SOME INSECTICIDE STUDIES ON THE RICE STEM BORER *DIOPSIS THORACICA* WESTW. (DIPTERA:DIOPSIDAE)

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by

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D E C L A R A T I O N

I hereby declare that, except for references to other people's work which have been duly cited, this thesis is the result of my own original work carried out from August 1972 to August 1973, and that it has not been presented elsewhere for another degree.

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Date: 6th February, 1975

ABSTRACT

Insecticide bioassays were carried out on *D. thoracica* (a) adults by topical application of 1 µl drop of toxicant on their nota; and (b) eggs by dipping them into insecticidal solutions for 30 min. LC50 values obtained from the tests on the adults were: Gamma BHC, 26.40mg/l; Pirimiphos methyl (PP511) 28.00 mg/l; Sevin, 67.00 mg/l and Malathion, 380.00 mg/l. Values obtained from the eggs tests were: PP511, 1.30 mg/l; Sevin, 5.30 mg/l; gamma BHC, 18.30 mg/l and Malathion, 83.00 mg/l.

A field trial was carried out to assess the efficacies of PP51 gamma BHC, Sevin, Basudin (Diazinon) and Bidrin in controlling Diopsid borer infestation in a rice farm on the Accra Plains. Spra applications were made of 0.04% active ingredient solutions of the insecticides at 5, 8, and 11 weeks after transplanting rice variety

All the insecticides reduced the borer infestation compared to the control plots at the pre-heading stage of the rice plants. Gam BHC and Bidrin treatments reduced the borer infestation more than th other insecticides after the second spray application. Stem borer infestation during the heading period was low in all the experiment plots and yields from the treated plots were not different from the control. Borer-induced tillering in the rice may have compensated for the effect of borer infestation on yield.

The rate of penetration of ^{14}C-PP511 into the abdominal cuticle of D. thoracica adult females was determined by topical application of 0.01 μCi (0.693 μg) of the insecticide in cyclohexane solvent. The maximum rate of penetration, 3.1%/min (186%/h) occured 11.5 min after application when 28% of the applied dose had penetrated the insect's cuticle. Toxic effects of the insecticide became evident about 60 min after application and became acute thereafter.

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ABBREVIATIONS AND SYMBOLS

a.i.	active ingredient
Anon.	Anonymous
A.R.S.	Agricultural Research Station at Kpong
^{14}C	labelled carbon
¢	Cedi
cm	centimetre
cpm	counts per minute
Fig.	Figure
gamma BHC	gamma Benzene hexachloride
g	gram
h	hour
ha	hectare
ICI Company Ltd.	Imperial Chemical Industries Company Limited
IRRI	International Rice Research Institute, Los Banos, Laguna Philippines
kg	kilogram
kg/ha	kilogram per hectare
km	kilometre
LC50	Lethal Concentration to kill 50% of the test insects
ld-p line	log dosage-probit line
log	logarithm

m	metre
mCi	milliCurie
mg	milligram
ml	millilitre
mm	millimole
mg/l	milligram/litre
min	minute
No.	number
ppm	parts per million
PP511	Pirimiphos methyl
rpm	revolutions per minute
SD	standard deviation
Sy.x	standard deviation of probits about the ld-p line
Westw.	Westwood
X^2	Chi square
λ x.y	standard deviation of log dosages about the ld-p line
µCi	micro Curie
µg	microgram
µl	microlitre
%/min	percent per minute
**	significant at 1% level
*	significant at 5% level
%	percent; percentage
°C	degree Centigrade

I. INTRODUCTION

In his quest for food and raw materials, man has had to put large acreages of land under cultivation. This has invariably provided favourable conditions for the rapid multiplication of pests which not only reduce yields but also serve as vectors of diseases. All these result in economic loss to the farmer.

Paddy is one of the most extensively grown cereals in the world. Grist (1965) records that its cultivation falls within latitudes 49° North and 40° South; the chief limiting factor to its growth being water supply. It has been the principal source of carbohydrate for the peoples of Asia for centuries and in recent times, it has come to occupy an increasingly important position in the diet of West Africans. Ghartey (1970) stated that annual rice consumption in Ghana had been estimated at 80,000 tons, nearly 50% of which was imported at a cost of ¢7.8 million. The cost of rice importation had also been estimated to rise to ¢11 million by 1975 if domestic production did not increase.

The stem boring larvae are among the most serious pests of rice all over the world. They infest the rice plant at all its stages of growth and in the tropics where temperature variation is slight and multiple cropping is practised, they maintain a high population throughout the year. With such a situation, effective means must be found to control them. Grist (1965) has listed effective pest control in paddy as being second in importance only to improved drainage and irrigation in improving yield.

Pest control has been a problem from ancient times. This is evidenced by the numerous accounts of the ravages of locusts in Biblical times. From those times, man has had to devise ways and means of controlling pests on his crops. These mainly consisted of cultural practices, such as planting at times most likely to avoid the onslaught of particular pests, or growing mixed crops to make conditions unfavourable for any particular insect to increase in number to epidemic proportions. Apart from some instances of successful biological control of insect pests, such as the control of cottony cushion scale insect _Icerya purchasi_ Maskell by the ladybird _Rodolia cardinalis_ Muls. in California (U.S.A.), insecticides have had to be used to control pests wherever extensive cropping is done.

In recent times many insecticides have been produced. They vary in their physical and chemical properties, their cost of production, mode of application, toxicity and residual effects. It is therefore desirable to carry out tests to find the most effective insecticides against any pest in any given condition. In a pest control or insecticide evaluation exercise, it is necessary first of all to measure the level of tolerance of the pest to different insecticides and to select the most potent ones. These may then be tested in the field before being recommended for use by farmers.

Work for this thesis involved laboratory determination of the tolerance of D. thoracica adults (the predominant rice stem borer in the Accra Plains; Van Halteren, 1970; Abu, 1972), to four insecticides, namely: Gamma BHC, Sevin, Malathion and Pirimiphos methyl or PP511 (a new organophosphorus insecticide). Ovicidal effects of these insecticides on D. thoracica eggs were also determined. A field experiment was carried out to verify the efficacies of gamma BHC, Sevin, PP511, Bidrin and Basudin or Diazinon in controlling stem-borer infestation of rice at Dahwenya, in the Accra Plains. The rate of penetration of labelled PP511, into the abdominal cuticle of D. thoracica adult females was also determined.

2. REVIEW OF LITERATURE

2.1: Diopsis thoracica Westw. (Diptera:Diopsidae)

Rice stem borers belong to the two insect orders: Lepidoptera and Diptera. In West Africa, damage caused by Diopsis species (Diptera:Diopsidae) on swamp rice is considered to be more serious than that caused by lepidopterous borers which have been recorded as major pests of rice all over the world (Anon, 1970).

Shortly after hatching, the stem boring larvae bore into rice stalks where they stay until they become adults. The larvae feed on the plant material causing death or injury to the plant, resulting in little or no crop being produced.

Decamps (1956) first reported Diopsis thoracica Westwood, as one of the principal enemies of rice in Northern Cameroun and ranked it first among insect pests of rice in the Benue river valley. The following year (1957), he published an account of the biology and morphology of fifteen species of the family Diopsidae in Northern Cameroun, of which seven were said to be pests, in varying degrees, on graminaceous plants.

Three years' observations conducted in several West African countries by Brenière (1969), brought out the importance of three Diopsis species on rice. D. thoracica Westw., was by far the most abundant in Casamance (Senegal), the Ivory Coast and Sierra Leone; D. apicalis Dalm. (Synonymous to D. tenuipes Westw.) was associated with the first in certain parts of the Ivory Coast and D. collaris Westw. occurred in Casamance. Akinsola (1970) reported D. thoracica and D. apicalis as mild pests of rice in Nigeria. Van Halteren (1970) noted that there was a virtual absence of moth (lepidopterous) borers at the University of Ghana Agricultural Research Station at Kpong (in the Accra Plains), and stated that D. thoracica appeared to be the most important pest of rice there. He however doubted the need for any control measures against this pest because it appeared to cause little damage.

Abu (1972) gave an account of the bionomics of Diopsis species on rice in the Accra Plains. He stated that of the three species of Diopsis (D. thoracica Westw., D. tenuipes Westw. and D. ichneumonea Dahl.) found in rice-growing areas of the Accra Plains, D. thoracica occurred in far greater numbers than the others. It constituted 73% of the total adult Diopsis species collected from April 1971 to April 1972 in 24 bi-weekly collections at Dahwenya. He suggested that D. thoracica caused most of the initial damage to rice since D. tenuipes, the second in order of abundance (constituting 24% of the total adult Diopsid population collected at the same period) had the tedency of infesting already damaged plants. He also found that stalk infestation by Diopsis species during the same period, reached 48% in the rainy season at Dahwenya, while infestation by lepidopterous (moth) borers never exceeded 2.9%. Artificial infestation of rice with D. thoracica larvae showed that a single larva could destroy 3 - 6 stalks in its life. Stem borer infestation induced compensatory tillering, but this phenomenon did not appear to have any appreciable effect on yield if the crop was heavily infested.

Contrary to Van Halteren (1970), Abu (1972) believed that such a level of Diopsid infestation during the rainy season (48%) could lead to heavy losses in yield, and therefore attacks by Diopsis species on rice could not be ignored completely.

2.2: Tolerance of D. thoracica to insecticides

From Abu's (1972) account on Diopsid infestation of rice in the Accra Plains, it appears that there was need for chemical control of the pest; especially during the rainy season. In a systematic study of the chemical control of a pest, a laboratory evaluation of the potencies of different insecticides would be a necessary initial determination before field trials are carried out.

Finney (1964) has defined the term bioassay (or biological assay) as the measurement of the potency of any stimulus, physical, chemical or biological, physiological or psychological by means of reactions which it produces in living matter.

All insecticide bioassay procedures fall in either of two main categories (a) taking the toxicant to the insect, such as "topical application", and (b) taking the insect to the toxicant such as "dipping". In accounts on such bioassay procedures, emphasis were placed on the adherence to some pre-test, test and post-test conditions of the insect and the environment (Busvine, 1957; Shepard, 1958, 1960; Hoskins and Craig, 1962).

Busvine (1957) referred to these as intrinsic and extrinsic factors respectively. The intrinsic factors relate to the species and stage specificities (i.e. age, sex and size) of the test insects. The extrinsic factors relate to both rearing and testing temperatures, humidity, type of diet and recent feeding or starvation, population density and illumination.

No indication was obtained of any insecticide bioassay having been carried out on Diopsis thoracica.

