SUSCEPTIBILITY OF LARVAL STAGES OF *MAMESTRA BRASSICAE* L. (LEPIDOPTERA: NOCTUIDAE) TO INSECTICIDES

by

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To my parents

in affection and gratitude

ABSTRACT

The susceptibility of various larval stages of *Mamestra brassicae* (L.) to DDT and malathion has been investigated using topical, injection, feeding and contact methods of application. The relative toxicity (LD_{50}/mg body wt.) of malathion was fairly constant between larval stages with the topical and injection methods of application. However, when malathion was applied in the diet the VI instar was less susceptible than the V. With DDT applied topically, the II, III and V instars had similar LD_{50} values; greater values were obtained for the IV and VI instars. The LD_{50} values for the contact application method indicated that the VI instar was the least susceptible to DDT and malathion, while the II instar was the most susceptible to DDT. The III instar was the most susceptible stage to malathion.

The relationship between the mode of administration of the insecticides and their relative toxicities is discussed. The differences between the susceptibility of the V and VI instar larvae to malathion applied in the food was investigated further.

Comparison was made of the metabolism and degradation of malathion-^{14}C in extracts of the guts of V and VI instar larvae. It was found that the rate of hydrolysis of malathion to non-toxic metabolites was significantly greater in the VI than in the V instar. A large proportion of the hydrolytic activity in the gut extracts was shown to be due to carboxylesterase activity. The rate of oxidation of malathion to its more toxic metabolite malaoxon was found to be three orders of magnitude slower than the rate of hydrolysis in both V and VI instars. Non-enzymatic hydrolysis of malathion-^{14}C under the alkaline conditions in the gut of the larvae was not considered to be an important factor in the toxicity of the pesticide.

The effect of DDT and malathion on the rate of feeding of the IV, V and VI instar larvae was studied using two methods. In a gravimetric method, the amount of untreated cabbage leaf-disc consumed was greatest in the VI instar and least in the IV. The presence of DDT and malathion reduced the amount of leaf consumed particularly for the IV instar larva. Similar results were obtained with a "leaf-sandwich" method although the gravimetric method was considered to be the more accurate. The effects of the rate of feeding on insecticide toxicity are discussed.

The feeding behaviour of the I, II and III instar larvae was also examined. DDT and malathion were not found to have a repellent effect although they did result in a reduction in the amount of leaf-disc consumed.

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INTRODUCTION

Insect pests continue to cause some of the greatest losses to the agricultural industry. Of an estimated 35% loss in crop yield caused by all pests, over the last two decades worldwide, insect pests accounted for approximately 14%, with the greatest losses occurring in the less developed countries (Hayes, 1975).

It is not surprising therefore that despite an increasing awareness of the disadvantages of insecticides (Bennett *et al.*, 1972; Edwards, 1973; O'Donnell, *et al.*, 1975; Southwood, 1979), their use in the developed world continues to increase substantially (Roberts, 1975; Graham-Bryce, 1975; Green *et al.*, 1977). In the rest of the world, the use of insecticides has increased at an even faster rate as more and more countries attempt to develop their economies (Ware, 1978). Even with the increasing usage of alternative cultural and biological methods of control, pesticides are likely to remain in the forefront of the battle against insects for the foreseeable future (George, 1975). It is imperative therefore that the highest priority should be given to research and development for the more efficient use of insecticides in particular in integrated control programs (Way, 1977).

Among recent trends have been the development of selective insecticides (Corbett, 1974; George, 1975) and the more selective application of existing insecticides to the most vulnerable stage(s) in the life cycle of the target insect species. This latter approach seems worthwhile since within the same developmental stage, considerable differences in sensitivity to insecticides can be encountered (Busvine, 1971). Furthermore, it has been shown that even within the same larval stage variation in weight can affect the susceptibility to insecticides (Way, 1954). By far the

commonest trend reported in the literature is that an increase in weight or larval instar number is associated with a decrease in susceptibility to insecticides (Table 1).

The only recorded example in which increasing weight gave almost a constant response to insecticides is found in the work of Gast (1959). He observed that when the organochlorine endrin was applied topically to the larvae of the lepidopteran *Protoparce sexta* (Johan) of weight ranges 2000-2900, 4000-4900 and 6000-6900 mg no appreciable changes occurred in their susceptibility (LD_{50} values: 1.4, 1.8 and 1.9 µg mg^{-1} body wt. respectively).

An example of an insect species where an increase in weight or instar number resulted in increased susceptibility to insecticides is *Choristoneura fumiferana* (Lepidoptera) where the introduction of the novel insecticide Dimilin in the diet of the II to VI instar larvae; resulted in EC_{50} value of 91.0 ppm for the II instar compared to 8.3 ppm and 3.7 ppm for the V and VI instars respectively (Granett *et al.*, 1977).

The above data show that the relationship between the developmental stage and/or weight of an insect and its susceptibility to an insecticide can be complex. The present work has been devoted to such an investigation in *Mamestra brassicae* L. (Lepidoptera: Noctuidae). The Lepidoptera and in particular the noctuids include many of the most important insect pests in world agriculture (Stapley *et al.*, 1969), and *M. brassicae* is a well-known polyphagous pest in much of Western Europe (Montagne, 1977), parts of U.S.S.R., Japan and Asia (Bonnemaison, 1965), although in the United Kingdom it is a minor pest (Montagne, 1977).

Although *M. brassicae* is associated with numerous crops including

TABLE 1 : Examples of decreasing susceptibility to insecticides associated with increasing weight, or instar number of larva or nymphs of various insect species.

Insect species	Instar or weight ratio*	Insecticide	LD_{50} ratios	Mode of application	Reference
Lacanobea oleracea (L.) (Lepidoptera)	I (1)	DDT	1	Spray	Mukerjea, 1953
	II (15)	"	2	"	
	III (60)	"	3	"	
	IV (580)	"	8	"	
	V (2550)	"	50	"	
Tenebrio molitor (L.) (Coleoptera)	1	"	1	"	"
	2	"	2	"	
	5	"	3	"	
	9	"	9	"	
	12	"	12	"	
	24	"	24	"	
Periplaneta americana (L.) (Orthoptera)	1	"	1	"	"
	2	"	2	"	
	7	"	3	"	
	17	"	5	"	
	29	"	7	"	
	48	"	8	"	

TABLE 1 : Continued..

Insect species	Instar or weight ratio*	Insecticide	LD_{50} ratios	Mode of application	Reference
L. oleracea (Lepidoptera)	1	DDT	1	Stomach poison	Way, 1954
	1.3	"	2	"	
	1.8	"	4	"	
Heliothis virescens (F.) (Lepidoptera)	1	"	1	Topical	Gast, 1959
	6	"	2	"	
	12	"	3	"	
L. oleracea (Lepidoptera)	I (1)	Pyrethrins	1	Spray	Mukerjea, 1953
	II (5)	"	3	"	
	III (71)	"	14	"	
	IV (558)	"	19	"	
	V (2571)	"	35		
T. molitor (Coleoptera)	1	"	1	"	"
	3	"	3	"	
	6	"	5	"	
	8	"	14	"	
	12	"	28	"	
	25	"	40	"	

TABLE 1 : Continued.

Insect species	Instar or weight ratio*	Insecticide	LD_{50} ratios	Mode of application	Reference
P. americana	1	Pyrethrins	1	Spray	Mukerjea, 1953
	2	"	2	"	
	7	"	5	"	
	16	"	5	"	
	32	"	7	"	
	53	"	10	"	
H. virescens	1	Phosdrin	1	Topical	Gast, 1959
	6	"	2	"	
Sitophilus granarius (L.) (Coleoptera)	I	Methyl bromide	1	Fumigant	Howe & Hole, 1966
	IV	"	1.4	"	
Trichoplusia ni (Hubner) (Lepidoptera)	III	Chlordimeform	1	Topical	Crecelius *et al.*, 1976
	V	"	2	"	
T. ni	III	Demethyl-Chlordimeform	1	"	"
	V		1.3	"	

* Where appropriate instar number is given in Roman numerals, the weight ratio in Arabic numerals.

carnations and chrysanthemums under glass (Miles *et al.*, 1948), peas, sugar-beet, sunflower, maize, tobacco, peppers and lucerne (Dochkova, 1971; Hadziskevic, 1973; Oku, *et al.*, 1973; Straka, 1974; Chekemenev, 1975; Verigin, 1975); the Brassicae such as Brussel sprouts and cabbages appear to be its preferred host plants (Hussey *et al.*, 1969; Montagne, 1977). The organochlorine DDT is used extensively for its control (Ionescu *et al.*, 1969; Miles, 1948) together with several organophosphate insecticides including mevinphos, methyl parathion (Dochkova, 1971), and azinphos methyl (Rygg *et al.*, 1975).

The current work has centered on the susceptibility of the various larval instars of *M. brassicae* to DDT (1, 1, 1-Trichloro-2, 2-Bis (p-chlorophenyl) ethane) and the organophosphate insecticide malathion (O, O-dimethyl-S- (1, 2-dicarbethoxyl ethyl phosphodithioate)) under laboratory conditions using four methods of insecticide application, namely topical, injection feeding and contact. In addition, some of the physiological and behavioural factors underlying toxicological differences between instars have been investigated.

The mechanism of DDT poisoning has not yet been clearly worked out. However, it is known to act on axonal transmissions (O'Brien, 1967; Brooks, 1979; Narahashi *et al.*, 1979) with the result that the affected nerve fibers produce repetitive discharge in response to a single stimulus. Secondly, the nerve cords suffer a "prolongation of afterpotential" (Matsumura, 1975).

