th coefficients by their standard errors and it indicates that temperature, wind speed, and solar radiation are better "predictors" of total bites than is relative humidity. Because of this, attention was then concentrated on these three remaining meteorological variables.

(U) In the following section, the regression on the number of captures in baited traps is considered. Only 32 trials, Trials A-7, A-10 through A-31, and A-33 through A-41, were judged to have suitable

ring-pairs--that is, volunteer trios and baited pig-baited trap circles on the same day. Therefore, in the remainder of the present section, attention is confined to these trials.

(C) On these 32 trials, the regression,

$$y = a + bW + cT$$

where y is the total number of mosquitoes captured in baited traps, was fitted and gave:

$$a = -46.57,$$

$$b = +6.93,$$

$$c = +1.90, \text{ and}$$

$$s^2_e = +3277.56 \text{ (29 degrees of freedom).}$$

The quantity s^2_e is the sum of squares of deviations divided by the degrees of freedom.

(C) Again, on these 32 trials, the regression

$$y = a + bW + cV \quad ,$$

wherein wind speed and solar radiation were considered, gave:

$$a = +47.132,$$

$$b = -5.474,$$

$$c = +27.639, \text{ and}$$

$$s^2_e = +1023.63 \text{ (29 degrees of freedom).}$$

Comparison of these two s^2_e quantities (1023.63 and 3277.56) shows that wind speed and solar radiation (W x V) is a better predictor than is wind speed and temperature (W x T).

<u>Regressions Including Baited Trap Capture Totals</u>

(C) Using the 32 trials listed above, the regression

$$y = a + bT + cB$$

was fitted, where y is the total number of bites and B is the total

This gave:

$$a = -37.391,$$

$$b = +0.873,$$

$$c = +1.716, \text{ and}$$

$$s^2_e = +841.34 \text{ (29 degrees of freedom).}$$

The regression

$$y = a + bW + cB,$$

wherein wind speed and baited trap captures were considered, gave:

$$a = +48.99,$$

$$b = -5.441,$$

$$c = +1.855, \text{ and}$$

$$s^2_e = +640.11 \text{ (29 degrees of freedom).}$$

(C) The comparison of these s^2_e quantities (640.11 and 841.34) shows that temperature, when baited-trap captures were considered, was not as good a predictor as wind speed. In fact, the regression

$$y = a + bW + cV + dB,$$

wherein wind speed, solar radiation, and baited trap captures were considered, gave:

$$a = 42.282,$$

$$b = -5.232,$$

$$c = 6.174,$$

$$d = 1.829, \text{ and}$$

$$s^2_e = 659.18 \text{ (28 degrees of freedom).}$$

The comparison of these s^2_e quantities (659.18 and 640.11) shows that solar radiation, even when wind speed and baited-trap captures are considered, had no utility as a predictor of total bites.

(C) Finally, the regression [illegible]

$$y = a + bB \quad ,$$

wherein the number of bites and baited trap captures alone were considered, gave:

$$a = 16.598,$$

$$b = 2.089, \text{ and}$$

$$\sigma^2_e = 869.81 \text{ (30 degrees of freedom).}$$

(C) Any linear regression analysis must be interpreted with caution. Because "true" relationships are seldom linear, predictions based on such regressions are likely to be imprecise or even inaccurate. However, throughout these analyses, it was seen that the coefficients of W (wind speed) were consistently in the range of from -5 to -7. This suggests that an increase of 1 mile per hour in wind speed (in the range of speeds considered in these trials) is associated with an approximate decrease of six bites in a 10-man ring of 30-foot diameter and with an exposure time of 30 minutes.

(C) Biting activity seems to be fairly well correlated with wind speed and solar radiation, decreasing with an increase in the former and increasing with an increase in the latter. Moreover, biting activity is positively correlated with baited-trap totals, especially when wind speed is taken into account. Finally, under the conditions of these trials and in the time period involved, a single mosquito in a baited trap appears to be equivalent to about two bites on a human subject (in the sense that the coefficients of B in the above analyses ranged about the value 2.0). The trap capture and bites-received data were then examined to ascertain whether traps could be used to replace humans in future field trials. For consistency, the same 35 trials used in the previous trap analyses were studied. The appertaining data, summarized from Table 3, are presented in Table 22.

