THE TOXIC EFFECTS OF NAPHTHALENE ON BRUCHUS OBTECTUS AND TENEBRIO MOLITOR IN VARIOUS STAGES OF DEVELOPMENT[1]

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INTRODUCTION

Although naphthalene has been in use as an insecticide for about 50 years, knowledge of its toxic effects is based mainly on investigations conducted under uncontrolled conditions. Very little is known of the physiological effects of naphthalene on insects. In late years naphthalene has been found useful against an increasing variety of insect pests, but it is probably most effective in killing or repelling insects found in houses, greenhouses, stored products, and in the soil.

The period of exposure necessary to kill the immature forms of the Japanese beetle in a saturated atmosphere of naphthalene depends on the temperature (Fleming and Baker (*3*)).[2] It ranges from 12 hours at 80° F. to 120 hours at 50°. The relative humidity of the atmosphere also influences insecticidal action on the larvae, mortality increasing with an increase in relative humidity.

Herrick and Griswold (*5*) found that naphthalene inhibited the development of the eggs of clothes moths, no eggs hatching after an exposure of 14 days in an enclosed space at room temperatures. Hartzell and Wilcoxin (*4*) observed that naphthalene was toxic to the eggs of the red spider mite. Read (*8*) showed by laboratory experiments that at least 8 hours' exposure of red spider mite eggs to a saturated atmosphere was required to prevent hatching. A study of the comparative resistance of the larva, protonymph, deutonymph, and adult female to naphthalene vapor showed that there was a slight increase in resistance as the stages advanced from larva to adult. Fleming and Baker (*3*) found the resistance of immature stages of the Japanese beetle to increase in the following order: (1) larvae, (2) eggs, (3) pupae. It was observed by Mercier (*7*) that, when the pupae of the fly *Calliphora erythrocephala* Meig. had been exposed to naphthalene vapor, some of the first-generation descendants of the treated flies showed malformations, but in the second generation these malformations did not appear. Shull, Riley, and Richardson (*10*) found that naphthalene produced no apparent effects on the coagulation of the blood nor on the appearance of the blood cells in the oriental cockroach (*Blatta orientalis* L.): Fleming and Baker (*3*) observed that eggs and larvae of the Japanese beetle exposed to naphthalene vapor became reddish in color, the intensity of the color depending on the period of exposure. The vapor appeared to have a paralyzing effect on the larvae of the Japanese beetle. Toscano

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[2] Reference is made by number (italic) to Literature Cited, p. 712.

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Rico (*11*) also observed the paralyzing effect of naphthalene vapor on the parasitic roundworm (*Ascaris lumbricoides* L.). This paralysis was followed by death.

In view of the more extended use which might be made of naphthalene in the future, a clearer knowledge of the toxic effects of this material would be of much value to entomologists. For this reason investigations on the toxicity of naphthalene to various stages of the bean weevil and yellow mealworm have been conducted under controlled conditions, and the findings are here presented.

METHODS

The apparatus used in the experiments was a modification of that used by Lehman (*6*). Humidity was controlled by passing air through saturated solutions of sodium chloride to give the air stream a relative humidity of approximately 70 percent. The apparatus was placed in a chamber the temperature of which was held constant at 25° C. by the use of a bimetallic thermoregulator with a resistance unit as a source of heat. In all experiments air was passed through this apparatus at the rate of 14 liters per hour. To determine whether saturation of the air was obtained, U-tubes containing naphthalene were weighed and placed in the system and then reweighed after the air had passed through them for a definite length of time. The loss in weight of the U-tubes was the amount of naphthalene taken up by the air. The amount volatilizing in a definite volume of air checked closely with the calculations for the amounts of naphthalene in a saturated atmosphere published by Roark and Nelson (*9*).

Before the beginning of each experiment air was passed over the naphthalene for about one-half hour. The insects were then placed in the flasks and allowed to remain there the desired length of time in contact with a constant stream of air saturated with naphthalene. Flasks containing control insects were similarly treated except that they were not exposed to naphthalene vapor. At the conclusion of each experiment the insects were removed and placed in a rearing chamber at a constant temperature and humidity. All insects in their various stages before and after treatment were kept in the constant-temperature chamber at 25° C. with a relative humidity of approximately 60 percent.