The mechanism of malathion poisoning on the other hand is fairly well known. As in other organophosphate pesticides malathion inhibits the enzyme acetyl-cholinesterase (AchE) (Corbett, 1974). Inhibition of this enzyme results in an accumulation of the neurotransmitter acetylcholine

(Ach) at the post synaptic membrane of cholinergic synapses which are then unable to return to their original (resting) state (O'Brien, 1967; Corbett, 1974). Thus, unlike the organochlorine insecticides which affect transmission of nerve impulses within the nerve cells, organophosphate insecticides affect transmission of nerve impulses from one cell to another (Burt, 1970). Unlike vertebrates and some invertebrates AChE is located only in the central nervous system of insects (Usherwood *et al.*, 1968; McDonald, 1975) and their neuromuscular junctions are mediated by non-cholinergic systems. The target enzyme AChE - appears very early in embryogenesis, and in *M. brassicae* AChE was detected in eggs 60 h after oviposition (Takeshi, 1957), although it may not yet be functional; the amount of enzyme increasing until eclosion (Smallman *et al.*, 1969). Similar work relating to possible changes in AChE activity in the larval stages of this species is lacking.

As both DDT and malathion are neurotoxic they would be expected to affect various sensory processes in insects (Brooks, 1979), and might therefore influence feeding. In the present work the effects of DDT and malathion on various aspects of feeding in *M. brassicae* have been studied.

1) Effects of DDT and malathion on the rate of feeding of *M. brassicae*

It might be expected that the greater the rate of pesticide uptake the greater its toxicity due to increased accumulation of pesticide or its toxic metabolites at target sites. In this case, the rate of uptake of pesticide would exceed the rate of its degradation and excretion. On the other hand, the presence of an insecticide in the diet may have an antifeeding effect thereby acting to reduce the rate of its own intake. For example, a pronounced inhibition of gut amylase and protease activity in *Spodoptera littoralis* (L.) larvae fed on sugar-beet leaves treated with

an organotin compound led Ishaaya *et al* (1974) to suggest that organotin compounds inhibited assimilation of food; the subsequent blockage of the gut preventing further uptake of treated leaves (antifeedant effect).

The effect of DDT and malathion on the rate of feeding of *M. brassicae* larvae in relation to their toxicity was therefore examined. Work on the effects of pesticides on the rate of feeding of lepidopterous insects is lacking. However, in aphids it has been shown that a general reduction in the amount of liquid imbibed occurs in the presence of various pesticides, especially systemic organophosphates; the reduction usually being related to the pesticide concentration (Parry & Ford, 1971). More importantly, it has also been possible to relate differences in susceptibility to differences in the rates of pesticide uptake in two resistant strains of *Myzus persicae* (Sulz) (Parry *et al.*, 1967) and between two other aphid species, *Brevicoryne brassicae* and *M. persicae* (Temple, 1980). In both instances, the species or strain feeding at the greater rate was the more susceptible.

2) Effect of DDT and malathion on feeding behaviour of the early instar larvae of *M. brassicae*

The use of behavioural studies to augment toxicological work is not uncommon. For example, resistance in some strains of the housefly *Musca domestica* (L.) has been described as being due to their avoidance of malathion baits (Fay *et al.*, 1958).

In the case of *M. brassicae* full toxicological investigations could not be done on the I, II or III instar larvae due to their relatively small size. The feeding behaviour of these early instars was therefore studied and the effects of DDT and malathion on such factors as site selection and time taken to initiate feeding examined. Later experiments were extended to include the effects of low doses of DDT and malathion on

the quantity of various crops consumed when these were offered as different treatment combinations in choice chambers.

Finally, some biochemical factors which may influence the toxicity of malathion to *M. brassicae* were investigated.

1) Effect of pH on the degradation of malathion

Malathion is most unstable at pH values < 5 and > 8 (Matsumura, 1975) and Fest *et al* (1973) showed that the products of hydrolysis of malathion varied with pH. At alkaline pH values the pesticide undergoes a peculiar intermolecular 'C - S' cleavage to produce dithiophosphate and a fumaric acid ester, while in acidic media the 'P - S' cleavage occurs with the formation of γ-mercapto-succinic acid. *In vivo*, the influence of pH on for example gut enzymes must also be considered. The variation in the pH of various body fluids in different larval instars of *M. brassicae* was therefore examined, and the affect of pH on the rate of hydrolysis of Malathion-^{14}C determined.

2) Metabolism of malathion

The rate of metabolism of an insecticide within an insect species is of major importance in determining its toxicity, and enhanced degradative metabolism of an insecticide is the most common mechanism conferring resistance among insects (Corbett, 1974).

Malathion like many other organophosphate insecticides is converted both to more toxic compounds through desulphuration to its oxygen analogue (malaoxon) and to relatively non-toxic compounds by hydrolytic and other reactions (O'Brien, 1967; Corbett, 1974). In the case of malathion, degradative hydrolysis due to carboxylesterase activity is particularly important (Matsumura, 1975; Dauterman, 1976). In the present work the

relationship between the toxicity of malathion when applied in the diet to V and VI instar larvae of *M. brassicae* and the rate and route of metabolism of malathion in gut extracts of these instars was compared.

MATERIALS AND METHODS

A. CULTURING OF *MAMESTRA BRASSICAE*

A.1. Breeding

M. brassicae larvae were from a culture originally obtained from the Glasshouse Crops Research Institute and bred in the laboratory since May 1978. The culture was maintained at 21 ± 1°C (range 19-23°C) with an R.H. of approximately 50% and a daylength of 16 h. The basic culturing procedure was as follows:

Eggs. Eggs were initially disinfected with 5% (v/v) formalin at room temperature in one of two ways:

(i) Fumigation:- Eggs were placed in a sealed container with formalin impregnated cotton wool for 7 h.

(ii) Immersion:- Eggs were immersed in 5% (v/v) formalin for 5 min and then washed for 2 min in distilled water.

Both methods were found to be satisfactory but in later generations the more rapid immersion method was used. The eggs were then placed on moist filter paper in 230 cm^3 plastic containers for hatching.

Larvae. The first instar larvae were transferred into plastic sandwich boxes (25.0 x 8.5 x 5.3 cm) lined with dry tissue paper and supplied with fresh cabbage leaves. The sandwich boxes were disinfected with 10% (v/v) formalin prior to use. Each container was filled with dry sawdust to a depth of approximately 1.0 cm before lining it with tissue paper. This was to ensure that the container was as dry as possible and to provide burrowing and pupation sites for the fifth, sixth and prepupal stages.

After two generations had been successfully bred; the duration of the various stages was studied.

Pupae. Pupated insects were promptly collected and sexed. Up to fifty pupae (approx. 25♀ + 25♂), were kept in disinfected plastic sandwich boxes containing 1.0 cm of moist peat. The pupae were then covered with a thin layer of moist peat which was kept moist with distilled water. The number of pupae and dates of pupation were noted and observations were also made daily for any emerging adults.

Adults. The moths were kept in plastic sandwich box containers lined with tissue paper. Oviposition sites were made from grease-proof paper. Adults were fed on a 10% (v/v) honey solution in distilled water, which was impregnated onto cotton wool. The cotton wool was changed daily and no more than twenty moths were kept per container. Observations were made for any eggs laid and for any mortalities that occurred. All eggs were collected, disinfected and kept on moist filter paper until they hatched.

A.2. Relationship between the cast head capsule width and the stage of development of the larva of *M. brassicae*

Head capsule measurements were made to conclusively establish the number of instars present in the larval stages of *M. brassicae* and to identify these instars.

Sixty first (I) instar larvae which hatched on the same day were placed individually in plastic Petri dishes (5.0 cm diameter) lined with filter paper and supplied with fresh cabbage leaves. Larvae were observed daily under a binocular microscope for cast head capsules and these were removed and measured with a micrometer eye-piece. Body weight measurements were also made for each instar two days after moulting and any morpho-

TABLE A.1 : Mean width of cast head capsules (mm) of the different larval instars of *M. brassicae*.

Larval instar	Mean width of cast head capsule (mm)	Exact 95% C.L. (mm)	
		Lower	Upper
I	1.224	1.224	1.224
II	2.111	2.069	2.153
III	3.233	3.163	3.303
IV	4.814	4.736	4.892
V	7.681	7.503	7.859
VI	12.842	12.674	13.010

phological changes were noted.

The results showed there were six instars in the larval stages of *M. brassicae* (Table A.1). No overlap in the width of the head capsule was found and in all subsequent experiments, the larval instars were selected mainly on the basis of the width of the head capsule.

B. TOXICOLOGICAL STUDIES

B.1. Calibration of the Arnold electric microapplicator and Agla all-glass syringe

An Arnold electric microapplicator (Arnold, 1965) with an Agla all-glass syringe bearing a bent (approx. 90^{o}), 25G hypodermic needle was used to deliver the marked volumes of 0.1; 0.2; 0.3; 0.5 and 1.0 µl of malathion-^{14}C in risella oil (specific activity = 6.6 m Ci/mMol) containing 3210 dpm 1.0^{-1} µl onto small pieces (approx. 5 x 7 mm) of aluminium foil. Each piece of foil was placed into a scintillation vial with Bray's (10 cm^3) scintillant (Bray, 1960) and the vials counted in a Beckman LS 250 Scintillation Counter calibrated against n-hexadecane-^{14}C for 100 min or a 2σ error of 0.5% whichever was the shorter.

The percentage of the stated volume actually delivered by the applicator declined with decreasing volume (Table B.1). However, the variance of the volumes actually delivered was considered to be satisfactorily small and an appropriate correction factor was employed for the calculation of the LD_{50} values (± 95% C.L.).