According to Hoskins and Craig (1962), the results of insecticide bioassays almost always are expressed in the log dosage - probit system and the properties of the log dosage - probit (ld-p, hereafter) line are of prime importance in any consideration of the value of a method or comparison of two more methods used. The slope of the ld-p line expresses the magnitude of variability in susceptibility of the test population as measured by the procedure involved. A steep line means that a population has a small variation in dosage over the response interval; that it is relatively homogeneous in susceptibility. Conversely, a flatter line results from the use of a population varying widely in susceptibility. For a given chemical used on a chosen species, the slope of the ld-p line should be the same for all procedures if the dose bears a constant relation to dosage over the range used, and the mode of action is the same. Different slopes may be an indication of different modes of action, however if auxiliary chemicals are present in some procedures and not in others, they may change the course of toxic action. To obtain a straight line, two more important conditions must be satisfied: (a) the population (of test insects) must have a normal distribution of susceptibility to the toxicant concerned; the samples actually used being representative of the population; (b) over the range used in a test, the effective doses which gave rise to toxic action must be in a constant ratio (k) to the applied dosage (dose = k x dosage).

The most fundamental information yielded by a bioassay is the Median Lethal Dosage (MLD) expressed as LC50, LD50 or LT50, depending on the method of measuring dosage (whether as concentration, dose or time to kill 50% of the test population). This determines the sensitivity and sets the midpoint of the range of dosages that may be profitably used. Hoskins and Craig (1962) stated that the ratio: Standard deviation of LC50/LC50 was an indication of the variation to be expected in the median lethal dosage. They also stated that the scattering of experimental points about the best ld-p line was the most useful measure of variability inherent in a bioassay procedure. It is indicated by the standard deviations of probits and log dosages about the ld-p line: i.e. S y.x and λ x.y respectively. Either of the two standard deviations may be used in any of the general applications of bioassay to measure the spread of susceptibility. Sy.x is more useful in determining changes in susceptibility whereas λx.y is more appropriate for evaluating the suitability of the methods for residue determination or screening. The ratios Sy.x/LC50 and λ x.y/LC50 are more significant measures of variability of results from a chosen procedure since they do not depend on the units of dosage used.

Bliss (1934) stated that aside from an increased accuracy in calculating dosage - mortality curves over a more extended range of mortalities than has been practicable with the usual asymmetrical "S" curve, this type of presentation (i.e. log dosage - probit system of presentation) has led to the following advantages:

(1) test of the proposed theory of toxic action that (a) the variation in susceptibility among individuals is normal and (b) that the effectiveness of the dosage increases as its logarithm,

(2) a closer scrutiny of experimental technique to determine if the organisms exposed to each dosage were truly equivalent, and if the amounts administered were uniformly proportional to the effective dosage over the entire range covered by the experiment,

(3) the disclosure of a change in the mode of lethal action with certain poisons over different sections of the dosage range, indicated by an abrupt change in slope, and

(4) a simple method of expressing in the slope of a straight line, the relative uniformity or diversity between individuals in their susceptibility to a poison.

2.3: Efficacies of Pirimiphos methyl, Sevin, Gamma BHC, Basudin (or Diazinon) and Bidrin in controlling Rice stem-borer infestation

In rice stem-borer control programmes parameters used in estimating the efficacies of insecticides in controlling borer damage include percentage "dead heart" and "white head" formations. Formation of "dead heart" or "stem rot" is caused by the stem-boring larva feeding within the central shoot of the plant and results in its death and drying up. Infestations during the heading period results in the formation of "white heads" or discoloured panicles with empty or partially filled grains, often with secondary fungal attack (Plate 3, page 46). Other parameters used are percent infested tillers, number of productive panicles per hill and yield (IRRI report, 1963).

Various insecticides have been used to control Asian rice stem-borers. Of these 0.04% Endrin has been reported to be the most effective in India (Rao and Israel, 1964). In field trials at the International Rice Research Institute (IRRI) in the Philippines, with compounds showing ovicidal and larvicidal properties in the laboratory, Pathak (1964), reported that foliar applications of Endrin, Imidan and Lebaycid at 0.04%, 0.09% and 0.05% respectively, applied at 400-500 gallons per hectare effectively controlled dead heart formation. All the treated plots had significantly lower percentages of white heads and produced higher yields than the controls.

Akinsola's (1970) experiment with five insecticides namely: Sevin 85, Murfotox 80, Dimecron 20, Gammexane 50 and Sumithion 50 applied as sprays on the 8th, 12th, 15th and 18th weeks after transplanting proved ineffective in controlling borer damage at Badeggi, in Northern Nigeria. Morgan (1973) found that although none of the twelve insecticides used in his trials significantly reduced percentage dead heart formation over the control, Sumithion, D.D.V.P. (dimethyl-2-dichlorovinyl phosphate), Pantrin, E.P.N. (ethyl p-nitrophenyl thionobenzene phosphate) and Aldrin appeared to be more effective than gamma BHC (gamma 1,2,3,4,5,6, hexachloro-cyclohexane), the insecticide commonly used to control rice stem borers in Sierra Leone.

Kwai and Engman (1973) of Dahwenya rice settlement farm, stated that insecticides recommended against rice stem-borers were E.P.N., Kitazin, gamma BHC, Malathion, and Metasystox. The settler-farmers are advised to spray their seedlings in the nursery against leaf-eating caterpillars and their fields when the stem borer population is high, but they do not take the advice because of the extra cost and labour involved. At the University of Ghana Agricultural Research Station, Kpong, rice pests are controlled with Gammalin 20 (20% gamma BHC). A concentration of 100 ml insecticide in 4 gallons of water is used and the volume of spray is estimated to range from 25 to 30 gallons per acre (0.1% gamma BHC at 284.4 to 341.2 litres per hectare).

Spraying is done when the borer population is observed to be high (Johnson, 1973). No quantitative estimates have been made of the efficacies of insecticides in controlling stem-borers at both places.

On timing of insecticide applications, Pathak (1964) reported that treatments during the first 50 days (7 weeks) after transplanting were not important in preventing crop losses. If the crop was not protected beyond 50 days, grain yield declined sharply. He attributed this to the inability of the plants to compensate for lost tillers by producing new ones. Such tillers, even if produced, remained in the vegetative phase and did not mature by harvest time. It was found that plants were most susceptible to damage from the 7th to 11th weeks after transplanting, beyond which the borers might not prefer the crop for oviposition.

2.4: Rate of Penetration of labelled Pirimiphos methyl into the abdominal cuticle of *Diopsis thoracica* adult females

Radioisotopes offer an excellent tool for research on problems that previously had been insoluble or difficult to attack by conventional methods; their uses in entomology cover many physiological and toxicological studies, such as the penetration, distribution and breakdown of insecticides in insects (Jenkins and Hasset, 1950).

The measurement of the rate of penetration of an insecticide through an insect's cuticle is a desirable initial step in a general study of the toxicology of the insecticide on the insect. This is because permeability of the integument is listed as one of the variety of factors which determine the tolerance (or natural resistance) of insects to insecticides (Hoskins and Gordon, 1956). Sternburg and Kearns (1950) have reported that when DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) was applied on resistant houseflies, it was degraded mainly in the cuticle-hypoderm, and that very little DDT or DDE (1,1, dichloro-2,2bis(p-chlorophenyl ethylene) was found in the interior parts of the fly.

The insect cuticle has been described by Richards (1958) as a heterogeneous asymmetrical membrane composed of sublayers of considerable chemical and physical complexity. Armstrong et al (1951) found that the first stage of pick-up of insecticide by an insect was simple solution of the insecticide in the waxy covering of the epicuticle; and that structural effects played an important part in the penetration of insecticides through the cuticle.

Sun (1968) observed a dynamic state of penetration and detoxification of insecticides in insects; and stated that the combined effects of the rates of penetration and detoxification mainly determined the toxicity of insecticides to different species, as well as susceptible and resistant strains of the same species. He also observed that organophosphorus insecticides penetrated into houseflies more readily than non-organophosphorus insecticides, and that their maximum rates of penetration were much higher and could be reached in a shorter period after application; quick knockdown (or immobilising effect) of some carbamates indicated their rapid penetration, and quick recovery from them indicated that they were not only reversible cholinesterase inhibitors, but were also quickly detoxified. He concluded that when the activity of insecticides was viewed on a dynamic basis, species specificity, resistance, synergism, knockdown and speed of toxic action could be explained by differences in their rates of penetration and detoxification.

The experiments to be described now, were designed:

1. (a) to measure the tolerance or susceptibility of D. thoracica adults to gamma BHC, Malathion, Sevin (common organochlorine, organophosphorus and carbamate insecticides respectively) and Pirimiphos methyl or PP511 (a new organophosphorus insecticide) by "topical application" on their nota.

(b) to measure the ovicidal effects of the same insecticides on D. thoracica eggs by "dipping"

2. to determine the efficacies of PP511, gamma BHC, Sevin and two other organophosphorus insecticides: Bidrin and Basudin or Diazinon in controlling rice stem borer infestation in the field, and

3. to measure the rate of penetration of labelled Pirimiphos methyl (the only labelled insecticide that was obtained) into the abdominal cuticle of D. thoracica adult females.

3 EXPERIMENTAL

3. TOLERANCE OF DIOPSIS THORACICA ADULTS AND EGGS TO PIRIMIPHOS METHYL, GAMMA BHC, SEVIN AND MALATHION

The experiment was carried out to determine the tolerance of D. thoracica adults and eggs to Pirimiphos methyl or PP511, gamma BHC, Sevin and Malathion. The tests on the adults were carried out by topical application of 1 µl drop of toxicant on their nota. Tests on the eggs were carried out by 'dipping' the eggs into solutions of the insecticides.

Preliminary tests were earlier done with each insecticide to determine the range of concentrations to use.

3.1: Materials: Adult Diopsis thoracica (Plate 1, page 16) were collected from Dahwenya irrigated rice farm (40 km from Legon, in the Accra Plains) by sweeping the foliage of the rice plants with a handnet. The insects were kept in 45 x 45 x 70 cm cages covered with 2 mm wire mesh on all sides except the bottom, which was wooden. They were fed with 30% glucose solution which they sucked from a filter paper soaked in the solution. Three potted rice plants were placed in the cages for the insects to rest on.

Gravid females were selected from the cages, and batches of ten of them were kept with five males in lantern glass globes with plastic mesh covering the tops and held over potted rice seedlings. The insects were fed on organic matter in paddy-field water (their natural food) poured over the soil in the pots. Eggs (Plate 2, page 17)

PLATE I DIOPSIS THORACICA ADULT (2X).

PLATE II *DIOPSIS THORACICA* EGG (X1)

collected from the leaf surfaces of the seedlings were used for ovicidal tests.

The insecticides used in the experiments were:

(a) Pirimiphos methyl or PP511 (50% 2 diethylamino-6-methyl pyrimidin-4-yl dimethyl phopshorothionate), a broad spectrum organophosphorus insecticide of slight systemic action (ICI technical data sheet, 1970).

(b) Gamma BHC (26% gamma 1,2,3,4,5,6 hexachlorocyclohexane), an organochlorine insecticide which is reported to be an extreme stomach poison with persistent contact toxicity and fumigant action (Spencer, 1968).

