

ematics are referred to Aikat and Das (1976) and Pichon (1983).

Owing to space limitations the full program listings cannot be given here (SINFIT is 219 lines, SINCOM is 108 lines), but the print-outs obtained are shown in Figs. 1 and 2 (the printer was set to compressed print mode). The data used are for microfilarial periodicities by Harinasuta et al., cited in full by Aikat and Das (1976). Note that results from only one of the data sets are printed out to illustrate SINFIT (Fig. 1), but both data sets have been plotted to show how plots can be superimposed (Fig. 2). The analysis of variance performed by SINCOM appears at the bottom of Fig. 2.

For any readers interested in having the programs, full listings will be provided on request (please specify whether for HP85 or HP86). Readers wishing to obtain programs already recorded should send appropriate media (tape cassette for HP85, 3½" micro-flexible disc for HP86).

Aikat and Das (1976) and Pichon (1983) provide considerably more information on the details of the calculations and interpretation.

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#### References Cited

- Aikat, T. K. and M. Das. 1976. A modified statistical method for analysis of periodicity of microfilariae. WHO/FIL/76.142, 12 pp. (mimeographed document).
- Pichon, G. 1983. Crypto-periodicity in *Mansonella ozzardi*. Trans. R. Soc. Trop. Med. Hyg. 77:331-333.
- Sasa, M. and H. Tanaka. 1972. Studies on the methods for statistical analysis of the microfilarial periodicity data. S. E. Asian J. Trop. Med. Publ. Hlth. 3:518-536.
- Sasa, M. and H. Tanaka. 1974. A statistical method for comparison and classification of the microfilarial periodicity. Jap. J. Exp. Med. 44:321-346.

### SUSCEPTIBILITY OF *CULEX NIGRIPALPUS* TO SEVERAL ISOLATES OF *PLASMODIUM HERMANI* FROM WILD TURKEYS IN FLORIDA<sup>1</sup>

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Wild turkeys (*Meleagris gallopavo*) in Florida have been found to be infected with a malarial parasite, *Plasmodium hermani* (Forrester et al. 1974, Telford and Forrester 1975). Four species of Florida mosquitoes (*Culex nigripalpus* Theobald, *Cx. salinarius* Coquillett, *Cx. restuans* Theobald and *Wyeomyia vanduzeei* Dyar and Knab) have been shown to be susceptible to infection to the Fisheating Creek isolates (P-27 and P-41) of *P. hermani* (Young et al. 1977; Nayar et al. 1980, 1981a, 1981b). Additionally, two of these mosquito species (*Cx. nigripalpus* and *Wy. vanduzeei*) were found susceptible to the Lochloosa isolate (L-5) of this parasite (Nayar et al. 1980). Only slight differences were observed in the rate of infection between isolates P-27 and P-41, but no significant dif-

ferences were observed in the number of oocysts per midgut of *Cx. nigripalpus*, fed on poult infected with three isolates (P-27, P-41 and L-5) (Table 1). Recently, two new isolates of *P. hermani* (W-1 and Lc-1) have been obtained from wild turkeys from other areas of Florida. Distinct differences were observed in their infectivity to *Cx. nigripalpus*. We now report the differences between these two isolates in the rate of infection and in the number of oocysts per midgut and compare them with that of the Lochloosa isolate (L-5).

Three-to-five-day-old females of colonized *Cx. nigripalpus* (Vero Beach) were tested for susceptibility to *P. hermani*. Larval mosquitoes were reared and adults maintained as described by Nayar and Pierce (1977). The various isolates of *P. hermani* were obtained from wild turkeys by subinoculation of heparinized blood into 2 to 3-week-old domestic turkey poults following the method of Forrester et al. (1974). Isolates P-27 and P-41 were obtained from an adult female turkey on July 29, 1975 and from an adult male on August 2, 1979, respectively, from Fisheating Creek near Palmdale, Glades County, isolate L-5 was obtained from a juve-

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Table 1. Susceptibility of *Culex nigripalpus* to isolates of *Plasmodium hermani* from wild turkeys in Florida.

