THE EFFECTS OF VARIOUS INSECTICIDES APPLIED TO A TERRESTRIAL MODEL ECOSYSTEM OR FED IN THE DIET ON THE SERUM CHOLINESTERASE LEVEL AND REPRODUCTIVE POTENTIAL OF COTURNIX QUAIL

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DOCTOR OF PHILOSOPHY

by

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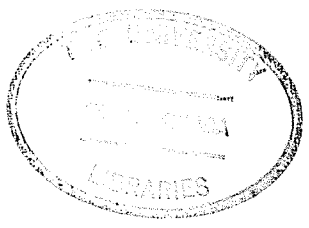

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

The study was initiated to determine the effects of five commonly used insecticides on the serum cholinesterase level and certain reproductive parameters of coturnix quail, when exposed to an overhead spray application in a model ecosystem, or fed a treated diet.

A four component model ecosystem proved satisfactory for evaluating the effects of insecticides on target and nontarget species. Dimethoate and fenitrothion applied to model ecosystems at recommended field rates produced 70 and 20 per cent reductions respectively in the serum cholinesterase level of female coturnix quail. Malathion, carbaryl and methomyl exhibited no significant effect on the serum cholinesterase levels of quail. None of the five insecticides tested in the model ecosystems appeared to affect bird behavior or egg production.

A diet containing 10, 40 or 80 ppm dimethoate during a 12 day treatment period produced an 85, 89 and 92 per cent reduction in the serum cholinesterase levels of treated birds, with recovery occuring within 11 days on an untreated diet. However, only the 80 ppm dietary level of dimethoate produced a reduction in food consumption, body weight and egg production over the 12 day treatment period. Recovery from the insecticide treatment was rapid when the birds were changed to an untreated diet.

Feeding a diet containing methomyl at 10, 40 and 80 ppm, or dimethoate at 10 or 40 ppm for 30 days had no significant effect on egg production of quail. Dimethoate at 80 ppm in the diet inhibited egg production

for 24 days, after which egg production continued on a sporadic basis for the remaining six days of treatment. Dimethoate at 10, 40 and 80 ppm in the diet caused a reduction in egg fertility, whereas methomyl had no effect on any of the reproduction parameters monitored. Only dimethoate at 80 ppm in the diet produced ataxia, trembling and other behavioral abnormalities in coturnix quail.

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TABLE OF CONTENTS

Chapter Page

LIST OF FIGURES

LIST OF TABLES

Table

Page

INTRODUCTION

Insecticides have become an intrinsic part of the environment of most living organisms. With the introduction of the organochlorine compound DDT, and it's widespread usage from 1947 to 1960, great steps were made in reducing populations of disease carrying insects and crop pests. Unfortunately, DDT and other organochlorine compounds were found to have adverse effects on the reproduction of certain avian species and to undergo biological magnification in biotic food chains. The organophosphate and carbamate insecticides which have been adopted as substitutes for the organochlorine compounds have a much shorter half-life in the environment, with rapid excretion from nontarget vertebrates. The organophosphate and carbamate insecticides are well known as cholinesterase inhibitors, and monitoring cholinesterase levels in the blood and brain tissue of living organisms has become a means of determining the presence of these insecticides in various environments. However, the effects of organophosphate and carbamate insecticides on the reproduction of avian species has not been well documented.

The model ecosystem approach for the evaluation of toxic chemicals in the environment is a recent development. A small terrestrial-water system has been developed and used with success, with data derived from this model system closely approximating that obtained from field experimentation. Unfortunately, this small model ecosystem does not permit long term experimentation using higher vertebrates due to its restricted size.

Coturnix quail have become increasingly popular for use in pesticide research. These birds exhibit moderate susceptibility to insecticides; and their short generation interval of eight weeks makes them useful in chronic feeding and population studies. Coturnix quail, being Galliforme birds, are representative of species found inhabiting nearly all agricultural areas of North America. As coturnix quail are small, easy to manage and omnivorous, they make an ideal top component for a large terrestrial model ecosystem. The objectives of this thesis were:

(1) To develop a terrestrial model ecosystem for the evaluation of insecticides on target and nontarget organisms.

(2) To determine the effects of certain insecticides on the cholinesterase level and certain reproductive parameters of an avian species.

Chapter 1

REVIEW OF THE LITERATURE

Organochlorine Compounds and Avian Species

The egg shell thinning phenomenon of organochlorine compounds in certain bird species has been well documented (Ratcliffe, 1967, 1970; McLane and Hall, 1972). Sublethal levels of DDT in the diet were shown to cause delayed ovulation in birds (Jefferies, 1967; Peakall, 1970). Theories on the physiological mechanism of reproductive abnormalities caused by organochlorine insecticides center around either a decreased supply of calcium to the egg shell or decreased use of calcium by the egg shell gland (Cooke, 1973). DDT did not affect clutch size of finches (Jefferies, 1971), sparrow hawks (Porter and Weinmeyer, 1969), or mallards (Heath et al, 1969). Peakall (1967) and Lehman et al, (1974) have reported the effects of DDT on enzyme levels in birds.

Organochlorine compounds have been found to induce changes in the normal behavior of birds (Jefferies, 1967; and Dahlgren et al, 1970). McEwen and Brown (1966) noted changes in normal breeding behavior of dieldrin treated sharp-tailed grouse released in the field. Kreitzer and Heinz (1974), fed endrin to young coturnix quail and found that chicks exhibited a lower avoidance response than untreated chicks, but that behavior returned to normal after two days on untreated food.

Grey partridges fed dieldrin in the diet produced eggs exhibiting an increase in dead embryos and hatchability of fertile eggs decreased from 82 to 73 per cent (Neill et al, 1971). DeWitt (1955) demonstrated

that hatchability of quail eggs from birds fed 10 ppm DDT in the diet was 50 per cent below that of untreated birds. However, Azevedo et al (1965) found DDT at 200 ppm in the diet had no effect on egg hatchability for quail.

Survival of chicks from eggs laid by quail fed 200 ppm DDT or endrin at one ppm in the diet was lower than for untreated birds (DeWitt, 1956). Chick mortality was not recorded by Neill et al (1971) for grey partridge fed three ppm dieldrin, or for pheasants fed 10 or 100 ppm DDT in the diet (Azevedo et al, 1965).

Azevedo et al (1965) reported that pheasants fed DDT in the diet produced fewer eggs that untreated birds, due to aversion of birds to treated food. Similar observations on behavior were made by French and Jefferies (1969) and Jefferies and French (1971, 1972) for pigeons dosed with capsules containing DDT.

Carbamate and Organophosphate Insecticides and Avian Species

Metabolism of Carbamates and Organophosphate Insecticides

Unlike the organochlorine insecticides which tend to accumulate in the tissues of living organisms, the carbamate and organophosphate insecticides are rapidly metabolized. The metabolic breakdown of the insecticide carbaryl by vertebrates and invertebrates has been studied intensively by Dorough and Casida (1964), Kuhr (1970) and Paulson et al (1970). Methylcarbamate insecticides are rapidly metabolized in mammals, with major metabolic transformation usually occuring within 12 hours of oral dosing. Excretion is rapid, with 80 per cent of a given dose eliminated in the urine and up to 15 per cent in the feces, 24 hours after treatment, (Dorough, 1970). Metabolism of consumed carbamates is virtually complete with little of the parent compound being excreted. Metabolism involves hydrolysis of the carbamic acid ester and excretion of the sulfur and/or

glucuronide conjugates (Dorough, 1970).

Organophosphate insecticides are rapidly hydrolysed by carboxyamidase in vertebrates (Hassen et al, 1969). Fenitrothion is absorbed through the gut of mammals (Douch et al, 1968) and excreted in the urine (90 per cent) and feces (10 per cent) within three days (Hollingworth et al, 1967). Dimethoate is likewise rapidly absorbed from the gut of rats (Sanderson and Edson, 1964) and excretion by cattle in the urine was 90 per cent of an oral or intramuscular dose within 24 hours (Dauterman et al, 1959).

Effects of Carbamate and Organophosphate Insecticides on Avian Reproduction

Egg Production

Haegele and Tucker (1974) found that carbaryl and parathion reduced the egg shell thickness of coturnix quail, but reported a similar reduction in shell thickness could be induced by starving the birds for 36 hours. Technical dimethoate at 30 ppm in the drinking water had no effect on egg production of hens (Sherman et al, 1963). Coturnix quail fed 50 ppm Azodrin in the diet exhibited a decreased food consumption and egg production was completely inhibited after the fourth day of feeding (Shellenberger et al, 1966). Sherman et al (1971) reported that the organophosphate insecticide SD-9020 severely depressed egg production in coturnix quail fed at the rate of 800 ppm in the diet, but that 400 ppm had no effect on egg production. Similar trends were reported by English (1975), with coturnix quail fed Abate at 80 ppm exhibiting reduced egg production, while those fed 32 ppm Abate in the diet, maintained normal egg production.

Hatchability

Shellenberger (1966) reported that eggs from coturnix quail fed Azodrin and Bidrin at 0.5 and 5 ppm in the diet exhibited no reduction in

hatchability. The organophosate compounds SD-9020, SD-8280, SD-8430 and SD-8211, when fed to adult coturnix quail, had no effect on the fertility or hatchability of eggs and no abnormal embryos or chicks occurred, even at levels of 800 ppm in the diet (Sherman et al, 1971).

Chronic Feeding

Sherman et al (1963) reported an overall food consumption and weight gain reduction for hens treated with 30 ppm dimethoate in the drinking water. Dimethoate at 100 ppm in the feed of pigeons and pheasants caused no mortality or toxic symptoms over a treatment period of up to 42 days (Bunyan et al, 1969). English (1975) observed no adverse effects on three generations of coturnix quail fed Abate at 32 ppm in the diet, but birds fed 80 ppm exhibited a slower growth rate, lower organ weight and reduced reproductive potential.

Behavioral Effects

Pigeons treated with Phosdrin exhibited reduced response rates to stimuli when dosed at levels below which external somatic symptoms of poisoning occur (Lewis et al, 1973). Sherman and Ross (1961) reported that the predominant symptom of acute organophosphate poisoning was lethargy, and was usually accompanied by excessive salivation and/or ataxia.

