SELECTION OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY FOR RESISTANCE TO GROWTH INHIBITOR

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ABSTRACT. Selection pressure designed to produce 75% mortality was applied to alternate generations of *Culex pipiens quinquefasciatus* Say larval populations using the growth inhibitor Monsanto-0585 (2, 6-di-t, butyl-4-(α,α-dimethyl benzyl) phenol) through 20 generations. Statistical analysis of LC_{50} determinations revealed that the Mon-0585 selected population had significantly more variation ($P<0.05$) between generations than did the unselected population. Significant differences ($P<0.05$) in variation existed between the 3rd, 7th, 9th, 11th, 13th and 19th generations of the selected compared to the unselected population. The LC_{50} (ppm) increased by ca. 4x at near the midpoint of the selective regime (F_9–F_{11}) and returned to ca. the same level observed for the F_1 by the F_{15} to F_{17} generations. No significant differences ($P>0.05$) existed between the amount of variation that occurred between the 1st, 5th, 15th, and 17th generations of selected compared to the unselected population. There was no significant difference ($P>0.05$) in the amount of variation between generations of the selected and unselected populations when the LC_{50} determinations for both were analyzed. Thus, resistance was not demonstrated in the selected population through 20 generations.

The extensive use of organochlorine and organophosphate insecticides to control mosquito populations has frequently led to the development of resistance to these compounds in many parts of the world. Several chemical compounds have been developed which inhibit insect development. This study was designed to determine if resistance to the growth inhibitor, Monsanto-0585 (2, 6-di-t, butyl-4-(α,α-dimethyl benzyl) phenol), could be established in an organochlorine and organophosphate suscepible laboratory population of *Culex pipiens quinquefasciatus* Say.

MATERIALS AND METHODS. Approximately 1,000 *C. p. quinquefasciatus* adult females were removed from the laboratory colony and blood-fed on a chicken host. The blood-engorged mosquitoes were equally divided into Colony A (unselected strain) and Colony B (selected strain).

Egg rafts were collected and transferred to 28 x 17 x 4.5 cm enamel pans containing water for hatching and larval development. A temperature of 24±2° C and a 9 hr photoperiod were maintained in the rearing and testing room. Each pan of larvae was aerated continously, and 0.25 g of finely ground commercial rabbit pellets was added daily as food for the larvae. After the larvae had molted to the 2nd instar, 10 ml of 11% brewer's yeast solution was added to each pan.

Larvae used for selection were removed by random dipping from the Colony B larval population. All remaining Colony B larvae were destroyed. The pupae surviving selection were transferred into a 6 x 8 cm jar containing dechlorinated water. This jar was then placed inside of a 17 x 18 cm carton with screened lid for confining the adults, which were supplied raisins for food. Egg rafts from newly emerged blood-fed adults of the selected colony (B) were reared through successive generations as previously described. Mosquitoes from the unselected colony (A) were reared by the same procedures but without selection.

Baseline levels of susceptibility were determined for 20 generations in the unselected strain; however they were determined only for every other generation in the selected colony due to the length of time required to complete susceptibility and/or selection tests. Thus, a baseline was established for the odd numbered generations and the even numbered generations were exposed to Mon-0585 selection pressure in the selected colony. Otherwise, the concentration required to cause 75% mortality determined for the F_1

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population would have been used for each succeeding generation. Mortality caused by Mon-0585 was determined after all mosquitoes exposed to treatment had died or emerged as adults.

A 3 lb AI/gal emulsifiable concentrate formulation of Mon-0585 was used in this study. Five or more concentrations of Mon-0585 were used for the determination of the baseline for each generation. The untreated checks and each concentration were replicated four times. Fifty 2nd instar larvae per test concentration were randomly taken from the colonies and placed in 28 x 17 x 45 cm enamel pans that contained 1.0 liter of water which had been treated with the desired concentration of Mon-0585 for each baseline test. The larvae were exposed to the selected concentrations of Mon-0585 continuously from early 2nd instar through the 4th instar. No additional Mon-0585 was added to the pans after the initial treatment. During the selection and the baseline tests, the water in each test pan was aerated continuously and the larvae were fed 0.25 g of finely ground commercial rabbit pellets per day.