Bean weevils (*Bruchus obtectus* Say) were reared on red kidney beans at a temperature of 25° C. and relative humidity of 60 percent. The cultures were started at weekly intervals so that a new batch of adults might emerge every week.

Larvae of the yellow mealworm (*Tenebrio molitor* L.) were reared on a dry, complete dog food in the form of a meal, while the adults were fed once a week on dog biscuits, canned dog food, and banana skins. The cultures were kept at a constant temperature of 27.5° C. and relative humidity of 70 percent.

EFFECTS OF NAPHTHALENE ON EGGS OF THE BEAN WEEVIL AND YELLOW MEALWORM

A series of experiments was undertaken to determine the toxicity of naphthalene vapor to the eggs of insects. An attempt was made to determine whether the toxicity varied with the age of the eggs and whether any general physiological effects were produced.

Bean weevil and yellow mealworm eggs, none of which were over 1 day old, were exposed to naphthalene vapor for varying periods of time. The percentage of eggs hatching in the checks was very constant, averaging 90 percent or more (table 1). Naphthalene was toxic to the eggs, the number which failed to hatch varying directly with the period of exposure to the vapor. The large mealworm eggs were affected much more readily when not more than 1 day old than were the small eggs of the bean weevil of the same age. Approximately the same lethal effects were obtained with eggs of the mealworm with only one-half the length of exposure given the bean-weevil eggs.

TABLE 1.—*Toxicity of naphthalene* [1] *to eggs of the bean weevil and the yellow mealworm at different time exposures*

Exposure	Trials	Check		Treated	
		Total eggs	Hatched	Total eggs	Hatched
BEAN-WEEVIL EGGS 0 TO 1 DAY OLD					
Hours	*Number*	*Number*	*Percent*	*Number*	*Percent*
1	3	360	96	1,006	87
2	3	400	91	1,000	80
3	9	1,000	93	2,300	45
4	4	600	91	1,200	14
5	4	500	92	1,300	6
6	4	400	95	1,200	.4
MEALWORM EGGS 0 TO 1 DAY OLD					
½	2	200	90	600	85
1	3	300	89	700	72
1½	3	300	89	800	48
2	3	300	91	1,000	25
2½	3	300	85	900	8
3	5	455	91	735	1

[1] Naphthalene-saturated air stream at 25° C.; relative humidity, 70 percent.

To determine the relation between the stage of development of an insect egg and its susceptibility to the vapor of naphthalene, eggs of both the bean weevil and the mealworm were exposed for 3-hour periods at different ages (table 2). A minimum of 500 eggs was used at each stage of development and several replicates and checks were run with each experiment. The percentage of bean-weevil eggs killed increased with age until the eggs were 4 to 5 days old, when the peak was reached. From that time on toxicity decreased and when the eggs were ready to hatch (6 to 7 days old) very few were killed. The reaction of mealworm eggs to naphthalene vapor was almost the reverse of that of the bean-weevil eggs. The gas was most lethal to eggs 1 day old, with a sudden decrease in toxicity thereafter and a slight increase just before hatching (7 to 8 days old). Newly emerged larvae showed greater resistance than did the eggs to the vapors of naphthalene. These results indicate that the age or stage of development of an insect egg influences its susceptibility to naphthalene.

TABLE 2.—*Age and percentage daily loss in weight of bean weevil and yellow mealworm eggs in relation to the toxicity of naphthalene vapors*

Age of eggs	Eggs dead after 3-hour exposure		Daily loss in weight of untreated normal eggs		Age of eggs	Eggs dead after 3-hour exposure		Daily loss in weight of untreated normal eggs	
	Bean weevil	Mealworm	Bean weevil	Mealworm		Bean weevil	Mealworm	Bean weevil	Mealworm
Days	*Percent*	*Percent*	*Percent*	*Percent*	*Days*	*Percent*	*Percent*	*Percent*	*Percent*
0 to 1	55	99	2.72	7.83	5 to 6	68	11	3.48	1.38
1 to 2	70	83	2.71	2.08	6 to 7	22	15	----------	1.68
2 to 3	71	38	2.77	1.51	7 to 8	----------	23	----------	2.04
3 to 4	81	12	3.67	1.18	8 to 9[1]	----------	0	----------	1.68
4 to 5	90	10	4.56	1.18	9 to 10[1]	----------	----------	----------	.96

[1] Larvae.

