RESULTS AND DISCUSSION. The results of the tests are presented in Table 1. A comparison of the LC-50's and LC-90's obtained with the various materials and species indicated that parathion was the most toxic insecticide, followed by lindane, DDT, and malathion, respectively. Bayer 29493 (*o,o*-dimethyl *o*-[4-(methylthio)-*m*-tolyl] phosphorothioate) was tested against *Culex tarsalis* and *Culiseta inornata* only, the results were comparable with those obtained with parathion.

Larvae of *Culiseta inornata* were the most difficult to kill, followed by those of *Culex erythrothorax, C. tarsalis,* and the *Aedes* spp., respectively. *Aedes nigromaculis* was slightly more sensitive to all of the toxicants than *A. dorsalis* and *A. melanimon.* There were no clear-cut differences in susceptibility indicated between these last two species.

Differences in laboratory techniques, laboratory temperatures, and sources of larvae (whether obtained as eggs, first, or fourth instar) probably vitiate any valid comparison with results obtained fron untreated areas, such as those in Californi in the early and mid-1950's. It is, howeve interesting to note that the results obtaine in Nevada generally fall within or belo the ranges reported by various writers fo the same materials from uncontrolled area in California. As an example, the follow ing LC-50 ranges were reported for *Cule tarsalis* larvae by Gjullin and Peters (1952 and Gjullin and Isaak (1957); DD 0.0093–0.0154 p.p.m.; lindane, 0.0118–0.0 p.p.m.; malathion, 0.02–0.035 p.p.m.; an parathion, 0.0032–0.0052 p.p.m. It can b concluded from this study that resistan to insecticides appears to be no immedia problem in the mosquitoes of Nevada.

Literature Cited

GJULLIN, C. M., and ISAAK, L. W. 1957. Pr ent status of mosquito resistance to insecticid in the San Joaquin Valley in California. Mo quito News 17(2):67–70.

GJULLIN, C. M., and PETERS, R. F. 195 Recent studies of mosquito resistance to insect cides in California. Mosquito News 12(1):1–

TRAPPING OVERWINTERING ADULTS OF THE MOSQUITOE *CULEX TARSALIS* AND *ANOPHELES FREEBORNI*

ROBERT F. HARWOOD [1]

INTRODUCTION. There continues to be considerable interest in finding natural overwintering sites of *Culex tarsalis* Coq. This is desirable in order to determine definitely whether the viruses of western equine and St. Louis encephalitis survive through the winter in this mosquito. As *C. tarsalis* is involved in summer maintenance and transmission of these viruses, it is also desirable to kno when the mosquito emerges from wint diapause, and how large a populati has successfully overwintered. The fin ings related here are primarily direct towards these latter problems. Observ tions on *Anopheles freeborni* Aitken a included since this mosquito was fr quently encountered in overwinteri quarters.

STUDY AREA AND METHODS. The i vestigation took place in the U. S. Fi and Wildlife Service's Columbia Wildl Refuge near Othello, Washington. T refuge lies approximately twenty mil

[1] Associate Entomologist, Washington State University. This study was supported by a grant (E–2253) from the National Institutes of Health, U. S. Public Health Service. Washington Agricultural Experiment Stations Scientific Paper 2169, conducted under Project 1434.

southeast of the overwintering area studied by Rush *et al.* (1958), is topographically and climatically similar to their area, and has been previously described in a study of summer resting sites (Harwood and Halfhill 1960). The landscape is generously endowed with basalt outcroppings, with talus slopes of some depth at the base of cliffs. Mammalian burrows, especially those of the yellow-bellied marmot, *Marmota flaviventris avara* Bangs, are commonplace in many localities.

