LABORATORY SELECTION OF *CULEX NIGRIPALPUS* THEOB. FOR RESISTANCE TO PARIS GREEN

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ABSTRACT. Laboratory selection of *Culex nigripalpus* at the LD_{80} level for 31 generations resulted in no significant increase in tolerance to paris green when compared to the unselected laboratory colony.

Between 1921 and 1943 paris green was used extensively as a mosquito larvicide, particularly against anopheline mosquitoes in anti-malarial programs. The introduction of DDT in the 1940's revolutionized mosquito control and all but eliminated the use of paris green for this procedure; but with the advent of mosquito resistance to DDT in the 1950's, paris green was reintroduced as a mosquito larvicide in granular form (Rogers and Rathburn, 1958) and used widely for the control of larvae of most mosquito species. It proved to be both an effective and safe larvicide giving excellent control with as little as 0.75 of a pound of actual paris green per acre without harm to fish and most other aquatic organisms. It was also shown that it does not increase the arsenic content in the soil or water of mosquito larval habitats. (Bishop 1940; Rathburn 1966).

Although used extensively for more than twenty years there has never been a documented case of mosquito larval resistance to paris green; however, resistance or the appearance of resistance has been reported for lead arsenate with the codling moth, *Carpocapsa pomonella* (Hough, 1928), the peach twig borer, *Anarsia lineatella* (Summers, 1949) and for arsenic with a South African tick *Boophilus decoloratus* (Whitnal, 1949). Even though reports of resistance of insects to arsenic compounds are meager and absent in most uses this does not mean that the development of resistance of mosquito larvae to paris green could not occur. Therefore, research was initiated to determine if it would be possible to select a paris green resistant strain of mosquitoes in the laboratory. This paper reports the results of that research.

METHODS

The *Culex nigripalpus* used in this study were from a laboratory colony that has been maintained since 1965. The 1st generation used for these tests was designated F_1. To avoid possible mixing of the selected and unselected strains and because of limited space in the insectary, selected specimens were maintained in 45 x 45 x 45 cm. cages in the testing laboratory and unselected specimens were maintained in one 90 x 90 x 90 cm. cage in the insectary. The adult mosquitoes were offered blood from anesthesized chickens 3 times weekly and had continuous access to a 5 percent sugar solution. Three generations of selected adults were maintained at all times in the event the youngest generation died out before all tests could be completed.

SELECTION TESTS. Egg rafts were collected weekly in bowls of infusion water placed in each cage. To facilitate handling and counting, the rafts were transferred to 400 ml. plastic beakers of tap water, 18 rafts per beaker. One ml of a suspension consisting of 24 g. of liver powder and 16 g of brewer's yeast in 1000 ml. of tap water was added as food, and the beakers were transferred to the insectary for rearing. When hatched the larvae in each beaker were placed in circular enameled pans containing 4000 ml. of tap water which was constantly aerated. The larvae were fed 2 ml of the food suspension daily. They were reared to the 3rd instar at a temperature of 29±2 °C at which time the larvae in each pan were washed in tap water and placed in plastic lined metal testing pans containing 4000 ml. of tap water.

Since paris green is insoluble in water, a technique which had been developed previously for dosage-mortality tests was used. This consisted of suspending 10 mg. of 90 percent paris green, which had been screened through a 325 mesh sieve, in 2000 ml. distilled water by means of an electric stirrer. While being stirred 50 to 75 ml. of the suspension was pipetted into the testing pans containing the larvae and the pans were held at room temperature. The water temperature in the pans ranged from 20 to 29° C (average 24.5° C). Early in the testing program it was shown that the amount of paris green necessary to obtain the desired 80 percent mortality in the selection tests had to be increased in tests conducted at lower temperatures. Therefore there was a variation in the amount of paris green suspension added to the pans.

