SUSCEPTIBILITY OF THE BLACK PORTUGESE MILLIPEDE, *OMMATOIULUS MORELETII* LUCAS (DIPLOPODA:IULIDAE) TO INSECTICIDES

A thesis presented in fulfilment of the requirements of the Degree of Master of Agricultural Science,
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SUMMARY

The black Portugese millipede, *Ommatoiulus moreletii* Lucas appears in plague numbers in autumn and, to a lesser extent, spring in several areas of South Australia including the Mount Lofty Ranges near Adelaide.

The millipedes invade domestic dwellings, fouling food, water and bedding, an unpleasant nuisance by their presence alone.

The use of chemical insecticides forms an integral part of most millipede control strategies, but the range of insecticides recommended for such use is very limited.

Millipedes are most likely to encounter dried insecticide residues on surfaces such as the walls and paths around domestic dwellings and businesses, or on soil or leaf litter.

A biological assay technique was developed in which millipedes were exposed to non-porous and chemically inert Whatman GF/A glass filter papers treated with known amounts of insecticide, in closed disposable petri dishes.

Septene^{R} Liquid (500 g/L carbaryl) was chosen as the reference treatment as carbaryl is recommended as a barrier treatment at the rate of 1.2 grams/square metre (Birks 1979).

Statistically significant differences in response (between males and females) for both knockdown and moribundity were attributed to the difference in weights between the sexes, males weighing only about half that of females of similar stadial age.

The KT_{50} and MT_{50} for *O. moreletii* exposed to 1,375.4 mg carbaryl/m^2 (approximating the recommended rate of 1,200 mg carbaryl/m^2) are:

KT_{50} = 1,126 $\pm$ 29.35 minutes $grams^{-1}$;

MT_{50} = 1,127 $\pm$ 73.27 minutes $grams^{-1}$.

To compare the efficacy of Septene^R Liquid with that of other formulated insecticides against *O. moreletii* a biological assay experiment was conducted in which knockdown and moribundity of the millipedes exposed to a number of cost related concentrations of eleven insecticidal formulations were recorded.

The most effective knockdown and moribundity agents against *O. moreletii* compared on the basis of concentration of active constituent were the synthetic pyrethroid formulations, viz., Decis^R 25EC, Grenade^R 200EC, Ripcord^R 200EC and Baythroid^R H.

When the cost of treatment formed the basis of comparison of effectiveness against *O. moreletii* the cheaper carbamate insecticides X-18 Carbaryl, Baygon^R 80WP, Baygon^R 200 and Ficam^R W were the most effective formulations for knockdown and moribundity.

The organophosphate insecticides Lorsban^R 50EC and Lorsban^R 25WP were cost effective moribundity agents against *O. moreletii*, but performed poorly as knockdown agents.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, this thesis contains no material previously published or written by any other person, except when due reference is made in the text of the thesis.

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1. INTRODUCTION

The term "biological assay" has been defined broadly as "the measurement of the potency of any stimulus, physical, chemical or biological, physiological or psychological, by means of the reactions which it produces in living matter" (Finney 1952).

Pest species may be subjected to biological assays in order to determine the potency of a control agent, or the susceptibility of a pest species (Busvine 1971a).

Measurements of susceptibility are primarily concerned with the detection and measurement of resistant strains of pest species.

Measurements of potencies are used in both basic and applied research concerning the development of new control agents, or the selection of the most effective amongst existing available ones.

When a control agent, e.g., a pesticide, must be selected for use against a particular pest, the most important consideration is the ultimate efficacy of the control agent in the field. Comparatively precise measurements of toxicity, such as the topical application or injection of measured doses to individuals, will provide less information than experiments which include various factors which may affect the potency of the pesticide in the field. These factors include the use of complete formulations of toxicants rather than active ingredients in solvent only, and, in the case of residual contact toxicants, the use of surfaces such as wood, concrete or plant foliage.

Data from field trials provide the best criteria for the evaluation of a control agent. These trials may be comparatively slow and expensive, and may not always provide conclusive results because of the difficulties in providing adequate replication.

Laboratory tests therefore provide a suitable means of eliminating all but the two or three most promising candidate control agents.

The black Portugese millipede, *Ommatoiulus moreletii*, occurs in plague numbers in autumn and spring in several areas of South Australia, thereby constituting a revolting nuisance (Baker 1978a).

This project is designed to study the effects of a wide range of commercially available formulated insecticides against the black Portugese millipede, *O. moreletii*, under laboratory conditions.

2. BIOLOGY AND ECOLOGY OF *O. MORELETII*

To develop a meaningful laboratory method for determining the susceptibility of *O. moreletii* to insecticides, consideration must be given to the general biology and ecology of the millipede.

The biology and ecology of *O. moreletii* in South Australia has been extensively studied by Baker (1976, 1978a, 1978b, 1978c, 1979a, 1979b).

2.1 DISTRIBUTION AND ABUNDANCE

O. moreletii, a native of Spain and Portugal, became established in South Australia prior to 1953 on lower Eyre Peninsula, and prior to 1964 in the Mount Lofty Ranges near Adelaide. *O. moreletii* has subsequently appeared in other areas of South Australia, Victoria and Western Australia.

The distribution and dispersal of *O. moreletii* in South Australia has been reported by Baker (1978a).

Although comparatively rare in South-Western Europe, *O. moreletii* exists in large numbers in Australia. Baker (1978a) considered that *O. moreletii* in Australia represented a permanent eruption of an introduced species.

Outbreaks spread at a rate of up to 200 metres in radius per year. The activities of man appear to be a major factor in the establishment of new outbreaks.

2.2 LIFE CYCLE

The life cycle of *O. moreletii* in South Australia has been reported by Baker (1976, 1978b) and summarised in Figure 2.1.

After mating in autumn to early winter, females dig a hole a few centimetres deep in the soil, in which they lay approximately 200 eggs. The eggs hatch to give legless, immobile pupoids, each enclosed within a membrane.

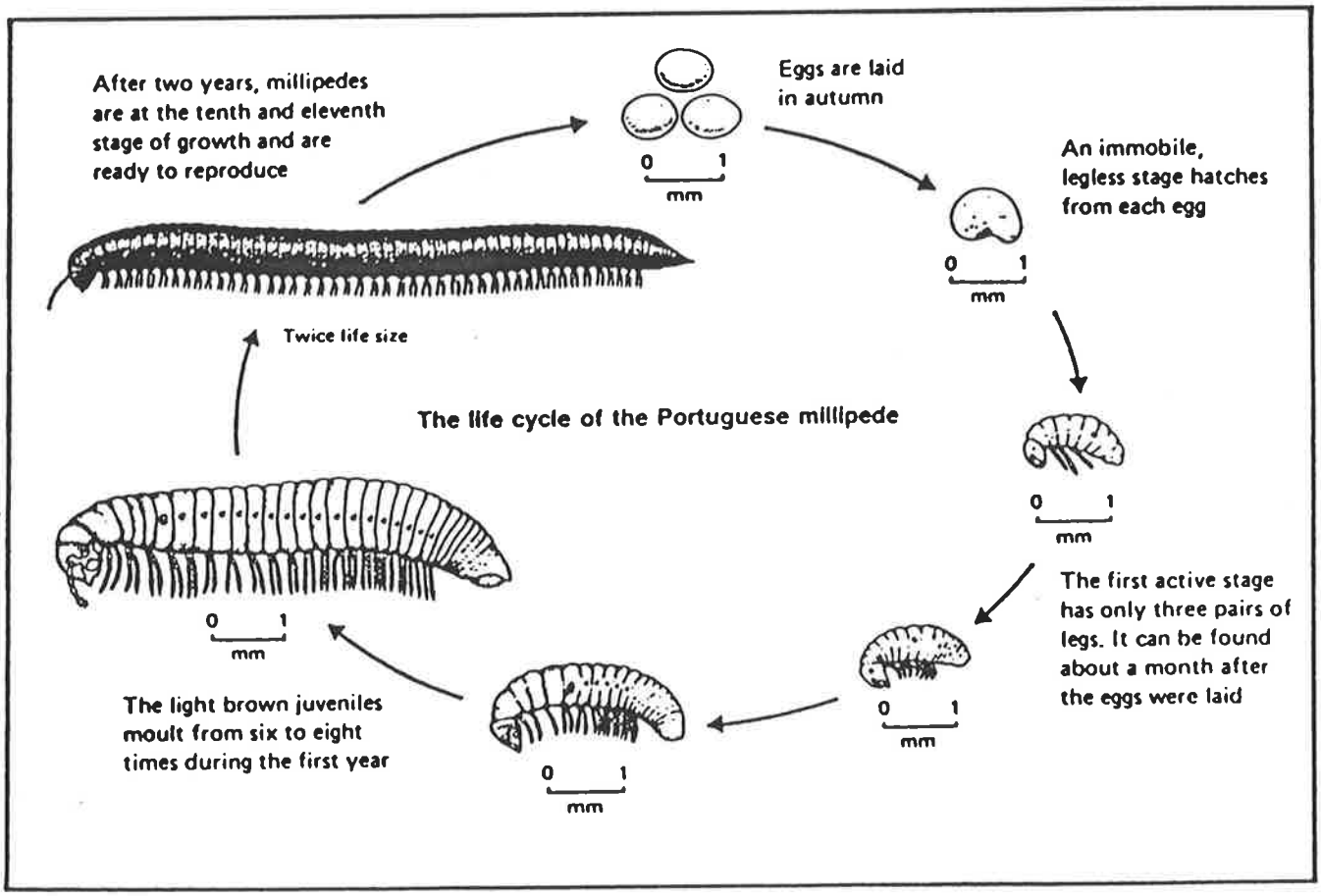
The pupoids subsequently moult to six-legged larvae, which are considered the first stadium of development. A series of moults follows, in which the body size and the number of diplo-segments increase. The seventh, eighth or ninth stadium is reached in the first year, while the tenth or eleventh stadium is reached after two years. Usually, individuals older than about one year moult only in spring and summer, so after three years the twelfth and thirteenth stadia are reached.

Individuals in the tenth, eleventh and subsequent stadia are usually dark purple to black, and those in younger stadia are mostly light brown.

Sexual maturation of females generally occurs by the tenth or eleventh stadium, while sexual maturation of males occurs from the eighth to the twelfth stadia, but predominantly by the tenth or eleventh stadium.

FIGURE 2.1

The Life Cycle of *O. moreletii* in South Australia
(after Hurley and Birks 1985)



In *O. moreletii*, as in some other Iuliform millipedes, the first mature stadium continues to moult. Therefore, the adult population may be composed of individuals of a number of different stadia.

Adult males are periodomorphic, alternating between a sexually functional form and an intercalary non-functional form with regressed sexual appendages. Males are sexually functional from late summer to spring, and intercalary for the remainder of the year.

Sixteen stadia of *O. moreletii* have been identified.

2.3 ACTIVITY

The activity patterns of *O. moreletii* in South Australia have been reported by Baker (1976, 1979a, 1979b).

O. moreletii spends the summer inactive in underground burrows and cool moist sites on the soil surface. The millipedes are active in autumn and, to a lesser extent, in spring.

The autumn peak corresponds with the start of the breeding season and is also related to increases in rainfall. The spring peak is related to increases in temperature.

Adults are much more active than juveniles, and the activity of these adult millipedes bring the species into conflict with man.

Active millipedes "en masse", especially around domestic dwellings, have been described as a "revolting nuisance" (Baker 1978a).

The millipedes invade domestic dwellings, fouling food, water and bedding. Dead and dying millipedes may discolour floor coverings and give rise to objectionable odours. Home garden crops such as tomatoes, potatoes, strawberries and melons may also be damaged.

3. CONTROL OF *O. MORELETII*

Control measures employed against *O. moreletii* include garden hygiene, the use of physical barriers and chemical insecticides (Hurley and Birks 1985).

Successful control usually involves the adoption of these methods in supportive combination, e.g., garden hygiene plus physical barriers where possible, plus application of chemical insecticides where and when necessary.

Biological control, using a parasite or predator, may provide a long-term control solution (Baker 1979).

3.1 CHEMICAL CONTROL

Chemical insecticides are useful in reducing the nuisance posed by hordes of active millipedes by providing a chemical barrier to exclude them from domestic dwellings and other buildings where their presence is considered objectionable.

Chemicals may also be used to control millipedes where they shelter and breed. In many cases, this approach is impractical as breeding areas are often in comparatively inaccessible and ecologically sensitive areas such as small creeks and water courses, and their immediate environs. The extensive use of chemical insecticides in these areas would be unwise.

In the period 1st July, 1984 to 30th June, 1985, the Department of Agriculture registered a number of insecticides under the Agricultural Chemicals Act, 1955-1978 for millipede control. The registered products and their directions for use are given in Table 3.1.

The range of registered products for millipede control is limited.

The carbaryl based products are the only ones that are effective outdoors as protective insecticidal barriers. The mixture of propoxur and dichlorvos, Baygon^{R}, is effective for this purpose indoors only. Baysol^{R} containing methiocarb, and the carbaryl products are effective in reducing millipede numbers in compost heaps, leaf litter and the like. Maxwell's Millipede Spray containing pyrethrins is useful only where the spray makes direct contact with the millipedes because pyrethrins degrade quickly on exposure to oxygen and light (Hartley and West 1969a).

3.2 OTHER CONTROL MEASURES

Garden hygiene measures such as reducing the amount of leaf litter and mulch in which millipedes live and feed, will reduce millipede numbers in the immediate vicinity.

Physical barriers may be used to deny crawling millipedes access to homes and other buildings. Such barriers include vertical glass panels and galvanised iron channel traps (Hurley and Birks 1985).

TABLE 3.1

Products Registered under the Agricultural Chemicals Act, 1955-1978 for Millipede Control in South Australia

Active Ingredient	Product and Company	Directions for Use
carbaryl (800 g/kg)	X-18 Carbaryl Insecticide Agchem Pty. Ltd.	Spray 50 grams in 5 litres of water over 30 m^2. Spray source of infestation and paths and walls around buildings to a height of 1 metre to form a protective barrier.
carbaryl (800 g/kg)	CRG Codling Moth Spray Chemical Recovery Co.	Spray 5 grams of powder in 4 litres of water.
carbaryl (100 g/l)	CRG Liquid Carbaryl Chemical Recovery Co.	Spray 500 mls in 5 litres of water over 40 m^2. Spray infestations, and paths and walls to a height of 1 metre to form a protective barrier.
carbaryl (50 g/kg)	Carbaryl Garden Dust Hortico (Aust.) Pty. Ltd.	Dust lightly wherever millipedes are noticed.
propoxur (20 g/kg) dichlorvos (3.5 g/kg)	Baygon Household Insecticide Surface Spray Bayer Australia Ltd.	Thoroughly spray surfaces inside the home, where millipedes enter to maintain an insecticide barrier.
methiocarb (20 g/kg)	Baysol Snail & Slug Killer Bayer Australia Ltd.	Sprinkle thinly in areas where millipedes occur, approximately 100 pellets per square metre.
pyrethrins (0.2 g/l) piperonyl butoxide (0.8 g/l)	Maxwell's Millipede Spray AJM Industries	Spray directly onto millipedes.

4. LABORATORY METHODS FOR TESTING INSECTICIDES AGAINST MILLIPEDES

Various laboratory methods have been used to evaluate the effects of insecticides against millipedes.

A number of these methods are reviewed herein, with special reference to the general principles of biological assay, including specific details of the experimental techniques employed, and the conditions such as the temperature and relative humidity in which the experiments were conducted.

The suitability of each method for the evaluation of the efficacy of insecticides for controlling *O. moreletii* is also discussed.

4.1 DIPPING

Dipping methods involve the complete immersion of the millipedes in solutions or suspensions of insecticides for standard times.

The results of such investigations are often expressed as the relation between the concentration of insecticide in the dip and the toxic response, usually percentage mortality for a given immersion period.

The rate of entry and the total amount of insecticide entering the millipedes are reflected in the toxic response. Penetration of the insecticide takes place both during immersion

of the millipedes in the insecticidal dip, and subsequently from residual fluid remaining on the millipedes after removal from the dip.

Dipping has been used to determine the contact effect of seventeen insecticidal formulations on three species of millipedes, viz., *Spinotarsus fiedleri*, *Poratophilus pretorianus* and *P. robustus* (Fiedler 1965). Different cohorts of millipedes were dipped in solutions of the insecticides ranging in concentrations from 0.05-0.2% active constituent for 30 seconds. Subsequently a fan was used to dry the millipedes. They were then placed in glass petri dishes containing a thin layer of clean, dry river sand and observed for up to eight days during which time the number of mortalities were recorded. Fresh pieces of potato were supplied as food, the temperature was kept at 25° C. and the relative humidity varied between 60 and 65%.

4.2 BAITS

Baiting methods involve the mixing of insecticides at known concentrations into a suitable substrate.

Millipedes are offered known weights of the bait and allowed to feed on it. After a set time, the millipedes are removed to clean holding containers and observed for mortality. The remaining bait is weighed to determine the dose consumed.

Fiedler (1965) used baits consisting of coarse maize meal mixed with 0.2% active constituent for seventeen formulations of different insecticides against three species of millipede, viz., *S. fiedleri*, *P. pretorianus* and *P. robustus*. Mortalities were recorded after one, two and three days exposure to the baits.

4.3 TREATED SOIL

The method of using treated soil involves the addition of insecticide to known volumes of soil, and then exposing the test animals to the soil. These methods do not adequately differentiate between dermal and oral toxicity.

The effectiveness of nine different insecticides applied as soil drenches were tested against the millipede *Ox. gracilis* by Edwards and Gunn (1961). Flower pots were half filled with soil and a known volume of insecticide solution was watered onto the soil in each pot.

Millipedes were exposed to the treated soil for a given period and percent mortalities were recorded.

Biernaux *et al.* (1973, 1974) evaluated the effectiveness of a number of insecticides against millipedes using a similar "pot method".

A fine spray of insecticide of known volume or a known weight of granules was evenly applied to a known volume of soil in a shallow dish. The insecticide was incorporated

into the soil which was then placed in flower pots. An equal number of millipedes was introduced to each pot four days later, and after four days exposure they were removed and kept in a high humidity insecticide-free environment. Each millipede was examined regularly for signs of poisoning for a period of up to thirty days. Four classifications of poisoning were used:

(1) normal;

(2) slightly intoxicated: animals able to move, but in an abnormal or hesitant way;

(3) severely intoxicated: animals unable to move from place to place, but retaining some ability to move; and

(4) dead: animals unable to move or respond to any stimuli, provocation or excitement.

4.4 TREATED SURFACES

The method of using treated surfaces involves the application of known amounts of insecticide to a particular surface, e.g., glass or filter paper on which the millipedes are exposed.

The millipedes become contaminated with a dose of insecticide while crawling over the treated surface. The dose may be expressed on the basis of duration of exposure or concentration of insecticide.

Busvine (1971b) however, warns that:

> "It can be quite misleading to speak of the residue on a substrate as a 'dose'. The relations between the insecticide deposit and the amount contaminating the insect can be quite complex, so that one cannot assume the dose to be a simple function of the rate of deposit."

Rust and Reierson (1977) used treated filter papers to determine the effectiveness of thirteen formulations of different insecticides against two millipede species *Ox. gracilis* and *Bollmaniulus sp.*

Field collected millipedes were stored in the laboratory in covered plastic boxes containing soil, leaves and bark, with damp folded paper towels for additional moisture and slices of potato for food.

Whatman No. 1 filter papers treated with either 0.1-0.3 cc powdered insecticide or 0.5 mls liquid insecticide were placed in 9 cm diameter petri dishes in which three to five millipedes per dish were confined.

Intoxication was evidenced by paralysis, usually a yellowish secretion and loss of the strong spiral reflex. The criteria used to determine knockdown were paralysis and the inability of the millipede to right itself within three minutes.

During the experiments, millipedes were held at 24° C. and 30-50% relative humidity or at 15° C. and 69-77% relative humidity.

4.4.1 Filter Paper Selection

Whatman cellulose filter papers have been used extensively as surfaces on which to expose insects in general, and millipedes in particular to insecticides.

Georghiou and Gidden (1965) reported a loss of activity of propoxur (a carbamate insecticide) residues due to sorption by the fibres of the Whatman cellulose filter papers used. These losses were minimised by the use of glass fibre filter papers, e.g., Whatman GF/A's.

In addition, Barlow and Hadaway (1968) reported that the contact toxicities of insecticide residues on materials composed of cellulose, including cellulose filter papers, were often dependent on the relative humidity prevailing before and during testing. Non-porous surfaces, such as glass filter papers, do not respond in this manner to humidity changes.

Therefore significant advantages are inherent in the use of such filter papers, as a medium for testing the efficacy of insecticides using a treated surface, especially where humidity may vary.

4.5 EVALUATION AND SUITABILITY OF LABORATORY METHODS

In order to select the most appropriate laboratory method for the evaluation of insecticides against *O. moreletii*, consideration of the circumstances in which millipedes will encounter insecticides under field conditions in South Australia is necessary.

The current uses of insecticides for millipede control (Section 3.1) indicate that millipedes are likely to:

(i) encounter an insecticidal barrier;

(ii) contact or consume insecticidally treated soil, leaf litter, compost and the like;

(iii) encounter insecticidal baits;

(iv) be sprayed directly with insecticide.

Dipping millipedes in insecticidal solutions or suspensions in the laboratory may be considered as an exaggerated equivalent of direct contact by spray droplets with millipedes in the field.

This direct contact is considered to be a comparatively rare event compared with contact between dry spray residue on soil or other surfaces, such as paths, walls, compost and leaf litter, and the millipedes.

Dipping methods were therefore not considered further.

Baiting methods were not considered, primarily because of the lack of commercially available products other than Baysol^{R}

(active constituent, methiocarb). Baysol^{R} is already being used against millipedes in South Australia with some success.

In addition, the formulation of technical insecticides in a suitable carrier, if attempted, would have required the testing of bait suitability and stability. Time was not available to undertake this type of work.

Exposure of millipedes to insecticidally treated soil or surfaces, such as filter papers, provide the most useful laboratory method of testing the responses of millipedes to insecticides because this method mimics closely the most important way in which millipedes acquire doses of insecticide in the field mentioned earlier.

The use of filter papers in petri dishes exhibit the following practical advantages over the use of soil in pots.

Filter papers are comparatively easy to handle and to treat with insecticides. Being manufactured, they provide a consistent surface of known characteristics. Glass filter papers are also physically and chemically inert with respect to the insecticides applied to them.

Although soil is a realistic surface on which to expose millipedes to insecticides, it is more difficult to use and is neither physically nor chemically inert.

Although the effects of different surfaces on the efficacy of insecticides applied to them must be taken into account in the final development of insecticides for use against millipedes, insecticides should first be screened on surfaces which do not mask their efficacy as a consequence of the particular physical or chemical characteristics of the surface.

The laboratory method used in this thesis will therefore be based on the exposure of millipedes to insecticidally treated glass filter papers held in petri dishes.

5. GENERAL METHODS

5.1 FIELD COLLECTION OF *O. MORELETII*

O. moreletii were collected from three sites in the Adelaide Hills over a period of four months from April, 1984. All three collection sites consisted of the banks and adjacent surrounds of small water courses, abundant in moist leaf litter.

The three sites were:

(1) The Sturt River and environs (Plate 5.1).
2.6 km southwest of Stirling adjacent to Sturt Valley Road.

(2) An unnamed small creek and environs (Plate 5.2).
0.6 km west of Stirling adjacent to Braemar Terrace.

(3) An unnamed small creek and environs (Plate 5.3).
1.1 km along Gandy's Gully Road, Stonyfell.

Leaf litter and associated decaying debris were searched and any millipedes located were collected using forceps and were placed directly into clear plastic bins measuring 26 cm long x 19 cm wide x 6 cm deep containing approximately 1000 cubic centimetres of moist Johnson's Orchid Potting Mix (Appendix I).

Between 100 and 200 millipedes were placed in each bin.

PLATE 5.1

O. MORELETII COLLECTION SITE (1)

The Sturt River and Environs
2.6 km southwest of Stirling adjacent to Sturt Valley Road

PLATE 5.2

O. MORELETII COLLECTION SITE (2)

An Unnamed Small Creek and Environs
0.6 km west of Stirling adjacent to Braemar Terrace

PLATE 5.3

O. MORELETII COLLECTION SITE (3)

An Unnamed Small Creek and Environs
1.1 km along Gandy's Gully Road, Stonyfell

5.2 LABORATORY CULTURE OF *O. MORELETII*

The bins containing the collected millipedes were kept in a laboratory where the temperature varied from 20^{o} C. to 23^{o} C.

High relative humidities were retained in the storage bins containing the millipedes by using a double layer of damp tissue over the Johnson's Orchid Potting Mix. Limited ventilation was afforded through mesh inserts in the storage bin lids.

The temperatures and humidities used in the laboratory culture of the millipedes correspond to favourable temperatures and humidities for *O. moreletii* (Baker 1980).

The millipedes were fed regularly with fresh potato slivers so they were not starved (Baker 1976, Fiedler 1965, and Rust and Reierson 1977). (Plates 5.4 and 5.5).

An acclimatisation period of at least 48 hours after collection was provided before any of the millipedes were used in experiments.

In several storage bins, a number of millipedes became infested with the mite *Histiostoma feroniarium* or infected by an unidentified nematode, or both (Appendix II).

PLATE 5.4

O. MORELETII IN THE STORAGE BINS

The millipedes were held in clear plastic bins containing moist Johnson's Orchid Potting Mix. High relative humidities were retained in these bins by using a double layer of damp tissue over the potting mix. The millipedes were fed regularly with potato slivers so they were not starved. Between 100 and 200 millipedes were placed in each bin.