Cholinesterase Inhibition

The carbamate and organophosphate insecticides are well known cholinesterase inhibitors (Metcalfe and March, 1949; Lowell, 1962; Sanderson and Edson, 1964; and Sherman et al, 1971). The anti-cholinesterase activity of carbamate compounds tends to be reversible, whereas the organophosphates act as irreversible inhibitors, (O'Brian, 1957). The oxon metabolite of dimethoate is 75 to 100 times as potent as the parent

compound at inhibiting rat brain acetyl cholinesterase (Hassan et al, 1969). Walker (1971) mentions that plasma cholinesterase in chicks is more rapidly inhibited by maloxon, but recovery from inhibition is more rapid than from inhibition by the parent compound, malathion.

Coturnix quail fed Azodrin at five ppm in the diet had whole blood cholinesterase completely inhibited in males, and females showed only nine per cent of the enzyme level of untreated birds. Male quail fed Bidrin at 0.5, 5 and 50 ppm in the diet exhibited complete enzyme inhibition whereas females treated at the 0.5 ppm rate still exhibited an enzyme activity of four per cent (Shellenberger et al, 1966), with a return to near normal enzyme activity occurring after a two week post-treatment period on untreated feed.

Holland et al (1969) proposed that determination of acetyl cholinesterase levels in fish would be a useful technique for monitoring the presence of cholinesterase inhibiting compounds in aquatic systems. Findlay et al, (1974) monitored the effect of an aerial application of fenitrothion on nontarget species in a forest environment, using serum acetyl cholinesterase levels in coturnix quail as a parameter. Birds penned in exposed areas exhibited a 62.5 per cent reduction in acetyl cholinesterase activity eight hours postspray. Partial recovery occurred by two days posttreatment and enzyme levels were reported to have returned to normal five days postspray.

It should be noted that cholinesterase levels tend to fluctuate with time within a population of animals (Gibson et al, 1969). A high level of exposure to insecticides that may cause the death of one individual, may not cause the death of others treated in a similar manner.

Model Ecosystems

First reported use of a model ecosystem for determining the fate of pesticides in a multi-system food chain was made by Metcalfe et al, (1971). This small system contained a terrestrial-aquatic interface with plant and animal components to make up a multi-component food chain. Metcalfe et al (1971) reported fairly good reproduceability of results between similar model systems and that results from the model ecosystem approximate conditions observed in the field.

This model ecosystem approach, using the small ecosystem developed by Metcalfe et al (1971) has since been used for experimentation by Sanborn and Yu (1973) and Yu et al, (1975).

Coturnix Quail as a Laboratory Animal

The coturnix quail has been gaining popularity as a laboratory animal, and information on the biological parameters of this bird have been documented by Ivey and Padgett, 1959; Wetherbee, 1961 and Wilson et al, 1962. Since the reproductive cycle of coturnix quail is short and the birds are adaptable, these small galliforme birds are being widely used as an avian representative for laboratory testing of insecticides (Shellenberger and Newell, 1965; Sherman et al, 1971, Ludke, 1974 and English, 1975). Attempts to produce populations of quail homozygous for certain traits has met with little success (Shellenberger and Newell, 1965), with inbreeding resulting in a drastic reduction in bird vitality and fecundity. Thus researchers are at present forced to work with populations of birds exhibiting considerable variability with respect to many of the parameters used in pesticide research.

Chapter 2

MATERIALS AND METHODS

Construction of a Terrestrial Model Ecosystem

A terrestrial model ecosystem was designed to simulate an agricultural field with a multicomponent food chain. The system consisting of soil, plants, invertebrates and vertebrates, was designed to provide the top component, coturnix quail, with adequate forage for a period of up to 10 days.

The basic bottom bench structure of the model ecosystem was constructed of asbestos board with a fiberglass bottom. Inside measurements of the bench were 332 cm long by 74 cm wide and 20 cm high. This bench structure was supported by a stand made of five cm diameter steel pipe. The high sides prevented the birds from seeing out, thus alleviating the problem of the birds running back and forth along the edges trying to escape.

A removable screen top to fit on the bench was constructed of clear cedar to prevent warping. Although 332 cm long, this top structure was light enough for one person to handle with ease. The sides, ends and top were covered with nylon netting (3 mm mesh). The netting for the ends and top was held in place with tacks, to allow entry to the system. The netting on the sides was fastened in place with staples.

The inside of the bench was lined with six mil plastic sheeting to prevent any seepage of water. The excess plastic, left hanging loose from the bench, could be raised above the outer sides of the screen top

(Fig. 1) to prevent spray drift during application and possible contamination of adjacent model ecosystems.

Prior to placing the soil in the bench, an underground irrigation system was installed consisting of two plastic tubes running the length of the bench and 25.4 cm from the sides. Tubes were 10 mm in diameter, with two mm holes at 15 cm intervals along the tube length. A plumber's T, with the tubing connected was installed at one end of the bench, with the opposite ends of the irrigation tubes stoppered with corks. Water was added to the bench by attaching one end of the plumber's T to rubber tubing connected to a five gallon pail located above the bench with water flow controlled by an adjustable clamp.

Components

Light was provided by a fluorescent light bank running the full length of the bench. These light banks were adjusted to the height of the plant growth in the benches and could be raised high enough to allow for spray application when the wooden screen top was in place. The light bank provided 5000 foot candles at the soil surface when in position four inches above the wooden top.

Soil used in the model ecosystem was a clay loam, with high organic matter content. Trials using various soil depths indicated that a depth of 6.3 cm was adequate to provide good plant growth. This depth of soil retained adequate moisture, using a three day irrigation interval, and the soil did not create a disposal problem at the end of an experiment.

A wide variety of plant species were tested for use in the model ecosystem. The plant species that exhibited the best growth under these growing conditions and was palatable to coturnix quail, was rapeseed (Brassica napus). As the birds tended to trample the lower leaves of

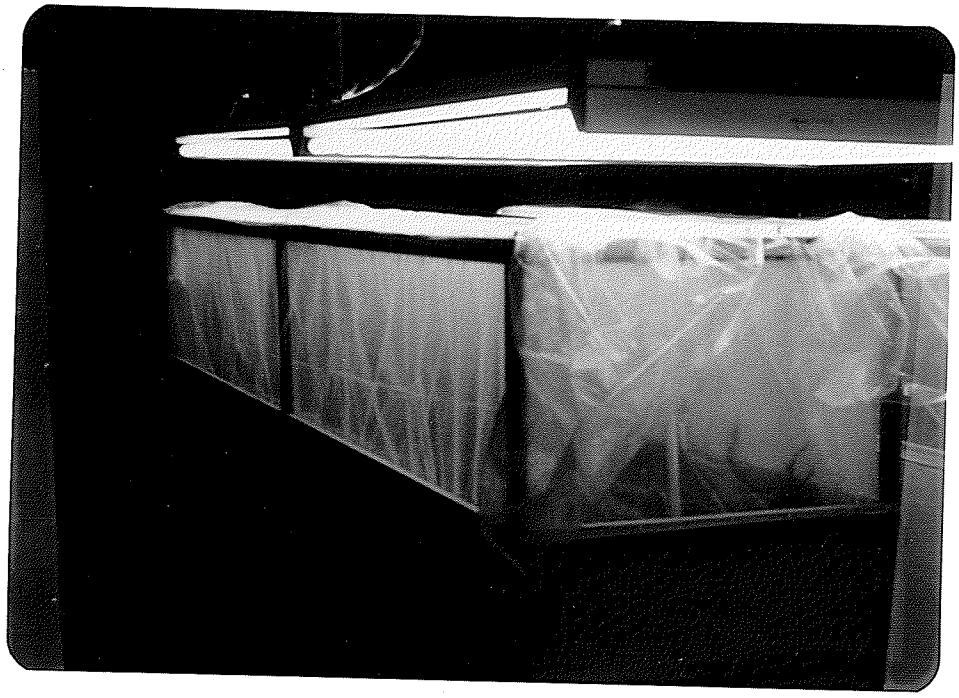

Fig. 1. Terrestrial model ecosystem: plastic sheeting raised

the rapeseed plants, or break off leaves, wheat (Triticum sp.) was interplanted to give extra stability to the rape plants and provide cover as well as extra food for the birds as the lower leaves of the rape plants were consumed.

The seeds of rapeseed and wheat were planted alternately every 15 cm, in four rows spaced 15 cm apart. This allowed for 88 rapeseed and 88 wheat plants per bench. The growth rate of these plants under an 18 hour light: six hour dark regime produced plants that were large enough (Fig. 2) 40 days after sowing to provide food for four birds for 7 to 10 days of experimentation.

Drinking water was provided for the birds with a one quart water pot placed in each model ecosystem, with a total exposed water surface area of 60 sq. cm. Water pots were placed in such a manner that the water surface was exposed to spray application and not covered by plant growth.

A total of four identical model ecosystems were constructed as described above. In addition, two smaller versions, 209 cm long by 74 cm wide and 20 cm high were utilized for conditioning of birds or for the maintenance of control birds during the experiments.

The green peach aphid [Myzus persicae (Sulzer)] was utilized as an invertebrate component, providing extra food for the quail and allowing for the determination of chemical effectiveness. Grasshoppers [Melanoplus bivittatus (Say)] were utilized in one experiment as an additional component. Although fairly easy to culture in the laboratory, grasshoppers were collected from the field as needed for experimentation.

For those experiments where more than four birds were to be maintained in each model ecosystem, five gm of chick starter per bird was placed in a corner of each ecosystem each day.

Fig. 2. Growth of rapeseed and wheat plants in a terrestrial model ecosystem

Quail used in all experiments in the model ecosystem were adult female coturnix quail (Coturnix coturnix japonica), 16 to 20 weeks of age. Females were found to be better foragers and less excitable than males, with egg production providing another parameter for monitoring. Birds were placed in the model ecosystems a minimum of three hours prior to experimentation; and in most cases could not easily be found amongst the foliage (Fig. 3) prior to chemical spraying.