(C) This table shows that the average number of bites received was over three times the average baited trap captures and over four times the average nonbaited trap captures. However, the variation between individual trials is excessive, and the lack of any consistent relationship is quite evident. It is clear that the number of bites received in any one trial could hardly be predicted from the trap recovery data, and the present traps can not be used to replace humans in the field assessment of A. aegypti behavior.

TRIAL NUMBER	BAITED TRAP CAPTURES	NONBAITED TRAP CAPTURES	NUMBER OF BITES RECEIVED	TRIAL NUMBER	BAITED TRAP CAPTURES	NONBAITED TRAP CAPTURES	NUMB OF BI RECEI
A- 7	27	26	50	A-28	0	3	2
A- 8	24	6	7	A-29	6	0	6
A- 9	4	6	9	A-30	3	3	1
A-10	20	10	59	A-31	0	4	34
A-11	19	28	99	A-33	21	2	103
A-12	12	19	47	A-34	38	10	66
A-13	24	2	104	A-35	25	0	86
A-15	4	2	53	A-36	3	5	13
A-16	20	10	70	A-37	6	0	48
A-17	23	29	76	A-38	7	3	32
A-18	34	44	44	A-39	5	0	6
A-19	0	0	0	A-40	8	0	52
A-20	7	24	23	A-41	16	9	41
A-21	8	7	14	A-51	16	10	20
A-22	22	16	90	A-52	15	5	3
A-23	10	18	23	Total	470	332	153
A-24	7	9	0				
A-25	4	4	12	Average			
A-26	15	7	28	per			
A-27	9	14	15	trial	13.4	9.4	4

*Summarized from Table 3.

(C) BW 459 was conducted to determine whether or not Aedes aegypti, a domestic, house-loving, tropical mosquito, could be field tested under the climatic conditions of a hot, dry desert environment. Results show that the initial biting instincts of this mosquito are not impaired or appreciably modified by release into a dry climate. It is further evident that this vector can be used for initial primary effects against troops in the open in desert areas. The corollary effect of the desert environment upon mosquito longevity was not investigated in this phase, and it is suspected that longevity would be greatly shortened. However, for primary target effects, this is not of great significance and may even be an advantage in certain situations.

(C) The lower temperature limit for vector activity in the non-cold resistant strain of A. aegypti has been reported to be 59°F (1). However, bites were recorded in 11 of the 14 trials conducted at temperatures below 59°F; therefore, it would appear that this lower limit is set too high. These trials are tabulated in Table 23.

TABLE 23: Summary of Biting Data for Trials Conducted at Temperatures Less than 59°F, BW 459 (CONFIDENTIAL)

TRIAL NUMBER	AMBIENT TEMPERATURE (°F)	AVERAGE WIND SPEED (mph)	TOTAL NUMBER OF BITES ON HUMANS	NUMBER OF HUMANS REPORTING BITES (x/10)
A-21	56.7	4.7	14	3
A-24	55.8	2.4	0	0
A-25	58.5	6.1	12	3
A-30	58.1	6.4	1	1
A-33	57.7	3.2	103	9
A-36	56.5	6.8	13	5
A-39	58.5	5.2	0	0
A-43	54.3	5.1	25	7
A-44	54.3	9.4	2	2
A-45	53.9	ca. 5.0*	14	6
A-46	53.1	ca. 4.0*	15	8
A-47	56.9	3.1	21	5
A-48	57.2	7.0	1	1
A- 9	57.8	11.2	ND**	ND
A-50	57.3	9.5	0	0

*Approximate data--field equipment malfunction; data from Dog Area station.

**No data--premature munition function.

at higher temperatures [illegible]
is operative at these low temperatures that is not evident in the meteorological parameters being measured (cf. Trials A-21, A-24, and A-25). Ground temperature and ultra-violet radiation data will be investigated in BELLWETHER-II.

(C) A. aegypti has been consistently cited in the literature as a crepuscular biter (viz. dawn and dusk biter) in nature; however, the trials of BW 459 were, as a rule, conducted during the general midday period. This fact did not seem to impair their biting propensity but, in view of the probable desirability of nighttime delivery, further field trials must be conducted to ascertain whether or not the biting rate is increased by early morning or late evening release. In the present test, one trial, A-15, was conducted just at sunset on the same day that Trials A-13 and A-14 were accomplished. Only 53 bites were reported in the sunset trial against 104 and 138, respectively, in the two preceding trials.

