Ecological and Nutritional Studies on *Coleomegilla maculata* De Geer (*Coleoptera: Coccinellidae*). III. The Effect of DDT, Toxaphene, and Endrin on the Reproductive and Survival Potentials[1]

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ABSTRACT

Adult lady beetles *Coleomegilla maculata* De Geer, were treated with DDT, endrin, toxaphene, or a toxaphene-DDT mixture. DDT had no effect on longevity, increased oviposition by ⅔ but reduced the number of F_1 progeny produced by ¼; it was equally toxic to diapausing and active beetles. DDT was significantly more toxic to beetles collected from Baton Rouge than to those collected at Boyce, Louisiana but both populations were heterogeneous in their response to it. Endrin decreased longevity but had no effect on oviposition or survival of F_1; it was equally toxic to diapausing and active beetles; the experimental population was homogeneous in its response to endrin. Toxaphene decreased longevity and prevented oviposition; the diapausing beetles withstood higher doses of toxaphene than the active beetles; the experimental population was heterogeneous in its response to toxaphene. Toxaphene-DDT mixture exhibited strong synergistic action, decreased longevity, and decreased reproduction about ⅓. It had no effect on survival potential of the F_1. The experimental population was homogeneous in response to the mixture.

A log time-probit line technique was devised as a means of expressing the effect of a poison on longevity of an organism under laboratory conditions. This technique is discussed.

Few studies have been reported on the effect of insecticides on the reproductive and survival potential of insects. None of these studies has dealt with entomophagous insects.

Friedrichs and Steiner (1930) stated that the older larvae of the pine moth, *Bupalus piniarius* L., were not always killed by arsenical dusts. Pupae of poisoned larvae were noticeably smaller, and the fertility of females produced from them was impaired. Breeding experiments with eggs collected in stands treated the previous year showed that 3.4% of the resultant larvae pupated compared to 19% in the nonpoisoned groups. Nenyukov and Tareeva (1931) concluded that incomplete poisoning affects metabolic processes in insects and would probably reduce their reproductive power. Beard (1960) stated that insecticides applied to populations should be considered as ecological factors affecting insect numbers. He found that DDT-treated house fly, *Musca domestica* L., populations showed a modified reproductive biology disadvantageous to the flies. The various population patterns indicated that different insecticides differed in their long-range effects and that their influence was not limited to their lethal action in merely removing a portion of the flies. Sublethal doses of DDT increased the reproductive potential of the granary weevil, *Sitophilus granarius* (L.) about 20%; however, mortality was high and the total number of offspring that reached adulthood was much lower than in the untreated cultures (Kuenen 1958). Pickett and Patterson (1963) studied the effect of arsenates on the fecundity of some Diptera. Their results showed varying degrees of reduced fecundity when sublethal doses of arsenic were included in the food. Egg production was greatly reduced when the newly emerged adults were fed sublethal doses of arsenates. An orchard test confirmed the laboratory results. Knutson (1959) showed that dieldrin changed the reproductive potential of treated house flies. Kenaga (1965) stated that triphenyl tins suppress or control reproduction in the house fly; the German cockroach, *Blattella germanica* (L.); and the confused flour beetle, *Tribolium confusum* Jacquelin du Val. The compounds sterilized the adults well below the lethal concentration. In the house fly, females were sterilized at lower concentrations than males. Kenaga further stated that some derivatives produce easily reversible control of reproduction; but some do not, depending to some extent on the dose. Bartlett (1963) studied the contact toxicity of some pesticide residues to hymenopterous parasites and coccinellid predators. He stated that the effect of each pesticide upon most kinds of adult parasitic Hymenoptera could be anticipated with a high degree of reliability while the effect upon predatory coccinellids was much less predictable.

The study reported here was concerned with the effect of 3 insecticides on the reproductive and survival potential of the lady beetle *Coleomegilla maculata* De Geer.

