

OVERWINTERING *CULEX PIFIENS PIFIENS* IN THE OHIO-MISSISSIPPI RIVER BASIN 1962-1967^{1, 2}

JACK HAYES³

ABSTRACT. Attempts were made to isolate SLE Virus from *C. pipiens* collected in the Ohio-Mississippi River Basin each winter from 1962 through 1967. No virus was isolated. The various species of hibernating mosquitoes are described in numerous sites. The duration and

breaking of hibernation in *pipiens* is documented. The occurrence of gonotrophic dissociation in a natural hibernating site was demonstrated as well as the ability of *pipiens* to hibernate successfully without blood.

INTRODUCTION. In 1962 The Center for Zoonoses Research initiated studies on hibernating mosquitoes in an effort to determine if St. Louis encephalitis (SLE) was overwintering in *Culex pipiens pipiens*.

The main objective of the overwintering studies was to isolate SLE virus. In addition it was hoped to learn which species of mosquitoes hibernated in the Ohio-Mississippi River Basin, the nature of the hibernating sites, and the duration of hibernation. The work of Eldridge (1968) prompted field experiments to determine if gonotrophic dissociation⁴ was demonstrable in natural hibernating sites and to learn if females could hibernate successfully without having had a blood meal.

Hibernating sites were located throughout the Ohio-Mississippi River Basin in both natural and man-made situations. Man-made sites occurred in many metropolitan areas. Natural sites included limestone caves located along the Mississippi River, caves and sink holes along the Ohio River, and sandstone escarpments in the Shawnee National Forest.

METHODS AND MATERIALS. Mosquitoes were collected from hibernating sites⁵ by either an oral or hand operated battery powered mechanical aspirator. All mosquitoes were placed in a dry ice chest and frozen for future species determination. Specimens were maintained throughout the identification process on a cold table (a modified freezing compartment from an ordinary household refrigerator). Processing of mosquitoes for virus isolation was done by the procedure described by Kokernot *et al.* (1969).

For experimental studies on gonotrophic dissociation, progeny from *pipiens* taken from known hibernating sites were utilized. This was done to eliminate any possibility of using *quinquefasciatus* or *molestus*. Terminalia of reared progeny were mounted and measured and sample female progeny were tested for autogeny; all specimens were typical *pipiens* (Barr, 1957). Females collected the second week of February from known hibernating sites in McLeansboro were taken into the laboratory and none of these laid eggs; they had to take blood in order to produce eggs. All progeny obtained for Experiments I and II were obtained in this manner. There was no special treatment of the progeny; they were reared at room temperature with no special lighting.

RESULTS. No SLE virus was isolated during the study period from any hiber-

¹ This investigation was supported in part by U.S. Public Health Service Research Grant CC00037, from the National Communicable Disease Center, Atlanta, Georgia.

² This research was conducted at The University of Illinois Dixon Springs Agricultural Center.

³ Asst. Professor, School of Medicine, Texas Tech University, Lubbock, Texas 79409.

⁴ Gonotrophic dissociation is the taking of a blood meal and not developing eggs, as opposed to gonotrophic concordance, the development of eggs after a blood meal.

⁵ A hibernating site is any place that can protect the mosquito from prolonged freezing temperature.

nating mosquito. The lowest temperature recorded at which mosquitoes successfully survived was 48° F. This particular site was a 50' deep escarpment. Hibernating sites in metropolitan areas include outdoor toilets, basements, outbuilding foundations, and some storm sewers. Natural sites include caves, escarpments, hollow trees, sink holes and animal burrows. The most unusual collection sites were auger holes—holes three feet in diameter bored up 60' in length into coal seams in strip mines (Fig. 1). Table I illustrates

the number of species collected from different hibernating sites in the Ohio-Mississippi River Basin during 1965.

Culex pipiens remains in hibernation until suitable environmental conditions exist that permit exodus from the hibernating site. In the fall, females frequently move on warm nights after hibernation is initiated. Following the 1964 SLE outbreak in McLeansboro adult resting collections were made from November to May. In late November of 1964 every female possible was collected



FIG. 1.—Auger hole collecting. 60' to the rear of the auger hole thousands of *Culex pipiens* mosquitoes survived the winter. Note: Cave-in of roof makes collection hazardous.