B.2. Chemicals

DDT (1, 1, 1-Trichloro-2, 2-Bis(p-chlorophenyl) ethane) (technical grade) obtained from I.C.I. Plant Protection Ltd., was recrystallized by

TABLE B.1 : Calibration of the Arnold electric micro-applicator.

Test volumes (µl)	Mean volume actually delivered ± S.E. (µl)	Range of volumes delivered (µl)
1.0	0.75 ± 0.01	0.73 - 0.76
0.5	0.34 ± 0.01	0.29 - 0.37
0.3	0.19 ± 0.01	0.15 - 0.21
0.2	0.11 ± 0.01	0.10 - 0.15
0.1	0.05 ± 0.005	0.04 - 0.06

the method of West and Campbell (1950) to produce DDT crystals with a melting point of 109.5-110°C. Test solutions of DDT were prepared in Risella 17 oil (Shell). Control experiments were treated with R.17 oil only.

Malathion (O, O-dimethyl-S-(1, 2-dicarbethoxyl ethyl phosphodithioate) (94-96% v/v pure) was obtained from Albright and Wilson Ltd., and diluted to experimental concentrations using technical grade olive oil. Control experiments were treated with olive oil only.

Malathion-^{14}C (specific activity 6.6 m Ci/mMol^{-1}, purity 99%) was obtained from the Radiochemical Centre, Amersham in benzene solution. The benzene was evaporated under nitrogen and stock solutions prepared in redistilled acetone. Analytical grade samples of unlabelled malathion and malaoxon (the oxygen analogue of malathion) (99% pure) were obtained from the Pest Infestation Control Laboratory, Slough.

B.3. Bioassay experiments

The average weight of larvae for each assay was recorded. All treated insects were kept at a temperature of 26 ± 1.5°C, approximately 50% R.H. and 16 h daylength.

B.3.a. Topical application

An Agla micrometer glass syringe was half filled with insecticide solution and a bent 25G hypodermic needle fitted. The syringe was then attached to the Arnold electric micro-applicator. Between 0.1 µl and 1.0 µl (marked volumes) of the insecticide was applied to the dorsal surface of any of the three thoracic segments of the larva. The second and third instar larvae were the most active and it was necessary to restrain the larvae by chilling for a period up to 2 min on crushed ice before the

insecticide was applied.

The volumes applied were 0.1, 0.2, 0.3, 0.5 and 1.0 (µl) for the second, third, fourth, fifth and sixth instar larvae respectively. Treated larvae were kept in plastic containers (230 cm^3) lined with tissue paper and supplied with fresh food. Twenty larvae were dosed at each concentration and each experiment was replicated three times. Mortality was assessed after 24 h.

B.3.b. Injection method of application

All equipment was autoclaved before use. Larvae intended for injection were starved for 3 h prior to treatment, then anaesthetized with diethyl ether until signs of slight anaesthesia were observed. Hyamine 1622 solution (0.5% w/v) was used to clean and sterilize the needle tip while in use and also to clean the underside of the larvae.

The desired volumes of the insecticides were delivered with an Agla all-glass syringe bearing a 30, 31 or 33 gauge straight hypodermic needle. For injection, each larva was supported on a microscope stage covered with filter paper. One of the second prolegs was grasped with a pair of forceps and pulled towards the needle tip. The penetration was made as shallow as possible and the required volume of insecticide injected. The needle was then carefully withdrawn and the tip of the proleg squeezed with the forceps immediately the tip of the needle was out.

Treated larvae were placed in plastic containers lined with tissue paper and allowed up to 1 h to recover from the anaesthetic. Those which failed to recover in this time were discarded. Fresh food was supplied and the insects kept at 26.0 ± 1.5°C for 24 h when mortality was assessed. Twenty individual larvae were injected with a given concentration



PLATE I : The "leaf-sandwich" method.

a. VI instar larva eating the "sandwich".

and each experiment was replicated three times. No results were obtained for injections made on the third instar larvae due to their small size. Up to 100% mortality (range 45% - 100%) was obtained in the control experiments. No attempt was made to inject first or second instars. Thus, the method was found to be suitable only for the fourth, fifth and sixth instars.

B.3.c. Feeding method of application

A modification of the leaf "sandwich" method of Toppozada *et al* (1964) was used.

In preliminary experiments, two leaf discs, 5.0 mm and 7.0 mm in diameter were cut out of untreated cabbage leaves. The required volume of insecticide was applied to the 5.0 mm disc by means of the Arnold electric micro-applicator. The 7.0 mm disc was then placed onto the treated disc so that the insecticide was the filling of the "sandwich". The leaf discs stuck together because of the oils used as insecticide solvents. These "sandwiches" were offered to the larvae individually by laying them on the floor of a 5.0 mm diameter Petri dish (Plate I). Larvae were starved for 4 h before being placed in individual Petri dishes. The larvae were kept at 23 ± 1.0^{o}C until complete consumption of the discs had been observed, otherwise the larvae were discarded after 24 h. The larvae were then transferred to plastic containers (230 cm^{3}) with fresh untreated cabbage leaves. Twenty individually treated larvae for a given treatment were grouped together. Each experiment was replicated three times. Since all larvae did not consume discs within the same time the groupings into twenty per container was based, as much as possible, on the time taken to finish the bait. Mortality was recorded 24 h after such times.

Preliminary observations showed that 15 h was required by the third and fourth instar larvae to consume 45-60% of the sandwich as measured by leaf area; and over 36 h in some of the malathion treatments, by which time the leaf discs had dried up. In the main experiment therefore, several modifications were made.

Firstly, the initial period of starvation, was extended to 24 h for the third and fourth instars, and to 8-10 h for the fifth and sixth instars. It was found that starvation above 10 h resulted in cannibalism among the fifth and sixth instars.

Secondly, instead of a "sandwich", only the smaller disc with the insecticide was presented to the third and fourth instar larvae. This modification was necessary as the early instars could not chew through a single cabbage leaf, let alone a "sandwich". These single leaf discs with the insecticide were placed on the floor of a Petri dish with the insecticide treated surface facing upwards in order to prevent loss of insecticide to the container. This procedure had the disadvantage that larvae could contact the insecticide before consuming the leaf disc. With these modifications the "sandwich" method was found to be most suitable for the fifth and sixth instar larvae. However, not all the third and fourth instar larvae consumed the whole disc even after 48 h confinement.

B.3.d. Contact method of application

Different concentrations of DDT and malathion were mixed with twice their volume of acetone (G.P.R. grade). This volatile constituent was added to enable the oil solvents with the insecticide (Section B.2) to spread evenly over the filter papers (9.0 or 11.0 cm diameter) used as substrates. A pipette was used to deliver 1 cm^3 of a given concentration of insecticide onto the filter paper in a spiral fashion. The latter was

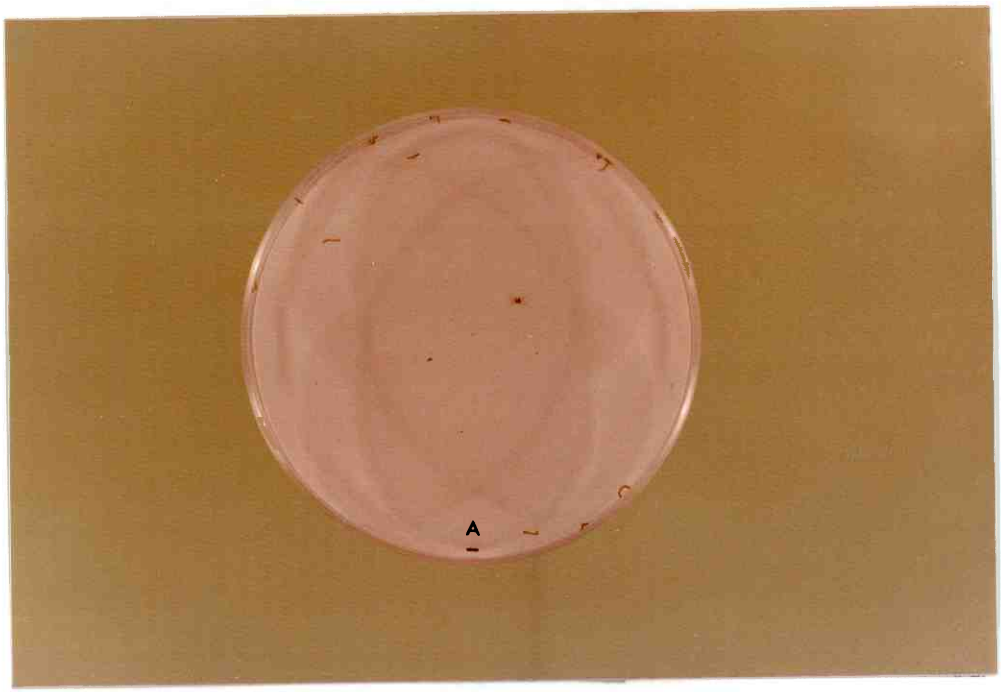


PLATE II : The contact application method, used for I to IV instar larvae, A.

