A SURVEY OF NORTH MISSISSIPPI MOSQUITOES FOR PATHOGENIC MICRO-ORGANISMS [1]

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ABSTRACT. A one-year survey in 3 counties of North Mississippi for pathogenic micro-organisms occurring in natural mosquito populations was undertaken. A variety of pathogens were isolated, representing several genera of microsporidian protozoans (*Thelohania, Stempellia,* and *Nosema*), fungal agents (*Coelomomyces* spp.), and bacteria (*Bacillus* spp. and *Spirillum* spp.). Adult light trap and larval samples produced 35 distinct species. Data on population densities throughout the period of study indicated peaks in adult and larval populations during June, August, and October.

Alternate insect control practices (biological, mechanical, etc.) are receiving more emphasis because of problems associated with insecticidal residues and with resistance. The use of presently available insecticides will undoubtedly be limited in the future. Biological control practices utilized alone or in conjunction with chemicals will be desirable.

Biological control of mosquitoes by viral, fungal, bacterial, and protozoan pathogens shows promise. Fungal infections alone have shown some success in controlling mosquito populations. After noting a 95 percent mortality of *Anopheles gambiae* Giles larvae caused by *Coelomomyces* sp. Muspratt (1962), Laird (1960, 1965), and Laird and Colless (1962) successfully introduced *Coelomomyces* sp. into natural habitats and later assessed an increase in *Aedes polynesiensis* larval infections from 13.6 percent (1960) to 37.1 percent (1963). Chapman *et al.* (1968) reported infection levels from September to June as ranging from 2–93 percent and averaging 57 percent in 10 mosquito species in Louisiana.

Microsporidia are among the most abundant mosquito pathogens, with the genus *Thelohania* being the most prevalent; *Nosema, Stempellia,* and *Plistophora* are less common. Marchoux and Simond (1906), Kellen and Wills (1962), and Kellen, Chapman *et al.* (1965, 1966) reported transovarial transmission of microsporidians in mosquitoes; this is indicative of their potential as control agents.

The possibility of using host-specific bacteria for mosquito control was first mentioned by Laveran (1902). Since then many micro-organisms have been found in the digestive tract and malpighian tubules. Kellen, Clark *et al.* (1965) isolated a strain of *Bacillus sphaericus* Heide from larvae of *Culiseta incidens* Thomson and later found 10 species of mosquitoes to be susceptible to this bacterium which produced fatal septicemias.

The only source of pathogens for biological control studies is the naturally occurring mosquito population. A widespread survey of mosquitoes must be undertaken to determine as many naturally occurring pathogens as possible.

A survey was initiated in North Mississippi to determine the endemic mosquito species and associated host-pathogens. The seasonal abundance of mosquitoes and evaluations of pathogen specificity were also investigated.

MATERIALS AND METHODS. Adult and larval mosquitoes were collected every 2 weeks from selected sites in Clay, Lowndes, and Oktibbeha Counties from March, 1972 to February, 1973. A Latin square design

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was used to randomize the collection day arrangement (Tuesday, Thursday, and Saturday) for the three counties. New Jersey light traps, which had been modified so that the potassium cyanide was completely enclosed, were utilized for adult collections. A trap was allowed to operate 1 night during each biweekly period at each of 6 permanent sites per county. All sites were adjacent to wooded areas, where possible. The traps were turned on in the late afternoon (approximately 4:00 p.m.) for collection. The collections were removed the next day and taken to the laboratory for identification and storage. Corroborative identification of all adults and larvae was made by Dr. R. F. Darsie, CDC, Atlanta, Georgia.

Five to 8 mosquito larval sampling sites were selected within a mile radius of each light trap location. These sites included a variety of different habitats, e.g., ponds, lakes, pools, artificial containers, sewage ditches, swamps, and temporary rainfilled pools. Larval surveys were made to compensate for any differences in attraction to light for separate species and for pathogen isolation. Larval densities were noted by counting the number of larvae per dip using a 4-inch diameter dipper. Dips were made at random in the various breeding sites. Larval samples were taken to the laboratory for identification, preservation, and for pathogen studies. All fresh material was studied visually and microscopically; those larvae which appeared to be diseased were dissected and examined for bacteria, fungi, viruses, and microsporidia. Pathogens were sent to WHO/International Reference Center—Diagnosis of Diseased Vectors, Columbus, Ohio, for further identification and verification.

