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I am submitting herewith a thesis written by Edgar Randall Merchant entitled "Comparative Toxicity of Petroleum and Two Synthetic Oils to the Large Milkweed Bug, Oncopeltus fasciatus." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

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COMPARATIVE TOXICITY OF PETROLEUM AND TWO
SYNTHETIC OILS TO THE LARGE MILKWEED
BUG, ONCOPELTUS FASCIATUS

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ABSTRACT

Effects of petroleum and two synthetic oils on mortality and reproduction of the large milkweed bug, Oncopeltus fasciatus (Dallas), were compared. Reproduction was investigated because of its importance in the maintenance of population levels and the sensitivity of the reproductive system to toxicants.

Adult milkweed bugs were dosed topically with the test oils to measure acute toxicity. Chronic toxicity was determined by mean survival after chronic exposure, starting with the fifth instar. The ability of sublethal levels of the test oils to affect reproduction was measured by the number of eggs laid and their percent hatch.

The two synthetic oils were found to be more acutely and chronically toxic than petroleum. Egg production was not affected by petroleum but was reduced approximately 20% by sublethal levels of both synthetic oils. Egg viability was not affected.

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CHAPTER I

INTRODUCTION

Insects are important in the functioning of most ecosystems. They are a dominant animal group, having more species than all other plant and animal species combined (Romoser, 1981). Insects are prominent in ecosystems as a source of food for other animals, as scavengers aiding the decomposition of plant and animal matter, as pollinators, and as soil enhancers that aerate and enrich soils.

Successful reproduction is of importance to the maintenance of any insect population, and as such, it is a critical target for the action of a toxicant. The success of insects is due in part to their relatively short life cycle and ability to increase rapidly in response to favorable conditions. Any factor that has a deleterious effect on reproductive parameters would likely have an impact on population growth.

Environmental safety evaluations for any new substance of unknown toxicity should include acute, chronic, and long-term reproductive studies. Acute toxicity tests determine the inherent ability of a substance to produce lethality, which facilitates comparison with other substances of known toxicities. Chronic toxicity testing involves repeated exposure of the test organism over a substantial portion of its lifetime, allowing for the expression of cumulative effects that might not be evident in an acute test. The reproductive system is likely to be sensitive to toxic agents (Casarett and Doull,

1975) and as such, should be included when evaluating the toxicity of unknown substances.

The evaluation of toxicity of complex chemical mixtures such as petroleum or synthetic oils presents special problems. Identification of the toxic components may be costly and time consuming. The biological activity of the toxic component may be modified by other components in the mixture. Synergistic and antagonistic interactions can not be excluded from consideration. Another problem is that the composition of the mixture may vary from batch to batch so that the results obtained with a particular sample may not be reproducible.

The purpose of this study was to compare the toxic and reproductive effects of petroleum and two synthetic oils on a terrestrial insect, the large milkweed bug, Oncopeltus fasciatus (Dallas). Three complex oil mixtures, crude petroleum and two synthetic oils, were selected for testing of their ability to affect reproduction. The acute toxicities of these three oils were determined by calculating the median lethal dose (LD₅₀) at 24 and 48 hours after topical application. Chronic toxicities were established by calculating mean survival over a 30-day period during contact with a thin film of the test oils. Effects on reproduction were determined by recording the duration of the fifth instar, number of eggs laid and the viability of these eggs.

Synthetic oils were selected for toxicity testing in conjunction with the operation of a synthetic fuel pilot plant for providing coal liquids. Data on the toxicity of synthetic oils from this small-scale

plant may be useful in improving the design and operation of commercial-scale facilities to reduce the toxicity of the product. Petroleum was included in this study for comparison since its toxicity has been better studied.

CHAPTER II

LITERATURE REVIEW

Reproductive Biology of *Oncopeltus fasciatus*

The large milkweed bug was originally termed *Lygaeus fasciatus* by Dallas in 1852, but was renamed *Oncopeltus fasciatus* by Stal in 1868. It is a seed bug in the family Lygaeidae in the order of true bugs Hemiptera. Its range extends over most of the U.S. south of the fortieth parallel and into Mexico and Brazil (Holmes, 1925). Annual migration is believed to occur, allowing the adult to survive the winter in a warm climate, and then to re-establish colonies further north during the summer (Simanton and Andre, 1935).

The milkweed bug is an excellent experimental animal and has been used in research since the 1930's. This insect is easy to raise in large numbers in the laboratory year around. Its large size facilitates many laboratory procedures, and it is resistant to disease. Its life cycle is sufficiently short (approximately 30 days at 30°C) to permit full life studies within a convenient time period. Many aspects of its biology, including reproduction, development, physiology and endocrinology, have been well studied.

Reproduction in the milkweed bug has been described by various authors and has been reviewed by Feir (1974). The sex of adult milkweed bugs can easily be determined. The male is usually smaller than the female and has a rounded genital capsule on the end of the posterior seventh abdominal segment; the female has a partially folded

ovipositor on this segment. The ventral side of the fourth abdominal segment of the female has a median protrusion on its posterior edge which is lacking on the male (Bonhag and Wick, 1953). Fifth instar females can be identified by black spots on the ventral side of the last two posterior segments while fifth instar males have black spots on the ventral side of only the last posterior segment (Feir, 1974).

Adult male milkweed bugs have two testes, each consisting of seven tubules arranged side by side. Morphologically abnormal testes are rare (Bonhag and Wick, 1953). Each tubule is divided by membranes into germ cells at various stages of development. The most anterior section contains the apical germarium and primary spermatogonia. Proceeding posteriorly, cysts are found containing secondary spermatogonia, primary spermatocytes and their secondary spermatocyte (each containing the haploid number of chromosomes, i.e., eight). These are followed by cysts containing spermatids in various stages of development. Spermatogonia and mature sperm fill the rest of the testicular tube and are not contained in a cyst (Bonhag and Wick, 1953). Each tubule contains about 18,000 sperm (Economopoulos and Gordon, 1971).

The testes develop during the last two instars and the early adult stage. There is a five-fold increase in testicular size during the fourth instar, mainly due to mitotic division of the spermatogonia. Meiotic division at the beginning of the fifth instar results in another three-fold increase in testicular size, plus spermatocyte differentiation. Maturation and production of sperm continues at a slower rate in the early adult (Economopoulos and Gordon, 1971).

Adult female milkweed bugs have two ovaries, each with seven ovarioles. As in most Hemiptera, the ovaries are telotrophic, with the trophocytes occupying an apical position in the ovaries. From anterior to posterior, the ovaries contain the germarium and trophocytes, the oogonia, and the young oocytes, still attached to the trophocytes by a nutritive cord. Enlargement of the oocytes in the vitellarium gives rise to a series of follicles which usually number four to six in a seven-day old adult (Bonhag and Wick, 1953).

Proliferation of the germ cells during the fourth instar of the female is similar to the fourth instar male. The ovarioles grow five times faster than the rest of the body, through mitotic divisions of the oogonia. During the last part of the fourth instar they differentiate into resting primary oocytes. Unlike the male, there is no further differentiation during the fifth instar: the primary oocyte remains in prophase until the mature egg is fertilized. The ovaries continue to grow during the fifth instar due to mitotic proliferation of the trophocytes which build up reserves for yolk formation.

The approximate duration of the egg stage at 30°C is four days and for each of the five stadia, six days. The adults live about 30 days at this temperature, although much variability is found. Longer photoperiod, higher temperatures, and lower population densities enhance reproduction and increase mortality (Feir, 1974). Temperature seems to be more important in development while photoperiod and density have more of an influence on mating and oviposition (Dingle, 1968).

The ovaries of the female contain approximately 11,000 eggs (Wick and Bonhag, 1955) and the testes of the male contain ca. 250,000 sperm (Economopoulos and Gordon, 1971). Males mate two or three days after molting to the adult and females five to twelve days. Frequent mating results in maximum egg production and fertility. Females lay 30 or more eggs per day after mating; virgin females have been known to lay small clusters of eggs at infrequent intervals (Gordon and Bandal, 1967). Sperm are deposited directly into the spermatheca by the male which permits the female to remain fertile for at least four weeks. Oviposition usually occurs five days after mating but can range from 1-15 days. The time between mating and oviposition increases with increasing age of the female while the percent egg hatch decreases (Richards and Kolderie, 1957).

The milkweed bug undergoes incomplete metamorphosis, the nymphs resembling the adults but lacking well-developed wings and reproductive organs. The five molts from first instar to adult are triggered by ecdysone produced in the prothoracic gland. This gland is stimulated by a hormone produced by neurosecretory cells in the brain and released from the corpora cardiaca. Juvenile hormone produced in the corpora allata prevents molting to the adult by early instars. Reduced levels of juvenile hormone in the fifth instar allows expression of adult characteristics in the next molt.

Effects of toxicants

A number of chemical as well as physical agents have been shown to be toxic to the large milkweed bug. Ultraviolet irradiation

(wavelength 254 nm) is especially effective in killing the egg stage (Beard, 1971). Aflatoxins, toxic metabolites of the fungus Aspergillus flavus, are toxic to adults when administered in their drinking water (Gaston and Llewellyn, 1980).

The toxicities of a variety of insecticides to the milkweed bug have been investigated. Jao and Gordon (1969) found a number of pyrethroids and carbamates to be toxic and suggested that detoxification in the milkweed bug is extremely slow. It is possible that its restricted diet and suctorial mode of feeding have not favored selection of a great variety of detoxication mechanisms found in many other insects (Gordon, 1961). The benzoylphenyl urea insecticide diflubenzuron is toxic to the milkweed bug presumably by blocking the terminal polymerization step of chitin formation in the integument. When applied to adults it also reduces egg production, egg hatchability, and viability of the first instar (Hajjar and Casida, 1978). Topical application with a triamide of phosphoric acid, tretamine, caused sterilization of adult male milkweed bugs with little effect on longevity, spermatogenesis, or mating (Economopoulos and Gordon, 1969).

Chemical disruption of reproduction in O. fasciatus has been demonstrated through interference with normal endocrine function. Recent discovery of a chemical in the plant Ageratum houstonianum that has anti-juvenile hormone activity on insects (Bowers et al., 1976) has led to investigations into its mode of action. Application of this chemical called precocene II (6,7-dimethoxy-2,2-dimethylchromene)

causes destruction of the corpora allata (Unnithan et al., 1977) and decreases levels of juvenile hormone causing precocious metamorphosis to the adult (Bowers et al., 1976). It also causes sterility in the adult female milkweed bug (Bowers et al., 1976) and reduced mating activity in the male (Walker, 1978). Masner et al. (1979) report that male milkweed bugs are fertile, even when precocene treatment inactivated the corpora allata.