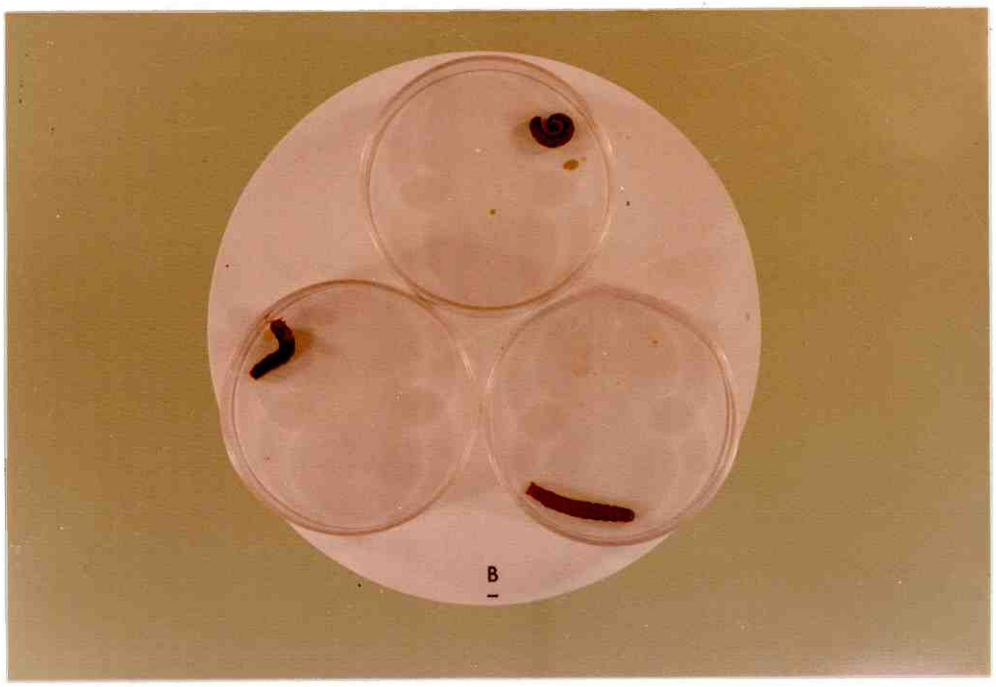


PLATE III : The contact application method used for the V and VI instar larvae, B.

supported on a bed of drawing pins for 2-5 min for the acetone to evaporate. A number of a particular instar was transferred onto treated filter papers and were confined by inverting a Petri dish over the filter paper (Plate II). This allowed observations to be made without disturbing the insects. At various time intervals the number of dead and paralysed larvae, as well as larvae which had moved onto the Petri dish were noted.

For each concentration sixty I, II, III and IV instar larvae and thirty V and VI instar larvae were treated. The number of replicates was varied according to the size of the larvae; three replicates were used for the I, II and III instars (20 per substrate); six replicates for the IV instar (10 per substrate); ten replicates for the V and VI instars (3 per substrate). In the V and VI instar larvae each larva was confined separately on the substrate to prevent cannibalism (Plate III). The average temperature for the experimental chamber was 18.0 ± 2°C (ranging from 15.0°C to 21.5°C). Two control treatments were employed. In one, filter papers were treated with acetone while in the other filter papers were treated with Risella 17 oil or olive oil in acetone, for DDT and malathion experiments respectively.

C. FEEDING EXPERIMENTS

C.1. Effects of DDT and malathion on the rate of feeding by larval instars of *M. brassicae* on cabbage leaf discs

C.1.a. Number of larvae (out of 50) which completely consumed insecticide-treated leaf discs

The procedure described for the feeding method of insecticide application (Section B.3.c.) was used. The number of individual larvae which had completely consumed the "sandwich" or single leaf disc was

recorded 0, 4, 8, 16, 20 and 24 h after presentation.

Fifty individuals for each of the IV, V and VI instars were tested at each concentration of insecticide, with the control treatments receiving Risella 17 oil (R.17 oil) or olive oil-treated leaf discs for DDT and malathion respectively.

C.1.b. Area (cm^2) of cabbage leaf disc consumed in 24 h by individual larvae of *M. brassicae*

C.1.b.i. Relationship between leaf area and leaf weight

The gravimetric method described by Šestak *et al* (1971) was used. Different areas were carefully cut out from sheets of A4 graph paper and weighed on a Mettler H20 balance (room temperature range 18-22°C). A graph of weight versus area was then plotted. The same batch of graph paper was used in the following series of experiments.

C.1.b.ii. Area (cm^2) of untreated cabbage leaf consumed in 24 h by larval instars of *M. brassicae*

The outlines of untreated cabbage leaf discs were traced onto A4 sheets of graph paper. The leaf discs were then placed individually in 9.0 cm diameter Petri dishes lined with moist filter paper containing larvae of *M. brassicae*. After 4, 8, 16 and 24 h the remaining leaf discs were removed, brushed clean and their outlines traced. The area consumed at a given time was then calculated.

In trial experiments, the number of larvae maintained in a Petri dish was varied according to their size. Thus, 3 IV instar, 2 V instar or 1 VI instar larvae were confined per Petri dish. Four replicates were used for each instar.

The result showed that there was insufficient leaf material for a 24 h exposure period. Hence, for the main experiment only one larva was confined per Petri dish for all instars and larger leaf discs were used. Four replicates were used per treatment.

After the 24 h exposure period outlines of the leaf discs on graph paper were weighed and converted to their corresponding areas by substituting into the established regression equation for the A4 graph paper.

C.1.b.iii. Area (cm^2) of insecticide-treated cabbage leaf disc consumed in 24 h by larval instars of *M. brassicae*

The method described in the previous section was followed except that DDT or malathion-treated cabbage leaf discs were used. The mean areas of the leaf discs were 11.18 cm^2 for each IV instar larva, 11.21 cm^2 for each V instar larva and 16.23 cm^2 for each VI instar larva.

Three µl of stock DDT or malathion solutions in Risella or olive oil respectively were uniformly deposited on each leaf disc prior to its introduction into the Petri dish. The concentrations of insecticide used were: 0.0005, 0.05, 0.5 and 2.0% (w/v) DDT, and 0.00125, 0.0125, 0.25, 1.25, 2.5, 5.0 and 10.0% (v/v) malathion. In addition, controls with the solvents alone were used. Each treatment was replicated four times.

C.2. Feeding behaviour of the early instar larvae of *M. brassicae*

C.2.a. Preliminary behaviour experiments

C.2.a.i. Choice of feeding site on cabbage leaves by the I instar larvae of *M. brassicae*

Five batches of eggs (45-50 eggs/batch) due to hatch within 2 h were each placed individually in plastic sandwich boxes (24.8 x 8.5 x 5.3 cm) approx. 2 cm from a leaf off a 6 week old cabbage plant. The newly hatched larvae were thus confronted with a choice of feeding either on the upper epidermis which was towards the light or on the lower epidermis. The distribution of the larvae was recorded 24 h after hatching. The feeding behaviour of the larvae was also observed at various time intervals.

C.2.a.ii. Choice of feeding site by I instar larvae of *M. brassicae* on potted cabbage plants

Five batches of egg-masses (22-30 eggs/batch) due to hatch within 1-2 h were attached with a honey paste to the lower or upper epidermis of leaves of 6 week old potted cabbage plants. The arrangement was such that each plant had two leaves, one bearing an egg-mass on the lower epidermis the other an egg mass on the upper epidermis. Leaves of similar size and similar position on the plant were selected so that environmental factors such as temperature, light incidence and wind direction were approximately equal.

The extent of wandering by the larvae in selecting the feeding sites was observed and the number and extent of feeding at the two different sites after 24 h recorded.

C.2.a.iii. Factors influencing the choice of feeding site by I and II instar larvae of *M. brassicae* on cabbage leaves

Two cabbage leaves were placed approximately 8.0 cm apart in a plastic sandwich box (24.8 x 8.5 x 5.3 cm), one with the lower epidermis downwards, the other with the upper epidermis downwards. Batches

of 30 I (3 days old) or II (2 days old) instar larvae were placed between the two leaves. Each treatment was replicated five times.

C.2.a.iv. Confirmation of the influence of light on the choice of feeding site by the early instar larvae of *M. brassicae*

Eight sandwich boxes (24.8 x 8.5 x 5.3 cm) were used. Three batches (30 per batch) of I and II instar larvae were put in separate boxes and a cabbage leaf introduced into each with the upper epidermis uppermost. These cages were kept in an incubator at 23°C for 24 h in total darkness. For controls, the remaining two boxes, each containing a batch of either I or II instar larvae and a cabbage leaf were maintained at 23°C in 16 h light and 8 h dark cycle for 24 h.

The number of larvae feeding or wandering on either side of the leaf was noted after 24 h.

C.2.a.v. Feeding behaviour of the early instar larvae of *M. brassicae* in the presence of insecticides

The surface areas of eighty similar sized cabbage leaves from 6 week old plants were measured on a Paton electronic planimeter. Various solutions (8 µl) of DDT and malathion were applied to the lower epidermis of 64 such leaves using an Arnold electric micro-applicator. The concentrations of insecticides used were:

1) DDT: Control (Risella 17 oil treated); 0.025%; 0.2%; 2.0% (w/v).

2) Malathion: Control (Olive oil treated); 0.00125%; 0.25%; 2.5% (v/v).

Four leaves were treated with each concentration of insecticide, pairs of which were lightly rubbed against each other on the treated surface.

Leaves were placed individually in sandwich boxes. Thirty, I or II instar larvae were placed on the untreated surface of the leaf in each container. Two controls were used for each larval stage, one with untreated leaves, the other with solvent-treated leaves. Each treatment was replicated four times.

The leaf areas were measured after 24 h. The difference between the two measurements was used as an indication of the extent of feeding. In addition, observations were made on:

1) whether the larvae would still choose the side of the leaf with the lower incidence of light even in the presence of insecticides,
2) time taken to start feeding on leaves with different treatments (observations were made every 10 min for 2 h), and
3) the larval mortality after 24 h.