PLATE 5.5

O. MORELETII FEEDING

The millipedes were fed regularly with potato slivers so they were not starved.

5.3 EXPERIMENTAL METHODS

5.3.1 Pre-Exposure Conditions and Handling *O. moreletii*

The millipedes selected for use in experiments were estimated, on the basis of size and colour, to be in stadium ten or eleven, the most numerous active stadia in autumn (Baker 1976). They were placed in "holding" disposable petri dishes, 9 cm in diameter each containing a Whatman No. 1 filter paper, for 12 to 24 hours during the day prior to exposure to insecticides.

Millipedes kept in holding dishes without filter papers fouled themselves with their own liquid and solid excrement. The use of filter papers prevented this.

Five millipedes of the same sex were placed in each petri dish.

The sexes were separated using differences in the appearance of the seventh diplo-segment. In the female, the legs of this diplo-segment remain ambulatory throughout life, while those of the male develop into gonopods. This differentiation is most commonly observed in the sixth stadium (Baker 1978b).

The holding dishes, in groups of twenty, were kept in covered clear plastic, mesh-based bins over

a tray of water, to maintain a high relative humidity and to prevent dessication of the millipedes.

Food was not given to the millipedes during the period from selection to exposure to the insecticides.

5.3.2 Preparation of Insecticidally Treated Filter Papers

The solutions of insecticide required were made up using distilled water as a diluent. Only freshly prepared solutions were used.

Single Whatman GF/A glass filter papers, 9 cm in diameter, were placed in disposable 9 cm diameter petri dishes.

A 1.75 ml aliqout of the desired dilution of insecticide was applied to each filter paper as a series of evenly distributed drops from a 2 ml graduated pipette. 1.75 mls is the water absorption volume of a 9 cm diameter GF/A filter paper (Whatman 1980).

The concentrations of insecticides on the glass filter papers were calculated in the following way: e.g., for Septene^{R} Liquid (500 g/l carbaryl) a 1/100 dilution provides a concentration of 5.00 mg carbaryl/ml. A 1.75 ml aliqout of this solution

contains 8.75 mg carbaryl. This aliqout is applied to a 9 cm diameter glass filter paper with an area of 6.3617×10^{-3} m^2. The resultant concentration of carbaryl on the glass filter paper is 1,375.4 mg a.c./m^2.

Each treated filter paper was prepared 12 to 24 hours immediately before use, to allow time for drying.

5.3.3 Exposure of *O. moreletii* to Insecticidally Treated Filter Papers

At the beginning of each experiment, the millipedes in each "holding" dish were transferred to the appropriate 9 cm disposable petri dish containing insecticide.

The type and concentration of the insecticides, and the duration of exposure varied, depending on the experiment.

During exposure in the closed petri dishes, the millipedes were kept in conditions identical to those used to hold them in preparation for testing, i.e., groups of twenty petri dishes were kept in closed, clear plastic bins with mesh bases over water.

Millipedes used as controls were likewise transferred to petri dishes containing untreated glass

filter papers moistened with 1.75 mls water only, and kept under similar conditions to those millipedes exposed to insecticides.

The nature and degree of intoxication of the millipedes in each dish was recorded at various intervals, using criteria developed and discussed in Experiments I and II.

The allocation of millipedes to treatments, the order in which treatments were exposed to insecticides and the location of treatments in the plastic bins, were random.

5.3.4 Post-Exposure Conditions and Handling of *O. moreletii*

When the exposure time had elapsed, each millipede was transferred back into its respective holding dish, and kept in conditions identical to those used both during preparation for exposure and during the exposure to insecticides.

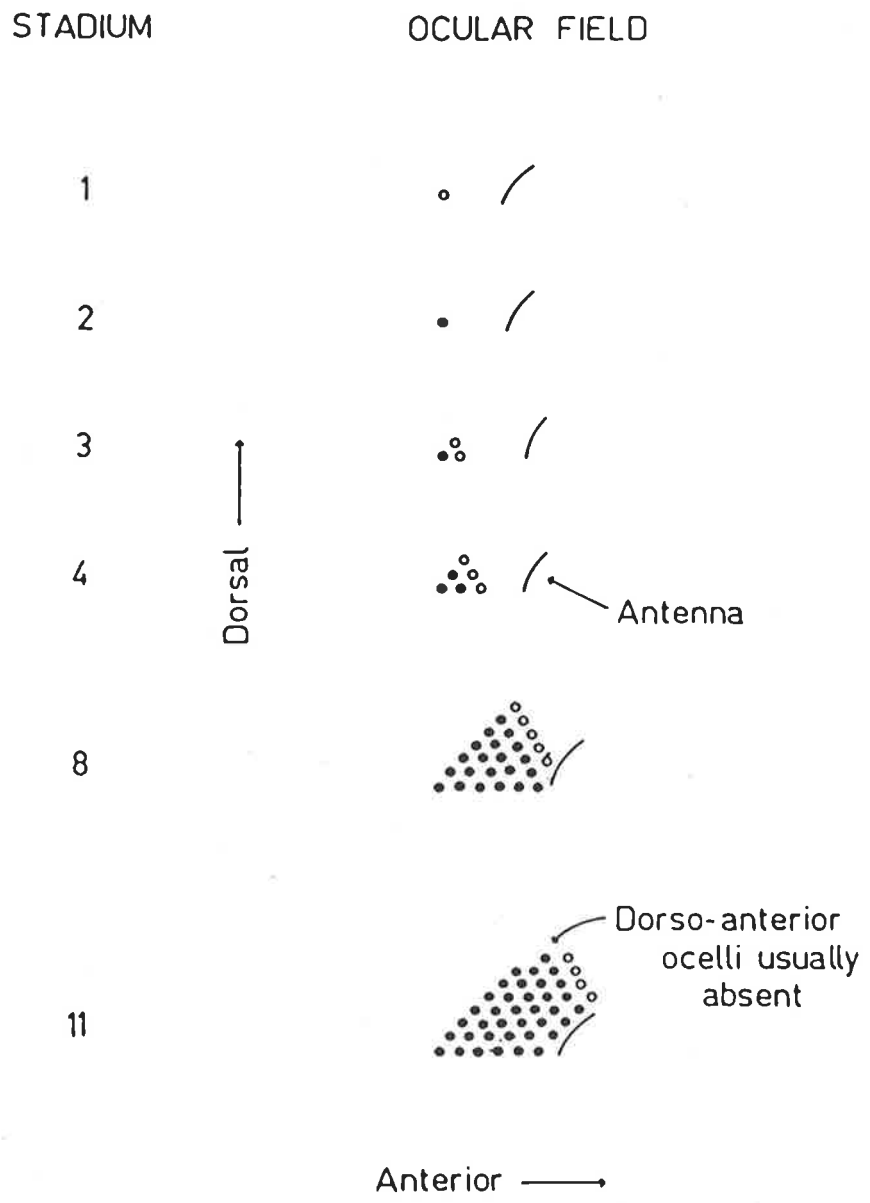
Food in the form of potato slivers was introduced 24 hours after the beginning of each experiment.

The stadia of the millipedes were determined using the ocular field method (Vachon 1947, Saundray 1952).

For *O. moreletii* from the second stadium onwards, counting the number of rows of ocelli from bottom left to top right of the ocular field and adding one gave the stadium of the individual (Baker 1976). The development in the ocular field of *O. moreletii* is shown diagrammatically in Figure 5.1.

FIGURE 5.1

Development of ocelli in the ocular field of *O. moreletii* (o = ocelli added at moult to the present stadium, ● = ocelli added at earlier moults). Six or seven rows of ocelli were added before their growth was obstructed by the antennae. The dorso-anterior ocelli were usually missing in the older stadia. From the second stadium onwards, counting the number of rows of ocelli from bottom left to top right of the ocular field and adding one gave the stadium of the individual (after Baker 1976).



5.3.5 Statistical Analyses

The crude data comprising the results of biological assays and quantal response tests in general are -

(i) the number of millipedes used;

(ii) the number of millipedes affected, e.g., knocked down or moribund;

(iii) the dose or dosage.

The proportion of millipedes affected and the dosage were transformed to probits and log dosage respectively, and critical dosage levels, e.g., KD_{50} (knockdown dose 50 percent) calculated using the GLIM (GLIM 3.11 (c) 1977, Royal Statistical Society London) program, MACRO PROB, developed by Mr. P.I. McCloud (Appendix III).

Probit regression lines were calculated using MACRO PROB and values with standard errors for the intercept, slope and 50 percent response point, e.g., KD_{50} were calculated. The standard errors for the 50 percent response points were approximate only as derivation from log values of 50 percent response point and standard error does not produce a confidence band with the 50 percent response point centrally located.

The percentage of total variation associated with the linear relationship between log dose and the probit transformation of proportion affected, e.g., knockdown or moribundity was calculated using the method of least squares.

GLIM was also used to compare pairs of data sets, e.g., knockdown or moribundity of male and female millipedes.

These comparisons were made in two ways. The slopes and intercepts of the respective probit regression lines were compared in which case the null hypothesis, Ho was:

(i) that the two intercepts of the probit regression lines being compared were equal;

(ii) that the two slopes of the probit regression lines being compared were equal.

Secondly only the intercepts of the probit regression lines were compared, with the additional constraining requirement of parallel slopes for the regression lines incorporated in the analyses. For this case, the null hypothesis, Ho was that the two intercepts of the probit regression lines being compared were equal.

The t-test was used to test the abovementioned hypotheses.

For valid comparisons of toxicity of two insecticides, the probit regression lines should be parallel, otherwise the relative potency will vary with the mortality level chosen. If the regression lines cross, one insecticide will be more toxic above the intersection, and less toxic below it.

Before comparisons of toxicity between insecticides were made, the equality of the slopes of the respective probit regression lines was tested.

The null hypothesis Ho, was that the slopes of all probit regression lines being compared were equal.

The F-test was used to test this hypothesis.

For groups of data for which the probit regression lines were not significantly different another GLIM program MACRO LDCOM developed by Mr. P.I. McCloud (Appendix IV) was used to calculate values with standard errors for the intercept, common slope and 50 percent response point, e.g., KD_{50} for each data set compared.

The level of significance for all the statistical tests detailed in this sub-section was $p = 0.05$.

6. EXPERIMENT 1

Preliminary Observations of the Intoxication and Moribundity of *O. moreletii* from Exposure to Insecticides from Three Different Chemical Groups

6.1 INTRODUCTION

In experiments with residual deposits of contact insecticides on glass filter papers two variables, rate of deposit and duration of exposure contribute to the actual dose which affects the millipedes.

Bendiocarb is effective as a barrier treatment against the millipede *Bollmaniulus sp.* (Rust and Reierson 1977). In their preliminary laboratory experiments the KT_{50} (knock-down time 50 percent) for nymphal *Bollmaniulus sp.* exposed to bendiocarb at a concentration of 393 mg a.c./m^2 was 1.0 hours at 24° C. and 58 $\pm$ 5 percent relative humidity.

The selection of an exposure time of one hour for this experiment was based on the above information.

Three insecticides, from different chemical groups were selected for testing, viz., Septene^R Liquid containing carbaryl, a carbamate insecticide; Lorsban^R 50EC, containing chlorpyrifos, an organophosphate; and Decis^R 25EC, containing deltamethrin, a synthetic pyrethroid.

Carbaryl applied as a barrier treatment at the rate of 1.2 grams a.c./m^2 is effective against *O. moreletii* in South Australia (Birks 1979).

The rates of carbaryl deposits for this experiment were selected so the rate of 1.2 grams a.c./m^2 was within the range of rates used.

The rates of chlorpyrifos deposits selected were the same as those rates selected for carbaryl.

The intrinsic insecticidal potency of synthetic pyrethroid insecticides is usually significantly higher than the most effective organophosphates and carbamates against most economically important insect orders (Ruscoe 1977). Therefore the rates of deltamethrin selected for this experiment were 1/100th of those rates selected for carbaryl and chlorpyrifos.

A variety of criteria have been used to assess knockdown and moribundity in millipedes (Biernaux *et al.* 1973, 1974; Rust and Reierson 1977; Lue and de la Cruz 1978) (Section 4), but none have been developed specifically for *O. moreletii*.

This preliminary experiment was designed to:

(i) discover the rate of deposit and duration of exposure of *O. moreletii* to carbaryl under laboratory conditions, which reflect the known efficacy of carbaryl in the field;

(ii) observe if the symptoms of intoxication of *O. moreletii* exposed to insecticides from different chemical groups differ;

(iii) observe if males and females differ in response to the different insecticides;

(iv) determine criteria for the assessment of intoxication of *O. moreletii*, e.g., knockdown and moribundity.

6.2 MATERIALS AND METHODS

In general, the materials and methods used in this experiment were similar to those materials and methods detailed in Section 5, except that food was withdrawn only one hour before the millipedes were exposed to the treated filter papers and was reintroduced thirty-two hours after exposure.

The three insecticidal formulations used were:

(1) Septene^{R} Liquid (500 g/L carbaryl)

(2) Lorsban^{R} 50EC (500 g/L chlorpyrifos)

(3) Decis^{R} 25EC (25 g/L deltamethrin)

Fifteen chemical treatments were used. These treatments were obtained by serially diluting each of the three insecticidal formulations with distilled water, producing five different concentrations of each insecticide (Table 6.1).

Each treatment was tested separately with male and female millipedes estimated on the basis of size and colour to be in stadium ten or eleven, and replicated once for each sex. Five millipedes were used in each replicate.

Two male and female replicates were also used as controls giving a total of 85 male and 85 female millipedes used in this experiment.

The duration of exposure of each millipede to the insecticidally treated filter paper was one hour.

TABLE 6.1

Dilution Factors and Concentrations of the Three Insecticides used in Experiment 1

Insecticide	Dilution Factor	Conc. of Insecticide mg a.c./m^2
Septene^R Liquid 500 g/L carbaryl	1/10 1/50 1/250 1/1250 1/6250	13,754.00 2,750.80 550.16 110.03 22.01
Lorsban^R 50EC 500 g/L chlorpyrifos	1/10 1/50 1/250 1/1250 1/6250	13,754.00 2,750.80 550.16 110.03 22.01
Decis^R 25EC 25 g/L deltamethrin	1/50 1/250 1/1250 1/6250 1/31250	137.54 27.51 5.50 1.10 0.22

As no decision had yet been made on discriminating the various symptoms and stages of intoxication, each millipede was classified arbitrarily into one of three broad groups, depending on its behaviour:

(1) unaffected - exhibiting normal behaviour;

(2) affected - exhibiting abnormal behaviour;

(3) moribund - exhibiting no movement.

Millipedes move about by pushing with their legs. The power to push is achieved by the use of gaits in which the back strokes of the legs are of much longer duration than the forward stroke. These leg movements are co-ordinated in a wave-like manner. As they walk about millipedes also tap the surface over which they move with the tips of their antennae testing its nature with sensory hairs and chemo-receptive sensillae (Cloudsley-Thompson 1958).

Exploratory activities, with legs and antennae functioning in a co-ordinated fashion as described above were observed in *O. moreletii*. Other normal activities of *O. moreletii* were, being tightly coiled in a testing or defensive spiral and standing, which was often accompanied by preening. Stimulation of *O. moreletii* with a sharp probe induced intense bursts of writhing and wriggling, followed by escape or adoption of the coiled spiral position.

Moribund *O. moreletii* were those which failed to respond to stimulation with a sharp probe.

The condition of each millipede whether unaffected, affected or moribund, was recorded at initial exposure to the treated filter papers, and after one, two, four, eight, 24 and 48 hours respectively.

During this experiment, the temperature ranged from 20 to 22^{o} C.

6.3 RESULTS

The observations of intoxication of *O. moreletii* by the various insecticides are detailed in Appendix V and are summarised as follows.

Carbaryl

Only one millipede became affected from exposure to carbaryl.

This millipede was one of those males exposed to carbaryl at the highest concentration of 13,754 mg a.c./m^2 and was affected after one hour, remaining so for at least eight hours. Recovery had taken place by the time that the 24 hour observation was made.

Neither the male millipedes exposed to the lower concentrations of carbaryl, i.e., 2,750.80, 550.16, 110.03 and 22.01 mg a.c./m^2, nor the female millipedes exposed to any of the concentrations of carbaryl used exhibited any signs of intoxication at any time.

The abnormal behaviour, i.e., symptoms of intoxication exhibited by the male millipede affected by carbaryl at the highest concentration of 13,754 mg a.c./m^2 were, in order of observation:

(i) unco-ordinated jerky movements of the antennae;

(ii) paralysis of the antennae;

(iii) unco-ordinated jerky body and leg movements;

(iv) mild spasmodic writhing, coiling and uncoiling.

Chlorpyrifos

Male and female millipedes became affected as a result of exposure to a number of the concentrations of chlorpyrifos. Males appeared to become affected more quickly than females.

Male millipedes first became affected after two hours when two of the males exposed to chlorpyrifos at the highest concentration of 13,754 mg a.c./m^2 and three of the males exposed at the lowest concentration of 22.01 mg a.c./m^2 were affected.

Subsequently males also became affected after four hours at 550.16 mg a.c./m^2 and after eight hours at 110.03 mg a.c./m^2.

Two of the female millipedes exposed to chlorpyrifos at the highest concentration of 13,754 mg a.c./m^2 were, after four hours, the first females affected. Subsequently females also became affected after eight hours at 550.16 mg a.c./m^2 and after 24 hours at 2,750.80 mg a.c./m^2. The females exposed at the lowest concentrations of 110.03 and 22.01 mg a.c./m^2 failed to exhibit any signs of intoxication at any time.

The observations that some of the males exposed at these lowest concentrations of chlorpyrifos of 110.03 and 22.01 mg a.c./m^2 became affected, while the females so exposed did not, and that males became affected more quickly than females, suggest that males may be more susceptible to chlorpyrifos than females.

The conditions of the millipedes after 48 hours are tabulated in Table 6.2.

This data shows that most male millipedes were affected or became moribund at all concentrations of chlorpyrifos, their reaction to chlorpyrifos apparently independent of the concentration of insecticide.

TABLE 6.2

The Effects on Millipedes of One Hour Exposure to Various Concentrations of Chlorpyrifos (Lorsban 50EC) at 24 Hours after Initiation of Exposure

Sex	Concentration of Chlorpyrifos mg/m^2				
	13,754.00	2,750.80	550.16	110.03	22.01
Males	4M	1A,3M	5M	4M	1A,4M
Females	4M	1M	1A,1M	0	0

The number entered is the number of millipedes per treatment (out of five) which are:

A = affected

M = moribund

However, the reaction of female millipedes to chlorpyrifos appears to be directly related to the concentration of insecticide.

Most female millipedes were affected at the highest concentration of chlorpyrifos of 13,754 a.c./m^2 and at the two lowest concentrations of chlorpyrifos, i.e., 110.03 and 22.01 mg a.c./m^2 no female millipedes were affected.

The abnormal behaviour, i.e., symptoms of intoxication exhibited by the male and female millipedes affected by chlorpyrifos were, in order of observation:

(i) unco-ordinated jerky movements of the antennae;

(ii) paralysis of the antennae;

(iii) unco-ordinated jerky body and leg movements;

(iv) mild spasmodic writhing, coiling and uncoiling;

(v) intense spasmodic writhing, coiling and uncoiling, often accompanied by a yellow secretion (millipedes now unable to move from one place to another);

(vi) near total paralysis, i.e., limited response in legs and body to stimulation with a sharp probe;

(vii) no movement in response to stimulation with a sharp probe.

The first four signs of intoxication of the millipedes affected by chlorpyrifos were similar to the signs of intoxication of the single millipede affected by carbaryl.

Deltamethrin

Male and female millipedes became affected from exposure to several concentrations of deltamethrin.

The male and female millipedes exposed to deltamethrin at the highest concentration of 137.54 mg a.c./m^2 were all affected after one hour, and after 48 hours all were still affected, one male being moribund.

Male millipedes, however, may be more susceptible to deltamethrin than females, because three of the male millipedes exposed to deltamethrin at 27.51 mg a.c./m^2 were affected after two hours while none of the female millipedes exposed at this concentration of deltamethrin exhibited any signs of intoxication at any time.

Two of the affected male millipedes exposed to deltamethrin at 27.51 mg a.c./m^2 recovered after four hours while the third recovered after 24 hours.

None of the male or female millipedes exposed to the lower concentrations of deltamethrin, i.e., 5.5, 1.1 and 0.22 mg a.c./m^2 were affected at any time.

The abnormal behaviour exhibited by the male and female millipedes affected by deltamethrin was similar to that shown by the millipedes affected by carbaryl and chlorpyrifos, except that the millipedes affected by deltamethrin first became highly agitated, moving about constantly, and then exhibited symptoms of intoxication similar to those shown by millipedes affected by carbaryl and chlorpyrifos.

Comparisons of efficacy between carbaryl, chlorpyrifos and deltamethrin were not an aim of this experiment because the criteria for comparison of efficacy were under development. However, using the arbitrary classification, non-affected, affected and moribund, deltamethrin appears the most effective insecticide, followed by chlorpyrifos, with carbaryl the least effective insecticide.

Neither abnormal behaviour nor mortality were observed in the control treatments, i.e., exposure to glass filter papers not treated with insecticide.

The stadial ages of each millipede as determined by the ocular field method are detailed in Appendix VI. The mean stadial age of the millipedes was 9.9 ± 0.774.

6.4 DISCUSSION

In experiments studying the effects of insecticide on millipedes a proportion of test millipedes often die from natural or other causes which cannot be attributed to the insecticide. An estimate of this mortality may be obtained from the mortality of the millipedes in "control" replicates, where they are treated identically to the test millipedes but are not exposed to the insecticide.

Appreciable mortality of control millipedes will affect the precision of the results and the following corrected percent mortality, Px, may be estimated with Abbott's formula (Abbott 1925):

$$Px = \frac{Po - Pc}{100 - Pc} \times 100$$

where Po = observed percent mortality, and

Pc = control percent mortality.

Abbott's formula is widely used to analyse data from numerous standardised tests for determining the level of insecticide resistance in populations of insects. For these tests correction for control mortality is not justified when the control replicate mortality is less than 5 percent; if it exceeds 20 percent, the test should be repeated and efforts made to reduce or eliminate the causes of such mortality (FAO 1969).

The mortality of millipedes in the control treatment for this experiment was zero, which suggests that both the environmental conditions under which the millipedes were kept and the collection and handling procedures adopted were adequate.

The millipedes exposed to the insecticide treated filter papers exhibited various degrees of intoxication, ranging from minor, temporary effects to total paralysis and death. Generally numerous categories are used to describe different degrees of intoxication, but the less categories used, the easier the interpretation and statistical analyses of the results (Busvine 1971d).

Lue and de la Cruz (1978) used the two categories, knockdown and death, to record the intoxication of the millipede *Ox. gracilis* when exposed to the insecticide mirex. Biernaux *et al.* (1973, 1974) used three categories, viz., slightly intoxicated, severely intoxicated and dead, to record the effects of a range of insecticides against millipedes in soil. Rust and Reierson (1977) used only the one category, knockdown, to record the effects of a range of insecticides on two millipede species.

To determine the most appropriate criteria for assessing the effects of insecticides on *O. moreletii*, decisions need to be made on the critical symptoms of intoxication on which control of the pest is based.

Where insecticides are used as chemical barriers to exclude millipedes from domestic dwellings and other buildings, the critical stages of intoxication are, the loss of co-ordination thereby preventing movement from one area to another, i.e., knockdown, and the loss of the ability to recover from intoxication, i.e., moribundity.

For a chemical barrier to be effective fast knockdown is required, to prevent millipedes moving through the barrier. High moribundity is also necessary to prevent millipedes recovering, especially when removed from contact with the insecticide, e.g., intoxicated millipedes are often swept from domestic paths treated with insecticide.

In situations where millipedes are likely to remain in contact with insecticidal material for longer times than on a chemical barrier, such as in compost heaps and leaf litter, fast knockdown is of less importance than high moribundity.

The above argument suggests that the following two criteria for assessing the intoxication of *O. moreletii* exposed to insecticide treated filter papers require further investigation.

(1) knockdown - sufficient intoxication to prevent movement from one area to another;

(2) moribundity - sufficient intoxication to prevent recovery.

Based on the foregoing criteria for assessing the intoxication of *O. moreletii* exposed to insecticide, the criterion for assessment of recovery may be the regaining of the ability to move from one area to another.

6.5 CONCLUSIONS

The one hour exposure time used in this experiment was insufficient to cause any intoxication of *O. moreletii* exposed to carbaryl at concentrations up to twice the rate of carbaryl used effectively in the field, i.e., 1200 mg a.c./m^2.

Male *O. moreletii* appear to be more susceptible to deltamethrin and chlorpyrifos than females.

The *O. moreletii* which became affected by chlorpyrifos and deltamethrin exhibited similar symptoms of intoxication, except that those *O. moreletii* affected by deltamethrin became highly active before the onset of symptoms of intoxication common to both insecticides. These common symptoms were in order of occurrence:

(i) unco-ordinated jerky movements of the antennae;

(ii) paralysis of the antennae;

(iii) unco-ordinated jerky body and leg movements;

(iv) mild spasmodic writhing, coiling and uncoiling;

(v) intense spasmodic writhing, coiling and uncoiling, often accompanied by a yellow secretion (millipedes now unable to move from one place to another);

(vi) near total paralysis, i.e., limited response in legs and body to stimulation with a sharp probe;

(vii) no movement in response to stimulation with a sharp probe.