Quail Maintenance

Quail used in test experiments were from the coturnix quail colony maintained by the Department of Entomology, University of Manitoba. This breeding population was maintained in a chick brooder (Hawkins Million Dollar Hen Inc.), with a ratio of 10 males to twenty females in each layer. A light regime of 18 hours light: 6 hours dark was provided. Birds were maintained on a diet of commercial chick starter (21 per cent protein) and water was supplied by two automatic water cups per cage layer.

Eggs for incubation were collected daily and stored at $4^{\circ}C$ for a maximum of 10 days. A Robbins incubator, set at a temperature of $33^{\circ}C$ and 87 per cent relative humidity was used for incubation. Eggs were left in the incubator trays until hatching had started, at which time the eggs were transferred to hatching trays. Once dry, the chicks were moved to a specially constructed brooder. Heat was provided by 60 watt light bulbs and relative humidity was maintained inside the brooder at 85 to 90 per cent by closing the air vents and placing wet paper towels in jars of water in the corners of the brooder.

Feed was provided for the first week by placing ground chick starter on paper towels under the light bulbs, and in egg trays in the center of the brooder. One quart water pots, with stones in the bowls to keep the

Fig. 3. Coturnix quail foraging in a terrestrial model ecosystem

chicks from drowning, were kept filled to the lip of the bowl and provided at the rate of one water pot for every 50 chicks. By providing high humidity, finely ground food and easy access to water, chick mortality during the critical first seven days was reduced to 10 per cent compared to 40 per cent for those maintained under brooder conditions suitable for chickens. Chicks were kept in the brooder for 14 days, by which time they were well feathered and could be transferred to the breeding cages.

Conditioning of Birds

Quail taken from the breeding cages and placed in model ecosystems, divided layer brooders or rat cages, need time to adjust to their new surroundings. During the process of adjustment to all environments, the birds would freeze in one spot for hours at a time, or fly wildly into the sides of the cage or model ecosystem. Food and water consumption was greatly reduced for several days, and when the birds did start foraging, it was observed that they consumed large amounts of soil, small stones and twigs, with some birds dying from impacted gizzards. Birds to be tested in the model ecosystem were conditioned for three weeks prior to experimentation. For the first week the birds were fed grit (Hartz Mountain) and leaves of lettuce and rapeseed plant while maintained in the breeding cages. Metal trays containing one inch of soil in which rapeseed plants were growing were placed in the breeding cage during the second week of conditioning, with birds to be used in an experiment in the model ecosystems. One week prior to experimentation in the model ecosystem, quail to be used for a test, along with two spares, were placed in a smaller version of the model ecosystem, which was densely planted to rapeseed and clover. Birds in this conditioning bench were offered grit and chick starter free choice and provided with two, one quart water pots. As the

rapeseed plants were consumed, fresh rapeseed plants and lettuce leaves were added daily to ensure a continuous supply of forage.

Birds for experimental feeding trials in the divided layer cages were conditioned in these cages for one week before experimentation. Quail to be maintained in the rat cages required a conditioning period of at least two weeks before experimentation. Since confinement in the small rat cages upset the birds greatly for several days, reducing intake of food and water and causing a drop in egg production, 20 per cent more birds were conditioned than were used for experiments. Only the properly conditioned birds were used in experiments.

Experimental Cages for Feeding Trials

Two types of cages were used during experimentation:

A) Divided-layer brooders; standard chick brooders 297 cm long by 73 cm wide by 20 cm high, with each of the top four layers divided in half by a screen divider to provide eight separate cages. Each cage was equipped with a single water cup and 76 cm long feeder.

B) Standard rat cages; rat cages, 24 cm long by 18 cm wide by 18 cm high, held in a cage stand, were used to house individual birds. Each cage was equipped with a 116 ml glass jar for water and a similar jar for food.

Insecticide Treatments

The selection of insecticides for testing was based on usage in agriculture and forest insect control. Insecticides used in the test experiments (Table 1) were commercial formulations as provided by the manufacturer. Application rates tested in the model ecosystem were per acre dosages recommended for agricultural crops and forest spray operations, or multiples of recommended rates. Rates of chemicals fed to caged birds were based on possible environmental levels of consumption (Findlay et al, 1974; English, 1975) or multiples of same.

Table 1.
Formulation and rate of insecticides evaluated in model ecosystem experiments

Insecticide	Group	Formulation[1]	Rate (oz ai/acre)
Malathion	Organophosphate	50 EC	8
Carbaryl	Carbamate	80 S	10
Dimethoate	Organophosphate	40 EC	16
Methomyl	Carbamate	20 EC	3.6
Fenitrothion	Organophosphate	97 EC	4

[1]EC - emulsifiable concentrate; S - soluble powder

Insecticide Applications

Model Ecosystems

Insecticides were applied to the model ecosystems using a one gallon Green Cross pressure sprayer equipped with an adjustable nozzle. The insecticides were applied evenly over the bench (plastic raised) in such a manner as to minimize loss by contact with the screen top. After spray application, the plastic was lowered to allow normal ventilation.

Experimental Feeding Trials

Insecticides were added to dry feed by mixing the required amount of toxicant in 100 ml of acetone and pipetting the mixture onto 1.0 kg of chick starter. After the acetone had evaporated, the feed was thoroughly mixed and added to 4.0 kg of untreated feed and manually mixed. Treated feed was mixed weekly and stored in sealed plastic bags at 4°C.

Insecticide Efficacy

The effectiveness of insecticide applications was determined by evaluating the control of aphid populations on the rapeseed plants. Aphid population densities were determined prespray and six hours, two days, five days and where applicable, ten days postspray. Five leaves were sampled from each model ecosystem at each sampling period and the number of aphids recorded.

Dimethoate Analysis

Sampling

Ecosystem components collected for analysis were rapeseed, grasshoppers and quail. Rapeseed leaves were randomly collected four hours prespray and ten days postspray (collections were made from the remaining lower leaves). Ten leaves were collected as a sample from each model ecosystem replicate. Sixteen grasshoppers were collected from each model ecosystem four hours prespray and six hours postspray. Two quail were collected

from each treated model ecosystem ten days postspray, while three birds were sampled from the untreated control. All birds were killed by decapitation and individually stored for analysis. All samples were stored in poly bags at -20°C until analysed.

Extraction of Dimethoate from Rapeseed Tissue

Ten gm of rapeseed tissue, 30 gm of Na_2SO_4 and 25 ml of 1% glacial acetic acid were homogenized together in a Sorval omni-mixer for five minutes at a setting of four. The liquor was then filtered through glass wool into a separatory funnel and washed with three 15 ml volumes of acetonitrile. The rape tissue was then returned to the blender and the process repeated twice. The homogenate was extracted twice more with 25 ml acetonitrile, the acetonitrile portions were combined in a round bottom flask and evaporated to dryness on a rotary evaporator. The residue was taken up in acetone and transferred to a septum vial and stored at 1°C until analyzed.

Extraction of Dimethoate from Grasshopper and Quail Tissue

Grasshopper samples (16 insects weighing approximately two gm), 10 gm of Na_2SO_4 and 25 ml of acetonitrile (or 10 gm of quail tissue, 40 gm of Na_2SO_4 and 50 ml of acetonitrile) were blended in a Sorval omni-mixer for five minutes at a setting of four. The extract was then filtered through glass wool into a round bottom flask. The tissue samples were extracted twice more with 50 ml acetonitrile. The three filtrates were combined in the round bottom flask and the extract was evaporated to dryness on a rotary evaporator at 40°C. The residue was taken up in acetone and transferred to a septum vial for storage at 1°C to await derivatization.

The analysis of dimethoate and its oxon metabolite was carried out using a Tracor Micro Tek MT 220 gas chromatograph equipped with a Melpar

Flame Photometric detector operated in the phosphorus mode (526 rm) as described by Sarna et al, 1976.

Serum Cholinesterase Analysis

Blood was collected from quail be decapitation or by vein puncture. Blood samples were collected in micro hermatocrit capillary tubes. Serum was obtained by centrifugation of the tubes for five minutes at a setting of five in a standard centrifuge. The tubes were then tightly sealed with crivac and stored at -20°C until analysed.

Cholinesterase activity in the serum was measured following the method of Ellman et al (1961), with analysis conducted on a Zeiss Spectrophotometer using prepared reagent from Boehringer Mannheim Corporation. Serum samples were diluted 1:10 prior to analysis at a machine operating temperature of 40°C. All samples were done in duplicate. Acetyl cholinesterase activity is expressed in milli Units/ml of serum.

Standardization Experiments

Since the results of most experimental trials were interpreted on the basis of quail serum cholinesterase fluctuations, preliminary experiments were conducted to ascertain the extent of cholinesterase fluctuations on untreated and treated birds. Blood samples were collected every five days from six female quail fed an untreated diet for 35 days to determine the range of fluctuations in serum cholinesterase levels. The birds were maintained in individual rat cages for the duration of the experiment.

Results indicated that serum cholinesterase levels tend to fluctuate over a period of time (Table 2), with a mean fluctuation ranging from 65 to 400 mU/ml from sample period to sample period occurring for birds under these experimental conditions. Birds #1 and #5, maintained constant enzyme levels for 15 days, but by day 25 bird #1 exhibited an increase of 800 units, while bird #5 showed a drop of 700 units. The other four

Table 2.
Serum cholinesterase levels (mU/ml) of coturnix quail maintained on an untreated diet for thirty-five days

Bird Number	Days on Untreated Diet						
	5	10	15	20	25	30	35
1	2090	2090	2090	2090	2330	2020	1310
2	2540	2480	2480	2650	2230	2530	-[1])
3	1970	1300	1980	1970	1690	1350	1970
4	2430	2080	1950	2080	1640	2430	1950
5	1950	1950	1950	1950	1250	1530	1630
6	2840	2800	2310	2310	2200	2040	2646
$\bar{X}$	2304	2207	2372	2175	1983	2065	2002
SD	326	338	396	240	524	339	343

[1]Bird eggbound

birds underwent less drastic but more sporadic changes in enzyme level over the 35 day treatment period.

The length of time required for serum cholinesterase levels in quail to recover following treatment with a cholinesterase inhibiting insecticide (fenitrothion) was determined. A gelatin capsule containing fenitrothion at 25 mg/kg (LD 50 level) was given orally to twelve, 20 week old female quail. Blood samples were collected four hours pretreatment and 2,4,12 and 24 hours posttreatment.

