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Colonization and Production of a Dieldrin-Resistant Strain of *Anopheles quadrimaculatus* Say[1]

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Abstract

A dieldrin-resistant field strain of *Anopheles quadrimaculatus* Say from Cleveland, Mississippi, has been established under insectary production. Three-day-old females show high dieldrin resistance; 60-minute exposures to 0.8% through 8% dieldrin-Risella oil papers result in less than 10% mortality in 24 hours, while 480-minute exposures to 4% papers killed only 50%. Susceptible females are killed by 60-minute exposures to 1.6% dieldrin-Risella oil papers.

Initial attempts in 1956 and 1957 to colonize a dieldrin-resistant field strain of *Anopheles quadrimaculatus* Say from Cleveland, Mississippi were unsuccessful. Field-collected blood-engorged females produced eggs which were shipped airmail to the laboratory and reared to adults by standard insectary procedures. Twenty-four-hour mortality values for the resultant 3-day-old adult females with 30-minute exposures to residual deposits of 25, 100, and 200 mg. of dieldrin per square foot averaged 5%, a level comparable to that of the field adults (Mathis *et al.* 1956). Only an occasional insectary-reared female fed on the hare or human hosts provided, and the few eggs laid gave rise only to susceptible individuals.

Studies were continued in 1958. Since the biting rate of inseminated females was found to be about three times greater than that of unmated females (Terzian & Stahler 1949), mating studies were conducted starting with a susceptible laboratory strain. Adult males and females were separated when less than 24 hours old and kept apart

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until 3 days old. At that time they were recombined in a small (18″×18″×18″) cage 3 hours before sunset. The first observed activity of the mating cycle, waving of the antennae by the males, occurred 4 minutes before sunset (meridian lighting—11 foot-candles). At sunset the males began waving their hind legs vertically (meridian lighting —8 foot-candles). Shortly after this, the males swarmed in the center of the cage and the females ceased resting on the sides and top of the cage, and entered the swarm. Mating in flight commenced 5 minutes after sunset (meridian lighting—4.8 foot-candles). The copulating pairs moved about the cage, often falling to the bottom. Copulation was rapid, not lasting more than 10 or 15 seconds with the mating pair oriented head to head, the male underneath. At the peak of mating, as many as 12 pairs were observed per minute out of a total of 120 females and 140 males in the cage. Mating continued until the meridian lighting was less than 0.01 foot-candle. In a repeated study essentially the same results were obtained.

When 3-day-old adults of the resistant strain were compared in separate cages with those of the susceptible strain, the latter strain mated shortly after sunset; however, no mating was observed in the resistant strain. Although the males reacted in about the same manner as the susceptible males, the females did not respond but instead remained on the sides and top of the cage. These studies were repeated several times. Although the susceptible strain mated freely each time, the resistant strain has never been observed mating.

The resistant adults were removed after each mating experiment and placed in a large (3′×3′×3′) cage on a screened porch. Pupae reared from eggs received from Cleveland, Mississippi were added to this cage. Since one of the best field sources of the blood-engorged females had been pig houses, and since Horsfall (1955) notes that "Pigs, dogs, and, to a varying extent, fowls were often chosen as sources for blood meals," a young, newly-weaned pig was tried as a host. The pig was placed in the large colony cage nightly and an oviposition pan was provided. Viable eggs were produced.

The pig was somewhat unsatisfactory as a host (a) since adult mosquitoes escaped when the pig was introduced and when it was removed from the colony cage through a cloth sleeve and (b) since rapid weight gain necessitated a new host animal approximately every 6 weeks. After 4 months, further attempts were made to use a hare as the host animal. A special cage was used to present both the pig and the hare to the females. It was observed that the females fed on the animal that was presented first, thus a hare was adopted successfully as the only source of blood. The females engorged readily and no decrease in egg production occurred.

Since initially only 10% of the larvae completed pupation, relatively few adults were available for determining dieldrin susceptibility of the insectary-produced resistant strain. Over several generations, however, 3-day-old females of the resistant and the susceptible strains were compared in the laboratory by a method developed for resistance measurements (Mathis *et al.* 1959) using 60-minute exposures to various dieldrin-Risella oil-impregnated papers prepared by the World Health Organization. Results (table 1) showed that dieldrin resistance had been retained by the strain since they were only slightly affected by the 1.6% dieldrin-Risella oil papers, which killed all of the susceptible strain.

Since the mortalities of the 3-day-old resistant females (table 1) were less than 10% with 60-minute exposures for 0.4% through 8% dieldrin, longer exposure periods were tried. Accordingly, 3-day-old resistant females were exposed to 4% dieldrin-Risella oil papers for periods ranging from 60 to 480 minutes (table 2). The maximum exposure killed about half of the females exposed, again indicating high resistance.