The first four common symptoms of intoxication were also exhibited by the one male *O. moreletii* affected by carbaryl.

Sufficient intoxication to prevent movement from one area to another, i.e., common symptom (v), requires further investigation as the criterion for assessment of knockdown of *O. moreletii* exposed to insecticides.

Sufficient intoxication to prevent response to the prodding of a sharp probe, i.e., common symptom (vii), requires further investigation as the criterion for assessment of moribundity of *O. moreletii* exposed to insecticides.

7. EXPERIMENT II

Knockdown and Moribundity of *O. moreletii* Exposed for Eight Hours to Three Insecticides from Different Chemical Groups

7.1 INTRODUCTION

The results of Experiment I indicate that the exposure time of one hour is insufficient to cause any intoxication of *O. moreletii* exposed to carbaryl, even at the rate of 2,750.8 mg a.c./m^2, which is more than twice the rate of 1,200 mg a.c./m^2 of carbaryl which is known to be effective in the field.

This short exposure time also did not allow conclusions to be drawn on the complete range of symptoms of intoxication of *O. moreletii* affected by carbaryl, and on the influence of the sex of *O. moreletii* on the efficacy of carbaryl.

This experiment was designed to:

(i) discover the rate of deposit and duration of exposure of *O. moreletii* to carbaryl under laboratory conditions, which reflect the known efficacy of carbaryl in the field;

(ii) verify the applicability to carbaryl of the criteria for the assessment of knockdown and moribundity proposed in Experiment I;

(iii) observe any differences in response between males and females to the different insecticides;

(iv) compare the response of *O. moreletii* to insecticides from three different chemical groups.

7.2 MATERIALS AND METHODS

In general, the materials and methods used in this experiment were similar to those materials and methods detailed in Section 5, except that food was withdrawn only one hour before the millipedes were exposed to the treated filter papers, and was re-introduced after eight hours.

The three insecticide formulations were:

(1) Septene^{R} Liquid (500 g/L carbaryl)

(2) Lorsban^{R} 50EC (500 g/L chlorpyrifos)

(3) Decis^{R} 25EC (25 g/L deltamethrin)

Four chemical treatments were used. These treatments were obtained by serially diluting Septene^{R} Liquid with distilled water producing two different concentrations of carbaryl, and by diluting Lorsban^{R} 50EC and Decis^{R} 25EC once each with distilled water, producing single concentrations of chlorpyrifos and deltamethrin respectively. The dilution factors and concentrations of insecticide for each treatment are detailed in Table 7.1.

Each treatment was tested separately with male and female millipedes estimated on the basis of size and colour to be in stadium ten or eleven and replicated three times for each sex. Five millipedes were used in each replicate.

TABLE 7.1

Dilution Factors and Concentrations of the Three Insecticides used in Experiment II

Treatment	Formulation	Dilution Factor	Conc. of Insecticide mg a.c./m^2
S1	Septene^R Liquid 500 g/L carbaryl	1/10	13,754.00
S2		1/100	1,375.40
L	Lorsban^R 50EC 500 g/L chlorpyrifos	1/1250	110.03
D	Decis^R 25EC 25 g/L deltamethrin	1/250	27.51

Two male and female replicates were also used as controls giving a total of seventy male and seventy female millipedes used in this experiment.

The duration of exposure of each millipede to the insecticidally treated filter paper was eight hours.

The condition of each millipede whether unaffected, knocked down, moribund, or recovered was recorded at initial exposure to the treated filter paper, and at thirty minute intervals for six hours and after seven, eight, 24 and 48 hours respectively.

The criteria for assessing the intoxication of *O. moreletii* exposed to insecticides were:

(i) knockdown - intense spasmodic writhing, coiling and uncoiling, often accompanied by a yellow secretion (millipedes now unable to move from one place to another);

(ii) moribundity - no movement in response to stimulation with a sharp probe;

(iii) recovery - millipedes again able to move from one place to another.

During this experiment, the temperature ranged from 20 to 22^{o} C.

Probit analyses were performed separately on the male and female knockdown data for each treatment using the GLIM program, MACRO PROB, developed by Mr. P.I. McCloud.

7.3 RESULTS

The signs of intoxication common to male and female millipedes affected by carbaryl, chlorpyrifos and deltamethrin were, in order of observation:

(i) unco-ordinated jerky movements of the antennae;

(ii) paralysis of the antennae;

(iii) unco-ordinated jerky body and leg movements;

(iv) mild spasmodic writhing, coiling and uncoiling;

(v) intense spasmodic writhing, coiling and uncoiling, often accompanied by a yellow secretion (millipedes now unable to move from one place to another);

(vi) near total paralysis, i.e., limited response in legs and body to stimulation with a sharp probe;

(vii) no movement in response to stimulation with a sharp probe.

In addition to these signs of intoxication, male and female millipedes affected by deltamethrin first became highly agitated, moving about constantly, and then exhibited symptoms of intoxication similar to those detailed above.

The similarity of symptoms of intoxication of millipedes affected by carbaryl with those symptoms of intoxication of millipedes affected by chlorpyrifos or deltamethrin verifies

the applicability to carbaryl of the criteria for the assessment of knockdown and moribundity and recovery proposed in Experiment I.

The temporal observations of the knockdown of *O. moreletii* for the four treatments, S_1, S_2, L and D are given in Appendix VII.

The observations after 24 and 48 hours of the knockdown, moribundity and recovery of *O. moreletii* for these treatments are given in Appendix VIII.

The results of the probit analyses performed separately on the male and female knockdown data for each treatment are given in Table 7.2 along with the moribundity of the male and female millipedes 48 hours after exposure to the insecticidal treatments.

The effect of exposure time on the knockdown response of the millipedes for each insecticidal treatment is displayed, with respective probit regression lines included, in Figure 7.1.

TABLE 7.2

Results of the Probit Analyses of the Knockdown Data for Male and Female *O. moreletii* Exposed for Eight Hours to Four Insecticide Treatments, viz., Treatment S_1 (13,754.0 mg carbaryl/m^2), Treatment S_2 (1,375.4 mg carbaryl/m^2), Treatment L (110.03 mg chlorpyrifos/m^2) and Treatment D (27.51 mg deltamethrin/m^2) and the Moribundity after 48 Hours of Male and Female Millipedes so Exposed

Treatment	KT_{50} (minutes)	Log KT_{50}	Slope of Probit Line	Intercept of Probit Line	Percent of Variance Accounted for	Percent Moribundity at 48 Hours
S_1 Male	151.3 ± 18.34	2.180 ± 0.0526	3.855 ± 0.7447	- 3.404 ± 1.625	78.7	100.0
S_1 Female	251.2 ± 10.09	2.400 ± 0.0174	6.398 ± 1.264	-10.36 ± 2.950	77.9	53.3
S_2 Male	135.1 ± 17.95	2.131 ± 0.0577	2.884 ± 0.5799	- 1.144 ± 1.174	79.8	100.0
S_2 Female	143.0 ± 7.458	2.155 ± 0.0227	7.384 ± 0.3946	-10.91 ± 0.8528	98.9	93.3
L Male	241.8 ± 6.687	2.383 ± 0.0120	14.24 ± 0.4654	-28.94 ± 1.117	99.5	100.0
L Female	280.2 ± 6.328	2.447 ± 0.0098	15.53 ± 2.976	-33.02 ± 7.222	86.7	86.7
D Male	60.36 ± 6.668	1.781 ± 0.0480	6.461 ± 0.9132	- 6.509 ± 1.677	94.2	13.3
D Female	70.94 ± 9.084	1.851 ± 0.0556	5.917 ± 1.460	- 5.951 ± 2.681	83.7	6.67

FIGURE 7.1

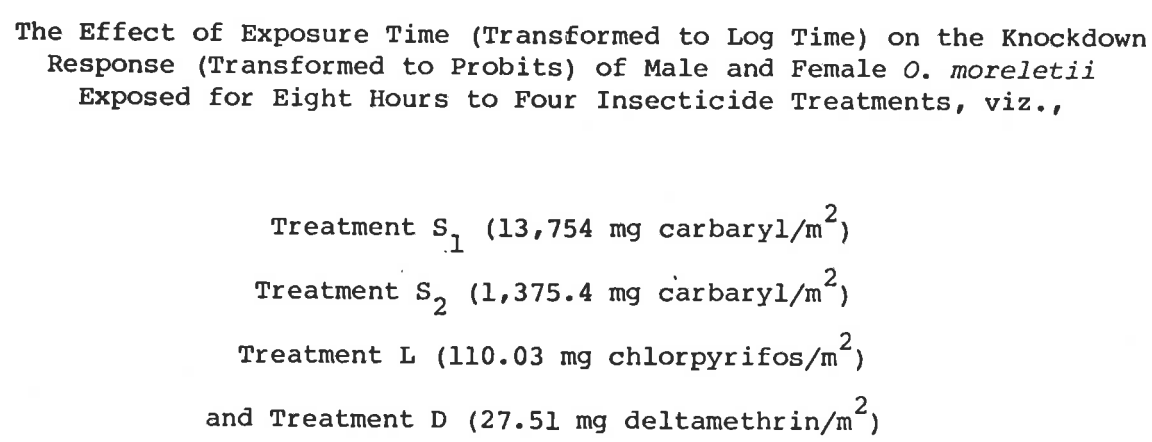
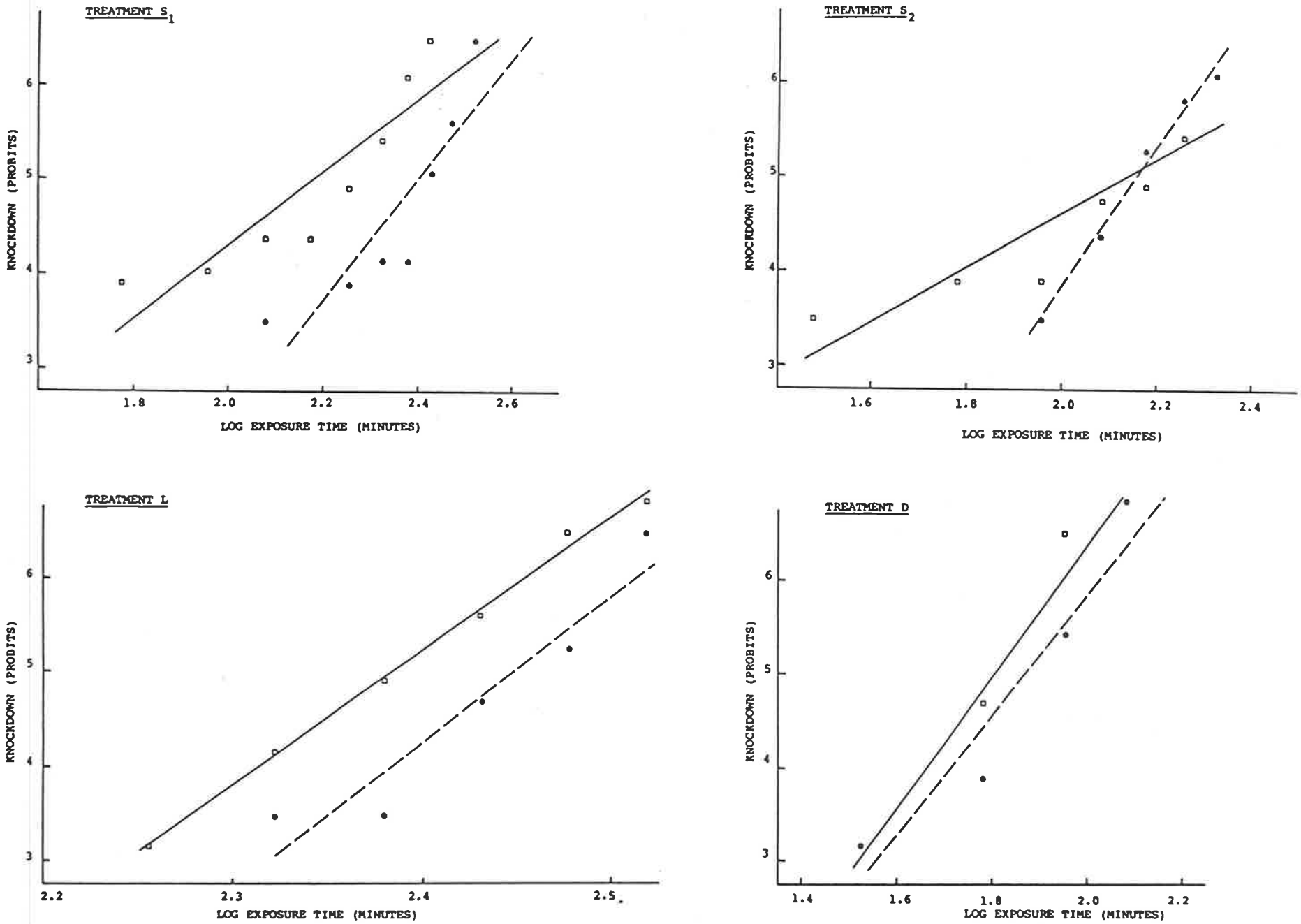

The Effect of Exposure Time (Transformed to Log Time) on the Knockdown Response (Transformed to Probits) of Male and Female O. moreletii Exposed for Eight Hours to Four Insecticide Treatments, viz.,
Treatment S_1 (13,754 mg carbaryl/m^2)
Treatment S_2 (1,375.4 mg carbaryl/m^2)
Treatment L (110.03 mg chlorpyrifos/m^2)
and Treatment D (27.51 mg deltamethrin/m^2)
The graphical characters represent the responses of male (□) and female (●) millipedes. The probit regression lines calculated from the male (——) and female (– –) data for each treatment are included.

FIGURE 7.1


TREATMENT S_1
KNOCKDOWN (PROBITS)
LOG EXPOSURE TIME (MINUTES)
TREATMENT S_2
KNOCKDOWN (PROBITS)
LOG EXPOSURE TIME (MINUTES)
TREATMENT L
KNOCKDOWN (PROBITS)
LOG EXPOSURE TIME (MINUTES)
TREATMENT D
KNOCKDOWN (PROBITS)
LOG EXPOSURE TIME (MINUTES)

Carbaryl

Eight hours exposure to carbaryl at the rate of 1,375.4 mg a.c./m^2, which approximates the rate of 1,200 mg a.c./m^2 of carbaryl effective against *O. moreletii* in the field, caused knockdown in all, and moribundity in most millipedes so exposed.

However, the knockdown time and moribundity after 48 hours of the male and female *O. moreletii* exposed to carbaryl did not appear to be dependent on the concentration of insecticide.

The KT_{50} of the male millipedes exposed to the higher concentration of carbaryl of 13,754 mg a.c./m^2 (Treatment S_1) was 151.3 $\pm$ 18.34 minutes. This KT_{50} was similar to those of the males and females exposed to the lower concentration of 1,375.4 mg a.c./m^2 of carbaryl (Treatment S_2), which were 135.1 $\pm$ 17.95 minutes and 143.0 $\pm$ 7.458 minutes respectively.

The KT_{50} of the female millipedes exposed to 13,754 mg a.c./m^2 of carbaryl was 251.2 $\pm$ 10.09 minutes, which was longer than the KT_{50}'s of both the male and female millipedes exposed to carbaryl at the lower concentration of 1,375.4 mg a.c./m^2.

The moribundity of the male millipedes exposed to both concentrations of carbaryl, 13,754 mg a.c./m^2 and 1,375.4 mg a.c./m^2 were 100 percent after 48 hours, while the moribundity of the females were 53.3 percent and 93.3 percent respectively.

Chlorpyrifos

The knockdown time and moribundity after 48 hours of the male and female millipedes exposed to chlorpyrifos at a concentration of 110.03 mg a.c./m^2 (Treatment L) suggest that males are more susceptible to chlorpyrifos than females.

The KT_{50}'s for millipedes exposed to chlorpyrifos were 241.8 $\pm$ 6.687 minutes for males and 280.2 $\pm$ 6.328 minutes for females. The moribundities after 48 hours for males and females were 100 percent and 86.7 percent respectively.

Deltamethrin

The knockdown times of the male and female millipedes exposed to deltamethrin at a concentration of 27.51 mg a.c./m^2 (Treatment D) suggest similar susceptibility to the insecticide, but the moribundity of these millipedes after 48 hours suggests that males are more susceptible to deltamethrin than females.

The KT_{50}'s for male and female millipedes exposed to deltamethrin were 60.36 $\pm$ 6.668 minutes and 70.94 $\pm$ 9.084 minutes respectively and the moribundities after 48 hours were 13.3 percent for males and 6.67 percent for females.

Comparisons of the KT_{50}'s of the four treatments indicate that deltamethrin (Treatment D) produced the fastest knockdown of the millipedes, followed by carbaryl (Treatment S_2 and then Treatment S_1), with chlorpyrifos (Treatment L) producing the slowest knockdown.

Comparison of the moribundities after 48 hours of the four treatments reveal that carbaryl (Treatment S_2) produced the highest moribundity, followed by chlorpyrifos (Treatment L), and carbaryl (Treatment S_1) with deltamethrin (Treatment D) producing the lowest moribundity.

The fast knockdown of the millipedes produced by deltamethrin and the high moribundity produced by chlorpyrifos suggest that these insecticides should be further investigated as control agents for *O. moreletii*. This suggestion is strengthened by consideration of the concentrations of the insecticides used in this experiment. The lower concentration of carbaryl used was 12.5 x and 50 x higher than those used for chlorpyrifos and deltamethrin respectively.

Differences in susceptibility to the insecticides between male and female millipedes were not consistent.

The KT_{50}'s for the millipedes in Treatments S_2 and D suggest similar susceptibility of males and females to the insecticides. However, the KT_{50}'s of the millipedes in Treatments S_1 and D, and the moribundity after 48 hours for all treatments suggest that males are more susceptible to the insecticides than females.

No abnormal behaviour or moribundity was observed in the control treatments, i.e., exposure to glass filter papers not treated with insecticide.

The stadial ages of each millipede as determined by the ocular field method are detailed in Appendix IX. The mean stadial age of the millipedes was 10.3 ± 0.698.

7.4 DISCUSSION

As the external signs of intoxication were similar for the three insecticides tested, similar responses probably also extend to other insecticides in the respective chemical groups.

The similarity of response between the organophosphate, chlorpyrifos, and the carbamate, carbaryl, was not unexpected. The mode of action of both of these types of insecticide involves the inhibition of the enzyme, cholinesterase. This enzyme hydrolyses acetylcholine generated in myoneural junctions during the transmission of motor commands.

When cholinesterase is inhibited the accumulated acetylcholine interferes with the muscle co-ordination. Interference with the muscles of vital organs may eventually lead to death (Hartley and West 1969b).

Synthetic pyrethroids are also neuroactive insecticides. Both the peripheral and central nervous system are affected, but the precise mechanism of such effects is not yet known. Pyrethroids act on axonal transmission, which is poorly understood compared to the action of cholinesterase (Corbett 1974).

The initial phase of intense agitation which preceded other signs of intoxication of the millipedes affected by deltamethrin is common among pyrethroids (Herve 1982).

In some types of indirect dosage systems, e.g., insects confined on residues of contact insecticides, mortality is equally a function of time of exposure to insecticide, or the concentration of insecticide in the environment. Thus the dosage rate and exposure time for a given effect may be interchangeable, introducing the possibility of dosing by time of exposure to a single concentration of insecticide (Busvine 1971d).

The determination of the KT_{50}'s for each treatment in this experiment is an example of dosing by time of exposure to a single concentration of insecticide.

The condition of each millipede whether unaffected or knocked down, was recorded at initial exposure to the treated filter paper, and at thirty minute intervals for six hours thereafter.

However, the KT_{50}'s so obtained should be viewed with reservations.

In the determination of the KT_{50}'s the time of exposure (a measure of the dose) and the time of knockdown (a measure of the effect of the dose) are confounded. Also, the serial observations on the same treatment are statistically limited by the results of the previous observation. Had a series of treatments been exposed for different times the observations would have been independent.

7.5 CONCLUSIONS

The symptoms of intoxication were similar for millipedes exposed to carbaryl, chlorpyrifos and deltamethrin except that a phase of intense agitation preceded other signs of intoxication in the case of deltamethrin.

The criteria for knockdown and moribundity and recovery based on the results of Experiment I were suitable to assess the effects on millipedes of all the insecticides tested.

Eight hours exposure to carbaryl was sufficient to cause knockdown in all, and moribundity in most millipedes

at the rate of 1,375.4 mg a.c./m^2, which approximates the rate of 1,200 mg a.c./m^2 of carbaryl which is effective against *O. moreletii* in the field.

The KT_{50} time and level of moribundity for male millipedes, appeared to be shorter and higher respectively, than those estimates for females in most treatments, but further studies are required to determine whether these differences are statistically significant.

The quick knockdown of *O. moreletii* produced by deltamethrin and the high moribundity produced by chlorpyrifos suggest that these insecticides should be further investigated as control agents for *O. moreletii*.

8. EXPERIMENT III

Development of a Biological Assay to Measure the Toxicity of Septene^{R} Liquid (Carbaryl) Against *O. moreletii*

8.1 INTRODUCTION

Experiments I and II provided laboratory methods for handling and exposing millipedes to insecticide treated filter papers without inducing control mortality of the millipedes.

These experiments also provided a set of criteria for the assessment of knockdown, moribundity and recovery for *O. moreletii* exposed to insecticides from the three major groups of insecticides, i.e., organophosphates, carbamates and synthetic pyrethroids.

The range of exposure time at which carbaryl is effective in the laboratory at rates of application effective in the field has been established as between one and eight hours. The determination of a more precise time requires the development of a suitable biological assay.

Observations from Experiments I and II also suggest that male and female millipedes may respond differently to insecticides. Data to substantiate such differences may also be obtained through the development of a suitable biological assay.

This experiment was designed, through the development of a biological assay technique, to:

(i) further specify the response of *O. moreletii* to Septene^{R} Liquid (carbaryl) at the rate known to be effective in the field by determination of the KT_{50} (knockdown time 50 percent) and MT_{50} (moribundity time 50 percent);

(ii) further examine the differences in response to carbaryl between male and female millipedes.

8.2 MATERIALS AND METHODS

The materials and methods used in this experiment were similar to those materials and methods detailed in Section 5.

The insecticide formulation used was Septene^{R} Liquid (500 g/L carbaryl).

The Septene^{R} Liquid was diluted 1/100 with distilled water, and the rate of application of carbaryl was 1,375.4 mg a.c./m^2.

Five treatments were used. These treatments were obtained by using five different exposure times, viz., 60, 120, 180, 300 and 420 minutes.

Each treatment was tested separately with male and female millipedes estimated on the basis of size and colour to be in stadium ten or eleven, and replicated five times for each sex. Five millipedes were used in each replicate.

Five male and female replicates were also used as controls giving a total of 150 male and 150 female millipedes used in this experiment.

The condition of each millipede whether unaffected, knocked down or moribund, was recorded at initial exposure to the treated filter paper, and during exposure, depending on the duration of exposure at thirty minute intervals up to 360 minutes and after 420 minutes. The criteria for assessment of knockdown and moribundity were the same as those used in Experiment II.

After 24 and 48 hours the condition of each millipede, whether unaffected, knocked down, moribund or recovered, was also recorded.

During this experiment the temperature ranged from 20 to 23^{o} C.

Probit analyses were performed separately on the male and female knockdown and moribundity data using the GLIM program MACRO PROB developed by Mr. P.I. McCloud.

The knockdown and moribundity data for each sex were further analysed by comparing the probit regression lines in two ways using GLIM. The slopes and intercepts of the probit regression lines were compared (Method 1) and secondly only

the intercepts of the probit regression lines were compared, but with the additional constraining requirement of parallel slopes of the regression lines incorporated in the analyses (Method 2).

8.3 RESULTS

The observed knockdown and moribundity of male and female *O. moreletii* exposed to carbaryl are summarised in Appendix X and detailed in Appendix XI.

Probit analyses were performed separately on the male and female knockdown and moribundity data and the results are given in Table 8.1. The effect of the different treatments (exposure times) on the knockdown and moribundity of the male and female millipedes exposed to carbaryl is also shown with probit regression lines included in Figure 8.1.

The KT_{50}'s for *O. moreletii* exposed to carbaryl at the rate of 1,375.4 mg a.c./m^2 were:

for males 136.7 $\pm$ 11.25 minutes;

for females 148.7 $\pm$ 13.99 minutes.

The MT_{50}'s after 48 hours were:

for males 127.7 $\pm$ 8.63 minutes;

for females 182.0 $\pm$ 16.16 minutes.