By two hours posttreatment, seven of the twelve birds were dead and another was nearly dead. The remaining four birds exhibited a mean serum cholinesterase level of 2.1 per cent of pretreatment levels (Table 3). The enzyme level remained very low at four hours posttreatment, but increased to 8.7 per cent of the pretreatment level by 12 hours posttreatment. Twenty-four hours after dosing, the enzyme level increased to 21.2 per cent of the pretreatment level.

Two, four and 12 hours after treatment, the quail exhibited ataxia, excessive salivation and gasping. Twenty-four hours after treatment the quail no longer exhibited any visible signs of fenitrothion poisoning and were feeding actively, even though the serum cholinesterase level was only 21.2 per cent of the pretreatment level.

Chapter 3

EXPERIMENTAL

PART A

Model Ecosystem Experimentation

Malathion

A. Aphid Control

Malathion at 8 oz ai/acre provided good control of aphids on the rapeseed plants (Table 4). By two days postspray, no aphids were present in the treated model ecosystems, but were still abundant on plants in the untreated system.

B. Effects on Serum Cholinesterase Level

Malathion applied as an overhead spray to four replicate model ecosystems at the rate of 8 oz ai/acre had no apparent effect on the serum cholinesterase levels of coturnix quail maintained in the model system for five days (Table 5). Blood samples for this experiment were collected by decapitation, sampling one bird from each model system at each sampling period. There were no observable differences between the foraging activity and general behavior of treated and untreated birds. Egg production of the treated birds continued through the experiment with an average hen day production (Appendix I) of 75.

Carbaryl

A. Aphid Control

Carbaryl at 10 oz ai/acre did not provide complete control of aphids on the rapeseed plants. The aphid populations on the upper leaves, out of

Table 4.
Aphid populations on rapeseed before and after malathion[1] treatment to four model ecosystems

(Means and standard deviations for 5 leaves per system)

Treatment	Prespray	Postspray 6 hours	Postspray 2 days	Postspray 5 days
Malathion	12±5	5.4±4	0	0
Control	9.8±6	7.6±4	12.4±4	22±7

[1]Malathion applied at 8 oz ai/acre

Table 5.
Serum cholinesterase levels (mU/ml) of coturnix quail before and after malathion[1] treatment to four model ecosystems

(Means and standard deviations for 4 birds)[2]

Treatment	Prespray	Postspray 6 hours	Postspray 2 days	Postspray 5 days
Malathion	1810±236	1860±227	1965±160	1755±133
Control	1935±253	1875±178	1965±188	1790±288

[1]Malathion applied at 8 oz ai/acre
[2]One bird collected from each model ecosystem at each sampling time and blood collected by decapitation

reach of the quail, was reduced 70 per cent by two days postspray (Table 6) compared to pretreatment levels, but the infestation had increased by 10 days postspray to 60 per cent of the pretreatment level (33 per cent of the population on the untreated plants).

B. Effects on Serum Cholinesterase

Carbaryl applied as an overhead spray to four replicate model systems at the rate of 10 oz ai/acre had no appreciable effect on the serum cholinesterase activity of coturnix quail (Table 7). Enzyme levels of eight birds were monitored on an individual basis by the vein puncture technique for a period of 10 days, with cholinesterase levels fluctuating within normal limits. Behavior of treated birds appeared normal as compared to untreated birds and egg production continued throughout the duration of the experiment (80 hen day production).

Dimethoate

A. Aphid and Grasshopper Control

The aphid population present on the rapeseed plants prior to treatment had disappeared by two days postspray (Table 8). No reinfestation had occurred by the termination of the experiment on the tenth day. Sixty grasshoppers which had been introduced to each model ecosystem one hour prespray were dead by 24 hours posttreatment. Dead and dying grasshoppers were rapidly consumed by the quail.

B. Effects on Serum Cholinesterase Levels

Dimethoate was applied as an overhead spray to three replicate model ecosystems at the rate of 16 oz ai/acre. By two days posttreatment the serum cholinesterase levels were significantly reduced to 30.3 per cent of pretreatment levels (Table 9). Untreated birds maintained steady cholinesterase levels throughout the duration of the experiment. Birds

Table 6.
Aphid populations before and after carbaryl[1] treatment to four model ecosystems

(Means and standard deviation for 5 leaves per system)

Treatment	Prespray	Postspray 6 hours	2 days	5 days	10 days
Carbaryl	10±3.5	4±3	3±2	5±4	6±3
Control	9±3.6	8±4	14±5	12±4	19±4

[1]Carbaryl applied at 10 oz ai/acre

Table 7.
Serum cholinesterase levels (mU/ml) of individual[1] coturnix quail before and after carbaryl[2] treatment to four model ecosystems

Treatment	Bird No.		Prespray	Postspray 6 hours	2 days	5 days	10 days
Carbaryl	1		2430	2325	1730	2150	1980
	2		1680	1990	1250	1600	2400
	3		1975	1750	1040	1875	2430
	4		2310	2200	1975	2400	1965
	5		1975	2030	2030	2000	2310
	6		2760	2420	2775	2500	2400
	7		2430	2310	1525	2530	2535
	8		2400	2350	2200	2300	2300
		$\bar{X}$	2240	2172	1816	2169	2289
		SD	321	214	518	306	195
Control	9		2450	2325	1990	1900	2500
	10		1950	1900	2150	2200	1950
		$\bar{X}$	2200	2113	2070	1950	2240
		SD	250	213	79	250	293

[1]Blood samples collected by vein puncture
[2]Carbaryl applied at 10 oz ai/acre

Table 8.
Aphid populations before and after dimethoate[1] treatment to three model ecosystems

(Means and standard deviations for 5 leaves per system)

Treatment	Prespray	Postspray			
		6 hours	2 days	5 days	10 days
Dimethoate	17±8	16±2	0	0	0
Control	18±8	19±7	28±10	28±12	18±10

[1]Dimethoate applied at 16 oz ai/acre

Table 9.
Serum cholinesterase levels (mU/ml) of individual[1] coturnix quail before and after dimethoate treatment to three model ecosystems

Treatment	Bird No.		Prespray	Postspray			
				6 hours	2 days	5 days	10 days
Dimethoate	1		1790	1500	800	900	1500
	2		1560	1200	300	1590	1800
	3		1300	1350	400	1430	1135
	4		1300	1200	300	530	950
	5		1500	1400	470	1200	2100
	6		1300	1300	900	900	1200
		$\bar{X}$	1421	1307	532[a]	925[a]	1447
		SD	190	108	219	317	399
Control	7		1850	1790	1800	2000	1800
	8		1400	1450	1700	1500	1450
	9		1800	1750	1750	1600	1700
		$\bar{X}$	1683	1663	1750	1700	1650
		SD	201	151	40	216	147

[1]Blood samples collected by vein puncture

[2]Dimethoate applied at 16 oz ai/acre

[a]Significantly different (ANOV, $P \leq 0.05$) from prespray value

#1, 4 and 6 still exhibited a low enzyme activity five days postspray. Serum cholinesterase activity returned to near normal limits in all the birds with the exception of #4, by 10 days posttreatment.

None of the birds exhibited any apparent external symptoms of insecticidal poisoning. Behavior remained normal for the duration of the experiment, with the birds foraging actively. Egg production continued normally through the experiment, with an average hen day production of 83.

C. Residues in Ecosystem Components

Leaves of rape plants collected ten days postspray from the treated model systems (ten leaves sampled from each of three model ecosystems) contained dimethoate residues ($\bar{x}$ 9.6 ppm). Two of the rapeseed samples also contained the oxon metabolite ($\bar{x}$ 2.3 ppm). Dead and dying grasshoppers collected six hours postspray contained residues of both dimethoate ($\bar{x}$ 18 ppm) and dimethoxon ($\bar{x}$ 12 ppm). Thus rapeseed and grasshoppers being consumed by the quail contained both dimethoate and dimethoxon. Quail collected at the end of the 10 day ecosystem study were submitted to residue analysis. Residues of dimethoate were detectable in only birds #5 and #1, where residue levels were 10 and 7 ppm respectively. No dimethoxon was recovered from tissue of any quail.

Methomyl

A. Aphid Control

Populations of aphids in the treated model ecosystems were reduced by the methomyl spray by the two day sampling period (Table 10). By five days postspray, all aphids had been eliminated from the model ecosystems.

B. Effects on Serum Cholinesterase Levels

When applied to four replicate model ecosystems at the rate of 3.6 oz ai/acre, methomyl had only a slight but non-significant effect on serum

cholinesterase levels of coturnix quail six hours posttreatment.

Four birds were sampled four hours prespray, and at six hours, two days and five days postspray, with blood collected by decapitation. Serum cholinesterase levels of treated birds were lower (11 per cent) than for prespray values for the six hour sampling time (Table 11), but had returned to pretreatment levels two days after treatment. The treated birds showed no signs of insecticide poisoning, with foraging continuing normally. Egg production continued throughout the duration of the experiment with an average hen day production of 75.

Fenitrothion

A. Aphid Control

Within six hours after treatment, fenitrothion had reduced the aphid populations in the treated model ecosystems by 90 per cent (Table 12). Two days posttreatment sampling revealed no aphids remaining on the treated rapeseed plants, whereas aphid populations on the untreated plants were so high as to be reducing the vigor of the plants.

B. Effects on Serum Cholinesterase Levels

When applied to four replicate model ecosystems at a rate of 4 oz ai/acre, fenitrothion caused a slight but non-significant reduction in the average serum cholinesterase level by six hours posttreatment (Table 13). Bird #'s 1, 2, 6 and 4 exhibited a drop in enzyme level of 36, 30, 22 and 33 per cent, respectively, at six hours postspray, with enzyme levels returning to pretreatment levels by two days posttreatment. Bird #'s 3 and 5 exhibited no significant drop in enzyme level by six hours postspray, but by the two day sampling period the enzyme level had dropped by 47 and 30 per cent of the pretreatment levels respectively. Enzyme levels for both birds #5 and #3 were within normal limits by five days posttreatment.