Juvenile hormone from the corpora allata is known to be necessary for normal egg development (Johansson, 1958). Treatment of milkweed bug nymphs with precocene resulted in precocious females having well formed but nonfunctional ovaries (Bowers, et al., 1976). Bowers also found that treatment of newly emerged adult females prevented ovarian functioning. Exposure of inseminated adult females to precocene caused yolk reabsorption and degeneration of the ovaries to the undeveloped condition. All of the above effects were reversed upon the application of exogenous juvenile hormone, i.e., fertility was restored (Bowers, et al., 1976).

Effects of Fossil-Derived Oils

Previous studies have investigated the toxicities and reproductive effects of petroleum and synthetic oils on aquatic organisms. Short term effects of crude petroleum spills on marine populations include a heavy initial kill, inhibition of the recolonization of the oil-contaminated sediments, and eventual resettlement by the more resistant fauna (Blumer and Sass, 1972). Long-term effects of a crude

petroleum spill on the saltmarsh crab include behavioral disorders, locomoter impairment and population decline. Full recovery of the population can take as long as seven years (Krebs and Burns, 1977).

Sampling around offshore oil wells, a presumed source of crude petroleum, showed some morphological abnormalities in the gonads of a number of fish species, although no evidence was presented that the fish were actually exposed to crude petroleum leaks (Stott, et al., 1981).

Laboratory studies have shown that exposure to crude petroleum can interfere with reproduction. Ingestion of crude petroleum seriously reduced the rate of oviposition and the viability of fertilized duck eggs (Holmes, et al., 1978; Coon and Dieter, 1981). Short term exposure to high concentrations of water soluble fractions of aromatic heating oil caused a significant reduction in the life span, and in the number of eggs produced by an estuarine copepod (Berdugo, et al., 1977). Similar results were obtained with two species of amphipods (Lee et al., 1977).

The toxic effect of synthetic oils on a few species has been investigated. Studies by Giddings, et al. (1982) showed that synthetic oils were more toxic than petroleum to three aquatic organisms: the fresh water alga Selenastrum capricornutum, the zooplankton Daphnia magna and the midge larva Chironomus tentans. Some work has been done with synthetic oils on terrestrial insects. Acridine, found in the tar from coal gasification, has been shown to be highly toxic to cricket eggs (Walton, 1980). Treatment of the milkweed bug with

shale oil results in the development of males with morphologically abnormal testes (Walton, et al., 1982). These findings suggest that synthetic oils from coal may interfere with some aspect of reproduction in insects.

Comparative Composition of Test Oils

In general coal-derived synthetic oils have a higher concentration of aromatic and polar compounds, and have a higher nitrogen content than petroleum (Dooley, et al., 1980). They also contain higher levels of acidic components such as phenols and cresols, some of which appear in the low boiling distillate. Like petroleum, saturated paraffins dominate the low boiling fractions of coal-derived synthetic oils (Braunstein, 1977). Synthetic oils produced from coal by the H-Coal process contain paraffins, olefins, monocyclic and polycyclic aromatic compounds and phenols with aromatic compounds constituting the largest percent (Griest, et al., 1983), (Table C-2, Appendix C).

Population Biology

The rate of growth of any population can be defined simply as: $\frac{dN}{dt} = (b-d)N$ where b =birth rate, d =death rate, and N =number of individuals in the population. In practice $(b-d)$ is usually replaced by the constant " r ", the intrinsic rate of increase. The intrinsic rate of increase is usually modified by the physical or biotic environment so that under natural conditions the birth rate equals the death rate and the population size remains constant about a mean.

Milkweed bug populations exhibit a high intrinsic rate of increase, $r=0.0861$ under laboratory conditions (Dingle, 1968). In response to longer days, rising temperatures, and low population densities, early reproduction is promoted which increases r . This is highly adaptive for natural populations of milkweed bugs that migrate north every spring to colonize previously empty habitats. In the fall, population growth slows (lower r) for migration south to more favorable conditions (Dingle, 1968).

Slight changes in fecundity have pronounced effects on the growth rate of populations, more so than phenotypic switches in characteristics such as development rate and longevity (Lewontin, 1965). The effect of a reduction in fecundity would have on either laboratory or field populations of the large milkweed bug is not known. In any study, factors affecting fecundity such as food supply, photoperiod, population density, frequency of mating, and temperature would have to be considered. Nicholson (1954) showed that in closed laboratory populations of the sheep blowfly Lucilia cuprina, destruction of 50 percent of the eggs laid, more than doubled the mean adult population.

CHAPTER III

ACUTE TOXICITY

Materials and Methods

Insects. Stock colonies of the large milkweed bug, Oncopeltus fasciatus (Dallas), were started from field collections in Roane County, Tennessee, in the fall of 1980. Additional field collections were made during succeeding fall seasons and added to the stock colonies to maintain their genetic diversity. Each colony was housed in a clear plastic box, (40 x 25 x 15 cm) with a lid having small holes for ventilation. Dried seeds from the milkweed plant (Asclepias syriaca) and tap water were provided and changed weekly. The colonies were kept in incubators at $28 \pm 1^\circ\text{C}$ with a 16-hr photoperiod. Fluorescent gold light (wavelength > 500 nm) was used to prevent photooxidation of the test oils. Small cubes of cotton were provided for egg laying.

Oils. Two samples of unrefined H-Coal synthetic oil were collected October 29, 1981, from the H-Coal Synthetic Fuel Pilot Plant in Catlettsburg, Kentucky, for the Department of Energy's H-Coal Health and Environmental Program (Cowser, 1980). The first sample (#887) was a light weight oil produced under conditions of high temperature and pressure which contained high levels of phenols (Giddings, et. al., 1982). The second sample (#888), a heavy weight oil contained lower levels of phenols (Giddings, et al., 1982). These oils are not necessarily representative of commercial H-Coal products. Both synthetic

oils and a sample of crude petroleum (CRM 3.5) were obtained through the US EPA/DOE Fossil Fuels Research Materials Facility located at the Oak Ridge National Laboratory, Oak Ridge, Tennessee. All samples were stored in glass containers without light at 2°C.

Acute toxicities. Acute toxicity of the two synthetic oils was determined by topical application of the oils to the adult milkweed bugs followed by observations for mortality at 24 and 48 hrs. One hundred sixty-two adults that had molted from the fifth instar within the previous 7 days were separated from the stock colonies for experimentation. Ten were selected at random, anesthetized with CO₂ gas, and weighed individually to obtain an average weight (53.3 ± 9.3 mg). They were returned to the original group which was then randomly distributed into 8-oz. plastic-coated cardboard containers with plastic lids. There were six bugs per container; a total of 27 containers per oil tested were used. Each container was supplied with a few milkweed seeds and a piece of cotton soaked with water. Three containers were randomly assigned to each of the nine treatment groups which consisted of a control and doses of 10, 15, 20, 25, 30, 35, 40, and 45 mg of oil per g insect body weight.

The six bugs in each container were anesthetized with CO₂ gas, and the test oil was applied to the abdominal sternum using a hypodermic syringe attached to a power-driven ISCO microapplicator. Calculations of delivery volumes were based on the mean insect body weight, and the density of each oil as determined by weighing known volumes of the oils in Van Slyke volumetric flasks. Densities of the

oils were found to be: petroleum, 0.9273 ± 0.0047 g/mL; synthetic oil #887, 0.9344 ± 0.0032 g/mL; and synthetic oil #888, 0.9874 ± 0.0029 g/mL. A test group of bugs was treated with a volume of a non-toxic light mineral oil (density 0.8472 ± 0.0029 g/mL) equal to the largest volume of the test oil dispensed. This allowed determination of mortality due to equal volumes of a chemically inert oily substance on the body surface.

The treated bugs were held under the same conditions as the stock colony but in a separate incubator. Mortality was recorded after 24 and 48 hours. Two trials were performed to verify the results.

The viscosity of crude petroleum prevented it from being applied undiluted. Para-dioxane was used as a solvent for crude petroleum in a ratio of three parts crude petroleum to one part p-dioxane by weight. P-dioxane is a highly volatile, clear liquid which is a good solvent for non-polar liquids such as petroleum. The same procedures were used for dosing with the 3:1 mixture of crude petroleum and p-dioxane as with the H-Coal oils except that two instead of three containers of bugs were used at each dose. Doses used were: control, 20, 30, 40, 50, 60, 80, and 100 mg petroleum per g insect body weight. A test group of bugs was treated with 100 mg/g of the solvent p-dioxane to determine if the solvent was toxic.

Statistical analyses. The data were analyzed by the probit analysis method (Finney, 1971). Control mortality was corrected for using Abbott's formula (Abbott, 1925). A graphical representation was produced by plotting the probability transformation (probit) of the

percent mortality versus the logarithmic transformation of the dose. A line was fit to the data by the least squares method of linear regression, and the coefficient of determination (r^2) was calculated to indicate how closely the line fit the experimental data. A lethal dose to 50% of the population, LD₅₀, was computed from the equation for the line.

The significance of the difference between the LD₅₀ values for two experiments on the same oil was determined by the method of Stanley (1963); where the difference was not significant, the data were combined for recalculation of the LD₅₀ based on the pooled data. Ninety-five percent confidence limits were calculated (Sokol and Rohlf, 1969) in order to compare LD₅₀ values among experiments using different oils.

Results

The application of light mineral oil did not increase mortality over that of the untreated control group. Para-dioxane did not cause any additional mortality when applied at doses equal to the largest dose used as a solvent for petroleum.

For both synthetic oils the difference in LD₅₀ values between trials 1 and 2 were statistically insignificant (Stanley, 1963) after both 24 and 48 hours. LD₅₀ values and regression statistics calculated from the combined data from trials 1 and 2 are given in Table 1. A graphical representation of the results for both oils is given in Figures 1 and 2.

Table 1. LD₅₀ values and regression statistics for adult *O. fasciatus* 24 and 48 hours after treatment with synthetic oils #888 and #887.