METHODS AND MATERIALS.—Beetles for most of the tests were collected at Boyce, La., from their diapausing quarters, mostly under loose bark and debris near the trunks of large pecan trees growing in cotton fields. The beetles were in aggregations of hundreds and sometimes thousands. They were brought to the laboratory and divided into 2 groups. Group 1 was held at 15.5°C in ½-gal cartons with some debris and bark so that the beetles would remain in diapause. Beetles in group 2 were separated from the debris, placed in ½-gal cartons with food and water and held at 26.7°C under a 16-hr photoperiod provided by a high pressure mercury vapor lamp.[2] This treatment was designed to eliminate diapause. Mating was the criterion used to decide when the beetles were no longer in diapause. In some of the treatments laboratory-reared beetles, from parents collected at Baton Rouge, La., were used.

An artificial diet and a rearing technique used in previous studies were used for feeding and rearing the insect in these studies. (Atallah and Newsom 1966).

One μliter of acetone solution containing the desired amount of insecticide was applied to the scutellum of each beetle by a calibrated syringe, the plunger

[1] Portion of a dissertation presented by the senior author for the Doctor of Philosophy degree, Louisiana State University. Accepted for publication May 31, 1966.

[2] General Electric Code H 100-SP4 Mercury Vapor Light.

Table 1.—The comparative effect of DDT on active and diapausing *C. maculata* collected at Boyce, La., February 1963. Beetles held at 26.7°C and observed for 168 hr.

Days after treatment	μg of toxicant/beetle											
	10		20		30		40		60		Check	
	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total
	Diapausing											
1	6	60	14	60	13	52	21	60	29	60	0	60
2	11	60	14	60	13	52	21	60	29	60	0	60
3	11	60	14	60	13	52	21	60	29	60	0	60
5	14	60	14	60	14	52	21	60	31	60	0	60
7	14	60	15	60	16	52	23	60	33	60	0	60
	Active											
1	3	60	7	60	12	60	15	60	24	60	0	60
2	9	60	14	60	14	60	20	60	29	60	0	60
3	9	60	16	60	14	60	21	60	30	60	0	60
5	9	60	17	60	21	60	27	60	34	60	0	60
7	11	60	17	60	22	60	32	60	27[a]	60	0	60

[a] Seven beetles recovered after having been considered dead on the 5th-day count.

of which was driven by a micrometer. The treated beetles were then placed in 1-pint cartons, 10 in each, supplied with food and water, and held at 26.7°C. Counts of live and dead beetles were made at 0.0, 1.0, 2.0, 3.0, 5.0, and 7.0 days. For interpretation of the toxicity data the methods of Abbott (1925) and Finney (1952) were used.

Counts at the end of 72 hr were used for computing log dose-probit lines. For each dosage, time versus mortality was drawn in a log time-probit line that illustrated the relative effect of the dosage on longevity. All the probit analyses were programmed on an IBM 1640 digital computer at the University Computer Center.

The effects of the sublethal treatments on the biotic potential (ability of a population to reproduce and survive, i.e., natality versus mortality) of the survivors were determined. They were placed in pairs in cartons and provided with food and water. The reproductive potential (natality rate) was measured by recording the number of eggs produced by each female until she died. To determine the effect of the treatment on the survival potential (ability of the organism to withstand environmental resistance) of the progeny, the F_1 larvae were reared in single tubes and the number of larvae reaching the adult stage was recorded.

The chemicals used were DDT, endrin, and toxaphene. They were tested on both diapausing and active beetles. A toxaphene-DDT mixture (2:1) was tested only on active beetles.

Table 2.—The comparative effect of DDT on active and diapausing *C. maculata* collected at Boyce, La., February 1963. Beetles held at 26.7°C and observed for 72 hr.

Dose, μg/beetle	Diapausing			Active		
	Dead	Total	% mort.	Dead	Total	% mort.
0	0	80	0.00	0	80	0.00
10	13	80	16.25	10	80	12.50
20	18	80	22.50	18	80	22.50
30	18	80	22.50	17	80	21.25
40	30	80	37.50	24	80	30.00
60	39	80	48.75	40	80	50.00

RESULTS.—*Toxicity of DDT to* C. maculata *collected at Boyce, La.*—Data obtained from treating diapausing and active adults with DDT are summarized in Tables 1 and 2. Log dose-probit lines for diapausing and active beetles are illustrated in Fig. 1. The slopes of the 2 lines indicate that there was great heterogeneity in resistance to DDT in the experimental population. The LD_{50} was 6482 mg/kg for active beetles and 6329 mg/kg for diapausing beetles. The difference was not significant.