Table 10.
Aphid population before and after methomyl[1] treatment to four model ecosystems

(Means and standard deviations for five leaves per system)

Treatment	Prespray	Postspray		
		6 hours	2 days	5 days
Methomyl	17±8	12±2	3±3	-
Control	15±4	14±3	19±6	50±9

[1]Methomyl applied at 3.6 oz ai/acre

Table 11.
Serum cholinesterase levels (mU/ml) of coturnix quail before and after methomyl[1] treatment to four model ecosystems

(Means and standard deviations for 4 birds[2])

Treatment	Prespray	Postspray		
		6 hours	2 days	5 days
Methomyl	1785±187	1580±131	1780±44	1740±256
Control	2025±541	1900±254	1850±154	1905±330

[1]Methomyl applied at 3.6 oz ai/acre
[2]Blood samples collected by decapitation

Table 12.
Aphid population before and after fenitrothion[1] treatment to four model ecosystems

(Means and standard deviations for 5 leaves per system)

Treatment	Prespray	Postspray 6 hours	Postspray 2 days	Postspray 5 days
Fenitrothion	19±6	1.8±2	-	-
Control	18±5	19±3	27±8	26±20

[1]Fenitrothion applied at 4 oz ai/acre

Table 13.
Serum cholinesterase levels (mU/ml) of individual[1] coturnix quail before and after fenitrothion[2] treatment to four model ecosystems

Treatment	Bird No.		Prespray	Postspray 6 hours	Postspray 2 days	Postspray 5 days	Postspray 10 days
Fenitrothion	1		2230	1436	2425	1995	1300
	2		1860	1245	2645	1415	1360
	3		1415	1395	800	1620	1750
	4		2140	1400	2034	2056	2348
	5		1575	1475	1090	1510	1680
	6		1750	1350	1730	1730	1230
	7		1315	1300	1064	950	1290
	8		2130	2000	1625	950	1680
		$\bar{X}$	1802	1452	1677	1466	1663
		SD	326	219	624	393	316
Control	9		1900	1800	1800	1500	1920
	10		1290	1290	1250	1010	1290
	11		1500	1455	1740	1500	1900
		$\bar{X}$	1562	1514	1639	1337	1704
		SD	253	213	235	232	294

[1]Fenitrothion applied at the rate of 4 oz ai/acre
[2]Blood samples collected by vein puncture

Fenitrothion at the 4 oz ai/acre rate had no noticeable effects on quail behavior. The birds foraged actively and egg production continued at an average hen day production rate of 75.

PART B

Short Term Feeding Studies

A study was initiated to determine the effects of a dietary intake of dimethoate insecticide on the food consumption, body weight and serum cholinesterase levels of coturnix quail. Treatments were 10, 40, and 80 ppm dimethoate in the feed and an untreated control, the 10 and 40 ppm levels corresponding to residue levels found in the model ecosystems and in the field. Four female quail, 16 weeks of age, were selected for each treatment from a group of birds with consistently high egg production. Each treatment consisted of four replicate birds specially conditioned prior to experimentation. Birds were individually maintained in rat cages, with separate food and water containers. Food consumption, body weight and serum cholinesterase levels were recorded for each bird every three days, during six days pretreatment, 12 days treatment, and at four, 11 and 16 days posttreatment. Egg production was recorded daily throughout the entire period.

Food Consumption

Consumption of food by quail maintained on a control diet averaged 18.5 gm per bird per day. Similar food consumption occurred for birds maintained on the diet containing 10 ppm dimethoate (Table 14). Those birds fed 40 ppm dimethoate in the diet exhibited a 19 per cent drop in food consumption at the six day treatment sampling period. Food consumption remained below normal for the remaining treatment period, with a return to pretreatment levels by four days on an untreated diet. Birds fed a diet containing 80 ppm dimethoate exhibited a reduction of 22.7 per cent

Table 14.
Effects of various levels of dietary dimethoate on daily food consumption (gm) of coturnix quail

(Means and standard deviations for 4 birds/treatment)

Dimethoate Diet (ppm)	Pretreatment (days)			Treatment (days)				Posttreatment (days)		
	6	3	0[1]	3	6	9	12	4	11	16
0	17±2	17±3	18±2	20±2	17±1.7	16±2	20±0.8	19±3.3	19±2	22±2
10	20±1.4	19±3.4	21±1.2	19±1.3	21±3	20±3	21±1.3	19±1.8	19±1.6	16±6[2]
40	20±1.6	21±1.7	21±2.3	21±2	17±1.6	16±2.8[a]	16±2.4[a]	21±2.3	21±0.8	19±1.4
80	23±2	23±1.8	22±3	17±2.5[a]	12±1.6[a]	11±0.8[a]	10±1.4[a]	19±2	24±1.2	23±1.5

[1]Weigh in one hour prior to changing diet

[2]One bird egg bound

[a]Significantly different (ANOV, $P \leq 0.05$) from pretreatment value

in food consumption after three days on a treated diet and further decreased to 54.5 per cent of the pretreatment level after 12 days on the treated diet. Recovery was rapid for birds treated at the 80 ppm level, with an average daily food consumption of 19 gm by four days post-treatment.

Body Weights

Body weights remained constant for those birds maintained on the untreated and the 10 ppm dimethoate diet, however, birds fed the 40 ppm dimethoate diet exhibited a 9.0 per cent reduction in weight after 12 days on a treated diet, while those maintained on the 80 ppm level experienced a 20 per cent weight reduction (Table 15). Birds maintained on the 40 and 80 ppm diets exhibited a continued increase in weight when returned to an untreated diet, reaching pretreatment levels by 16 days posttreatment. The mean weight value for birds on the 10 ppm diet exhibited a reduction in the posttreatment period due to one of the birds being egg bound and subsequently going off feed.

Serum Cholinesterase Levels

Serum cholinesterase levels of the birds during the pretreatment period, and the control birds, remained fairly constant and within pre-treatment limits (Table 16). Once placed on a treated diet, all birds exhibited an immediate and dramatic reduction in serum cholinesterase level, regardless of the level of intake. Those birds treated with 10 ppm dimethoate in the diet exhibited an 85 per cent reduction in enzyme level, while those birds maintained on the 40 and 80 ppm diets showed enzyme level reductions of 89 and 92 per cent respectively of pretreatment levels. Four days after returning to an untreated diet, enzyme levels were still reduced for all treated birds. The birds on the 10 ppm diet had pretreatment cholinesterase levels by 11 days posttreatment. Those

Table 15.
Effects of various levels of dietary dimethoate on body weight (gm) of coturnix quail

(Means and standard deviations of 4 birds/treatment)

Dimethoate Diet (ppm)	Pretreatment (days)			Treatment (days)				Posttreatment (days)		
	6	3	0[1]	3	6	9	12	4	11	16
0	136±17	140±16	139±12	135±13	139±11	146±14	135±12	129±16	141±16	138±16
10	131±11	128±7	131±9	120±11	119±8	129±5	127±10	128±3	129±16	111±20[2]
40	122±12	132±15	122±5	118±4	112±6	113±8	111±3[a]	114±4	128±15	130±19
80	126±3	130±9	129±5	112±9[a]	104±8[a]	106±11[a]	103±7[a]	108±10[a]	125±10	130±9

[1]Birds weighed two hours prior to treatment

[2]One bird egg bound

[a]Significantly different (ANOV, $P \leq 0.05$) from pretreatment value

Table 16.
Serum cholinesterase levels (m U/ml)[1] of coturnix quail before, during and after dietary dimethoate treatment

(Means and Standard Deviations for 4 birds/treatment)

Dimethoate Diet (ppm)	Pretreatment (Days)			Treatment (days)				Posttreatment (days)		
	6	3	0[2]	3	6	9	12	4	11	16
0	1712±110	1750±150	1629±80	1675±192	1878±151	1703±211	1679±81	1747±267	1641±271	1750±269
10	1972±256	1960±255	1924±257	374±122[a]	303±59	292±53	300±31	342±47	2000±210	2020±196
40	2384±226	2362±294	2359±303	259±57	259±85	195±30	189±29	255±68	1697±686	1889±386
80	1775±496	1770±526	2046±514	198±18	159±28	171±56	147±17	156±38	1534±480	1548±338

[1]Acetyl cholinesterase activity expressed as mU/ml of serum as in method of Ellman et al (1961).

[2]Blood samples collected one hour prior to treatment with a contaminated diet.

[a]All treatment values significantly different (ANOV $P \leq 0.05$) from pretreatment values.

birds maintained on the 40 and 80 ppm diets continued to exhibit reduced enzyme levels of 20 and 25.4 per cent respectively after 16 days on the untreated diet.

Egg Production

Egg production of the control birds remained at a high level throughout the experiment with 30 eggs produced during the 12 day treatment period (Table 17). Similar egg production levels occurred for the birds maintained on a diet containing either 10 or 40 ppm dimethoate, with 31 and 28 eggs produced respectively. However, those birds maintained on the 80 ppm diet ceased egg production after seven days on the treated diet, and produced only nine eggs during the entire treatment period, compared to 30 for the control birds. By eight days posttreatment, egg production for those birds on the 80 ppm diet had returned to pretreatment levels and remained there for the duration of the posttreatment period.

PART C

Effects of Chronic Uptake of Dimethoate and Methomyl on Various Reproductive Parameters of Coturnix Quail

Feeding studies were initiated to determine the chronic effects of continuously feeding an organophosphate or a carbamate insecticide in the diet on the reproductive potential of coturnix quail. The organophosphate insecticide dimethoate at 10, 40 and 80 ppm, the carbamate insecticide methomyl at 10, 40 and 80 ppm or an untreated diet were each fed to four replicates of seven female and three male coturnix quail. Egg production data were recorded for six days pretreatment, during the 30 day treatment period and for 12 days posttreatment. Egg hatch data were recorded for six days pretreatment, for the 30 day treatment period,

Table 17.
Daily egg production of coturnix quail maintained on diets containing various levels of dimethoate

(Totals for 4 birds/treatment)

Dimethoate Diet (ppm)	Pretreatment (days)						Treatment Period (days)												Posttreatment (days)														
	6	5	4	3	2	1	1	2	3	4	5	6	7	8	9	10	11	12	(Total)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	3	3	3	3	3	4	0	3	2	2	2	3	3	3	3	3	4	2	(30)	4	4	4	[1]	-	-	-	3	2	2	2	2	2	0	2	1
10	3	4	4	1	4	3	3	1	1	4	4	2	3	3	4	1	4	1	(31)	3	2	2	-	-	-	-	2	3	2	3	3	2	3	3	3
40	3	2	3	1	3	2	3	4	0	2	2	3	2	4	3	1	3	2	(29)	1	4	3	-	-	-	-	1	2	2	1	4	1	2	3	1
80	4	3	4	3	4	3	2	1	2	1	2	1	0	0	0	0	0	0	(9)	0	0	0	-	-	-	-	2	2	3	3	4	1	4	3	3

[1] No samples collected for 4 days

but for 42 days posttreatment. The experiment was conducted in divided standard chick brooders. Birds were disturbed as little as possible during the experiment to prevent stress induced reproductive abnormalities as reported by English (1975). Eggs were collected daily, and incubated as described in the methods section.