Overwintering mosquitoes were found by using hibernation traps. These were placed over suspect sites, while the weather was generally below freezing, in February of 1959, 1960, and 1961. Traps placed over burrows were of the type developed for that purpose (Harwood and Halfhill 1960). Traps used for other suspect locations were modifications whereby the same type of trap chamber was placed on a tripod with 1½ foot legs, and was surrounded by a black polyethylene tarpaulin. Such tarpaulin traps were placed on talus slopes (Fig. 1), or over other suspect areas such as brush piles. The edges of each tarpaulin were held down tightly by rocks or earth.

FIG. 1.—Black polyethylene tarpaulin, with coffee can trap chamber, placed on talus slope. Columbia Wildlife Refuge, Washington.

In theory, mosquitoes emerging under the areas covered by a trap fly up and towards light, thereby entering the trap chamber. It is obvious that those emerging near the outer margins of tarpaulins held down with rocks might discern more light and escape towards the open air. With traps of standardized dimensions one might assume this escape factor to be constant.

During the first winter of study it was found that the tarpaulin type of trap was prone to catch emerging spiders. These often webbed across the throat of the chamber, and decimated the catch if they entered the trap chamber. This problem was practically eliminated by generously coating the tripod legs and under surfaces of the tripod base with motor oil. It was also found that the standard 4 mil black polyethylene film would tear to a limited extent when buffeted against rocks by strong gusts of wind characteristic of springtime in the study area. In the final winter of study, 6 mil film was used; it only rarely wore through, and never tore extensively.

RESULTS. *1959*—Six tarpaulin traps 10 x 10 feet in size were set out over rock outcroppings bearing fissures. These outcroppings were shallow, unlike talus slopes at the base of cliffs, and were not as prevalent as talus slopes. One trap yielded a single female of *C. tarsalis,* and another yielded two females of *A. freeborni*.

Sixteen traps were placed over mammalian burrows. A single female of *C. tarsalis* was obtained from a burrow located at the base of a basalt cliff. Three burrows in soil and at the base of cliffs yielded a total of seven female *A. freeborni*.

1960—Eight tarpaulin traps (8 x 10½ feet) were set out on talus slopes, and two (10 x 10 feet) traps were placed over small brush piles. Fifty-four traps were placed over mammalian burrows, including some dug directly into earth, some at the base of cliffs, and some dug between piles of loose rock. The trap chambers were emptied and replaced March 25, while the weather was still quite cool, and re-examined May 6 when the temperature no longer fell below freezing.

Four tarpaulin traps were located at various heights on a W-facing talus slope, thereby subject to warming by the afternoon sun. All traps yielded mosquitoes on the first examination. The traps at the top and bottom of the slope each contained 2 ♀ *C. tarsalis,* that at the bottom of the slope also had 2 ♀ *A. freeborni.* The two traps located near the middle of the slope yielded 11 ♀ *C. tarsalis* plus 1 ♀ *A. freeborni,* and 13 ♀ *C. tarsalis* plus 3 ♀ *A. freeborni.* The lowest trap yielded 3 additional ♀ *A. freeborni* on the second examination.

In another area two tarpaulin traps were placed on a S-facing slope, and two on a NNE-facing slope. One trap on each slope yielded mosquitoes, the S-facing one having 1 ♀ *C. tarsalis* at first examination, and the NNE-facing one having 1 ♀ *C. tarsalis* and 2 ♀ *A. freeborni* at second examination.

The two brush pile locations yielded no mosquitoes, though large numbers of a great variety of other insects were trapped. Spiders were very much in evidence in the brush piles.

None of the fifty-four burrows trapped yielded any *C. tarsalis.* Eight of the burrows contained *A. freeborni.* Results of burrow trapping are listed in Table 1. Burrows are categorized by principal occupant, and by topographic characteristics (dug into bare earth, at base of cliffs, or into earth within rock piles). Marmot burrows at cliff bases and in rock piles were definitely most productive for *A. freeborni.* Burrows of woodrats were situated in similar locations, but are considerably smaller than marmot burrows and proved unproductive. A single male *A. freeborni* was taken at the first date of examination, and a sizable proportion of the total catch did not emerge until after March 25.