Isolates of <i>P. hermani</i>	No. passages in domestic turkeys prior to use in experiments	No. mosquitoes dissected/no. of lots dissected	Percentage midguts infected			Oocysts/midgut			Reference
			Mean	Range	P	Mean ± S.E.	Range	P	
P-27	9	52/3	67.1	30-100		9.0 ± 0.8	1-35	Young et al. 1977	
P-27	12	184/7	62.6	29-90		10.2 ± 0.9	1-69	Nayar et al. 1981a	
P-41	2	66/3	78.8	20-100	<0.05	9.1 ± 1.1	1-62	Nayar et al. 1981b	
P-41	6*	111/5	77.9	25-100		9.3 ± 1.2	3-60	Nayar et al. 1980	
L-5	11	73/3	79.3	30-100		11.2 ± 1.0	2-55	Nayar et al. 1980	
L-5	36	121/5	88.4	50-100		12.8 ± 1.0	1-54		
W-1	11	29/3	98.3	89-100	>0.05	41.3 ± 3.9	21-77	<0.001	
Le-1	4-6	61/4	90.2	72-100	>0.05	23.0 ± 2.8	2-89	<0.001	

\* Includes 1 mosquito passage.

nile female at the Lochloosa Wildlife Management Area, Alachua County, on February 23, 1972; isolate W-1 was obtained from an adult female at Waukeelah, Jefferson County on May 22, 1980; and isolate Le-1 was obtained from an adult female at the Talquin Wildlife Management Area, Leon County on June 14, 1983. From the third through the fifth days of peak parasitemia, lots of approximately 50 to 100 female *Cx. nigripalpus* were allowed to feed to repletion on poultlets inoculated with *P. hermani* isolates L-5, Le-1 and W-1. After the blood meal the mosquitoes were maintained under a 12-hr, light:dark cycle at 27° C, at RH 75% and with access to 10% sucrose solution. From each lot of mosquitoes a maximum of 25 midguts were examined for oocysts. Oocysts were counted between 11 to 13 days after the infective blood meal. Salivary glands were examined for sporozoites between 16 to 18 days after the infective blood meal. Differences between percentages of midguts infected and number of oocysts per midgut were compared by unpaired student *t*-tests. When *P* was less than 0.05, the differences were considered to be significant.

Table 1 shows that *Cx. nigripalpus* is comparatively more susceptible to the two newest isolates (W-1 and Le-1) of *P. hermani* than to the L-5 isolate. As compared to the Lochloosa isolate, the two new isolates had similar mean infection rates which varied from 88.4 to 98.3% but differed in the number of oocysts found on the midguts. Mosquitoes fed on poultlets infected with the Leon County (Le-1) isolate had almost twice, and Waukeelah (W-1) isolate almost four times the number of oocysts as those fed on poultlets with the Lochloosa (L-5) isolate. The two new isolates of *P. hermani* obtained from the panhandle area of northern Florida were more infectious than previously obtained isolates P-27, P-41 and L-5. Furthermore, repeated pas-

sage of the turkey malaria in domestic turkeys after isolation from wild turkeys did not change the number of oocysts produced per midgut of *Cx. nigripalpus* (Table 1). Sporozoites of all isolates were recovered from appropriately infected mosquitoes. Huff et al. (1959), obtained similar results by feeding *Culex pipiens* Linn and *Culex tarsalis* Coquillett on two isolates (P and B) of *Plasmodium relictum* obtained from domestic pigeons from different geographical regions. They concluded that *Cx. tarsalis* was more susceptible to isolate P than to isolate B regardless of the vertebrate host or manner of transmission to the host, and that *Cx. pipiens* was more susceptible to isolate B.

Immediately prior to blood feeding of *Cx. nigripalpus* females, the number of gametocytes per 10,000 red blood cells present in thin blood smears varied in different isolates: L-5 had 1-5 males and 5-20 females, W-1 had 1-8 males and 2-8 females, and Le-1 had 2-9 males and 2-10 females. When the number of oocysts per midgut produced were compared to the number of gametocytes, we found no correlation between the number of gametocytes present per 10,000 red blood cells and the number of oocysts observed on the midguts of infected mosquitoes. Infection of mosquitoes always occurred, regardless of the number of gametocytes observed as long as at least some gametocytes were present in thin blood smears made immediately prior to blood feeding of mosquitoes.

#### References Cited

- Forrester, D. J., L. T. Hon., L. E. Williams, Jr. and D. H. Austin. 1974. Blood protozoa of wild turkeys in Florida. *J. Protozool.* 21:494-497.
- Huff, C. G., D. F. Marchbank and T. Shiroishi. 1959. Susceptibility and resistance of avian and mosquito hosts to strains of *Plasmodium relictum* isolated from pigeons. *J. Protozool.* 6:46-51.

