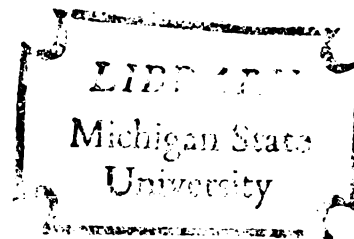




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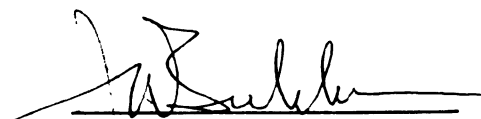
Macro-Arthropod Cryptozoan Predators:
DDT Metabolism and Food Chain Studies

presented by

Gary Voyle Manley

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of the requirements for

Ph.D. degree in Entomology


Major professor

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ABSTRACT

MACRO-ARTHROPOD CRYPTOZOAN PREDATORS: DDT METABOLISM AND FOOD CHAIN STUDIES

By

Gary Voyle Manley

Due to the abundance and persistence of DDT in the environment, many investigations have been carried out on its disappearance and metabolism. Included among numerous papers showing that organisms are able to metabolize DDT is recent work at Michigan State University which indicates that soil inhabiting Collembola and Acarina may play important roles in this phenomenon.

In light of these observations, it seemed worthwhile to look at the ability of other litter and soil arthropods to clean up their own environments. The project described here was designed to study the fate of DDT in a natural system once it becomes a part of the invertebrate food chain. Information was sought on (1) those arthropods which were the major feeders on Collembola and (2) movement of DDT in the macro-arthropod predator food chain.

The chemical was introduced directly into the invertebrate food chain by feeding laboratory reared DDT-resistant Collembola at the rate of 100,000 parts per million DDT in their food before they were released into experimental field plots. At selected intervals, all of the macro-arthropod fauna was randomly sampled from sub-plots and

chromatographically analyzed for DDT and its metabolites. Because of the small levels of pesticide used (less than 5 grams per acre) the pesticide was not followed beyond the arthropod predator food chain.

Enclosed study plots, five by five meters square, were located in a well-drained beech and hard-maple forest near Michigan State University. Live Collembola were released by sprinkling them over the leaf litter surface at dusk. The first samples were taken the following morning, and later samples at selected intervals thereafter.

Op' DDT, pp' DDT and pp' DDE were fed to Collembola which were released in the field. Each of the materials acted differently. Significant amounts of DDT were metabolized into DDE. In fact, some of the most efficient arthropod forms were able to convert virtually all of the ingested DDT to DDE within a few hours.

Op' DDT appears to be metabolized in several different ways, while pp' DDT appears to be almost entirely converted to pp' DDE. However, it would appear that given time a significant part of both op' and pp' DDT will be metabolized to pp' DDE.

Op' DDT degradation appears to occur through formation of pp' DDT, from which pp' DDE is formed. Due to the absence of pp' DDT evidence on the GLC trace, some arthropods appear to metabolize op' DDT directly to pp' DDE. As suggested by the speed at which some arthropods can convert pp' DDT to pp' DDE, however, detectable levels of pp' DDT are apparently never reached.

Some of the arthropods best adapted for metabolism of pp' DDT are spiders of the families Hahniidae, Thomisidae, and Agelenidae. Staphylinid beetles appear to be one of the important feeders on

Collembola populations, and in these studies accumulated large amounts of the pesticide. Within twelve hours they had more DDE than DDT in their bodies.

Movement of the pesticide in the food chain was very rapid and encompassing. Within a period of twelve hours, the pesticide had moved to virtually all of the arthropods in the plots.

Pp' DDE appears to be the most stable isomer in the environment. When pp' DDE was introduced into the plots, the arthropods accumulated large amounts, larger than either op' or pp' DDT. No conversion products or metabolites of pp' DDE were identified by the GLC analysis.

Spiders were found to account for the greatest number of Collembola eaten. Chilopoda appear to have the second greatest predatory impact on released Collembola.

MACRO-ARTHROPOD CRYPTOZOAN PREDATORS:
DDT METABOLISM AND FOOD CHAIN STUDIES

By
Gary Voyle Manley

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INTRODUCTION

Due to the abundance and persistence of DDT in the environment, many investigations have been carried out on its disappearance and metabolism. Included among numerous papers showing that organisms are able to metabolize DDT is recent work at Michigan State University which indicates that soil inhabiting Collembola (Butcher, Kirknel and Zabik, 1969) and Acarina [Aucamp and Butcher (in press)] may play important roles in this phenomenon.

In light of these observations, it seemed worthwhile to look at the ability of other litter and soil arthropods to clean up their own environment. The project described here was designed to study the fate of DDT in a natural system once it became a part of the invertebrate food chain. The project, as related to the macro-arthropod predators, had two major goals. One was to study the ability of various cryptozoan fauna to degrade DDT in its different forms, and to find out which degradation pathways of metabolism were most important for the study animals. The second goal was to learn about the most important predators of the cryptozoan community studied, and gain further insight into forest litter food chains. These two goals are related when the total community is considered.

The project was entirely field-oriented, for the following reasons: (1) no field work of this specific nature had been done in the past. By working in the field, a wider scope of animals became

available to study. The ability of many animals to degrade DDT could be studied, and various groups could be compared; (2) previous work has suggested that micro-flora in the gut of arthropods play an important role in metabolism of DDT. In the field, the micro-flora of the gut would be more natural than could be produced in the laboratory. This is of particular interest in respect to DDD; (3) what happens to DDT in a natural system is of special interest and (4) a study of the effects of the cryptozoan predators on the community could only be carried out in the field.

The prime objective of this project was to study the effect of the cryptozoan fauna on DDT rather than the effect of the pesticide on the arthropods. For this reason, a method had to be devised for introducing sublethal amounts of DDT into the food chain without contaminating the non-Collembola feeding forms directly. This was successfully accomplished by feeding the chemical to DDT-resistant Collembola and then releasing them into the field plots. The Collembola which was chosen as a pesticide carrier for the project was Folsomia candida (Willem). In addition to being highly resistant to DDT in all its forms, the species has a relatively short life cycle (about 21 days); is easy to rear; is able to adjust rapidly and can tolerate moving and handling.

The arthropod population of the study area was checked for background levels of DDT, DDE, and DDD prior to beginning the study. Since detectable levels of DDT, DDE, and DDD were absent from all arthropods tested the assumption was made that pesticide levels present in the arthropods were those introduced during the present study.

METHODS AND MATERIALS

Collembola Rearing

For the purpose of this project, Collembola were reared in plastic boxes 25 x 35 centimeters and 10 centimeters high. A mixture of fifty percent plaster-of-paris and 50 percent charcoal was poured into the box to a depth of three to five centimeters. After this substrate hardened, Collembola were introduced and fed by sprinkling powdered yeast over the surface of the container two or three times a week. All Collembola were reared at temperatures ranging between 70 and 80 degrees fahrenheit. During the days immediately before release the Collembola were cooled to 50 degrees fahrenheit.

At the time of field release, Collembola were anesthetized with carbon dioxide and emptied into weighing containers. After being weighed, they were moved to rearing containers and fed yeast containing 100,000 parts per million of the appropriate pesticide. Yeast containing the pesticide was sprinkled over the entire surface of the container, and the Collembola were allowed to feed at 70 degrees fahrenheit.

DDT was added to the yeast by dissolving in acetone. After the DDT was completely dissolved the proper weight of yeast was added. The solution was mixed and allowed to stand overnight. The following day the acetone was evaporated and the yeast re-powdered and fed to the

Collembola. The Collembola were allowed to feed on the yeast containing DDT for two days. Following this they were fed untreated yeast for two days. On the evening of the fourth day after feeding, they were sprinkled over the surface litter of the field plots at dusk. Before being released, they were conditioned for field release by being kept at 50 degrees fahrenheit for about twenty-four hours.

Actual levels of DDT in the bodies of released Collembola generally ranged between one to two thousand parts per million at the time of release. However, on a per acre basis the actual rate of pesticide application was very low when compared with standard application levels, being less than five grams per acre. Principally because of this low level of DDT application pesticide leaving the invertebrate food chain could not be traced. Adding to the problem of following the DDT beyond the predator food chain was the fact that DDT had been degraded to several components; each of which left the food chain in amounts smaller than the original level of DDT.

Field Plots

The site chosen for field studies in this investigation was a level, well-drained mesophytic beech and hard-maple forest situated in Williamston Township, Ingham County, T4NR1E Sec. 4.

The dominant canopy species of the forest are beech (Fagus grandifolia), and hard-maple (Acer saccharum). Other species included are black cherry (Prunus serotina), ironwood (Ostrya virginiana), red oak (Quercus rubra), and dogwood (Cornus florida).

Enclosed plots were five by five meters. The enclosure consisted of a piece of sheet metal on the bottom which extended about

four inches into the soil and about four inches above the soil. Above the metal a screen extended another eighteen inches. The top of the enclosure was left open. The bottom metal piece and the screen on top were built as separate eight foot sections that came apart, so they could easily be separated. This method of construction allowed the plots to be moved about in the woods for each new release. The interior of the enclosure was gridded off at each one-half meter interval with string so that samples could be randomly selected and located rapidly.

Plots were set up two to five days before sampling began. This period was intentionally kept short so that populations and conditions inside the plots would be as close to outside conditions as possible.

Sampling

Randomly selected sub-samples twenty-five by twenty-five centimeters square were taken from the five by five meter field plots at preselected times during the duration of the study (Appendix II). Ten to twelve sample days were selected for each release. A one quarter meter strip immediately adjacent to the wall of the plot was left unsampled. Also not sampled were two one quarter meter strips at right angles to the sides across the middle of each plot. A six-inch board was laid on these strips during sampling. This acted as a walkway.

A relatively uniform sample was taken by pushing a metal box into the leaf litter. Then a knife was used to cut around the inside of the box after which the leaf and humus layer to the depth of mineral soil was scraped off by hand and placed in a plastic bag. Plastic

bags containing the samples were transferred to a styrofoam ice box for transporting back to the laboratory.