TABLE 8.1

Results of Probit Analyses on the Knockdown and Moribundity Data for Male and Female *O. moreletii* Exposed for Various Times to Carbaryl at the Rate of 1,375.4 mg a.c./m^2

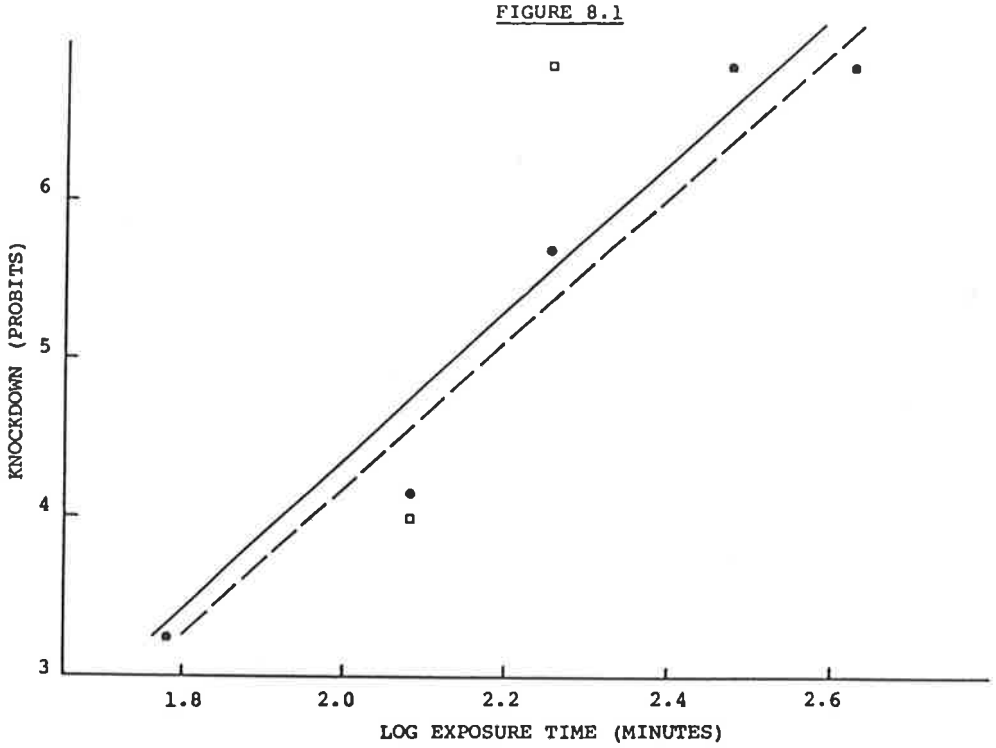
	Males	Females
KT_{50} (minutes)	136.7 ± 11.25	148.7 ± 13.99
log KT_{50}	2.136 ± 0.0357	2.172 ± 0.0409
slope of probit line	4.689 ± 1.306	4.603 ± 0.6176
intercept of probit line	-5.014 ± 2.954	-4.999 ± 1.397
percent of variance accounted for	74.8	93.2
MT_{50} (minutes)	127.7 ± 8.630	182.0 ± 16.16
log MT_{50}	2.106 ± 0.0293	2.260 ± 0.0386
slope of probit line	4.027 ± 1.138	3.586 ± 0.2540
intercept of probit line	-3.578 ± 2.575	-3.106 ± 0.5747
percent of variance accounted for	74.7	98.0

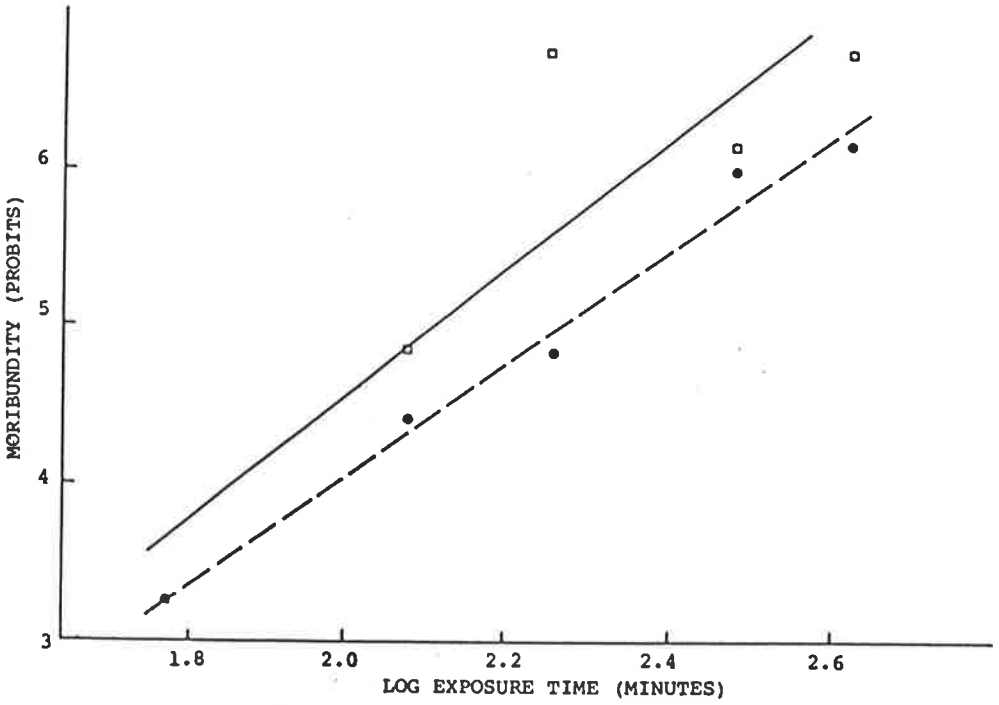
FIGURE 8.1

The Effect of Exposure Time (Transformed to Log Time) on the Knockdown and Moribundity (both Transformed to Probits) of Male and Female *O. moreletii* Exposed to 1,375.4 mg carbaryl/m^2 for Five Different Times (Treatments) viz., 60, 120, 180, 300 and 420 Minutes

The graphical characters represent the responses of male (□) and female (●) millipedes. The probit regression lines calculated from the male (——) and female (– –) data for each treatment are included.

FIGURE 8.1


KNOCKDOWN (PROBITS)
6
5
4
3
1.8
2.0
2.2
2.4
2.6
LOG EXPOSURE TIME (MINUTES)


MORIBUNDITY (PROBITS)
6
5
4
3
1.8
2.0
2.2
2.4
2.6
LOG EXPOSURE TIME (MINUTES)

The differences in knockdown and moribundity between male and female *O. moreletii* exposed to carbaryl were not significant at p = 0.05.

Comparison of the male and female knockdown data using Method 1 revealed no significant difference in the intercept (t_6 = 0.0047) or the slope (t_6 = 0.0598) of the respective probit regression lines.

Comparison of these data using Method 2 also did not reveal any significant difference in the intercept (t_7 = 0.448) of the respective probit regression lines at p = 0.05.

Comparison of the male and female moribundity data revealed no significant difference in the intercept (t_6 = 0.179) or the slope (t_6 = 0.417) using Method 1, and in the intercept (t_7 = 1.897) using Method 2 of the respective probit regression lines at p = 0.05.

Neither abnormal behaviour nor moribundity were observed in the control treatments, i.e., exposure to glass filter papers not treated with insecticide.

The stadial age of each millipede as determined by the ocular field method are detailed in Appendix XII. The mean stadial age of the millipedes was 10.0 $\pm$ 0.716.

8.4 DISCUSSION

The statistically insignificant differences (p 0.05) between male and female millipedes responses, i.e., knockdown and moribundity to carbaryl may be real or they may be biased because of the limited amount of experimental data generated.

For a number of treatments in this experiment the knockdown and moribundity responses to carbaryl of the millipedes tested were either nil or total response.

Three of the five dosage times (treatments) used in this experiment, viz., 420, 300 and 180 minutes produced 100 percent knockdown in the male millipedes so exposed. The longest of these dosage times also produced 100 percent moribundity in the males and 100 percent knockdown in the female millipedes so exposed.

The shortest dosage time of 60 minutes produced no knockdown in males, and neither knockdown nor moribundity in female millipedes exposed for this time.

The data from treatments for which the millipedes either did not respond at all, or responded totally cannot be used in probit analyses without adjustment.

Arbitrary adjustments were made. 0 was adjusted to 1, and 25 was adjusted to 24, adjustments which may not have been consistent with the actual response. These arbitrary adjustments therefore may have been a major source of error.

For probit analyses using five data points, ideal levels of response, i.e., knockdown or moribundity for these points would be approximately 10, 35, 50, 65 and 90 percent. When designing this experiment, it was not known at what exposure times these levels of response would occur, so the times were selected arbitrarily between the known limits of one and eight hours. As previously stated, many of the knockdown and moribundity responses for this experiment were nil or total, clearly indicating that the exposure times selected were far from ideal.

The level of the data derived from this biological assay would be improved and by the adoption of exposure times closely aligned to the specific response levels listed above.

The level of these data could also be improved by increasing the number of millipedes used. This may be achieved by either increasing the number of millipedes used per treatment, or by increasing the number of treatments while keeping the number of millipedes per treatment constant, or both.

8.5 CONCLUSIONS

The KT_{50}'s and MT_{50}'s for male and female millipedes exposed to 1,375.4 mg/m^2 carbaryl have been determined as:

for males: $KT_{50} = 136.7 \pm 11.25$ minutes

$MT_{50} = 127.7 \pm 8.63$ minutes

for females: KT_{50} = 148.7 $\pm$ 13.99 minutes

KT_{50} = 182.0 $\pm$ 16.16 minutes

Differences in knockdown and moribundity between male and female *O. moreletii* exposed to carbaryl have not been statistically substantiated.

9. EXPERIMENT IV

Refinement of a Biological Assay to Measure the Toxicity of Septene^{R} Liquid (Carbaryl) Against *O. moreletii*

9.1 INTRODUCTION

Busvine (1971c) considered that males of most species of insect are more susceptible to contact insecticides than females.

A possible reason for such differences in response to insecticides between the sexes is the greater body weight of females compared to males for many species.

Body size also influences surface area, and because absorption of insecticides frequently involves the surfaces of tissues Moore (1909) has suggested that surface area would be a more reliable measure to use to account for size differences than weight.

Differences in respiratory metabolism (Forgash 1956) and behaviour (David and Bracey 1946) have also been proposed as explanations in specific cases for differences in responses between males and females.

Male *O. moreletii* are approximately half the weight of females of the same stadial age, e.g., the mean weight of males in stadia ten and eleven is 0.12 grams compared to 0.24 grams for females in the same stadia.

The aims of this experiment are to determine:

(i) if the difference in response to insecticide observed in Experiment II between male and female millipedes is statistically significant, and

(ii) if the response is different, whether it may be ascribed to the difference in their respective body weights, especially as the mean body weights of males and females in the same stadia differ.

9.2 MATERIALS AND METHODS

In general, the materials and methods used in this experiment were similar to those materials and methods detailed in Section 5.

Similar to Experiment III, the insecticide used was Septene^{R} Liquid (500 g/L carbaryl), diluted 1/100 with distilled water and applied at a rate of 1,375.4 mg a.c./m^2 of carbaryl to the filter papers.

Fourteen treatments were used. These treatments were obtained by using fourteen different exposure times, viz., 250, 200, 170, 140, 120, 100 and 70 minutes for males and 420, 280, 220, 190, 170, 140 and 90 minutes for females, which approximate the 90, 75, 60, 50, 40, 25 and 10 percent moribundity response exposure times calculated for male and female millipedes respectively exposed to carbaryl in Experiment III.

Each treatment was tested with male or female millipedes estimated on the basis of size and colour to be in stadium ten or eleven, and replicated five times. Five millipedes were used in each replicate.

Five male and female replicates were also used as controls giving a total of 200 male and 200 female millipedes used in this experiment.

The 25 millipedes in each treatment were weighed collectively and their average weight determined.

The condition of each millipede whether unaffected, knocked down or moribund was recorded at the initial time of exposure, and at the termination of exposure using the same criteria for assessment of knockdown and moribundity as in Experiment II.

After 24 and 48 hours the condition of each millipede whether unaffected, knocked down, moribund or recovered was also recorded.

During this experiment the temperature ranged from 20 to 23^{o} C.

Probit analyses were performed separately on the male and female knockdown and moribundity data using the GLIM program MACRO PROB.

These data were further analysed by comparing the probit regression lines for each sex in two ways also using GLIM. The slopes and intercepts of the probit regression lines were

compared (Method 1) and secondly, only the intercepts of the probit regression lines were compared, but with the additional constraining requirement of parallel slopes of the regression lines incorporated in the analyses (Method 2).

9.3 RESULTS

The observed knockdown and moribundity of male and female *O. moreletii* exposed to carbaryl are summarised in Appendix XIII and detailed in Appendix XIV.

The knockdown and moribundity data were analysed without regard for the difference in body weight between male and female millipedes in the same stadia, i.e., dosage = time of exposure (minutes) as in Experiment III, and also with dosage adjusted to account for this difference in weight, i.e., dosage = time of exposure/average millipede weight (minutes $grams^{-1}$).

Analyses of Data Using Dosage = Time of Exposure

Probit analyses were performed separately on the male and female knockdown and moribundity data. The results of these analyses are shown in Table 9.1. The effect of the different treatments on the knockdown and moribundity of males and females exposed to carbaryl is shown with probit regression lines included, in Figure 9.1.

The KT_{50}'s for *O. moreletii* exposed to carbaryl at the rate of 1,375.4 mg a.c./m^2 were:

for males: 151.6 $\pm$ 3.96 minutes:

for females: 230.6 $\pm$ 7.21 minutes.

TABLE 9.1

Results of Probit Analyses on the Knockdown and Moribundity Data for Male and Female *O. moreletii* Exposed for Various Times to Carbaryl at the Rate of 1,375.4 mg a.c./m^2

For these analyses dosage = time of exposure

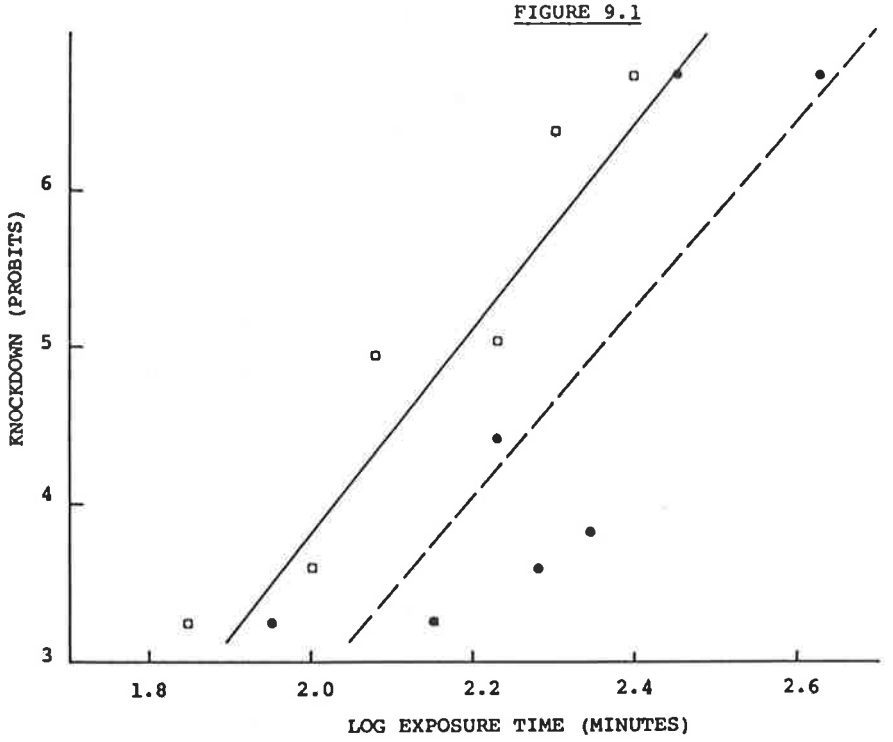
	Males	Females
KT_{50} (minutes)	151.6 ± 3.956	230.6 ± 7.209
log KT_{50}	2.1807 ± 0.0113	2.3629 ± 0.0136
slope of probit line	6.604 ± 1.826	6.109 ± 1.754
intercept of probit line	-9.400 ± 3.925	-9.435 ± 4.029
percent of variance accounted for	66.8	65.0
MT_{50} (minutes)	127.7 ± 9.229	321.4 ± 142.5
log MT_{50}	2.1062 ± 0.314	2.5071 ± 0.1925
slope of probit line	5.733 ± 1.389	3.819 ± 0.9100
intercept of probit line	-7.075 ± 2.987	-4.575 ± 2.091
percent of variance accounted for	72.8	73.5

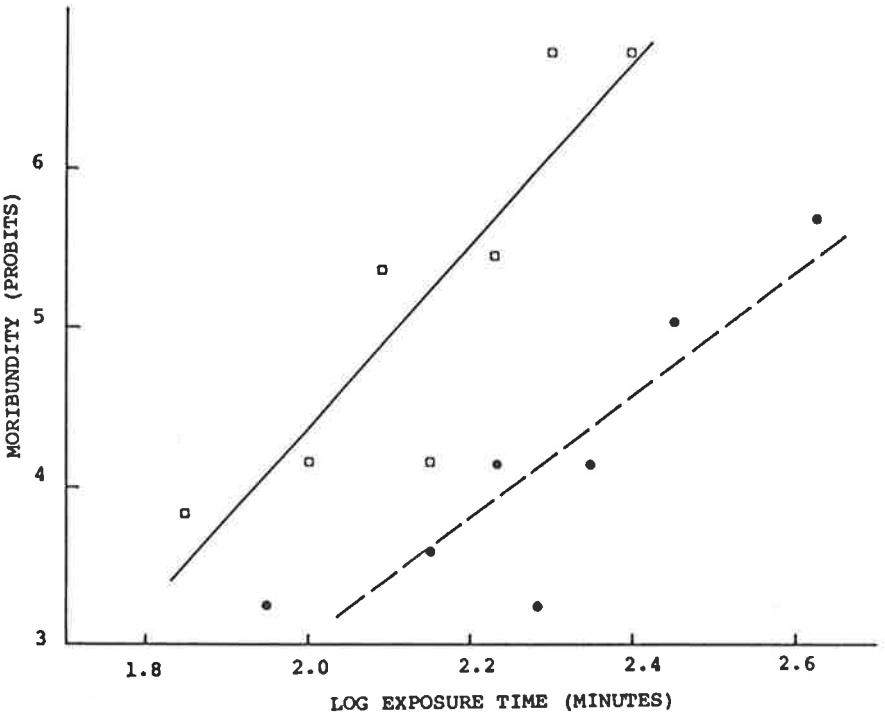
FIGURE 9.1

The Effect of Dosage (Exposure Time Transformed to Log Time) on the Knockdown and Moribundity (both Transformed to Probits) of Male and Female *O. moreletii* Exposed to 1,375.4 mg Carbaryl/m^2 for Various Times

The graphical characters represent the responses of male (□) and female (●) millipedes. The probit regression lines calculated from the male (——) and female (– –) data for each treatment are included.

FIGURE 9.1


KNOCKDOWN (PROBITS)
6
5
4
3
1.8
2.0
2.2
2.4
2.6
LOG EXPOSURE TIME (MINUTES)


MORIBUNDITY (PROBITS)
6
5
4
3
1.8
2.0
2.2
2.4
2.6
LOG EXPOSURE TIME (MINUTES)

The MT_{50}'s were:

for males: 127.7 ± 9.23 minutes:

for females: 321.4 ± 142 minutes.

Differences in knockdown and moribundity, between male and female *O. moreletii* exposed to carbaryl were not significant when compared using Method 1, but were significant when compared using Method 2 at $p = 0.05$.

Comparison of the male and female knockdown data revealed no significant difference at $p = 0.05$ in the intercept ($t_6 = 0.01$) or the slope ($t_6 = 0.19$) of the respective probit regression lines using Method 1. Similarly comparison of the male and female moribundity data using this method revealed no significant difference at $p = 0.05$ in the intercept ($t_6 = 0.69$) or the slope ($t_6 = 1.17$) of the respective probit regression lines.

However, comparison of the knockdown and moribundity data between males and females revealed significant difference in the intercepts ($t_7 = 2.33$ and $t_7 = 5.23$) of the probit regression lines for knockdown and moribundity respectively at $p = 0.05$.

Analyses of Data Using Dosage = Time of Exposure/ Average Millipede Weight

The results of the probit analyses performed separately on the male and female knockdown and moribundity data are shown in Table 9.2. Figure 9.2 shows the effect of the different treatments on the knockdown and moribundity of males and females exposed to carbaryl, with probit regression lines included.

The KT_{50}'s for *O. moreletii* exposed to carbaryl at the rate of 1,375.4 mg a.c./m^2 were:

for males: $1{,}285 \pm 35.35$ minutes $grams^{-1}$;

for females: 952.2 ± 77.90 minutes $grams^{-1}$.

The MT_{50}'s were:

for males: $1{,}073 \pm 83.56$ minutes $grams^{-1}$;

for females: $1{,}390 \pm 839.8$ minutes $grams^{-1}$.

The differences in knockdown and moribundity between male and female *O. moreletii* exposed to carbaryl were not significant at $p = 0.05$.

Comparison of the male and female knockdown data using Method 1 showed no significant difference at $p = 0.05$ in the intercept ($t_6 = 0.44$) or the slope ($t_6 = 0.33$) of the respective probit regression lines. Similarly comparison of these data using Method 2 did not reveal any significant difference in the intercept ($t_7 = 1.67$) of these probit regression lines at $p = 0.05$.

TABLE 9.2

Results of Probit Analyses on the Knockdown and Moribundity Data for Male and Female *O. moreletii* Exposed for Various Times to Carbaryl at the Rate of 1,375.4 mg a.c./m^2

For these analyses dosage = time of exposure/ average millipede weight

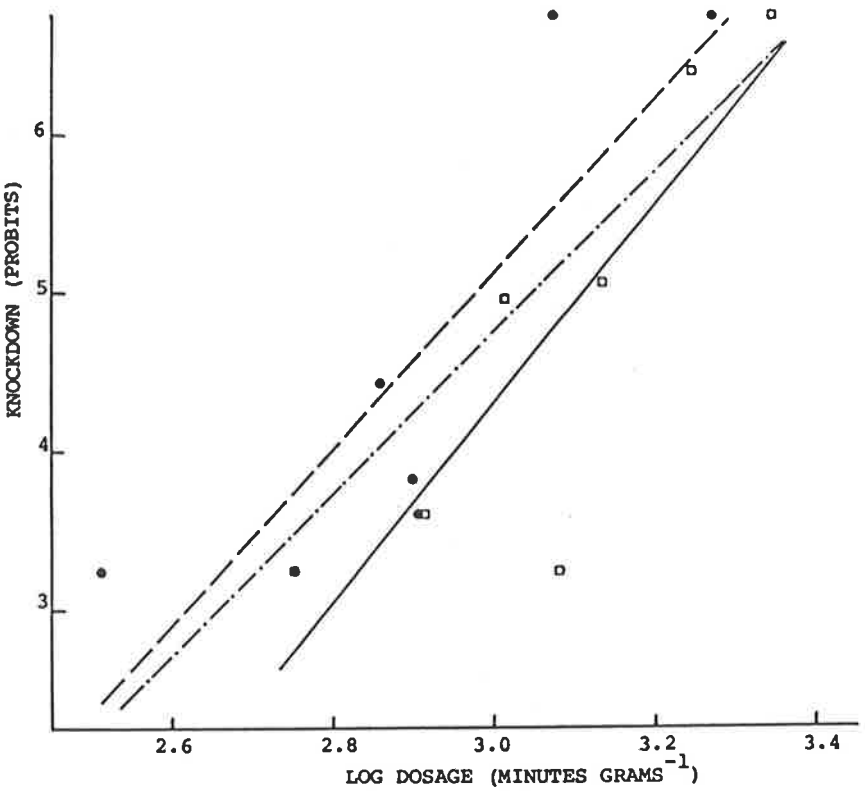
	Males	Females	Males + Females Combined
KT_{50} (minutes $grams^{-1}$)	1,285 ± 35.35	952.2 ± 77.90	1,126 ± 29.35
log KT_{50}	3.109 ± 0.0119	2.979 ± 0.0355	3.052 ± 0.0113
slope of probit line	6.281 ± 1.699	5.528 ± 1.534	5.126 ± 1.073
intercept of probit line	-14.53 ± 5.225	-11.47 ± 4.457	-10.64 ± 3.210
percent of variance accounted for	67.9	66.6	62.7
MT_{50} (minutes $grams^{-1}$)	1,073 ± 83.56	1,390 ± 839.8	1,127 ± 73.27
log MT_{50}	3.031 ± 0.0338	3.143 ± 839.8	3.052 ± 0.0282
slope of probit line	5.460 ± 1.281	3.387 ± 0.8451	4.510 ± 0.7037
intercept of probit line	-11.55 ± 3.938	-5.644 ± 2.455	-8.764 ± 2.105
percent of variance accounted for	74.1	71.5	75.5

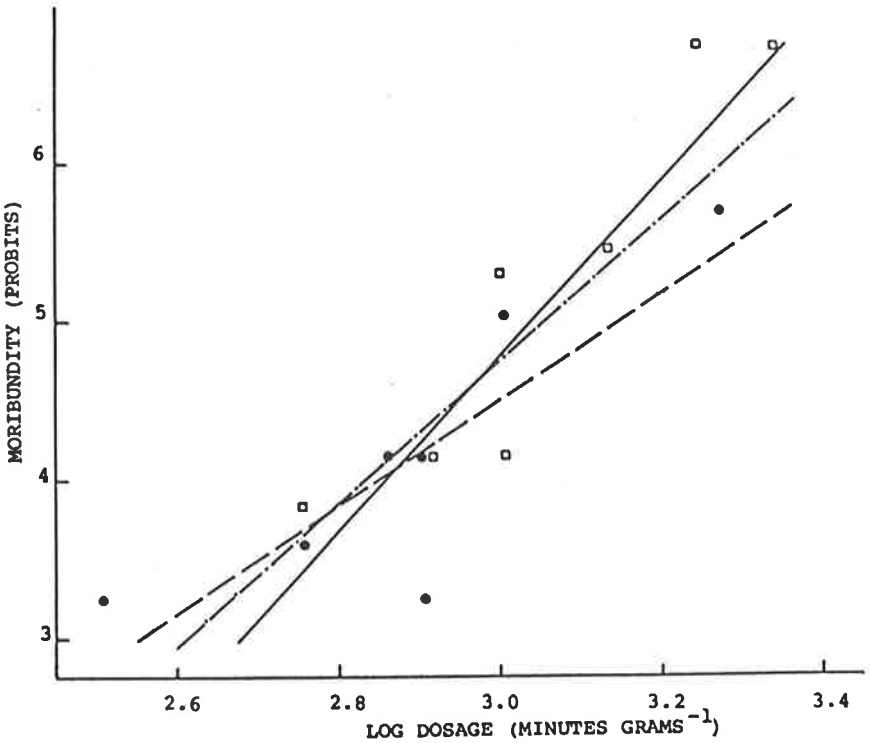
FIGURE 9.2

The Effect of Dosage (Exposure Time/Average Millipede Weight Transformed to Logs) on the Knockdown and Moribundity (both Transformed to Probits) of Male and Female *O. moreletii* Exposed to 1,375.4 mg Carbaryl/m^2 for Various Times

The graphical characters represent the responses of male (□) and female (●) millipedes. The probit regression lines calculated from the male (——), female (– –) and combined male plus female (–·–) data for each treatment are included

FIGURE 9.2


KNOCKDOWN (PROBITS)
6
5
4
3
2.6
2.8
3.0
3.2
3.4
LOG DOSAGE (MINUTES GRAMS^{-1})


MORIBUNDITY (PROBITS)
6
5
4
3
2.6
2.8
3.0
3.2
3.4
LOG DOSAGE (MINUTES GRAMS^{-1})

Comparison of the male and female moribundity data revealed no significant difference in the intercept (t_6 = 1.31) or the slope (t_6 = 1.38) using Method 1, or in the intercept (t_7 = 0.93) using Method 2 for the respective probit regression lines at p = 0.05.