- Nayar, J. K. and P. A. Pierce. 1977. Utilization of energy reserves during survival after emergence in Florida mosquitoes. *J. Med. Entomol.* 14:54-59.
- Nayar, J. K., M. D. Young and D. J. Forrester. 1980. *Wyeomyia vanduzeei*, an experimental host for wild turkey malaria, *Plasmodium hermani*. *J. Parasitol.* 68:166-167.
- Nayar, J. K., M. D. Young and D. J. Forrester. 1981a. *Culex restuans*: An experimental vector for wild turkey malaria, *Plasmodium hermani*. *Mosq. News* 41:748-750.
- Nayar, J. K., M. D. Young and D. J. Forrester. 1981b. *Plasmodium hermani*: Experimental transmission by *Culex salinarius* and comparison with other susceptible Florida mosquitoes. *Exp. Parasitol.* 51:431-437.
- Telford, S. R., Jr. and D. J. Forrester. 1975. *Plasmodium (Huffia) hermani* sp. n. from wild turkeys (*Meleagris gallapavo*) in Florida. *J. Protozool.* 22:324-328.
- Young, M. D., J. K. Nayar and D. J. Forrester. 1977. Mosquito transmission of wild turkey malaria, *Plasmodium hermani*. *J. Wildl. Dis.* 13:168-169.

#### AN INJECTION METHOD FOR SPRAYING BIOLOGICAL CONTROL AGENTS AND A MONOMOLECULAR SURFACE FILM FOR CONTROL OF IMMATURE MOSQUITOES<sup>1</sup>

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Biological control agents against immature mosquitoes are replacing a significant percentage of conventional chemical toxicants at many mosquito control districts with little, or no, modification of the established methods for dispensing chemicals. However, the standard method for mixing the biological agents with water in spray tanks causes problems when the entire contents of the tank are not used the same day. For example, the larvicidal material tends to settle to the bottom of the tank, allowing contamination by proteinaceous anaerobic bacteria which often produce noxious odors and rapidly decrease the larvicidal potency. An attempt to avoid these problems by the use of a liquid chemical injection valve is described below.

<sup>1</sup> Mention of a brand name or proprietary product does not constitute a guarantee or warranty by Lee County Mosquito Control District, and does not imply its approval to the exclusion of other products that may also be suitable.

Tests with biocontrol agents were conducted using a Dema<sup>2</sup> Model #203 chemical injection valve and tests with a monomolecular surface film were performed with Dema Model #202C. The injector was installed on a small trailer on the pressure side of the pumping mechanism (Fig. 1). The pumping mechanism was a John Bean Pump (Model R-5) capable of delivering 5 G.P.M., driven by a 4 hp Briggs and Stratton engine.

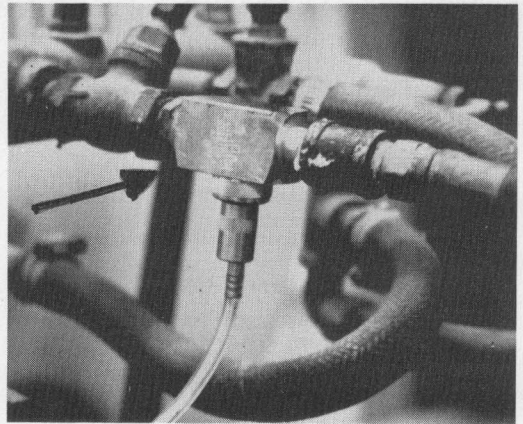


Fig. 1. A close-up view of the Dema injection valve installed.

The water volume sprayed from the nozzle was measured with a 50 liter jug and timed by a stopwatch. Output in liters/min. were measured at 5- and 10-minute intervals. One hundred ml of larvicidal concentrate in a graduated cylinder was sucked into the injection valve and timed by a stopwatch. Small changes in the suction rates were corrected by manipulation of the metering screw on the injection body capable of increasing or decreasing the flow rate 10-fold.

Test formulations were *Bacillus sphaericus*, strain 1593 (B.s. 1593) consisting of prespore and sporulating bacterial cells of ca.  $2 \times 10^9$  c/ml; *Bacillus thuringiensis*, H-14 used as a 5% aqueous suspension of a slurry product provided by Sandoz, Inc.<sup>3</sup>, yielding ca.  $1.5 \times 10^9$  c/ml; *Metarhizium anisopliae* var. *anisopliae* was used as a spore suspension of ca.  $6.2 \times 10^9$

<sup>2</sup> A series of Dema liquid chemical injectors are manufactured by Dema Engineering Company, 10020 Big Bend Boulevard, St. Louis, MO 63122.

<sup>3</sup> Sandoz, Inc., Crop Protection, 480 Camino Del Rio South, San Diego, CA 92108.

<sup>4</sup> Arosurf<sup>®</sup> MSF (=ISA-20E=Arosurf 66-E2) is a product of Sherex Chemical Company, Inc., Post Office Box 646, Dublin, OH 43017.