Bird Mortality

Dimethoate at 10 and 40 ppm and the three dietary levels of methomyl had no effect on the viability of coturnix quail. Any mortalities occurring on these treatments were due to the birds being egg bound or injuring themselves in the cages. A total of 14 birds died (35 per cent) during the 30 day treatment on 80 ppm dimethoate compared to a total of five for all other treatments combined (Table 18). Ten of the birds which died on the 80 ppm treatment were emaciated at the time of death. Of the remaining four birds, three died from injuries sustained in the cage and one died from unknown causes. Dissection of all dead birds revealed no gross morphological changes of internal organs, when compared to untreated birds, however three of the birds which had been treated with 80 ppm dimethoate exhibited fluid in the lungs.

Behavior

Birds receiving dimethoate at 10 and 40 ppm and all methomyl treatments exhibited no signs of insecticidal poisoning. After ten days on the 80 ppm dimethoate diet, the birds tended to group together at the rear of the cages, with feathers ruffled, trembling noticeably (Table 19). After 20 days the birds exhibited ruffled feathers, very minimal activity and some ataxia. Birds were gasping, foaming at the mouth and occasionally trying to regurgitate. After 30 days most birds moved slowly and with difficultly, foaming at the mouth and attempting to regurgitate. Food

Table 18.
Mortality rate of coturnix quail fed dimethoate, methomyl, or an untreated diet[1].

Treatment (ppm)	Treatment Period (Days) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30	Total
Dimethoate 10	- -	0
40	- -	0
80	- - - - - - - ♀ - ♂ ♂ - - - ♀ - ♂ - - - - ♀ ♂ ♀ ♀ - ♂ - ♂ -	14
Methomyl 10	- - - - - - - - - - ♂ - - - - - - - - - - - - - - - - -	1
40	- - - - - - - - - - ♂ - - - ♀ - - - - - - - - - - - - -	2
80	- - - - - - - - - - - - - - ♀ - - - - - - - - - - - - -	1
Control	- - - - - - - - - - - - - - - ♀ - - - - - - - - - - - -	1

[1]each symbol (♀) represents one bird

Table 19.
Symptoms of insecticide poisoning in coturnix quail during and after treatment with dimethoate at 80 ppm in the diet for 30 days.

	Degree of insecticide induced symptoms[1]				
	Treatment Period (days)			Posttreatment Period (days)	
Symptoms	10	20	30	5	10
ataxia	-	sl	sev	-	-
trembling	sl	m	sev	sl	-
ruffled	m	sev	sev	sl	-
retiring	m	sev	sev	sev	-
foaming	-	sl	sl	-	-
gasping	-	m	sev	m	-
regurgitation	-	sl	sl	-	-
leg paralysis	-	-	m	-	-

[1] sl; less than 40 per cent of birds affected
m: 40 to 80 per cent of birds affected
sev: 80 to 100 per cent of birds affected

consumption for birds maintained on the 80 ppm dimethoate diet was 45 per cent of the control bird consumption.

Five days after being returned to an untreated diet, the birds from the 80 ppm dimethoate feeding level continued to show some disinclination to move and continued to gasp, while other symptoms regressed quickly. By ten days posttreatment the birds behaved normally, as compared to the control birds, and daily food consumption returned to pretreatment levels.

Egg Production

Hen day egg production of the control birds fluctuated from 68 to 86. The treated birds fed all diets containing methomyl and dimethoate at 10 and 40 ppm exhibited similar fluctuations (Figs. 4 & 5). Birds fed 80 ppm dimethoate in the diet showed a rapid reduction in egg production during the first six days on the treated diet, and by nine days egg production had ceased. However, after 24 days on a treated diet egg production commenced again on a sporadic basis, even though birds were exhibiting definite signs of organophosphate poisoning.

When the birds were returned to an untreated diet, egg production levels recovered quickly. Within six days hen day production increased from 3 to 15, and further increased to 38 by 12 days posttreatment. Twenty-one days after returning to an untreated diet, hen day production (74) had returned to the pretreatment level.

Per cent Hatch

The per cent hatch tended to fluctuate greatly within as well as between treatments (Tables 20 - 26). None of the methomyl treatments nor the 10 ppm dimethoate treatment exhibited a significantly lower hatchability compared to pretreatment levels. Eggs from birds fed 40

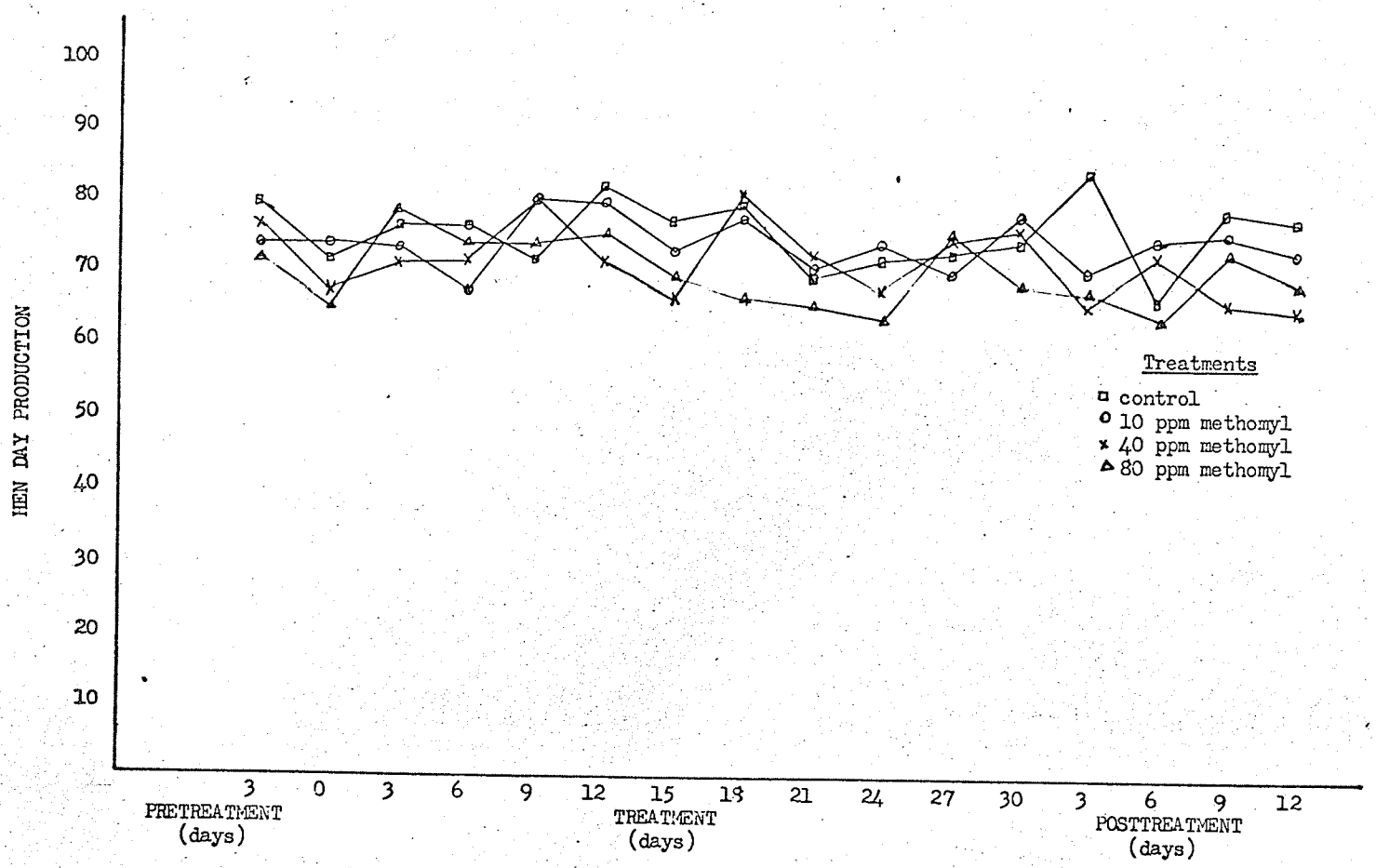

Fig. 4. Egg production[1] of coturnix quail for 6 days pretreatment, when fed 10, 40 or 80 ppm methomyl for 30 days, and for 12 days posttreatment.
[1])mean hen day production - eggs collected for three days prior to each date