(c) Sevin (85% 1-Naphthyl-n-methylcarbamate). A carbamate, reported to be a contact insecticide of good residual and systemic properties (Spencer, 1968).

(d) Malathion (50% 0,0-Dimethyl S-1-2-di-(ethoxycarbamyl phosphorodithioate), an organophosphorus contact insecticide (Spencer, 1968).

Pirimiphos methyl was an emulsifiable concentrate, and the other three insecticides were wettable powders.

3.1.2: Method:

(i) Topical application of the four insecticides on the nota of D. thoracica adults

Concentrations of insecticides (Tables 1a,b,c, and d) used in the tests were prepared with 5% olive oil in acetone as solvent.[1]

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1 The solvent used in insecticide bioassays at the Cocoa Research Institute, Tafo, Ghana.

Ten D. thoracica adults were put in a petri dish which contained a piece of cotton wool soaked in 30% glucose solution and a piece of filter paper about ¼ of the area of the dish bottom. The insects fed on the glucose solution and the filter paper absorbed their excrements to keep the dish dry.

The insects were immobilised with ether soaked in a piece of cotton wool and placed in the dish for 1 min. The anaesthesised insects were then arranged in a semi-circle for ease of application of the toxicant. Each insect was examined to see if it was recovering from the anaesthesia before 1 µl of toxicant was deposited from a microsyringe on its notum. Possible recovery of the insect from the anaesthesia was detected by the beating of the wings and/or the halteres.

A treatment in which solvent alone was applied was carried out and was taken as a standard control test. All treatments were carried out in the laboratory at ambient temperatures ranging from 25°C to 29°C, and were replicated five times. Mortality counts were made 24 h after treatment, and insects incapable of movement by that time were considered as dead. Percentage mortality was calculated by expressing the number of dead insects as a percentage of the total number of insects exposed to the insecticide.

3.1.3: Method

(ii) Dipping of D. thoracica eggs into solutions of the four insecticides

Distilled water was used as solvent in preparing the various concentrations of insecticide used in this experiment.

Ten eggs, laid within 24 h were put into a glass vial 1 x 5 cm, with the aid of a horsehair brush. The eggs were covered with a piece of muslin cloth (to prevent them from floating) and enough insecticidal solution was then poured into the vial to cover the muslin cloth. The eggs remained in the solution for 30 min. After this time, the contents of the vial were poured into a dish and the eggs were picked with the horsehair brush onto a piece of filter paper in a petri dish. A standard control test was carried out using distilled water as solution. All treatments were replicated five times.

Records of percentage hatching were taken from the third day after treatment, when the eggs started to hatch, to the seventh day. Percentage mortality was calculated by expressing the number of unhatched eggs as a percentage of the total number of eggs exposed to the insecticide.

3.1.4: Statistical treatment of data

(1) Except where stated otherwise analyses of data were based on methods discussed by Finney (1964),

(2) Percentage mortality values were transformed to probits and plotted against log dosages (i.e. log concentration) to give

regression lines of probits on log dosages or log dosage-probit (ld-p) lines.

(3) Lethal concentrations of toxicants to kill 50% of the test population (LC50) were read from the ld-p lines (i.e. dosage values corresponding to probit value of 5). LC50 is the most important parameter for comparing the potencies of insecticides in a bioassay. With the LC50 values, the relative toxicities of the insecticides, with that of the least potent insecticide as 1, were determined.

(4) Chi square (X^2) values were calculated for the ld-p lines of the insecticides. These values indicated whether the experimental points were within limits of random variation at a particular level of significance; that is, whether the ld-p lines were adequate representations of the data (or experimental points).

(5) The regression coefficient of probits on log dosages (i.e. the slope or "b") of each ld-p line was determined. The slope of the ld-p line measures variability in susceptibility of the test population; a steep line (high b) is preferable to a line with gentle slope (low b) since a change in response to a given change in dosage is greater with the steeper line.

(6) The standard deviation of LC50 for each insecticide was calculated. The ratio, standard deviation of LC50/LC50, which indicates the variation to be expected in the median lethal dosage (Hoskins and Craig, 1962) was calculated for each insecticide.

This ratio does not depend on the unit of dosage of the insecticide used and therefore affords a common basis for comparing the variations in LC50s of the insecticides.

(7) The standard deviations of probits and log dosages about the ld-p line, Sy.x and λ x.y respectively (Hoskins and Craig, 1962), were calculated for each insecticide test. These parameters measure the scattering of experimental points about the ld-p line. They measure the effects of imperfect techniques considered as errors in mortalities and dosages respectively. Sy.x is more appropriate when susceptibility is being studied and λ x.y is more appropriate for evaluating the suitability of methods for screening of insecticides. The ratios Sy.x/LC50 and λ x.y/LC50, which are better measures of variability of results from a bioassay procedure than Sy.x and λ x.y themselves (Hoskins and Craig, 1962), were also calculated. These ratios do not depend on the unit of dosage of the insecticide used and therefore afford a common basis for comparing the insecticide tests.

RESULTS AND DISCUSSION

3.2.1: Ds. theracica adults

(a) Primary data

Percentage mortalities of the test insects increased with increasing dosage concentrations (Tables 1a, b, c, and d). The graph of transformed percentage mortalities (probits) against log dosages (Fig. 1, page 29) shows that gamma BHC had the lowest LC50 value of 26.40 mg/l (or ppm) and was therefore the most potent of the four insecticides against D. thoracica adults. It was followed closely by PP511 with LC50 value of 28.00 mg/l. Sevin had an LC50 value of 67.00 mg/l and Malathion, the least potent of the insecticides had an LC50 value of 380.00 mg/l. The slopes of their respective ld-p lines (in probits/log unit of dosage) are 3.03, 1.82, 2.18 and 2.37.

Table 1

Tolerance of D. thoracica adults to (a) Pirimiphos methyl (b) gamma BHC (c) Sevin and (d) Malathion as determined by topical application of 1 µl drop of toxicant on their nota with 5% olive oil in acetone as solvent

(a) Pirimiphos methyl - D. thoracica adults test.

Dosage in ppm (mg/l)	Total No. of insects used	Number killed	% Mortality	Empirical probits
0	50	0	0	0.000
5	50	6	12	3.825
10	50	10	20	4.158
20	50	18	36	4.641
30	50	28	56	5.151
40	50	30	60	5.253
60	50	36	72	5.582

(b) Gamma BHC - *D. thoracica* adults test

Dosage in ppm (mg/l)	Total No. of insects used	Number killed	% Mortality	Empirical probits
0	50	0	0	0.000
20	50	17	34	4.587
30	50	27	54	5.100
40	50	35	70	5.524
50	50	41	82	5.915
60	50	44	88	6.175
100	50	48	96	6.750

(c) Sevin - *D. thoracica* adults test

Dosage in ppm (mg/l)	Total No. of insects used	Number killed	% Mortality	Empirical probits
0	50	0	0	0.000
20	50	6	12	3.825
50	50	15	30	4.475
100	50	32	64	5.368
150	50	38	76	5.716
200	50	42	84	5.994
250	50	48	96	6.750

(d) Malathion - *D. thoracica* adults test

Dosage in ppm (mg/l)	Total no. of insects used	Number killed	% Mortality	Empirical probits
0	50	0	0	0.000
80	50	3	6	3.445
100	50	7	14	3.919
200	50	17	34	4.587
300	50	19	38	4.694
500	50	28	56	5.151
800	50	38	76	5.706
1000	50	42	84	5.994
1500	50	43	86	6.080

(b) Secondary data

Several parameters (3.1.4, page 20) were derived from the ld-p lines drawn from the transformed primary data (i.e. percentage mortalities and dosage concentrations). These yielded secondary data (Table 2, page 27) which are discussed in detail hereafter.

Table 2

Derived (secondary) data from the ld-p lines of *D. thoracica* adults - insecticide tests

Insecticide	LC50±SD mg/l	x^2 (df)	SD of LC50 / LC50	Sy. x in probits	λ x.y in logs	Sy.x / LC50 in units	λ x.y / LC50 in units
PP511	28.00±2.78	1.44 (4)	0.099	0.115	0.055	0.164	0.127
gamma BHC	26.40±1.66	0.49 (4)	0.063	0.079	0.026	0.121	0.046
Sevin	67.00±5.84	3.99 (4)	0.087	0.282	0.125	0.167	0.289
Malathion	380.00±27.65	7.37 (6)	0.073	0.210	0.088	0.023	0.203

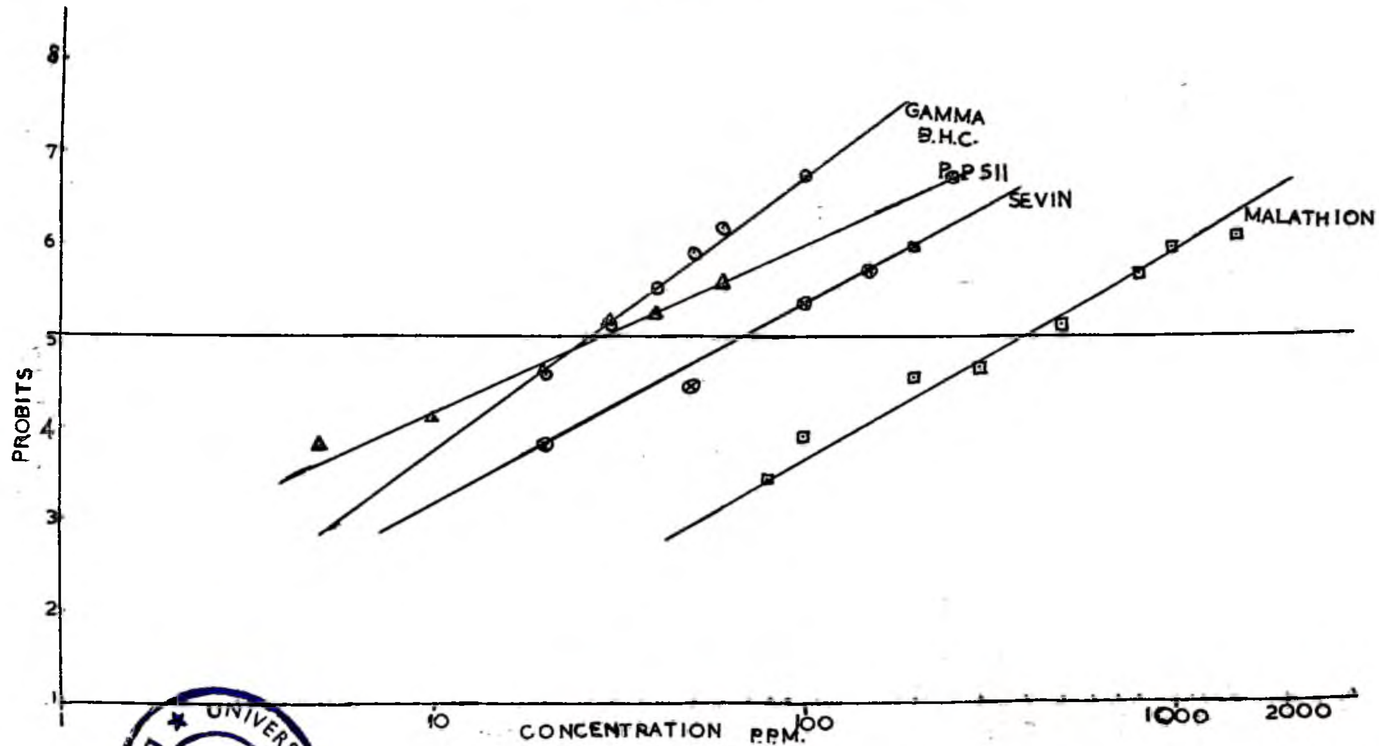
The straight ld-p lines obtained for the insecticides Fig. 1, page 29) suggest that susceptibility of the insects to the insecticides followed the normal distribution. This is an indication that the insect population (from which the samples were taken) was entirely susceptible to the insecticides and did not comprise any appreciable number of insects of hybrid or resistant genotypes; otherwise, the ld-p lines would have had inflections at points on the lines corresponding to the proportion of each genotype in the insect population (Hoskins and Craig, 1962). Effective doses were more or less in constant ratios to the dosages applied and there was no change in the mode of lethal action of each insecticide over the range of dosages applied.