Cotton (*2*) has shown that the effects of fumigants on insects can be correlated with their respiratory metabolism. During the course of their development the insect eggs which were used in these experiments continuously lost weight, presumably through the loss of water and carbon dioxide. Since both water and carbon dioxide are the end products of respiratory metabolism it is believed that the percentage daily loss in weight of the eggs can be used as a relative measure of the rate of respiratory metabolism.

Normal bean weevil and mealworm eggs were cleaned and weighed on a chemical balance and then placed in closed containers over a saturated solution of ammonium nitrate (60 percent relative humidity) at a temperature of 25° C. The eggs were weighed at 24-hour intervals until they hatched, and the percentage of daily loss in weight obtained (table 2). The data indicate that there is a definite relation between weight loss of the eggs (respiratory metabolism) and the lethal effects of naphthalene. A greater weight loss (respiratory metabolism) was associated with increased mortality of the eggs.

Naphthalene vapor in sublethal dosages prolonged the developmental period of bean weevil eggs. The mean number of days required to complete incubation for bean weevil eggs of different ages which survived exposure for 3 hours to a naphthalene-saturated atmosphere is shown in table 3.

TABLE 3.—*Mean number of days required to complete incubation for bean weevil eggs of different ages which survived 3-hour exposure to a naphthalene-saturated atmosphere*

Age of eggs when treated	Incubation period		Age of eggs when treated	Incubation period	
	Untreated	Treated		Untreated	Treated
Days	*Days*	*Days*	*Days*	*Days*	*Days*
1	8.1	9.4	5	7.7	9.1
2	8.0	9.2	6	7.8	8.8
3	7.8	9.1	7	8.0	8.4
4	7.8	9.6			

Exposure in early stages of development delayed hatching of those eggs that survived for more than a day. The greatest delay occurred when eggs were 3 to 4 days old at about which time naphthalene was most toxic (table 2). When the eggs were ready to hatch (6 to 7 days old) the vapor delayed hatching very little. Those larvae that survived 3-hour exposure to naphthalene were apparently uninjured. The incubation period of the eggs of the mealworm was not significantly affected by the naphthalene treatment. However, the treated eggs, previously creamy white in color, assumed a pink or reddish-brown shade, this color being more prominent in the eggs which had been exposed for the longest period. An examination of the newly emerged larvae from such eggs indicated that this discoloration occurred in the large mid-intestine. No discoloration was observed in other parts of the body as Fleming and Baker (*3*) had found in larvae of the Japanese beetle. The larvae appeared normal in all other respects and the colored matter disappeared after a few days.

EFFECTS OF NAPHTHALENE ON ADVANCED STAGES OF THE YELLOW MEALWORM AND ADULTS OF OTHER SPECIES

Previous data (table 2) showed that after the mealworm eggs had hatched, there was a decided increase in the resistance of the larvae to the lethal effects of naphthalene vapor. Since considerable variation in size was noted among larvae of the same age in the stock cultures, treatments were made with different sized larvae of the same age to determine whether the toxicity of naphthalene vapor was related to the size of the larvae. Larvae of different ages but of the same size were also treated to see whether the age of the larvae affected their susceptibility to naphthalene vapor. After treatment larvae were placed in the constant temperature and humidity cabinet for 48 hours, when the numbers of living and dead insects were recorded. The data (table 4) show that both weight and age influenced larval susceptibility to naphthalene. Sublethal exposures to naphthalene vapor of the newly emerged larvae of the mealworm did not affect subsequent growth as measured by gain in weight.

TABLE 4.—*Comparative toxicity of naphthalene to various stages of the yellow mealworm with special reference to size and age of larvae*

16-HOUR EXPOSURES

Stage	Age	Treated	Weight (average)	Weight loss (24 hours)	Dead
		Number	*Milligrams*	*Percent*	*Percent*
Larvae	2 days	50	0.47	5.95	100
	1 month	50	.77		98
	2 months	50	1.53		86
	do	25	8.37	2.62	32
	3 months	25	4.85		60
	do	25	11.32		48
	do	25	13.47	2.70	32
	do	25	20.32	2.12	16
	4 months	25	5.46	2.71	48
	do	25	36.00		4

24-HOUR EXPOSURES

Stage	Age	Treated	Weight (average)	Weight loss (24 hours)	Dead
Larvae	Full grown	50	152.70	0.28	20
Prepupae		50		2.26	94
Pupae	0 to 2 days	50		.62	38
	6 to 8 days	50			40
	8 to 10 days	30		1.19	86.6
Adults	0 to 7 days	30		2.06	100