Time after treatment (hr.)	LD ₅₀ (mg oil/g bug)	95% C.L. ^a	y-intercept	Slope	r ²
<u>Synthetic Oil #888</u>					
24	22.69	21.68-23.71	0.16	3.57	0.98
48	16.35	13.80-19.36	0.17	3.98	0.89
<u>Synthetic Oil #887</u>					
24	11.51	8.77-15.97	0.36	4.38	0.93
48	11.18	9.68-13.83	-0.14	4.90	0.96

^a 95% confidence limits around the LD₅₀

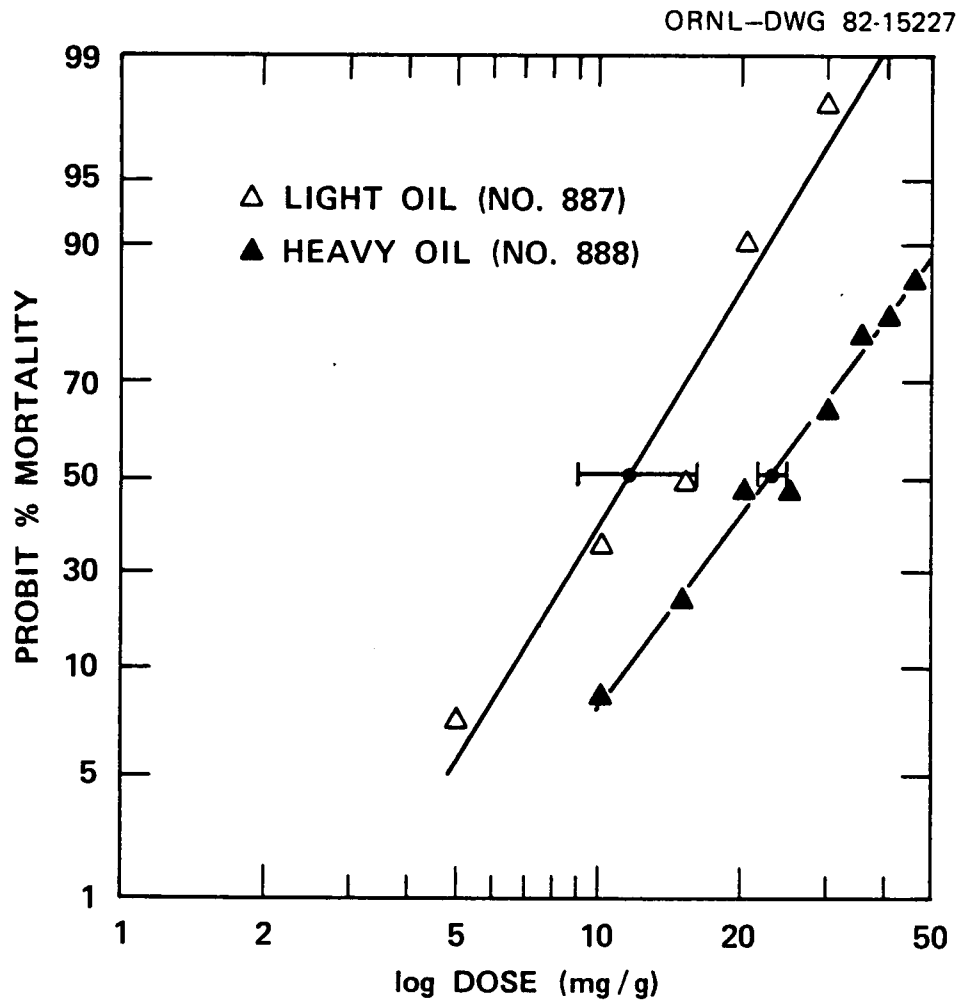


Figure 1. Acute toxicity of two synthetic oils applied topically to adult O. fasciatus, 24 hours after application.

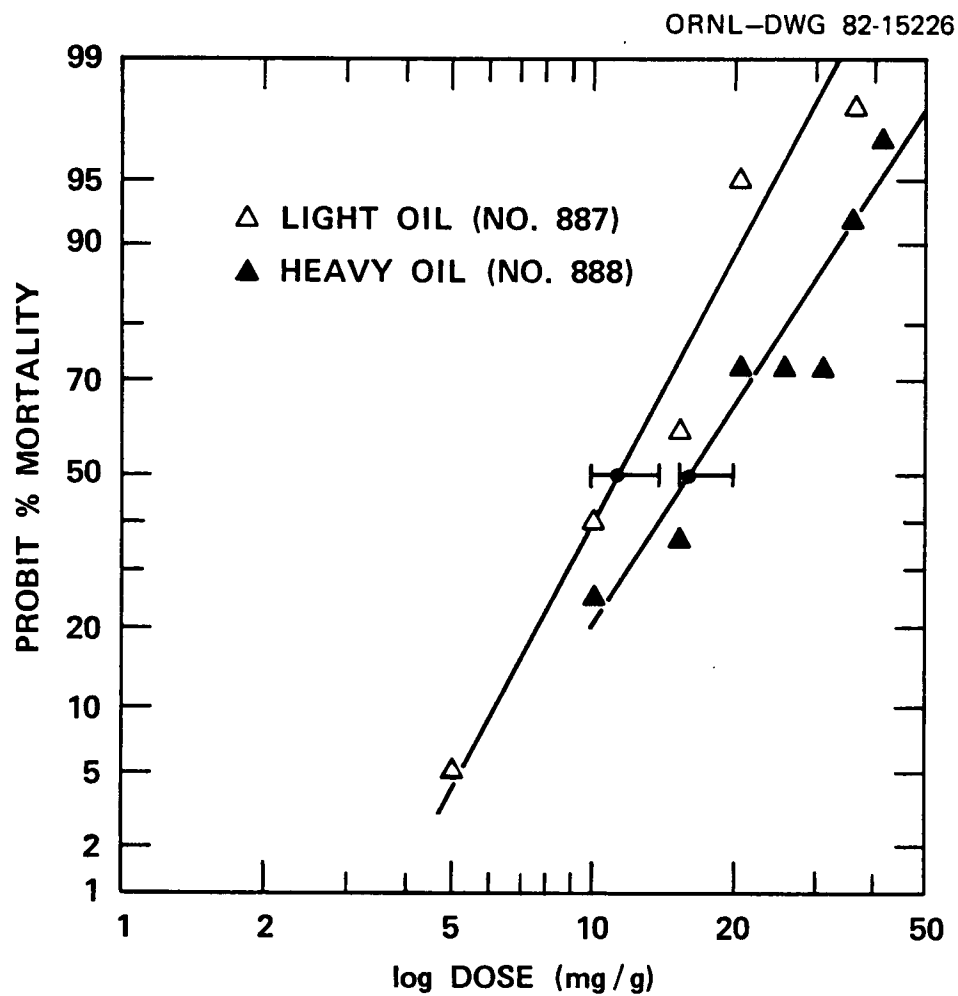


Figure 2. Acute toxicity of two synthetic oils applied topically to adult O. fasciatus, 48 hours after application.

The LD₅₀ for crude petroleum was estimated to be approximately 100 mg/g. Its high viscosity and low toxicity prevented a more precise determination of its acute toxicity. Undiluted petroleum was too viscous to be used in the microapplicator, and after dilution with p-dioxane, exceedingly large volumes were necessary to produce a toxic effect.

Discussion

The high phenolic content of synthetic oil #887 may be partially responsible for its greater toxicity than #888, since phenols are known to be toxic (Loomis, 1978). The water soluble fraction of synthetic oil #887 was found to be more acutely toxic than the water soluble fraction of #888 to crustacean Daphnia magna, the midge Chironomus tentans, and the alga Selenastrum capricornutum. The higher acute toxicity of the water soluble fraction of #887 compared with the water soluble fraction of #888 showed a strong correlation with the phenolic content (Giddings, et al., 1982).

For synthetic oil #888, the LD₅₀ decreased significantly between 24 and 48 hours. In contrast, the LD₅₀ for synthetic oil #887 changed very little after an additional 24 hours. One possible explanation for this would be detoxication or excretion of phenols in #887. Thus after the high initial mortality, few additional deaths would occur due to the rapid elimination of the phenols. The unknown toxic component in synthetic oil #888, although not as toxic initially as the toxic component in oil #887, may be more difficult to detoxify or eliminate and thus continue to exert a toxic effect over a longer

period. Another possible explanation for the difference in toxicity between the two synthetic oils might be a more rapid cuticular penetration by the toxicants in #887.

Comparing the 24-hour LD₅₀ values, synthetic oils #888 and #887 were approximately four and nine times more acutely toxic than crude petroleum, respectively. In general, crude H-Coal synthetic oils contain a higher concentration of polar and aromatic compounds than crude petroleum. The total amount of polar and aromatic compounds by weight percent in crude petroleum was 15.6 (Dooley, et al., 1980). For H-Coal synthetic oils #887 and #888, these weight percentages were 68.5 and 88.5, respectively (Griest, et al., 1983). These differences could serve as a starting point for fractionation of the test oils to determine the toxic component or components in each.

CHAPTER IV

CHRONIC TOXICITY

Materials and Methods

Oils. Test containers consisting of glass petri dishes, 9 cm in diameter, were prepared by pipeting 2 mL of a solution of the test oil dissolved in methylene chloride into the dish and swirling in a fume hood until the methylene chloride evaporated. This left a thin film of the test oil spread on the bottom of the petri dish.

A stock solution of 2 mg synthetic oil #888 per mL of methylene chloride was prepared and diluted in series to give solutions of 1 mg/mL and 0.5 mg/mL. All solutions were stored in the dark at 2°C. Since 2 mL of each dilution was pipeted into each dish, the doses per dish were 4, 2, and 1 mg. These doses were equivalent to 60, 30, and 15 μg of oil per cm^2 surface area of petri dish, respectively. The same doses were used for synthetic oil #887; doses of 750, 450, and 150 $\mu\text{g}/\text{cm}^2$ were used for petroleum. Based on the results of the acute toxicity test, doses of petroleum approximately ten times those of the synthetic oils would be necessary to cause a reduction in survival. Solvent control dishes for all of the test oils were prepared by evaporating 2 mL of methylene chloride per dish.

Insects. Mature fourth instars were separated from the stock colony. Within 24 hours of molting, the newly emerged fifth instars were separated by sex and placed in the treated glass petri dishes. Three males and three females were placed in each treated dish and

covered with a glass lid. Fresh milkweed seeds and water were provided and changed weekly.

Control and oil treated bugs were held under the same conditions as the stock colony but in a separate incubator. Observations for mortality were made daily. Every seven days the surviving bugs were transferred to new petri dishes with a fresh dose of the test oil. The tests were terminated after 30 days.

For synthetic oil #888, four petri dishes (3 males and 3 females each) were used per dose. For synthetic oil #887, two trials were run; the first with two dishes per dose and the second with four dishes per dose. Multiple trials were necessary due to the limited number of bugs at the correct stage at any one time. Three separate trials were performed for petroleum. The first consisted of five control dishes and four dishes at $150 \mu\text{g}/\text{cm}^2$. The second trial had four control dishes and four at a dose of $750 \mu\text{g}/\text{cm}^2$. The third trial consisted of nine control dishes, and seven each at 150, 450, and $750 \mu\text{g}/\text{cm}^2$.