Sorting Samples

Collections were hand sorted by placing each sample in a metal container with a one-half inch hardware cloth mesh bottom. These were shaken onto a white table cover. Animals were collected immediately as they fell onto the cover. Each animal was placed in a holding container until all samples were sorted. After sorting was completed the arthropods were anesthetized with carbon dioxide so they could be identified and weighed. After being weighed, each group was placed in a vial and covered with 0.5 ml. of hexane. Arthropods were ground in the vial with a glass rod and quick frozen until analysis could be completed with the gas chromatograph.

Analysis

Samples were analyzed on a Beckmann GC-4 gas chromatograph with an electron capture detector. A six foot by 1/16 inch I.D. pyrex column was packed with 11% DC 200, 3% QF-1, 60/80 GCQ: temperature was 230 degrees centigrade. The helium flow through the column was 40 ml/min. Concentrations were calculated using peak height and were based on wet weights of all material analyzed. Standards were injected at the beginning of each run, after every ten to fifteen samples, and at the end of the run. Minimum detectable level for the instrument was 0.01 part per million for DDT and .003 parts per million for DDE. Minimum detectable levels in the arthropod tissue was 0.001 parts per million for DDT and 0.001 parts per million for DDE.

RESULTS AND DISCUSSION

Introduction

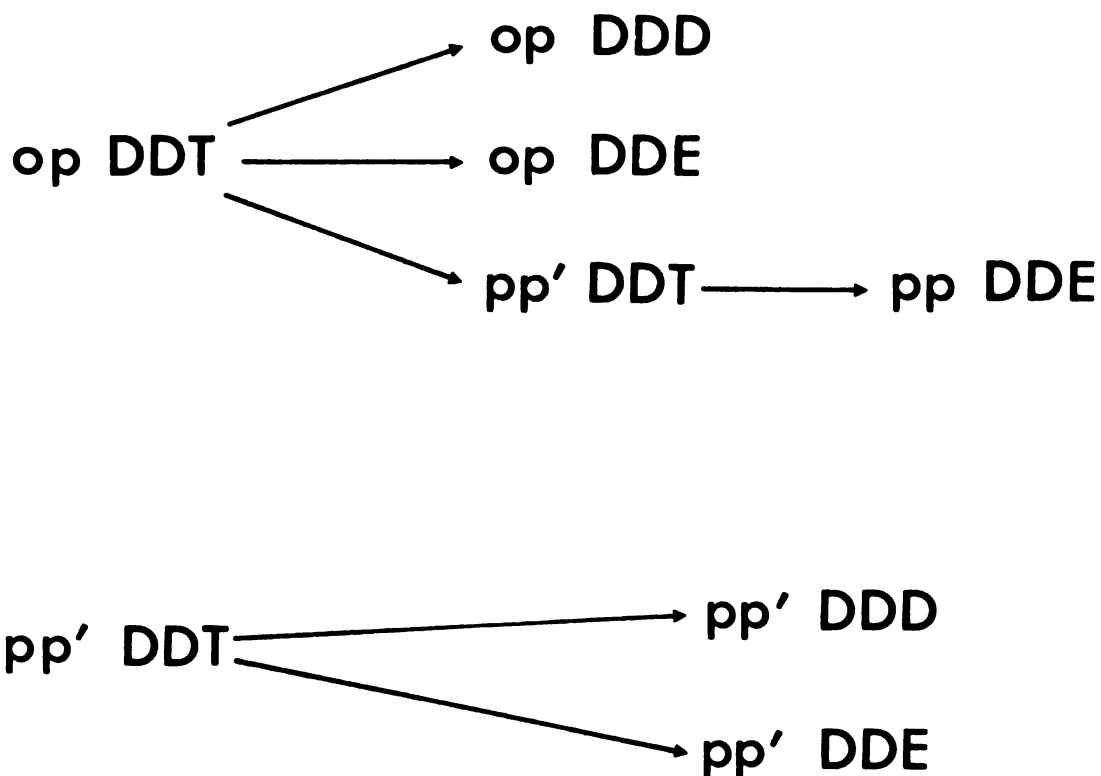
Many arthropods are found in the forest litter. However, the cryptozoan predators can basically be divided into four classes as follows: Araneida, Chilopoda, Coleoptera larva, and Coleoptera adults. For purposes of studying both the ability of various predators to metabolize DDT and the effects of various arthropods on the cryptozoan community, both taxonomic and weight class divisions were used. The relative size of the predator was often more important than the particular species group to which it belonged. On the other hand, it was found that some species did vary from the normal pattern of their weight class. Weight classes were most important in studying predatory effects, and taxonomic determinations were of major importance in studying metabolism ability.

Op' DDT, pp' DDT and pp' DDE were each fed to Collembola which were released in the field. Each of the materials acted differently when released into the cryptozoan food chain.

The degradation pathways used by the various arthropods of the forest litter were studied. Op' DDT metabolism products are more varied than are those of pp' DDT. Op' DDT is metabolized in several different directions, while pp' DDT is mostly converted to pp' DDE Figure 1. However, given time a significant part of both op' and pp' DDT will be metabolized to pp' DDE, by converting op' DDT to pp' DDT

**Apparent Degradation
Pathways of DDT Metabolism in a
Forest Invertebrate Litter Food Chain**

FIG. 1



and then to pp' DDE Figure 1. Pp' DDT is an important intermediate step in the breakdown of op' DDT. Some groups of cryptozoan predators used one degradation pathway and not another, or they could metabolize more rapidly by one of the pathways than could other species. The degradation pathways for DDT appear to be relatively consistent within species and taxonomic groups of arthropods analyzed in this study, as suggested by figures nine through twelve.

The introduction of pp' DDE into the food chain by means of Collembola was used to study the predation by various species in the plots. Weight class analysis proved to be the most useful parameter in this part of the study.

Degradation of pp' DDT to pp' DDE

Pp' DDT metabolism to pp' DDE is the most important pathway used by the cryptozoan predators when pp' DDT is introduced into the food chain. As suggested by the diversity in metabolic degradation among the macro-arthropod fauna presented in figures three through eight, the variety of degradation pathways used (Figures nine through twelve), the lower degradation ability of the average of all arthropods (Figure 5b), and the longer length of time found necessary for Collembola to degrade DDT (Butcher, 1969) facts would indicate that the ability to degrade pp' DDT to pp' DDE is a characteristic common to most all the macro-cryptozoan arthropod predators sampled.

The ability to degrade pp' DDT varied both within taxa and between taxa. Differences in degradation of pp' DDT among the cryptozoan fauna are mostly a matter of degree. Over a period of a few days DDT is degraded beyond detectable levels (below 0.01 ppm) and

the only detectable traces of the pesticide left is that of pp' DDE.

The percent of arthropods fed on pp' DDT which had measurable levels (above 0.01 ppm) of pp' DDT and pp' DDE (Figure 2b) after twelve hours reveals that DDE is present almost as often as is DDT; emphasizing the speed at which metabolism starts. This is a contrast to those which are fed on op' DDT in which no pp' DDE is present at the beginning (12 hours) of the sample sequence.

The most rapid converters of pp' DDT among the arthropod cryptozoan predators are Thomisidae (Figure 3a), Elateridae larva (Figure 8b), and Carabidae (Figure 5a). These three groups, for the most part, accounted for no DDT. This indicates that pp' DDT was degraded to pp' DDE immediately. At the first sampling twelve hours after release, most of the arthropods were only beginning to degrade the material, but the above forms contained only DDE in their systems.

Staphylinidae (Figure 6) were also very rapid converters of pp' DDT but showed some trace of DDT for the first three days after release, after which time they contained only DDE. These animals appear to have degraded most of the DDT after twelve hours. This ability to rapidly degrade DDT is most striking in the small beetles of this family. Some of the larger Staphylinidae did not show this rapid rate of metabolism; but they were not abundant enough in the plots to permit a valid assumption to be made.

A major group of predators are the spiders, which numerically may be the most important degraders among the cryptozoan predators. The spiders were divided into three classes based on live weight as

follows: small, medium, and large. For the purpose of metabolism studies, hahniids will not be included with the small spiders, the group with which they would be classed on a weight basis. The small spiders (Figure 7b) showed the least (and most varied) ability to degrade the pesticide. This diversity may be explained by the fact that this was a grouping of heterogeneous forms, including several families and uncommon species which varied from sample to sample. These studies suggest that some of the small spiders are inefficient degraders of the pesticide. Hahniidae (Figure 7a) on the other hand showed an above average ability to metabolize DDT; more closely related to that of the medium-sized spiders (Figure 3b).

Outside of the thomisids, the medium-sized class appears to be the best metabolizer of pp' DDT among the Araneae. The data suggest they are efficient convertors of pp' DDT to pp' DDE, but somewhat slower than the fastest forms of cryptozoan predators. After twenty-four to thirty-six hours, the amount of pp' DDE is greater than the amount of pp' DDT, but it is not until the eighth sample before the DDT drops under detectable levels.

Chilopoda (Figure 4) were also found to metabolize pp' DDT, but appear to take longer than spiders to convert completely to DDE. Chilopoda are significant degraders of the pesticide in the community, particularly the larger forms which are the best degraders of DDT. The small forms (less than six months old) can be considered inefficient.

The average ratio of DDE to DDT for all arthropods (Figure 5b) is considerably below that for the most efficient metabolizers. The averages omit Staphylinidae figures because of the extremely high ratio for DDE conversion found in that group.