Full-grown larvae, prepupae, pupae, and adults were exposed to naphthalene vapor for a period of 24 hours (table 4). Forty-eight hours after exposure the larvae and adults were examined and the number of dead recorded. Those unable to crawl were considered as dead. The pupae and prepupae which survived treatments were allowed to develop into adults; those that developed into abnormal adults (undeveloped elytra and pupal abdomens) were recorded as dead. Full-grown larvae were the most resistant to lethal effects of naphthalene vapor and adults were the least resistant. The prepupae showed marked susceptibility to the vapor, whereas the pupae showed marked resistance until a few days before emerging as adults, when their susceptibility increased greatly. It is interesting to note that the stages which lost the greatest weight in 24 hours were the ones to which naphthalene was the most toxic. About 48 hours after treatment the affected pupae began to turn dark until they became chocolate colored, failed to respond when touched, and finally died. The reaction to naphthalene vapor was very gradual and the nervous and muscular systems were not the first tissues to be affected.

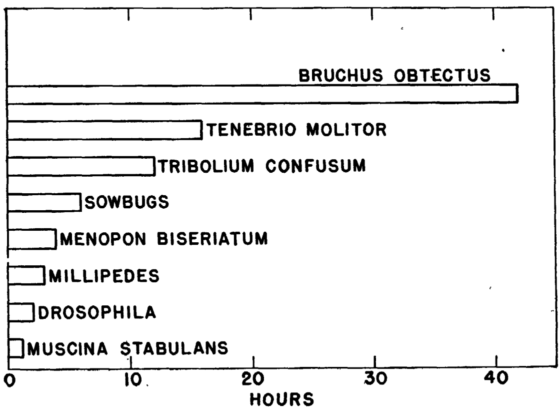

FIGURE 1.—The period of exposure required to kill approximately 100 percent of the species indicated.

Adults emerging from pupae subjected to sublethal dosages produced eggs which were not significantly different in number or fertility from the checks. A 6-hour treatment of adult mealworms had no effect on the fertility of eggs which surviving individuals laid, but it had a decided effect on the number produced. Control specimens produced 5.4 eggs per day per female, while in the treated lot 1.8 eggs per day per female were obtained.

For purposes of comparison several species of adult insects and closely related organisms were exposed to naphthalene vapors for varying periods of time and examined 48 hours after treatment. The time required to kill approximately 100 percent of the insects is shown in figure 1. Diptera were the most susceptible to naphthalene and Coleoptera the least susceptible of the insects used; in general it appeared that the most active insects were the most susceptible.

EFFECTS OF NAPHTHALENE INJECTIONS ON YELLOW MEALWORM PUPAE

It was thought that the injection of naphthalene into insects might yield some added information concerning its toxicity. Mealworm pupae were selected for this experiment because they were uniform in size (weight) and were easily handled.

Olive oil was used as a solvent for naphthalene since injection tests with olive oil alone had shown it to be nontoxic to the pupae when injected in much larger amounts than those used with naphthalene. The injection apparatus was similar to that used by Campbell (*1*). For making injections a rubber tube 8 cm long was attached to the pipette and a screw clamp was attached about 2 cm from the other end. The pipette was filled by suction and the screw clamp tightened. The pupae were held ventral aspect upward and the point of the glass needle was inserted near the lateral margin between the fourth and fifth abdominal segments. The point was directed cephalad for a distance of about 3 mm parallel to the longitudinal axes of the pupae. Pressure was then applied to the rubber tube by the thumb and forefinger which drove the solution into the pupae. The bore of the needle was too small for any blood to enter and the capillary attraction of the small bore prevented the solution from being sucked back when the pressure was released on the rubber tube. After every one or two injections the screw clamp was loosened to release the tension on the liquid in the capillary and then tightened again. The injected pupae were then placed in the constant-temperature cabinet for observation.