Dead individuals were removed from the pans at each 24 hr observation period. All live pupae were removed from the pans and held in small souffle cups that were covered with a screened lid. A maximum of 20 pupae were held in one souffle cup and observed at 24 hr intervals.

Total mortalities (larvae, pupae, intermediates and abnormal adults) were adjusted, when necessary, by Abbott's (1925) formula and plotted on logarithmic-probit graph paper. Baselines for appropriate generations were established by eye-fitted lines, and the LC_{50}, LC_{75} and LC_{90} were estimated from this line.

The amount of Mon-0585 required to produce 75% mortality in the selected generation of Colony B was derived from the baseline established during the previous unselected generation of Colony B. Selec-

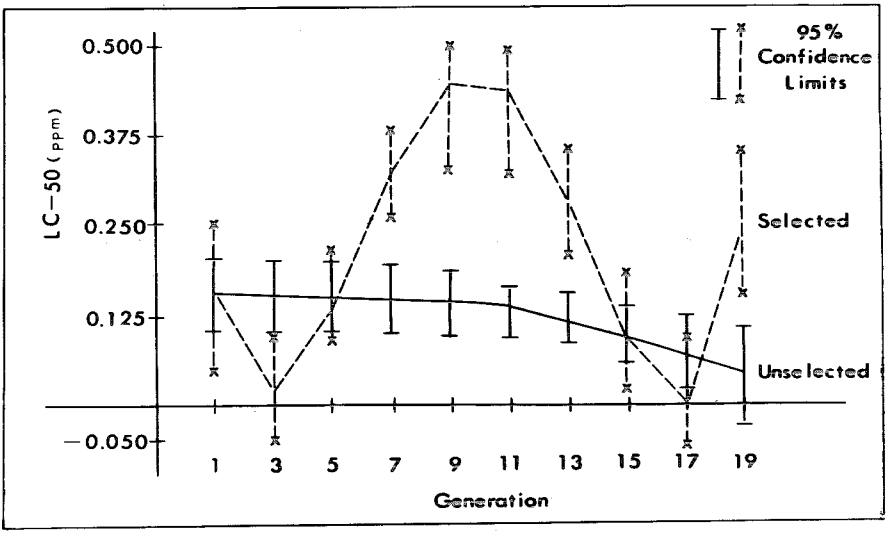

FIG. 1. Regression lines and 95% confidence limits, shown for LC_{50} for Mon-0585 selected and unselected populations of *C. p. quinquefasciatus*.

tion pressure was applied to 20 replications of fifty 2nd instar larvae (1,000 larvae exposed) per alternate generation. The larvae were exposed to the concentration of Mon-0585 expected to cause 75% mortality continuously from early 2nd through the 4th instar. No additional Mon-0585 was added to the pans after the initial treatment. Dead individuals were removed from the pans at each 24 hr observation period. Live pupae were removed from the treatment pans and held in small souffle cups containing dechlorinated water. The pupae were observed at 24 hr intervals. Survivors of this selection were allowed to complete development and, therefore, propagate the next generation.

A stepwise regression of LC_{50} and LC_{90} on generation number (Service, 1972) was conducted, using the maximum R^2 procedure, where the linear, quadratic, cubic and quartic effects of generation number were included. This procedure was used to evaluate the LC_{50} and LC_{90} data of Mon-0585 selected and unselected populations of *C. p. quinquefasciatus*.

RESULTS AND DISCUSSION. The Mon-0585 selected population had significantly more ($P<0.05$) variation between generations than did the unselected population of *C. p. quinquefasciatus* when the LC_{50} determinations for both were analyzed. There was no significant difference ($P>0.05$) in the amount of variation between the generations of the selected and unselected populations when the LC_{90} determinations for both were analyzed.