(S) Preliminary indoor biting rates have been determined by the U. S. Army Chemical Corps Biological Laboratories (4). They found an over-all mean of 2.40 bites per vector in trials involving 100- to 400-square foot rooms, 15-minute exposure duration, and varying ratios of vector/host density. These indoor rates were not approached in the BW 459 outdoor studies. The higher indoor biting rates can be ascribed, in part at least, to the confinement of the vectors and to the complete absence of wind. In the 39 trials used in the analyses of variance, a total of 1555 bites were reported for an average outdoor biting rate of 0.40 bites per vector in the 30-minute time period with a 10/1 vector host ratio.

CONCLUSIONS

(S) Using uninfected, virgin female Aedes aegypti mosquitoes in Phase A of BW 459, the results obtained within the ranges of conditions encompassed in these trials indicate that:

1. It is feasible to test this mosquito under hot, dry, desert conditions, at least for the initial primary time period, and to assess the effects of various meteorological variables upon biting activity.

2. Although many of these trials produced erratic and unpredictable results, it would appear from the analysis of these data that each of the meteorological variables studied--wind speed, temperature, relative humidity, and solar radiation--exert a significant influence on the biting activity of the A. aegypti mosquito, and all would have to be considered as important parameters in any model designed to predict

manifested only in terms of [illegible]
other; wind speed alone had a direct effect upon biting activity. Moreover, within the ranges of conditions encompassed in these trials, it appears that wind speed was the most important factor affecting biting activity.

3. An increase of 1 mile per hour in the ambient wind speed was associated with a decrease of approximately six bites in a 15-foot radius circle with 10 volunteers over a 30-minute time interval.

4. The data suggest that the previously determined lower temperature limit of 59°F for vector biting activity of th non-cold resistant strain is placed too high; however, at these lower temperatures some other factor(s), at present unknown, produce erratic results.

5. A 30-minute sampling period is sufficient to encompass an average of 80 per cent of the expected initial primary biting activity.

6. Guinea pig-baited traps captured one and one-half times as many mosquitoes as did nonbaited traps.

7. Whereas, on the over-all average, a single mosquito in a baited trap was equivalent to two bites on a human, excessive intertrial variation precludes replacing human samplers with traps to determine biting rate activity.

8. With a vector-host ratio of 10:1, in only a very few trials did 100 per cent of the volunteers report bites. The mean percentage of test subjects bitten lay between 60 and 70 per cent.

9. The over-all average outdoor biting rate for this vector was 40 bites per 100 mosquitoes in the time period studied.

10. No evidence of crepuscular-period biting preference was obtained in these trials.

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APPENDIX

EXPOSITION OF STATISTICAL METHODS

THREE-FACTOR ANALYSIS OF VARIANCE WITH UNEQUAL OBSERVATIONS PER CELL

(C) The method for the 3-factor analysis of variance with unequal observations per cell is given below. Tables 5, 6, and 7 in the text, where the unequal observations per cell occurred, are repeated here, for convenience, as Tables 1, 2, and 3.

TABLE 1: Three-way Table of Means for Wind Speed, Temperature, and Relative Humidity Analyses (CONFIDENTIAL)

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Relative Humidity		High Relative Humidity		Low Relative Humidity		High Relative Humidity	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low Temperature	0.7562	4	1.2770	5	-*	0	0.3654	10
High Temperature	1.8848	9	0.5190	1	1.1780	6	0.3443	4

*Missing combination (high wind speed, low temperature, and low relative humidity).

TABLE 2: Three-way Table of Means for Wind Speed, Temperature, and Solar Radiation Analyses (CONFIDENTIAL)

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Solar Radiation		High Solar Radiation		Low Solar Radiation		High Solar Radiation	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low Temperature	0.4488	5	1.7915	4	0.4792	5	0.2516	5
High Temperature	1.3407	4	2.0200	6	1.0033	6	0.6062	4

CATEGORY	LOW WIND SPEED				HIGH WIND SPEED			
	Low Solar Radiation		High Solar Radiation		Low Solar Radiation		High Solar Radiation	
	mean	number of trials	mean	number of trials	mean	number of trials	mean	number of trials
Low Relative Humidity	1.1268	6	1.8897	7	1.3655	4	0.8030	2
High Relative Humidity	0.2820	3	2.0193	3	0.4220	7	0.2967	7

(U) Table 2 will be analysed first. A simple analysis of variance of the 39 trials is performed, and the results are shown in Table 4.