1961—As previous results indicated animal burrows did not harbor significant numbers of *C. tarsalis,* and brush piles did not appear productive, only tarpaulin tests on talus slopes were conducted. Two main types of information were sought; (1) the effect of slope exposure on numbers of mosquitoes overwintering and time of emergence, and (2) the relationship of emergence to rising temperatures. Unfortunately, talus slopes which essentially faced the four major compass points could not be obtained in one limited location. All traps were standardized at 5 x 8 feet. One group of eight traps was placed at varying heights on the same W-facing slope tested the previous winter, and eight traps were similarly placed on each of three slopes which faced S, NNE, and ENE, some three miles away. The trap chambers were emptied and replaced at approximately weekly intervals, starting March 11, when possible, but the tarpaulins were left undisturbed. No col-

TABLE 1.—*Anopheles freeborni* females overwintering in animal burrows, Columbia Wildlife Refuge, Washington, 1960

	Earth		Cliff base		Rocks	
	Mar. 25	May 6	Mar. 25	May 6	Mar. 25	May 6
Marmot	13–4–1*	13–3–0	6–0–9†	6–3–1	10–5–13	10–4–11
Woodrat	..	..	4–0–0	4–0–0	12–3–0	12–2–0
Badger	7–3–1	7–4–0	..	..	..	..
Rabbit	2–0–0	2–0–0	..	..	..	..

* First number refers to number of burrows in this category, second to number of burrows pushed aside or dug around by burrow inhabitant, third to total number of mosquites collected.

† One male.

lection was made one week after March 18 because of rain and wind storms. Traps were not examined the week after April 14 because no mosquitoes were found on that date. The last collection occurred on April 29.

A total of 35 ♀ and 1 ♂ *C. tarsalis,* and two ♀ and one ♂ *A. freeborni* was captured. The numbers of female *tarsalis* at each collection and dates of collection are indicated in Figure 2. Official U. S. Weather Bureau maximum and minimum air temperatures at Othello, Washington, seven to ten miles from the study area, are included in this figure in order to relate mosquito emergence to environmental temperatures. Mean minimum daily soil temperatures for four nearby locations at the six-inch level were also obtained from April 12 through May 13. This reading is not included in Figure 2; however, minimum daily temperature at the six-inch level fluctuated markedly between 47 and 58 degrees Fahrenheit.

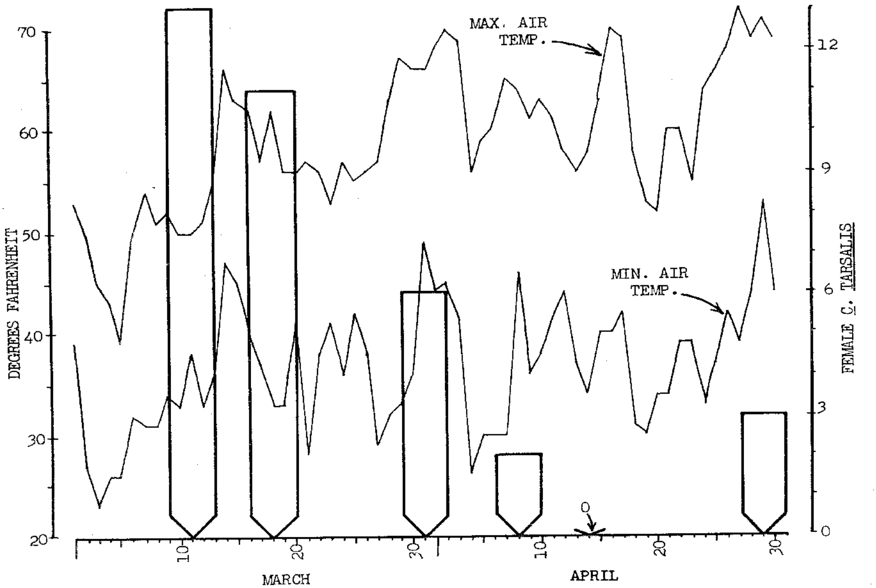

FIG. 2.—Emergence of *C. tarsalis* from talus slopes, 1961. Eight traps, 5 x 8 ft., placed on each of four slopes essentially facing major compass points. Height of each shield-shaped figure indicates number of mosquitoes captured, the bottom of the figure relating to the date traps were examined.