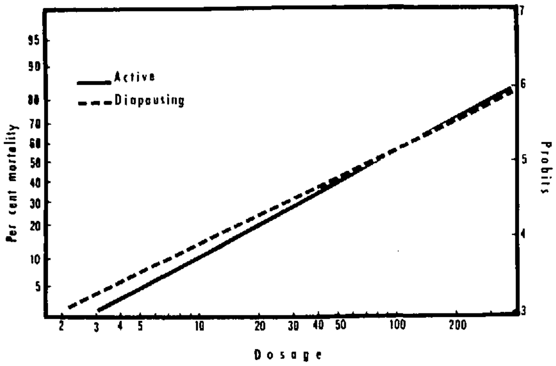
Effect of DDT on Longevity.—Log time-probit lines for treatments at 10, 30, and 60 μg/beetle in diapause are shown in Fig. 2 and for active beetles in Fig. 3.

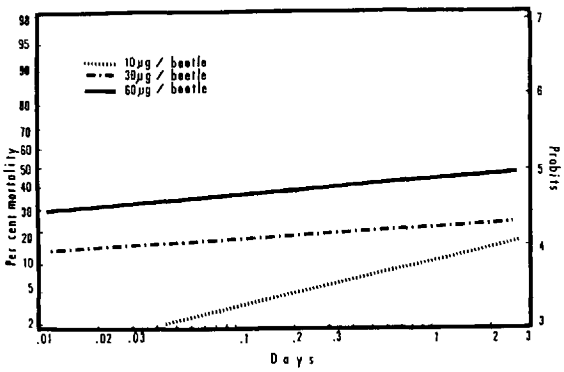
Although the log time-probit line is theoretical, it gives an indication of the effect of treatment on longevity of the adults. The slope of the line indicates the effect of the insecticide on the longevity of the beetle when natural mortality is eliminated, the steeper the slope, the greater the effect.

Effect of DDT on Reproductive and Survival Potentials.—Adult males and females that survived the 40 μg DDT treatment (LD_{34}) were kept together, whether diapausing or active, in ½-gal ice cream cartons and provided with food and water. The untreated animals were handled similarly. Whenever a male and female copulated, they were isolated in a 1-pint ice cream carton with food and water which provided conditions appropriate for oviposition (Atallah 1966).

Within 10 days 20 pairs were isolated from the check, while among the survivors of the treatment, no copulation had taken place. The treated beetles did not feed on the diet for 2 days after treatment, but most of them started feeding on the 3rd day.

The treated adults recovered, copulated, and started laying eggs 16 days after treatment. A record of the number of egg batches and the number of eggs per batch was kept for each pair of treated and untreated beetles until all the females died (February through July). The eggs were collected at 2-hr intervals from 7 AM until 11 PM, except for a few times during the 5-month period. Most of the eggs were laid during May and June.





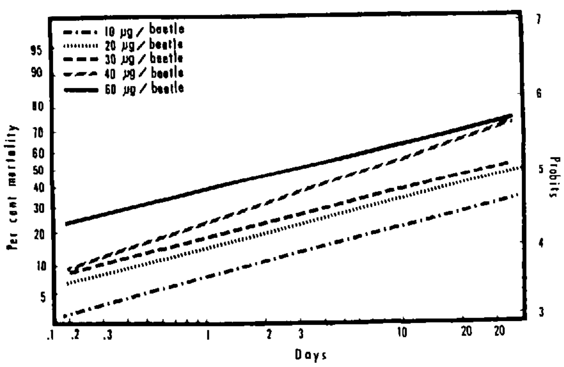


FIG. 1. (top)—Log dose-probit lines for DDT-treated *C. maculata* based on 72-hr observations. Boyce, La., February 1963.

FIG. 2. (center)—Log time-probit lines for DDT-treated *C. maculata* (diapausing) collected at Boyce, La., February 1963.

FIG. 3. (bottom)—Log time-probit lines for DDT-treated *C. maculata* (active) collected at Boyce, La., February 1963.