C.2.b. Feeding of early instar larvae of *M. brassicae* in different choice chambers

C.2.b.i. Description of choice chamber

The basic choice chamber consisted of a 9.0 cm diameter Petri dish lined with moist filter paper (Whatman No. 1) on which was placed a treated or untreated leaf disc. Leaf discs were cut out of leaves from standard aged cabbage, Brussel sprout (10–14 weeks) or Bean (6–10 weeks) plants using a 2.75 cm diameter cork-borer. For the III instar whole Bean leaves were used. Brussel sprout leaves were substituted for cabbage leaves in some experiments due to unavailability of cabbages of standard age.

Where treated leaves were used, 2.0 µl of DDT or malathion solution was applied to the leaf disc with an Arnold micro-applicator

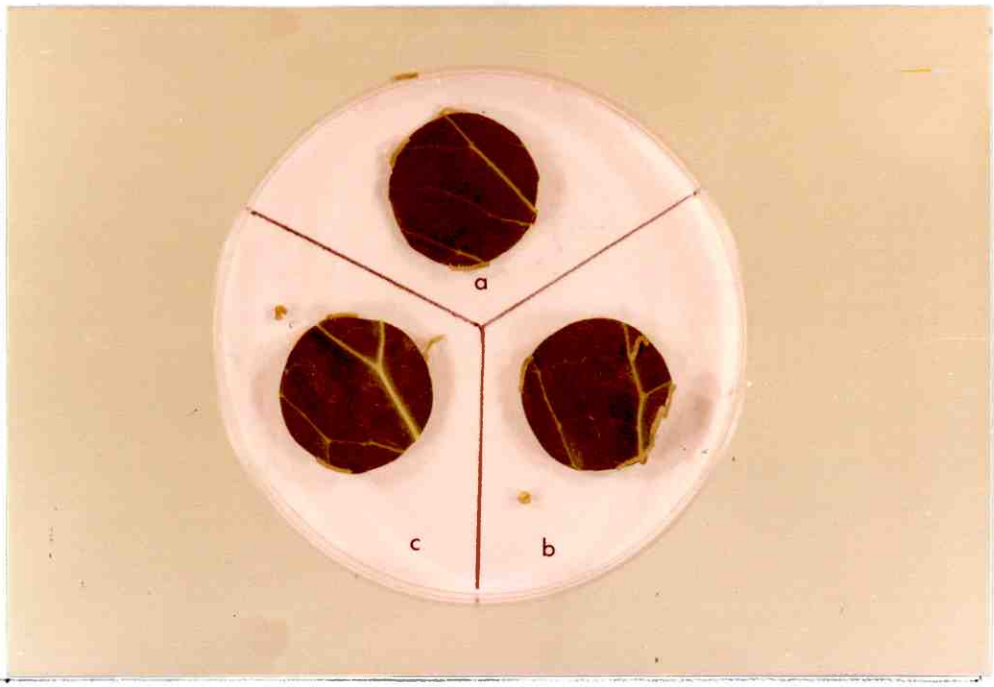


PLATE IV : The "Three-way" choice chamber.

a. Untreated cabbage leaf disc.

b. Olive oil-treated cabbage leaf disc.

c. Risella 17 oil-treated cabbage leaf disc.

fitted with an Agla all-glass syringe and a 25G hypodermic needle. Two leaf discs with the same concentration of pesticide were gently rubbed against each other to effect a more even distribution of insecticide. The treated leaf discs were introduced into the choice chamber with the treated surface downwards.

Each leaf disc was weighed on a Mettler H20 balance before placement in a choice chamber. The final weight was recorded after 24 h exposure and the amount of leaf consumed calculated. All experiments were conducted at 26 ± 1.8°C, an R.H. of approximately 45-50%, and a daylength of 16L-8D.

C.2.b.ii. Feeding of the early instar larvae of *M. brassicae* in a "Three-way" choice chamber

The basic choice chamber was divided into three approximately equal parts. Two leaf discs from Brussel sprout or Bean leaves were treated with 2.0 µl olive oil and Risella 17 oil (R.17 oil) respectively, weighed and introduced into the choice chamber with their treated surface downwards. An untreated leaf disc was also added to each choice chamber (Plate IV).

Fifteen larvae (I, II or III instar) were added per chamber with five replicates per treatment. The amount of leaf disc consumed was determined after 24 h.

C.2.b.iii. Feeding of the early instar larvae of *M. brassicae* in a "Two-way" choice chamber

The basic choice chamber was divided into two equal halves with a solvent-treated control leaf disc placed in one half and a pesticide-treated leaf disc in the other (Plate V). Both leaf discs were placed

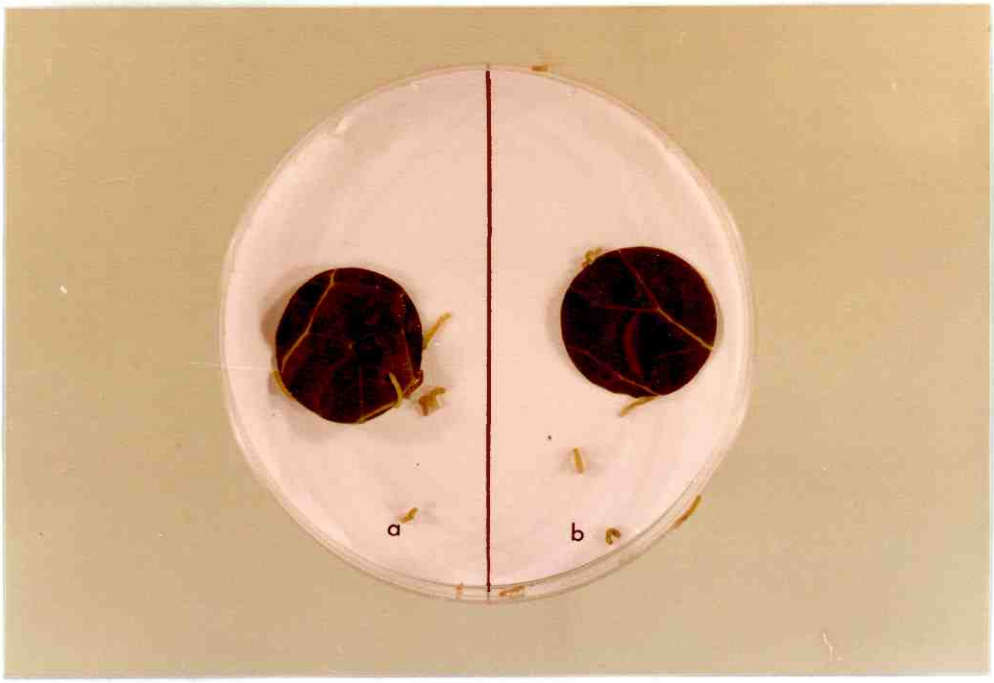


PLATE V : The "Two-way" choice chamber.

a. Control (R.17 oil or Olive oil) treated cabbage leaf disc.

b. Insecticide (DDT or Malathion) treated cabbage leaf disc.

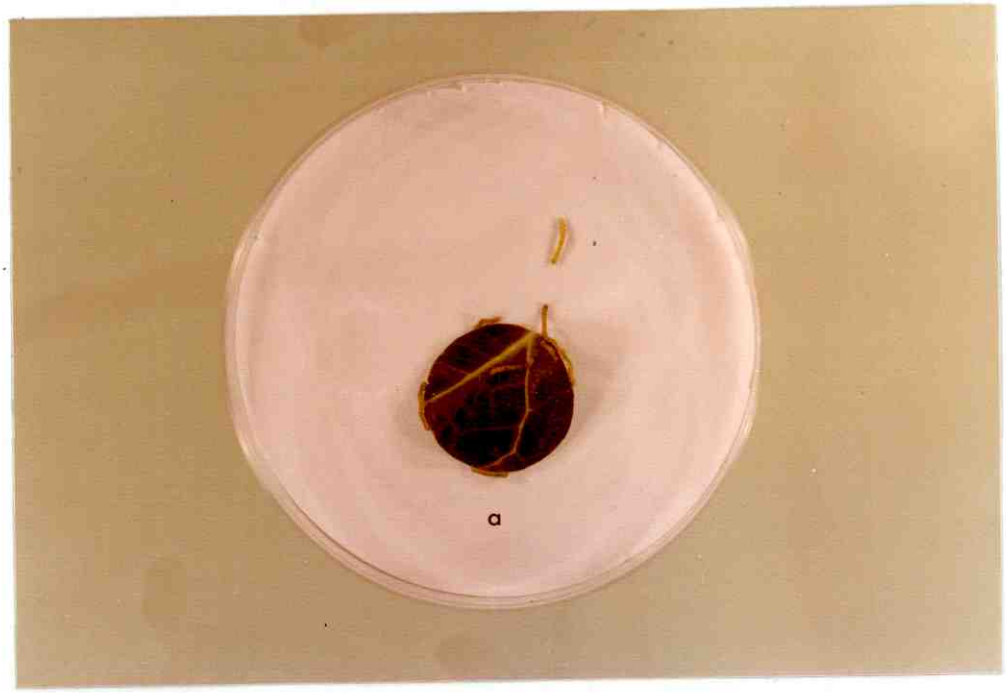


PLATE VI : The "One-way" choice chamber.

a. Insecticide (DDT or Malathion) treated cabbage leaf disc.

with the treated-surface downwards. Choice chambers were initially set up with the following arrangements:

1) Untreated control v. R.17 oil; R.17 oil v. 0.0002% (w/v) DDT; R.17 oil v. 0.0025% (w/v) DDT.