TABLE 1.—Species distribution in various overwintering habitats collecting October 1964–March 1965

	Wells cisterns	Rock shelters, escarpments	Fults cave*	Auger holes	Outbuildings basement foundation	Storm sewers**
<i>C. pipiens</i> complex	2684	250	2844	4338	753	2961
<i>Culex erraticus</i>	60	1072	20	15	0	0
<i>Culex territans</i>	0	1	0	0	0	2
<i>Anopheles quadrimaculatus</i>	2	0	0	2	0	11
<i>Anopheles punctipennis</i>	3	233	2	69	7	40
<i>Uranotaenia sapphirina</i>	0	1	0	0	0	0

* Fults Cave is a large cave in contrast to small rock shelters, escarpments. Fults Cave: Opening about 30' across and 15' high, leading back to 2 crawl ways communicating with a larger interior room

** These collections were from storm sewers in the McLeansboro, Illinois area.

from Site 49. The next week, after a few days of warm weather this site was "reinfested" by numerous females—evidence of movement from other sites. This observation was made several times in similar situations.

Movement from hibernating sites can result in the death of many individuals by the return of cold weather and the mosquitoes being trapped in unsuitable locations.

The breaking of hibernation was best documented in 1967. Winter temperatures showed an unseasonable warm spell from January 21 to 25, 1967. Thousands of *pipiens* mosquitoes, observed in Fults Cave in December 1966, were almost completely absent when the cave was checked again the first week in February 1967. Similarly, temporary hibernating shelters in the shallow escarpments in Pounds Hollow (Hardin County, Illinois) which were void of any living mosquitoes by December 31, 1966 were again "replenished" with *pipiens* when visited the first week of February 1967 after the "warm spell." In previous years mosquitoes had been observed in hibernating sites throughout March and well into April.

Postulation as to the overwintering maintenance of SLE virus in the Ohio-Mississippi River Basin led to the collection of hibernating *pipiens* for attempted virus isolation. In 1963, over 15,000 *pipiens* were processed and no virus was isolated. The inability to utilize accumu-

lated fat makes the survival of *quinque fasciatus* impossible during the typical winters of the Ohio-Mississippi River Basin area (Tekle 1960). The autumnal reduction of blood feeding and increased sugar feeding leads one to believe there would be little likelihood of virus surviving the winter in *pipiens*. Substantiating this is the absence of any gravid females collected in hibernation. Several hundred females were taken from hibernation, dissected and all were nulliparous. However, the laboratory experiments of Eldridge, 1968, demonstrated the existence of gonotrophic dissociation in *pipiens*. Eldridge's works prompted two simple field experiments: (1) whether *pipiens* could survive the winter on a sugar solution with no blood, and (2) whether gonotrophic dissociation could be demonstrated in a natural hibernating site. Parson's Cave (Pope County, Illinois) was used for both of the experiments. This cave was one in which mosquitoes had been observed each winter (November through March) from 1962 through 1966 and was unquestionably a suitable hibernating site.

EXPERIMENT I. On March 28, 1967, progeny reared from *pipiens* females collected from hibernating sites in McLeansboro, Illinois were placed in Parson's Cave. The progeny had been given a sugar solution (10cc of Karo syrup diluted in 100cc of distilled water) for 5 days before placement in hibernation. No special lighting was employed and

specimens were maintained at room temperature. The sugar solution was removed before transportation to the hibernating site. The mosquitoes were left in the cave in a 1-foot square gauze-covered cage with a solid plywood bottom, and observed weekly until all were dead. Males survived in hibernation for as long as 58 days and females survived for 113 days. The cave temperature on December 28, 1967 ranged from 53° to 55° F. and remained in this range until April 15. From April 15 to May 20, the temperature ranged from 56° to 58° F. Water droplets (condensation) formed inside the cage and probably contributed to the mortality. Nonetheless, a survival time of 113 days substantiates the female's ability to hibernate on sugar alone.

EXPERIMENT II. This experiment consisted of placing engorged females into Parson's Cave and checking what proportion, if any, would exhibit gonotrophic dissociation. Again, the source of material was progeny reared from *pipiens* females taken in hibernation. It was found that the female progeny engorged most readily when they had previously fed on sugar. Females reared and offered chickens directly did not feed as readily as did those given sugar and then offered a blood meal 3 to 4 days after the sugar solution had been removed. All attempts to get the mosquitoes to feed in the cave failed. The initial attempt was to permit feeding on a restrained chicken overnight and transporting engorged females to the cave the next morning. Only 1 of 68 females exhibited gonotrophic dissociation and this female could have fed just prior to being transported while the others, if they fed early in the evening, would have been exposed to higher temperatures for as long as 8 hours.