Upon hatching, the 1st-instar larvae were separated and reared individually. The numbers of larvae that pupated and adults that emerged were recorded. The effect of DDT on the F_1 of treated beetles was determined from these data. The summarized data are given in Table 3.

There were 67% more eggs laid by the treated than by untreated females, in spite of the fact that 1 of the treated females laid no eggs. The number of larvae produced was significantly higher in the treated than in the check unless a 1-in-10 chance occurred in sampling. In this particular instance the 10% level of significance has a "biological" significance.

Twenty-one percent of F_1 larvae from treated beetles pupated compared to 53 in the check. Mortality was higher during the larval and prepupal stages than in any other developmental stage. The percentage of pupae which gave rise to adults was 96.3 in the checks and 89.2 in the treatment.

The differences found between the DDT-treated beetles and the check may have been caused by a genetic difference obtained as a result of selection by DDT, the presence of DDT or its metabolites in the beetles and their progeny, or both. In either case the inducing factor was DDT.

DDT and DDE were found in the body, feces and eggs of the treated beetles (Atallah and Nettles 1965).

Toxicity of DDT to C. maculata *Collected at Baton Rouge, La.*—In July 1962, 20 adults were collected at Baton Rouge, brought to the laboratory, and reared on aphids and artificial diets. Toxicity tests were run on 7th- and 8th-generation progeny from these beetles. Summarized data obtained from this test are given in Table 4.

The slope of the log time-probit line in Fig. 4 indicates that the effect of DDT on the longevity of beetles of the Baton Rouge strain is greater than the effect on the Boyce strain.

The log dose-probit line for this experiment is shown in Fig. 5. The slope of the line indicates more homogeneity in response to DDT treatment in the Baton Rouge population than in the Boyce population. The LD_{50} was 13.7 μg/beetle (1125 mg/kg), which is significantly less than that for the Boyce population. This result suggests that *C. maculata* is not "naturally resistant" to DDT, but that resistance has developed in the Boyce strain, or selection for susceptibility in the Baton Rouge laboratory population occurred during the rearing program.

Toxicity of Endrin to C. maculata *Collected at Boyce, La.*—Data obtained from topical treatment of diapausing and active adults with endrin are shown in Table 5. The log dose-probit lines for diapausing and active beetles are illustrated in Fig. 6. The LD_{50} was 5.8 μg/active beetle (472 mg/kg) and 5.6 μg/diapausing beetle (455 mg/kg). The slope of the line

Table 3.—The effect of 40 μg of DDT/beetle on the reproductive and survival potentials of *C. maculata* collected at Boyce, La., February 1963.

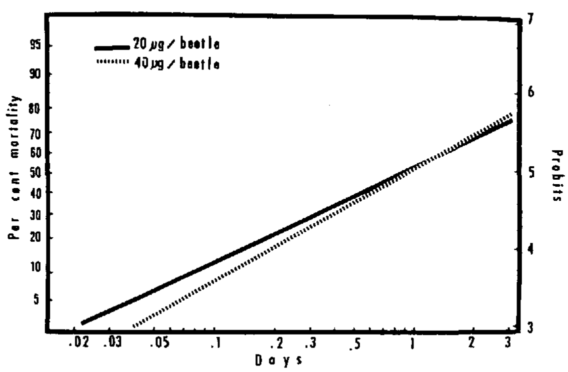
Category	No. pairs	No./pair				
		Egg batches	Eggs	Larvae	Pupae	Adults
Check	6	5.0±2.6	35.2±15.7	24.7± 7.6	13.5±3.4	13.0±3.2
Treated	6	6.2±3.5	58.8±31.3	46.2±24.6	10.8±6.1	9.7±4.8

Table 4.—Toxicity of DDT to 7th- and 8th-generation progeny of *C. maculata* collected at Baton Rouge, La., July 1962 and maintained in the laboratory until March 1963.

Days after treatment	μg of DTT/beetle							
	20		40		60		Check	
	Dead	Total	Dead	Total	Dead	Total	Dead	Total
0	0	30	0	30	0	30	0	30
1	18	30	17	30	30	30	0	30
2	19	30	18	30	30	30	0	30
3	21	30	23	30	30	30	0	30
5	26	30	27	30	30	30	0	30
7	26	30	27	30	30	30	0	30
14	29	30	29	30	30	30	0	30

indicates a homogeneous response to endrin in both active and diapausing animals.