Probit analyses were performed on the combined male and female data corrected for body weight, for both knockdown and moribundity, there being no significant difference in response between male and female *O. moreletii* on a weight corrected basis. These results are also shown in Table 9.2. The effect of the different treatments on the knockdown and moribundity of males and females exposed to carbaryl is also shown with probit regression lines included in Figure 9.2.

The KT_{50} and MT_{50} for *O. moreletii* exposed to carbaryl at the rate of 1,375.4 mg a.c./m^2 were:

KT_{50} = 1,126 $\pm$ 29.35 minutes $grams^{-1}$;

MT_{50} = 1,127 $\pm$ 73.27 minutes $grams^{-1}$.

Neither abnormal behaviour nor moribundity were observed in the control treatments, i.e., exposure to glass filter papers not treated with insecticide.

The stadial age of each millipede as determined by the ocular field method are detailed in Appendix XV. The mean stadial age of the millipedes was 10.1 $\pm$ 0.782.

9.4 DISCUSSION

The greater body weight of adult females compared to that of adult males has accounted for differences between the sexes in susceptibility to insecticides for a number of insects.

Female *Drosophila melanogaster* are 1.86 times as tolerant as males to DDT applied topically. When the doses are corrected for body weight this ratio falls to 1.17 (Kerr 1954). Female locusts *Schistocerca gregaria* and *Locusta migratoria* are also more tolerant than males, but this difference no longer appears when the doses are calculated in terms of unit body weight (MacCuaig 1956).

The finding of this experiment that male and female *O. moreletii* respond similarly to exposure to carbaryl when dosages are corrected for body weight is of practical benefit. In future experiments male and female knockdown data may be pooled, as could the moribundity data, provided the data are corrected for the relative weights of the male and female millipedes.

9.5 CONCLUSIONS

The KT_{50}'s and MT_{50}'s for male and female *O. moreletii* exposed to 1,375.4 mg carbaryl/m^2 are:

for males: KT_{50} = 151.6 $\pm$ 3.956 minutes;
MT_{50} = 127.7 $\pm$ 9.229 minutes.

for females: KT_{50} = 230.6 $\pm$ 7.209 minutes;
MT_{50} = 321.4 $\pm$ 142.5 minutes.

The differences in responses between male and female millipedes are statistically significant at p = 0.05 using Method 2 as the means of comparison.

The KT_{50}'s and MT_{50}'s for male and female *O. moreletii* exposed to carbaryl were also determined (with data corrected for the different body weights of male and female millipedes), i.e., dose = time of exposure/average millipede weight.

In this case the values of the KT_{50}'s and MT_{50}'s are:

for males: KT_{50} = 1,285 $\pm$ 35.35 minutes $grams^{-1}$;
MT_{50} = 1,073 $\pm$ 83.56 minutes $grams^{-1}$.

for females: KT_{50} = 952.2 $\pm$ 77.90 minutes $grams^{-1}$;
MT_{50} = 1,390 $\pm$ 839.8 minutes $grams^{-1}$.

Compared on this basis, the differences in response between male and female millipedes are not statistically significant at p = 0.05.

The statistically significant difference in response between male and female millipedes is accounted for by the difference in body weight of the sexes.

The KT_{50} and MT_{50} for *O. moreletii* (combined male and female data corrected for body weight) are:

$$KT_{50} = 1{,}126 \pm 29.35 \text{ minutes grams}^{-1};$$

$$MT_{50} = 1{,}127 \pm 73.27 \text{ minutes grams}^{-1}.$$

10. EXPERIMENT V

Comparison of the Toxicity of Septene^{R} Liquid (Carbaryl) with that of Ten Other Insecticide Formulations Against *O. moreletii*

10.1 INTRODUCTION

Fenemore (1982) stated that the following aspects require consideration when the most effective insecticide is being selected for a particular purpose:

(i) effectiveness for the pest to be controlled;

(ii) toxicity;

(iii) formulation and method of application most suited to the circumstances;

(iv) timing of application in relation to the stage of development of the pest and stage of growth of the crop;

(v) the need for repeat applications;

(vi) selectivity for beneficial organisms, particularly parasites and predators of the pest to be controlled;

(vii) cost.

The most important aspects for selection of insecticides to form treated barriers around areas to be protected, e.g., domestic dwellings, are efficacy, including residual life of the insecticide, toxicity and hazard to the applicator and to non-target organisms and the cost of treatment.

The scope of this thesis does not permit a study of the residual life of insecticides, which may vary depending on the surface and the environmental conditions to which the insecticides are applied and subjected respectively, but it does allow a range of formulated insecticides to be tested against field collected *O. moreletii* in the laboratory.

Among the range of formulated insecticides to be tested several formulations of the same active constituent are included because the physical properties of the formulation of an insecticide, and the material to which it is applied have a considerable influence on the availability of the active ingredients to insects, and hence on the ultimate efficacy of the formulation (Hadaway, Barlow and Duncan 1963).

The aims of this experiment are to:

(i) compare the efficacy of various formulated insecticides to control *O. moreletii* with carbaryl, the standard insecticide for control of millipedes, on the basis of cost of treatment and the concentration of active constituent;

(ii) observe if the efficacy of different formulations of the same insecticide to control *O. moreletii* differs.

10.2 MATERIALS AND METHODS

In general, the materials and methods used in this experiment were similar to those materials and methods detailed in Section 5.

Eleven insecticide formulations were used. These formulations are listed in Table 10.1.

Fifty five chemical treatments were used. These treatments were obtained by serially diluting each formulation with distilled water to concentrations corresponding to 4 x, 2 x, 1 x, ½ x and ¼ x the cost of applying Septene^{R} Liquid at the rate of 1,375.4 mg carbaryl/m^{2}. The cost of each insecticidal formulation relative to Septene^{R} Liquid is also listed in Table 10.1.

The dosages for each treatment were corrected for body weight so that male and female knockdown data could be pooled, as could the moribundity data, as discussed in Experiment IV. The concentration of each insecticide used for males was half that used for females for each treatment, because males are approximately half the weight of females (of the same stadia); the dosage per body weight of millipede was then similar for each sex.

The concentrations of active constituents for each of the eleven insecticidal formulations used, for each relative cost, are detailed in Table 10.2. Septene^{R} Liquid is used as the example for the pooling of male and female sub-treatments, e.g., S_1F pooled with S_2M, S_2F with S_3M, S_3F with S_4M, S_4F with S_5M and S_5F with S_6M.

TABLE 10.1

The Insecticide Formulations used in Experiment V, with their Relative Costs, Compared to the Cost of Septene^{R} Liquid (500 g/L Carbaryl)

Product	Active Constituent	Cost per 100 g a.c. ($)	Cost Ratio
Septene^{R} Liquid	500 g/L carbaryl	1.92	1.0
X-18 Carbaryl	800 g/kg carbaryl	1.20	0.63
Baygon^{R} 80WP	800 g/kg propoxur	6.79	3.54
Baygon^{R} 200	218 g/L propoxur	6.69	3.48
Ficam^{R} W	800 g/kg bendiocarb	18.91	9.85
Lorsban^{R} 50EC	500 g/L chlorpyrifos	3.00	1.56
Lorsban^{R} 25WP	250 g/kg chlorpyrifos	7.49	3.90
Decis^{R} 25EC	25 g/L deltamethrin	164.80	85.83
Grenade^{R} 200EC	200 g/L cyhalothrin	62.16	32.38
Ripcord^{R} 200EC	200 g/L cypermethrin	50.00	13.02
Baythroid^{R} H	100 g/kg FCR 1272	-	43.74*

* The cost ratio for Baythroid^{R} H was the average of the three other synthetic pyrethroids: Decis^{R}, Ripcord^{R} and Grenade^{R} because Baythroid^{R} H is still under development.

TABLE 10.2

The Concentrations of Active Constituents for each of the Eleven Insecticidal Formulations used for each Relative Cost

Septene^{R} Liquid is used as the Example for the Pooling of Male and Female Sub-Treatments, e.g., S_1F Pooled with S_2M, S_2F with S_3M, S_3F with S_4M, S_4F with S_5M and S_5F with S_6M

Relative Cost	4x	2x	1x	1/2x	1/4x	1/8x
Septene^{R} Liquid (S) (500 g/L carbaryl) conc. mg a.c./m^2 sub-treatment example	5,501.6 S_1F	2,750.8 S_2F S_2M	1,375.4 S_3F S_3M	687.7 S_4F S_4M	343.9 S_5F S_5M	172.0 S_6M
X-18 Carbaryl (X) (800 g/kg carbaryl) conc. mg a.c./m^2	8,733.8	4,366.9	2,183.5	1,091.7	545.9	273.0
Baygon^{R} WP (BP) (800 g/kg propoxur) conc. mg a.c./m^2	1,554.2	777.1	388.6	194.3	97.1	48.6
Baygon^{R} 200 (BE) (218 g/L propoxur) conc. mg a.c./m^2	1,580.9	790.5	395.2	197.6	98.8	49.4
Ficam^{R} W (F) (800 g/kg bendiocarb) conc. mg a.c./m^2	558.4	279.2	139.6	69.8	34.9	17.5
Lorsban^{R} 50EC (LE) (500 g/L chlorpyrifos) conc. mg a.c./m^2	3,526.5	1,763.3	881.6	440.8	220.4	110.2
Lorsban^{R} 25WP (LP) (250 g/kg chlorpyrifos) conc. mg a.c./m^2	1,411.2	705.6	352.8	176.4	88.2	44.1
Decis^{R} 25EC (D) (25 g/L deltamethrin) conc. mg a.c./m^2	64.1	32.1	16.03	8.01	4.01	2.0
Grenade^{R} 200EC (G) (200 g/L cyhalothrin) conc. mg a.c./m^2	170.0	85.0	42.5	21.25	10.63	5.32
Ripcord^{R} 200EC (R) (200 g/L cypermethrin) conc. mg a.c./m^2	412.6	206.3	103.2	51.58	25.79	12.9
Baythroid^{R} H (H) (100 g/kg FCR1272) conc. mg a.c./m^2	125.7	62.85	31.43	15.71	7.86	3.93

The duration of exposure for each millipede on the insecticidally treated filter paper was 270 minutes based on the MT_{50} for females of 278.3 minutes which occurred with carbaryl in Experiment IV, using combined male and female moribundity data (see Table 9.2).

The male and female millipedes used in each treatment were estimated on the basis of size and colour to be in stadium ten or eleven. Ten millipedes, five males and five females, were used in each replicate. Each treatment was replicated three times. Equipment and manpower restrictions allowed only one replicate to be exposed at a time.

Three replicates of males and females were also used as controls, giving a total of 840 male and 840 female millipedes used in this experiment.

The millipedes in each replicate of each treatment were weighed to determine the average weights of the five male and the five female millipedes making up the replicate. This weighing was done twelve to 24 hours before the millipedes were exposed to the insecticidally treated filter papers.

The condition of each millipede, whether unaffected, knocked down or moribund was recorded at the initial time of exposure, at thirty minute intervals up to three hours and after four hours using the same criteria for assessment of knockdown and moribundity used in Experiment II.

After 24 and 48 hours, the condition of each millipede whether unaffected, knocked down, moribund or recovered was also recorded.

During this experiment the temperature ranged from 20 to 22^{o} C. for the first and second replicates and from 20 to 21^{o} C. for the third replicate.

Probit analyses were performed on the combined male and female data corrected for body weight for knockdown and moribundity for each formulated insecticide using the GLIM program MACRO PROB.

For valid comparisons of toxicity the probit regression lines should be parallel, otherwise the relative potency will vary with the mortality level chosen. The slopes of groups of probit regression lines were compared for similarity using GLIM, with significance at $p = 0.05$ determined using the F-test.

Comparisons of the KD_{50}'s, KT_{50}'s, MD_{50}'s or MT_{50}'s of probit regression lines of similar slope were made using the GLIM program MACRO LDCOM developed by Mr. P.I. McCloud.

10.3 RESULTS

The modification of dosage adopted in this experiment, i.e., males were exposed to insecticide at half the concentration of active constituent to which females were exposed in the same treatments, was satisfactory because the difference between the average weight of males and half the average weight of female millipedes used in this experiment were not significant at $p = 0.05$, allowing the pooling of male and female data.

The mean weight of the male and female sub-treatments each made up of five millipedes was 0.5723 ± 0.0708 and 1.1771 ± 0.1616 respectively.

To determine the efficacy of the various formulated insecticides to control *O. moreletii* probit analyses were first performed individually for each formulated insecticide on the following data sets:

(i) knockdown data, for dosage calculated at the same cost of treatment;

(ii) moribundity data, for dosage calculated on the basis of cost of treatment;

(iii) knockdown data, for dosage calculated on the basis of concentration of active constituent;

(iv) moribundity data, for dosage calculated on the basis of concentration of active constituent.

The results of these probit analyses are shown in Table 10.3 and Table 10.4 for knockdown and moribundity respectively, and are examined in detail and compared in the following sub-sections.

(i) Knockdown Data Compared at the Same Cost of Treatment

The data for this comparison are composed of the observations of knockdown made at thirty minute intervals for 240 minutes of the same batches of millipedes.

These batches of millipedes were exposed at concentrations of the various insecticidal formulations equivalent in cost to that of applying Septene^{R} Liquid at the rate of 1,375.4 mg carbaryl/m^2, i.e., treatments $S_3F + S_4M$, $X_3F + X_4M$, etc.

These data are summarised in Appendix XVI and the results of the probit analyses for the data are shown in Table 10.3.

Comparison of all KT_{50}'s together was not possible as the slopes of the eleven respective probit regression lines were significantly different ($F_{10,35} = 3.298$) at $p = 0.05$.

However, comparisons of the KT_{50}'s within two groups were possible.

TABLE 10.3

The Results of Individual Probit Analyses of the Knockdown Data for *O. moreletii* Exposed to Various Insecticide Formulations

Dosages are calculated for the same cost of treatment,
i.e., dosage = time of exposure/average millipede weight (minutes $grams^{-1}$)

and on the basis of concentration of active constituent,
i.e., dosage = concentration of active constituent/average millipede weight (mg m^{-2} $grams^{-1}$)

Maximum KD_{50}'s are proposed where probit analyses are not possible

TABLE 10.3

Dosages Calculated for the same Cost of Treatment	Insecticide Formulation				
	S	X	BP	BE	F
KT_{50}	741.8 ± 89.88	245.7 ± 14.86	228.1 ± 19.43	228.5 ± 12.19	249.5 ± 21.84
log KT_{50}	2.8703 ± 0.0526	2.3904 ± 0.0263	2.3581 ± 0.0370	2.3589 ± 0.0232	2.3971 ± 0.0380
slope of probit line	3.964 ± 0.4194	5.186 ± 0.6233	4.432 ± 0.3459	5.423 ± 1.126	5.144 ± 0.6299
intercept of probit line	-6.379 ± 1.159	-7.397 ± 1.571	-5.451 ± 0.8760	-7.792 ± 2.853	-7.331 ± 1.609
percent variance accounted for	95.7	94.5	97.6	84.7	94.3
Dosages Calculated on the Basis of Concentration of Active Constituent					
KD_{50}	11,951 ± 13,613	< 2,349	< 422.9	< 424.4	350.4 ± 23.09
log KD_{50}	4.0774 ± 0.4947	-	-	-	2.5445 ± 0.0286
slope of probit line	0.8028 ± 0.3636	-	-	-	3.001 ± 0.9614
intercept of probit line	1.727 ± 1.376	-	-	-	-2.636 ± 2.718
percent variance accounted for	49.2	-	-	-	68.6

Dosages Calculated for the same Cost of Treatment	Insecticide Formulation					
	LE	LP	D	G	R	H
KT_{50}	666.2 ± 26.86	631.1 ± 20.64	426.7 ± 21.07	571.0 ± 23.29	335.1 ± 23.78	304.0 ± 21.08
log KT_{50}	2.8236 ± 0.0175	2.8001 ± 0.0142	2.6302 ± 0.0215	2.7566 ± 0.0177	2.5251 ± 0.0308	2.4829 ± 0.0301
slope of probit line	8.050 ± 1.080	8.950 ± 0.7481	5.316 ± 0.5653	7.127 ± 0.5376	5.931 ± 1.222	5.613 ± 0.8329
intercept of probit line	-17.73 ± 3.026	-20.06 ± 2.099	-8.982 ± 1.550	-14.65 ± 1.518	-9.977 ± 3.298	-8.939 ± 2.223
percent variance accounted for	93.2	97.3	94.6	97.8	84.9	91.7
Dosages Calculated on the Basis of Concentration of Active Constituent						
KD_{50}	10,606 ± 31,367	2,240 ± 619.7	6.2558 ± 14.41	118.92 ± 14.83	161.0 ± 55.94	19.86 ± 27.13
log KD_{50}	4.0256 ± 1.2844	3.3502 ± 0.1202	0.79629 ± 1.0004	2.0753 ± 0.0542	2.2068 ± 0.1509	1.2980 ± 0.5932
slope of probit line	0.4142 ± 0.2859	2.137 ± 0.2078	1.203 ± 0.3513	2.646 ± 0.2186	2.285 ± 0.6280	1.513 ± 0.4588
intercept of probit line	3.333 ± 1.035	-2.158 ± 0.6651	4.042 ± 0.6618	-0.4913 ± 0.5041	-0.0431 ± 1.683	3.035 ± 0.9960
percent variance accounted for	21.6	96.3	72.8	97.3	75.4	71.2

TABLE 10.4

The Results of Individual Probit Analyses of the Moribundity Data for *O. moreletii* Exposed to Various Insecticide Formulations

Dosages are calculated on the basis of cost of treatment^{-1}
i.e., dosage = relative cost x 100/average millipede (grams^{-1})
where the relative cost of applying Septene^{R} Liquid
at the rate of 1,375.4 mg carbaryl/m^2 = 1

Dosages are also calculated on the basis of concentration of active constituent
i.e., dosage = concentration of active constituent/average millipede weight (mg m^{-2} grams^{-1})

Maximum MD_{50}'s are proposed where probit analyses are not possible

TABLE 10.4

Dosages Calculated on the Basis of Cost of Treatment	Insecticide Formulation				
	S	X	BP	BE	F
MD_{50}	129.87 ± 204.9	<107.57	120.08 ± 133.97	0.0 ± 0.00016	234.0 ± 24.89
log MD_{50}	2.1135 ± 0.6852	-	2.0795 ± 0.4845	-6.790 ± 419.8	2.369 ± 0.0462
slope of probit line	0.8238 ± 0.2039	-	1.281 ± 0.4833	0.0273 ± 0.2431	2.676 ± 0.8489
intercept of probit line	3.259 ± 0.5412	-	2.336 ± 1.291	5.185 ± 0.6482	-1.339 ± 2.278
percent variance accounted for	79.3	-	60.1	32.8	69.1
Dosages Calculated on the Basis of Concentration of Active Constituent					
MD_{50}	1,787 ± 2,818	<2,349	466.5 ± 520.7	0.0 ± 0.00065	326.7 ± 34.8
log MD_{50}	3.252 ± 0.6851	-	2.669 ± 0.4847	-6.172 ± 417.9	2.514 ± 0.0462
slope of probit line	0.8238 ± 0.2039	-	1.281 ± 0.4833	0.0274 ± 0.243	2.676 ± 0.8489
intercept of probit line	2.321 ± 0.7714	-	1.582 ± 1.573	5.169 ± 0.7918	-1.727 ± 2.400
percent variance accounted for	79.3	-	60.1	32.8	69.1

Dosages Calculated on the Basis of Cost of Treatment	Insecticide Formulation					
	LE	LP	D	G	R	H
MD_{50}	<104.56	<105.93	1,471 ± 3,232	1,367 ± 1,575	623.3 ± 130.1	582.2 ± 106.0
log MD_{50}	-	-	3.168 ± 0.9542	3.136 ± 0.5004	2.795 ± 0.0906	2.765 ± 0.0790
slope of probit line	-	-	1.363 ± 0.6155	1.702 ± 0.3664	3.232 ± 0.7498	2.494 ± 0.0958
intercept of probit line	-	-	0.6841 ± 1.640	-0.3384 ± 0.9797	-4.033 ± 2.000	-1.896 ± 0.2553
percent variance accounted for	-	-	49.4	83.7	81.5	99.4
Dosages Calculated on the Basis of Concentration of Active Constituent						
MD_{50}	<921.79	<373.73	235.8 ± 518.3	580.7 ± 666.1	642.9 ± 134.1	183.0 ± 33.28
log MD_{50}	-	-	2.3725 ± 0.9546	2.7640 ± 0.4982	2.808 ± 0.0906	2.2624 ± 0.0790
slope of probit line	-	-	1.362 ± 0.6154	1.704 ± 0.3664	3.234 ± 0.7492	2.494 ± 0.0954
intercept of probit line	-	-	1.768 ± 1.159	0.2901 ± 0.8457	-4.083 ± 2.009	-0.6434 ± 0.2071
percent variance accounted for	-	-	49.4	83.7	81.5	99.4

Comparison of all KT_{50}'s in group A made up of formulations -

Septene^{R} Liquid (S),

X-18 Carbaryl (X),

Baygon^{R} 80WP (BP),

Baygon^{R} 200 (BE),

Ficam^{R} W (F),

Decis^{R} 25EC (D),

Ripcord^{R} 200EC (F),

Baythroid^{R} H (H),

was possible as the slopes of the eight respective probit regression lines were not significantly different ($F_{6,26} = 0.787$) at $p = 0.05$.

The results of the probit analyses using a common slope for all probit regression lines for group A are shown in Table 10.5 and the KT_{50}'s are compared at $p = 0.05$ in Figure 10.1.

Comparison of the KT_{50}'s at $p = 0.05$ for group A shows that the most cost effective formulations were X, BP, BE and F. H was as effective as X, BP and F, while R was only as effective as F and H. D was less cost effective than all other formulations except S which was the least cost effective knockdown formulation.