2) Untreated control v. olive oil; olive oil v. 0.0005% (v/v) malathion; olive oil v. 0.00125% (v/v) malathion; olive oil v. 0.0125% (v/v) malathion.

Twenty larvae (I, II or III instar) were added to each choice chamber and three replicates were used per treatment.

In a further experiment two concentrations of DDT (0.0002 and 0.0025% (w/v)) were compared directly using both Brussel sprout and Bean leaves. Twenty larvae were added to each choice chamber with five replicates per treatment.

C.2.b.iv. Feeding of the early instar larvae of *M. brassicae* in a "One-way" choice chamber

The basic choice chamber containing single leaf disc from Brussel sprout or Bean leaf was used (Plate VI).

Two concentrations of DDT (0.0002% or 0.0025% (w/v)) were examined for both Brussel sprout and Bean leaves.

Twenty larvae were added to each choice chamber and each treatment was replicated five times. The amount of leaf consumed after 24 h was determined.

D. BIOCHEMICAL STUDIES

D.1. pH studies

D.1.a. pH measurement of the body fluids of larvae of *M. brassicae*

The pH of a) regurgitated gut fluid, b) mid-gut fluid, and c) haemolymph, was determined as follows.

Individual larvae were disturbed by a gentle touch with a ca mel hair brush and the fluid ejected from the mouthpart collected in capillary tubes. This fluid was taken as regurgitated gut fluid. The larva was then restrained by chilling on crushed ice and a shallow wound made on its lateral side with a sharp 30G hypodermic needle, and a sample of haemolymph fluid taken. The larva was then dissected, the haemolymph thoroughly washed off with distilled water and the specimen dried with tissue paper. The mid-gut was removed onto a microscope slide and the contents of its lumen collected with a capillary tube.

The pH of each sample was recorded immediately after collection using pH papers of ranges 6.0-8.0 or 8.0-10.0. The pH of 40 individual larvae was measured for each of the IV, V and VI instars. An attempt to measure pH of the above fluids using a microelectrode was unsuccessful due to the small volumes available.

D.1.b. Stability of malathion under alkaline conditions

D.1.b.i. Radiochemical method

Tris (hydroxymethyl) aminomethane buffers (0.05 M) were prepared at pH 7.9; 8.3 and 8.8 (all at 25°C). Phosphate (Na_2HPO_4-NaH_2PO_4) buffers (0.1 M) were prepared at pH 6.5 and 7.9 (at 25°C). pH 7.9 was duplicated to check for any differences between the two buffers.

Malathion-^{14}C in redistilled acetone (20 µl) was applied with a Terumo glass syringe (Shandon Southern) to the bottom of a Quickfit glass tube and the acetone evaporated off at room temperature. A mean of 147,000 d.p.m. (3.3 µg malathion) was then added to the tube, buffer solution added (0.5 cm^3) and the mixture incubated at 25°C for 3 h in a shaking water-bath. The reaction was terminated by the addition of Analar grade chloroform (5.0 cm^3) followed by phosphate buffer (0.1 M, ph 7.5; 2.0 cm^3). The tubes were mechanically shaken for 5 min, centrifuged at 2,500 r.p.m. for 5 min and the organic (chloroform) fraction discarded. The chloroform extraction procedure was then repeated. (This extraction procedure was chosen after a series of preliminary experiments.)

A sample of the aqueous phase (1.0 cm^3) was then transferred into a scintillation vial containing Bray's scintillant (10 cm^3). Vials were counted in a Beckman LS-250 Liquid Scintillation counter for 50 min or to a 2σ error or 0.5% whichever was the shorter.

D.1.b.ii. Bioassay method

Fourth (IV) instar larvae of *Aedes aegypti* (L) were used for bioassay.

Twelve batches of thirty larvae were placed in distilled water (49.25 cm^3, pH 7.0) and aqueous solutions of malathion (1, 2, 3, 4, 5 ppm v/v; 0.25 cm^3) added. Larval mortality was compared with that of control larvae in distilled water after 24 h. Each treatment was replicated twice. The data was subjected to Probit analysis using a computer program (Minitab). The LC_{50} value obtained was 0.043 ppm (see Appendix D, Table D.2).

Subsequently, Tris buffers (0.1 M) were prepared at pH

values 7.0, 7.7, 8.3, 8.6, 8.8 (at 25°C). Thirty IV instar larvae of *A. aegypti* were placed in 49.25 cm^3 of each buffer solution and 0.75 cm^3 of a stock malathion solution (2.5 ppm v/v) in distilled water added to give the approximate LD_{50} concentration of 0.043 ppm. Larval mortality was compared with control larvae at various time intervals up to 24 h. Each treatment was replicated twice. No difference in control mortality due to pH (range 7.0-8.8) was observed.

D.2. Metabolism of malathion by gut homogenates from V and VI instar larvae of *M. brassicae*

D.2.a. Radiochemical method

Dissected midgut portions (0.003 g wet weight) from standard aged V and VI instar larvae were separately homogenized in phosphate buffer, pH 7.5 (0.1 M, 1.5 cm^3) in an all-glass homogenizer (2.0 cm^3) on crushed ice. The crude homogenates were stored on ice until required.

Malathion-^{14}C in redistilled acetone (40 µl) was applied to the bottom of each Quickfit glass tube and the acetone evaporated off at room temperature. A mean of 294,000 d.p.m. (6.62 µg malathion) was added to each tube. Gut homogenates (0.5 cm^3) were added to test tubes and phosphate buffer (0.1 M, pH 7.5; 0.5 cm^3) to control tubes and the tubes incubated at 25°C for 15 min in a shaking water-bath. The concentration of the gut homogenate and the incubation period were selected after a series of preliminary experiments, using incubation periods of up to 1 h with gut concentrations of 0.003-0.3 g gut 1.5 cm^{-3}.

The reaction mixture plus additional phosphate buffer (2.0 cm^3) was extracted twice with chloroform (5.0 cm^3) as described previously except that the chloroform fraction was collected and dried over anhydrous

sodium sulphate (1 g). Samples (1.0 cm^3) of both the aqueous and the chloroform phases were added to scintillation vials containing Bray's scintillant (10 cm^3).

Radioactivity was counted as described above. Because of quenching due to chloroform the organic fraction vials had to be recounted following the addition of known amounts of radioactivity in order to calculate the efficiency of counting. The remaining chloroform fraction (approx. 9.0 cm^3) was stored in sealed ampules at -20°C for further analysis.

The organic (chloroform) fraction was analysed by thin layer chromatography (tlc). Three solvent systems were used initially to separate malathion and malaoxon (Kadoum, 1970; personnal communication Radiochemical Centre, Amersham). These were:

I - Hexane: Acetic acid: Ether (15:3:2).

II - Benzene: Hexane: Acetic acid (4:4:2).

III - Hexane: Acetone (4:1).

Analytical grade samples (approx. 20 µg) of malathion and malaoxon in redistilled acetone were spotted onto aluminium backed silica gel 60 plates (Merck) and the plates run at room temperature in a particular solvent system. The plates were then removed, air-dried and sprayed with 2, 6-dibromo-N-chloro-p-quinoneimine (DCQ, 10.5% w/v) in redistilled acetone (Kadoum, 1970); and heated at 110°C for 20 min. The following Rf values were obtained for malathion and malaoxon.

TABLE D.1 : Rf values obtained for solvent systems I, II and III.

Solvent system	Rf value	
	Malathion	Malaoxon
I	0.36	0.07
II	0.49, 0.59, 0.59, 0.65 $\bar{x}$ = 0.58	0.23, 0.23, 0.29, 0.29 $\bar{x}$ = 0.26
III	0.26	0.08

Solvent system II gave the best separation for malaoxon from the starting point and was used for the analysis of the experimental samples.

Approximately 2 cm^3 of the chloroform extract from each experiment was concentrated to about 20 µl in a 0.3 cm^3 glass Reacti-vial (Pierce) at 40°C under a gentle flow of oxygen-free nitrogen. The total concentrated sample was then applied to the tlc plate with a Terumo syringe. Standards of analytical grade malathion and malaoxon were also applied to the plates.

Following tlc the parts of the plate with the standards were cut out and sprayed with DCQ reagent to visualize the spots. These strips were then realigned with the rest of the plate and the bands containing malathion-^{14}C and malaoxon-^{14}C marked with a pencil. The bands for each fraction were then scraped into separate scintillation vials with Bray's (10 cm^3) and counted and the activity of each expressed as a proportion of the total activity on the tlc plate. Total malathion and malaoxon present was then calculated from the total activity found in the organic fraction following the extraction process.

D.2.b. Bioassay methods

D.2.b.i. Cholinesterase (ChE) assay

Ten heads of VI instar larvae were weighed (mean total weight 0.1 g) and homogenized in cold Phosphate buffer (NaH_2PO_4-Na_2HPO_4), pH 8.0 (0.1 M; 1.5 cm^3) in an all-glass homogenizer on crushed ice. The crude homogenate was centrifuged for 3 min at 1,500 r.p.m. and the supernatant used as the source of ChE activity.

Guts from the decapitated larvae were washed free of haemolymph with distilled water and excess fluid removed by drying the guts on tissue paper. The midguts were collected, weighed (approx. 0.3 g), homogenized in phosphate buffer (0.1 M, 1.5 cm^3) and centrifuged as described above. Extracts were stored on ice until required or prepared fresh.

Gut extract (0.5 cm^3) was incubated with 20 ppm malathion for 1 h at 25°C. Head extracts (0.5 cm^3) was then added to the malathion-gut extract and this mixture incubated for a further 10 min at 25°C.