The Effect of Endrin on Longevity.—The effect of endrin on the longevity of diapausing beetles is illustrated in Fig. 7. At a very low dosage ($LD_{0.4}$) endrin had no effect on longevity of the beetles which survived the treatment. However, when the dosage was LD_5 (2 μg endrin/beetle) an effect on longevity became obvious. Beyond this point the higher the dosage, the greater the effect of endrin on adult longevity.



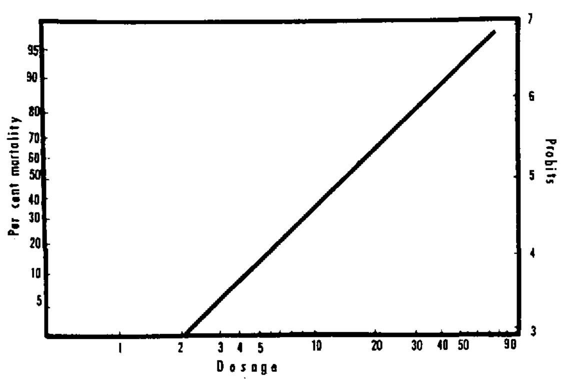
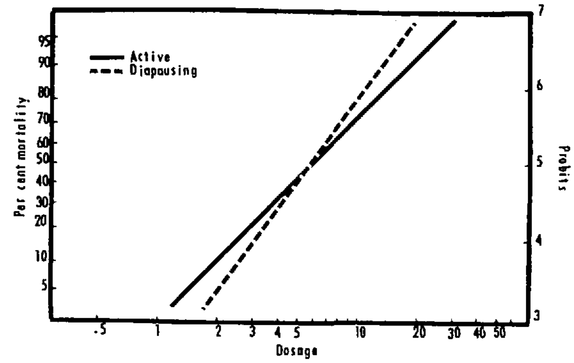
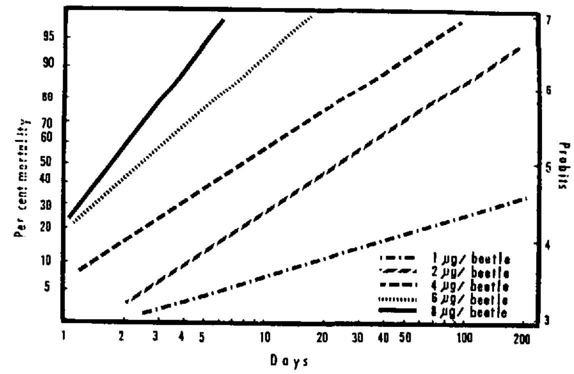


FIG. 4. (top)—Log time-probit lines for DDT-treated *C. maculata* (active) collected at Baton Rouge, La., and reared in the laboratory for 8 generations.

FIG. 5. (bottom)—Log dose-probit line for DDT-treated *C. maculata* (active) collected at Baton Rouge, La., and reared in the laboratory for 8 generations.





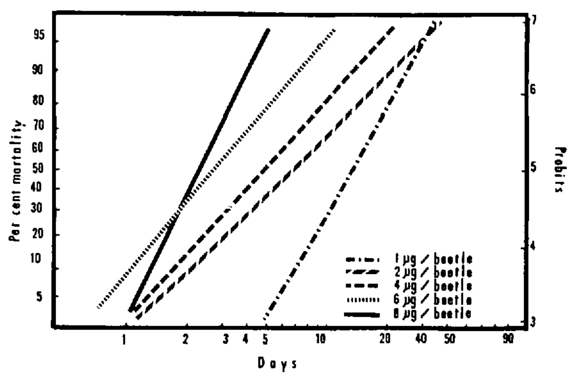


FIG. 6. (top)—Log dose-probit lines for endrin-treated *C. maculata* based on 72-hr observations. Boyce, La., February 1963.

FIG. 7. (center)—Log time-probit lines for endrin-treated *C. maculata* (diapausing) collected at Boyce, La., February 1963.