TABLE 10.5

The Results of Probit Analyses of Knockdown of *O. moreletii* Exposed to the Formulated Insecticides in Groups A and B at Concentrations Equivalent in Cost to that of Applying Septene^{R} Liquid at the Rate of 1,375.4 mg Carbaryl/m^2, using a Common Slope for the Probit Regression Lines within each Group

Group A was made up of the following formulations: Septene^{R} Liquid (S), X-18 Carbaryl (X), Baygon^{R} 80WP (BP), Baygon^{R} 200 (BE), Ficam^{R} W (F), Decis^{R} 25EC (D), Ripcord^{R} 200EC (R) and Baythroid^{R} H (H)

Group B comprised the following formulations: Lorsban^{R} 50EC (LE), Lorsban^{R} 25WP (LP) and Grenade^{R} 200EC (G)

TABLE 10.5

Formulation	Intercept	Slope	Log KT_{50}	KT_{50}
Group A				
S	-9.384 ± 0.6980	5.054 ± 0.2479	2.846 ± 0.0227	701.2 ± 36.61
X	-7.066 ± 0.6408	5.054 ± 0.2479	2.387 ± 0.0248	244.0 ± 13.95
BP	-7.019 ± 0.6438	5.054 ± 0.2479	2.378 ± 0.0301	238.8 ± 16.56
BE	-6.863 ± 0.6440	5.054 ± 0.2479	2.347 ± 0.0202	222.3 ± 10.32
F	-7.102 ± 0.6493	5.054 ± 0.2479	2.394 ± 0.0268	248.0 ± 15.29
D	-8.266 ± 0.6924	5.054 ± 0.2479	2.625 ± 0.0210	421.5 ± 20.35
R	-7.614 ± 0.6858	5.054 ± 0.2479	2.496 ± 0.0296	313.1 ± 21.33
H	-7.449 ± 0.6783	5.054 ± 0.2479	2.463 ± 0.0296	290.4 ± 19.76
Group B				
LE	-18.00 ± 1.079	8.184 ± 0.3840	2.810 ± 0.0152	646.2 ± 22.58
LP	-17.92 ± 1.080	8.184 ± 0.3840	2.800 ± 0.0141	630.8 ± 20.46
G	-17.63 ± 1.087	8.184 ± 0.3840	2.765 ± 0.0163	581.7 ± 21.78

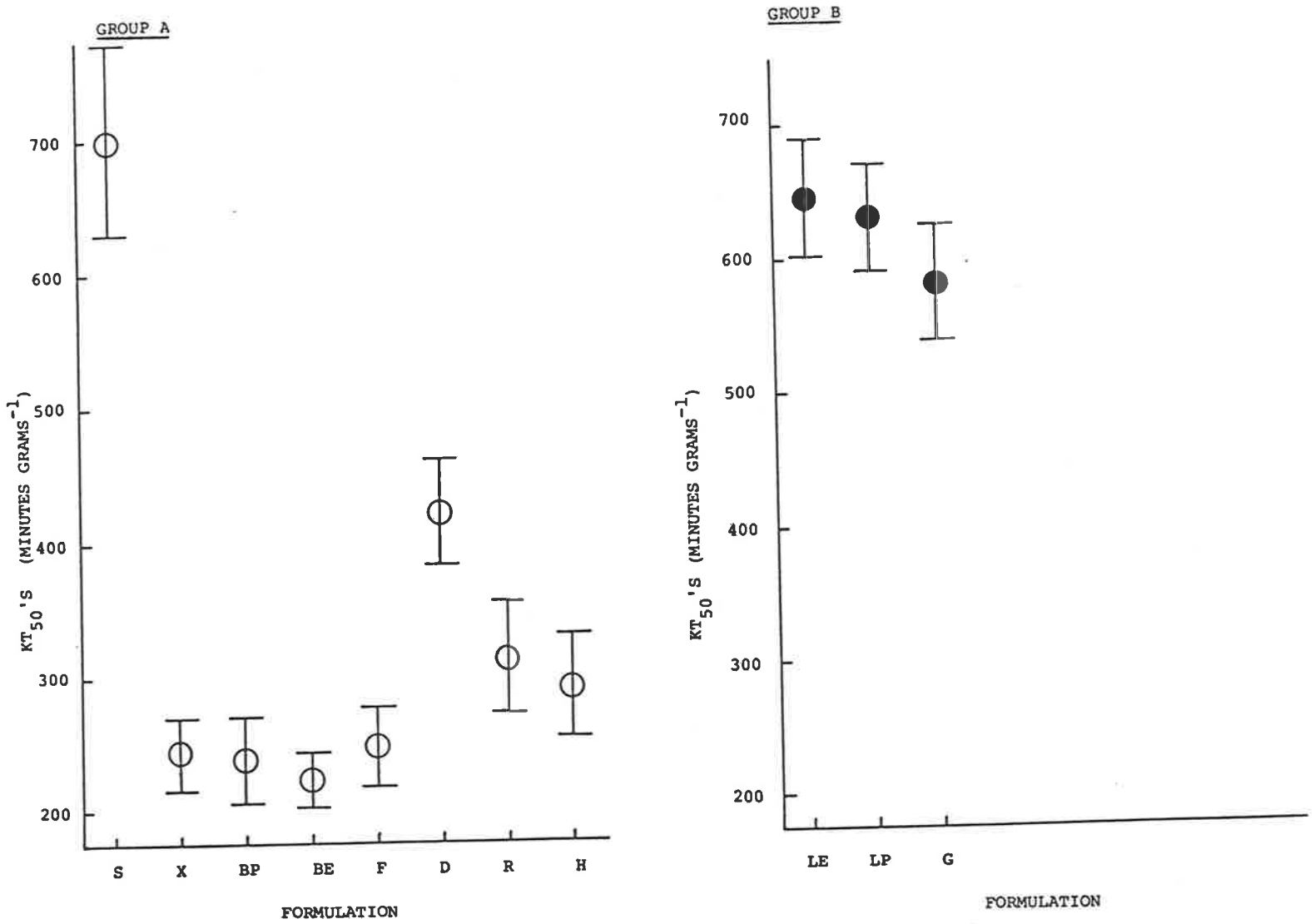
FIGURE 10.1

The KT_{50}'s with Confidence Limits (p = 0.05) for Knockdown of *O. moreletii* Exposed to the Formulated Insecticides in Groups A and B at Concentrations Equivalent in Cost to that of Applying Septene^R Liquid at the Rate of 1,375.4 mg Carbaryl/m^2

Group A was made up of the following formulations:
Septene^R Liquid (S), X-18 Carbaryl (X), Baygon^R 80WP (BP), Baygon^R 200 (BE), Ficam^R W (F), Decis^R 25EC (D), Ripcord^R 200EC (R) and Baythroid^R H (H)

Group B comprised the following formulations:
Lorsban^R 50EC (LE), Lorsban^R 25WP (LP) and Grenade^R 200EC (G)

FIGURE 10.1


GROUP A
700
600
500
400
300
200
KT_{50}'S (MINUTES GRAMS^{-1})
S
X
BP
BE
F
D
R
H
FORMULATION
GROUP B
700
600
500
400
300
200
KT_{50}'S (MINUTES GRAMS^{-1})
LE
LP
G
FORMULATION

Comparison of the KT_{50}'s in group B made up of formulations Lorsban^{R} 50EC (LE), Lorsban^{R} 25WP (LP) and Grenade^{R} 200EC (G) was also possible as the slopes of the three respective probit regression lines were not significantly different ($F_{2,9} = 2.623$) at $p = 0.05$.

The results of the probit analyses using a common slope for all probit regression lines for group B are also shown in Table 10.5 and the KT_{50}'s are compared at $p = 0.05$ in Figure 10.1.

Comparison of the KT_{50}'s for group B shows that all three formulations LE, LP and G are similarly cost effective.

N.B. The KT_{50}'s obtained in these analyses should be viewed with the reservations expressed in Section 7.4.

(ii) Moribundity Data Compared on the Basis of Cost of Treatment

The data for this comparison are composed of the observations of moribundity made 48 hours after exposure to insecticide for each treatment.

Dosage = relative cost x 100/average millipede weight where the relative cost of applying Septene^{R} Liquid at the rate of 1,375.4 mg carbaryl/m^2 = 1.

These data are summarised in Appendix XVII and the results of the individual probit analyses for the data are shown in Table 10.4.

Probit analyses on several moribundity data sets, viz.,

X-18 Carbaryl (X),

Lorsban^{R} 50EC (LE), and

Lorsban^{R} 25WP (LP),

were not possible because of the total moribundity of millipedes in most treatments. For these formulations, maximum MD_{50} values have been proposed. (Table 10.4)

The probit analysis for Baygon^{R} 200 (BE) did not show the expected positive linear relationship between moribundity (probits) and log dosage, and was consequently not included in the subsequent comparisons of MD_{50}'s.

Comparison of the MD_{50}'s for the remaining formulations, viz.,

Septene^{R} Liquid (S),

Baygon^{R} 80WP (BP),

Ficam^{R} W (F),

Decis^{R} 25EC (D),

Grenade^{R} 200EC (G),

Ripcord^{R} 200EC (R), and

Baythroid^{R} H (H),

was not possible as the slopes of the seven respective probit regression lines were significantly different ($F_{6,21}$ = 2.576) at p = 0.05.

However, comparisons of the MD_{50}'s within two groups were possible.

The MD_{50}'s for S, BP, D and G (group C) were compared as the slopes of the probit regression lines for each formulation were not significantly different ($F_{3,12}$ = 0.683) at p = 0.05.

Table 10.6 shows the results of the probit analyses using a common slope for all regression lines for group C, while the MD_{50}'s are compared at p = 0.05 in Figure 10.2.

Comparison of the MD_{50}'s at p = 0.05 for group C shows that the more cost effective formulations were S and BP, while D and G were less effective moribundity agents.

The MD_{50}'s for F, R and H (group D) were also compared as the slopes of the probit regression lines for each formulation were not significantly different ($F_{2,9}$ = 0.339) at p = 0.05.

Table 10.6 also shows the results of the probit analyses using a common slope for all regression lines for group D while the MD_{50}'s are compared at p = 0.05 in Figure 10.2.

Comparison of the MD_{50}'s at p = 0.05 for group D shows that F was the most cost effective formulation while R and H were less effective moribundity agents.

(iii) Knockdown Data Compared on the Basis of Concentration of Active Constituent

The data for this comparison are composed of the observations of knockdown made after 150 minutes of the 270 minute exposure time had elapsed for each treatment.

Dosage = concentration of active constituent/ average millipede weight.

These data are summarised in Appendix XVIII and the results of the individual probit analyses for the data are shown in Table 10.3.

TABLE 10.6

The Results of Probit Analyses of Moribundity of *O. moreletii* Exposed to the Formulated Insecticides in groups C and D for which Dosage = Relative Cost x 100/Average Millipede Weight, where the Relative Cost of applying Septene^R Liquid at the Rate of 1,375.4 mg Carbaryl/m^2 = 1, using a Common Slope for the Probit Regression Lines within each Group

Group C was made up of the following formulations:
Septene^R Liquid (S), Baygon^R 80WP (BP), Decis^R 25EC (D) and Grenade^R 200EC (G)

Group D comprised the following formulations:
Ficam^R W (F), Ripcord^R 200EC and Baythroid^R H (H)

TABLE 10.6

Formulation	Intercept	Slope	Log MD_{50}	MD_{50}
Group C				
S	2.030 ± 0.5883	1.293 ± 0.2136	2.297 ± 0.1013	198.3 ± 46.28
BP	2.305 ± 0.5917	1.293 ± 0.2136	2.085 ± 0.1291	121.5 ± 36.11
D	0.8678 ± 0.5904	1.293 ± 0.2136	3.197 ± 0.1301	1,572 ± 470.9
G	0.7429 ± 0.5921	1.293 ± 0.2136	3.293 ± 0.1441	1,964 ± 651.8
Group D				
F	-1.665 ± 0.9788	2.799 ± 0.3561	2.382 ± 0.0319	240.8 ± 17.66
R	-2.891 ± 0.9736	2.799 ± 0.3561	2.820 ± 0.0309	660.0 ± 46.91
H	-2.698 ± 0.9728	2.799 ± 0.3561	2.751 ± 0.0485	563.1 ± 62.94

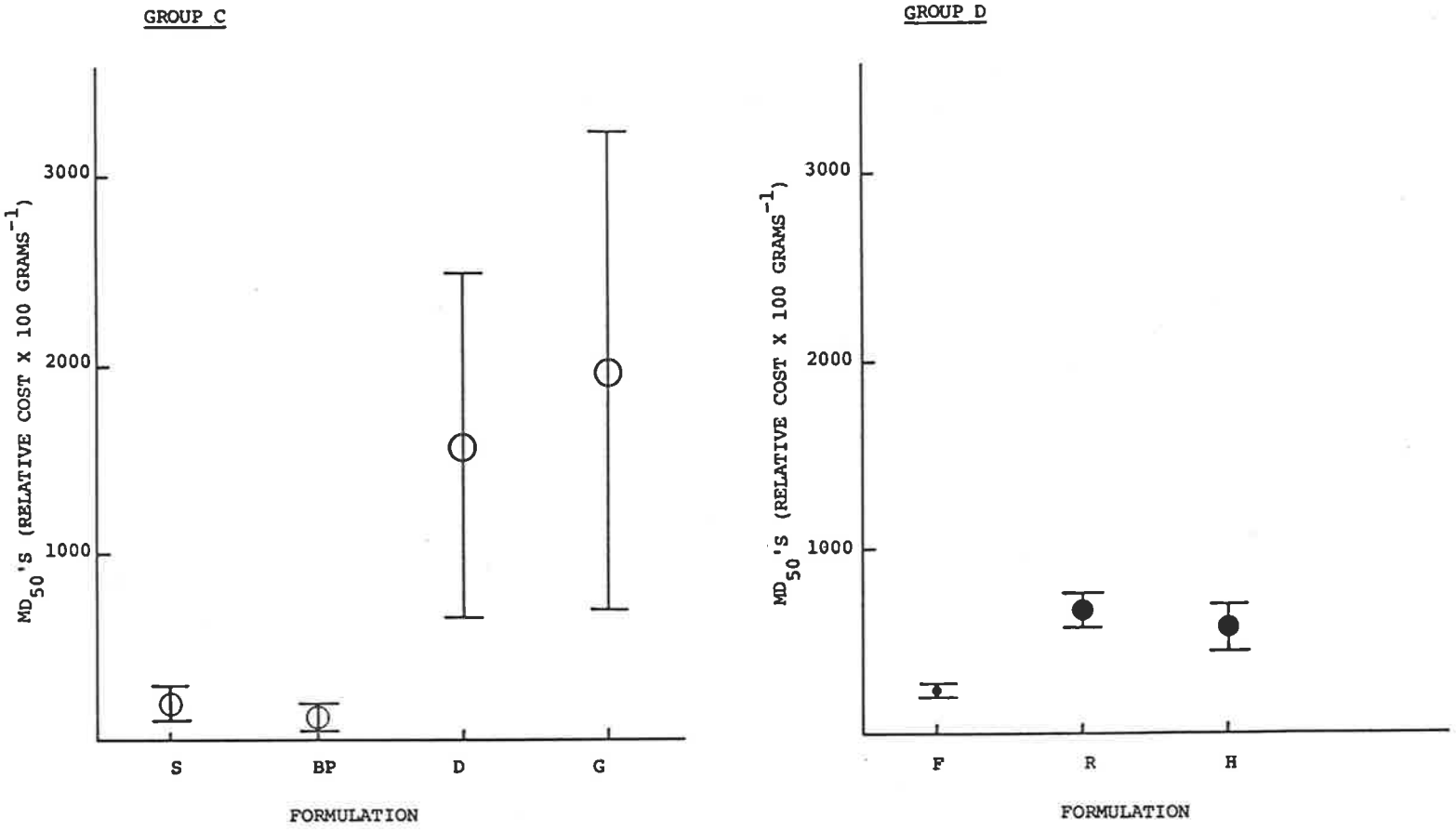
FIGURE 10.2

The MD_{50}'s with Confidence Limits (p = 0.05) for Moribundity of *O. moreletii* Exposed to the Formulated Insecticides in Groups C and D for which Dosage = Relative Cost x 100/Average Millipede Weight, where the Relative Cost of applying Septene® Liquid at the Rate of 1,375.4 mg Carbaryl/m^2 = 1, using a Common Slope for the Probit Regression Lines within each Group

Group C was made up of the following formulations:
Septene® Liquid (S), Baygon® 80WP (BP), Decis® 25EC (D) and Grenade® 200EC (G)

Group D comprised the following formulations:
Ficam® W (F), Ripcord® 200EC and Baythroid® H (H)

FIGURE 10.2


GROUP C
GROUP D
MD_{50}'S (RELATIVE COST X 100 GRAMS^{-1})
3000
2000
1000
S
BP
D
G
F
R
H
FORMULATION
FORMULATION

Probit analyses on several knockdown data sets, viz.,

X-18 Carbaryl (X),

Baygon^{R} 80WP (B), and

Baygon^{R} 200 (BE),

were not possible because of the total knockdown of millipedes in most treatments. Maximum KD_{50} values have been proposed for these formulations.

The expected positive linear relationship between knockdown (probits) and log dosage did not occur for Lorsban^{R} 50EC (LE) and was consequently not included in the subsequent comparisons of KD_{50}'s.

Comparison of the KD_{50}'s for the remaining formulations, viz.,

Septene^{R} Liquid (S),

Ficam^{R} W (F),

Lorsban^{R} 25WP (LP),

Decis^{R} 25EC (D),

Grenade^{R} 200EC (G),

Ripcord^{R} 200EC (R), and

Baythroid^{R} H (H),

was not made as the slopes of the seven respective probit regression lines were significantly different ($F_{6,21} = 2.383$) at $p = 0.07$, although not at $p = 0.05$.

However, comparisons of the KD_{50}'s within two groups were possible.

The KD_{50}'s for F, LP, G and R (group E) were compared as the slopes of the probit regression lines for each formulation were not significantly different ($F_{3,12} = 0.427$) at $p = 0.05$.

Table 10.7 shows the results of the probit analyses using a common slope for all regression lines for group E while the KD_{50}'s are compared at $p = 0.05$ in Figure 10.3.

Comparison of the KD_{50}'s at $p = 0.05$ for group E shows that the most effective treatments were G and R. F was less effective than G and R, but more effective than LP, the least effective knockdown agent.

The KD_{50}'s for S, D and H (group F) were also compared as the slopes of the probit regression lines for each formulation were not significantly different ($F_{2,9} = 0.824$) at $p = 0.05$.

Table 10.7 shows the results of the probit analyses incorporating a common slope for all

TABLE 10.7

The Results of Probit Analyses of Knockdown of
O. moreletii Exposed to the Formulated Insecticides
in Groups E and F for which
Dosage = Concentration of Active Constituent/Average Millipede Weight,
using a Common Slope for the Probit Regression Lines
within each Group

Group E was made up of the following formulations:
Ficam^{R} W (F), Lorsban^{R} 25WP (LP),
Grenade^{R} 200EC (G) and Ripcord^{R} 200EC (R)

Group F comprised the following formulations:
Septene^{R} Liquid (S), Decis^{R} 25EC (D) and Baythroid^{R} H (H)

TABLE 10.7

Formulation	Intercept	Slope	Log KD_{50}	KD_{50}
Group E				
F	-1.290 ± 0.8137	2.519 ± 0.2786	2.497 ± 0.0279	313.8 ± 20.19
LP	-3.372 ± 0.9149	2.519 ± 0.2786	3.323 ± 0.0544	2,105 ± 263.5
G	-0.2038 ± 0.6742	2.519 ± 0.2786	2.066 ± 0.0472	116.3 ± 12.64
R	-0.6623 ± 0.7744	2.519 ± 0.2786	2.248 ± 0.0543	176.9 ± 22.11
Group F				
S	0.3493 ± 0.8572	1.169 ± 0.2239	3.978 ± 0.1040	9,496 ± 2,274
D	4.104 ± 0.4426	1.169 ± 0.2239	0.7665 ± 0.2954	5.841 ± 3.973
H	3.769 ± 0.5040	1.169 ± 0.2239	1.053 ± 0.3225	11.30 ± 8.395

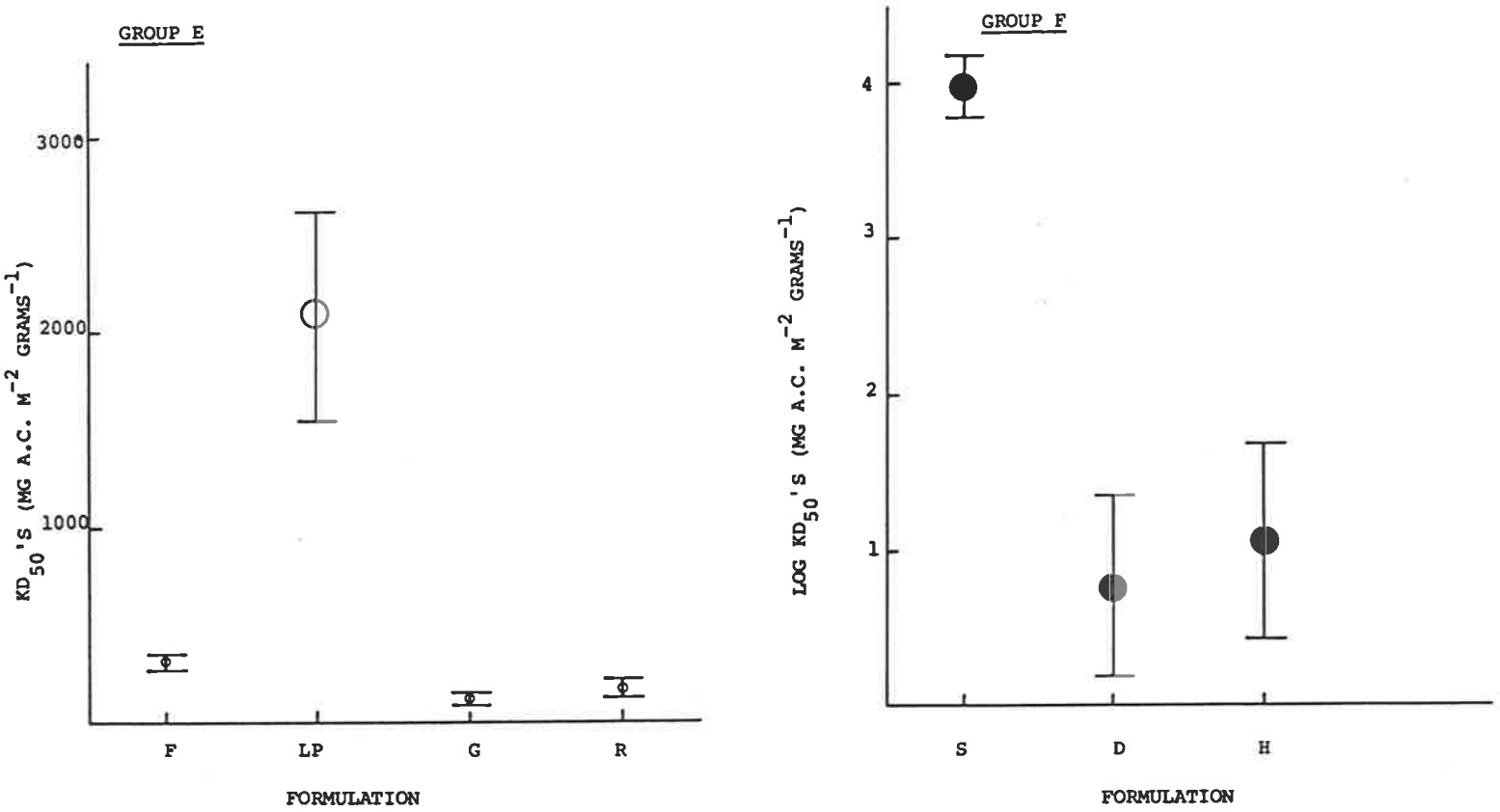
FIGURE 10.3

The KD_{50}'s with Confidence Limits (p = 0.05) for Knockdown of *O. moreletii* Exposed to the Formulated Insecticides in Groups E and F for which Dosage = Concentration of Active Constituent/Average Millipede Weight, using a Common Slope for the Probit Regression Lines within each Group

Group E was made up of the following formulations:
Ficam^{R} W (F), Lorsban^{R} 25WP (LP),
Grenade^{R} 200EC (G) and Ripcord^{R} 200EC (R)

Group F comprised the following formulations:
Septene^{R} Liquid (S), Decis^{R} 25EC (D) and Baythroid^{R} H (H)

FIGURE 10.3


GROUP E
3000
2000
1000
KD_{50}'S (MG A.C. M^{-2} $GRAMS^{-1}$)
F
LP
G
R
FORMULATION
GROUP F
4
3
2
1
LOG KD_{50}'S (MG A.C. M^{-2} $GRAMS^{-1}$)
S
D
H
FORMULATION

regression lines for group F, while the KD_{50}'s are compared at p = 0.05 in Figure 10.3.

Comparisons of the KD_{50}'s at p = 0.05 for group F shows that the more effective formulations were D and H while S was the least effective knock-down agent.

(iv) Moribundity Data Compared on the Basis of Concentration of Active Constituent

The data for this comparison are composed of the observations of moribundity after 48 hours for each treatment.

Dosage = concentration of active constituent/ average millipede weight.

These data are summarised in Appendix XVIII and the results of the individual probit analyses for the data are shown in Table 10.4.

Probit analyses on several moribundity data sets, viz.,

X-18 Carbaryl (X),

Lorsban^{R} 50EC (LE), and

Lorsban^{R} 25WP (LP),

were not possible because of the total moribundity of millipedes in most treatments. For these formulations maximum MD_{50} values have been proposed.

The probit analysis for Baygon^{R} 200 (BE) did not show the expected positive linear relationship between moribundity (probits) and log dosage, and was consequently not included in the subsequent comparisons of MD_{50}'s.

Comparison of the MD_{50}'s for the remaining formulations, viz.,

Septene^{R} Liquid (S),

Baygon^{R} 80WP (BP),

Ficam^{R} W (F),

Decis^{R} 25EC (D),

Grenade^{R} 200EC (G),

Ripcord^{R} 200EC (R), and

Baythroid^{R} H (H),

was not possible as the slopes of the respective probit regression lines were significantly different ($F_{6,21} = 2.576$) at $p = 0.05$.

However, comparisons of the MD_{50}'s within two groups were possible.

The MD_{50}'s for S, BP, D and G (group G) were compared as the slopes of the probit regression lines for each formulation were not significantly different ($F_{3,12} = 0.683$) at $p = 0.05$.

Table 10.8 shows the results of the probit analyses using a common slope for all regression lines for group G while the MD_{50}'s are compared at p = 0.05 in Figure 10.4.

Comparison of the MD_{50}'s at p = 0.05 for group G shows that BP, D and G were similarly effective while S was the least effective insecticide formulation.

The MD_{50}'s for F, R and H (group H) were compared as the slopes of the probit regression lines for each formulation were not significantly different ($F_{2,9}$ = 0.339) at p = 0.05.

Table 10.8 also shows the results of the probit analyses using a common slope for all regression lines while the resultant MD_{50}'s are compared at p = 0.05 in Figure 10.4.

Comparison of the MD_{50}'s at p = 0.05 for group H shows that H was more effective than F which was more effective than R.