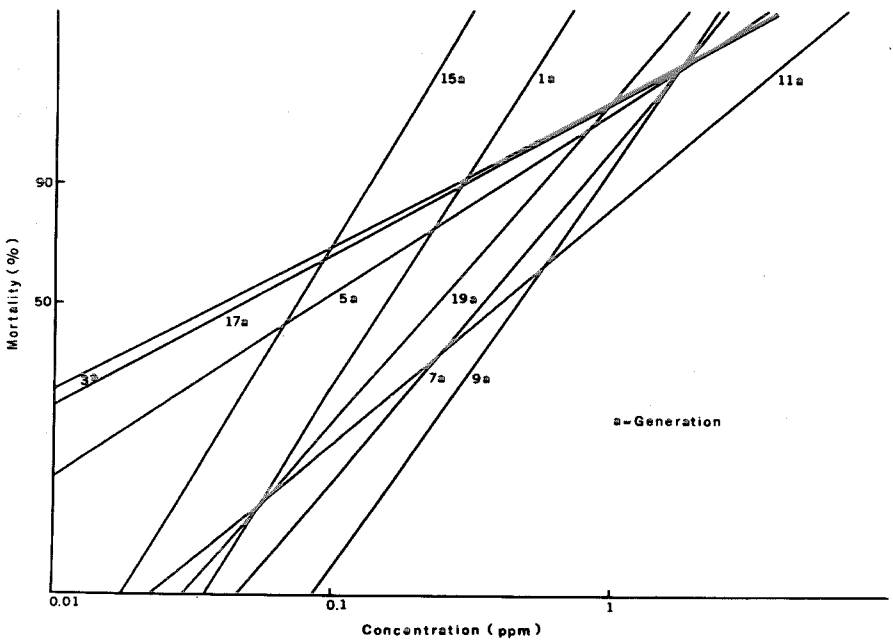

FIG. 2. Dosage-response lines for Mon-0585 selected population of *C. p. quinquefasciatus*.

Figure 1 shows the regression lines and 95% confidence limits determined for LC_{50} values through 20 generations of Mon-0585 selected and unselected *C. p. quinquefasciatus* populations. The 95% confidence limits of the 1st, 5th, 15th and 17th generations of selected and unselected populations overlapped indicating that no significant difference ($P>0.05$) occurred between these generations of selected and unselected populations. The 95% confidence limits of the 3rd, 7th, 9th, 11th, 13th and 19th generations of selected and unselected populations did not overlap indicating significant differences ($P<0.05$). The 75% selection pressure caused an increase in the amount of variation in the susceptibility of the selected population from the 7th through the 13h generations. However, by the 15th generation the selected population regression line returned to that level of variation seen earlier in generation 1 and 5. This type of response was reported by George and Brown (1967) in the selection of *Aedes aegypti* (L) with a chemosterilant. They observed a decrease in the percent sterility at the F_5 generation but sterility was higher in the F_6 generation than in the previous five generations. Our data indicate a definite pattern in the selected population ($R^2=.97$) as compared to the unselected where there was a slight decline in the LC_{50} from the first generation through the 20th generation.

From these data it is evident that a cyclic pattern has emerged. Thus, it is possible that a similar pattern would occur in future generations of selection. A similar cyclic pattern was observed in the