ChE activity was measured by the colorimetric method of Ellman *et al* (1961) using a Beckman DB spectrophotometer at 412 nm with a constant cell temperature of 25°C. The reaction mixture in the test cuvette was: 3.0 cm^3, 0.1 M phosphate buffer pH 8; 0.1 cm^3, head-gut extract; 0.1 cm^3, 0.01 M S-dithiobisnitrobenzoic acid (B.D.H. Ltd.,) in phosphate buffer pH 8 with added sodium bicarbonate ($NaHCO_3$, 1 mg cm^{-3}); 0.1 cm^3, 0.2 M Acetylcholine iodide (B.D.H. Ltd.,) in phosphate buffer, pH 7.0, 0.1 M; the reference cuvette contained 0.1 cm^3 distilled water in place of the enzyme extract.

The ChE activity of the head-gut extract mixture exposed to

malathion was compared with that of control extracts incubated without malathion, and with head and gut extracts incubated with 20 ppm malathion for only 10 min before assay. Activity was recorded for 10 min for two batches per treatment.

An attempt was made to inactivate ChE activity in the gut homogenates of VI instar *M. brassicae*, following pre-incubation with malathion in order to simplify this bioassay system. Fresh gut homogenates (approx. 0.5 cm^3) were heated for either 5 or 10 min at 35, 40, 50, 60, 70, 80 and 90°C in a water bath. After cooling to 25°C, ChE activity was determined.

D.2.b.ii. Bioassay method using *Aedes aegypti*

A standard curve (Appendix D, Table D.9) was obtained for malathion against the IV instar larvae of *A. aegypti* as follows.

Seven groups of IV instar larvae of *A. aegypti* were put in distilled water (50 cm^3), and dosed separately with stock malathion solutions (5, 10, 20, 30, 40, 50 ppm v/v; 0.1 cm^3). Control treatments received distilled water (0.1 cm^3) only. Mortality was determined after 24 h. Three replicates were used for each treatment. The data was subjected to probit analysis using the computer program described earlier. The approximate LD_{50} value obtained was 0.03 ppm.

To test if the gut extract of the VI instar larva of *M. brassicae* was toxic to the IV instar larvae of *A. aegypti*, gut extract (prepared as in Section D.2.b.i) (0.1 cm^3) was added to batches of 30 IV instar larvae of *A. aegypti* in distilled water (50 cm^3). Gut extracts of different ages were used and their toxicities were assessed after 24 h.

The results demonstrated that gut extracts stored for 1, 2,

3, 4 and 24 h on ice were non-toxic to the IV stage larvae of *A. aegypti*.

Malathion in distilled water (90 ppm v/v; 0.1 cm^3) was then incubated with freshly prepared gut extract (0.5 cm^3) of VI instar larvae of *M. brassicae* at 25°C for 2 or 24 h. Two groups of 30 IV instar stage *A. aegypti* were prepared as above and dosed either with malathion-gut extract mixture (0.1 cm^3) or with 15 ppm (v/v) malathion (0.1 cm^3) to give the approximate LD_{50} concentration. The number of dead larvae was noted after 24 h. Four replicates were used for each treatment.

D.3. Effect of Triphenyl phosphate (T.P.P.) on the hydrolysis of Malathion-^{14}C by gut homogenates of VI instar larvae of *M. brassicae*

Malathion-^{14}C in redistilled acetone (40 µl) was added to glass tubes as described in Section D.2.a.

In the normal treatment, gut extract (0.003 g, 1.5 cm^{-3}; 0.25 cm^3) was added to each tube plus phosphate buffer, pH 7 (0.1 M; 0.25 cm^3). In the T.P.P. treatment, gut extract (0.25 cm^3) was added together with phosphate buffer (0.25 cm^3) containing 2 x 10^{-4} M T.P.P. Control incubations with only phosphate buffer (0.5 cm^3) were also carried out.

The experimental conditions, extraction and counting procedures were as described in Section D.2.a.

D.4. *In vitro* inhibition of cholinesterase (ChE) activity from heads of VI instar larvae of *M. brassicae* by malathion and malaoxon

Extracts of heads from VI instar larvae of *M. brassicae* were prepared as described in Section D.2.b.i. Known volumes of analytical grade malathion and malaoxon in redistilled acetone were added to small glass tubes, the acetone allowed to evaporate and the pesticide diluted to the required concentration by the addition of head extract in phosphate buffer

pH 8.0 (0.1 M). The head extracts were then pre-incubated in the pesticide for 15 min at 25°C before assay for ChE activity. Untreated head extracts were also incubated for 15 min at 25°C prior to assay.

The pesticide concentrations used were: 1.5×10^{-5}, 3×10^{-5}, 1×10^{-4}, 1×10^{-3} M for malathion and 1×10^{-6}, 5×10^{-6}, 1.5×10^{-5}, 1×10^{-4} M for malaoxon.

ChE activity was determined on aliquots of the pre-incubated head extract (0.1 cm^3) using the method of Ellman *et al* (1961) described in Section D.2.b.i.

RESULTS

A. CULTURING OF *MAMESTRA BRASSICAE*

A.1. Breeding

The duration of the developmental stages of *M. brassicae* showed a considerable degree of overlap (Table A.2). In general, however, the I-IV larval instars were of a similar duration, the V and in particular the VI instars were somewhat longest.

The mean body weight increased exponentially with increasing instar number, with the major increase occurring between the IV to VI instars. No overlap was found in the 95% confidence limits for the different instars (Table A.3).

B. TOXICOLOGICAL STUDIES

The results for all the bioassay experiments were analysed by a computer program using the Maximum Likelihood Iterative Procedure described for Probit analysis by Finney (1952).

B.1. Determination of LD_{50} values for DDT and malathion when applied topically to the larval instars of *M. brassicae*

The LD_{50} values for DDT and malathion when applied topically to the II to VI instar larvae are shown in Tables B.2 and B.3. With this method of application as well as in the injection and feeding methods, the LD_{50} values have been calculated as µg insecticide mg^{-1} insect body weight. In addition, the 95% confidence limits (C.L.) are given.

Due to the general lack of parallelism between probit-regression lines statistical analysis was not possible. Instead, the "relative susceptibility" obtained as a ratio of LD_{50} values for successive larval instars (Hewlett,

TABLE A.2 : Mean duration (days) of the developmental stages of *M. brassicae*.

Developmental stage	Mean duration (days)	Exact 95% C.L. (days)	
		Lower	Upper
Egg	4.18	2.21	6.14
Larval instars			
I	3.28	1.06	5.50
II	3.07	0.86	5.28
III	3.12	0.98	5.27
IV	2.90	0.55	5.22
V	4.55	2.13	6.97
VI	5.90	3.61	8.19
Prepupal	4.36	2.15	6.57
Total larval duration	27.24	25.91	28.57
Pupae*	18.63	16.73	20.53
Adults*	17.01	10.81	23.21
Total lifespan	62.85	57.38	68.32

* The sexes were not separated in these studies.

TABLE A.3 : Mean body weight (mg) measurements of the larval instars of *M. brassicae*.

Larval instar	Mean body weight (mg)	Exact 95% C.L. (mg)	
		Lower	Upper
I	0.49	0.09	0.89
II	3.45	2.49	4.41
III	6.77	6.53	7.01
IV	27.99	27.31	28.67
V	130.88	129.70	132.06
VI	304.60	301.22	307.98

TABLE B.2 : LD_{50} values (± 95% C.L.) obtained for the topical application of DDT to the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg) Lower	Upper	Regression equation	Slope B ± S.E.	*Change in susceptibility
II	3.45 ± 1.00	0.0301	0.0087	0.0073	0.0103	Y = 8.0506 + 2.4985X	2.4985 ± 0.2553	1.00
III	6.77 ± 1.05	0.0474	0.0070	0.0054	0.0086	Y = 7.8561 + 2.0907X	2.0907 ± 0.2368	1.24
IV	27.99 ± 2.09	0.3878	0.0138	0.0112	0.0171	Y = 6.1365 + 1.6521X	1.6521 ± 0.1396	0.50
V	130.88 ± 4.38	0.9673	0.0074	0.0043	0.0103	Y = 5.7367 + 1.3495X	1.3495 ± 0.1962	1.86
VI	304.61 ± 8.41	4.6365	0.0152	0.0128	0.0183	Y = 5.4565 + 2.1856X	2.1856 ± 0.1983	0.48

* Calculated as a ratio of LD_{50} values (µg mg^{-1}) for successive larval instars.

TABLE B.3 : LD_{50} values (± 95% C.L.) obtained for the topical application of malathion to the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg)		Regression equation	Slope B ± S.E.	Change in susceptibility
				Lower	Upper			
II	3.45 ± 1.00	0.0127	0.0037	0.0009	0.0095	Y = 6.2849 + 0.8048X	0.8048 ± 0.1194	1.00
III	6.77 ± 1.05	0.0495	0.0073	0.0026	0.0169	Y = 6.3746 + 1.0202X	1.0202 ± 0.1288	0.51
IV	27.99 ± 2.09	0.3168	0.0113	0.0024	0.0383	Y = 5.8626 + 1.1120X	1.1120 ± 0.2005	0.64
V	130.88 ± 4.38	1.3406	0.0102	0.0023	0.0292	Y = 5.3992 + 0.9872X	0.9872 ± 0.1891	1.11
VI	304.61 ± 8.41	3.8630	0.0127	0.0037	0.0391	Y = 5.2126 + 0.7450X	0.7450 ± 0.1254	0.80

per. comm.) was calculated.