X^2 value for each insecticide's ld-p line shows that its experimental points were within limits of random variation ($P < 0.05$). The ld-p lines of the insecticides therefore adequately represent their experimental points.

The ld-p line of gamma BHC has the steepest slope of 3.03, followed in decreasing order of steepness by those of Malathion, 2.37; Sevin, 2.18; and PP511, 1.82. With a log unit increase in dosage for each insecticide, gamma BHC would kill the insects 1.09, 1.39 and 1.66 times more effectively than the other three insecticides respectively.

FIG I

LOG DOSAGE—PROBIT LINES OF PP511, GAMMA B.H.C, SEVIN AND MALATHION TOPICALLY APPLIED ON D. THORACICA ADULTS



Malathion with an LC50 value of 380.00 mg/l was the least potent insecticide against D. thoracica adults. It was followed in increasing order of potency by Sevin, 67.00 mg/l; PP511, 28.00 mg/l and gamma BHC, 26.40 mg/l. Their relative toxicities, with the LC50 of Malathion as 1.00 were Sevin, 5.65; PP511, 13.57; and gamma BHC, 14.39.

Gamma BHC was the most potent insecticide and its ld-p line was also the steepest of the four ld-p lines. It would be necessary to use a smaller gamma BHC dosage than the other insecticides to kill the same number of insects; and also a log unit increase in its dosage would kill more insects than the other insecticides with the same increase in their dosages. Gamma BHC was therefore the best insecticide against the insect. PP511 followed gamma BHC closely in potency but its ld-p line's slope was the lowest of the four ld-p lines. Relatively greater increase in PP511 dosage would be required to kill the same proportion of the insect as compared to Sevin and Malathion. PP511 was, however, sufficiently more toxic (with lower LC50 value) than Sevin and Malathion that these latter insecticides, although having steeper ld-p lines, would not be more useful.

There are at least three general applications of insecticide bioassays in use in entomology. They are: (a) the use of insects in place of physical or chemical methods to determine the amount of insecticide in animal or plant tissues. This is necessitated by regulations which require food for sale to contain only permissible levels of insecticide, (b) screening of chemicals to determine their insecticidal properties and (c) determination of the levels of susceptibility or resistance in an insect population. This is needed particularly in pest control programmes of public health and agriculture. Also the form of resistance in insects (whether biochemical, genetic or any other) can be found with the aid of insecticide bioassays. From the results of the bioassay tests, it is apparent that the Diopsis thoracica population at Dahwenya (where the insects were collected) was quite susceptible to the insecticides tested on them. The insects can therefore be used as material to measure the insecticidal content of plant or animal tissues and also to screen chemicals for their insecticidal properties. Gamma BHC and PP511, which were the two more potent of the four insecticides tested on the insects, should be preferred for the control of the pest.

Gamma BHC had the minimum variation in median lethal dosage, with standard deviation of LC50/LC50 value of 0.063. It was followed by Malathion with a value of 0.073, Sevin, 0.087 and PP511, 0.099. The same procedure and insects from the same population were used in each test, therefore the SD of LC50/LC50 values should reflect on the accuracy of dosage applications and records of their effects (i.e. mortalities). The SD of LC50/LC50 values for the insecticides are of about equal magnitude; all the tests therefore appear to have been done with about the same level of accuracy.

Sy.x/LC50 and λ x.y/LC50 values measure the effects of imperfect techniques considered as errors in mortalities and dosages respectively. Errors in techniques would come from (a) dosage applications (b) records of mortality (c) stability of the insecticides in the solvent which could affect the correctness of dosage concentrations (d) breakdown of insecticides in the insects which could affect their toxicities and (e) differences in the groups of test insects.

The minimum error in techniques occurred in the Malathion test with Sy.X/LC50 value of 0.023. The gamma BHC test followed with a value of 0.121 indicating that the gamma BHC test had 5.27 times more error as compared to that of Malathion. PP511 and Sevin tests had about the same amount of error with respective Sy.x/LC50 values of 0.164 and 0.167. These tests had 7.13 and 7.27 times more error respectively as compared to that of Malathion.

As regards λx.y/LC50 values, the gamma BHC test with its value of 0.046 appears to have had the least error followed by those of PP511, Malathion and Sevin with respective values of 0.127, 0.203 and 0.289. These tests appear to have had 2.82, 4.51 and 6.42 times more error respectively as compared to that of gamma BHC. Gamma BHC and PP511 tests appear to have had less error than that of Malathion, but with regard to Sy.x/LC50 values the Malathion test appears to have had the least error of the four insecticide tests. Greater value should be placed on Sy.x/LC50 values than λx.y/LC50 values because Sy.x is more appropriate when susceptibility to toxicants is being studied. This was essentially what the bioassay tests measured.

The effects of the various sources of error on the LC50 values could not be ascertained, however Sy.x/LC50 values for all the tests are low and therefore their LC50 values appear to be within acceptable order of accuracy.

3.2.2: D. thoracica eggs

(a) Primary data

The eggs of *Diopsis thoracica* had a natural (or control) mortality of 28%. Percentage mortalities of the treated eggs increased with increasing dosage concentrations (Tables 3a,b,c and d). The graph of the insecticides' ld-p lines (Fig. 2, page 34) shows that PP511 had the lowest LC50 value of 1.30 mg/l, indicating that it was the most potent of the four insecticides against the eggs. Sevin followed, in decreasing order of potency, with LC50 value of 5.30 mg/l, then gamma BHC, 18.30 mg/l, and Malathion, 83.00 mg/l. The slopes (in probits/log unit of dosage) of their respective ld-p lines are 0.73, 1.36, 1.97 and 4.59.

FIG 2 LOG DOSAGE PROBIT LINES OF PP511, GAMMA BHC, SEVIN AND MALATHION EXPOSED TO D. THORACICA EGGS BY DIPPING

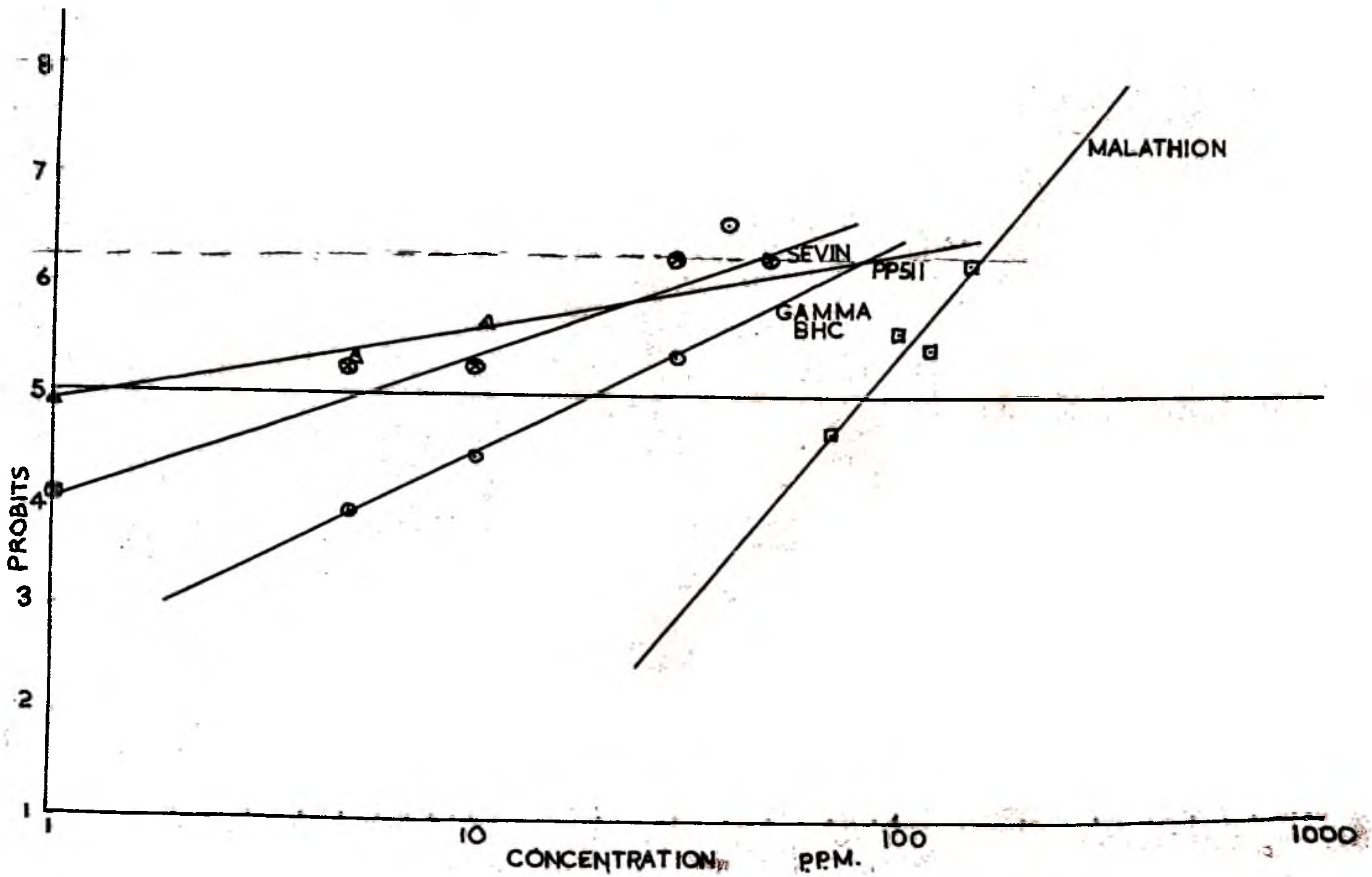


Table 3

Tolerance of *D. thoracica* eggs to (a) PP511 (b) Gamma BHC (c) Sevin and (d) Malathion as determined by dipping the eggs into solutions of the toxicants for 30 minutes with distilled water as solvent