TABLE 4: Analysis of Variance of Data in Table 2 (UNCLASSIFIED)

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Among cells	7	15.508737	
Within cells	31	14.138573	0.456083
Total	38	29.647310	

(C) The 7 degrees of freedom among cells is then subdivided into three main effects, three 2-factor interactions, and one 3-factor interaction. This subdivision cannot be orthogonal, because of the inequality of cell numbers. It is done as follows:

(1) Main effects. Each of the three main effects is essentially an estimate of the expected value of the difference between trial at the upper level of the corresponding meteorological condition and trials at the lower level.

$$\frac{[illegible] - [illegible]}{\frac{1}{5} + \frac{1}{5}} + \frac{[illegible] - [illegible]}{\frac{1}{5} + \frac{1}{4}} + \frac{[illegible]}{\frac{1}{6} + \frac{1}{4}} + \frac{[illegible]}{\frac{1}{4} + \frac{1}{6}}$$

$$= \frac{0.0304}{0.400} - \frac{1.5399}{0.450} - \frac{0.3374}{0.417} - \frac{1.4138}{0.417} = -7.545521 \quad \text{, and}$$

$$\frac{1}{0.400} + \frac{1}{0.450} + \frac{1}{0.417} + \frac{1}{0.417} = 9.518386 \quad .$$

Then the estimate of the effect of wind speed is

$$\frac{-7.545521}{9.518386} = -0.792731 \quad ,$$

the mean square for wind speed, with 1 degree of freedom, is

$$(0.792731)(7.545521) = 5.9816 \quad \text{, and}$$

the standard error of the estimate of the main effect (-0.792731) is

$$\frac{\sqrt{0.456083}}{\sqrt{9.518386}} = \frac{0.67534}{3.0852} = 0.2189$$

where 0.456083 is the within-cell mean square from the above preliminary analysis of variance.

(b) Temperature

$$\frac{1.3407 - 0.4488}{\frac{1}{4} + \frac{1}{5}} + \frac{2.0200 - 1.7915}{\frac{1}{6} + \frac{1}{4}} + \frac{1.0033 - 0.47[illegible]}{\frac{1}{6} + \frac{1}{5}} + \frac{0.6062 - 0.2516}{\frac{1}{4} + \frac{1}{5}}$$

$$= \frac{0.8919}{0.450} + \frac{0.2285}{0.417} + \frac{0.5241}{0.367} + \frac{0.3546}{0.450} = 4.746027 \quad \text{, and}$$

$$\frac{1}{0.450} + \frac{1}{0.417} + \frac{1}{0.367} + \frac{1}{0.450} = 9.567322$$

Then the estimate of the effect of temperature is

$$\frac{4.746027}{9.567322} = 0.496066 \quad ,$$

the standard error of the estimate of the main effect is

$$\frac{0.67534}{9.567320} = 0.2113$$

where $0.67535 = \sqrt{0.456083}$ has been computed as above.

(c) Solar Radiation

$$\frac{1.7915 - 0.4488}{\frac{1}{4} + \frac{1}{5}} + \frac{2.0200 - 1.3407}{\frac{1}{6} + \frac{1}{4}} + \frac{0.2516 - 0.4792}{\frac{1}{5} + \frac{1}{5}} + \frac{0.6062 - 1.0033}{\frac{1}{4} + \frac{1}{6}}$$

$$= \frac{1.3427}{0.450} + \frac{0.6733}{0.417} - \frac{0.2276}{0.400} - \frac{0.3971}{0.417} = 3.091517 \quad \text{, and}$$

$$\frac{1}{0.450} + \frac{1}{0.417} + \frac{1}{0.400} + \frac{1}{0.417} = 9.518386 \quad .$$

Then the estimate of solar radiation effect is

$$\frac{3.091517}{9.518386} = 0.324794 \quad ,$$

the mean square for solar radiation effect is

$$(0.324794)(3.091517) = 1.0041 \quad \text{, and}$$

the standard error of the estimate is

$$\frac{0.67534}{\sqrt{9.518386}} = 0.2189 \quad .$$

(2) Two-factor interactions. If two factors, A and B, are considered, and

HH denotes trials at upper level of A, upper level of B,
HL dentoes trials at upper level of A, lower level of B,
LH denotes trials at lower level of A, upper level of B, and
LL denotes trials at lower level of A, lower level of B,

then the corresponding 2-factor interaction estimates are as follows:

$= \frac{1}{2}$ [effect of B at upper level of A - effect of B at lower level of A]. These estimates and their standard errors will not be computed, as was done for the main effects, because it shall suffice to merely note their signs, whether positive or negative, and to compute the mean squares for purposes of testing significance.