METABOLISM OF DDT

FIG. 2a op' DDT

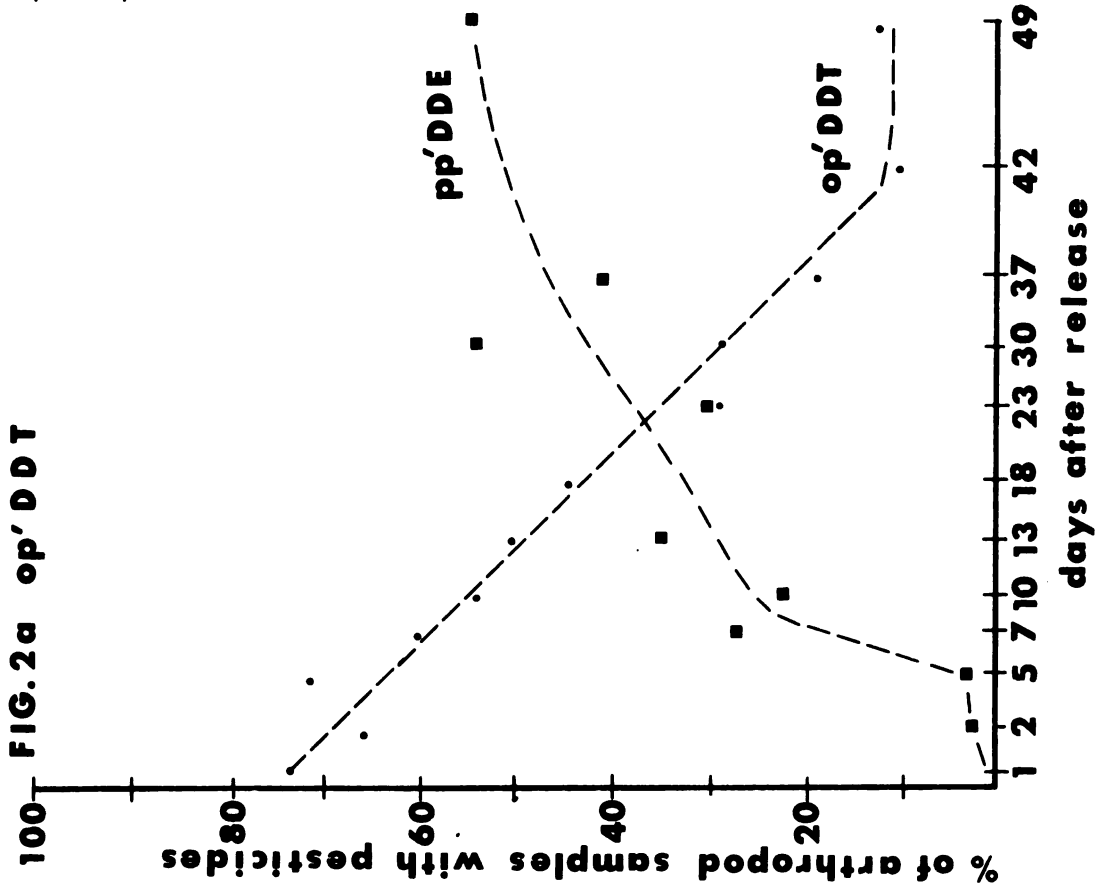
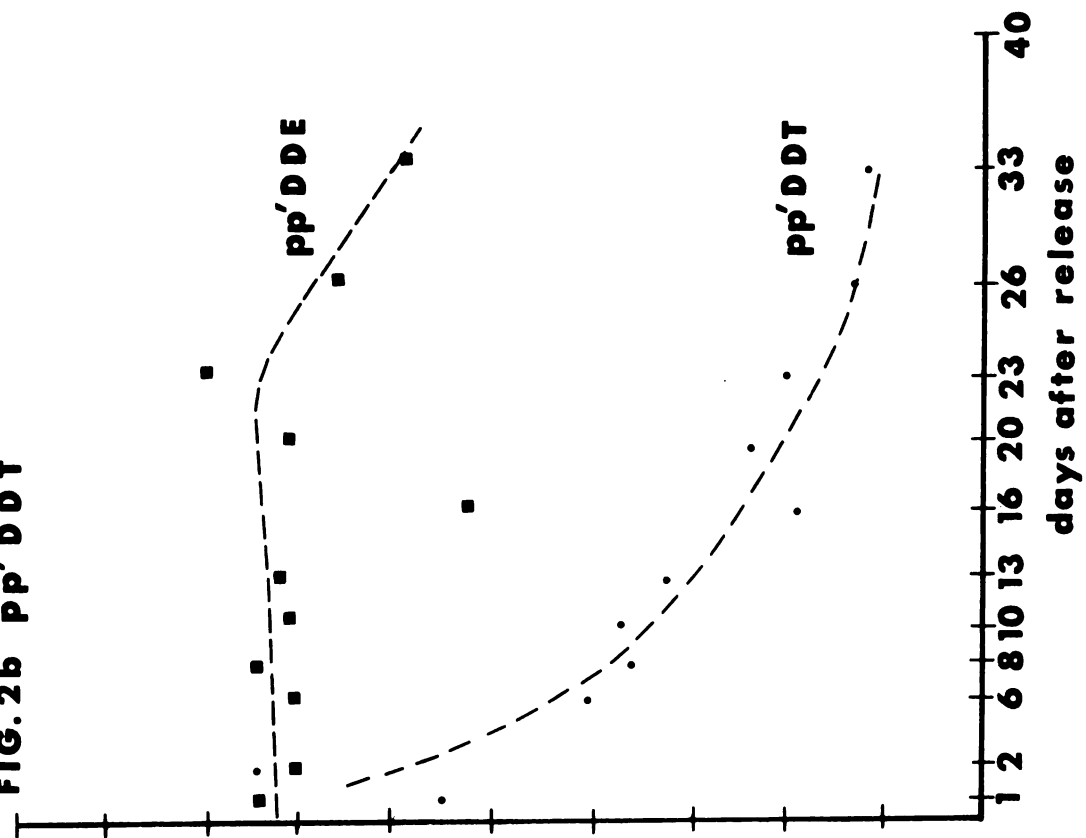
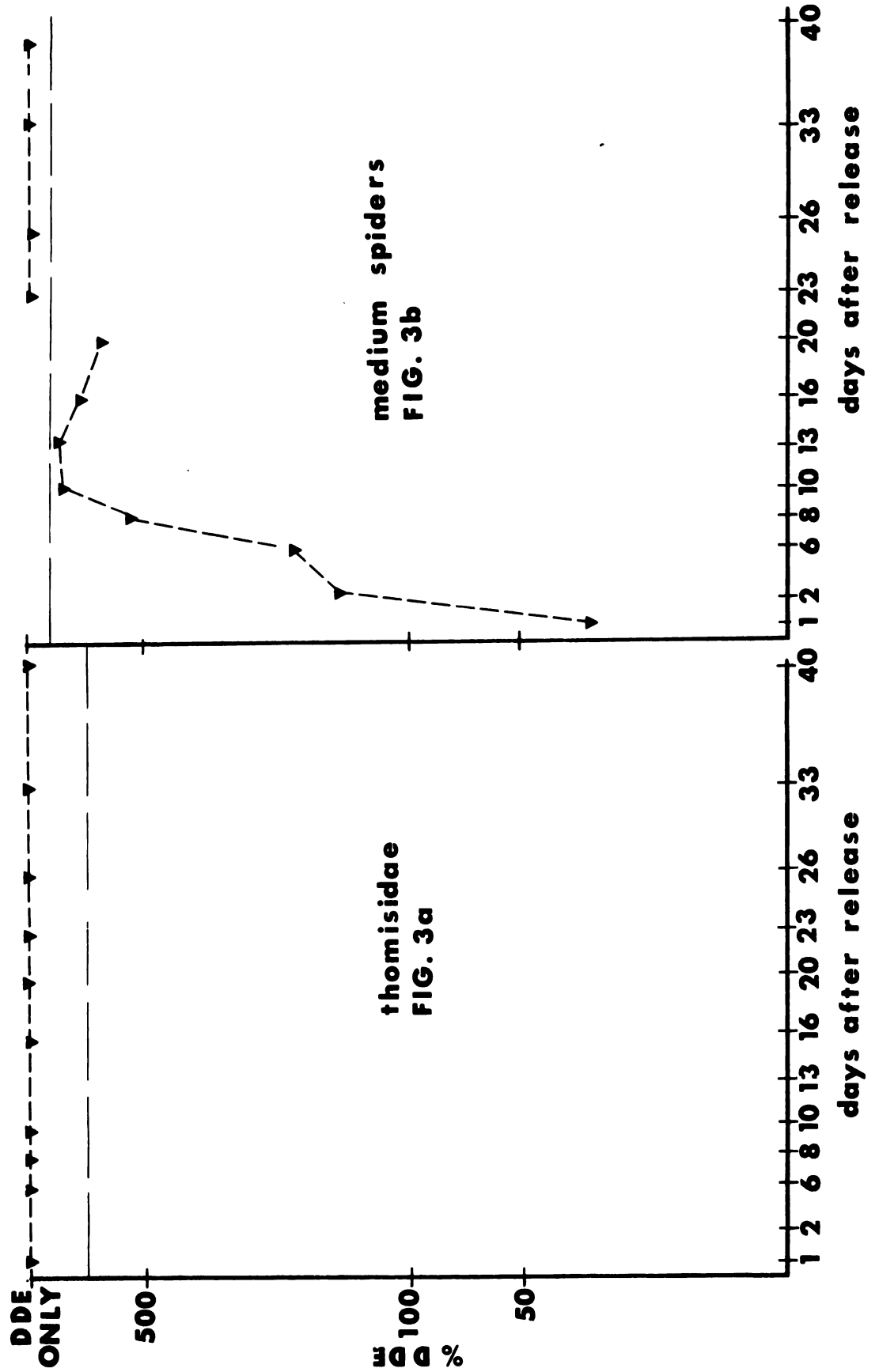
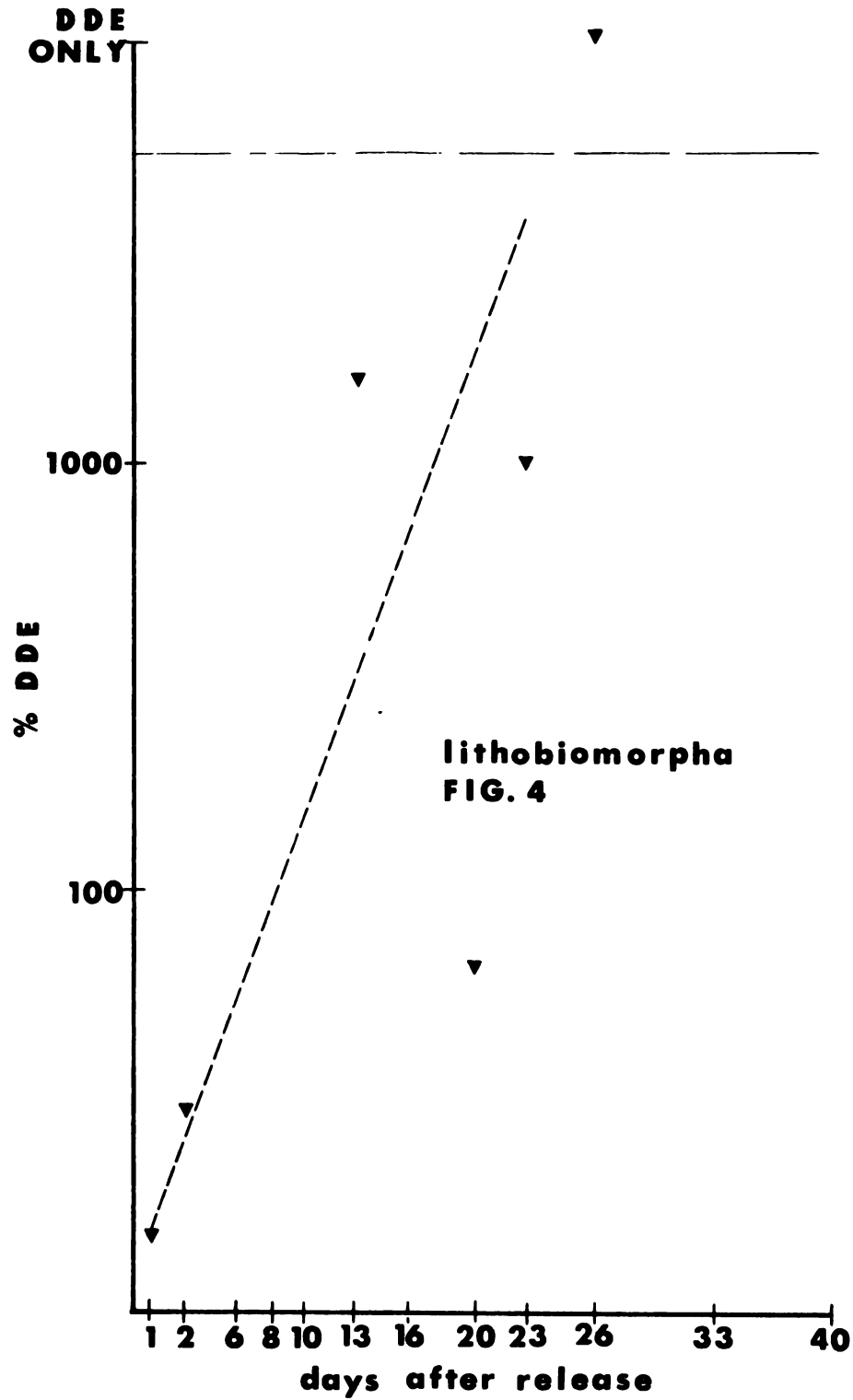