FIG. 8. (bottom)—Log time-probit lines for endrin-treated *C. maculata* (active) collected at Boyce, La., February 1963.

The log time-probit lines for active beetles are shown in Fig. 8. The slopes of the lines indicate that any of the endrin treatments used will affect the longevity of the beetles but that the effect is greater on active than on diapausing beetles.

The Effect of Endrin on Reproductive and Survival Potentials.—Adult males and females which survived the 2- and 4- μg treatments whether diapausing or active were held together in ½-gal ice cream cartons

Table 5.—Toxicity of endrin to diapausing and active adults of *C. maculata* collected at Boyce, La., February 1963. Beetles held at 26.7°C and observed for 168 hr.

	µg/beetle											
	1		2		4		6		8		Check	
Days after treatment	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total
					Diapausing							
0	0	60	0	60	0	60	0	60	0	60	0	60
1	1	60	0	60	3	60	10	60	15	60	0	60
2	1	60	3	60	12	60	24	60	31	60	0	60
3	1	60	3	60	14	60	34	60	45	60	0	60
5	2	60	6	60	22	60	45	60	59	60	0	60
7	4	60	11	60	28	60	51	60	59	60	0	60
					Active							
0	0	30	0	30	0	30	0	30	0	30	0	30
1	0	30	1	30	1	30	3	30	2	30	0	30
2	0	30	2	30	3	30	9	30	8	30	0	30
3	0	30	5	30	9	30	15	30	20	30	0	30
5	1	30	11	30	15	30	23	30	30	30	0	30
7	3	30	16	30	20	30	27	30	30	30	0	30

Table 6.—The effect of sublethal doses of endrin on the reproductive and survival potentials of *C. maculata* collected at Boyce, La., February 1963.

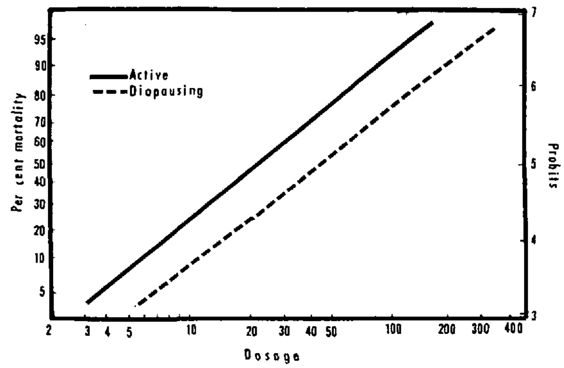
Category	No. pairs	No./pair				
		Egg batches	Eggs	Larvae	Pupae	Adults
Check	6	5.0±2.6	35.2±15.7	24.7±7.7	13.5±3.5	13.0±3.2
Treated	6	4.5±1.6	31.2± 6.6	21.8±4.9	14.5±2.9	14.2±2.9

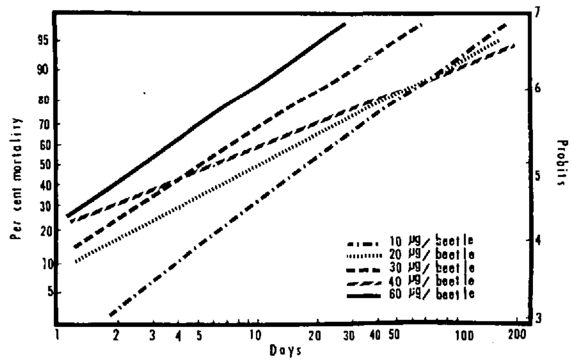
Table 7.—Toxicity of toxaphene to diapausing and active adults of *C. maculata* collected at Boyce, La., February 1963. Beetles held at 26.7°C and observed for 168 hr.