(a) Wind speed x temperature

$$\frac{1.0033 - 0.4792 - 1.3407 + 0.4488}{\frac{1}{6} + \frac{1}{5} + \frac{1}{4} + \frac{1}{5}} + \frac{0.6062 - 0.2516 - 2.0200 + 1.7915}{\frac{1}{4} + \frac{1}{5} + \frac{1}{6} + \frac{1}{4}}$$

$$= \frac{-0.3678}{0.8167} + \frac{0.1261}{0.8667} = -0.3049 \text{ (negative)} \quad \text{, and}$$

$$\frac{1}{0.8167} + \frac{1}{0.8667} = 2.3782 \quad .$$

The mean square is

$$(-0.3049)^2/2.3782 = 0.0391.$$

(b) Wind speed x solar radiation

$$\frac{0.2516 - 0.4792 - 1.7915 + 0.4488}{\frac{1}{5} + \frac{1}{5} + \frac{1}{4} + \frac{1}{5}} + \frac{0.6062 - 1.0033 - 2.0200 + 1.3407}{\frac{1}{4} + \frac{1}{6} + \frac{1}{6} + \frac{1}{4}}$$

$$= \frac{-1.5703}{0.8500} - \frac{1.0764}{0.8334} = -3.1390 \text{ (negative)} \quad \text{, and}$$

$$\frac{1}{0.8500} + \frac{1}{0.8334} = 2.3764 \quad .$$

The mean square is

$$(-3.1390)^2/2.3764 = 4.1463.$$

(c) Temperature x solar radiation

$$\frac{2.0200 - 1.3407 - 1.7915 + 0.448}{\frac{1}{6} + \frac{1}{4} + \frac{1}{4} + \frac{1}{5}} + \frac{0.6062 - 1.0033 - 0.2516 + 0.4792}{\frac{1}{4} + \frac{1}{6} + \frac{1}{5} + \frac{1}{5}}$$

$$= \frac{-0.6634}{0.867} - \frac{0.1695}{0.817} = -0.9727 \text{ (negative)} \quad \text{, and}$$

The mean square is

$$(-.9727)^2/2.3774 = 0.3980 \quad .$$

(3) Three-factor interaction. A 3-factor interaction is difficult to interpret because it is the result of a change in the interaction of factors A and B from one level of factor C to another. That is, it estimates

$$\tfrac{1}{8}\,(HHH - HHL - HLH + HLL - LHH + LHL + LLH - LLL)$$

$$= \tfrac{1}{8}\left[\tfrac{1}{2}\,(HHH - HLH - LHH + LLH) - \tfrac{1}{2}(HHL - HLL - LHL + LLL)\right]$$

$$= \tfrac{1}{8}\Big[(A \times B \text{ interaction at upper level of } C)$$

$$-(A \times B \text{ interaction at lower level of } C)\Big] \quad .$$

Wind speed x temperature x solar radiation

$$\frac{0.6062 - 1.0033 - 0.2516 + 0.4792 - 2.0200 + 1.3407 + 1.7915 - 0.4488}{\frac{1}{4} + \frac{1}{6} + \frac{1}{5} + \frac{1}{5} + \frac{1}{6} + \frac{1}{4} + \frac{1}{4} + \frac{1}{5}}$$

$$= \frac{0.4939}{1.6834} = 0.2934 \text{ (positive)} \quad .$$

The mean square is

$$(0.4939)^2/1.6834 = 0.1449 \quad .$$

This completes the computations of the analysis of Table 2.

(U) The analysis of Table 3 is similar, substituting relative humidity for temperature. This is the commonest type of analysis of 3-factor classifications; each factor is at two levels and all eight cells are present.

(U) The first of the three tables (Table 1) is special because, besides having unequal observations per cell, one of the cells is missing. This analysis will be described in some detail.

(U) First, a simple analysis of variance is performed (Table 5), dividing the 38 degrees of freedom into those "among cells" (6 degrees of freedom) and those within cells (32 degrees of freedom). Note that the total sum of squares is the same for all three analyses.

TABLE 3. Analysis of Variance by Wind Speed, Temperature, and Relative Humidity (UNCLASSIFIED)

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Among cells	6	13.837225	
Within cells	32	15.810085	0.494065
Total	38	29.647310	

(C) The next step is to subdivide (non-orthogonally) the 6 degrees of freedom among cells into three main effects and three 2-factor interactions. The fact that a cell is missing means that the 3-factor interaction cannot be assessed, as has been stated. The meaning of main effects and 2-factor interactions is the same as previously discussed.