Because of the large numbers of mosquito larvae tested at one time (approximately 30,000–50,000), actual counts of the larvae in each pan were not possible. Therefore, the 24-hour mortality in each pan was estimated twice by each of two individuals and averaged for each generation. The dead larvae were then removed and the live larvae were washed and placed in pans of clear tap water in a water bath at 29° C. Pupae from these larvae were placed in emergence bowls in the appropriate cage for emergence. One pan at each treatment was left untreated as a check. No marked check mortality was observed.

An average of 1226 egg rafts per generation was used to produce the 50 pans of larvae which were treated in each generation. Counts were kept of the number of pupae added to the cage of adults of each generation since it was felt that a minimum of 5000 adults per cage was necessary to assure an adequate egg supply for the following generation.

DOSAGE-MORTALITY TESTS. The methods used to obtain the larvae for the dosage-mortality tests were similar to those used for the selection tests, except that newly-laid eggs were collected weekly, hatched individually in 50 ml. plastic beakers containing tap water and food, and the larvae were reared to 3rd instar in 23 by 28 cm. flat enameled pans without aeration.

A stock suspension of paris green was made by stirring 5 mg. of 90 percent paris green which had been previously screened through a 325 mesh sieve in 2000 ml. of distilled water. While being stirred, 0.1, 0.2, 0.4, 0.8 and 1.6 ml. of the suspension were pipetted into 50 ml. plastic beakers each containing 10 3rd instar larvae in 25 ml. of tap water. The tests were conducted in a water bath at 27±0.5° C. Each test consisted of 4 replications of 5 treatments plus the check to which was added 1.0 ml. of distilled water. Six tests were conducted with every other generation, beginning with the 3rd generation, over a period of two to four weeks.

RESULTS

The selection tests were not designed to measure the degree of resistance, however it was assumed that an increase in the amount of paris green needed to produce the desired 80 to 85 percent mortality indicated a reduction in susceptibility and possibly the development of resistance. For the first few generations the amount of paris green required to produce 80 to 85 percent mortality decreased, but after the first year it became evident that this was a dosage-mortality response related to water temperature. As shown in Fig. 1, there was an inverse relationship between water temperature and the amount of paris green needed for the desired mortality. Since the selection tests were not conducted at a constant temperature, this fluctuation could also be correlated with time of year, and since the difference occurred over a small range in temperature, it may be of importance in field use of paris green.

An estimated 5–10 percent delayed treatment mortality occurred after the surviving larvae were placed in the pans for pupation. Therefore, although the mortality at 24 hours ranged from 74 to 88 percent (average 82 percent) the final mortality was approximately 80–98 percent (average 90 percent). This necessitated using a rather large number of

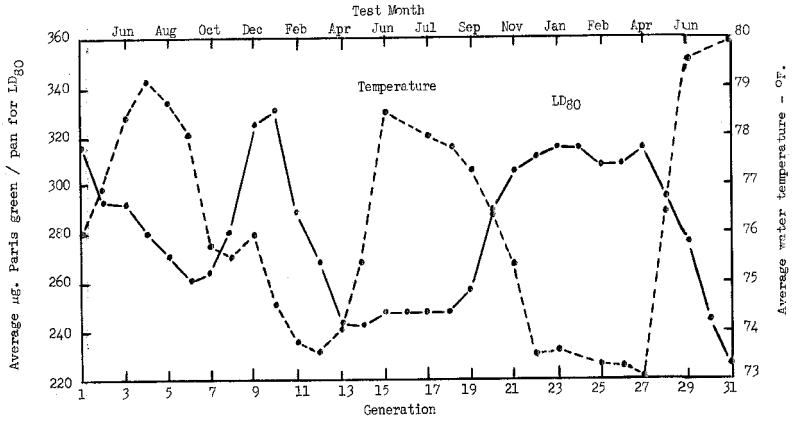

FIG. 1.—Effects of water temperature on the toxicity of paris green to the larvae of *Culex nigripalpus* Theob. in selection tests.

larvae to obtain sufficient adults for the next generation.