(a) PP511 - *D. thoracica* eggs test

Dosage ppm (mg/l)	No. of eggs used	No. unhatched after 7 days	% Mortality	Corrected* % mortality	Empirical probits
0	50	14	28	-	-
1	50	31	62	47.2	4.930
5	50	37	74	63.9	5.356
10	50	41	82	75.0	5.674

(b) Gamma BHC - *D. thoracica* eggs test

Dosage ppm (mg/l)	No. of eggs used	No. unhatched after 7 days	% Mortality	Corrected* % mortality	Empirical probits
0	50	14	28	-	-
5	50	19	38	13.9	3.915
10	50	24	48	27.8	4.411
30	50	37	74	63.9	5.356
50	50	48	96	94.4	6.589

*Percentage mortality was corrected using Abbotts formula (Finney, 1964):

$$\text{Corrected \% mortality} = \frac{\text{Observed \% mortality} - \text{Control \% mortality}}{100 - \text{Control \% mortality}} \times 100$$

(c) Sevin - D. thoracica eggs test

Dosage ppm (mg/l)	No. of eggs used	No. unhatched after 7 days	% Mortality	Corrected* % mortality	Empirical probits
0	50	14	28	-	-
1	50	20	40	16.7	4.034
5	50	35	70	58.3	5.210
10	50	35	70	58.3	5.210
30	50	46	92	88.9	6.221
50	50	46	92	88.9	6.221

(d) Malathion - D. thoracica eggs test

Dosage ppm (mg/l)	No. of eggs used	No. unhatched after 7 days	% Mortality	Corrected* % mortality	Empirical probits
0	50	14	28	-	-
70	50	27	54	36.1	4.644
100	50	40	80	72.0	5.583
120	50	38	76	66.7	5.432
150	50	46	92	88.9	6.221

*Percentage mortality was corrected using Abbotts formula (Finney, 1964):

$$\text{Corrected \% mortality} = \frac{\text{Observed \% mortality} - \text{Control \% mortality}}{100 - \text{Control \% mortality}} \times 100$$

(b) Secondary results

The same parameters (3.1.4, page 20) derived from the ld-p lines of the adults' tests were derived from the ld-p lines of the eggs' tests. These yielded secondary data which are shown in Table 4, page 38.

The straight ld-p lines obtained from the tests (Fig. 2, page 34) indicate that susceptibility of the eggs to the toxicants followed the normal distribution. The eggs were entirely susceptible to the insecticides and did not include partly or fully resistant types. Lethal action of each insecticide was the same over the range of dosages applied, and effective doses were also more or less in constant ratios to the applied dosages.

X^2 value for each insecticide's ld-p line shows that its experimental points were within limits of random variation ($P < 0.05$). The ld-p lines of the insecticides therefore adequately represent their experimental points.

The ld-p line of Malathion has the steepest slope (in probits/log unit of dosage) of 4.59, followed by those of gamma BHC, Sevin and PP511 with respective slopes of 1.97, 1.36 and 0.73. For a log unit increase in dosage, Malathion should give the greatest increase in egg mortality of the four insecticides. It should kill 2.33, 3.37 and 6.29 times more D. thoracica eggs than the other three insecticides respectively.

Table 4

Derived (secondary) data from the ld-p lines of the insecticides - *D. thoracica* eggs tests

Insecticide	LC50±SD mg/l	x^2 (df)	SD of LC50 / LC50	Sy.x in probits	λ x.y in logs	Sy.x / LC50 in units	λ x.y / LC50 in units
PP511	1.30±1.07	0.19 (1)	0.823	0.073	0.105	2.308	0.121
gamma BHC	18.30±2.94	5.35 (2)	0.161	0.471	0.256	1.005	0.598
Sevin	5.30±1.29	3.52 (3)	0.243	0.218	0.157	1.623	0.363
Malathion	83.00±6.95	4.12 (2)	0.084	0.259	0.063	0.125	0.143

The least potent of the four insecticides against D. thoracica eggs was Malathion with an LC50 value of 83.00 mg/l. It was followed in increasing order of potency by gamma BHC with LC50 value of 18.30 mg/l; Sevin, 5.30 mg/l and PP511, 1.30 mg/l. Their relative toxicities with LC50 of Malathion as 1.00 were: gamma BHC, 4.53; Sevin, 15.66; and PP511, 63.84. Malathion was the least potent insecticide, but its ld-p line had the steepest slope. At concentrations to kill 90% of the test insects (i.e. LC90 values, which correspond to probit value of 6.28; Fig. 2) the following values were estimated for the four insecticides: Malathion, 168.00 mg/l; gamma BHC, 80.00 mg/l; Sevin, 46.00 mg/l and PP511, 78.00 mg/l. Their respective LC50s would have to be increased 2.02 times for Malathion, 4.36 times for gamma BHC, 8.68 times for Sevin and 60.00 times for PP511 to kill 90% of the test insects. Malathion's dosage would require the smallest relative increase of the four insecticides to kill 90% of the test insects; though it would still remain the least potent of the four insecticides against D. thoracica eggs.

Malathion had the minumum variation in median lethal dosage of the four insecticides with SD of LC50/LC50 value of 0.084. It was followed by gamma BHC, Sevin and PP511 with respective SD of LC50/LC50 values of 0.161, 0.243 and 0.823. These values represent 1.92, 2.89 and 9.80 times more variation in their respective LC50 values as compared to that of Malathion. The same procedure and eggs from the same insect population were used in each test. Therefore the SD of LC50/LC50 values would reflect on the accuracy of dosage applications and records of their effects (i.e. mortalities) on the insects. The Malathion test appears to be the most accurately done of the four insecticide tests. Those of gamma BHC and Sevin appear to be of moderate accuracy and that of PP511 appears to be of the least accuracy.

Errors in techniques used in these insecticide bioassays would come mainly from (a) dosage applications, (b) records of egg mortalities, which were probably affected by fungal attack on some of the eggs and (c) stability of the insecticides in the solvent, which could affect the correctness of dosage concentrations.

The effects of imperfect techniques considered as errors in mortality were least in the Malathion test. The test had a Sy.x/LC50 value of 0.125. Gamma BHC, Sevin and PP511 tests followed with respective values of 1.005, 1.623 and 2.308. These tests had 8.04, 12.98 and 18.46 times more error as compared to the Malathion test.

As regards λ x.y/LC50 values which measure the same effects of imperfect techniques as errors in dosages, the PP511 test had the least error with a value of 0.121. Malathion, Sevin and gamma BHC tests followed with respective values of 0.143, 0.363 and 0.598. These tests had 1.18, 3.00 and 4.92 times more error than the

PP511 test respectively. Compared by the order Sy.x/LC50 values in which the PP511 test appeared to have the biggest error in technique, λx.y/LC50 value for the PP511 test was the minimum of all the insecticide tests. It therefore appeared to have the minimum error in technique. The Sevin test also appeared to have smaller error than the gamma BHC test. Dosage concentrations of PP511 were probably the most accurately prepared of all the insecticides and those of Sevin were probably more accurately prepared than those of gamma BHC. Both λ x.y/LC50 and Sy.x/LC50 values which measure errors in techniques in dosages and mortality respectively should be regarded as being of equal importance in measuring errors in the tests. This is because dosage applications and records of mortality appeared to be equally important as sources of error in the tests.

The effects of the various sources of error on the LC50 values could not be ascertained. λ x.y/LC50 values for all the tests appear to be low. Therefore, in terms of dosages, their LC50 values appear to be within an acceptable order of accuracy. The Sy.x/LC50 values for PP511, Sevin and gamma BHC however appeared to be relatively high. Therefore, in terms of egg mortalities, their LC50 values would probably require some modifications.

3.3: Concluding Remarks

(1) The adults and the eggs are the exposed stages in the life cycle of *Diopsis thoracica* (the larvae stay inside rice stalks almost all the time) and can therefore be attacked more directly with insecticidal sprays. Gamma BHC, PP511 and Sevin, in the order, would be recommended for the control of the adults. The same insecticides would be suitable against the eggs too, except that Sevin should be preferred to gamma BHC, because it was the more potent ovicide of the two insecticides. It is not usual to apply insecticides in the field solely for the control of insect eggs; an application of a combination of gamma BHC and PP511 (the two most potent insecticides against the adults and eggs respectively) would probably be most effective in controlling this rice pest.

(2) The field-collected *Diopsis thoracica* adults used in the topical application bioassays were not sexed. They were collected several km from Legon, and in captivity most of them barely survived beyond two weeks. All the adults (which were apparently of the same size) had to be used to obtain enough numbers for all the tests.

(3) No tests were made with the larvae because of their high natural mortality outside the rice plant. Newly hatched larvae hardly survived for 1 h outside the host and all attempts to feed them with artificial food proved futile.

4. THE EFFICACIES OF PIRIMIPHOS METHYL (PP511), GAMMA BHC, SEVIN, BASUDIN (DIAZINON) AND BIDRIN IN CONTROLLING STEM BORER INFESTATION OF RICE IN THE FIELD

Three of the insecticides tested on D. thoracica adults and eggs in the laboratory: PP511, gamma BHC and Sevin, and two other organophosphorus insecticides: Bidrin and Basudin were tested against stem borers in a rice farm at Dahwenya. This was to find out how results obtained in the laboratory would apply under field conditions. Malathion was not tested in the field because it was found from the laboratory tests to be too weak against the insect.

According to Abu (1972), Diopsis species would thrive throughout the year so long as there was rice or other suitable host plants. He found that Diopsid infestation of rice at Dahwenya was highest during the major rainy season (March to July). This field experiment was carried out in this season from April to August, 1973.

4.1: Materials and methods

4.1.1: Materials: A rice variety known as G.20 was used in this experiment. It is a medium-sized grain rice, introduced into Ghana by the Nationalist Chinese Agricultural Mission to Ghana, a few years ago. It matures in about 120 days from sowing and has a moderately high tillering capacity, growing to a maximum height of about 125 cm.

Insecticides used in this experiment were: (a) Pirimiphos methyl or PP511, (b) Gamma BHC, (c) Sevin, (d) Basudin or Diazinon (60% O, O-diethyl O- (-2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate) an organophosphorus compound of high insecticidal and possibly acaricidal properties (Spencer, 1968) and (e) Bidrin (24%

dimethyl-2-dimethyl carbamoyl-1-methylvinyl phosphate) an organophosphorus insecticide of systemic properties (Martin, 1968).