RESULTS AND DISCUSSION. Twenty-five species were collected as adults and 33 species were collected as larvae. There was a total of 35 species (Table 1). Only adult specimens of *Culex tarsalis* Coquillett, *Culiseta melanura* Coquillett, and *Coquillettidia perturbans* (Walker) were collected. Conversely, only larvae of *Aedes atlanticus* Dyar and Knab, *A. cinereus* Meigen, *A. dupreei* Coquillett, *A. tormentor* Dyar and Knab, *Culex pilosus* Dyar and Knab, *Orthopodomyia alba* Baker, *O. signifera* Coquillett, *Psorophora cyanescens* Coquillett, *Toxorhynchites septentrionalis* Dyar and Knab, or *Uranotaenia lowii* Theobald were found.

In those counties surveyed 34 species were collected in Clay County, 31 in Oktibbeha County, and 29 in Lowndes County. The greatest overall population density was in Lowndes County. *Culiseta inornata* Williston, *Culex restuans* Theobald, and *Aedes vexans* Meigen were more common from November to May while the *Anopheles* spp. prevailed from April until October. *Psorophora confinnis* Lynch Arribálzaga and *Culex erraticus* Dyar and Knab were most prevalent during the period July to November.

Adult and larval population densities were noted throughout the year, and both showed similar fluctuations (Figure 1). Adult studies were based upon the total number of adult mosquitoes trapped during each month and the average number of mosquitoes caught per trap during each month. The adult population peaked in June, August, and October. These peaks are attributed to rainfall. In January only a few adults were trapped, but thereafter, the numbers caught began rising to higher levels again. The presence of adult mosquitoes in February was probably due to the appearance of warmer temperatures at night which allowed more adult flight activity.

Thelohania spp. (order Microsporidia) were the predominant pathogens associated with larval specimens (Table 2). *Coelomomyces* spp., *Bacillus* spp., *Spirillum* spp., *Nosema* spp., and *Stempellia* spp. were less commonly isolated. Other workers have obtained similar results. Chapman *et al.* (1968) found many species of mosquitoes serving as hosts to microsporidians (mainly *Thelohania,* but also *Stempellia, Plistophora,* and *Nosema*). More specifically, Chapman *et al.* (1969) reported 18 species to be infected with *Thelohania* spp. Also, Chapman *et al.* (1970) found 23 species of anophelines to be hosts to microsporidians.

Coelomomyces spp., the most promising of the fungal pathogens, have been found to infect and kill mosquitoes in at least 11 genera (Chapman *et al.*, 1972). Chapman *et al.* (1969) reported 15 mosquito species as hosts of *Coelomomyces* spp.

Bacterial pathogens have been isolated from a number of species of mosquitoes. Reeves and Garcia (1970) reported a bicrystalliferous *Bacillus* sp. in *Aedes* species. Chapman *et al.* (1972) found a vibrio-like bacterium which infected 17 species of mosquitoes naturally.

Pathogenic micro-organisms may play

TABLE 1.—Summary of adult and larval collections from March, 1972 to February 28, 1973.

Species	Lowndes Co.		Clay Co.		Oktibbeha Co.		Number adults trapped			
	L[1]	A[2]	L	A	L	A	Lowndes	Clay	Oktibbeha	Total
Aedes aegypti	+	+	—	+	+	+	1	0	1	2
A. atlanticus	+	—	+	—	+	—	0	0	0	0
A. canadensis	+	—	+	+	+	—	0	1	1	2
A. cinereus	—	—	+	—	—	—	0	0	0	0
A. dupreei	—	—	+	—	—	—	0	0	0	0
A. tormentor	+	—	+	—	+	—	0	0	0	0
A. triseriatus	+	—	+	+	+	—	0	1	0	1
A. vexans	+	+	+	+	+	+	133	220	58	411
Anopheles barberi	—	—	+	+	—	+	0	4	5	9
A crucians complex[3]	+	+	+	+	+	+	432	27	46	505
A. punctipennis	+	+	+	+	+	+	11	27	31	69
A. quadrimaculatus	+	+	+	+	+	+	414	37	117	567
Culex erraticus	+	+	+	+	+	+	160	108	90	357
C. nigripalpus	—	—	+	+	+	+	0	1	1	2
C. pilosus	—	—	+	—	—	—	0	0	0	0
C. pipiens quinquefasciatus	+	+	+	+	+	+	47	71	12	130
C. restuans	+	+	+	+	+	+	100	120	68	288
C. salinarius	+	+	+	+	+	+	70	89	69	228
C. tarsalis	—	—	—	+	—	—	0	1	0	1
C. territans	+	+	+	+	+	—	1	2	0	3
Culiseta inornata	+	+	+	+	+	+	44	34	49	127
C. melanura	—	+	—	—	—	—	2	0	0	2
Coquillettidia perturbans	—	+	—	—	—	+	28	0	5	33
Orthopodomyia alba	—	—	+	—	+	—	0	0	0	0
O. signifera	+	—	+	—	+	—	0	0	0	0
Psorophora ciliata	+	+	+	+	+	+	10	55	12	72
P. confinnis	+	+	+	+	+	+	188	392	141	721
P. cyanescens	+	—	+	—	+	—	0	0	0	0
P. discolor	+	+	+	+	+	+	3	26	4	33
P. ferox	+	—	+	+	+	+	0	2	1	3
P. howardii	+	—	+	+	+	+	0	12	1	13
P. varipes	+	—	+	+	+	—	0	2	0	2
Toxorhynchites rutilus septentrionalis	+	—	+	—	+	—	0	0	0	0
Uranotaenia lowii	+	—	+	—	+	—	0	0	0	0
U. sapphirina	+	+	+	+	+	+	64	26	9	101
Unidentified[4]	..	..	..	..	..	..	36	54	80	170