In an attempt to shorten the time between blood feeding and placement in the hibernating cave, newly emerged females which had been maintained on a sugar solution for 5 days, then starved

for 3 to 4 days were placed with a chicken at 5:00 p.m. and every hour throughout the night females that had fed to repletion were removed and placed in the cave within 2 hours. At the end of 2 weeks, all surviving females were returned to the laboratory for examination. Only 3 of 84 surviving females exhibited gonotrophic dissociation. These 3 females were left at room temperature for 3 days, then dissected. None had evidence of ovarian development. The temperature in the cave during the experiment ranged from 56° to 57° F.

DISCUSSION. *Culex pipiens pipiens* is the sole member of the *C. pipiens* complex that was found hibernating in the Ohio-Mississippi River Basin (Hayes, 1973). The absence of virus in this species coupled with the cessation of blood feeding as fall approached leads one to conclude that *pipiens* is not involved in the overwintering of SLE virus. Further support of this theory is the finding that all females taken in hibernation are nulliparous with Stage I ovaries. On the other hand the discovery of gonotrophic dissociation by Eldridge in the laboratory and the author's demonstration of this phenomenon in nature establish the possibility of SLE Virus being maintained by hibernating *pipiens*.

Studies should be done in controlled natural hibernating sites to demonstrate whether or not laboratory infected *pipiens* can survive the winter and remain infective the following spring.

ACKNOWLEDGMENTS. The author thanks Dr. Botha de Meillon for his valuable guidance and encouragement throughout the course of this work and Dr. Bruce Eldridge for his suggestions and criticism during the preparation of this manuscript. The technical assistance of Ms. Tina Henshaw and Ms. Glenda Williamson is also gratefully acknowledged.

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EVALUATION OF ABATE INSECTICIDE FORMULATIONS AS LARVICIDES AGAINST *ANOPHELES GAMBIAE* IN NORTHERN NIGERIA

RUSSELL E. FONTAINE¹ AND PHILIP ROSEN²

The effectiveness of new synthetic organic insecticides as larvicides for the control of *An. gambiae*, the principal malaria vector in Africa, has not been previously evaluated in Nigeria. In view of the interest in larval control as a supplementary measure for house residual spraying in malaria control, Abate (OMS-786)³ was evaluated in field plot tests under dry and wet season conditions during 1970-1971 by the World Health Organization Anopheles Control Research Unit, Number 1 at Kaduna, Nigeria.

LABORATORY EVALUATION

Prior to the selection of Abate for the field trials the susceptibility of *An. gambiae* to nine other insecticides was tested for LC₉₅ values using a standard insectary strain (Kankiya) third instar *An. gambiae* larvae.

The WHO test procedures were followed except that the range of susceptibility was determined from three concentrations of the technical material in ethanol instead of five and disposable, waxed monocupes were substituted for glass beakers. Each insecticide was tested at least twice with four replications of each test.

The test data are shown in Table 1.

TABLE 1.—Susceptibility of third instar *An. gambiae* larvae (Kankiya Strain) to ten insecticides, Kaduna, Nigeria, 1970.

Insecticide	LC 50	LC 95	Oral LD-50 mg/Kg (female white rats)
Carbaryl	0.30	0.760	500*
Mobam	0.33	0.480	178
Propoxur	0.14	0.270	116
Malathion	0.048	0.106	1000*
Iodofenphos	0.011	0.032	1600
Bromophos	0.016	0.022	2000
Abate	0.0074	0.019	13000*
Fenthion	0.0049	0.013	245*
Ciba C-14814	0.0020	0.008	1000
Dursban	0.0022	0.006	82*

¹ Project Leader, WHO Anopheles Control Research Unit II, P.O. Box 1426, Kisumu, Kenya.

² Project Leader, WHO Anopheles Control Research Unit I, P.O. Box 503, Kaduna, Nigeria.

³ OMS-786 American Cyanamid, Abate, CL-52160 o,o',o'-tetramethyl o,o'-thiodi-para-phenylene phosphorothioate.

* World Health Organization Technical Report Series No. 443. All other values taken from Vol. 11 WHO/VBC/71.11 August 1971.