Statistical analyses. Survival in days was recorded for each individual. Mean survival and standard errors were calculated at each dose for each trial. Mean survival at the same dose was compared between trials using a t-test (Sokal and Rohlf, 1981). Where the difference between the means in two trials at the same dose was not significant, the raw data were combined and a new survival and standard error were calculated. Differences in mean survival between different doses of the same oil were then compared first by their

standard errors, and second by analysis of variance and Duncan's Multiple Range Test (Duncan, 1955).

Results

Mean survival in the solvent control dishes was not significantly different from that of the untreated control. There was no apparent difference in mortality due to sex, so this parameter was disregarded during data analysis. For synthetic oil #887 and petroleum, the differences in mean survival between trials were not significant at any of the doses and the data were therefore combined for analyses.

Survivorship curves for all three test oils are shown in Figures 3-5. Mean survival and standard error are given for each test oil in Table 2. It appears that an exposure of 30 $\mu\text{g}/\text{cm}^2$ of synthetic oil #888 was necessary before there was a substantial reduction in mean survival. For synthetic oil #887, 60 $\mu\text{g}/\text{cm}^2$ was necessary to reduce mean survival from that of the control. An exposure of 150 $\mu\text{g}/\text{cm}^2$ of petroleum reduced mean survival. The increase in percent survival at 15 $\mu\text{g}/\text{cm}^2$ of #888 (Figure 3) and 30 $\mu\text{g}/\text{cm}^2$ of #887 (Figure 4) over that of the control was not statistically significant (Table 3).

The results of analysis of variance showed that variation in survival due to differences among dishes within each dose was not significant, but that the variation in survival due to different doses was ($p < 0.05$). The results of Duncan's Multiple Range Test are shown in Table 3. Since it took less synthetic oil #888 to reduce mean survival than synthetic oil #887, #888 can be considered more chronically toxic. Both synthetic oils were more chronically toxic than petroleum.

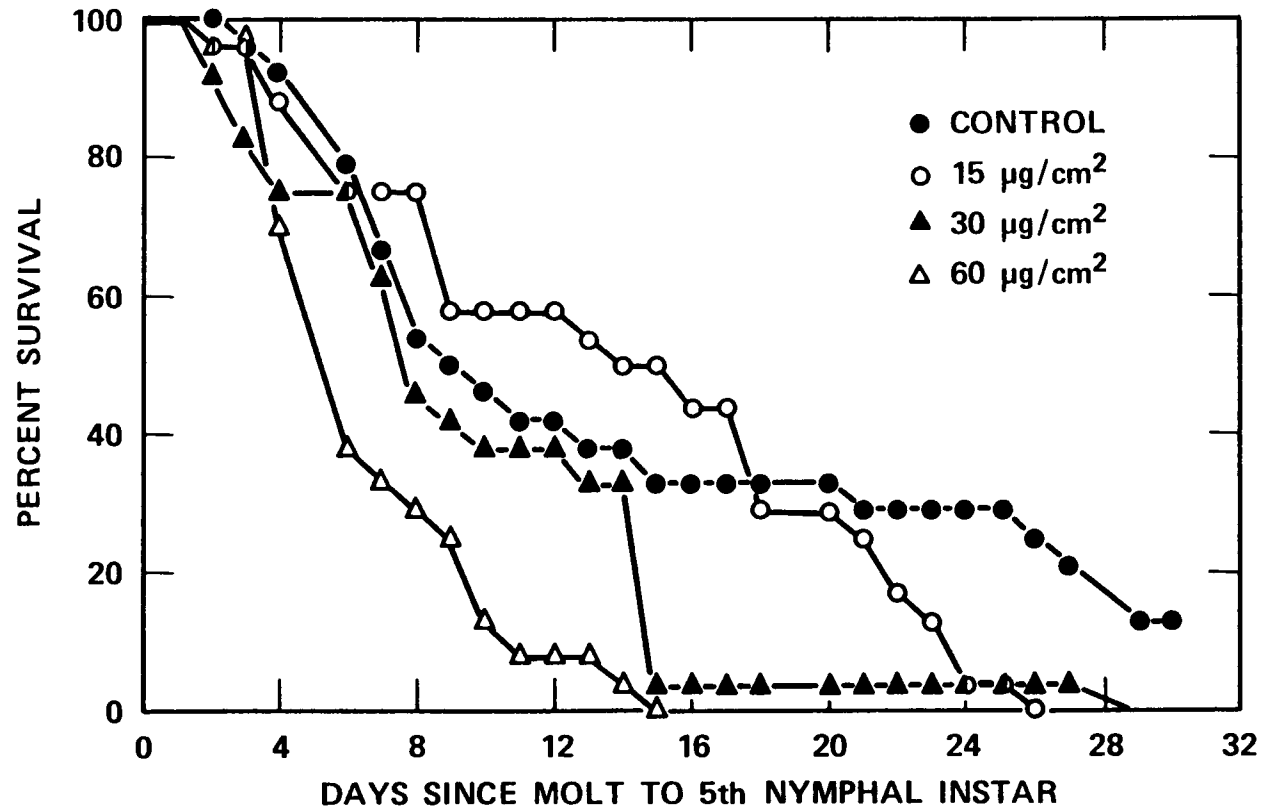


Figure 3. Percent survival of fifth instar *O. fasciatus* following 30 days of chronic exposure to synthetic oil #888. Treatment began at the molt to the fifth instar.

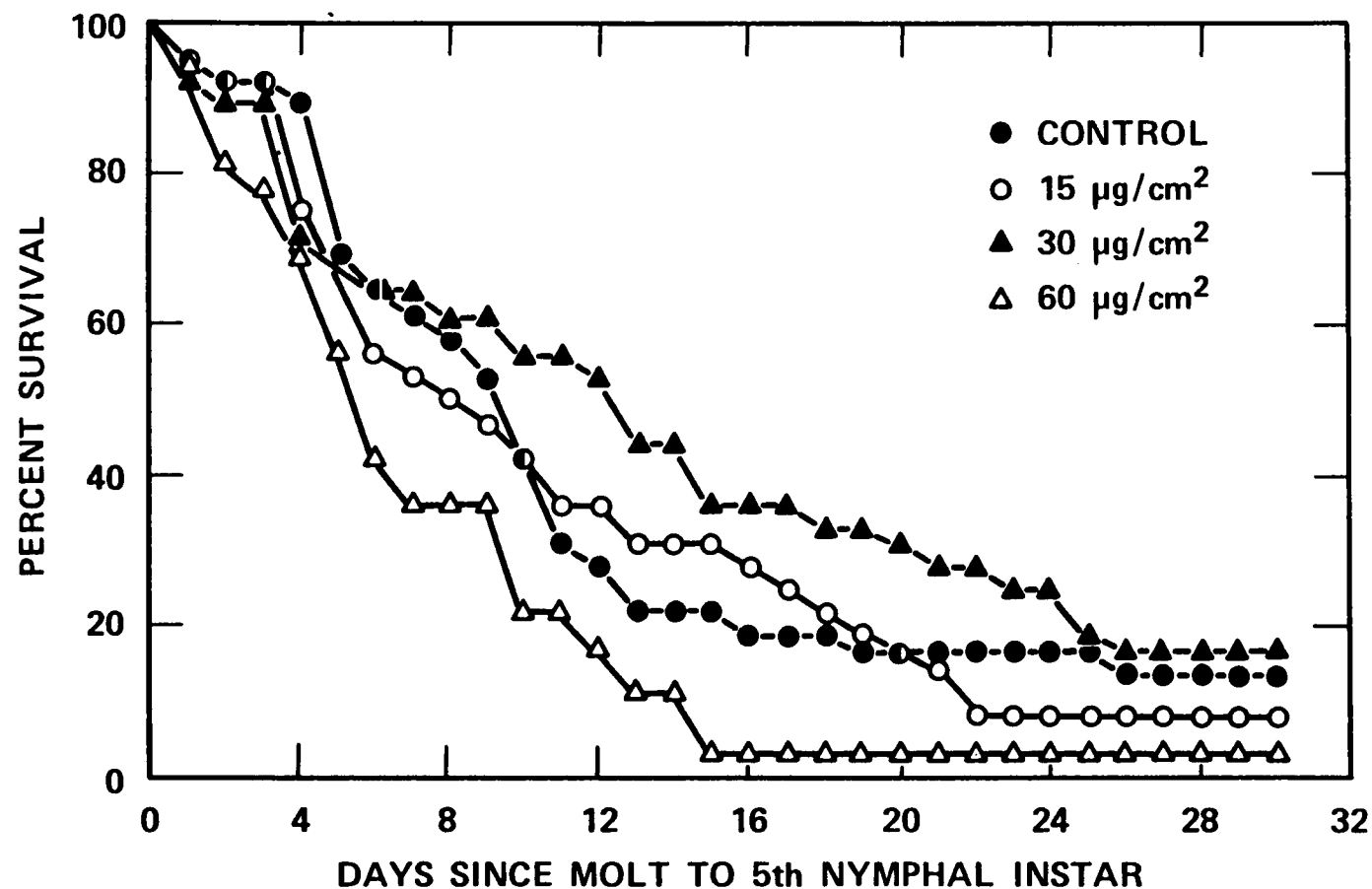


Figure 4. Percent survival of fifth instar *O. fasciatus* following 30 days of chronic exposure to synthetic oil #887. Treatment began at the molt to the fifth instar.