[1] Larvae.
[2] Adults.
[3] A mosquito complex including *Anopheles bradleyi, A. georgianus,* and *A. crucians* which are indistinguishable as adults.
[4] This term includes (a) those specimens which were mutilated beyond identification and (b) those male specimens which were not identified.

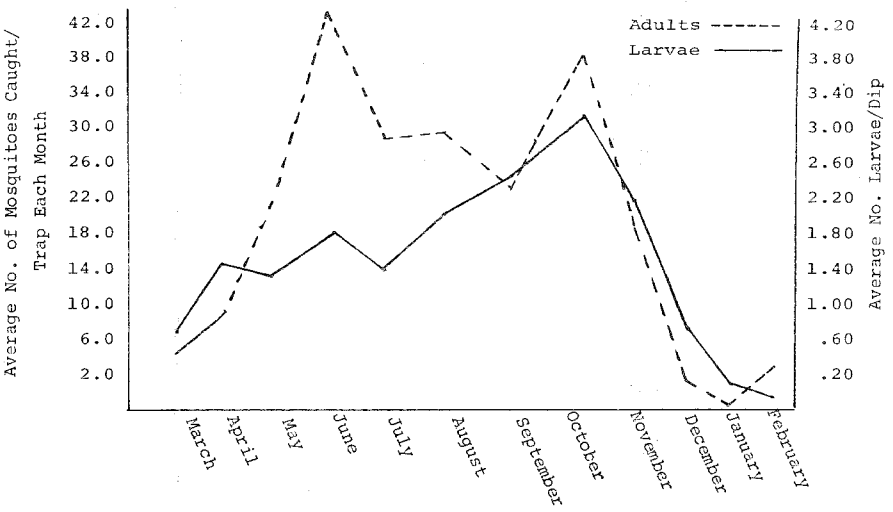

FIG. 1.—Larval and adult population density fluctuations throughout the year.

an important part in mosquito control programs. However, more knowledge of parasite and pathogen relationships with mosquitoes is needed, and little is understood about the factors favorable to pathogen development. Experts on mosquito diseases generally agree that the parasites and pathogens of mosquitoes are relatively host specific. However, species in several genera, such as *Thelohania* and *Coelomomyces,* infect a variety of mosquito species. Broad spectrum pathogens infecting several

TABLE 2.—Pathogen isolations from field collected mosquito larvae.

Mosquito species	Pathogen species	Counties where collected		
		Lowndes	Clay	Oktibbeha
A. quadrimaculatus	*Thelohania obesa* (Kudo)	+[1]	+	+
	Coelomomyces punctatus Couch	+	+	+
A. punctipennis	*C. lativattus* Couch & Dodge	—	+	—
	C. punctatus Couch	—	—	+
C. territans	*Thelohania opacitor* (Kudo)	+	+[2]	+
	Coelomomyces sp.	+	—	—
C. restuans	*Stempellia magna* (Kudo)	—	—	+
	Bacillus sp.	—	+	—
C. salinarius	*Bacillus* sp.	—	+	—
	Thelohania opacitor complex	+	—	+
C. pipiens quinquefasciatus	*Bacillus* sp.	—	+	—
	Vibrio sp.	+	—	—
C. erraticus	*Spirillum* sp.	+	—	—
	Thelohania minuta Kudo	—	—	+
Aedes canadensis	*T. opacitor*	—	—	+

[1] Indicates that the pathogen was isolated in the respective county.
[2] Indicates that the pathogen was not isolated in the respective county.

species would be ideal for general mosquito control.

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