FIG. 2b pp' DDT

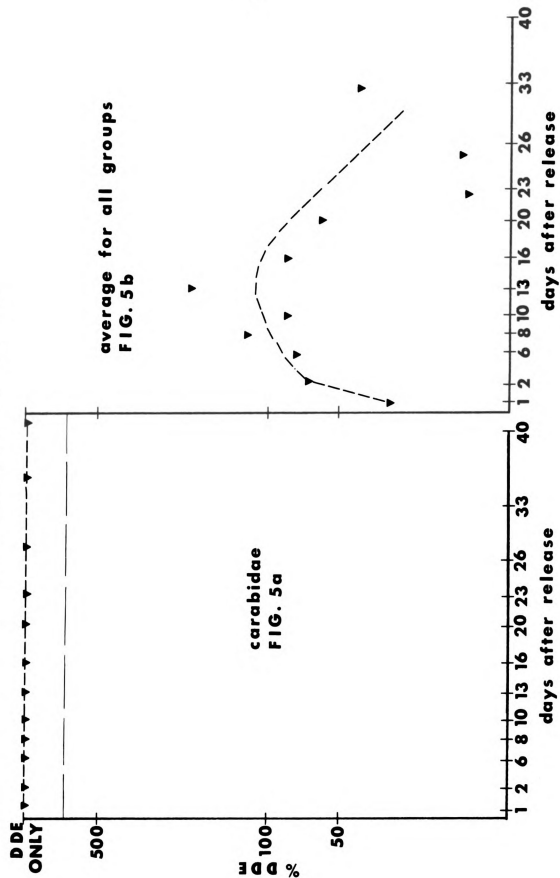


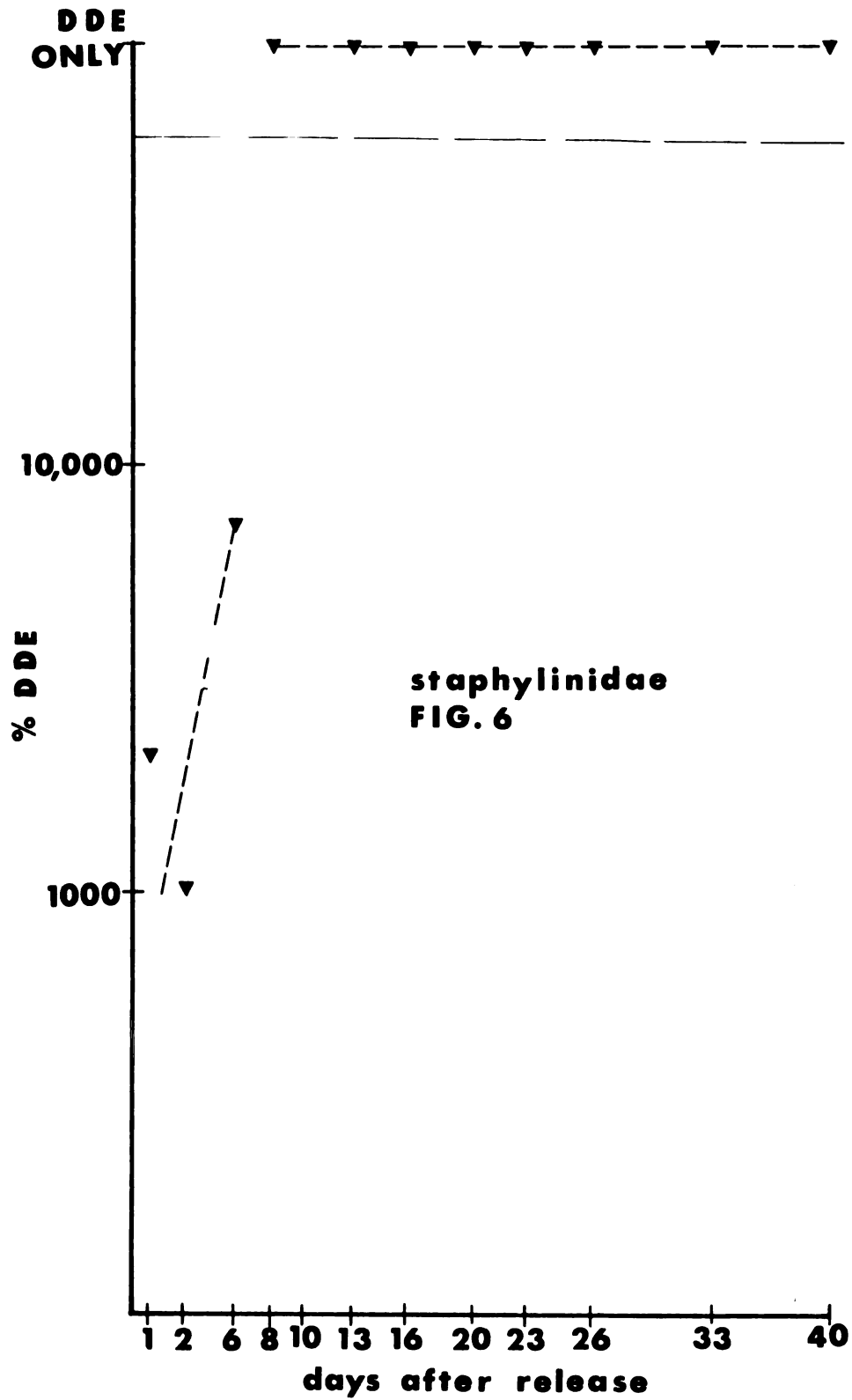
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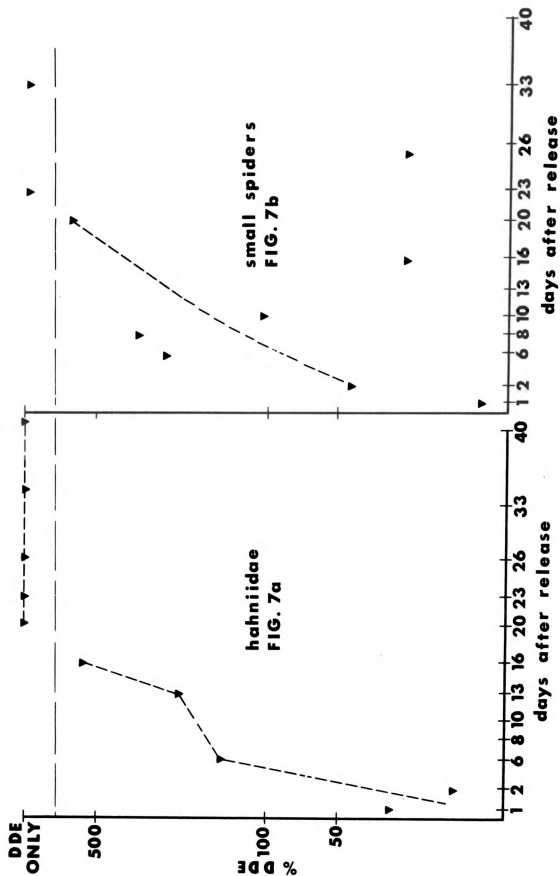
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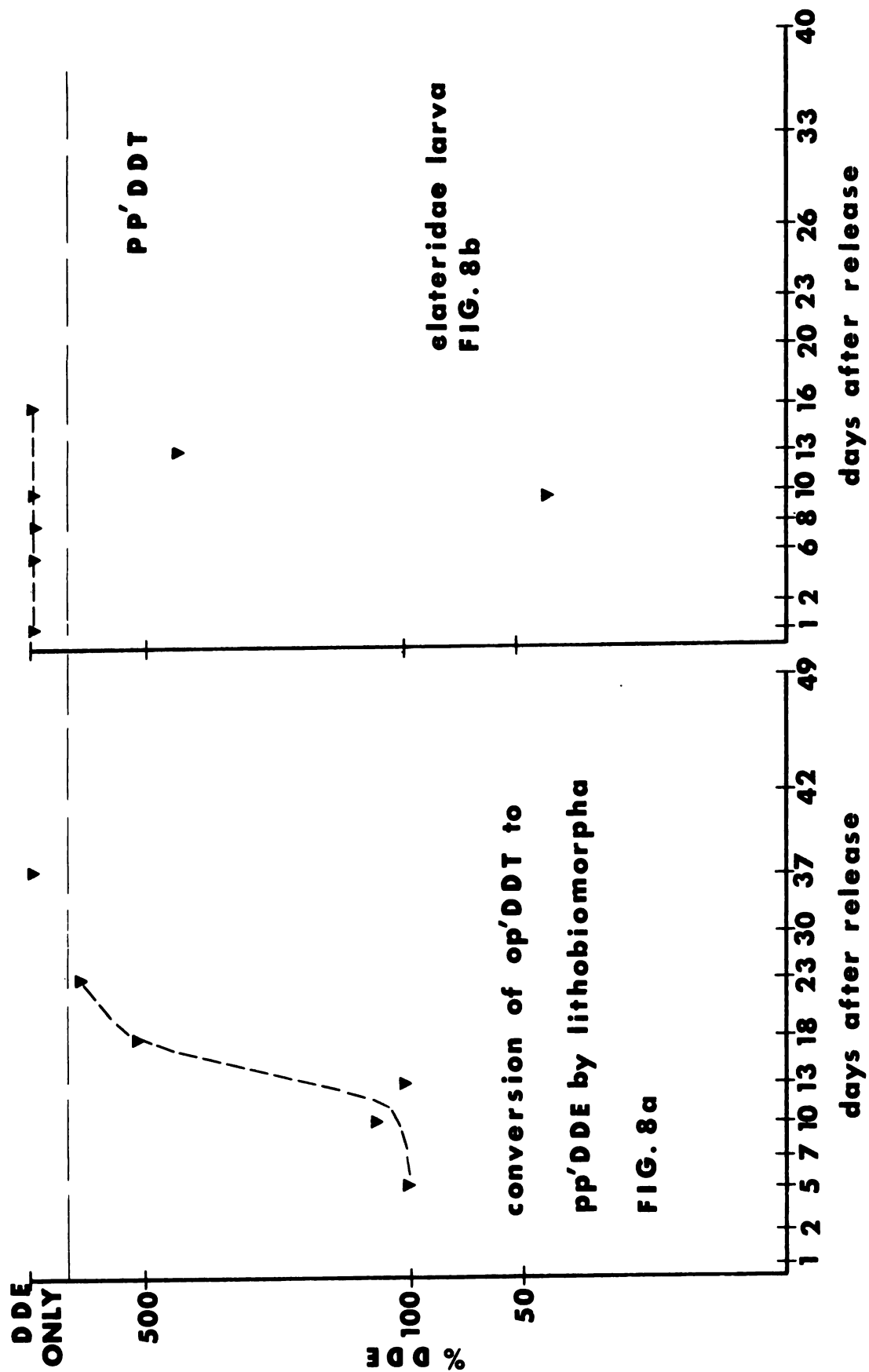