PP511, Basudin and Bidrin were emulsifiable concentrates; gamma BHC and Sevin were wettable powders.

4.1.2: Method: An 0.1 ha field was divided into 30 plots, each 6 x 4 m in size. Adjacent plots were separated by a path 0.75 m wide along their lengths and another path 0.5 m wide along their breadths. Seedlings of the rice variety G.20 were transplanted in the field 20 days after germination at a spacing of 25 x 25 cm, and at four seedlings per hill position. Four weeks after transplanting, the field was fertilized with 250 kg 15-15-15 fertilizer/ha. Three weeks later the whole field was weeded. A second fertilizer application at the same rate was made 8 weeks after transplanting. The experimental design was a completely randomized block which was replicated five times. Each replication consisted of six plots: five treated and one control.

The insecticides were applied with a knapsack sprayer and three spray applications were made: the first one was at 5 weeks after transplanting and subsequently at 3-week intervals. Each spray application was made at a concentration of 0.04% active ingredient (a.i.). This concentration was in keeping with those used in similar tests on rice stem borers at IRRI (IRRI, 1963, 1964 and 1965). To ensure a proper spray coverage, the plants were sprayed to runoff and the insecticidal solutions were sprayed at a rate of 300 gallons (or 1365 litres) per hectare.

Each of the 30 plots was divided into two subplots in the ratio 2:1, and samplings were made in the larger subplots to assess the intensity of stem borer infestation. This was done by counting tillers which had the "dead heart" or "white head" condition. Dead heart (or stem rot) formation is caused by the borer larva feeding in the central shoot of the plant, resulting in its death and drying up. White head formation is caused by the borer larva feeding in the rice stalk during the heading period. This results in the production of discoloured panicles with empty or partially filled grains, often with secondary fungal attack (Plate 3, page 46). Plants in the smaller subplots were harvested at maturity for yield determination.

Two samplings for dead heart formation were made. The first one was made 1 week after the first spray application and the second one was also made 1 week after the second spray application. Two weeks after the third spray application (10 days before harvest) a third sampling was made for tillers with white heads. At each sampling, ten hills (about 5% of the total plant population in the larger subplot) were taken. All the hills were selected by systematic sampling.

Plants in the smaller subplots were harvested 15 weeks after transplanting, when the crop appeared to be mature. Plants constituting the border row were not harvested. The paddy was threshed, winnowed and dried for 3 days in the sun, and for 12 hours in the oven at 55°C to 12 - 14% moisture content, before weighing.

PLATE III RICE PLANTS SHOWING (a) WHITE HEAD AND (b) DEAD HEART CONDITIONS

4.1: Results

Table 5, page 48, shows the mean number of tillers per hill, mean percentage dead hearts and white heads, mean number of productive panicles per hill and yield of paddy recorded for each treatment at various times after transplanting.

4.2: Discussion

The main rice stem borers encountered during the experiment were *Diopsis thoracica* Westw. and *Diopsis tenuipes* Westw. *Diopsis thoracica* was by far the most prevalent, and has been recorded to cause the greatest damage to swamp rice in the Accra Plains (Abu, 1972).

Various insecticides have been reported to be effective to varying degrees in reducing rice stem borer infestation in Asia (Pathak, 1964; Rao and Israel, 1964), and in parts of West Africa such as Sierra Leone (Morgan, 1973). Although insecticides have been used on a small scale to control rice stem borers in the Accra Plains (Kwai and Engman, 1973; Johnson, 1973), no systematic studies had been made on their relative effectiveness in controlling the borers in this area.

The effect of any foliar spray of insecticide against Diopsid stem borers would be: (a) to kill the adults and pupae which happen to be present at the time of application, (b) to kill exposed larvae which

Table 5

The effects of 0.4% a.i. PP511, gamma BHC, Sevin, Basudin and Bidrin sprayed at 300 gal (1,265 l)/ha at 5, 8 and 11 weeks after transplanting, on stem-borer infestation of rice variety G.20 at Dahwenya, April - August, 1973

Treatment	6 weeks after transplanting		9 weeks after transplanting		13 weeks after transplanting		Mean yield at maturity kg/ha
	Mean no. of tillers per hill	Mean % dead heart/hill	Mean no. of tillers per hill	Mean % dead heart/hill	Mean no. of productive panicles/ hill	Mean % white Head/hill	
PP511	62.1a	9.9 b	42.6a	5.3 b	29.2a	0.3 a	4431 a
Gamma BHC	60.0a	9.4 b	44.3a	3.6 c	30.3a	0.1 a	4686 a
Sevin	58.1a b	10.2 b	43.2a	5.6 b	29.8a	0.3 a	4374 a
Basudin	58.0a b	7.8 b	43.1a	5.0 b	31.0a	0.8 a	4224 a
Bidrin	54.7 b	8.5 b	44.9a	3.0 c	29.5a	0.3 a	4804 a
Control	60.3a	14.8 a	45.4a	9.5 a	28.7a	0.5 a	4573 a

Treatment means bearing the same letter are not significantly different at 5%. (Duncan's Multiple range test.)

may be newly hatched or older larvae moving from one stalk to another and (c) to kill eggs, with which the insecticides come in contact. The residual effect of the insecticide would serve to prevent oviposition and kill any larva or adult which may later come in contact with the insecticide. After spraying in the field insecticidal solutions collected between the sheaths and the inner stalks of the rice plants. These could seep down to reach larvae at the base of the stalks and kill them.

Observations of dead heart formation made one week after the first and second spray applications (i.e. at 6 and 9 weeks after transplanting respectively), showed that stem borer infestation was significantly more prevalent in the control plots than in the treated ones. At the first observation plots treated with Bidrin had significantly fewer tillers per hill than the control. However differences in percentage dead heart counts in all the treated plots were not statistically significant. All the insecticides appeared at that time to be equally effective in reducing the borer infestation. At the second observation differences in number of tillers per hill in all the experimental plots were not statistically significant. Percentage dead heart counts showed that gamma BHC and Bidrin treatments were significantly better in reducing stem borer infestation than Basudin, PP511 and Sevin treatments.

Bidrin is a systemic organophosphorus insecticide and was probably translocated to the inner plant tissues where it exerted its toxic effect on the larvae lodged inside the rice stalks.

Gamma BHC, being an organochlorine insecticide, would have relatively long residual effect, and would therefore provide control for a longer time than the other (non-organochlorine) insecticides. These properties probably accounted for the greater effectiveness of Bidrin and gamma BHC treatments over the other treatments.

At the 13th week after transplanting, differences in number of productive panicles per hill in all the experimental plots were not statistically significant. White head formation at that time was low in every plot and no insecticide treatment significantly reduced its occurence over the control. The plants were probably becoming so old by that time that the Diopsid borers no longer preferred them for oviposition. Abu (1972) found that D. thoracica larval infestation decreased with the age of the rice plant and that there were significant increases in yield between plants which were artifically infested when they were 21, 60 and 90 days old.

Yields obtained from the insecticide-treated plots were not significantly better than the control. The results mean that the insecticide sprays did not have any significant effect on the yield of the crop.

Pathak (1964) found that the rice plant was most susceptible to borer damage from the 7th to the 11th weeks after transplanting, and if the crop was not protected beyond the 7th week, yields declined sharply. Insecticidal applications in this experiment were made at the 5th, 8th and 11th weeks after transplanting. This covered the period when the crop was supposed to be most susceptible to borer damage. Observations of dead heart formation (Table 5) showed that the insecticidal sprays significantly reduced the borer infestation over the control during the pre-heading phase of the crop and infestation during the heading phase was generally low in all the plots. Nevertheless, yields obtained from the treated plots were not significantly better than that from the control. Probably, borer-induced compensatory tillering of the rice plants during the pre-heading phase, effectively offset the effect of borer infestation on yield. This phenomenon might have led Van Halteren (1970) to entertain doubts on the need for chemical control of D. thoracica on rice.

4.3: Summary

Five insecticides, Pirimiphos methyl or PP511, gamma BHC, Sevin, Basudin or Diazinon, and Bidrin were evaluated for their effectiveness against rice stem borer infestation in the Accra Plains. This was done by three spray applications of 0.04% active ingredient solutions of the chemicals in a rice farm at Dahwenya at the 5th, 8th and 11th weeks after transplanting.

Observations at the 6th and 9th weeks after transplanting showed that stem borer infestation was significantly reduced by these chemicals over the control in the pre-heading phase of the crop. At the first observation all the insecticides appeared to be equally effective in reducing the borer infestation. At the second

observation gamma BHC and Bidrin proved to be significantly more effective than the other three insecticides in reducing the borer infestation.

Stem borer infestation was low in all the plots during the heading phase of the crop. The plants were probably becoming too old for infestation by the predominant rice stem borer (D. thoracica) in the rice farm.

Yields obtained from the treated plots indicated that the insecticide sprays had no significant effect over the control. Borer-induced compensatory tillering in the crop might have effedtively offset the effects of borer infestation on yield.

5. RATE OF PENETRATION OF ^{14}C-PIRIMIPHOS METHYL INTO THE ABDOMINAL CUTICLE OF DIOPSIS THORACICA ADULT FEMALES.

^{14}C-Pirimiphos methyl, obtained from Imperial Chemical Industries Company of the United Kingdom, was used to measure rate of penetration of the chemical into the cuticle of *D. thoracica* adults. This determination appeared to be a useful initial step in a general study of the toxicology of the insecticide on this insect.

The method of measuring penetration of the insecticide was based on counting of radioactivity in the surface "washing" and in the "brei" at various times after topical application of the labelled insecticide on the insect's cuticle. This method has been used by several workers to measure the rate of penetration of other insecticides into different insects (Sternburg *et al*.. 1950; Armstrong *et al*., 1951; Lindquist *et al*.. 1951; and Hanna and Atalla, 1971).

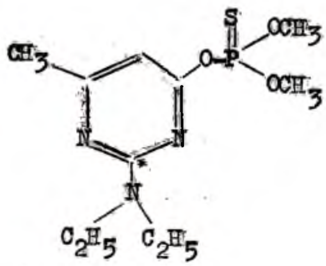
To ensure the maximum accuracy in this experiment, the materials had to be standardised as much as possible; also a relatively small number of insects were needed, therefore only *D. thoracica* adult females were used. A preliminary test was carried out to determine reasonable time intervals for adoption during the experiment.

5.1: Materials and method

5.1.1: Materials:

Diopsis thoracica species were collected from Dahwenya rice farm; and the females used in this experiment were selected by binocular examination of their external genitalia.