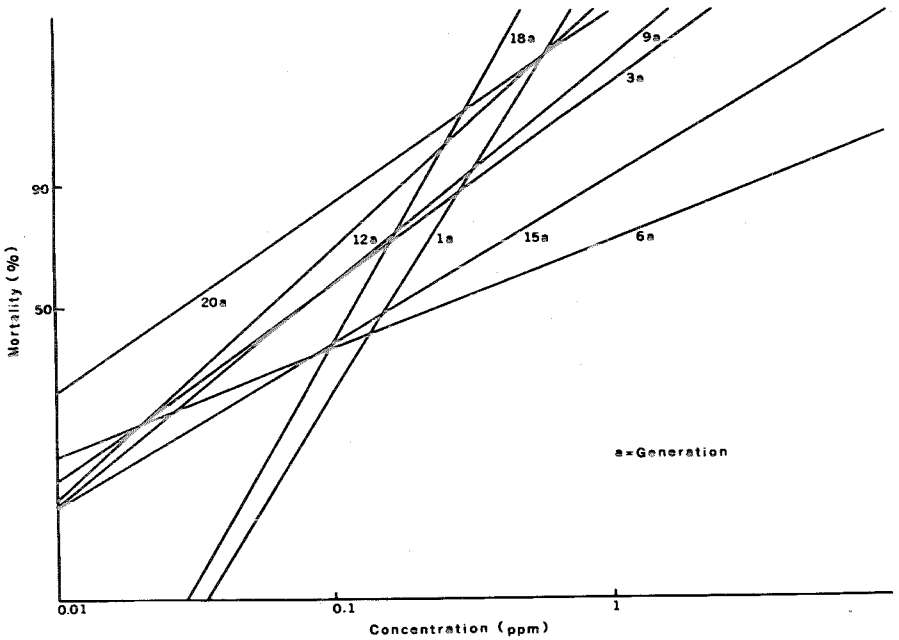

FIG. 3. Dosage-response lines for Mon-0585 unselected population of *C. p. quinquefasciatus*.

hempa selection of *A. aegypti* (George and Brown, 1967) where the percent sterility of the F_1 generation was 66%, F_4 generation 89%, F_5 generation 68% and the F_6 generation 91%.

The four-fold differences in susceptibility that existed between the selected and the unselected populations in the 9th and 11th generation were probably due to vigor tolerance. Figures 2 and 3 show the dosage-response lines of the selected and unselected populations, respectively. The F_{19} and F_{20} susceptibility levels (LC_{50}) for both populations reverted to approximately the same or became more susceptible than comparable F_1 generation population. Thus, these data indicate that resistance was not demonstrated in the selected population through 20 generations.

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INDUCTION OF HEAT-SENSITIVE LETHALS IN *CULEX TRITAENIORHYNCHUS* BY ETHYL METHANESULFONATE

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ABSTRACT. The virus vector mosquito, *Culex tritaeniorhynchus*, was treated with the mutagenic agent, ethyl methanesulfonate. Two temperature-sensitive conditional recessive sex-linked lethals and 22 morphological mutations were induced and recovered. By the use of these conditional lethals and appropriate genetic stocks, it is now possible to produce mosquitoes of only one sex (males) for a mass release program.

Conditional mutations have been useful in the analysis of development, genetic fine structure and gene action in a number of organisms (Foster and Suzuki, 1970; Suzuki, 1970; Loomis, 1969; Chovnick *et al.*, 1962). Moreover it has been suggested that these mutations, especially temperature-sensitive conditional lethals, may be employed in various ways in insect control programs (Knipling, 1960; LaChance and Knipling, 1962; Klassen *et al.*, 1970a, b; Sandler and Novitski, 1957; Curtis, 1968; Wehrhahn and Klassen, 1971; Whitten, 1971; Smith, 1971). Recessive heat-sensitive lethal factors has been isolated in the house fly, *Musca domestica*, by McDonald and Overland (1972).

In addition to the possibility of using temperature-sensitive conditional lethals themselves for control purposes, this class of lethals also can be used to produce adults of only one sex (males). In most field experiments involving the release of male insects, the separation of sexes can be difficult, expensive, time-consuming, and not completely effective. In the vector mosquito, *Culex tritaeniorhynchus*, a method for detecting temperature-sensitive lethals in the sex chromosomes is available (Sakai and Baker, 1972). In this species