METABOLISM OF PP' DDT

METABOLISM OF PP' DDT



METABOLISM OF DDT



Op' DDT Metabolism

When op' DDT was introduced into the cryptozoan food chain, it was found that the products of arthropod metabolism were more varied than was the case where pp' DDT was introduced (Figure 1). Various groups of arthropods followed different metabolic pathways of degradation. Faunal differences in pathways of op' DDT metabolism are for the most part a matter of degree. However, some groups formed more of one metabolic product than another. The data suggest that some groups of arthropods do not form some metabolites, at least not in detectable levels.

The cryptozoan predators studied which are capable of degrading op' DDT can be divided into groups on the basis of the pathway which is most important; or on the basis of certain pathways not used. Since most all arthropods form DDD at fairly consistent rates DDD need not be considered here as a pathway; it is discussed elsewhere.

On the basis of degradation pathways, the cryptozoan predators can be divided into three major groups. One, those which form op' DDE. Formation of op' DDE starts immediately after release of op' DDT fed Collembola (Figure 13b). Many of these arthropods also form pp' DDT and then pp' DDE, but usually at a slower rate when compared with other forms. The majority of spiders (Figure 9a, 10b, 10d) would fall into this group. Group two forms no op' DDE. All op' DDT goes to pp' DDT and then is converted to pp' DDE. The rate of conversion is intermediate, and significant levels of pp' DDT are found during most of the sample period, also small levels of op' DDT remains during the sample period (Figure 10c). The major fauna in this group are the

Chilopoda (Figure 10c, 11c). Group three would include those animals which appear able to convert op' DDT directly to pp' DDE. Some of the species found in this group frequently lack pp' DDT (Figure 9c) and may show no pesticide except pp' DDE (Figure 12b); even after twelve hours, at the time of the first sample. Many of these arthropods also build up larger amounts of pp' DDE and usually do not accumulate very large levels of pp' DDT. Facts indicate that all forms in this group degrade op' DDT to pp' DDT as an intermediate step but that pp' DDT is converted to pp' DDE so rapidly detectable levels are never reached. This line of thinking is further supported by the extremely rapid rate at which some forms convert pp' DDT to pp' DDE when pp' DDT is released in the field. Many of the arthropods which convert op' DDT to pp' DDE very rapidly are the same groups which are the most rapid converters of pp' DDT to pp' DDE. This accounts for the lack of pp' DDT in some forms. Included in group three are those forms which convert op' DDT to pp' DDT to pp' DDE very rapidly (Figure 10a). Many important predators are included in group three, including Carabidae (Figure 11a), Thomisidae (Figure 9b), Staphylinidae (Figure 10a), and Elateridae larva (Figure 9c).

The amount of time for various metabolites to reach detectable levels after release varies with the metabolite and the species involved. Pp' DDE varies considerably between species but, is the last product to appear, the last product to disappear and maybe the only detectable trace of the DDT introduction left by the end of the sample period (Figure 2a). Op' DDD is formed immediately and is the only metabolite to be found at its highest level during the first and second sample.

Op' DDE is found shortly after release as is pp' DDT (Figure 13b). The amount of pp' DDT in the arthropods varies as a percent of op' DDT depending on the rate of degradation from op' DDT to pp' DDT and the ability to degrade pp' DDT to pp' DDE. Those macro-arthropod predators which are rapid degraders of pp' DDT to pp' DDE never obtain large levels of pp' DDT while the slower metabolizing forms build up larger amounts of pp' DDT and maintain significant levels longer. For the most part, pp' DDT reaches maximum levels during the first half of the sample and then drops off.

Pp' DDE is the only material that constantly built up in the cryptozoan fauna during the entire study. This was found to be the case for both op' DDT and pp' DDT releases. All other metabolite levels peak during the sample periods and then level off. When op' DDT was released, op' DDE was formed immediately; increased during the first half of the sample, and then dropped off (Figure 13b). Not until about the fourth sample are significant amounts of pp' DDE found in the predators; other than group three (Figure 3b). By the end of the sampling, pp' DDE is the only pesticide left in detectable levels. By the fifth or sixth sample, some macro-arthropod predators contained only pp' DDE in detectable levels.

The data suggest that insofar as the macro-arthropod predators of the cryptozoa are concerned, the metabolism of op' DDT is more varied than is pp' DDT metabolism. Op' DDT degradation occurs by several different metabolic pathways, and large concentrations of any one metabolite are not formed, due to DDT being split up into several metabolic products. In addition, there is a dilution at each step in

MAJOR ROUTES OF OP' DDT METABOLISM

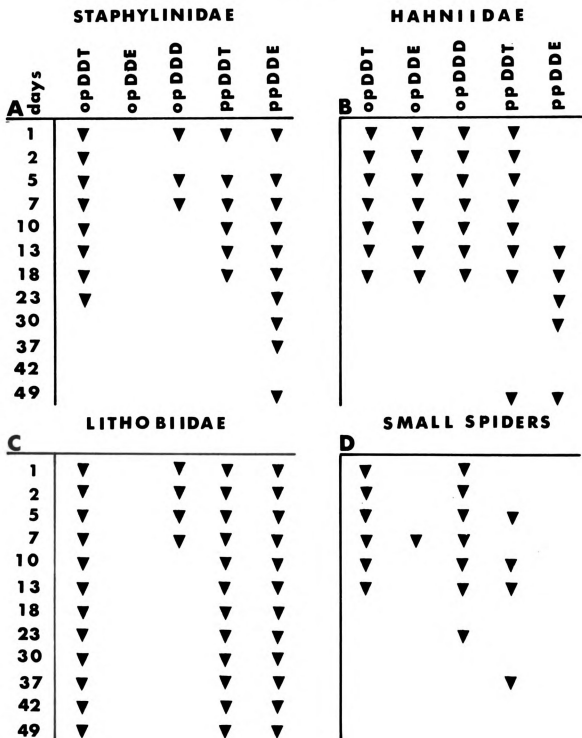
FIG. 9

MEDIUM SPIDERS						THOMISIDAE						
A	days	opDDT	opDDE	opDDD	ppDDT	ppDDE	B	opDDT	opDDE	opDDD	ppDDT	ppDDE
		▼	▼	▼	▼	▼		▼	▼	▼	▼	▼
	1	▼	▼	▼	▼							
	2	▼	▼	▼	▼	▼						
	5	▼	▼	▼								▼
	7	▼	▼	▼	▼	▼						
	10	▼	▼	▼	▼		▼					▼
	13	▼	▼	▼	▼	▼						▼
	18											▼
	23	▼	▼	▼								▼
	30					▼						
	37	▼			▼	▼						▼
	42											
	49					▼						

ELATERIDAE LARVAE						CANTHARIDAE LARVAE						
C		opDDT	opDDE	opDDD	ppDDT	ppDDE	D	opDDT	opDDE	opDDD	ppDDT	ppDDE
		▼			▼	▼		▼				
	1	▼			▼	▼		▼				
	2	▼			▼	▼						
	5	▼			▼	▼		▼	▼	▼	▼	
	7	▼				▼						
	10							▼	▼	▼	▼	▼
	13							▼	▼	▼	▼	▼
	18							▼		▼		▼
	23	▼				▼						
	30							▼	▼		▼	▼
	37					▼		▼				▼
	42											
	49					▼						