The labelled Pirimiphos methyl[1] (^{14}C-PP511; see formula below) had a specific activity of 4.4 mCi/mm, and the sample, which was supplied in cyclohexane solvent, had an activity of 20 µCi. This was diluted to 2 ml to give an activity of 0.01 µCi/µl.



The position of the radioactive carbon in the PP511 molecule is shown by the asterisk.

5.1.2: Method:

The insects were kept in groups of ten in petri dishes and fed with 30% glucose solution soaked in a piece of cotton wool, for at least 30 min.

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1 Source: Mr. D.M. Bowker, ICI, Plant Protection Ltd. Jealott's Hill Research Station, Bracknell, Birkshire, United Kingdom.

They were not immobilised with any chemical but were held in the fingers, in which position they swung their abdomen forward to project two spines on their scutella. This exposed the dorsal part of the abdomen where 1 µl of the labelled insecticidal solution was topically applied, with a microsyringe. Ten insects were treated for each of the following time intervals: 5, 15, 45, 60 and 90 min. Soon after each time interval, 2 ml cyclohexane were discharged over the dorsal abdomen to wash off unpenetrated insecticide into a glass bottle. The washings for each treatment were bulked. Each 'washed' insect was then put into a test tube containing 1 g clean sand, free of organic matter; 2 ml cyclohexane were added and the insect was ground thoroughly with a glass rod to extract the absorbed insecticide. One ml cyclohexane was discharged over the lower end of the glass rod to wash off insecticide on the rod into the test tube. Each test tube was then corked and centrifuged for 15 min at 1,500 rpm. 2 ml of the supernatent brei (out of a total of 3 ml brei) were drawn from each test tube and bulked in a glass bottle, for each treatment.

Countings of activities in the "wash" and the "brei" for the treatments were done at the Ghana Atomic Energy Commission laboratory at Kwabenya. A toluene based scintillant was prepared by adding 2.5 g 0.5% 2,5-diphenyloxazole (PPO) and 0.5 g 0-10% 1,4-bis-(2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP) to 500 ml toluene.

Ten ml scintillant were discharged into a counting vial and 1 ml sample (wash or brei) was added and gently shaken to mix the contents. Counting of radioactivity in each vial was done with a Packard Tri-Carb Liquid Scintillation Spectrometer, Series 314E. To determine the counting efficiency of the spectrometer, 1 µl radioactive insecticidal solution was discharged into a counting vial; 1 ml cyclohexane and 10 ml scintillant were added and the vial was gently shaken to mix the contents before its activity was counted. The mean of three such determinations was used to calculate the efficiency of counting on the spectrometer, using the following formula:

$$\frac{\text{mean counts per min (cpm) of 1 µCi of } ^{14}\text{C-PP511}}{\text{dpm of 1 µCi of pure radium } (= 2.22 \times 10^6)} \times 100$$

(Appendix 1).

Calculations made in this experiment were based on those discussed by Sun (1968).

(a) Percentage penetration of the toxicant was calculated from the difference between applied dose (µg/insect) and the amount of insecticide in the surface washing:

$$\frac{\text{"the difference"}}{\text{amount of toxicant applied}} \times 100$$

(b) Percentage penetration was plotted against time on an arithmetic scale, and a smooth curve was drawn through the points to the origin Fig. 3, page 58).

(c) On straight portions of the curve, the rate of penetration was calculated from the observed percentage penetration, thus:

$$\frac{P_2 - P_1}{t_2 - t_1} \text{ \% min.}$$

P_2 is the percentage penetration at time t_2

P_1 is the percentage penetration at time t_1

(d) A tangent ab (Fig.3, page 58) was drawn at the point where the percentage penetration curve begins to bend. The rate of penetration at this point (i.e. the slope of the tangent ab) was calculated as in (c).

(e) The rate of penetration at each point against its corresponding time was plotted, and a smooth curve was drawn through all the points (Fig. 4, page 59).

(f) Sun (1968) stated that the percentage of toxicant metabolised or detoxified was the difference between the percentage of material which penetrated, and the percentage of the original toxicant present in the treated insect.

The type of chemical represented by the activity recovered in the brei was unknown. The breakdown products of the insecticide in the insect and their relative toxicities were also unknown, and therefore it was not possible to comment on the percentage or rate of metabolism or detoxification of the insecticide in the insect.

FIG. 3 PERCENTAGE PENETRATION OF TOPICALLY APPLIED ^{14}C–PP511 ON *D. THORACICA* ADULT FEMALES

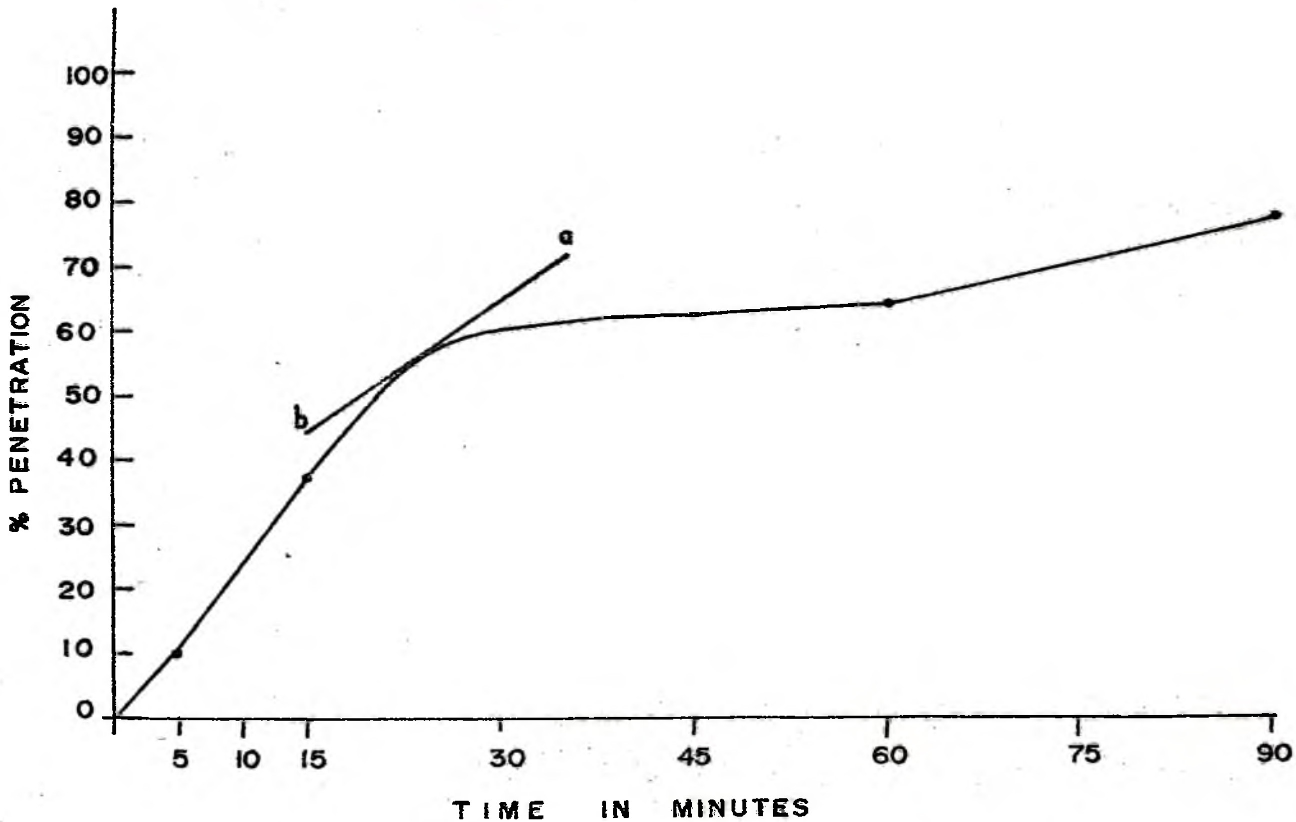
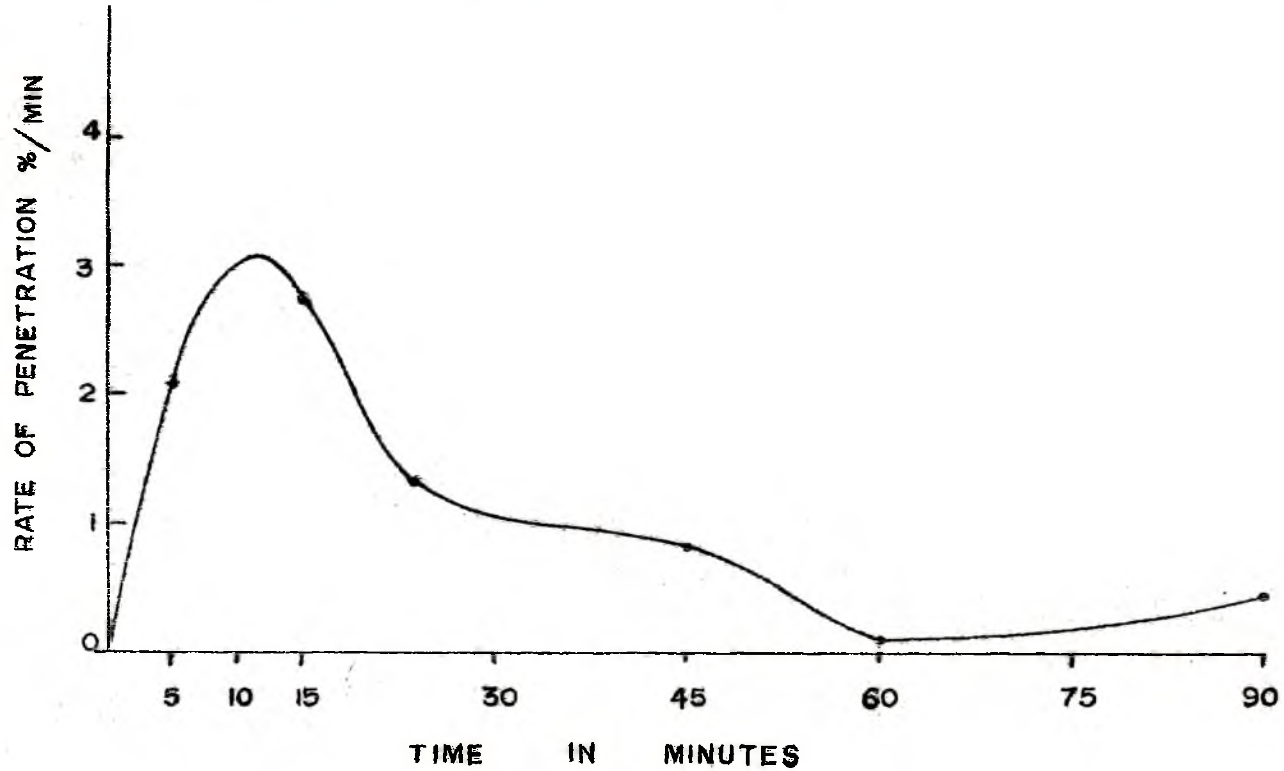


FIG. 4 RATE OF PENETRATION OF TOPICALLY APPLIED ^{14}C–PP511 ON D. THORACICA ADULT FEMALES



5.2: Results and Discussion

Table 6 Percentage and rate of penetration of 0.01µCi ^{14}C-PP511 topically applied on the dorsal abdomen of *D. thoracica* adult females.