TABLE 10.8

The Results of Probit Analyses of Moribundity of *O. moreletii* Exposed to the Formulated Insecticides in Groups G and H for which Dosage = Concentration of Active Constituent/Average Millipede Weight, using a Common Slope for the Probit Regression Lines within each Group

Group G was made up of the following formulations:
Septene^{R} Liquid (S), Baygon^{R} 80WP (BP),
Decis^{R} 25EC (D) and Grenade^{R} 200EC (G)

Group H comprised the following formulations:
Ficam^{R} W (F), Ripcord^{R} 200EC (R) and Baythroid^{R} H (H)

TABLE 10.8

Formulation	Intercept	Slope	Log MD_{50}	MD_{50}
Group G				
S	0.5582 ± 0.8231	1.293 ± 0.2136	3.436 ± 0.1013	2,728 ± 636.5
BP	1.543 ± 0.7126	1.293 ± 0.2136	2.674 ± 0.1290	472.1 ± 140.3
D	1.894 ± 0.4321	1.293 ± 0.2136	2.402 ± 0.1301	252.6 ± 75.65
G	1.225 ± 0.5169	1.293 ± 0.2136	2.920 ± 0.1441	832.1 ± 276.1
Group H				
F	-2.071 ± 1.029	2.799 ± 0.3562	2.526 ± 0.0319	336.1 ± 24.65
R	-2.926 ± 0.9780	2.799 ± 0.3562	2.832 ± 0.0309	679.1 ± 48.27
H	-1.292 ± 0.8019	2.799 ± 0.3562	2.248 ± 0.0485	177.0 ± 19.78

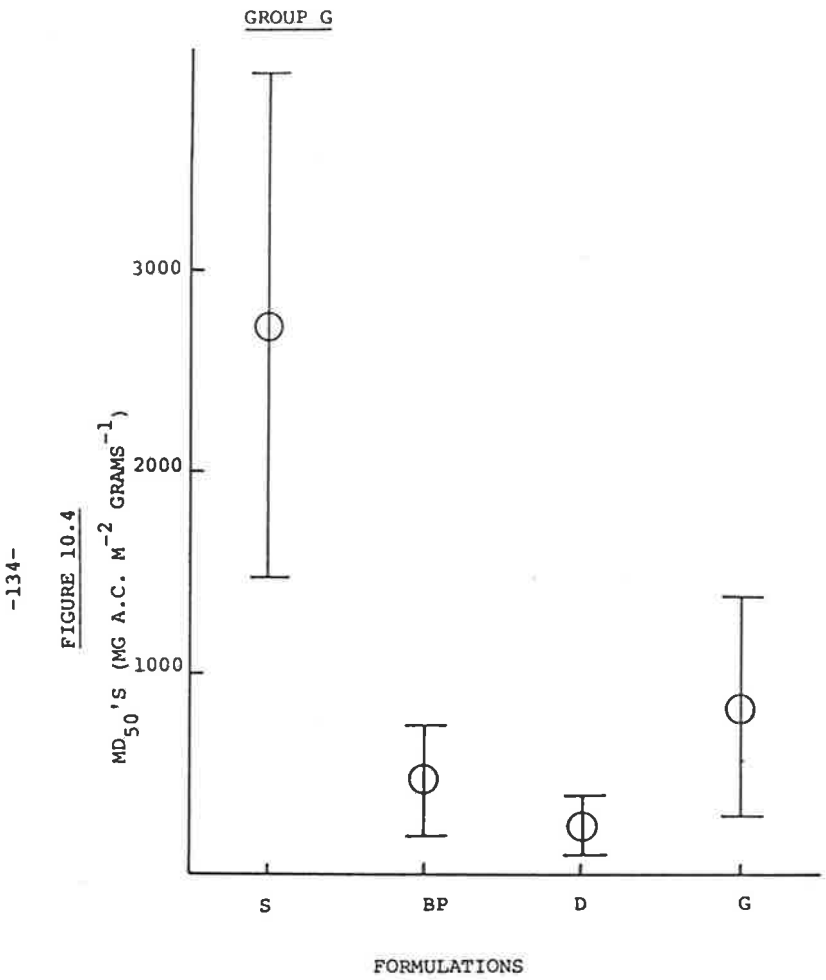
FIGURE 10.4

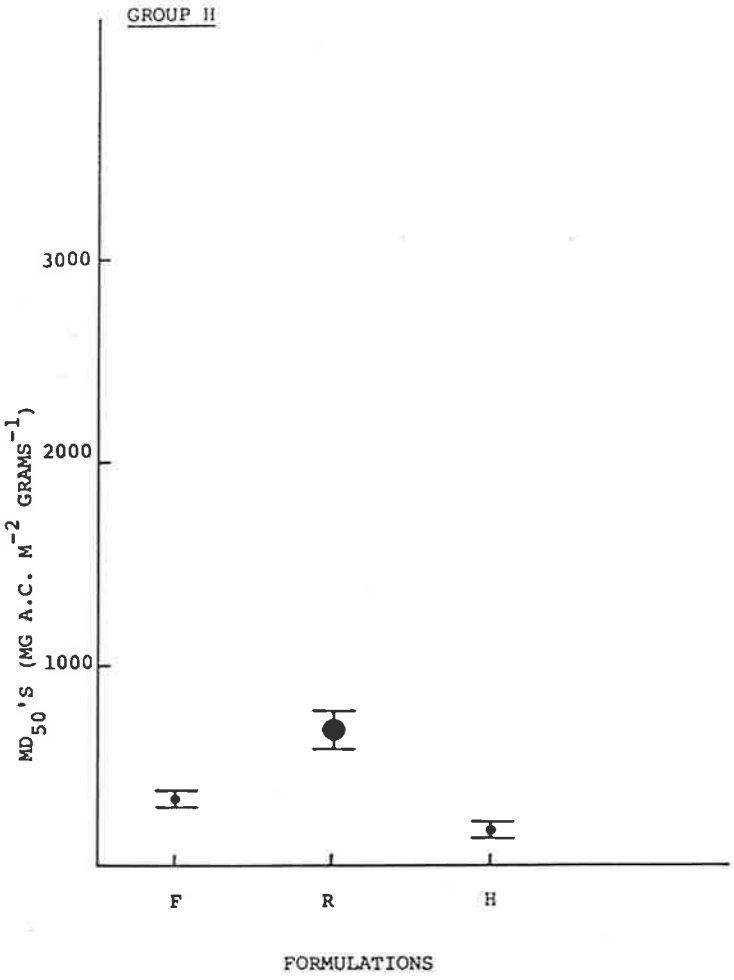
The MD_{50}'s with Confidence Limits (p = 0.05) for Moribundity of *O. moreletii* Exposed to Formulated Insecticides in Groups G and H for which Dosage = Concentration of Active Constituent/Average Millipede Weight, using a Common Slope for the Probit Regression Lines within each Group

Group G was made up of the following formulations:
Septene^{R} Liquid (S), Baygon^{R} 80WP (BP),
Decis^{R} 25EC (D) and Grenade^{R} 200EC (G)

Group H comprised the following formulations:
Ficam^{R} W (F), Ripcord^{R} 200EC (R) and Baythroid^{R} H (H)

FIGURE 10.4


GROUP G
3000
2000
1000
MD_{50}'S (MG A.C. M^{-2} GRAMS^{-1})
S
BP
D
G
FORMULATIONS


GROUP II
3000
2000
1000
MD_{50}'S (MG A.C. M^{-2} GRAMS^{-1})
F
R
H
FORMULATIONS

The comparisons of efficacy measured as knockdown and moribundity against *O. moreletii* of the eleven different formulated insecticides are summarised as follows:

(a) Septene^{R} Liquid (500 g/L carbaryl)

Septene^{R} Liquid compared very poorly with the other formulated insecticides and overall was the least effective formulation, its only advantage being comparatively low in cost.

Septene^{R} Liquid was the least effective formulation in its groups for knockdown (groups A and F) and moribundity compared on the basis of concentration of active constituent (group G), but was one of the most cost effective moribundity agents (group C).

(b) X-18 Carbaryl (800 g/kg carbaryl)

X-18 Carbaryl was more effective than Septene^{R} Liquid, the other carbaryl based formulation and compared favourably with other formulations for both knockdown and moribundity.

X-18 Carbaryl caused 100 percent moribundity in all treatments unlike Septene^{R} Liquid which achieved 80 percent moribundity only at the highest concentration of 5,501.6 mg carbaryl/m^2.

X-18 Carbaryl was also among the most cost effective knockdown formulation in group A for which Septene^{R} Liquid was the least cost effective formulation.

The effectiveness of X-18 Carbaryl as a knockdown agent is further supported by the total knockdown of millipedes exposed at the three highest concentrations of X-18 Carbaryl used.

(c) Baygon^{R} 80WP (800 g/kg propoxur)

Baygon^{R} 80WP was consistently among the most effective knockdown and moribundity agents.

Baygon^{R} 80WP was among the most cost effective knockdown and moribundity agents in group A and C respectively, and caused 100 percent knockdown at all but the lowest concentration of propoxur used after 150 minutes. Baygon^{R} 80WP was also one of the most effective in its group for moribundity compared on the basis of concentration of active ingredient (group G).

(d) Baygon^{R} 200 (218 g/L propoxur)

Baygon^{R} 200 was an effective knockdown agent, but knockdown and moribundity did not appear to be related to the concentration of propoxur at which the millipedes were exposed.

Baygon^{R} 200 caused knockdown as quickly as any formulation in group A (including Baygon^{R} 80WP) for which dosage was calculated as a function of exposure time. The knockdown and moribundity of millipedes exposed to Baygon^{R} 200 (where dosage was calculated as a function of concentration or cost of active constituent) were not compared with the other insecticidal formulations because Baygon^{R} 200 did not show the expected positive linear relationship between moribundity or knockdown (probits) and log dosage.

Therefore comparison of Baygon^{R} 200 with other products including Baygon^{R} 80WP was severely limited.

(e) Ficam^{R} W (800 g/kg bendiocarb)

Ficam^{R} W was among the most effective moribundity agents and one of the most cost effective knockdown agents.

Ficam^{R} W was the most cost effective formulation for moribundity in group D. It was also among the most effective knockdown and moribundity agents in group A (formulations compared at the same cost of treatment) and group H (dosage calculated as a function of concentration of insecticide) respectively.

(f) Lorsban^{R} 50EC (500 g/L chlorpyrifos)

Lorsban^{R} 50EC compared favourably with other formulations for moribundity, but knockdown did not appear to be related to the concentration of chlorpyrifos to which the millipedes were exposed.

Lorsban^{R} 50EC caused 100 percent moribundity in all treatments, but it did not show the expected positive linear relationship between knockdown (probits) and log dosage where dosage was calculated as a function of concentration of chlorpyrifos.

(g) Lorsban^{R} 25WP (250 g/kg chlorpyrifos)

Lorsban^{R} 25WP compared favourably with other formulations for moribundity, but compared poorly as a knockdown agent.

Lorsban^{R} 25WP caused 100 percent moribundity in all but one treatment, but was the least effective knockdown agent compared on the basis of concentration of active ingredient in group E.

Comparison of Lorsban^{R} 50EC with Lorsban^{R} 25WP was severely limited because both caused total or near total moribundity in all their respective treatments, and knockdown did not appear to be related to concentration of chlorpyrifos for Lorsban^{R} 50EC.

Comparison of these two formulations was only possible for knockdown at the same cost of treatment (group B) for which the two formulations performed similarly.

(h) Decis^{R} 25EC (25 g/L deltamethrin)

Decis^{R} 25EC was not among the most cost effective knockdown or moribundity agents because of its comparatively high cost, but when efficacy was compared on the basis of concentration of active constituent Decis^{R} 25EC was among the most effective knockdown and moribundity agents.

Decis^{R} 25EC was among the most effective knockdown and moribundity formulations in groups F and G respectively, compared on the basis of concentration of active constituent. However, Decis^{R} 25EC was second only to Septene^{R} Liquid as the least cost effective knockdown formulation, and with Grenade^{R} 200EC was the least cost effective moribundity agent in group C.

(i) Grenade^{R} 200EC (200 g/L cyhalothrin)

Grenade^{R} 200EC like the other synthetic pyrethroid insecticide Decis^{R} 25EC was not among the most cost effective knockdown or moribundity agents because of its comparatively high cost, but compared favourably with the other formulated insecticides on the basis of concentration of active constituent.

Grenade^{R} 200EC was among the most effective knockdown and moribundity formulations in groups E and G respectively, compared on the basis of concentration of active constituent. Grenade^{R} 200EC, however, was only as cost effective as Decis^{R} 25EC at causing moribundity in group C.

(j) Ripcord^{R} 200EC (200 g/L cypermethrin)

Ripcord^{R} 200EC, another synthetic pyrethroid also only compared favourably for both knockdown and moribundity with other formulations on the basis of concentration of active constituent, but not on cost of treatment.

Ripcord^{R} 200EC was less cost effective as a knockdown agent than X-18 Carbaryl, Baygon^{R} 80WP and Baygon^{R} 200 in group A and was less cost effective than Ficam^{R} W as a moribundity agent in group D. Ripcord^{R} 200EC was, however, among the most effective knockdown and moribundity formulations in groups E and H respectively compared on the basis of concentration of active constituent.

(k) Baythroid^{R} H (100 g/kg FCR 1272)

Baythroid^{R} H, like the three synthetic pyrethroids mentioned previously, compared favourably for both knockdown and moribundity on the basis of concentration of active constituent.

Baythroid^{R} H was among the most effective knockdown and moribundity formulations in groups F and H respectively. Little weight should be attached to the comparisons of knockdown and moribundity using cost of treatment because of the arbitrary way in which the cost for this formulation was calculated (see Table 10.1).

10.4 DISCUSSION

Hadaway, Barlow and Duncan (1963) reported that the initial toxicities of deposits of wettable powders of malathion and fenthion were greater than those emulsions on a given surface.

Similar effects were expected when comparing the efficacy against *O. moreletii* of the different formulations of the same active constituents in this experiment.

Greater superficial deposits are possible with wettable powders than with emulsifiable concentrates because the liquid emulsions are capable of wetting the surface of each glass fibre of the Whatman GF/A filter paper. Such an even distribution of active constituent is unlikely with the wettable powders because the retention efficiency of Whatman GF/A's is 1.6μm (Whatman 1980) and since wettable powders have a high proportion of particles only less than 5μm (Matthews 1979) many particles will be caught at or near the surface of the filter paper, biasing the distribution of active constituent towards the filter paper surface upon which the millipedes are exposed.

However, comparisons of efficacy against *O. moreletii* between pairs of different formulations containing the same active constituent, viz., Septene^{R} Liquid and X-18 Carbaryl,

Baygon^{R} 80WP and Baygon^{R} 200, and Lorsban^{R} 50EC and Lorsban^{R} 25WP, were inconclusive because the amount of data generated was limited.

Most treatments in which the millipedes were exposed to X-18 Carbaryl, Baygon^{R} 80WP, Lorsban^{R} 50EC or Lorsban^{R} 25WP, resulted in 100 percent moribundity of the millipedes so exposed. These data were not statistically comparable.

The knockdown and moribundity data for the millipedes exposed to Lorsban^{R} 50EC and Baygon^{R} 200 respectively did not show the expected positive linear relationship between knockdown or moribundity (probits) and log dosage, where dosage was calculated as a function of cost of treatment or concentration of active constituent. These data were not statistically comparable either.

Therefore the remaining data sets which could be compared were few and the influence on efficacy of the physical properties of the formulations was not determined.

An important aspect for selection of insecticides is toxicity or hazard to the applicator and non-target animals, particularly in the domestic situation where the insecticide is likely to be used by untrained personnel.

Table 10.9 lists the acute oral and dermal LD_{50}'s (for rats) for each insecticide active constituent, and the poison schedule for each formulation used in Experiment V.

TABLE 10.9

The Acute Oral and Dermal LD_{50}'s (for rats) for the Insecticide Active Constituents (Worthing 1983, Bayer El-8 42/844219) and the Poison Schedules of the Respective Formulations (NH & MRC 1985) used in Experiment V

Formulation	Active Constituent	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)	* Poison Schedule
Septene^{R} Liquid	500 g/L carbaryl	850	> 4000	6
X-18 Carbaryl	800 g/kg carbaryl	850	> 4000	6
Baygon^{R} 80WP	800 g/kg propoxur	90-128	800-1000	6
Baygon^{R} 200	218 g/L propoxur	90-128	800-1000	6
Ficam^{R} W	800 g/kg bendiocarb	40-156	566-600	6
Lorsban^{R} 50EC	500 g/L chlorpyrifos	135-163	c. 2000	6
Lorsban^{R} 25WP	250 g/kg chlorpyrifos	135-163	c. 2000	6
Decis^{R} 25EC	25 g/L deltamethrin	135-5000	> 2000	7
Grenade^{R} 200EC	200 g/L cyhalothrin	144-243	-	7
Ripcord^{R} 200EC	200 g/L cypermethrin	251-4123	> 2400	6
Baythroid^{R} H	100 g/kg FCR 1272	500-1200	> 5000	5

* The intrinsic toxicity and hazards of use of pesticides are signalled via the poison schedule. Schedules containing pesticides are, in increasing degree of hazard, 5, 6 and 7.

Schedule 7 poisons, including Decis^{R} 25EC and Grenade^{R} 200EC are too hazardous to be used by untrained personnel and could only be used in the domestic situation against millipedes by qualified Pest Control Operators.

The formulation which offers the greatest margin for safety to the applicator combined with efficacy against *O. moreletii* is Baythroid^{R} H.

This experiment, and all others in this thesis were conducted at approximately 20^{o} C. *O. moreletii* are likely to encounter insecticides at lower temperatures in the field.

Pyrethroids have a greater insecticidal effect when the temperature is lowered, i.e., they have negative temperature coefficient (Blum and Kearns 1956) so the favourable performance of the synthetic pyrethroids is likely to be further enhanced at lower temperatures.

10.5 CONCLUSIONS

The most effective knockdown and moribundity agents against *O. moreletii* compared on the basis of concentration of active constituent were the synthetic pyrethroid formulations, viz., Decis^{R} 25EC, Grenade^{R} 200EC, Ripcord^{R} 200EC and Baythroid^{R} H.

When the cost of treatment formed the basis of comparison of effectiveness against *O. moreletii* the cheaper carbamate insecticides X-18 Carbaryl, Baygon^{R} 80WP, Baygon^{R} 200 and Ficam^{R} W were the most effective knockdown and moribundity agents.

The organophosphate insecticides Lorsban^{R} 50EC and Lorsban^{R} 25WP were cost effective moribundity agents against *O. moreletii*, but they performed poorly as knockdown agents.

Comparison of the differences in efficacy against *O. moreletii* between different formulations containing the same active constituent, viz., Septene^{R} Liquid and X-18 Carbaryl, Baygon^{R} 80WP and Baygon^{R} 200 and Lorsban^{R} 50EC and Lorsban^{R} 25WP were inconclusive, so the influence on efficacy of the physical properties of the formulations was not determined.

11. FUTURE DIRECTIONS FOR RESEARCH

The laboratory biological assay developed in this thesis for *O. moreletii* will enable a wider range of insecticides to be screened relatively precisely and quickly for their efficacy against *O. moreletii* in the future.

However, the most promising control agents selected in such biological assays need to be evaluated in more expensive small-scale field trials.

Considering the data from experiments in this thesis, which products warrant further investigation as potential control agents for millipedes?

As potential barrier treatments, the synthetic pyrethroids were found the most effective knockdown and moribundity agents. Of the four tested, Baythroid^{R} H and Ripcord^{R} 200EC are the least intrinsically toxic and least hazardous to use. This is an important consideration as most millipede control measures would be carried out by untrained personnel.

Ripcord^{R} 200EC and Baythroid^{R} H should therefore be investigated as potential barrier treatments for millipede control.

However, when costs of treatments are considered, there was sufficient evidence to suggest that Ficam^{R} W and the Baygon^{R} products are also worthy of further study.

Although Lorsban^{R} 50EC and Lorsban^{R} 25WP performed poorly as knockdown agents, their performance as moribundity agents to millipedes suggests that they may be useful control agents where knockdown is a less important consideration of efficacy than moribundity. Such situations would include millipede breeding and feeding areas such as leaf litter and compost.

Thus Lorsban^{R} 50EC rather than the more expensive Lorsban^{R} 25WP should also be further investigated for millipede control.

APPENDIX I

Johnson's Orchid Potting Mix

Johnson's Orchid Potting Mix is a coarse, open potting mix composed chiefly of pine bark, rice husks and synthetic water absorbent Isolite^{R} beads.

This medium was found suitable for holding millipedes for at least one month, and being loose and clean, facilitated easy location and removal of millipedes for use in the experiments.

APPENDIX II

In several storage bins, a number of millipedes became infested with the mite, *Histiostoma feroniarium*, or a nematode (as yet unidentified) or both.

Plate A

Shows *H. feroniarium* mites and the unidentified nematodes on a millipede cadaver.

Plate B

Shows various stages of the unidentified nematode in the fluid surrounding a ruptured millipede cadaver.

Plate A *H. feroniarium* mites and an unidentified nematode on a millipede cadaver

Plate B Various stages of the unidentified nematode in fluid surrounding a ruptured millipede cadaver

APPENDIX III

MACRO PROB

The proportion of millipedes affected and the dosage were transformed to probits and log dosage respectively and critical dosage levels, e.g., KD_{50}, calculated using the GLIM program MACRO PROB developed by Mr. P.I. McCloud.

Probit regression lines were calculated using MACRO PROB and values with standard errors for the intercept, slope and 50 percent response point, e.g., KD_{50} were calculated

```
   MACRO PROB


$CALC LX = %LOG(X) / %LOG(10)
  :   P = Y / N
  :   Q = 1 - P
  :   EP = 5 + %ND(P)
  :   %L = %ND(%1/100) + 5

$YVAR EP
$FIT : +LX $
$DIS ER
$EXTRACT %PE
$CALC %C = %PE(1) : %B = %PE(2)
  :   %M = (%L-%C) / %B
  :   W = (%EXP(-0.5*(%FV-5)**2))**2/(2*%PI)/(P*Q)
  :   NW = N * W
  :   NWX = NW * LX
  :   NWX2 = NWX * LX
  :   %S = %CU(NW)
  :   %T = %CU(NWX)
  :   %X = %T / %S
  :   %Z = %CU(NWX2)
  :   %W = %Z - (%T**2) / %S
  :   %V = (1/%S + (%M-%X)**2 / W) / (%B**2)
  :   %E = %SQRT(%V)
  :   %O = 10 ** %M
  :   %U = %O * %LOG(10) * %E
$OUTPUT 6 130
$PRINT/"  X       LOG(X)       Y         N           EP              P          1-P"
$LOOK X LX Y N EP P Q $
$PRINT/" W         NW         NWLOG(X)        NWLOG(X)**2"
$LOOK W NW NWX NWX2$
$PRINT/" INTERCEPT =" %C "    SLOPE =" %B
$PRINT:" ESTIMATE OF LOG LD" *2 %1 " =" *5 %M
$PRINT:" ESTIMATE OF VARIANCE OF LOG LD" *2 %1 " =" *5 %V
$PRINT:" ESTIMATE OF STANDARD ERROR OF LOG LD" *2 %1 " =" *5 %E
$PRINT:" ESTIMATE OF LD" *2 %1 " =" *5 %O
$PRINT:" APPROXIMATE ESTIMATE OF THE SE OF THE LD" *2 %1 " =" *5 %U
$OUTPUT 6 80
$PLOT EP %FV LX $
```

APPENDIX IV

MACRO LDCOM

Comparisons of toxicity between different insecticides were made when the slopes of the respective probit regression lines were similar using the GLIM program MACRO LDCOM developed by Mr. P.I. McCloud

MACRO LDCOM calculated the values with standard errors for the intercept, common slope and 50 percent response point, e.g., KD_{50} for each data set compared

```
$MACRO LDCOM !
$CALC LX = %LOG(X) / %LOG(10) !
  :   P = Y/N !
  :   Q = 1-P !
  :   EP = 5 + %ND(P) !
  :   %L = %ND(%1/100) + 5 !
$YVAR EP !
$FIT : + %2 + LX - %GM !
$DIS ER !
$EXTRACT %PE !
$VAR 2 C M S T X2 Z W2 V E O U !
$CALC C(1) = %PE(1) : C(2) = %PE(2) : %B = %PE(3) !
  :   M = (%L - C) / %B !
  :   W = (%EXP(-0.5 * (%FV-5)**2))**2 / (2*%PI) / (P*Q) !
  :   NW = N * W !
  :   NWX = NW * LX !
  :   NWX2 = NWX * LX !
  :   S = 0 !
  :   S(%2) = S(%2) + NW !
  :   T = 0 !
  :   T(%2) = T(%2) + NWX !
  :   X2 = T/S !
  :   Z = 0 !
  :   Z(%2) = Z(%2) + NWX2 !
  :   W2 = Z - (T**2) / S !
  :   V = (1/S + (M-X2)**2 / W2) / (%B ** 2) !
  :   E = %SQRT(V) !
  :   O = 10 ** M !
  :   U = O * %LOG(10) * E !
$OUTPUT 6 130 !
$PRINT/"X      LOG(X)       Y          N          EP            P             1-P"!
$LOOK X LX Y N EP P Q $ !
$PRINT/"    W      NW       NWLOG(X)          NWLOG(X)**2 "!
$LOOK W NW NWX NWX2$!
$PRINT/"INTERCEPT "!
$LOOK C $!
$PRINT:"SLOPE = " %B!
$PRINT:"ESTIMATE OF LOG LD" *2 %1!
$LOOK M$!
$PRINT:"ESTIMATE OF VARIANCE OF LOG LD" *2 %1!
$LOOK V$!
$PRINT:"ESTIMATE OF STANDARD ERROR OF LOG LD" *2 %1!
$LOOK E$!
$PRINT:"ESTIMATE OF LD" *2 %1!
$LOOK O$!
$PRINT:"APPROXIMATE ESTIMATE OF THE SE OF THE LD" *2 %1!
$LOOK U$!
$VAR 1 SE
$CALC %D = (C(1)-C(2)) / %B !
  :  SE=%SQRT((1/S(1)+1/S(2)+(X2(1)-X2(2)-%D)**2/(W2(1)+W2(2)))/(%B**2)) !
$PRINT:"DIFFERENCE BETWEEN LD" *2 %1 " = " %D !
$PRINT:"STANDARD ERROR OF DIFFERENCE =" SE $!
$ENDMACRO !
$RETURN !
¢
```

APPENDIX V

The effects on male and female millipedes of one hour exposure to various concentrations of carbaryl in Experiment I

Time (hours)	Septene^{R} Liquid (500 g/L carbaryl) concentration in mg a.c./m^2				
	13,754.0	2,750.8	550.16	110.03	22.01
Male Data					
0	0	0	0	0	0
1	1A	0	0	0	0
2	1A	0	0	0	0
4	1A	0	0	0	0
8	1A	0	0	0	0
24	0	0	0	0	0
48	0	0	0	0	0
Female Data					
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
8	0	0	0	0	0
24	0	0	0	0	0
48	0	0	0	0	0

The number entered is the number of millipedes per treatment (out of five) which were:

A = affected

APPENDIX V (cont.)