Further tests were conducted with resistant adults less than 24 hours old. Adult males and females, exposed separately to various dieldrin-Risella oil-impregnated papers for 60 minutes, indicated that only about half of the adults succumbed at this age (table 3). A series of exposures, varying from 60 to 300 minutes, were conducted also with 4% dieldrin-Risella oil papers to determine the effect of exposure time upon mortality (table 4). Since a marked difference in resistance existed between 3-day-old and less-than-1-day-old females, 1- and 2-day-old fe-

Table 1.—Per cent mortality of 3-day-old female *Anopheles quadrimaculatus* from 60-minute exposures to various dieldrin-Risella oil-impregnated papers.

Per Cent Dieldrin-Risella Oil	24 Hour Mortality (%)	
	Susceptible Strain	Resistant Strain
8.0	—	6 (233)[a]
4.0	—	6 (208)
3.2	—	8 (275)
1.6	100 (184)	7 (283)
0.8	73 (179)	3 (266)
0.4	10 (188)	3 (277)
Control	0 (107)	3 (274)

[a] Numbers of individuals tested are shown in parentheses

Table 2.—Per cent mortality of 3-day-old resistant female *Anopheles quadrimaculatus* exposed to 4% dieldrin-Risella oil-impregnated papers for varying perods.

Exposure Period (Minutes)	24-Hour Mortality (%)
60	4 (744)[a]
120	11 (463)
240	25 (405)
480	50 (451)
Control	1 (512)

[a] Numbers of individuals tested are shown in parentheses.

Table 3.—Per cent mortality of less-than-24-hour-old resistant *Anopheles quadrimaculatus* from 60-minute exposures to various dieldrin-Risella oil-impregnated papers.

Per Cent Dieldrin-Risella Oil	24-Hour Mortality (%)	
	Males	Females
8.0	—	47 (147)[a]
4.0	61 (49)	36 (199)
3.2	58 (52)	50 (264)
1.6	42 (50)	34 (287)
0.8	35 (54)	28 (204)
0.4	—	20 (126)
Control	18 (45)	3 (264)

[a] Numbers of individuals tested are shown in parentheses.

Table 4.—Per cent mortality of less-than-24-hour-old resistant *Anopheles quadrimaculatus* exposed to 4% dieldrin-Risella oil-impregnated papers for varying periods.

Exposure Period (Minutes)	24-Hour Mortality (%)	
	Males	Females
60	48 (160)[a]	39 (263)
120	70 (161)	55 (125)
180	77 (159)	76 (431)
240	—	78 (252)
300	—	83 (132)
Control	5 (161)	3 (210)

[a] Numbers of individuals tested are shown in parentheses.

males also were exposed separately to dieldrin-Risella oil papers for 60 minutes (table 5). These tests showed that the response of the 1- and 2-day-old females was similar to that of the 3-day-old females.

One test has been run to determine the DDT-susceptibility of the strain. Three-day-old females of both the dieldrin-resistant and the susceptible strains, exposed to DDT-Risella oil papers for 60 minutes, showed quite comparable mortalities (table 6). The resistant strain is presently maintained under no selection, and, with improved rearing methods, about 20% of the larvae reach the pupal stage. The strain is now considered as established under insectary production.

Table 5.—Per cent mortality of 1-, 2-, and 3-day-old resistant female *Anopheles quadrimaculatus* exposed to various dieldrin-Risella oil impregnated papers for 60 minutes.

Per Cent Dieldrin-Risella Oil	24-Hour Mortality (%)		
	1-Day Old	2-Day Old	3-Day Old
8.0	7 (144)[a]	5 (200)	6 (233)
4.0	7 (126)	1 (202)	6 (208)
3.2	10 (123)	2 (206)	8 (275)
1.6	11 (128)	1 (203)	7 (283)
0.8	16 (131)	1 (193)	3 (266)
0.4	9 (132)	0 (179)	3 (277)
Control	1 (149)	1 (202)	3 (274)

[a] Numbers of individuals in test are shown in parentheses.

Table 6.—Per cent mortality of 3-day-old female *Anopheles quadrimaculatus* from 60-minute exposures to DDT-Risella oil-impregnated papers.

Per Cent DDT-Risella Oil	24-Hour Mortality (%)	
	Susceptible Strain	Resistant Strain
4	89 (75)[a]	82 (77)
3	—	67 (106)
2	22 (75)	67 (76)
1	0 (77)	16 (72)
Control	0 (75)	1 (78)

[a] Numbers of individuals tested are shown in parentheses.

References Cited

Horsfall, W. R. 1955. Mosquitoes—their bionomics and relation to disease. The Ronald Press Co., New York. p. 149.

Mathis, W., H. F. Schoof, K. D. Quarterman, and R. W. Fay. 1956. Insecticide resistance of *A. quadrimaculatus* in Bolivar County, Miss. Publ. Health Rept. 71(9): 876–9.

Mathis, W., H. F. Schoof, and R. W. Fay, 1959. Method for the field determination of susceptibility levels of adult mosquitoes. Mosquito News (In press).

Terzian, L. A., and N. Stahler, 1949. The effect of larval population density on some laboratory characteristics of *Anopheles quadrimaculatus* Say. Jour. Parasitol. 35: 487–95.

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