	µg/beetle											
	10		20		30		40		60		Check	
Days after treatment	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total
					Diapausing							
0	0	60	0	60	0	60	0	60	0	60	0	60
1	0	60	5	60	6	60	11	60	15	60	0	60
2	2	60	10	60	14	60	19	60	20	60	0	60
3	5	60	13	60	20	60	23	60	31	60	0	60
5	7	60	19	60	29	60	28	60	45	60	0	60
7	12	60	25	60	34	60	39	60	46	60	0	60
					Active							
0	0	60	0	60	0	60	0	60	0	60	0	60
1	3	60	13	60	22	60	19	60	11	40	0	60
2	9	60	24	60	35	60	39	60	21	40	0	60
3	12	60	28	60	40	60	47	60	27	40	0	60
5	18	60	37	60	50	60	58	60	38	40	0	60
7	26	60	41	60	55	60	60	60	39	40	0	60

with appropriate food and water. Untreated beetles were held in the same manner. The treated adults started mating 6 days after treatment. Six pairs from both the check and treatment were held in oviposition cages. A record for the number of egg batches and the number of eggs per batch was kept for each pair. Three factors might have interfered singly or collectively with the number of eggs laid by the treated females while not influencing the checks. They were: (1) the effect of the presence of endrin or its metabolites in the female's body; (2) selection might have occurred since not all individuals could survive the "sublethal" dose; (3) the effect on longevity of the beetles might have reduced the number of eggs laid by each female.

In any case endrin was the inducing agent. The





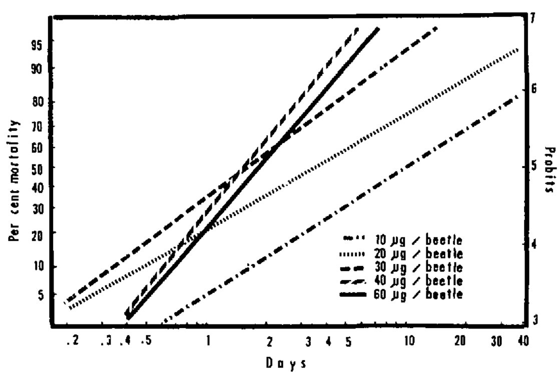


FIG. 9. (top)—Log dose-probit lines for toxaphene-treated *C. maculata* based on 72-hr observations. Boyce, La., February 1963.

FIG. 10. (center)—Log time-probit lines for toxaphene-treated *C. maculata* (diapausing) collected at Boyce, La., February 1963.

FIG. 11. (bottom)—Log time-probit lines for toxaphene-treated *C. maculata* (active) collected at Boyce, La., February 1963.

eggs were held until they hatched and larvae were reared individually to the adult stage. Data are summarized in Table 6. There was no significant difference between treated and untreated beetles.

Toxicity of Toxaphene to C. maculata *Collected at Boyce, La.*—Data obtained from treating diapausing and active adults with toxaphene topically are given in Table 7. The log dose-probit lines for diapausing and active beetles are illustrated in Fig. 9. The LD_{50} was 21.8 µg/active beetle (1789 mg/kg) and 44.2 µg/diapausing beetle (3619 mg/kg). These differences were statistically significant at the 0.05 level. The slopes of the lines indicate a heterogeneous response to toxaphene. This result suggests that the population may be in the process of developing resistance to toxaphene.

The Effect of Toxaphene on Longevity.—The log time-probit lines for diapausing beetles are shown in Fig. 10 and for active beetles in Fig. 11. All doses used affected the longevity of the diapausing beetles equally, but the higher the dose the greater the effect on longevity of active adults.

The Effect of Toxaphene on Reproductive and Survival Potentials.—Adult males and females which survived any of the treatments were held in their appropriate group in ½-gal ice cream cartons with food and water. The beetles started to copulate 15 days after treatment. Twenty pairs were isolated and held in oviposition cages. None of the females laid eggs. Egg laying was "normal" in untreated beetles.

The Toxicity of Toxaphene-DDT Mixture to C. maculata *Collected at Boyce, La.*—The effect of toxaphene-DDT mixture (2:1) on active adults was studied. A solution with a known concentration was first prepared for each of the insecticides. Appropriate amounts of the 2 solutions were mixed together so that each µliter of the resulting solution contained the desired dose per beetle. The doses used were 5:2.5, 10:5, and 20:10 µg/beetle. Data are summarized in Table 8.

The log dose-probit line for active beetles is illustrated in Fig. 12; the slope indicates homogeneity in response to the toxaphene-DDT mixture. The LD_{50} was 7.1–3.6 µg/active beetle (584 mg toxaphene and 292 mg DDT/kg.) According to the formula of Sun and Johnson (1960) the co-toxicity coefficient of the mixture equals 485, indicating a strong synergistic effect.