The effects on male and female millipedes of one hour exposure to various concentrations of chlorpyrifos in Experiment I

Time (hours)	Lorsban^{R} 50EC (500 g/L chlorpyrifos) concentration in mg a.c./m^2				
	13,754.0	2,750.8	550.16	110.03	22.01
Male Data					
0	0	0	0	0	0
1	0	0	0	0	0
2	2A	0	0	0	3A
4	3A,1M	0	4A	0	5A
8	3A,2M	1A	1A,4M	1A,1M	3A,2M
24	4M	2A,2M	5M	3M	1A,4M
48	4M	1A,3M	5M	4M	1A,4M
Female Data					
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
4	2A	0	0	0	0
8	5A	0	1A	0	0
24	2A,3M	1A	1M	0	0
48	4M	1M	1A,1M	0	0

The number entered is the number of millipedes per treatment (out of five) which were:

A = affected

M = moribund

APPENDIX V (cont.)

The effects on male and female millipedes of one hour exposure to various concentrations of deltamethrin in Experiment I

Time (hours)	Decis^{R} 25EC (25 g/L deltamethrin) concentration in mg a.c./m^{2}				
	137.54	27.51	5.5	1.1	0.22
Male Data					
0	0	0	0	0	0
1	5A	0	0	0	0
2	5A	3A	0	0	0
4	5A	1A	0	0	0
8	5A	1A	0	0	0
24	4A,1M	0	0	0	0
48	4A,1M	0	0	0	0
Female Data					
0	0	0	0	0	0
1	5A	0	0	0	0
2	5A	0	0	0	0
4	5A	0	0	0	0
8	5A	0	0	0	0
24	5A	0	0	0	0
48	5A	0	0	0	0

The number entered is the number of millipedes per treatment (out of five) which were:

A = affected

M = moribund

APPENDIX VI

The stadial age of the millipedes used in Experiment I determined by the ocular field method

Stadium	Number of Millipedes
8	6
9	37
10	100
11	22
12	5

The mean stadial age was 9.9 ± 0.774

APPENDIX VII

The knockdown of male and female millipedes exposed to 13,754 mg carbaryl/m^2 (Treatment S_1) for eight hours in Experiment II

λ	log λ	n	r (males)	r (females)
480	2.681	15	15	15
420	2.623	15	15	15
360	2.556	15	15	15
330	2.519	15	15	*14
300	2.477	15	15	*11
270	2.431	15	*14	* 8
240	2.380	15	*13	* 3
210	2.322	15	*10	* 3
180	2.255	15	* 7	* 2
150	2.176	15	* 4	* 1
120	2.079	15	* 4	* 1
90	1.954	15	* 3	0
60	1.778	15	* 2	0
30	1.477	15	0	0
0	-	15	0	0

λ = dosage time in minutes

n = number of millipedes exposed

r = number of millipedes knocked down

* = values used in probit analyses

APPENDIX VII (cont.)

The knockdown of male and female millipedes exposed to 1,375.4 mg carbaryl/m^2 (Treatment S_2) for eight hours in Experiment II

人	log 人	n	r (males)	r (females)
480	2.681	15	15	15
420	2.623	15	15	15
360	2.556	15	15	15
330	2.519	15	15	15
300	2.477	15	15	15
270	2.431	15	15	15
240	2.380	15	15	15
210	2.322	15	*13	*13
180	2.255	15	*10	*12
150	2.176	15	* 7	* 9
120	2.079	15	* 6	* 4
90	1.954	15	* 2	* 1
60	1.778	15	* 2	0
30	1.477	15	* 1	0
0	-	15	0	0

人 = dosage time in minutes

n = number of millipedes exposed

r = number of millipedes knocked down

* = values used in probit analyses

APPENDIX VII (cont.)

The knockdown of male and female millipedes exposed to chlorpyrifos/m^2 (Treatment L) for eight hours in Experiment II

人	log 人	n	r (males)	r (females)
480	2.681	15	15	15
420	2.623	15	15	15
360	2.556	15	15	15
330	2.519	15	*15 (14.5)	*14
300	2.477	15	*14	* 9
270	2.431	15	*11	* 5
240	2.380	15	* 7	* 1
210	2.322	15	* 3	* 1
180	2.255	15	* 0 (0.5)	0
150	2.176	15	0	0
120	2.079	15	0	0
90	1.954	15	0	0
60	1.778	15	0	0
30	1.477	15	0	0
0	-	15	0	0

人 = dosage time in minutes

n = number of millipedes exposed

r = number of millipedes knocked down

* = values used in probit analyses, the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX VII (cont.)

The knockdown of male and female millipedes exposed to 27.51 mg deltamethrin/m^2 (Treatment D) for eight hours in Experiment II

λ	log λ	n	r (males)	r (females)
480	2.681	15	15	15
420	2.623	15	15	15
360	2.556	15	15	15
330	2.519	15	15	15
300	2.477	15	15	15
270	2.431	15	15	15
240	2.380	15	15	15
210	2.322	15	15	15
180	2.255	15	15	15
150	2.176	15	15	15
120	2.079	15	*15 (14.5)	*15 (14.5)
90	1.954	15	*14	*10
60	1.778	15	* 5	* 2
30	1.477	15	* 0 (0.5)	* 0 (0.5)
0	-	15	0	0

λ = dosage time in minutes

n = number of millipedes exposed

r = number of millipedes knocked down

* = values used in probit analyses, the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX VIII

The condition of the male and female millipedes whether knocked down, moribund or recovered, exposed for eight hours to three insecticides from different chemical groups, 28 and 48 hours after exposure in Experiment II

Treatment	Concentration of Active Constituent	n	Response of Millipedes after 24 hours	Response Millipedes after 48 hours
S_1 male	13,754 mg carbaryl/m^2	15	15M	15M
S_1 female	13,754 mg carbaryl/m^2	15	9K,7M	3R,4K,8M
S_2 male	1,375.4 mg carbaryl/m^2	15	15M	15M
S_2 female	1,375.4 mg carbaryl/m^2	15	2K,13M	1R,14M
L male	110.3 mg chlorpyrifos/m^2	15	15M	15M
L female	110.3 mg chlorpyrifos/m^2	15	4K,13M	2K,13M
D male	27.51 mg deltamethrin/m^2	15	14K,1M	3R,12K
D female	27.51 mg deltamethrin/m^2	15	14K,1M	9R,6K

n = number of millipedes exposed

K = knocked down

M = moribund

R = recovered

APPENDIX IX

The stadial age of the millipedes used in Experiment I determined by the ocular field method

Stadium	Number of Millipedes
9	12
10	80
11	41
12	7

The mean stadial age was 10.3 ± 0.698

APPENDIX X

The knockdown and moribundity after 48 hours of male and female millipedes exposed to 1,375.4 mg carbaryl/m^2 for various times in Experiment III

Male Data

λ	log	n	r (knockdown)	r (moribundity)
420	2.623	25	*25 (24)	*25 (24)
300	2.477	25	*25 (24)	*22
180	2.255	25	*25 (24)	*24
120	2.079	25	* 4	*11
60	1.778	25	* 0 (1)	* 1

Female Data

λ	log	n	r (knockdown)	r (moribundity)
420	2.623	25	*25 (24)	*22
300	2.477	25	*24	*21
180	2.255	25	*19	*11
120	2.079	25	* 5	* 7
60	1.778	25	* 0 (1)	* 0 (1)

λ = dosage time in minutes

n = number of millipedes exposed

r = number of millipedes either knocked down or moribund

* = values used in probit analyses, the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX XI

Knockdown and moribundity observations for male and female millipedes exposed to 1,375.4 mg carbaryl/m^2 for one, two and three hours in Experiment III

Observation Time (minutes)	One Hour Exposure		Two Hour Exposure		Three Hour Exposure	
	Male	Female	Male	Female	Male	Female
0	0	0	0	0	0	0
30	0	0	0	0	3K	0
60	0	0	1K	0	6K	0
90	1K	0	2K	2K	10K	1K
120	1K	0	4K	5K	16K	1K
150	1K	0	8K	6K	22K	8K
180	1K	0	13K	6K	25K	19K
210	1K	0	14K	7K	25K	22K
240	1K	0	14K	7K	25K	20K,2R
270	1K	0	14K	7K	25K	21K,1R
300	1K	0	13K,1R	5K,2M	20K,5M	21K,1R
360	1K	0	13K,2R,1M	5K,2M	17K,8M	21K,1R,1M
420	1K	0	10K,4R,2M	5K,3M	14K,11M	19K,1R,3M
24 hours	1K	0	1K,4R,11M	2K,1R,5M	1K,24M	8K,4R,11M
48 hours	1M	0	5R,11M	1R,7M	1K,24M	1K,11R,11M

The number entered is the number of millipedes per treatment (out of 25) which were:

K = knocked down

M = moribund

R = recovered

APPENDIX XI (cont.)

Knockdown and moribundity observations of male and female millipedes exposed to 1,375.4 mg carbaryl/m^2 for five and seven hours in Experiment III

Observation Time (minutes)	Five Hour Exposure		Seven Hour Exposure	
	Male	Female	Male	Female
0	0	0	0	0
30	0	0	2K	0
60	1K	0	2K	0
90	3K	0	4K	0
120	8K	2K	8K	0
150	13K	6K	22K	5K
180	17K	11K	25K	12K
210	23K	15K	25K	13K
240	25K	18K	25K	19K
270	25K	22K	25K	25K
300	22K,3M	23K,1M	21K,4M	23K,2M
360	20K,5M	16K,8M	17K,8M	23K,2M
420	14K,11M	16K,8M	13K,12M	18K,7M
24 hours	3K,22M	2K,2R,20M	3K,22M	4K,2R,19M
48 hours	3K,22M	3R,21M	25M	3R,22M

The number entered is the number of millipedes per treatment (out of 25) which were:

K = knocked down

M = moribund

R = recovered

APPENDIX XII

The stadial age of the millipedes used in Experiment III determined by the ocular field method

Stadium	Number of Millipedes
8	1
9	46
10	177
11	67
12	8
13	1

The mean stadial age was 10.1 ± 0.716

APPENDIX XIII

The knockdown and moribundity after 48 hours of male and female millipedes exposed to 1,375.4 mg carbaryl/m^2 for various times in Experiment IV

Male Data

λ	log λ	wt (grams)	(λ/wt)	log (λ/wt)	n	r knockdown	r moribundity
250	2.3979	0.1136	2,200.70	3.343	25	*25 (24)	*25 (24)
200	2.3010	0.1136	1,760.56	3.246	25	*23	*25 (24)
170	2.2304	0.1248	1,362.18	3.134	25	*13	*17
140	2.1461	0.1168	1,198.63	3.079	25	* 1	* 5
120	2.0792	0.1168	1,027.40	3.012	25	*12	*16
100	2.0000	0.1212	825.083	2.916	25	* 2	* 5
70	1.8451	0.1232	568.182	2.754	25	* 1 (1)	* 3

Female Data

λ	log λ	wt (grams)	(λ/wt)	log (λ/wt)	n	r knockdown	r moribundity
420	2.6232	0.2248	1,868.33	3.271	25	*25 (24)	*19
280	2,4472	0.2360	1,186.44	3.074	25	*24	*13
190	2,2788	0.2360	805.085	2.906	25	* 2	* 0 (1)
220	2.3424	0.2760	797.101	2.902	25	* 3	* 5
170	2.2304	0.2332	728.988	2.863	25	* 7	* 5
140	2.1361	0.2472	566.343	2.753	25	* 0 (1)	* 2
90	1.9542	0.2780	323.741	2.510	25	* 0 (1)	* 1

λ = dosage time in minutes

wt = average millipede weight

n = number of millipedes exposed

r = number of millipedes either knocked down or moribund

* = values used in probit analyses, the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX XIV

Knockdown, moribundity and recovery observations of male and female millipedes exposed to 1,375.4 mg carbaryl/m^2 for various times in Experiment IV

Male Data

Observation time (t) (minutes)	Response at t = 0	Response at time (t)	Response after 24 hours	Response after 48 hours
70	0	0	1M	3M
100	0	2K	5M	5M
120	0	12K	16M	16M
140	0	1K	5M	5M
170	0	13K	2K,15M	17M
200	0	23K	25M	25M
250	0	25K	1K,24M	25M

Female Data

Observation time (t) (minutes)	Response at t = 0	Response at time (t)	Response after 24 hours	Response after 48 hours
90	0	0	1K	1M
140	0	0	1K	2M
170	0	7K	1K,2R,4M	2R,5M
190	0	2K	2R	2R
220	0	3K	1K,4M	5M
280	0	24K	9K,9R,8M	11R,13M
420	0	25K	10K,1R,14M	6R,19M

The number entered is the number of millipedes per treatment (out of 25) which were:

K = knocked down
M = moribund
R = recovered

APPENDIX XV

The stadial age of the millipedes used in Experiment IV determined by the ocular field method

Stadium	Number of Millipedes
8	6
9	69
10	214
11	96
12	15

The mean stadial age was 10.1 ± 0.782

APPENDIX XVI

Knockdown observations for male and female *O. moreletii* exposed to eleven different formulated insecticides compared at the same cost of treatment in Experiment V

The concentrations of each insecticide formulation corresponded in cost to the cost of applying Septene^{R} Liquid at the rate of 1,375.4 mg carbaryl/m^{2}

Formulation	λ	weight (grams)	λ/wt	n	r knockdown
S	240	0.2223	1,079.6	30	23
	180	0.2223	809.72	30	18
	150	0.2223	674.76	30	13
	120	0.2223	539.81	30	7
	90	0.2223	404.86	30	3
	60	0.2223	269.91	30	2
X	150	0.2428	617.79	30	30 (29)
	120	0.2428	494.23	30	28
	90	0.2428	370.68	30	26
	60	0.2428	247.12	30	20
	30	0.2428	123.56	30	0 (1)
BP	150	0.2359	635.86	30	30 (29)
	120	0.2359	508.69	30	28
	90	0.2359	381.52	30	26
	60	0.2359	254.35	30	20
	30	0.2359	127.17	30	3
BE	150	0.2355	636.94	30	30 (29)
	120	0.2355	509.55	30	29
	90	0.2355	382.17	30	29
	60	0.2355	254.78	30	23
	30	0.2355	127.39	30	0 (1)
F	150	0.2239	669.94	30	30 (29)
	120	0.2239	535.95	30	30 (29)
	90	0.2239	401.97	30	28
	60	0.2239	267.98	30	16
	30	0.2239	133.99	30	2
LE	240	0.2347	1,022.6	30	30 (29)
	180	0.2347	766.94	30	21
	150	0.2347	639.11	30	13
	120	0.2347	511.29	30	6
	90	0.2347	383.47	30	1

λ = dosage time in minutes
n = number of millipedes exposed
r = number of millipedes knocked down
( ) = the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX XVI (cont.)

Knockdown observations for male and female *O. moreletii* exposed to eleven different formulated insecticides compared at the same cost of treatment in Experiment V

The concentrations of each insecticide formulation corresponded in cost to the cost of applying Septene^{R} Liquid at the rate of 1,375.4 mg carbaryl/m^{2}

Formulation	λ	weight (grams)	λ /wt	n	r knockdown
LP	240	0.2326	1,031.8	30	29
	180	0.2326	773.86	30	26
	150	0.2326	644.88	30	13
	120	0.2326	515.91	30	6
	90	0.2326	386.93	30	1
D	240	0.2344	1,023.9	30	29
	180	0.2344	767.92	30	27
	150	0.2344	639.93	30	25
	120	0.2344	511.95	30	23
	90	0.2344	383.96	30	15
	60	0.2344	255.97	30	2
G	240	0.2239	1,071.9	30	29
	180	0.2239	803.93	30	26
	150	0.2239	669.94	30	23
	120	0.2239	535.95	30	11
	90	0.2239	401.97	30	4
R	180	0.2259	796.81	30	29
	150	0.2259	664.01	30	29
	120	0.2259	531.21	30	29
	90	0.2259	398.41	30	18
	60	0.2259	265.60	30	7
H	180	0.2428	741.35	30	29
	150	0.2428	617.79	30	29
	120	0.2428	494.23	30	28
	90	0.2428	370.68	30	22
	60	0.2428	247.12	30	7

λ = dosage time in minutes

n = number of millipedes exposed

r = number of millipedes knocked down

APPENDIX XVII

Moribundity observations for male and female *O. moreletii* exposed to eleven different formulated insecticides compared on the basis of cost of treatment in Experiment V

Dosage = relative cost x 100/average millipede weight, where the relative cost of applying Septene^{R} Liquid at the rate of 1,375.4 mg carbaryl/m^2 = 1

Formulation	λ	weight (grams)	λ/wt	n	r moribundity
S	400	0.2479	1,613.554	30	24
	200	0.2392	836.120	30	23
	100	0.2223	449.843	30	19
	50	0.2412	207.297	30	20
	25	0.2487	100.523	30	12
X	400	0.2360	1,694.915	30	30
	200	0.2397	834.376	30	30
	100	0.2428	411.862	30	30
	50	0.2236	223.614	30	30
	25	0.2324	107.573	30	30
BP	400	0.2330	1,716.738	30	30 (29)
	200	0.2322	861.326	30	24
	100	0.2359	423.908	30	17
	50	0.2226	224.618	30	23
	25	0.2296	108.885	30	15
BE	399.04	0.2320	1,720.000	30	20
	199.52	0.2276	876.626	30	16
	99.760	0.2355	423.609	30	19
	49.880	0.2365	210.909	30	15
	24.940	0.2328	107.131	30	20
F	400	0.2200	1,818.182	30	30 (29)
	200	0.2285	875.274	30	28
	100	0.2239	446.628	30	30 (29)
	50	0.2300	217.391	30	16
	25	0.2181	114.626	30	2
LE	400	0.2087	1,916.627	30	30
	200	0.2187	914.495	30	30
	100	0.2347	426.076	30	30
	50	0.2254	221.828	30	30
	25	0.2391	104.559	30	30

λ = dosage = % relative cost
n = number of millipedes exposed
r = number of millipedes moribund
( ) = the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX XVII (cont.)

Moribundity observations for male and female *O. moreletii* exposed to eleven different formulated insecticides compared on the basis of cost of treatment in Experiment V

Dosage = relative cost x 100/average millipede weight, where the relative cost of applying Septene^{R} Liquid at the rate of 1,375.4 mg carbaryl/m^2 = 1

Formulation	λ	weight (grams)	λ/wt	n	r moribundity
LP	400	0.2347	1,704.303	30	29
	200	0.2433	822.030	30	30
	100	0.2326	429.923	30	30
	50	0.2398	208.507	30	30
	25	0.2360	105.932	30	30
D	399.14	0.2456	1,625.163	30	21
	199.57	0.2274	877.617	30	10
	99.78	0.2344	425.683	30	3
	49.89	0.2312	215.787	30	2
	24.95	0.2292	108.857	30	5
G	401.30	0.2388	1,680.486	30	20
	200.65	0.2192	915.374	30	10
	100.32	0.2239	448.057	30	3
	50.16	0.2313	216.861	30	4
	25.08	0.2384	105.201	30	1
R	401.04	0.2434	1,647.658	30	29
	200.52	0.2356	851.104	30	21
	100.26	0.2259	443.825	30	3
	50.13	0.2214	226.423	30	1
	25.065	0.2378	105.404	30	0 (1)
H	400	0.2376	1,683.502	30	26
	200	0.2320	862.069	30	20
	100	0.2428	411.862	30	12
	50	0.2205	226.757	30	4
	25	0.2338	106.929	30	0 (1)

λ = dosage = % relative cost

n = number of millipedes exposed

r = number of millipedes moribund

( ) = the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX XVIII

Knockdown and moribundity observations for male and female *O. moreletii* exposed to eleven different formulated insecticides on the basis of concentration of active constituent in each treatment in Experiment V

Dosage = concentration of active constituent/average millipede weight, where the knockdown data were recorded after 150 minutes exposure (r_1) to the respective insecticides, while the moribundity data were recorded after 48 hours (r_2)

Formulation	λ	weight (grams)	λ/wt	n	r_1 knockdown	r_2 moribundity
S	5,501.6	0.2479	22,192.8	30	15	24
	2,750.8	0.2392	11,500.0	30	16	23
	1,375.4	0.2223	6,187.13	30	13	19
	687.7	0.2412	2,851.16	30	14	20
	343.9	0.2487	1,382.79	30	4	12
X	8,733.8	0.2360	37,007.6	30	30	30
	4,366.9	0.2397	18,218.2	30	30	30
	2,183.5	0.2428	8,993.00	30	30	30
	1,091.7	0.2236	4,882.38	30	29	30
	545.9	0.2324	2,348.97	30	27	30
BP	1,554.2	0.2330	6,670.39	30	30	30 (29)
	777.1	0.2322	3,346.68	30	30	24
	388.6	0.2359	1,647.31	30	30	17
	194.3	0.2226	872.87	30	30	23
	97.1	0.2296	422.91	30	24	15
BE	1,580.9	0.2320	6,814.2	30	30	20
	790.5	0.2276	3,473.2	30	27	16
	395.2	0.2355	1,678.1	30	30	19
	197.6	0.2365	835.52	30	29	15
	98.8	0.2328	424.40	30	25	20
F	558.4	0.2200	2,538.18	30	30 (29)	30 (29)
	279.2	0.2285	1,221.88	30	30 (29)	28
	139.6	0.2239	623.49	30	30 (29)	30 (29)
	69.8	0.2300	303.48	30	16	16
	34.9	0.2181	160.02	30	1	2
LE	3,526.5	0.2087	16,897.46	30	15	30
	1,763.3	0.2187	8,062.64	30	14	30
	881.6	0.2347	3,756.28	30	13	30
	440.8	0.2254	1,955.63	30	16	30
	220.4	0.2391	921.79	30	7	30

λ = dosage = mg a.c./m^2
n = number of millipedes exposed
r = number of millipedes knocked down (r_1) or moribund (r_2)
( ) = the numbers in brackets were substituted as approximations for the probit analyses where shown

APPENDIX XVIII (cont.)

Knockdown and moribundity observations for male and female *O. moreletii* exposed to eleven different formulated insecticides on the basis of concentration of active constituent in each treatment in Experiment V

Dosage = concentration of active constituent/average millipede weight, where the knockdown data were recorded after 150 minutes exposure (r_1) to the respective insecticides, while the moribundity data were recorded after 48 hours (r_2)

Formulation	λ	weight (grams)	λ/wt	n	r_1 knockdown	r_2 moribundity
LP	1,411.2	0.2347	6,012.78	30	23	29
	705.6	0.2433	2,900.12	30	19	30
	352.8	0.2326	1,516.77	30	13	30
	176.4	0.2398	735.61	30	5	30
	88.2	0.2360	373.73	30	0 (1)	30
D	64.1	0.2456	260.99	30	29	21
	32.1	0.2274	141.16	30	29	10
	16.03	0.2344	68.38	30	25	3
	8.01	0.2312	34.65	30	27	2
	4.01	0.2292	17.50	30	19	5
G	170.0	0.2388	711.89	30	30 (29)	20
	85.0	0.2192	387.77	30	28	10
	42.5	0.2239	189.82	30	23	3
	21.25	0.2313	91.87	30	12	4
	10.63	0.2384	44.59	30	3	1
R	412.6	0.2434	1,695.15	30	30 (29)	29
	206.3	0.2356	875.64	30	30 (29)	21
	103.2	0.2259	456.84	30	29	3
	51.58	0.2214	232.97	30	18	1
	25.79	0.2378	108.45	30	7	0 (1)
H	125.7	0.2376	529.04	30	30 (29)	26
	62.85	0.2320	270.91	30	30 (29)	20
	31.43	0.2428	129.45	30	29	12
	15.71	0.2205	71.25	30	22	4
	7.86	0.2338	33.62	30	17	0 (1)

λ = dosage = mg a.c./m^2

n = number of millipedes exposed

r = number of millipedes knocked down (r_1) or moribund (r_2)

( ) = the numbers in brackets were substituted as approximations for the probit analyses where shown

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