The results of the dosage-mortality tests are shown in Table 1. The resistance ratio shown in the last column is the LD_{50} of the selected strain divided by the LD_{50} of the unselected strain. An increase in this ratio with successive generations would indicate development of resistance. As the ratio of the third and thirty-first generations are almost identical, the data show that no resistance developed.

For the resistance ratio to be meaningful the regression lines of the selected and unselected larvae must be parallel or nearly so. The slope function is used as a measure of parallelism. This is the change in dose required to produce a unit standard deviation change in response along the regression line. In most generations,

TABLE 1.—Results of selection at the LD_{80} level by paris green of a laboratory colony of *Culex nigripalpus* Theob. for 31 generations as measured by the resistance ratio.

	LD_{50} µg/larvae		LD_{80} µg/larvae		Slope function[1]		
Generation	Sel.	Unsel.	Sel.	Unsel.	Sel.	Unsel.	RR[2]
3	0.14	0.09	0.62	0.42	3.3	3.2	1.5
5	0.17	0.10	0.69	0.43	3.1	3.1	1.7
7	0.19	0.14	0.73	0.54	2.9	2.8	1.3
9	0.14	0.11	0.45	0.37	2.6	2.6	1.3
11	0.18	0.16	0.77	0.71	3.2	3.2	1.1
13	0.09	0.07	0.42	0.36	3.4	3.4	1.2
15	0.18	0.10	0.68	0.35	2.8	2.6	1.8
17	0.21	0.16	0.95	0.66	3.2	3.0	1.3
19	0.19	0.16	0.90	0.54	3.0	2.6	1.2
21	0.34	0.23	1.22	0.77	2.7	2.6	1.5
23	0.24	0.16	0.70	0.47	2.3	2.3	1.5
25	0.38	0.26	1.89	1.22	3.5	3.5	1.5
27	0.19	0.14	0.59	0.14	2.3	2.3	1.4
29	0.24	0.18	0.54	0.41	1.9	1.9	1.3
31	0.28	0.18	0.81	0.41	2.3	2.0	1.6

[1] Slope function—$S=(LD_{84}/LD_{50}+LD_{50}/LD_{16})/2$.
[2] Resistance ratio—LD_{50} Sel./LD_{50} Unsel.

the slope function of the regression lines of the selected and unselected larvae are almost identical and in no case is the difference greater than 0.4, which would indicate highly divergent regression lines. The similarity and the goodness of fit of most of the dosage-mortality lines did not warrant the lengthy calculations necessary to establish confidence limits for the LD_{50}, LD_{90} and slopes of each of the 30 regression lines.

Shown in the top curve of Fig. 2 is the relationship of resistance ratios of successive generations. The regression line of the resistance ratio has a slope approaching zero ($b=0.002\pm0.006$) resulting in highly insignificant "t" and "F" values. Therefore, it may be assumed that this extremely small slope is the result of a random sampling from a population of zero slope, and there is little reason to suspect an increase in resistance ratio with increasing generations which would indicate development of resistance. Shown in the bottom curve of the Fig. 2 are the $LD_{50's}$ of the selected and the unselected larvae for each generation. Here also there is no evidence of a change in susceptibility between the selected and unselected larvae with successive generations which would also be an indication of the development of resistance.

CONCLUDING REMARKS

The ability or inability to develop resistance in laboratory populations of mosquitoes may give some insight into the same possibilities in field populations. Selection of a resistant strain in the laboratory would strongly indicate the probability of possible development of resistance in field populations of mosquitoes selected by the same insecticide. Likewise, failure to select a resistant strain in the laboratory strongly suggests that resistance will not develop in natural populations; however, prolonged inbreeding in the laboratory precludes assurance of this conclusion.