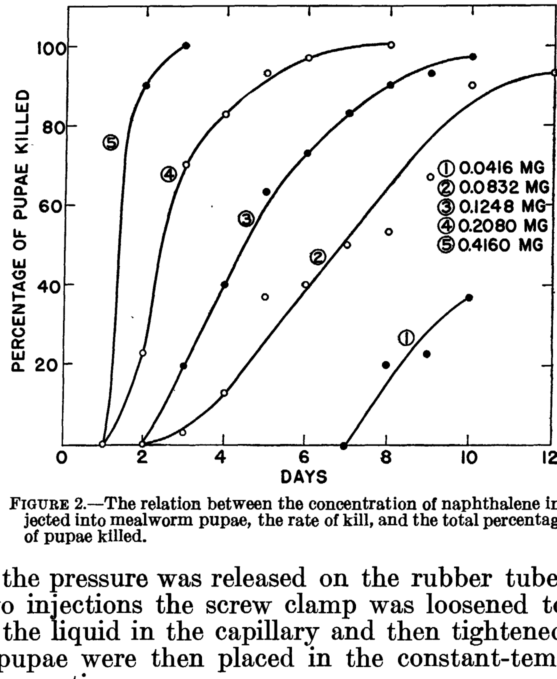

FIGURE 2.—The relation between the concentration of naphthalene injected into mealworm pupae, the rate of kill, and the total percentage of pupae killed.

Most of the injections were made without visible loss of blood. Pupae up to 2 days of age were used in all the injection experiments. Those that developed into abnormal adults were considered dead. Thirty pupae were used in each experiment. In the check, injected with olive oil alone, 10 percent failed to develop into normal adults. Figure 2 shows the relation between the concentration of naphthalene injected, the death rate, and the percentage of pupae ultimately killed.

The same toxic symptoms appeared in injected pupae as in pupae that were exposed for 24 hours to naphthalene vapor. Within a few days, the time depending upon the amount of material injected, the pupae began to turn dark but still responded to touch by wriggling. They lost all power of movement and were called dead when they finally became chocolate-colored. Examination of the body contents showed discolored plasma, fat, and muscles. All the tissues appeared to be undergoing disintegration. Naphthalene injected into the pupae did not produce materially different effects from those obtained with vapors.

From these injection experiments it seems that naphthalene vapor to be effective must be absorbed by the body tissues; that it is a slow-acting poison to the pupae of the mealworm; and that it affects other tissues before it affects the nervous or muscular systems. The fat bodies were probably the first tissues to be affected since they appeared to be partly disintegrated and discolored in treated larvae and pupae.

SUMMARY AND CONCLUSIONS

A study of the toxicity of naphthalene to the bean weevil (*Bruchus obtectus* Say) and the yellow mealworm (*Tenebrio molitor* L.) in their various stages of development is reported.

Experiments with the eggs of the bean weevil and the mealworm showed that toxicity varied with the age of the eggs. It was found that the rate of respiratory metabolism of the eggs measured by the percentage daily loss in weight was directly related to lethal dosages of naphthalene. An increased loss in weight was associated with a greater toxicity of naphthalene to the eggs. The development of bean weevil eggs was noticeably retarded by sublethal exposure to naphthalene vapor. The developmental rate of mealworm eggs was not significantly affected by sublethal exposures to naphthalene. Eggs and very young larvae of the mealworm when fumigated with naphthalene turned reddish brown, the color intensity varying directly with the period of exposure.

Experiments with larvae of the mealworm showed that the toxicity of naphthalene decreased with increased age and weight of the larvae. Sublethal exposures of mealworm eggs or young larvae to naphthalene did not affect subsequent growth.

Resistance to naphthalene varied greatly between instars of the more advanced stages of the mealworm. The order of susceptibility of all the stages of the mealworm to naphthalene from least to greatest resistance was: (1) Eggs, (2) young larvae, (3) adults, (4) prepupae, (5) pupae, (6) mature larvae. Increased respiratory metabolism as measured by percentage daily loss in weight appeared to be related to increased toxicity of naphthalene with the different stages of the mealworm.

In experiments with several species of adult insects and closely related organisms Diptera were most susceptible to naphthalene while Coleoptera were most resistant.

Injections of olive-oil solutions of naphthalene into mealworm pupae produced the same toxic effects as when the pupae were exposed to naphthalene vapor. Naphthalene was a slow-acting poison to the pupae even when injected into the body. The nervous and muscular systems were not the first tissues to be affected, as shown by the wriggling movements of darkened, dying pupae when touched. Fat bodies were probably the first tissues to be affected since they appeared to be partly disintegrated and discolored in treated larvae and pupae.

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