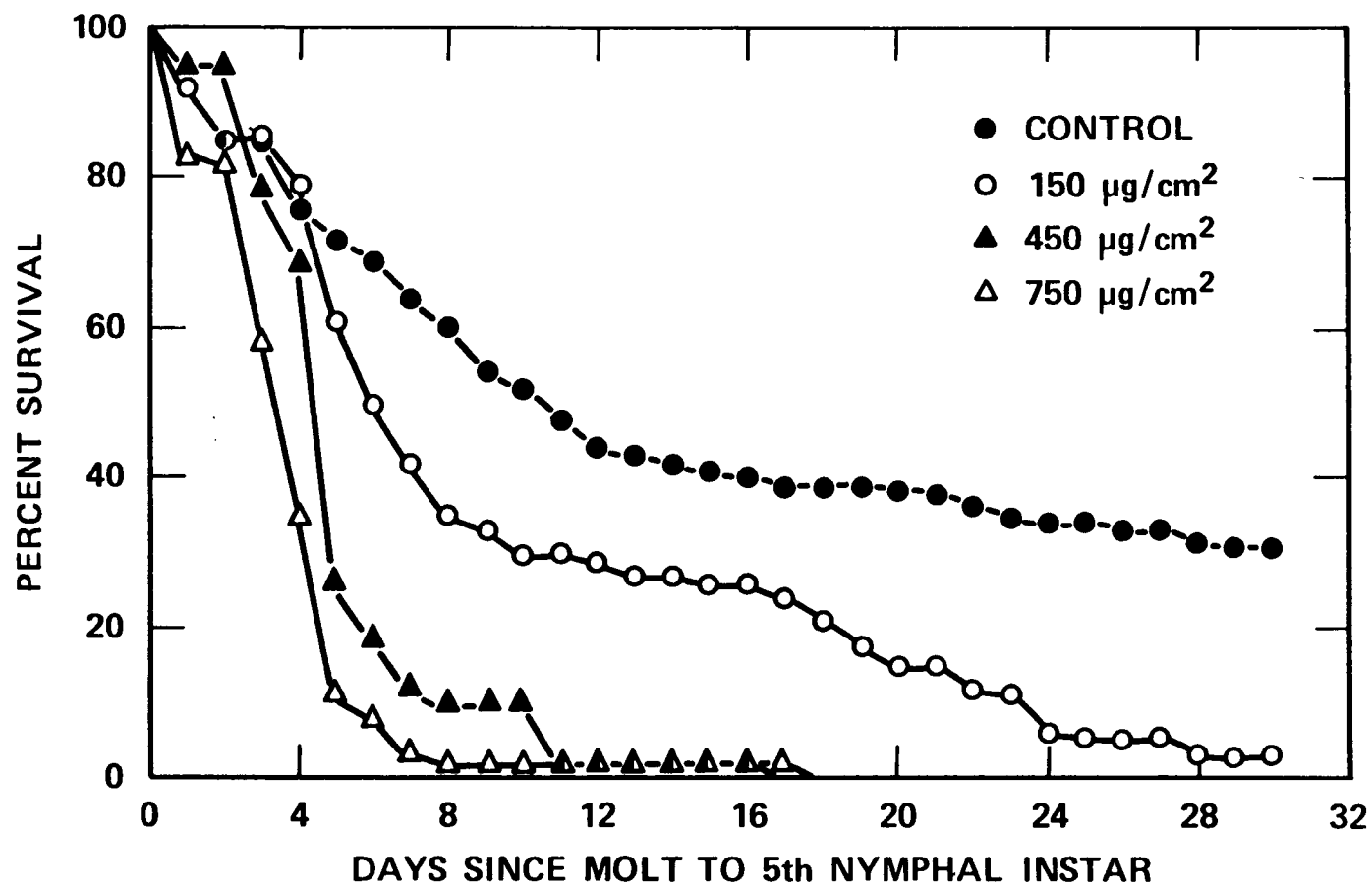


Figure 5. Percent survival of fifth instar O. fasciatus following 30 days of chronic exposure to crude petroleum. Treatment began at the molt to the fifth instar.

Table 2. Mean survival for *O. fasciatus* following 30 days of chronic exposure to the test oils.^a

Oil	Exposure ($\mu\text{g}/\text{cm}^2$)	N ^b	Mean Survival (days)	Range ($\pm$ standard error) of mean survival
888	0	24	12.8	10.7-14.9
	15	24	12.1	10.6-13.6
	30	24	7.9*	6.7- 9.1
	60	24	5.0*	4.3- 5.7
887	0	36	11.7	10.2-13.2
	15	36	10.9	9.5-12.4
	30	36	14.1	12.4-15.8
	60	36	7.5*	6.5- 8.5
Pet.	0	108	15.3	14.2-16.4
	150	66	10.0*	9.0-11.0
	450	42	5.4*	5.0- 5.8
	750	66	3.9*	3.6- 4.2

^a Exposure began at the onset of the fifth instar.

^b Number of bugs per exposure level.

* Mean is significantly different from the control at the 0.05 level.

Table 3. Duncan's Multiple Range Test on mean survival of O. fasciatus exposed chronically to crude petroleum and synthetic oils #888 and #887.

	<u>888</u>			
Dose ($\mu\text{g}/\text{cm}^2$)	60	30	15	0
Mean survival (days) ^a	<u>5.0</u>	<u>7.9</u>	<u>12.1</u>	<u>12.8</u>
	<u>887</u>			
Dose ($\mu\text{g}/\text{cm}^2$)	60	15	0	30
Mean survival (days)	<u>7.5</u>	<u>10.9</u>	<u>11.7</u>	<u>14.1</u>
	<u>Petroleum</u>			
Dose ($\mu\text{g}/\text{cm}^2$)	750	450	150	0
Mean survival (days)	<u>3.9</u>	<u>5.4</u>	<u>10.0</u>	<u>15.3</u>

^a Means that are underlined by the same line are not significantly different at the 0.05 level.

Discussion

Methylene chloride proved to be a good solvent for the test oils. The oils were readily soluble and chemical reactivity of the oils with methylene chloride is unlikely. The high volatility of the solvent made it possible to remove it from the treatment dishes before the bugs were added.

The relative order of toxicity of the two synthetic oils was reversed in this chronic test as compared with their acute toxicities. Synthetic oil #888 was found to be more toxic than #887 in the chronic test. The higher volatility of #887 may have resulted in a lower contact exposure than for #888 over the seven day period. The toxic components in #888 have yet to be determined, but the higher levels of polyaromatics as well as mono- and diaromatics (Griest, et al., 1983) may be important. The high levels of aromatic compounds in both synthetic oils, relative to petroleum (Dooley, et al., 1980), may also have been involved.

CHAPTER V

EFFECTS ON REPRODUCTION

Materials and Methods

Procedures similar to those used in testing the chronic toxicity of the test oils to the large milkweed bug were used to investigate potential effects of these oils on reproduction. Exposure levels were selected, based on the results of the chronic toxicity study, to have the maximum probability of affecting reproduction without causing significant mortality.

Oils. Solutions of 0.5 and 0.25 mg synthetic oil and 2.5 mg petroleum per mL of methylene chloride were prepared. As in the acute and chronic toxicity studies, approximately ten times as much petroleum as the synthetic oils was necessary to produce similar results. These solutions were stored in the dark at 2°C. Working in a fume hood, 2 mL of the solution was pipeted into each 9-cm glass petri dish and swirled to evaporate the methylene chloride solvent. The resulting exposure levels were 8 and 16 μg oil per cm^2 surface area of petri dish for the synthetic oils and 79 $\mu\text{g}/\text{cm}^2$ for petroleum. Control dishes were prepared by evaporating 2 mL of methylene chloride from each dish.

Insects. Fifth instars, 24 hours old or less, were obtained from the stock colony. Five individuals of the same sex were placed in each treated dish, which was then covered with a glass lid. Four

dishes of females and four dishes of males were prepared at each exposure level of synthetic oil #888. Five dishes of each sex per exposure level were used for both synthetic oil #887 and petroleum. More fifth instars were used at each dose for synthetic oil #887 and petroleum due to an increase in their availability. Fresh seeds and water were provided, and the bugs were returned to an incubator to be held at conditions identical to those of the stock colony. Observations were made daily for mortality and molting to the adult. The first 15 females that molted to the adult stage were removed daily and placed in individual 9-cm glass petri dish with a fresh application of the test oil. All of the males that molted to the adult stage were removed daily and placed in a 9-cm glass petri dish with a fresh application of the test oil. A maximum of five adult males were kept in each dish. This treatment insured that adults were exposed maximumly during early development of the adult reproductive system. On the third day after molting to the adult stage, one male was added to the petri dish with each female. Since it has been observed that egg production is about the same with either one or two males, only one male was used per female because female mortality is higher with two males (Gordon and Bandal, 1967). Males were not added until the third day to reduce female mortality caused by premature mating (Loher and Gordon, 1968). Fifteen male-female pairs were used per treatment level for each oil. A small cube of cotton for egg laying was added on the third day after the males were added. The cotton was replaced daily and the total number of eggs laid was recorded. These

eggs were kept separately in the same incubator and the percent hatch estimated after 5-7 days. The percent hatch was estimated to be within one of four classes: 0-25%, 26-50%, 51-75%, and 76-100%. The additional effort necessary to determine the actual percent hatch by counting individual first instars or empty egg shells was not found to be worthwhile since most egg clutches hatched successfully.

Every seven days, each male-female pair was moved to a new petri dish with fresh milkweed seeds and water and a fresh application of the test oil. Death of either individual of the pair ended egg collection from that pair. The test was terminated 30 days after the adult molt.

Statistical analyses. Mean duration of the fifth instar in the treatment and control groups was compared using a t-test (Sokal and Rohlf, 1981). Number of eggs laid by each female was averaged over day 10 to day 30 of adult life to estimate of the number of eggs laid per female per day. Where one individual of the male-female pair died, only the average egg production up to that time was used. These means were independent estimates of egg production at each dose. Non-homogeneous variance in mean egg production observed over treatment levels was corrected for using weighted estimates of mean egg production derived from the sample variance at each treatment level. This made it possible to apply the more appropriate weighted regression analysis rather than an unweighted regression analysis (Sokal and Roth, 1981). A second-order polynomial function of treatment level

was used to describe the fecundity relations for each of the synthetic oils.

To compare egg viability among exposure levels of the same oil, the fraction of the total number of days that egg hatch was in the 75-100% class was compared.

Results

An exposure of 16 $\mu\text{g}/\text{cm}^2$ of synthetic oil #887 caused about a one-day delay in the molt from the fifth instar to the adult (Table 4). There was no detectable delay at 8 $\mu\text{g}/\text{cm}^2$, nor with synthetic oil #888 or petroleum.

There was greater than expected mortality at the higher exposure levels of both synthetic oils. For oil #887 at 16 $\mu\text{g}/\text{cm}^2$, ten out of the original fifteen pairs survived to day 30 without one or both individuals dying. For synthetic oil #888 at 16 $\mu\text{g}/\text{cm}^2$, only three out of fifteen pairs survived the duration, although three pairs lost an individual the day before the end of the test.

The raw data on daily egg production are given in Appendix A. These data are summarized in Figure 6. Mean egg production at an exposure level of 79 $\mu\text{g}/\text{cm}^2$ petroleum did not differ significantly from the control. For oil #887, there was a decline in fecundity between treatment levels 8 and 16 $\mu\text{g}/\text{cm}^2$. For oil #888, this decline was between control and 8 $\mu\text{g}/\text{cm}^2$. From these data it appears that synthetic oil #888 caused a reduction in fecundity at a lower exposure level than #887.