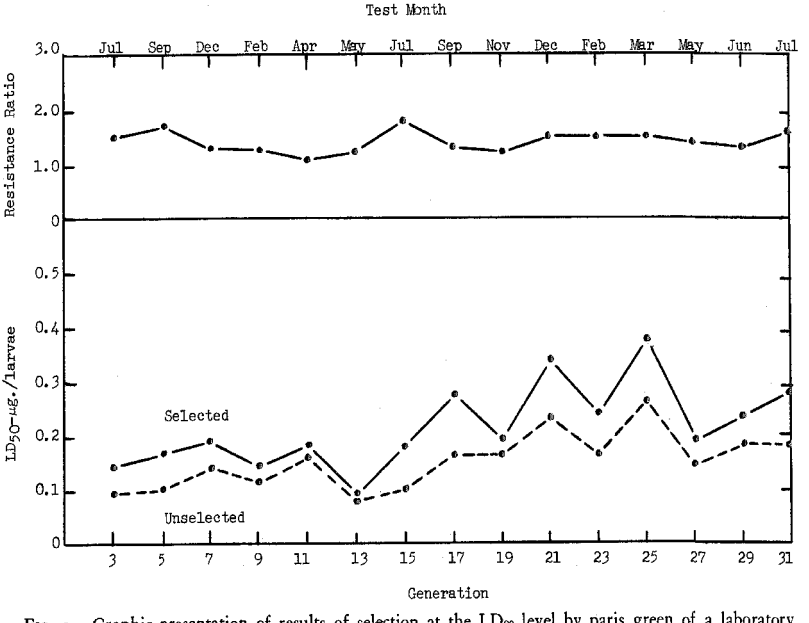

FIG. 2.—Graphic presentation of results of selection at the LD_{90} level by paris green of a laboratory colony of *Culex nigripalpus* Theob. for 31 generations.

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RELATIONSHIP OF DENSITY, LOCATION OF HOSTS, AND WATER VOLUME TO PARASITISM OF LARVAE OF THE SOUTHERN HOUSE MOSQUITO BY A MERMITHID NEMATODE[1]

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ABSTRACT. When the volume of water in six square outdoor ponds varied from 25 to 900, from 50 to 1800, and from 75 to 2700 liters, no significant differences in the mean incidence of parasitism by *Reesimermis nielseni* Tsai and Grundmann in *Culex pipiens quinquefasciatus* Say could be detected as a result of dilution. The incidence of parasitism was somewhat suppressed when most densities reached 0.4 host larvae per cc, but this reduction could be overcome by increasing the ratio of parasites to hosts at the time of exposure. Parasitism was higher in host larvae held in the corners of ponds of four or more square meters of surface area than when larvae were held at the edge or the middle of the ponds. Parasitism was lowest in larvae held in the middle of the ponds. Also, parasitism was higher when the hosts were held in screened containers that permitted a water surface-screen contact than when they were held in containers that prevented such contact.

Thus water volume was not a major factor in determining the incidence of parasitism by *R. nielseni,* and preparasitic *R. nielseni* exhibited thigmotactic and negatively geotactic behavior.

Reesimermis nielseni Tsai and Grundmann, a mermithid nematode parasite of mosquito larvae, is a promising biological control agent that has received considerable attention. Nevertheless, little is known about the way the infective stage (preparasitic nema) finds its host. An investigation was therefore conducted at Lake Charles, Louisiana, to delineate the importance of the densities of parasite and host on the incidence of parasitism by *R. nielseni* of *Culex pipiens quinquefasciatus* Say and to study the host seeking behavior of the preparasitic stage. In a previous laboratory study, parasitism by *R. nielseni* was observed to decrease by about 50 percent when water volumes were increased from 0.2 to 30 liters (Petersen and Willis, 1970). However, the factors of surface area, container shape, and depth of water were not considered. The present study was planned to compensate for these variables and was made in outdoor screened enclosures in ponds that permitted the use of greater volumes of water.

EFFECT OF DILUTION. Six ponds measur-

[1] In cooperation with McNeese State University, Lake Charles, Louisiana.