MAJOR ROUTES OF OP' DDT METABOLISM

FIG. 10



MAJOR ROUTES OF OP' DDT METABOLISM

FIG. II

CARABIDAE						CARABIDAE LARVAE						
A	days	opDDT	opDDE	opDDD	ppDDT	ppDDE	B	opDDT	opDDE	opDDD	ppDDT	ppDDE
	1	▼			▼	▼						▼
	2											
	5	▼			▼	▼		▼	▼	▼		
	7	▼		▼	▼	▼						
	10	▼			▼	▼		▼				▼
	13	▼										
	18											▼
	23	▼			▼	▼		▼	▼			▼
	30							▼				▼
	37											
	42							▼				▼
	49							▼				▼

LINOTENIA						DIPLOPODA						
C		opDDT	opDDE	opDDD	ppDDT	ppDDE	D	opDDT	opDDE	opDDD	ppDDT	ppDDE
	1	▼		▼	▼			▼				
	2				▼	▼		▼			▼	▼
	5	▼			▼	▼		▼			▼	▼
	7	▼				▼						
	10	▼			▼	▼		▼			▼	▼
	13							▼				
	18				▼	▼						
	23				▼	▼		▼				▼
	30	▼			▼	▼		▼			▼	▼
	37							▼			▼	▼
	42							▼				
	49	▼			▼	▼		▼			▼	▼

MAJOR ROUTES OF OP'DDT METABOLISM

FIG. 12

FORMICIDAE						APHODIUS						
A	days	opDDT	opDDE	opDDD	ppDDT	ppDDE	B	opDDT	opDDE	opDDD	ppDDT	ppDDE
	1	▼		▼								
	2											
	5							▼				▼
	7	▼		▼				▼				▼
	10	▼			▼	▼						
	13	▼		▼				▼				▼
	18											
	23											
	30	▼				▼						▼
	37											
	42											
	49											

PSEUDOSCORPIONS						
C	days	opDDT	opDDE	opDDD	ppDDT	ppDDE
	1	▼		▼		
	2					
	5	▼		▼		
	7	▼		▼		
	10	▼				
	13					
	18					

the system. During the metabolism of op' DDT by the arthropods small levels of several metabolites are found. In both cases, the principle product found during the terminal samples is pp' DDE.

Evaluation of Figures Nine through Twelve:
op' DDT Metabolism

9a. Medium-sized spiders accounted for an important pathway in metabolism of op' DDT through formation of op' DDE. Op' DDE was found to be common during the first half of the sample period and then disappeared. All isomers found during the study were accounted for by the medium-sized spiders. Pp' DDE was rare during the early samples (one spider had pp' DDE during sample two) but increased in amount and frequency of occurrence during the study. Pp' DDE was the most abundant isomer during the later samples. Pp' DDT was found throughout the samples in significant amounts but is most common during the first half of the sample period.

9b. Thomisidae do not represent the majority of spiders in respect to their ability to metabolize op' DDT. Thomisidae are very rapid converters of the pesticide. Pp' DDE was the only isomer found during the early samples when most arthropod fauna (all other spiders) are just beginning to form pp' DDE. Thomisidae, unlike other spiders, do not appear to form op' DDE. Three specimens of Thomisidae were found which did not conform to the above pattern of metabolism. These specimens were smaller and may have been a different species.

9c. Elateridae larvæ were rapid degraders of op' DDT. In the early samples op' DDT, pp' DDT, and pp' DDE are present. Pp' DDE soon becomes the major isomer. Pp' DDT is found only in the early

samples. Among insect larva analyzed, elaterid larva were the only group which did not show op' DDE.

9d. Cantharidae larvae, at one time or another, yielded all isomers of op' DDT. Pp' DDE does not appear to reach detectable levels until about the fifth sample, but after that time it is found consistently. Op' DDT appears to remain throughout the sample period as do most other isomers.

10a. Staphylinidae were rapid converters of op' DDT. No op' DDE was found but all other isomers appeared immediately. Pp' DDE continued to increase during the study and was the only isomer found after the eighth sample.

10b. Hanneidae has a metabolism pattern very similar to medium-sized spiders. Pp' DDE is not formed early but is the only isomer detected in the later samples.

10c. Lithobiidae was found to account for pp' DDT and pp' DDE immediately after release. Op' DDT and pp' DDT were observed during the entire sample period. Relatively large levels of pp' DDT were formed. Conversion to pp' DDE would appear to occur at a slower rate than was observed in some of the forms which metabolized more rapidly.

10d. Small sized spiders appear to form smaller amounts of both op' and pp' DDE than larger spiders. Pp' DDT was observed, so pp' DDE may have been present in small amounts. This was the most heterogeneous grouping of arthropods in the study. This may, along with their small size, account for the inconsistent results.

11a. Carabidae adults did not form detectable levels of op' DDE. Significant levels of pp' DDT and pp' DDE are formed shortly after release.

11b. Carabidae larvae, like cantharid larvae, showed op' DDE. It is interesting that carabid larva converted op' DDT to op' DDE but the adults did not. It would appear that the former have the ability to degrade pp' DDT to pp' DDE at a very rapid rate, since no pp' DDT was found.

11c. Linotenina (Geophilomorpha) has a metabolism pattern very similar to the Lithobiomorpha.

11d. Diplopoda appear to have metabolism abilities very similar to related myriapodous arthropods.

12a. Formicidae appear not to form op' DDE but use the pp' DDT to pp' DDE route of op' DDT metabolism.

12b. Aphodius was not collected consistently during the study, but results would indicate that they are good converters of op' DDT. Pp' DDE was formed immediately and was the only isomer accounted for.

12c. Pseudoscorpions contained pesticide only in early samples and did not appear to form metabolites other than op' DDD. The results are very inconclusive.

Op' DDT to op' DDE

Conversion of op' DDT among the macro-arthropod predators to op' DDE appears to be primarily a spider phenomenon (Figures 9a, 10b, 10d). Op' DDE was most common in spiders but did not reach large levels. The only other arthropod found to contain op' DDE was Cantharidae larvae (Figure 9d) and Carabidae larvae (Figure 11b).

In general, formation of op' DDE starts early, and the level of op' DDE increases as a percent of op' DDT during the early part of the sample period (Figure 13b) and then drops off. Levels of op' DDE

to op' DDT stay small and do not build up to concentration levels as in the case of pp' DDE. Average levels do not exceed ten percent, from three to six percent are most common. Isolated spiders may contain levels of op' DDE which are up to thirty percent that of op' DDT. Spiders of the families Agelenidae and Hahniidae appear to be the most important predators which follow this pathway. Worth noting is that the very rapid converters (group three) do not form any op' DDE.

Route of DDT to DDD

In a discussion of the degradation of DDT to DDD, the pp' DDT and op' DDT need not be separated. The level of DDD as well as the pattern of metabolism appears to be very similar (Figures 14a, b). Slightly higher levels of DDD were found when the pp' form was used and the rate of metabolism appeared to be a little more rapid also.

Previous work has shown that DDD is a metabolism product of a variety of microorganisms in the soil (Ko and Lockwood, 1968). Also suggested is that DDD is a product of DDT metabolism produced by micro-flora in the gut of organisms, rather than by arthropod enzymes. In studies of macro-arthropod predators of the cryptozoan community reported here, data suggest that DDD is a product of gut micro-flora metabolism and DDE is a metabolism product of the arthropod predators. This is assumed from the fact that op' and pp' metabolism of DDT to DDD is so similar and consistent over such a wide taxonomic range.

DDD in no cases approaches DDT levels in either percent concentration or part per million levels. The percent level of DDD appears to increase slowly during the first half of the sample period and then

drops off as DDT disappears from the system. DDD can generally be found to occur in all groups of animals at relatively low levels.