Time of exposure (min)	Activity in wash (µCi x 10^{-4})	Activity in brei (µCi x 10^{-4})	% Penetration	Rate of Penetration (%/min)
5	89.658	6.906	10.38	2.08
15	62.144	12.282	37.81	2.74
45	37.062	30.675	62.91	0.84
60	35.468	31.866	64.50	0.11
90	21.810	27.339	78.21	0.46

The amount of radioactivity outside the insect's cuticle (i.e. in the wash) decreased with time of exposure, whilst the amount inside the insect (i.e. in the brei) generally increased with time of exposure. The labelled insecticide therefore penetrated the insect's cuticle.

Percentage penetration of the labelled insecticide generally increased with time of exposure of the insect to the insecticide. The biggest increase in percentage penetration of 27.42% occured between the 5th and 15th minutes after application. It was during this period that the maximum or peak rate of penetration also occured (Fig. 4, page 59).

The maximum rate of penetration of the labelled insecticide was found to be 3.1%/min (186%/h), and it occurred 11.5 min after application, when 28% of the applied dose had penetrated the insects cuticle. The rate of penetration of the insecticide appeared to be quite fast, however it might have changed if a different dosage had been applied. This is because the rates of penetration of some insecticides have been found (DDT on houseflies by Sternburg et al., 1950 ; Malathion on American cockroaches by Matsumara, 1963) to increase with larger dosage applications. The quick penetration of PP511 into D. thoracica implies that it will reach the site of toxic action quickly and subsequently cause the early death of the insect.

In the course of the experiment, no insect died up to 60 min after application. One insect appeared to be dead by 75 min, and this number rose to 5 by 90 min. Toxic effect of the insecticide therefore began to be evident around 60 min after application and it appeared to be acute thereafter. Whether the death of some insects before 90 min affected the rate of penetration by that time was not ascertained. Hoffman et al (1950), have however found that absorption of DDT proceeded at about the same rate in dead as in living houseflies. It could be presumed that the death of the insects did not appreciably affect the rate of penetration of the insecticide within 90 min.

With the determination of the rate of penetration of PP511 into *D. thoracica* adults, other investigations can be made on other aspects of the toxicology of this insecticide on this insect. Experiments can be carried out to find out: (a) whether different formulations of the insecticide will depend on their rates of penetration for effectiveness against this insect, (b) what solvents and conditions will favour the penetration of the insecticide into the insect, (c) whether alterations in toxicity of the insecticide can be attributed to changes in its rate of penetration into the insect, and (d) the metabolic products of this insecticide in the insect, their rates of production and how they affect the toxicity of the insecticide also can be investigated.

5.3: Summary

The rate of penetration of ^{14}C-Pirimiphos methyl into the abdominal cuticle of *Diopsis thoracica* adult females was determined. This was carried out by topical application of 0.01 µCi (0.693 µg) of the insecticide in cyclohexane solvent, on the insect. The maximum rate of penetration (3.1%/min or 186%/h) occurred 11.5 min after application when 28% of the applied dose had penetrated the insect's cuticle. Toxic effect of the insecticide began to be evident around 60 min after application and it appeared to be acute thereafter.

6. INTEGRATING DISCUSSION AND CONCLUSIONS

The objectives of the experiments were to find answers to the possibility of insecticidal control of the rice stem borer *Diopsis thoracica* Westw., which has been reported to cause the greatest damage to swamp rice in the Accra Plains (Abu, 1972).

Insecticides have been used on a small scale to control Diopsid stem borers in this area (Kwai and Engman, 1973; Johnson, 1973), but no systematic studies had been made on their effectiveness in controlling the stem borers. New insecticides are continuously being developed and it is desirable to evaluate their potentialities as chemical control agents. In any such control or evaluation exercise, it will be necessary first of all to determine the levels of tolerance of the pest to different insecticides and then to select the more suitable ones. Insecticides to be used against Diopsid stem borers should either be powerful contact poisons with long residual effects, which will kill the adults, pupae, eggs and exposed larvae, as well as prevent further oviposition; or they should be systemic insecticides which will penetrate the plant tissues and kill larvae which are lodged inside the stalks, where they cause the actual damage.

Sevin appeared to be moderately weak against the insect in the laboratory, and was the least effective of all the insecticides in reducing the stem borer infestation in the field. PP511 appeared to be very toxic against the insect in the laboratory but was only slightly more effective than Sevin in reducing the borer infestation in the field. This shows an inconsistency in the laboratory and field performances of this insecticide. PP511 is reported to have limited persistence or residual effect (ICI technical data sheet, 1970) and therefore it would provide protection against the borers for only short periods after each spray application and thus would allow reinfestation between the applications. This may be the reason for its relative ineffectiveness in the field. Basudin (or Diazinon) was very effective in reducing borer infestation after the first spray application; but was only moderately effective after the second spray application. Being an organophosphorus insecticide like PP511, Basudin would have a relatively short residual effect and so it probably provided protection against the borers for only short periods after each spray application. This may be the reason for the apparent reduction in its effectiveness.

Gamma BHC was very toxic against D. thoracica adults and moderately toxic against the eggs in the laboratory. In the field experiment it effectively reduced stem borer infestation. This insecticide is reported to be powerful and has persistent contact toxicity (Spencer, 1968). It undoubtedly provided protection against the borers for a longer time than the other non-organochlorine insecticides. Its field performance was in line with expectation. Bidrin was very effective in reducing stem borer infestation in the field at the two pre-heading observations. Being a systemic insecticide, it probably penetrated the plant tissues to kill the larvae lodged inside the stalks and so prevented them from causing further damage.

It will be most desirable to determine the efficacies of other systemic insecticides in reducing Diopsid rice stem borer damage. From the foregoing account it is apparent that gamma BHC and Bidrin are worthy of recommendation for the control of Diopsid stem borers in the field.

In spite of the apparent effectiveness of some of the insecticides in reducing rice stem borer infestation, no insecticide treatment gave significantly higher yield than the control. Stem borer infestation induces compensatory tillering (Pathak, 1964; Van Halteren, 1970; and Abu, 1972); this phenomenon appears to have offset the effect of stem borer infestation on yield and led Van Halteren (1970) to entertain doubts on the need for chemical control of _Diopsis thoracica_ on rice in the Accra Plains.

PP511 was found to penetrate the insect's cuticle fast; it would therefore reach its site of action quickly to exert its toxic effect. It also appeared to be highly toxic against the insect in the laboratory test on the insect; therefore its penetration and toxicity were not limiting factors in its effect on the insect. The insecticide may be more effective in the field if it is used with a sticker to retain it for longer periods on the leaf surfaces.

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APPENDICES

Appendix 1

Efficiency of counting of radioactivity on the Packard Tri-Carb Liquid Scintillation Spectrometer.

Mean activity of 1 µl ^{14}C-PP511 = 14,770 cpm.

1 µl of the labelled insecticide (20 µCi in 2 ml cyclohexane solvent) had an activity of 0.01µCi

Therefore 0.01 µCi of ^{14}C-PP511 had the activity of 14,770 cpm.

1 µCi of ^{14}C-PP511 would have an activity of 1,477,000 cpm

1 µCi of pure radium has an activity of 2.22×10^6 dpm

Therefore efficiency of counting on the spectrometer

$$= \frac{1,477,000}{2,220,000} \times 100$$

$$= 66.53\%$$

Appendix II

Analysis of Variance table for number of tillers per hill at 6 weeks after transplanting rice variety G.20.

Source of Var.	df	SS	MS	Obs. F	Req. F 5%	Req. F 1%
Total for treatment	29	638.8				
Blocks	4	144.5	36.1			
Treatments	5	257.0	51.4	4.3**	2.71	4.10
Error	20	237.3	11.9			

** Significant at 1% level

Appendix III

Analysis of variance table for percentage dead heart formation at 6 weeks after transplanting rice variety G.20.

Source of Var.	df	SS	MS	Obs. F.	Req. F. 5%	Req. F. 1%
Total for treatment	29	376.72				
Blocks	4	106.87	26.72			
Treatments	5	154.15	30.83	5.33**	2.71	4.10
Error	20	115.70	5.78			

** Significant at 1% level.

Appendix IV

Analysis of variance table for number of tillers per hill at 9 weeks after transplanting rice variety G.20

Source of Var.	df	SS	MS	Obs. F.	Req. F. 5%	1%
Total for treatment	29	745.9				
Blocks	4	183.3	45.8			
Treatments	5	31.6	6.3	0.24	2.71	4.10
Error	20	531.0	26.6			

The results of number of tillers per hill at 9 weeks after transplanting are not significantly different at 5% level.

Appendix V

Analysis of variance table for percentage dead heart formation at 9 weeks after transplanting rice variety G.20.

Source of Var.	df	SS	MS	Obs. F.	Req. F. 5%	Req. F. 1%
Total for treatment	29	171.41				
Blocks	4	31.19	7.80			
Treatment	5	120.40	24.08	24.32**	2.71	4.10
Error	20	19.82	0.99			

** Significant at 1% level

Appendix VI

Analysis of variance table for number productive panicles per hill at 13 weeks after transplanting rice variety G.20.

Source of Var.	df	SS	MS	Obs. F.	Req. F. 5%	Req. F. 1%
Total for treatment	29	213.6				
Block	4	58.9	14.7			
Treatment	5	16.1	3.2	0.5	2.71	4.10
Error	20	138.6	6.9			

The results of number of productive panicles per hill at 13 weeks after transplanting are not significantly different at 5% level.

Appendix VII

Analysis of variance table for percentage white head formation at 13 weeks after transplanting of rice variety G.20.

Source of Var.	df	SS	MS	Obs. F.	Req. F. 5%	Req. F. 10%
Total for treatment	29	15.51				
Blocks	4	3.52	0.88			
Treatment	5	1.43	0.29	0.55	2.71	4.10
Error	20	10.56	0.53			

The results of percent white head formation at 13 weeks after transplanting of rice variety G.20 are not significantly different at 5% level.

Appendix VIII

Analysis of variance table for paddy yield in kg/ha.

Source of Var.	df	SS	MS	Obs.F.	Req. F. 5%	1%
Total for treatment	29	5,084,607.42				
Blocks	4	1,728,201.78	432,050.44			
Treatment	5	1,138,481.60	227,696.32	2.05	2.71	4.10
Error	20	2,217,924.04	110,896.20			

The results of paddy yield per hectare are not significantly different at 5% level.