(1) Main effects

(a) Wind speed

$$\frac{0.3654 - 1.2770}{\frac{1}{10} + \frac{1}{5}} + \frac{0.3443 - 0.5190}{\frac{1}{4} + \frac{1}{1}} + \frac{1.1780 - 1.8848}{\frac{1}{6} + \frac{1}{9}}$$

$$= -\frac{0.9116}{0.300} - \frac{0.1747}{1.250} - \frac{0.7068}{0.278} = -5.7209 \quad \text{, and}$$

$$\frac{1}{0.300} + \frac{1}{1.250} + \frac{1}{0.278} = 7.7304 \quad .$$

The estimate of effect of wind speed is

$$-\frac{5.7209}{7.7304} = -0.7401 \quad ,$$

the standard error of effect is

$$\frac{\sqrt{0.494065}}{\sqrt{7.7304}} = 0.2528 \quad \text{, and}$$

the mean square is

$$(0.7401)(5.7209) = 4.2340 \quad .$$

$$\frac{1.8848 - 0.7562}{\frac{1}{9} + \frac{1}{4}} + \frac{0.5190 - 1.2770}{\frac{1}{1} + \frac{1}{5}} + \frac{0.3443 - 0.3654}{\frac{1}{4} + \frac{1}{10}}$$

$$= \frac{1.1286}{0.361} - \frac{0.7580}{1.200} - \frac{0.0211}{0.350} = 2.4343 \quad .$$

The estimate of effect of temperature is

$$\frac{2.4343}{6.4605} = 0.3768 \quad ,$$

the standard error of effect is

$$\frac{\sqrt{0.494065}}{\sqrt{6.4605}} = 0.2765 \quad , \text{ and}$$

the mean square is

$$(0.3768)(2.4343) = 0.9172 \quad .$$

(c) Relative humidity

$$\frac{1.2770 - 0.7562}{\frac{1}{5} + \frac{1}{4}} + \frac{0.5190 - 1.8848}{\frac{1}{1} + \frac{1}{9}} + \frac{0.3443 - 1.1780}{\frac{1}{4} + \frac{1}{6}}$$

$$= \frac{0.5208}{0.450} - \frac{1.3658}{1.111} - \frac{0.8337}{0.417} = -2.0713 \quad , \text{ and}$$

$$\frac{1}{0.450} + \frac{1}{1.111} + \frac{1}{0.417} = 5.5204 \quad .$$

The estimate of effect of relative humidity is

$$\frac{-2.0713}{5.5204} = -0.3752 \quad ,$$

the standard error of effect is

$$\frac{\sqrt{0.494065}}{\sqrt{5.5204}} = 0.2992 \quad , \text{ and}$$

the mean square is

$$(0.3752)(2.0713) = 0.7772 \quad .$$

(a) Wind speed x temperature

$$0.3443 - 0.3654 - 0.5190 + 1.2770 = 0.7369 \text{ (positive)} \quad ,$$

$$\frac{1}{4} + \frac{1}{10} + \frac{1}{1} + \frac{1}{5} = 1.550 \quad \text{, and}$$

the mean square is

$$\frac{(0.7369)^2}{1.550} = 0.3503 \quad .$$

The fact that no estimate of this interaction is available from low relative humidity trials should be noted.

(b) Wind speed x relative humidity

$$0.3443 - 1.1780 - 0.5190 + 1.8848 = 0.5321 \text{ (positive)} \quad ,$$

$$\frac{1}{4} + \frac{1}{6} + \frac{1}{1} + \frac{1}{9} = 1.528 \quad \text{, and}$$

the mean square is

$$(0.5321)^2/1.528 = 0.1853 \quad .$$

(c) Temperature x relative humidity

$$0.5190 - 1.8848 - 1.2770 + 0.7562 = -1.8866 \text{ (negative)} \quad ,$$

$$\frac{1}{1} + \frac{1}{9} + \frac{1}{5} + \frac{1}{4} = 1.561 \quad \text{, and}$$

the mean square is

$$(1.8866)^2/1.561 = 2.2801 \quad .$$

The results of the three analyses are summarized in Tables 6, 7, and 8.