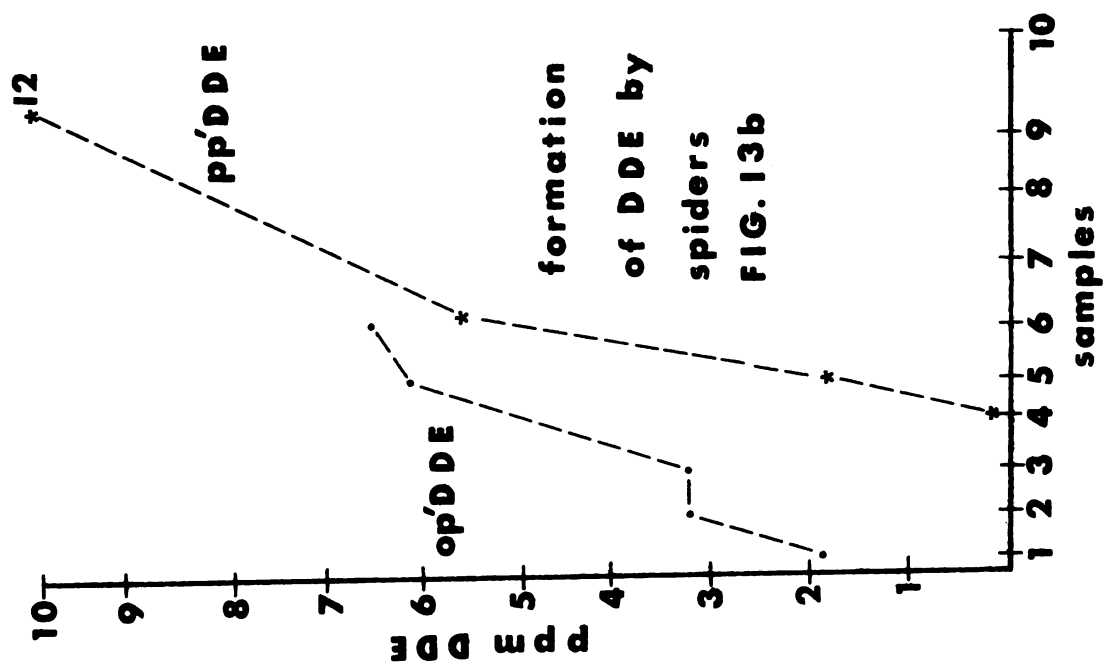
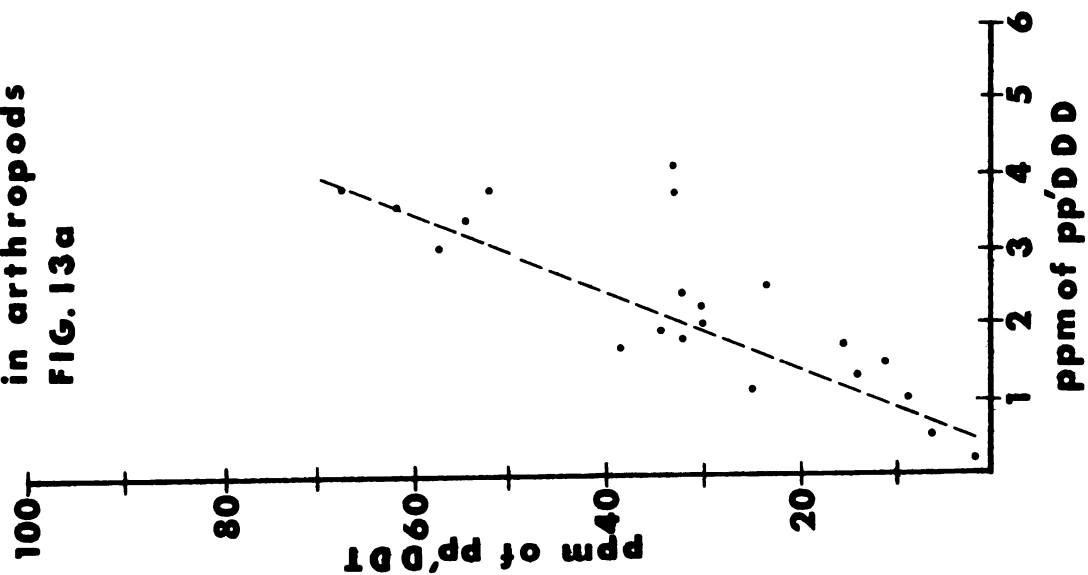
No group of arthropod predators studied accounted for more than any other group but a direct relationship was noted between the amount of DDT and DDD. Animals with a high level of DDT also contain a high level of DDD (Figure 13a). The percent of arthropods with DDT and DDD reveals that the pattern of their occurrence in the macro-arthropods is very similar. In both op' and pp' DDT introductions DDT drops off at about the same rate as DDT in time (Figure 14a,b). This is a reverse of the pattern noted for DDE. Also worthwhile noting is that the actual DDD levels are highest during the first samples. This is particularly evident in the pp' DDT release where DDT levels rapidly decrease due to the degradation to DDE.

These factors all suggest that DDD is a metabolism product of micro-flora in the gut of macro-cryptozoan predators produced only as long as DDT is present. It also appears that DDD is formed by the flora in the gut in proportion to the amount of DDT present.

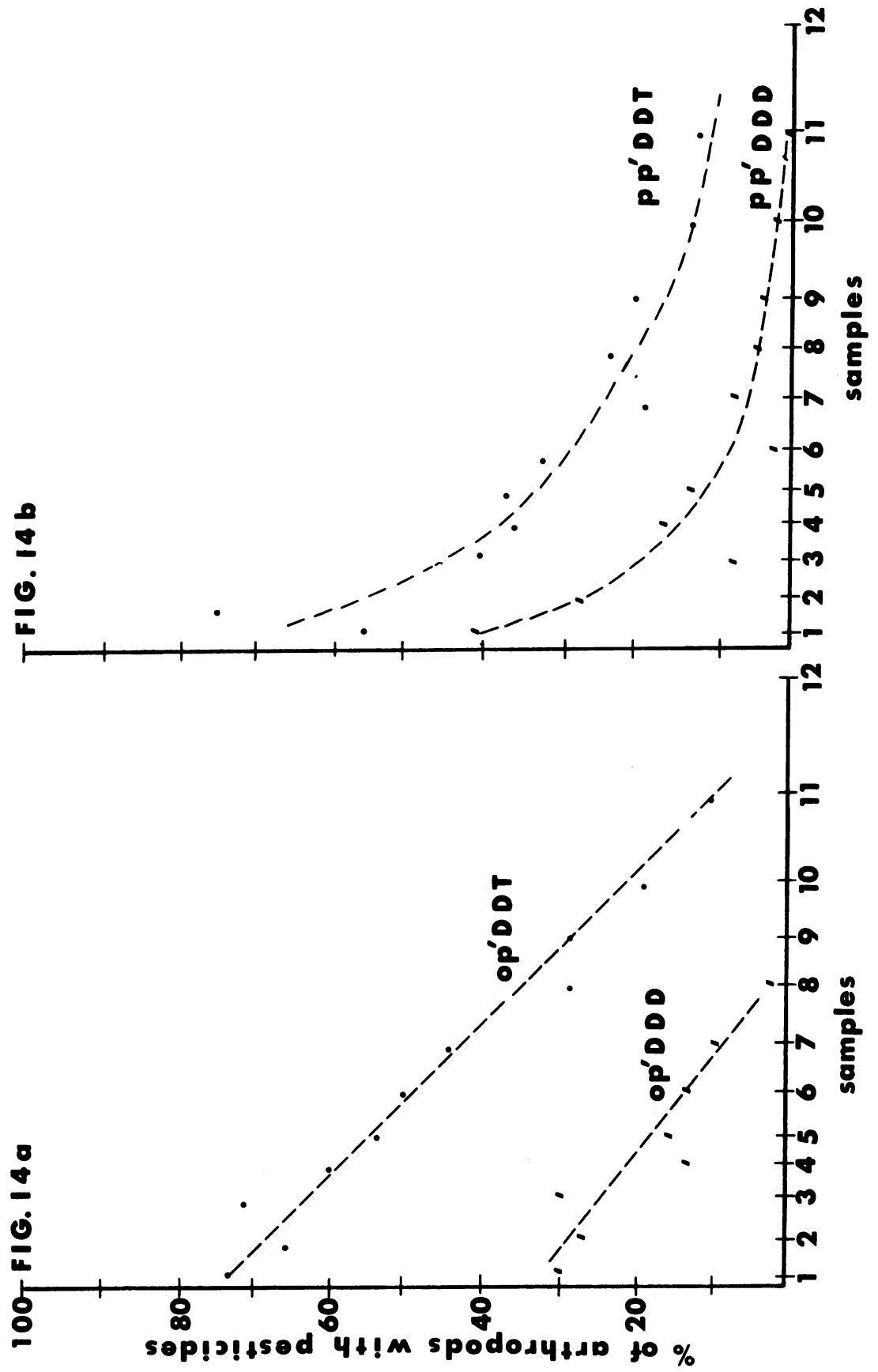
Pp' DDE Introduction

Pp' DDE when introduced into the food chain was found to be the most stable of the materials used. Macro-cryptozoan predators build up large amounts of the material in their bodies; much larger than found for either DDT introductions. The material was lost from the food chain during the study, but still remained in significant amounts at the end of the sample period. No recognized metabolites of DDE were found during the study. The major usefulness of the DDE introduction was to permit study of predatory tendencies, and efficiency of various cryptozoan predators within the community.

relationship of DDT to DDD
in arthropods
FIG. 13a



DISAPPEARANCE OF DDT AND DDD



The movement of DDE in the food chain was very rapid and encompassing. Within a period of twelve hours, the pesticide had moved to virtually all of the arthropods in the plots. 95% of the arthropods sampled contained DDE within twelve hours. While the part per million level increased after the twelve hours, the percent of collected animals with the pesticide did not. The same pattern was also observed in the other releases.

Ten major groups of cryptozoan fauna may be obtained from samples of the forest litter as follows: Araneae, Chilopoda, Coleoptera, Collembola, Diplopoda, Diptera, Hymenoptera, Lepidoptera larva, Orthoptera, and Pulmonata. For consideration of the predatory effects on the community, the first three groups are of major importance. The Araneae, Chilopoda and Coleoptera represent most all of the macro-cryptozoan predators and therefore are given most consideration in this study. Araneae are the most important. As a group, they form the most diverse fauna in terms of niches filled and predatory activities among the macro-cryptozoan fauna of the forest floor.

The important methods for determining the importance of an arthropod as a predator for this project was the amount of pesticide accounted for in terms of Collembola eaten (Table 1), as well as predator density and wet biomass. In order to estimate more accurately the number of Collembola eaten, spiders as well as some other groups such as the Chilopoda were divided into classes which were established on a weight basis. The divisions on weight proved very successful, but taxa must also be considered, as indicated by the hahniids which were a more aggressive predator than other members of their weight class.

TABLE 1

ESTIMATE OF NUMBER OF LABELED COLLEMBOLA EATEN, BASED UPON CONCENTRATIONS OF DDE RETAINED BY THE PREDATORS

	Ave. No. of Predator Animals Captured per Sample Period	Ave. No. of Labeled Collembola Eaten per Animal	Estimated No. of Labeled Collembola Eaten per Sample Period	Percent of Total Labeled Collembola Eaten by Predators Captured
Chilopoda large A	.83	6.46	5.36	9.15
medium B	1.50	6.88	10.32	17.63
small C	3.30	.51	1.68	2.8
Spiders				
Hahnidae	3.00	1.70	5.10	8.71
Thomisidae	.83	1.05	.87	1.40
Agelenidae A	1.10	6.07	6.67	13.10
B	1.50	3.44	5.16	8.80
C	1.30	1.17	1.52	2.59
Clubionidae A	.30			
B	1.50	3.89	5.83	10.00
Gnaphosidae	.30	.70	.21	0.35
Mixed small spiders	5.00	.94	4.70	8.00
Micryphantidae	3.00	.59	1.77	3.00
Staphylinidae	3.0	.50	1.50	2.56
Cantharidae larvae	.50	9.86	4.93	8.42
Insect Larvae	1.83	1.60	2.92	4.98
Estimated Total No. of Labeled Collembola Eaten by Those Predators Captured			58.52	

Estimates of Collembola eaten per animal and per sample period is listed in Table 1. Each sample period is equal to an area of one-half meter square. Estimates of Collembola eaten tend to be underestimated because losses due to excreta and breakdown are not accounted for. Table 1 also assumes no food chain magnification.