Table 4. Mean duration of the fifth instar of O. fasciatus exposed to the test oils at the beginning of this stage.

Oil	Dose ($\mu\text{g}/\text{cm}^2$)	N ^a	Mean duration (days)	Range ($\pm$ standard error) of mean duration
888	0	35	6.80	6.69-6.91
	8	38	7.00	6.88-7.12
	16	33	7.03	6.92-7.14
887	0	50	6.14	6.08-6.20
	8	49	6.22	6.15-6.29
	16	50	7.14*	6.72-7.65
Pet.	0	49	6.16	6.09-6.23
	79	44	6.07	6.03-6.11

^a Number of bugs at each dose.

* Mean is significantly different from the control at the 0.05 level.

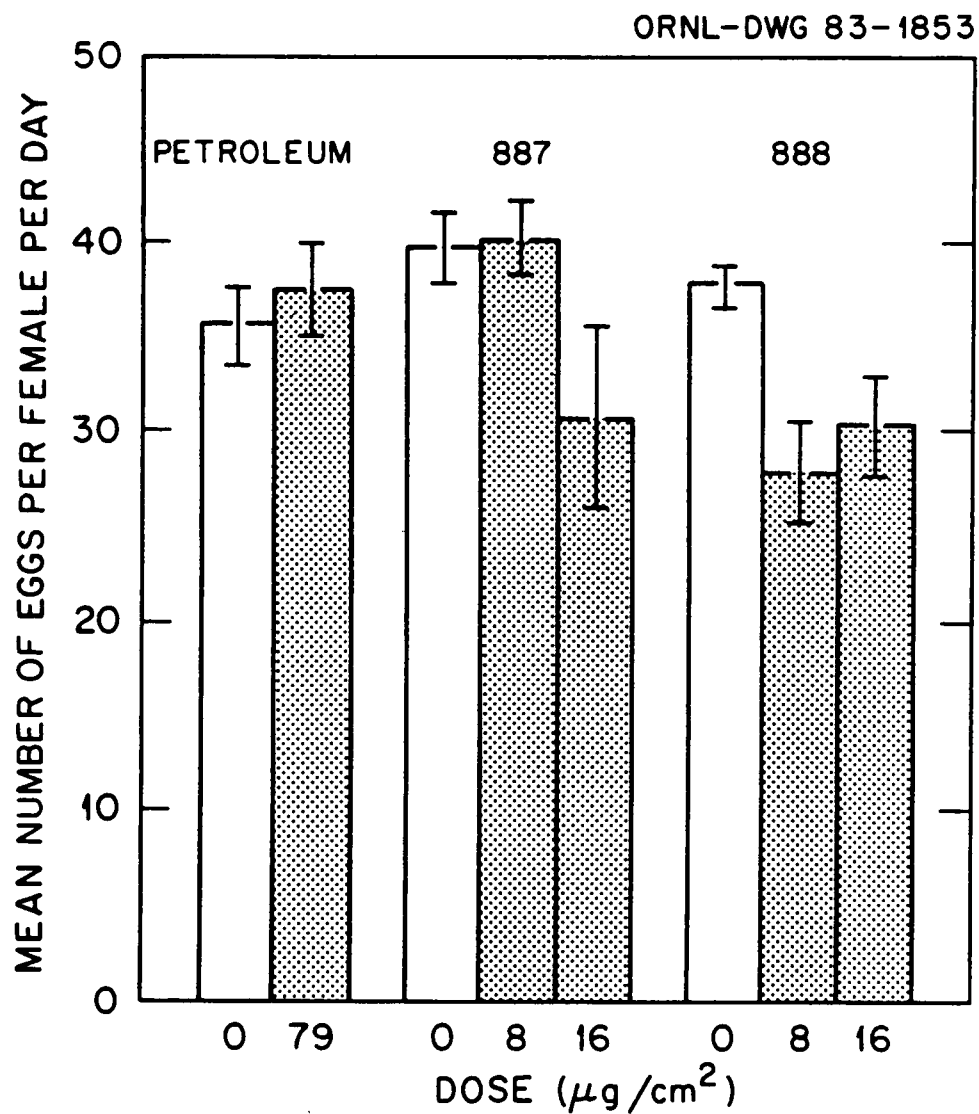


Figure 6. Mean daily egg production of *O. fasciatus* from day 10 to day 30 after the adult molt. A fresh application of the test oils was made every 7 days starting with the molt to the 5th instar.

Viability of the eggs that were laid, as measured by the percent hatch, was not affected at the exposure levels tested (Figure 7). Estimation of percent hatch for each male-female pair is given in Appendix B. Percent hatch was 75-100% on more than 80% of the days that eggs were laid. Variation in egg viability among oils is likely due to random differences among groups of adults that were used for each oil. The apparent reduction in viability of eggs at 8 $\mu\text{g}/\text{cm}^2$ of synthetic oil #888 was due to two pairs out of the fifteen that were apparently sterile. Normal numbers of eggs were laid but none ever hatched. Since the other thirteen pairs at this exposure level produced viable eggs, and no sterility occurred at 16 $\mu\text{g}/\text{cm}^2$, the overall effect on egg viability at this level was not considered different from that of the control.

Discussion

The milkweed bugs were exposed to the test oils during the stages when development and/or functioning of the reproductive system was most likely to be affected. Meiotic division of the sperm in the testes of the male, and mitotic proliferation of the trophocytes in the ovaries of the female, occur during the fifth instar and early adult (Bonhag and Wick, 1953).

Exposure levels of the test oils were selected for maximum probability of causing an adverse effect on reproduction without causing significant mortality. Sublethal levels were chosen for the reproductive toxicity test based on the results of the chronic

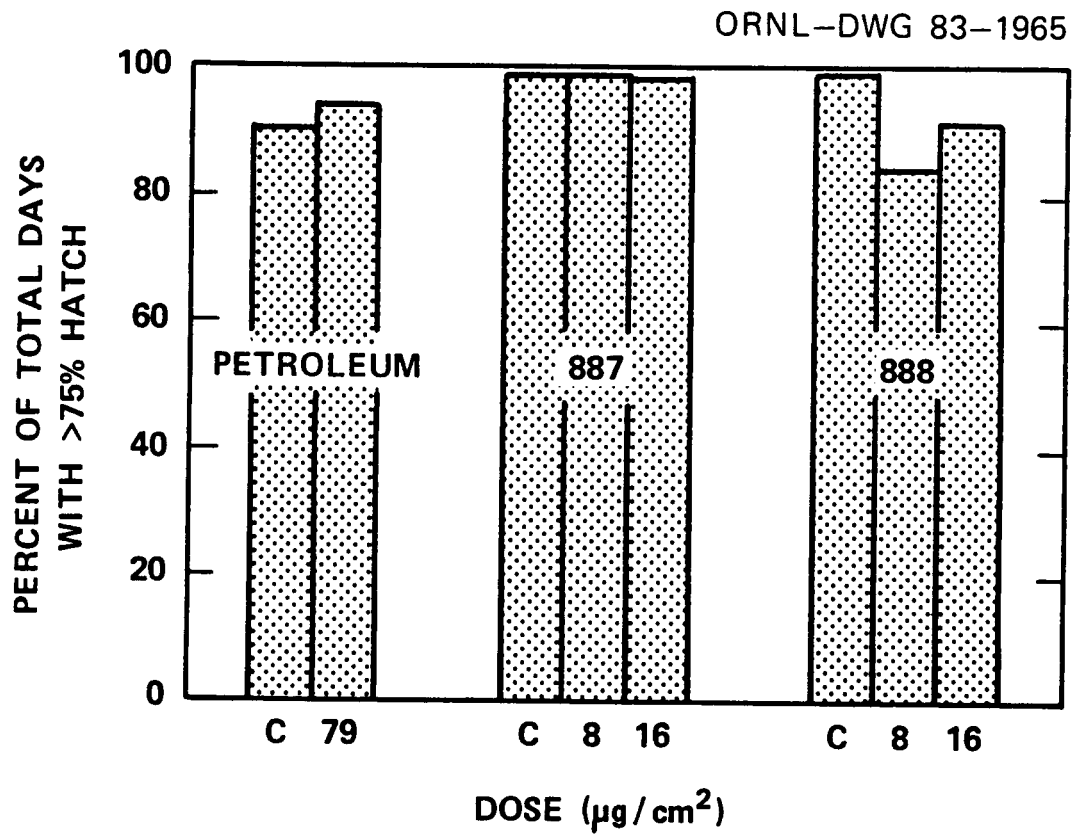


Figure 7. Viability of eggs from male-female pairs of *O. fasciatus* exposed repeatedly starting with the fifth instar.

toxicity test. The higher than expected mortality in the reproductive toxicity test at the higher level for both synthetic oils could have been the result of many factors. One reason may have been the longer exposure period during the reproductive study (36 days) versus the chronic toxicity study (30 days). The bugs in the reproductive study were subjected to one more application of the test oil than the bugs in the chronic toxicity test. Another reason may have been the higher frequency of mating in the reproductive study, which is known to reduce life expectancy (Lener, 1967). After the males and females had been combined in the chronic toxicity study, no attempt was made to control the male to female ratio. Some dishes ended up with two or three individuals of the same sex after all of the individuals of the other sex had died. This eliminated the stress associated with mating.

The duration of the fifth instar was investigated because preliminary testing had indicated that there might be a delay in the molt to the adult due to treatment. Although the observed delay of one day with the higher exposure of synthetic oil #887 may not appear important, a cumulative delay over all five molts from the egg to the adult could reduce the time that the adults are in the reproductive stage.

The approximate 20% reduction in egg production due to treatment with the synthetic oils could have important consequences for the viability of populations of milkweed bugs or other hemipterans. Testing field populations would be necessary to take into account all of the

complex physical and biological factors that control population levels.

The fact that the eggs laid were fertile and viable suggests that enough sperm were transferred upon mating to fertilize all of the eggs that were laid. Whether the reduction in egg production was caused by an effect of the synthetic oils on the male or the female or both can not be determined from this study. Possible modes of action could be a suppression of vitellogenesis in the female or a reduction in frequency of mating attempts by the male. Mating untreated females with treated males and vice versa should be performed to determine whether one or both sexes were being affected.

CHAPTER VI

CONCLUSIONS

The two synthetic oils tested were found to be more toxic to the large milkweed bug than crude petroleum. These two oils were more acutely and chronically toxic than petroleum and affected reproduction by reducing the number of eggs laid. The high aromatic and phenolic content of the synthetic oils is one possible reason for their higher toxicities.