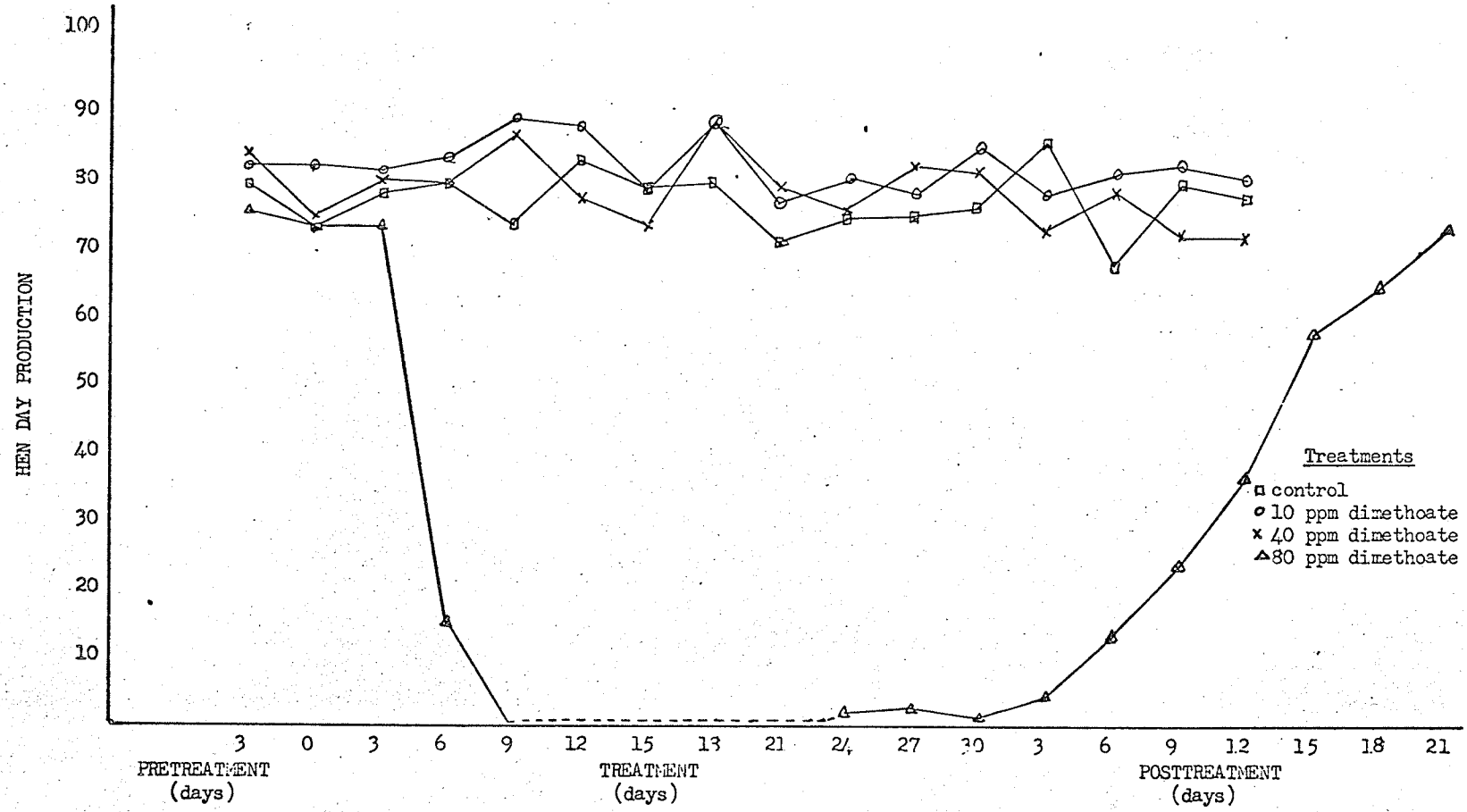

Fig. 5. Egg production [1] of coturnix quail for six days pretreatment, when fed 10, 40 or 80 ppm dimethoate for 30 days and for 12 days posttreatment (21 days for birds fed the 80 ppm diet).
[1] mean hen day production-eggs collected for three days prior to each date

Table 20.
Hatchability data of eggs from coturnix quail fed an untreated diet for 79 days

Sampling Period (days)	Eggs set	Hatch Data (per cent)[1] fertile	infertile	dead embryos	hatch	chick[2] survival
73	50	80	20	40	40	80
74	45	82	18	40	42	80
14	50	70	30	40	30	86
21	60	82	18	46	34	80
235	59	80	20	49	31	73
7	60	85	15	55	30	83
14	50	75	25	40	25	82
42	60	79	21	42	37	84

[1]Based on 4 replications
[2]Survival to 14 days after hatching
[3]Eggs collected for 3 consecutive days and combined for each replication prior to incubation
[4]Corresponds to seven days after start of treatment for insecticide diets
[5]Corresponds to two days prior to end of treatment for insecticide diets

Table 21.
Hatchability of eggs from coturnix quail fed a diet containing 10 ppm methomyl for 30 days

Sampling Period (days)	Eggs set	Hatch Data (per cent)[1] fertile	infertile	dead embryos	hatch	chick[2] survival
Pretreatment						
7[3]	50	72	28	45	27	85
Treatment						
7	60	78	22	40	38	86
14	50	78	22	50	28	78
21	57	82	18	54	28	93
28	60	70	30	32	38	90
Posttreatment						
7	49	75	25	45	30	75
14	57	77	23	33	44	80
42	50	80	20	38	42	90

[1]Based on 4 replications
[2]Survival to 14 days after hatching
[3]Eggs collected for 3 consecutive days and combined for each replication prior to incubation

Table 22.
Hatchability of eggs from coturnix quail fed a diet containing 40 ppm methomyl for 30 days

Sampling Period (days)	Eggs set	Hatch Data (per cent)[1] fertile	infertile	dead embryos	hatch	chick[2] survival
Pretreatment						
7[3]	50	84	16	42	42	85
Treatment						
7	60	83	17	41	42	92
14	50	82	18	54	28	85
21	59	72	28	42	31	77
28	60	82	18	34	48	79
Posttreatment						
7	49	80	20	38	42	81
14	57	74	26	37	37	95
42	49	84	16	30	54	84

[1]Based on 4 replications
[2]Survival to 14 days after hatching
[3]Eggs collected for 3 consecutive days and combined for each replication prior to incubation

Table 23.
Hatchability of eggs from coturnix quail fed a diet containing 80 ppm methomyl for 30 days

Sampling Period (days)	Eggs set	Hatch Data (per cent)[1] fertile	infertile	dead embryos	hatch	chick[2] survival
Pretreatment						
7[3]	50	80	20	55	25	83
Treatment						
7	59	86	14	58	28	85
14	50	78	22	50	28	78
21	52	76	24	53	23	97
28	59	77	23	50	27	86
Posttreatment						
7	50	84	16	58	26	81
14	57	76	24	42	34	83
42	50	72	28	40	32	93

[1]Based on 4 replications
[2]Survival to 14 days after hatching
[3]Eggs collected for 3 consecutive days and combined for each replication prior to incubation

Table 24.
Hatchability of eggs from coturnix quail fed a diet containing 10 ppm dimethoate for 30 days

Sampling Period (days)	Eggs set	Hatch Data (per cent)[1] fertile	infertile	dead embryos	hatch	chick[2] survival
Pretreatment						
7[3]	50	82	18	42	40	83
Treatment						
7	60	81	19	48	33	85
14	50	84	16	52	32	75
21	58	82	18	36	46	85
28	59	68	32[a]	43	25	86
Posttreatment						
7	50	70	30[a]	40	30	86
14	59	86	14	42	44	80
42	50	87	13	37	50	91

[1]Based on 4 replications
[2]Survival to 14 days after hatching
[3]Eggs collected for 3 consecutive days and combined for each replication prior to incubation
[a]Significantly different (ANOV, $P \leq 0.05$) from pretreatment value

Table 25.
Hatchability of eggs from coturnix quail fed a diet containing 40 ppm dimethoate for 30 days

Sampling Period (days)	Eggs set	Hatch Data (per cent)[1] fertile	infertile	dead embryos	hatch	chick[2] survival
Pretreatment						
7[3]	50	80	20	40	40	78
Treatment						
7	60	76	24	38	38	86
14	50	82	18	52	30	86
21	60	73	27	31	42	88
28	60	65[a]	35[a]	28	37	91
Posttreatment						
7	50	62[a]	38[a]	38	24[a]	91
14	56	82	18	36	46	76
42	50	80	20	30	50	89

[1]Based on 4 replications
[2]Survival to 14 days after hatching
[3]Eggs collected for 3 consecutive days and combined for each replication prior to incubation
[a]Significantly different (ANOV, $P \leq 0.05$) from pretreatment value

Table 26.
Hatchability of eggs from coturnix quail fed a diet containing 80 ppm dimethoate for 30 days

Sampling Period (days)	Eggs set	Hatch Data (per cent)[1] fertile	infertile	dead embryos	hatch	chick[2] survival
Pretreatment						
7[3]	50	85	15	40	45	87
Treatment						
7	5	40	60[a]	40	-	-
14	-	-	-	-	-	-
21	-	-	-	-	-	-
28	-	-	-	-	-	-
Posttreatment						
7	18	25[a]	75[a]	25[a]	-	-
14	53	43[a]	57[a]	26[a]	17[a]	89
42	50	70	30	25[a]	45	88

[1]Based on 4 replications
[2]Survival to 14 days after hatching
[3]Eggs collected for 3 consecutive days and combined for each replication prior to incubation
[a]Significantly different (ANOV, $P \leq 0.05$) from pretreatment values

ppm dimethoate, exhibited a hatchability of 24 per cent for the seven day posttreatment hatch compared to pretreatment level of 40 per cent (Table 25). However the hatch for eggs set from the collection made 14 days posttreatment was 46 per cent, within the range for eggs from the pretreatment col ection (40 per cent).

Hatchability for eggs from birds fed 80 ppm dimethoate in the diet was 17 per cent at the 14 day posttreatment period compared to 45 for pretreatment levels (Table 26). By 42 days posttreatment, hatchability had increased to 45, equal to the pretreatment level.

Chick Survival

Survival of chicks from all treatments was satisfactory. Chicks were maintained for ten weeks on standard rations until egg production commenced. No adverse effects of parental treatment were manifested in the chicks during the 10 week posthatch period.

Embryonic Death

The percentage of eggs in which the embryos died, was high for the methomyl, 10 and 40 ppm dimethoate and control treatments (Tables 20-25) with levels ranging from 30 to 58 per cent. Eggs collected from birds during the posttreatment period after receiving 80 ppm dimethoate exhibited a lower embryo mortality than for the pretreatment period; 25 per cent as compared to 40 per cent.

Egg Fertility

Examination of eggs which failed to hatch after incubation revealed that fertility (presence of some observable stage of embryo development) was high for the control amd methomyl treatments (Tables 20-23), throughout the duration of the experiment. The 10 and 40 ppm levels of dimethoate produced a reduction of 14 and 15 per cent respectively, as compared

to pretreatment levels in the fertility of eggs collected after 28 days on the treated diets. Egg fertility levels had returned to within normal limits 14 days after being returned to an untreated diet.