DISCUSSION AND CONCLUSIONS. Rush *et al.* (1958) reviewed the literature to show that *Culex tarsalis* might overwinter in earth cellars, animal burrows, and caves rather than man-made shelters. In their investigation they found considerable numbers some distance under the surface of talus slopes and rock piles. Results described here indicate that burrows, suspected by previous investigators as important, are not of much significance as overwintering sites of *C. tarsalis* where layers of rock are available. Bennington, Blackmore and Sooter (1958) believe that spring emergence of *C. tarsalis* coincides with a sudden inversion in soil temperature, resulting in the simultaneous appearance of large numbers of adult mosquitoes. While there may be an initial high incidence of emergence, the data summarized in Figure 2 indicate that emergence in the spring actually extends over a considerable period. The highest emergence recorded for March 11 might be a true peak, but could consist of a cumulative total from some time previous to that date.

It seems likely that emergence is affected by temperature of the air at or near the soil surface. No catch was obtained on April 14 (Figure 2) during a general lowering trend in air temperature, but additional adults were found on April 29 when air temperatures were steadily on the upswing. This activity of *C. tarsalis* in the spring in response to fluctuating temperatures is similar to fluctuating distribution in hibernation sites noted in the fall for this same mosquito by Trent (1960).

Anopheles freeborni shows some distinct differences from *C. tarsalis* in successful use of hibernation sites. While *C. tarsalis* was noted on only one occassion to overwinter in burrows, this frequently occurred with *A. freeborni*. *A. freeborni* also used talus slopes to a limited extent, but despite its relative abundance in the summer of 1960, it was not found in hibernation traps in as great numbers as *C. tarsalis*.

The direction in which talus slopes face has some effect on time of spring emergence. In 1959 and 1960, peak emergence in N-facing, and therefore colder, slopes was somewhat later than emergence from other slopes. Snow lingered longer on such slopes, providing visible evidence of colder conditions.

It is generally thought that males of *Culex* and *Anopheles* do not survive winter conditions, but this study indicates they may survive most of the winter if not into spring. Very likely the finding of males is a result of sampling environmental situations differing from those of previous investigators. It is of particular interest that the male of each species obtained in tarpaulin traps in 1961 was from the N-facing slope which would be colder and more stable, conserving energy reserves in hibernators.

The use of tarpaulin traps has certain advantages in studying the overwintering of mosquitoes under natural conditions. Such traps could be used to obtain overwintering mosquitoes to test for virus if they were frequently observed, since survival time of trapped mosquitoes is short. Estimates can be made of overwintering populations. Assuming capture of all hibernators under tarpaulin traps, the 35 female *C. tarsalis* from 32 traps (1,280 square feet) used in the winter of 1960–61 indicates an average of 1,190 females of this mosquito per acre of talus slope in the area studied. The eight traps (320 sq. ft.) situated on the W-facing slope yielded 18 female *C. tarsalis,* an average of 2,450 per acre for that locality.

There are possible drawbacks to the literal interpretation of catches with black polyethylene tarpaulin traps. The black cover might absorb sunlight and warm up hibernating mosquitoes sooner than normally takes place. However, on talus slopes an imperfect seal permits ready exchange of air under the tarpaulins. Possibly mosquitoes will not show completely normal reaction when most of the light is excluded; activity would thus be affected.