Synthetic oil #887, a light weight oil, was more acutely toxic than synthetic oil #888, a heavy weight oil, based upon their LD₅₀ values. Both synthetic oils were more acutely toxic than petroleum.

Synthetic oil #888 was more chronically toxic than #887 when comparing mean survival of the milkweed bugs in contact with a thin film of the oils for 30 days. Both synthetic oils were more chronically toxic than petroleum.

Sublethal levels of both synthetic oils caused a decrease in the numbers of eggs that were laid. Synthetic oil #888 was effective at a lower exposure level than #887 in causing this reduction. Treatment with petroleum did not affect egg production. Egg viability, as measured by percent hatch, was not affected: almost all of the eggs that were laid hatched successfully.

It is difficult to predict without further experimentation whether the observed effects on reproduction would have a serious impact on natural populations of the large milkweed bug. Many complex

physical and biological factors act together to control population levels. Because of this complexity, direct effects of toxicants on one population can influence other populations indirectly: community structure can be altered by the loss of one species and the consequent change in species diversity. The effect that a 20 percent reduction in egg production would have on this species depends upon the interaction of this reduction with other environmental stresses on the population. Full life cycle exposure to the oils, field testing, and evaluation of population dynamics will be necessary for predicting the effects of synthetic oils on milkweed bug populations.

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APPENDIXES

APPENDIX A

Table A-1. Daily egg production by male-female pairs of O. fasciatus during exposure to crude petroleum (control).

REP ^a	D10 ^b	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	41	40	20	30	30	24	40	34	34	42	33	26	30	37	47	45	34	25	43	42	42
2	0	78	40	40	43	37	41	0	36	74	13	46	43	0	38	72	0	36	25	0	42
3	53	58	65	0	47	48	99	0	55	99	0	67	47	50	100	43	61	0	47	0	24
4	43	50	55	33	41	37	35	36	43	56	38	38	13	45	42	43	39	31	51	37	50
5	41	0	28	0	34	52	35	0	48	31	0	64	44	0	65	26	32	31	29	34	37
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	37	33	29	10	60	38	31	28	26	25	0	28	9	15	20	55	28	29	27	6	19
8	32	73	44	45	40	0	87	38	46	35	14	17	21	19	55	50	8	14	8	1	24
9	39	12	22	13	15	0	3	15	0	0	0	0	0	0	51	43	31	33	38	36	32
10	44	28	28	0	34	0	30	36	36	39	39	38	39	40	8	76	35	37	38	1	68
11	39	40	56	0	57	0	68	0	62	38	31	0	40	36	30	65	36	0	32	41	31
12	114	16	46	51	43	37	41	49	48	43	40	48	41	12	84	36	57	37	42	43	42
13	46	86	43	0	0	34	72	0	46	82	35	0	49	46	43	89	44	38	14	38	42
14	20	96	60	40	46	39	56	42	56	55	5	36	37	44	44	65	42	42	45	42	14
15	69	40	46	37	42	0	75	39	0	82	0	33	43	39	71	35	39	30	49	0	40

a "REP" equals the replicate number.

b "D" equals the number of days since the adult molt.

Table A-2. Daily egg production by male-female pairs of O. fasciatus during exposure to crude petroleum (79 $\mu\text{g}/\text{cm}^2$).

REP	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	41	51	41	0	35	0	95	42	50	52	0	50	47	51	93	54	49	39	55	0	50
2	0	0	0	0	0	58	44	42	43	36	31	39	32	37	33	37	57	39	0	69	0
3	0	48	82	0	75	0	64	20	0	70	0	41	34	40	43	69	31	41	39	35	34
4	53	0	93	41	119	36	57	46	48	39	41	4	47	69	0	91	42	39	40	47	33
5	72	51	0	0	67	0	69	0	31	71	0	34	47	33	30	74	36	38	33	0	0
6	0	0	0	0	0	0	0	0	0	61	0	65	0	62	0	25	0	65	36	0	6
7	22	11	36	64	17	0	68	41	55	32	34	23	43	38	41	49	34	31	36	0	12
8	83	56	53	0	88	43	52	46	15	77	0	45	80	18	32	48	51	36	37	0	34
9	29	41	42	0	81	0	89	36	57	33	37	48	33	0	83	28	12	48	20	15	0
10	64	48	56	14	86	48	60	51	57	33	37	48	33	0	83	28	12	48	20	15	0
11	0	0	0	0	67	40	42	53	26	46	51	11	83	40	55	55	46	39	43	50	43
12	71	6	82	0	32	20	47	38	47	32	51	37	71	0	0	45	36	8	64	35	0
13	101	23	77	0	101	52	58	58	45	46	56	13	74	0	75	52	38	40	31	32	0
14	47	13	74	40	35	13	59	0	66	43	13	66	44	8	38	69	41	7	71	13	58
15	40	20	96	43	35	10	79	45	51	50	40	46	49	8	42	82	57	2	76	50	9

a "REP" equals the replicate pair number.

b "D" equals the number of days since the adult molt.

* Data collection was terminated when at least one individual died.

Table A-3. Daily egg production by male-female pairs of O. fasciatus during exposure to synthetic oil #887 (control).

REP ^a	D10 ^b	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	101	40	0	92	57	34	48	44	0	42	46	42	0	85	39	36	0	44	65	28	32
2	41	36	40	34	32	0	26	39	0	46	30	42	31	6	62	46	48	36	36	31	47
3	37	0	35	82	43	34	43	39	0	40	40	30	40	35	78	29	36	31	12	43	31
4	48	49	46	44	46	48	43	44	0	42	42	0	83	48	37	40	38	29	0	57	30
5	34	38	41	0	86	40	35	47	18	28	72	0	79	39	42	45	34	43	37	34	38
6	95	45	48	45	54	0	78	59	14	75	49	54	47	45	40	43	49	51	42	10	82
7	87	39	50	51	60	36	46	56	32	44	23	72	48	46	43	43	0	49	44	19	76
8	0	0	0	0	0	0	0	79	0	30	48	34	27	32	50	34	51	48	38	32	58
9	92	31	50	45	50	0	87	45	16	75	41	0	41	87	49	39	52	47	10	73	48
10	93	0	47	44	91	0	47	91	46	12	80	0	54	91	36	50	0	59	55	47	83
11	92	51	43	42	56	34	41	49	20	69	38	53	12	69	43	40	18	46	15	69	52
12	51	75	13	65	31	0	32	48	29	5	27	25	30	66	27	27	40	12	26	27	52
13	0	0	0	44	38	0	77	86	44	0	82	0	62	76	37	44	46	50	0	53	85
14	44	0	51	81	49	44	40	50	33	8	44	44	40	0	86	46	43	47	34	41	38
15	22	43	36	39	42	37	42	33	13	35	26	14	0	0	49	29	39	38	34	24	28

a "REP" equals the replicate pair number.

b "D" equals the number of days since the adult molt.

Table A-4. Daily egg production by male-female pairs of O. fasciatus during exposure to synthetic oil #887 (8 $\mu\text{g}/\text{cm}^2$).

Rep ^a	D10 ^b	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	29	48	0	46	74	0	43	82	31	8	71	10	38	76	38	44	0	87	8	33	87
2	46	44	38	44	47	41	43	60	38	45	41	49	39	47	45	43	43	14	66	40	44
3	92	35	38	55	58	39	55	58	41	45	41	30	43	38	33	35	33	31	28	35	43
4	0	0	0	55	42	34	39	47	30	39	0	9	0	0	25	15	0	0	0	0	0
5	81	19	77	40	61	43	45	64	35	52	49	19	50	77	39	52	50	11	39	44	70
6	47	27	5	64	42	8	51	45	44	33	40	45	10	58	52	29	45	39	36	15	65
7	42	41	47	36	51	43	44	48	0	93	0	63	64	46	47	42	38	37	38	8	76
8	74	0	0	82	80	0	80	17	0	48	45	43	88	86	0	40	44	0	56	39	84
9	35	52	69	52	46	36	45	46	47	44	54	43	88	0	0	112	48	34	32	76	60
10	39	41	42	32	38	34	36	46	35	44	35	11	71	0	0	88	31	43	0	35	70
11	74	0	85	0	79	0	71	38	0	29	58	7	67	0	73	35	0	71	31	35	38
12	37	30	78	0	89	0	74	45	0	60	34	43	39	72	49	43	44	44	29	55	50
13	72	0	74	0	79	0	48	76	0	71	0	40	27	61	33	33	0	45	40	25	39
14	83	0	83	0	58	0	62	33	33	30	22	0	39	63	30	34	49	31	12	55	14
15	84	0	54	91	57	43	42	15	49	0	0	105	25	64	92	46	63	52	56	53	48

a "REP" equals the replicate pair number.

b "D" equals the number of days since the adult molt.

Table A-5. Daily egg production by male-female pairs of O. fasciatus during exposure to synthetic oil #887 (16 µg/cm²).

Rep ^a	D10 ^b	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	84	0	85	0	60	0	47	15	0	0	22	19	16	11	0	0	0	16	0	0	*
2	52	0	0	35	0	0	0	0	52	42	83	0	53	59	94	42	56	52	48	48	.
3	75	0	0	97	74	0	41	38	43	39	42	0	110	0	93	0	36	15	37	0	51
4	47	0	52	40	83	0	45	50	0	38	33	0	42	75	28	28	28	0	62	0	60
5	72	0	62	0	69	0	44	60	0	39	40	35	42	36	56	30	39	33	12	39	65
6	0	0	0	0	52	11	31	52	0	39	40	29	26	36	56	46	45	0	39	76	39
7	71	45	0	84	47	44	47	47	34	43	47	44	44	50	40	41	18	78	0	57	97
8	135	42	44	52	51	48	50	50	48	52	51	48	55	53	55	32	37	37	0	33	41
9	58	0	41	35	0	103	0	84	0	32	39	35	44	84	40	76	20	0	68	42	27
10	77	0	85	32	44	0	54	0	84	31	40	0	107	50	44	47	41	52	39	46	41
11	89	25	26	47	95	0	49	96	0	84	0	59	90	52	47	84	41	52	39	46	27
12	0	48	49	0	93	42	55	52	21	87	49	49	15	90	61	44	56	45	50	50	52
13	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14	0	0	37	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	0	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

a "REP" equals the replicate pair number.

b "D" equals the number of days since the adult molt.

* Data collection was terminated when at least one individual died.

Table A-6. Daily egg production by male-female pairs of O. fasciatus during exposure to synthetic oil #888 (control).