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT	STANDARD ERROR OF EFFECT
Wind speed, W	1	4.2340	8.569	<5	-0.7401	0.2528
Temperature, T	1	0.9172	1.856	<20	0.3768	0.2765
Humidity, H	1	0.7772	1.573	NS*	-0.3752	0.2992
W x T	1	0.3503	<1	NS	positive	
W x H	1	0.1853	<1	NS	positive	
T x H	1	2.2801	4.615	<5	negative	
Error	32	0.4941		-		

*Not significant.

TABLE 7: Summary of the Results of the Analysis by Wind Speed, Temperature, and Solar Radiation (CONFIDENTIAL)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT	STANDARD ERROR OF EFFECT
Wind speed, W	1	5.9816	13.115	<1	-0.7927	0.2189
Temperature, T	1	2.3543	5.162	<5	0.4961	0.2183
Solar radiation, V	1	1.0041	2.201	<20	0.3248	0.2189
W x T	1	0.0391	<1	NS	negative	
W x V	1	4.1463	9.091	<1	negative	
T x V	1	0.3980	<1	NS	negative	
W x T x V	1	0.1449	<1	NS	positive	
Error	31	0.4561		-		

*Not significant.

Humidity, and Solar Radiation (CONFIDENTIAL)

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F-VALUE	APPROXIMATE PROBABILITY (%)	ESTIMATE OF EFFECT	STANDARD ERROR OF EFFECT
Wind speed, W	1	2.4202	5.801	<5	-0.5448	0.2262
Relative humidity, H	1	2.5865	6.200	<5	-0.5616	0.2256
Solar radiation, V	1	1.5764	3.779	<10	0.4061	0.2089
W x H	1	0.2288	<1	NS*	negative	
W x V	1	5.1580	12.364	<1	negative	
H x V	1	1.0133	2.429	<10	positive	
W x H x V	1	0.1435	<1	NS	negative	
Error	31	0.4172		-		

*Not significant.

ACCUMULATION OF BITES VERSUS TIME (WHITE'S METHOD)

(C) Suppose, in the i-th trial, n_i mosquitoes are released and x_{i1} x_{i2} ...x_{im} accumulated total mosquito bites are observed by the 10 test subjects in the human circle at times t_{i1} t_{i2} ...t_{im}. Under the assumptions that the successive differences of these x_{ij}'s ($d_{i2} = x_{i1}$, $d_{i2} = x_{i2} - x_{i1}$, . . . $d_{im} = x_{im} - x_{im-1}$) are statistically independent and Poisson distributed, the probability frequency of d_{ij} is

$$\frac{\left[n_i\ (\theta_{ij} - \theta_{ij-1})\right]^{d_{ij}} \exp\left[-n_i\ (\theta_{ij} - \theta_{ij-1})\right]}{d_{ij}\ !} \quad .$$

where θ_{ij} is a function of t_{ij}. The expectation of x_{ij} is $n_i\ \theta_{ij}$ and the plot of x_{ij} is $n_i\theta_{ij}$; the plot of x_{ij} versus t_{ij} gives some knowledge of the relationship between θ and t.

(U) After detailed consideration of the data it was found that the model

$$\theta_{ij} = \frac{\gamma_i}{2}\left[1 + \sin\ (\alpha_i + \beta_i \log t_{ij})\right] \quad ,$$

where α_i, β_i, and γ_i are parameters to be estimated, would reasonably fit.

$$x_{ij} = \frac{n_i \gamma_i}{2} \left[1 + \sin (\alpha_i + \beta_i \log t_{ij}) \right]$$

or,

$$y_{ij} = \alpha_i + \beta_i \log t_{ij} \quad .$$

where

$$y_{ij} = \arcsin \left(\frac{2x_{ij}}{n_i \gamma_i} - 1 \right) \quad .$$

(U) Estimates of $\hat{\alpha}_i$ and $\hat{\beta}_i$ may be such that $\hat{\alpha}_i + \hat{\beta}_i \log t_{ij}$ is less than $-\frac{\pi}{2}$ or greater than $+\frac{\pi}{2}$. Therefore, the model was modified so that, apart from error, when $\hat{\alpha}_i + \hat{\beta}_i \log t_{ij}$ was less than $-\frac{\pi}{2}$, then x_{ij} was defined as zero, and when $\hat{\alpha}_i + \hat{\beta}_i \log t_{ij}$ was greater than $\frac{\pi}{2}$, then x_{ij} was defined as equal to $n_i \gamma_i$.