Birds fed 80 ppm dimethoate in the diet ceased normal egg production during exposure to the insecticide. Those few eggs collected during the last six days of treatment were found to be infertile. By the end of the 30 day feeding period, all the males in one replication had died. The remaining females were placed in the other three replicated cages of the same treatment group, to ensure females were mated. After one week on the untreated diet egg fertility was 25 per cent and increased to 70 per cent after six weeks.

Chapter 4

DISCUSSION AND CONCLUSIONS

The terrestrial model ecosystem proved satisfactory for evaluating the effectiveness of insecticides for the control of target species and monitoring adverse effects on certain non-target species. The model ecosystem proved workable, with the soil, plant, insect and bird components all being readily available, easily established and maintained. Increasing the duration of light reduces the time necessary to produce suitable sized plants. Thus it is possible to conduct eight separate experiments in a 12 month period, using the same equipment, depending on duration of individual experiments. Reproducibility of results between the model ecosystem and field experiments is fairly reasonable, when compared to the results of Findlay et al, (1974). This similarity of results between field and model ecosystem experiments was well documented by Metcalfe et al (1971).

One of the faults of the terrestrial model ecosystem is the restricted number of components that can be maintained without adding additional food for the top component. Sample size is also restricted due to the finite nature of the system.

There is no doubt that this terrestrial model ecosystem would be useful for evaluating the effects of new insecticides, herbicides and fungicides on target and non-target species. The model ecosystem as described would be especially useful for tests involving radioactive labelled materials, due to the controlled conditions available.

Malathion, methomyl, dimethoate and fenitrothion gave adequate control of the target species, the green peach aphid, at the rates tested, which approximate those rates used for agricultural or forest spray operations. Carbaryl reduced aphid populations by only 75 per cent, with subsequent increases in insect populations occurring.

Dimethoate caused a 70 per cent reduction within two days in the serum cholinesterase level of quail, with a subsequent recovery by ten days posttreatment. This reduction in enzyme activity was similar to that reported by Findlay et al (1975) where fenitrothion at four oz ai/acre applied by aircraft to a forest ecosystem produced a 62.3 per cent suppression in enzyme activity for quail maintained in an exposed location. However, fenitrothion (4 oz ai/acre) tested in the model ecosystem produced only a slight reduction (20 per cent) in the average serum cholinesterase level of the birds tested, although individual birds exhibited up to a 57 per cent reduction in enzyme levels. As Findlay et al (1975) reported no significant decreases in enzyme levels for quail maintained under a tree canopy, it is reasonable to presume that those quail which were not actively foraging in the model ecosystem at the time of spray application would not receive appreciable levels of fenitrothion, being protected from dermal uptake by the rapeseed leaves. Due to the rapid breakdown of this insecticide (Yule and Duffy, 1972 ; Leuck and Bowman, 1969), 50 per cent of the applied chemical would have been volatilized or degraded within five days. Dead insects collected after the field application of fenitrothion contained residue levels of 5 to 13 ppm (Findlay et al, 1975). Residues of dimethoate in grasshoppers following treatment in the model ecosystem at 16 oz ai/acre averaged 18 ppm with the oxygen analogue residues averaging 12 ppm. Thus quail, on consuming dead or dying grass-

hoppers were exposed to considerable levels of dimethoate in the diet. Once the grasshoppers were consumed (the preferred food), the quail consumed leaves of rapeseed plants and aphids. As rapeseed plants contained an average of 9.6 ppm dimethoate at the 10 day posttreatment sampling period, quail were still exposed to dimethoate, yet serum cholinesterase levels had returned to pretreatment levels. This is likely due to the quail feeding on the lower leaves which were at least partly protected from direct exposure to the spray by the upper leaves of the plants. Two birds contained residues of 7 and 10 ppm dimethoate, at 10 days posttreatment, yet serum cholinesterase levels for these birds were within normal limits, while subsequent experiments indicated that a dietary intake of 10 ppm could cause a reduction in serum cholinesterase levels.

Birds in the model ecosystem which were exposed to dimethoate and exhibited a 70 per cent reduction in serum cholinesterase levels continued to forage actively with no ataxia or retiring behavior being manifested. Egg production remained within normal limits for the duration of the experiment.

Dimethoate at 10 ppm in the diet had no effect on the body weight of coturnix quail during a 12 day treatment period. Similarly, food consumption for birds maintained on a 10 ppm diet approximated that of untreated birds. Quail fed 40 and 80 ppm dimethoate exhibited a reduction in body weight of 9 and 20 per cent respectively, following a reduction of 15 and 54 per cent in daily food consumption. This corresponds to the results of Sherman et al (1963) who reported a reduction in food consumption and weight gain for hens treated with 30 ppm dimethoate in the drinking water and Shellenberger et al (1966) who demonstrated that quail

fed Azodrin at 50 ppm in the diet exhibited reduced food consumption. Food consumption had returned to pretreatment levels after four days when birds were returned to an untreated diet, with bird weights reaching pretreatment levels by 11 days posttreatment.

Egg production for quail fed 10 and 40 ppm dimethoate, and the untreated control, remained at a high level throughout the 12 day treatment period. However, birds fed the 80 ppm diet ceased production after six days on a treated diet. By eight days posttreatment, egg production had commenced, and continued for the duration of the experiment.

Quail fed either 10, 40 or 80 ppm dimethoate in the diet exhibited a rapid drop in serum cholinesterase levels within three days on a treated diet. Enzyme activity for birds treated at the 10 ppm dimethoate level exhibited an 85 per cent enzyme inhibition, with enzyme levels returning to pretreatment levels by 11 days posttreatment. Birds fed 40 or 80 ppm dimethoate exhibited an 89 and 92 per cent reduction respectively in enzyme activity, which corresponds to the results reported by Shellenberger et al (1966), who reported complete inhibition of whole blood cholinesterase in male coturnix quail fed Bidrin at 0.5 ppm in the diet. Shellenberger also demonstrated that female quail fed the same diet still exhibited an enzyme level of four per cent of pretreatment levels.

Chronic feeding studies to determine the effects of long term ingestion of dimethoate (10, 40 and 80 ppm) and methomyl (10, 40 and 80 ppm) indicated that only dimethoate at 80 ppm had an adverse effect on viability and reproduction of coturnix quail.

During the 30 day treatment period, 35 per cent of the birds fed 80 ppm dimethoate in the diet had died, compared to 2.5 per cent for the control birds. Birds fed the high dimethoate diet exhibited ataxia,

retiring behavior, excessive salivation and gasping. The first external sign of insecticide induced stress was the greatly reduced activity of birds, which manifested itself within five days, with other symptoms becoming noticeable after 10 days of treatment.

Egg production for birds fed 80 ppm dimethoate had ceased by four days on a treated diet. This is similar to the results of Shellenberger et al (1966) and Sherman et al (1971) and results described previously. However, egg production commenced again on the 24th day of treatment, and continued sporadically for the remaining six days of treatment. Recovery of egg production was rapid, with a hen day production of 30 after seven days and 74 within 21 days on an untreated diet. Thus even where birds exhibit severe symptoms of organophosphate poisoning, egg production was not totally inhibited.

Egg fertility was unaffected by the methomyl treatment, but was reduced (18 per cent) for the 10 and 40 ppm dimethoate diets after 28 days on the treated diets. No data was recorded for the 80 ppm dimethoate treatment due to near cessation of egg production, however, egg fertility had increased to 70 per cent within six weeks on an untreated diet. It would appear that egg fertility is reduced by intake of the organophosphate insecticide dimethoate, likely due to the poor coordination of the male birds preventing normal mating. Similar behavioral changes were reported by English (1975) for quail treated with 80 ppm Abate in the diet.

Per cent hatch and subsequent chick survival were unaffected by treatment, and varied greatly within treatments. Embryonic deaths were high for all treatments except for eggs from birds fed 80 ppm dimethoate and switched to an untreated diet, where eggs collected 7, 14 and 42 days posttreatment exhibited a low rate of embryonic deaths (25 per cent), compared to 40 to 55 per cent for control birds.

Chapter 5

SUMMARY

1. The terrestrial model ecosystem that was developed proved satisfactory for the evaluation of the effects of various insecticides on target and nontarget species. Results from experiments conducted in the model ecosystem approximated those obtained from field experimentation.
2. Dimethoate applied to model ecosystems as an overhead spray at recommended field rates produced a 70 per cent reduction in serum cholinesterase levels of coturnix quail, with fenitrothion causing a 20 per cent reduction. Behavior and egg production remained normal in spite of a reduced enzyme activity. Malathion, carbaryl and methomyl at recommended field rates had no significant effects on serum cholinesterase levels nor affected bird behavior or egg production.
3. Eighty ppm dimethoate in the diet caused a reduction in food consumption and body weight during a 12 day feeding period. Egg production stopped completely after six days of treatment, but had recovered fully eight days after returning to an untreated diet.
4. Serum cholinesterase levels of quail were reduced drastically within three days of initiation of a diet containing 10, 40 or 80 ppm dimethoate. Eleven days after being returned to an untreated diet, enzyme levels had returned to within normal limits.
5. A dietary intake of 80 ppm dimethoate for 30 days inhibited egg production for the first 24 days, with egg production continuing on

a sporadic basis for the remaining six days of treatment. Dimethoate at 10 and 40 ppm and methomyl at 10, 40 and 80 ppm had no significant effect on egg production.

6. Dimethoate at 80 ppm in the diet caused behavioral changes in coturnix quail, such as ataxia, retiring behavior, ruffled feathers, regurgitation, trembling and gasping. Dimethoate at 10 and 40 ppm and methomyl at 10, 40 and 80 ppm had no noticeable effect on bird behavior.

7. Dimethoate at 10 and 40 ppm in the diet caused a reduction in egg fertility, whereas methomyl at 10, 40 and 80 had no effect on egg fertility recorded during the 30 day treatment period. Dimethoate at the 80 ppm rate inhibited egg production during the 30 day treatment period, the reproduction parameters returning to pretreatment levels by 42 days posttreatment.

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Chapter 7

APPENDIX 1

Hen day production - total eggs laid divided by the number of live hens and expressed as a per cent.