The Effect of Toxaphene-DDT Mixture on Longevity.—The log time-probit lines for active beetles are shown in Fig. 13. Longevity was drastically affected by the mixture of toxaphene-DDT. The highest dose killed the beetles so rapidly that the lower end of the line moved up, while the upper points did not move up so fast because the percent mortality was already more than 90 on the 3rd day. This is the reason for the decreasing value of slope at a very high dose. Thus, the effect of an insecticide on the longevity of any insect should be better studied on low or moderate doses and not on high doses if the log time-probit line is to be used for interpreting the data.

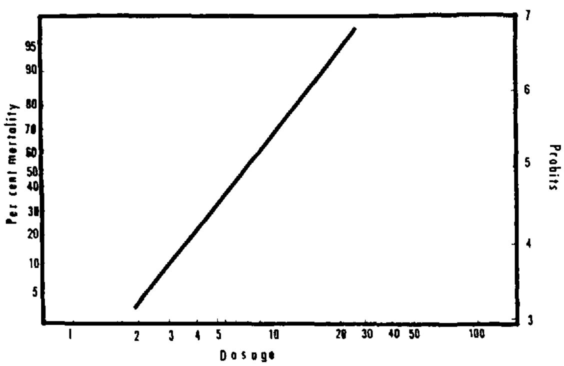
Table 8.—Toxicity of toxaphene-DDT mixture to active adults of *C maculata* collected at Boyce, La., January 1963. Beetles held at 26.7°C and observed for 168 hr.

Days after treatment	µg/beetle					
	5:2.5		10:5		20:10	
	Dead	Total	Dead	Total	Dead	Total
0	0	30	0	30	0	30
1	4	27	17	27	24	27
2	7	27	19	27	24	27
3	7	27	19	27	21	27
5	17	26	23	26	25	26
7	22	26	25	26	26	26

Table 9.—The effect of a sublethal dose of toxaphene-DDT mixture on the reproduction and survival potentials of *C. maculata* collected at Boyce, La., February 1963.

Category	No. pairs	No./pair				
		Egg batches	Eggs	Larvae	Pupae	Adults
Check	6	5.0±2.6	35.2±15.7	24.7±7.7	13.5±3.5	13.0±3.2
Treated	6	3.5±1.1	22.8± 5.7	16.2±4.3	8.0±1.4	7.8±1.6

The Effect of Toxaphene-DDT Mixture on Reproductive and Survival Potentials.—Survivors of treatment with 5–2.5 toxaphene-DDT mixture were held in ½-gal ice cream cartons and treated the same way as in the study of the effect of DDT on the reproductive and survival potentials. The data are summarized in Table 9. There was no significant effect of toxaphene-DDT treatment on the reproductive potential of the treated females. However, they laid 35% fewer eggs than the untreated beetles.



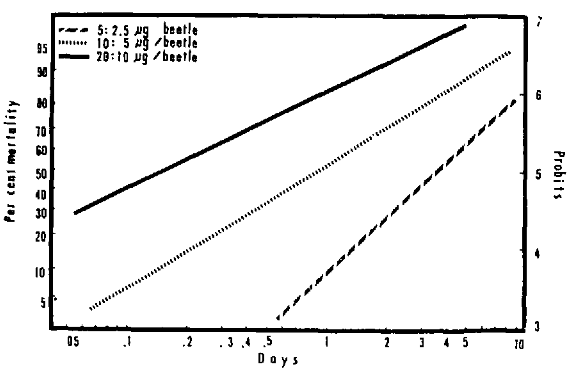


FIG. 12. (top)—Log dose-probit line for active *C. maculata* treated with toxaphene-DDT mixture based on 72-hr observations. Boyce, La., 1963.

FIG. 13. (bottom)—Log time-probit lines for active *C. maculata* treated with toxaphene-DDT mixture collected at Boyce, La., February 1963.

The toxaphene-DDT treatment reduced the survival potential of the F_1 and the progeny production of the treated beetles significantly at the 0.01 confidence level.

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