(C) For positive values of β_i, x_{ij} attains its maximum value, $n_i \gamma_i$, when $\hat{\alpha}_i + \hat{\beta}_i \log t_{ij} = +\frac{\pi}{2}$.
Hence, in the i-th trial, γ_i is the maximum number of bites per mosquito (if the trial were extended long enough) and t_{ij}^{**}, where

$$\log t_{ij}^{**} = \frac{+\frac{\pi}{2} - \alpha_i}{\beta_i}$$

is the time it would take to achieve this number of bites. Also, x_{ij} has its minimum value, 0, when

$$\hat{\alpha}_i + \hat{\beta}_i \log t_{ij} = -\frac{\pi}{2} \quad .$$

Hence, t_{ij}^{*}, where

$$\log t_{ij}^{*} = \frac{-\frac{\pi}{2} - \alpha_i}{\beta_i}$$

is the time taken after release for the biting process to begin.

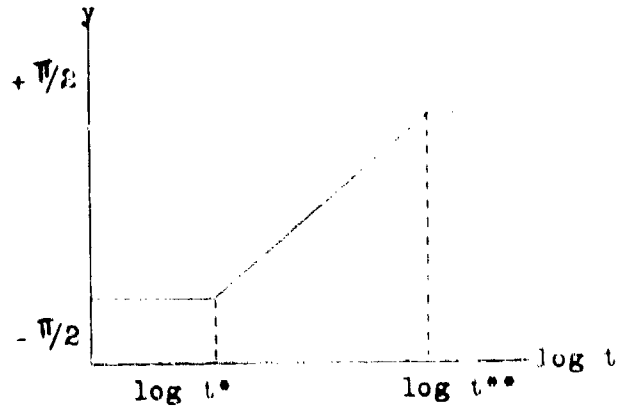

Fig. 1.- Plot of y_{ij} versus log t_{ij}.

(C) A point to observe in the above model is that if γ_i is guessed and if the proportion (p) of total bites observed in the i-th trial by the j-th time interval ($p_{ij} = \frac{x_{ij}}{n_i \gamma_i}$) is transformed so that y_{ij} = arsin ($2p_{ij} - 1$), then a plot of y_{ij} versus log t_{ij} should be approximately linear. If this plot is not linear but essentially concave upward, the value of γ_i should be increased. Similarly, if the plot is concave downward, γ_i should be decreased. By these means, a good initial guess of γ_i may be obtained. Thereafter, the process of estimation essentially consists of the iterative fitting of straight lines by the method of minimum chi-square and obtaining the residual chi-square values. These values are then plotted against guesses of γ_i until the minimizing γ is found.

AD_____ACCESSION_____

BW Branch, TD&A, Technical Operations, U.S. Army Chemical Corps Proving Ground, Dugway, Utah

Short Title: DPGR 259--PROJECT BELLWETHER-I

Technical Report DPGR 259--illus--tables--69 pp with 8 Unclassified abstract cards--SECRET--December 1960

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This volume reports on the results of the first of the current entomological trials conducted by Dugway Proving Ground. A total of 52 field trials was conducted to ascertain the effects on insect behavior of various meteorological parameters. These effects, their analysis, and other appertaining data are reported.

AD_____ACCESSION_____

BW Branch, TD&A, Technical Operations, US Army Chemical Corps Proving Ground, Dugway, Utah

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SECRET

DEPARTMENT OF THE ARMY
U. S. ARMY DUGWAY PROVING GROUND
DUGWAY, UTAH 84022-5000

REPLY TO
ATTENTION OF

STEDP-JA

1 February 1999

MEMORANDUM FOR Defense Technical Information Center, ATTN: DTIC-BCP, 8725 John J. Kingman Road, Suite 944, Ft. Belvoir, VA 22060-6218

SUBJECT: Removal of Distribution Markings

1. We recently had a request for a document entitled, *"Outdoor Mosquito Biting Activity Studies,"* ACCN AD 596046L, under the Freedom of Information Act (FOIA). While the document was unclassified, it still contained "limited distribution" markings.

2. The document has been reviewed by our Intelligence Office and a determination made that it can now be released to the general public.

3. Please remove the limited distribution markings from this document. The first two pages are enclosed for your information.

4. Please contact Ms. Teresa Shinton, FOIA Manager, or the undersigned if there are questions. Our telephone number is (435) 831-3716.

Encl
as

F. GIL BRUNSON
LTC, JA
Command Judge Advocate