Population density and biomass estimates consisted essentially of censusing active above-ground forms. They did not include members of burrowing groups such as earthworms or forms hibernating or estivating beneath the soil surface or in other secluded places. Moulder (1970) considers burrowing or hibernating forms of animals to be out of the reach of surface predators and therefore their contribution to energy flow within the litter zone is negligible. These were, for the most part, overlooked during this study.

The total importance of an animal in the community in terms of its ability to degrade a compound or clean up its environment involves many factors; some of which interact. Essentially the animals' abilities to degrade the substance is important; but in order for the species to be a good degrader the material must enter the arthropod. Therefore, the effectiveness of predators in the community as tools for environmental cleanup depends not only on their degradation ability but also on their ability as predators, their density, and their biomass in relation to the total community. In the case of pesticide breakdown, of particular importance to the total community is that some species enter the food chain of larger or more efficient degrading species, particularly if the species is a poor degrader of the compound and only builds it up in the body. Certain species may also be

harmful in terms of the total degradation picture if breakdown of the pesticide does not occur rapidly enough; particularly if susceptible to predation by vertebrates which can move the pesticide out of the community or into a community of other forms, and still poorer degraders.

Spiders as Predators

Moulder (1970) considers spiders to be clearly among the most important entomophagous predators in nature. The present study would certainly indicate that they are the most important among forest floor fauna. Part of the importance of spiders is due to their abundance in the forest community. Several workers have given estimates of forest spider populations. These include Bornebusch (1930), Van der Drift (1951), Turnbull (1960), Gasdorf (1963), Reichle and Crossley (1967), and Moulder (1970). Observations during the present study revealed a spider population compatible with the above estimates.

Total spiders accounted for 55% of the total Collembola eaten. Size class analysis revealed that small spiders had the greatest impact, accounting for 22.5% of the total Collembola eaten. Large spiders, due to their lower population density and slower growth and feeding rate, were least important of the three groups, accounting for 13.1% of the total Collembola eaten. Medium-sized spiders were responsible for 20.1% of the total Collembola eaten. Of small spiders, Hahniidae accounted for 38.7% of the Collembola eaten. The Hahniidae accounted for about two times as many Collembola per animal as did the other small spiders on the average. In some cases three times. It is interesting that the Collembola eaten per animal by the Hahniidae is

closer to that for the small Agelenidae, to which they are closer related, than it is to other small spiders. If Hahnidae were omitted from the small spiders, then medium-sized spiders become the most important Collembola feeders.

The present study indicates that spiders are the most important group of predators in the cryptozoan population. They have the largest biomass, largest density, and account for the largest number of Collembola eaten per arthropod of any of the macro-arthropods of the forest floor (Appendix I).

All predatory levels are represented by the spider fauna. They are therefore not dependent on smaller predators to get the pesticide to them. They feed at all trophic levels from the small detritus feeding mites, Collembola, and the larger Diptera larva to the largest invertebrates in the community. Of the litter fauna, spiders are perhaps the most consistent predators, being exclusively predaceous, and showing the best predatory structure in relation to prey and predator biomass. Many of the other forms are predators for the most part, but are also opportunists and may feed on most anything, thereby feeding in many different trophic levels including detritus and up to a prey size class that they can overpower. Spider predation is limited by force or lack of it on the part of the spider; and in some cases on repellency.

Generally the larger forms of predators accounted for more Collembola per animal than did the smaller forms. In the Chilopoda, the medium-sized specimens accounted for more Collembola than did the larger forms. This suggests that all size classes of Chilopoda feed

directly on Collembola. On the other hand, spiders show a positive relationship to size and Collembola eaten. This may be accounted for by larger spiders eating smaller predators instead of eating Collembola directly.

Small-sized classes of predators accounted for about the same number of Collembola per animal throughout the study but larger forms increased in numbers of Collembola eaten per animal through time. Large forms also retained the pesticide longer. When compared with larger forms, the smaller spiders showed much less variation in number of Collembola eaten per specimen and part per million of DDE. This further indicates that the smaller forms eat Collembola directly and larger forms obtain their pesticide from smaller predators.

Another indication that larger spider forms do not obtain their DDE level directly from Collembola is that medium-sized Agelenidae and medium-sized Clubionidae were identical in regards to Collembola eaten, yet these two forms of spiders have different feeding habits. Agelenidae depend on a web and for the most part wait for food. Clubionidae, on the other hand, move about in search of their food.

Effects of Chilopoda on Cryptozoan Populations

Chilopoda is the second most important group of predators, accounting for 30% of the total Collembola eaten. Like the spiders, Chilopoda are conveniently separated into three classes based on size. The large sized specimens are the adults of the population and are three years old or older. The medium-sized group are the two-year-old specimens for the most part. The small chilopoda are young that have hatched during the spring of the present year.

The small specimens are most abundant and account for 0.51 Collembola per animal, or 2.8% of the total Collembola eaten. The medium-sized Chilopoda accounted for 17% of the Collembola eaten; which is about double that of the larger Chilopoda. When animals which accounted for the most Collembola per sample were analyzed, medium-sized Chilopoda were number one 57% of the time. Chilopoda also accounted for the largest number of Collembola consumed by a single specimen (19.4).

In the study woodlot, Chilopoda of all size classes fed directly on Collembola. On a per animal basis, they are perhaps the most important Collembola predator. Laboratory studies have shown Chilopoda of all size classes to be efficient predators on Collembola.

Coleoptera Larva as Cryptozoan Predators

Coleoptera larvae were the only immature insects found to be of importance as cryptozoan predators of released Collembola. Important Coleoptera larvae consisted of Cantharidae, Staphylinidae, Carabidae, and Elateridae. Total Coleoptera larvae accounted for about 5% of the Collembola eaten.

Cantharid larvae were found to be aggressive predators and on an "average per specimen" basis accounted for more Collembola than any other arthropod in the study area.

Carabid larvae were also aggressive predators and, along with the Elateridae, were very efficient degraders of the pesticide.

Staphylinid larvae were for the most part very small and sampling techniques were not adequate to make an assessment of their value.

**Coleoptera Adults as
Cryptozoan Predators**

Numerous adult Coleoptera were collected from the plots including Hydrophilidae, Scaphidiidae, Cantharidae, Lampyridae, Elateridae, Cucujidae, Nitidulidae, Alleculidae, Scarabaeidae, Chrysomelidae, Curculionidae, Staphylinidae, and Carabidae.

Staphylinidae and Carabidae were the only adult beetles collected of importance as predators. They were also the most common.

For the purpose of analysis, Staphylinidae were divided into two size classes: large and small. The small beetles were the most important of the two groups in terms of numerical abundance, total number of Collembola eaten, and number of Collembola eaten per animal. Small Staphylinidae accounted for 2.56% of the total Collembola eaten.

CONCLUSION

The macro-arthropod cryptozoan fauna was found to be capable of degrading both *op'* and *pp'* DDT when it was introduced directly into the food chain with Collembola. The intermediate products of metabolism varied, depending on which isomer was used and which arthropod ingested the DDT; but the final products detected in both cases were *pp'* DDE.

Studies of important predators in the community revealed that spiders are the major cryptozoan predators in terms of Collembola eaten. Spiders were also found to be the most common predators of the macro-cryptozoan fauna.

The study provided information on the following: (1) characterization of the most important cryptozoan predators that feed on Collembola or Collembola predators; (2) an interpretation of the possible role of arthropods in degradation of DDT; (3) evidence that both *op'* DDT and *pp'* DDT can be degraded by biological systems; (4) abilities of metabolic conversion of DDT by the cryptozoan predators in their natural environment; and (5) possible metabolic pathways of DDT for a variety of forest arthropod predators.

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APPENDICES

APPENDIX I

CRYPTOZOAN PREDATORS IN ORDER OF IMPORTANCE

Total number of Collembola eaten

- I. Araneida
 - a. medium
 - b. small
 - c. large
- II. Chilopoda
 - a. medium
 - b. large
 - c. small
- III. Coleoptera larvae
- IV. Coleoptera adults

Number of Collembola eaten per animal (percent frequency in top 5)

- I. Chilopoda 57%
 - a. medium
 - b. large
 - c. small
- II. Spiders 29%
 - a. large
 - b. medium
 - c. small
- III. Coleoptera larvae 14%
- IV. Coleoptera adults

Total biomass ranking

- I. Spiders
 - a. large
 - b. medium
 - c. small
- II. Coleoptera larva
- III. Coleoptera adults
- IV. Chilopoda
 - a. large
 - b. medium
 - c. small

Frequency of animals per sample period

- I. Spiders
 - a. small
 - b. medium
 - c. large
- II. Chilopoda
 - a. small
 - b. medium
 - c. large
- III. Coleoptera adults
- IV. Coleoptera larva

APPENDIX II

SAMPLING TIMES

All graphs showing "samples" are based on the following sample information. Samples were figured from days after release of Collembola, the evening of release being zero, and the sample taken the following morning being equal to one.

op' DDT Introduction

Sample number	Days from release
------------------	----------------------

1	1
2	2
3	5
4	7
5	10
6	13
7	18
8	23
9	30
10	37
11	42
12	49

pp' DDT Introduction

Sample number	Days from release
------------------	----------------------

1	1
2	2
3	6
4	8
5	10
6	13
7	16
8	20
9	23
10	26
11	33
12	40

APPENDIX III

NUMBERS OF ARTHROPODS REPRESENTED BY METABOLISM GRAPHS

Type of Arthropod	Average Number of Arthropods Represented by "Samples" on Graphs	Range for Number of Arthropods Represented by "Samples" on Graphs
Carabidae	3.3	1-5
Carabidae larva	1.6	1-4
Staphylinidae	10.8	5-18
<u>Aphodius</u>	2.8	2-7
Elateridae larva	1.2	1-4
Cantharidae larva	2.7	2-8
Lithobiidae	4.8	1-16
<u>Linotenias</u>	1.2	1-2
Diplopoda	13.0	8-21
Small spiders	17.5	5-35
Medium spiders	15.4	8-25
Thomisidae	4.5	1-11
Hahniidae	4.5	1-9
Formicidae	6.3	1-10
Pseudoscorpions	3.6	2-4

Total number of animals analyzed equaled 3434.

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