REP	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	40	37	43	39	37	0	36	41	94	0	59	44	41	41	0	108	38	0	39	36	36
2	44	48	0	87	33	28	38	43	91	49	0	49	35	34	97	45	42	17	80	0	36
3	37	38	0	43	43	40	0	80	43	40	0	0	46	40	77	39	40	40	19	0	43
4	40	25	0	33	18	0	43	0	86	35	40	52	78	0	64	44	47	0	72	0	39
5	0	60	33	30	0	43	29	37	40	76	28	48	45	15	68	44	34	50	48	0	45
6	38	49	29	68	41	49	42	42	78	39	0	45	43	42	0	91	38	45	15	35	40
7	37	46	29	46	42	48	42	47	55	55	40	55	48	49	40	44	8	0	10	38	41
8	72	38	0	72	12	0	72	0	83	37	0	76	44	0	50	75	53	36	13	40	38
9	34	25	29	0	14	0	56	34	48	32	36	19	38	35	45	40	42	41	51	42	43
10	87	37	0	28	56	43	0	39	47	52	0	32	36	28	35	47	0	29	41	0	29
11	94	30	0	52	40	37	0	50	0	92	0	49	96	0	100	63	44	54	40	0	0
12	76	45	46	34	51	47	48	44	48	45	0	87	50	30	48	55	48	33	11	0	0
13	76	38	*	0	85	0	43	38	94	50	0	49	63	34	93	49	0	54	44	41	51
14	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

a "REP" equals the replicate pair number.

b "D" equals the number of days since the adult molt.

* Data collection was terminated when at least one individual died.

Table A-7. Daily egg production by male-female pairs of O. fasciatus during exposure to synthetic oil #888 (8 µg/cm²).

REP	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	0	0	0	0	0	0	0	0	0	29	37	0	0	75	33	45	26	7	58	0	23
2	35	37	34	0	37	22	0	43	83	46	0	72	0	35	49	66	40	38	0	42	0
3	72	42	0	39	41	43	58	0	48	49	64	0	47	55	44	49	39	31	35	38	38
4	60	0	0	72	34	67	0	66	40	44	0	46	30	43	57	58	0	75	10	44	70
5	69	27	0	69	17	32	27	0	30	30	37	0	28	36	49	40	28	36	27	12	51
6	54	28	28	35	14	0	47	32	30	26	28	20	0	30	67	31	29	42	28	38	31
7	0	77	0	20	74	0	36	47	67	37	35	43	24	0	49	69	0	44	39	35	44
8	36	45	0	42	77	0	52	37	90	52	0	42	89	0	46	71	0	35	22	0	91
9	0	0	0	0	34	33	56	17	65	44	0	48	19	0	8	0	0	0	0	0	0
10	11	19	0	37	9	18	0	0	68	12	0	24	0	23	3	23	24	7	35	0	54
11	67	33	27	0	22	0	28	31	24	29	9	0	0	38	0	92	39	28	31	0	36
12	35	44	26	24	26	39	0	67	53	33	23	36	29	37	43	52	26	38	32	0	81
13	60	30	39	31	37	0	33	33	90*	34	37	38	0	38	78	46	41	0	71	0	44
14	4	13	0	0	0	19	3	0	*	34	37	38	0	38	78	46	41	0	71	0	44
15	0	37	0	22	22	27	0	20	28	27	45	56	0	46	13	23	11	0	.	.	.

a "REP" equals the replicate pair number.

b "D" equals the number of days since the adult molt.

* Data collection was terminated when at least one individual died.

Table A-8. Daily egg production by male-female pairs of O. fasciatus during exposure to synthetic oil #888 (16 µg/cm²).

REP ^a	D10 ^b	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27	D28	D29	D30
1	0	0	0	0	0	32	0	0	0	0	0	0	43	47	0	72	31	0	21	29	43
2	45	39	0	35	39	42	67	38	48	40	0	46	65	56	45	44	0	49	41	0	83
3	42	52	0	28	56	40	40	0	58	21	40	39	0	42	68	42	45	40	33	0	84
4	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	8	16	11	16	48	0	0	37	61	39	0	35	34	24	17	62	0	31	32	25	.
8	0	0	0	0	50	0	72	7	76	37	16	78	0	44	33	53	.	.	.	.	.
9	44	23	30	29	32	0	27	35	35	37	22	27	0	39	26	39	24	0	46	27	.
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12	0	56	0	74	37	0	35	0	83	36	0	47	0	73	46	46	0	45	39	0	.
13	61	36	0	86	34	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

a "REP" equals the replicate pair number.

b "D" equals the number of days since the adult molt.

* Data collection was terminated when at least one individual died.

APPENDIX B

Table B-1. Number of days, during a 21 day period, that daily egg hatch was within each of four percentage classes.^{a, b}

Days per Hatch Class									
Pair 0-25% 26-50% 51-75% 76-100%					Pair 0-25% 26-50% 51-75% 76-100%				
<u>Petroleum: Control</u>					<u>Petroleum: 79 $\mu\text{g}/\text{cm}^2$</u>				
1	0	0	0	21	1	0	5	6	5
2	0	0	0	16	2	0	0	0	14
3	0	0	0	16	3	0	0	0	16
4	0	0	0	18	4	0	0	0	19
5	3	5	1	5	5	0	0	0	14
6	0	0	0	0	6	0	0	0	6
7	0	0	0	20	7	1	0	1	17
8	10	1	1	8	8	0	0	0	18
9	0	0	0	14	9	0	0	0	17
10	0	0	0	20	10	0	0	0	9
11	0	0	0	16	11	0	0	0	16
12	0	0	0	21	12	0	0	0	16
13	0	0	0	17	13	0	0	0	18
14	0	1	3	17	14	0	0	0	20
15	0	0	0	16	15	0	0	2	19
<u>887: Control</u>					<u>887: 8 $\mu\text{g}/\text{cm}^2$</u>				
1	0	0	0	17	1	0	0	0	17
2	0	0	0	19	2	0	0	0	20
3	0	0	0	19	3	0	0	1	19
4	0	0	0	18	4	0	0	0	9
5	0	0	0	18	5	0	0	0	20
6	0	0	0	20	6	0	0	0	20
7	0	0	0	21	7	0	0	0	18
8	0	0	0	12	8	0	0	0	14
9	0	0	0	19	9	0	0	0	17
10	0	0	1	15	10	0	0	0	17
11	0	0	0	20	11	0	0	0	14
12	0	0	1	19	12	0	0	0	17
13	0	0	0	14	13	0	0	0	14
14	0	0	0	19	14	0	0	0	16
15	0	0	0	19	15	0	0	0	17

Table B-1 (continued)

Days per Hatch Class										
Pair 0-25% 26-50% 51-75% 76-100%					Pair 0-25% 26-50% 51-75% 76-100%					
<u>887: 16 µg/cm²</u>					<u>888: Control</u>					
1	0	0	0	9	1	0	0	0	17	
2	-*	-	-	-	2	0	0	0	18	
3	0	0	0	17	3	0	0	0	17	
4	0	0	0	13	4	0	0	0	14	
5	0	0	0	14	5	0	0	0	18	
6	0	0	0	16	6	0	0	0	17	
7	0	0	0	19	7	0	0	0	20	
8	0	0	0	19	8	0	0	0	16	
9	0	0	0	15	9	0	0	0	19	
10	0	0	4	12	10	0	0	0	16	
11	0	0	0	17	11	0	0	0	16	
12	0	0	0	18	12	0	0	1	17	
13	-	-	-	-	13	0	0	0	15	
14	-	-	-	-	14	-	-	-	-	
15	-	-	-	-	15	-	-	-	-	
<u>888: 8 µg/cm²</u>					<u>888: 16 µg/cm²</u>					
1	0	0	0	9	1	-	-	-	-	
2	0	0	0	15	2	-	-	-	-	
3	0	0	0	18	3	-	-	-	-	
4	0	0	0	16	4	0	0	1	7	
5	17	0	0	0	5	0	0	5	10	
6	0	0	1	18	6	0	0	0	7	
7	0	0	0	16	7	0	0	2	14	
8	0	0	0	15	8	0	1	0	17	
9	0	0	0	9	9	-	-	-	-	
10	0	0	0	15	10	-	-	-	-	
11	15	0	0	0	11	0	0	0	16	
12	0	0	0	19	12	0	0	0	13	
13	0	0	0	17	13	0	0	0	4	
14	0	0	1	3	14	-	-	-	-	
15	0	0	0	13	15	-	-	-	-	

* Death of at least one individual of the pair.

a Data are listed for numbered pairs of O. fasciatus.

b Days with no egg production are excluded.

APPENDIX C

Table C-1. Analysis of acid fractions of water-soluble fractions of test oils.^a

Oil	Compound					Total
	Phenol	C ₁ -phenol	C ₂ -phenol	C ₃ -phenol	C ₄ -phenol	
Pet.	ND ^b	ND	ND	ND	ND	ND
887	41.0	752.7	736.1	205.1	140.1	1875.0
888	ND	ND	2.7	3.7	23.3	29.7

^a Values shown are measured in mg per L.

^b ND means not detectable.

Source: Giddings, J.M., S.E. Herbes, C.P. Allen, A.J. Stewart, P.J. Franco, J.L. Forte, W.K. Roy, and K.L. Daniels. 1982. Water soluble fractions (WSFs) of coal liquids: Phenolic analysis and aquatic toxicity, pp. 71-73. in K.E. Cowser, ed., Life Sciences Synthetic Fuels Semiannual Progress Report for the Period Ending December 31, 1981. ORNL/TM-8229. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Table C-2. Chemical class fractionation of synthetic oils #887 and #888.

Class	Weight % for Sample Oil	
	887	888
Volatiles	0.8	0.06
Ether Soluble Bases	3.2	4.7
Ether Soluble Acids	3.2	1.8
Total Insolubles	<0.01	0.1
Saturates	20.9	18.5
Mono/Di Aromatics	61.4	69.0
Polyaromatics	0.7	13.0
Residue	<0.01	0.12
Total Recovery	90.2	107.0

Source: Griest, W.H., M.P. Maskarinec, and B.A. Tomkins. 1983, in preparation. Management and chemical/physical characterization of process/product effluent from the H-Coal pilot plant at Catlettsburg, Kentucky. ORNL/TM. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

VITA

Edgar Randall Merchant was born January 26, 1956, in Jacksonville, Florida. He attended schools there and graduated from Terry Parker High School in June 1974. The following September he entered Florida State University and in March 1978 received the Bachelor of Science degree, cum laude, with a major in Biology.

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