Summary. Natural mosquito hibernation sites near Othello, Washington were studied. Traps were placed over mammalian burrows, and tarpaulin traps sampled other sites. *C. tarsalis* used talus slopes almost exclusively, and *A. freeborni* was found in such slopes and in burrows. More *A. freeborni* were found in marmot burrows at cliff bases or among rocks, than in bare earth. Mosquitoes emerged over an extended period, responding to fluctuating air and surface temperatures. In two winters of observation, 2 male *A. freeborni* and 1 male *C. tarsalis* survived at least into late winter. It is suggested that tarpaulin traps can be used to assess populations of *C. tarsalis* success-

ılly overwintering, and to determine me of spring emergence.

ACKNOWLEDGMENT. Particular recogni-on is due Mr. Paul Steel, then manager f the Columbia Wildlife Refuge, U. S. .W.S., for servicing the tarpaulin trap ıambers on several occasions during the ›ring of 1961. The U. S. Bureau of eclamation office in Othello, Washington rovided official U. S. Weather Bureau ecords of maximum and minimum air mperature, and minimum daily soil mperatures at the six-inch level were ›tained from the county agent's office Othello, Washington.

References Cited

BENNINGTON, E. E., BLACKMORE, T. S., and SOOTER, C. A. 1958. Soil temperature and the emergence of *Culex tarsalis* from hibernation. Mosq. News 18:297–8.

HARWOOD, R. F., and HALFHILL, J. E. 1960. Mammalian burrows and vegetation as summer resting sites of the mosquitoes *Culex tarsalis* and *Anopheles freeborni*. Mosq. News 20:174–8.

RUSH, W. A., BRENNAN, J. M., and EKLUND, C. M. 1958. A natural hibernation site of the mosquito *Culex tarsalis* Coquillett in the Columbia River Basin, Washington. Mosq. News 18:288–93.

TRENT, D. W. 1960. Observations on the hibernation of *Culex tarsalis* Coquillett in Utah Valley, Utah. Master's thesis, Department of Zoology and Entomology, Brigham Young University.

COLONIZATION OF SIX SPECIES OF MOSQUITOES IN JAPAN [1]

A. BURNS WEATHERSBY, CDR, MSC, USN

Division of Parasitology, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland

Laboratory colonies of mosquitoes are value to military Preventive Medicine nits for training personnel within the nit, ships or stations, and for determin-g insecticide resistance and relations to sease. To meet these needs colonization several species of mosquitoes was at-mpted at U. S. Navy Preventive Medi-ne Unit No. 8 at Yokosuka, Japan, in ugust 1955. Two species, *Armigeres Armigeres) subalbatus* (Coq.) and *nopheles (Anopheles) sinensis* Wied, ıve not been reported in laboratory lonization; *Culex (Culex) tritaenio-ynchus* Giles and *Aedes (Finlaya) togoi* Theo.) recently have been reported in ccessful colonization (Newsom, *et al.*, 56; Lien, 1959); and *Aedes (Stegomyia) albopictus* (Skuse) and *Culex (Culex) pipiens* Linn. have been in colonization for many years but *Culex (Culex) pipiens* var. *pallens* Coq. has not been reported in colonization. Species previously colonized are mentioned here because of the ease with which they adapted to laboratory conditions.

MATERIALS AND METHODS. Larvae of these mosquitoes were collected from various habitats on the Miura Peninsula. They were reared in the laboratory by well-established techniques (Trembley, 1955). The *Armigeres* and *Aedes* were reared in mouse jars and fed Purina guinea pig and dog chow and aeration of the cultures kept them free of surface scum. The *Anopheles* and *Culex* were reared in white enamel photographic pans. The *Anopheles* fed on finely ground Purina guinea pig chow and the *Culex* fed on the pellets. The adults were maintained initially in 14" x 18" screened cylin-

[1] The opinions and assertions contained herein the private ones of the writer and are not be construed as official or reflecting the views the Navy Department or the Naval Service at ge.