For DDT (Table B.2) it was found that the IV and VI instars were the least susceptible. The II, III and V instars had similar LD_{50} values with overlapping 95% confidence limits. Only the probit-regression lines for the III and VI instars were parallel as indicated by their slopes (Table B.2).

The results for malathion were much more variable than those for DDT as is apparent from their 95% confidence limits (Table B.3). The overlap of the 95% confidence limits suggested that susceptibility to malathion was fairly stable throughout the larval life of the insect.

The probit-regression lines were only parallel between the III and V instars.

B.2. Determination of LD_{50} values for DDT and malathion when injected into larval instars of *M. brassicae*

Comparable response levels were obtained for both DDT and malathion when they were administered by injection (Tables B.4 and 5) to the IV, V and VI instar larvae. In the DDT treatments (Table B.4), although the LD_{50} increased from 0.0036 to 0.0069 $\mu g\ mg^{-1}$ for the IV and VI instars respectively the overlap of the 95% confidence limits demonstrated that the three instars show similar susceptibility to DDT when injected. As in the data obtained from the topical application experiments, heterogeneity of response within instars (as indicated by the slope of the Probit-regression line) varied considerably (Table B.4).

In contrast to the results for DDT, the slopes of the regression lines obtained for injected malathion were reasonably parallel particularly between the V and VI instars (Table B.5). However, the response of the

TABLE B.4 : LD_{50} values (± 95% C.L.) obtained for the injection of DDT into the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg) Lower	Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
IV	27.99 ± 2.09	0.0998	0.0036	0.0004	0.0044	Y = 5.8785 + 0.6879X	0.6879 ± 0.0185	1.00
V	130.88 ± 4.38	0.6609	0.0051	0.0040	0.0061	Y = 6.1403 + 1.603 X	1.603 ± 0.1311	0.71
VI	304.61 ± 8.41	2.0940	0.0069	0.0041	0.0118	Y = 5.753 + 1.3589X	1.3589 ± 0.1943	0.74

TABLE B.5 : LD_{50} values (± 95% C.L.) obtained for the injection of malathion into the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg) Lower	Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
IV	27.99 ± 2.09	0.0931	0.0033	0.0012	0.0074	Y = 5.9204 + 0.7039X	0.7039 ± 0.078	1.00
V	130.88 ± 4.38	0.6552	0.0050	0.0009	0.0186	Y = 5.5352 + 0.7484X	0.7484 ± 0.1548	0.66
VI	304.61 ± 8.41	1.8100	0.0059	0.0021	0.0147	Y = 5.47 + 0.7613X	0.7613 ± 0.1069	0.85

three instars did show a similar trend to the DDT treatments. Hence, while the actual LD_{50} values (Table B.5) increased with larval age their 95% confidence limits suggested no real differences in their individual responses to injected malathion.

B.3. Determination of LD_{50} values for DDT and malathion when applied in the diet of the larval instars of *M. brassicae*

As for the topical and injection methods of application the toxicity data from the feeding of DDT and malathion showed a lack of homogeneity in the response of the instars (Tables B.6 and 7). The probit-regression lines for both pesticides having non parallel slopes. The LD_{50} values for DDT were similar for the V and VI instars with considerable overlap between their 95% confidence limits (Table B.6). However, no overlap was found between the 95% confidence limits for malathion (Table B.7) and the LD_{50} values suggested that the V instar was about 2.5 times as susceptible to malathion taken orally than the VI instar. This difference in response to orally applied malathion was investigated further (Section D).

B.4. Contact application of insecticide

B.4.a. Determination of LD_{50} values for the contact application of DDT and malathion to the larval instars of *M. brassicae*

Results from the contact method were evaluated differently from the other methods of pesticide application used. The LD_{50} values were expressed as the concentration of insecticide on the substrate to which a number of insects were exposed. The amount of pesticide picked up per larva being unknown.

In the contact experiments it was also not possible to fix one time after which LD_{50} values could be calculated for all of the six

TABLE B.6 : LD_{50} values (± 95% C.L.) obtained for feeding of DDT to the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg) Lower	Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
V	130.88 ± 4.38	1.6755	0.0128	0.0101	0.0168	Y = 5.4053 + 1.3217X	1.3217 ± 0.1242	1·00.
VI	304.61 ± 8.41	2.3783	0.0078	0.0031	0.0193	Y = 5.9025 + 1.8092X	1.8092 ± 0.4383	1.64

TABLE B.7 : LD_{50} values (± 95% C.L.) obtained for feeding of malathion on the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg) Lower	Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
V	130.88 ± 4.38	13.2940	0.1016	0.0887	0.1345	Y = 3.8861 ± 1.9843X	1.9843 ± 0.4187	1·00
VI	304.61 ± 8.41	74.5575	0.2448	0.2138	0.3872	Y = 4.1173 ± 0.8848X	0.8848 ± 0.2424	0.42

TABLE B.6 : LD_{50} values (± 95% C.L.) obtained for feeding of DDT to the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg) Lower	Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
V	130.88 ± 4.38	1.6755	0.0128	0.0101	0.0168	Y = 5.4053 + 1.3217X	1.3217 ± 0.1242	1·00.
VI	304.61 ± 8.41	2.3783	0.0078	0.0031	0.0193	Y = 5.9025 + 1.8092X	1.8092 ± 0.4383	1.64

TABLE B.7 : LD_{50} values (± 95% C.L.) obtained for feeding of malathion on the larvae of *M. brassicae* 24 h after treatment.

Larval stage	Mean weight ± S.E. (mg)	LD_{50}/ Larva (µg)	LD_{50}/ BWT (µg mg^{-1})	Exact 95% C.L. (µg/mg) Lower	Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
V	130.88 ± 4.38	13.2940	0.1016	0.0887	0.1345	Y = 3.8861 ± 1.9843X	1.9843 ± 0.4187	1·00
VI	304.61 ± 8.41	74.5575	0.2448	0.2138	0.3872	Y = 4.1173 ± 0.8848X	0.8848 ± 0.2424	0.42

instars. In these experiments observations commenced 1 h after confinement of larvae and LD_{50} values were established from the 24 h exposure period onwards. This was to allow a comparison with the results of the previous three methods of insecticide application. No observations were made beyond 48 h because after this period surviving larvae were observed to chew the substrate (pesticide-treated filter paper). In addition, mortalities of 60-100% were noted in the I and II instars after 24 h. For these early stages therefore observations were terminated after 24 h.

Table B.8 shows the toxicity data obtained after 24 h for the I to IV instar larvae and after 36 h for the III, IV and VI instar larvae. The LD_{50} values at 24 h (µg/substrate) show in general a decrease in susceptibility with increasing instar from the I to the IV instar stage. The LD_{50} value for the II instar being lower than might have been expected. Parallelism was shown between the probit-regression lines for the II and III; and the I and IV instars.

After 36 h exposure to DDT the trend was less clear but the VI was considerably less sensitive than the other instars tested. The slopes of the Probit-regression lines were only parallel between the III and VI instars (Table B.8).

After 48 h exposure to DDT no trend at all was apparent (Table B.9), the V instar being the least susceptible and the IV instar the most susceptible. The Probit-regression lines were only parallel between the V and VI instars.

Contact exposure to malathion for 24 h showed much clearer differences between the instars. The early instars (I to III) were the most susceptible with relatively little difference between their LD_{50}

TABLE B.8 : LD_{50} values (± 95% C.L.) obtained for the contact application of DDT to the larvae of *M. brassicae* after 24 h and 36 h exposure.

Exposure h	Larval instar*	Mean weight ± S.E. (mg)	LD_{50} (µg/substrate)	Exact 95% C.L. Lower	Exact 95% C.L. Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
24	I	0.49 ± 0.04	154.8	57.0	420.6	Y = 6.187 + 0.6555X	0.6555 ± 0.057	1.00
	II	3.45 ± 1.00	23.7	8.9	62.6	Y = 7.131 + 0.8115X	0.8115 ± 0.072	6.50
	III	6.77 ± 1.05	1,103.0	71.0	1,714.3	Y = 5.725 + 0.7570X	0.7570 ± 0.070	0.02
	IV	27.99 ± 2.09	1,950.0	1,037.0	3,667.0	Y = 5.456 + 0.6417X	0.6417 ± 0.065	0.57
36	III	6.77 ± 1.05	356.9	121.6	1,049.4	Y = 6.194 + 0.8249X	0.8249 ± 0.072	1.00
	IV	27.99 ± 2.09	166.9	90.4	307.6	Y = 6.042 + 0.5863X	0.5863 ± 0.053	2.140
	VI	304.61 ± 8.41	2,928.0	1,371.0	6,253.0	Y = 5.431 + 0.8084X	0.8084 ± 0.122	0.057

* Only larval instars with results obtainable at the stated exposure time are given.

TABLE B.9 : LD_{50} values (± 95% C.L.) obtained for the contact application of DDT to the larvae of *M. brassicae* after 48 h exposure.

Larval stage*	Mean weight ± S.E. (mg)	LD_{50} (µg/substrate)	Exact 95% C.L.		Regression equation	Slope B ± S.E.	Change in susceptibility
			Lower	Upper			
III	6.77 ± 1.05	142.4	88.4	230.0	Y = 6.619 + 0.877 X	0.877 ± 0.076	1.00
IV	27.99 ± 2.09	32.5	19.0	55.7	Y = 6.814 + 0.729 X	0.729 ± 0.063	4.38
V	130.88 ± 4.38	512.0	273.4	957.6	Y = 6.306 + 1.012 X	1.012 ± 0.128	0.06
VI	304.61 ± 8.41	350.4	189.8	648.0	Y = 6.542 + 1.059 X	1.059 ± 0.134	1.46

* Only larval instars with results obtainable at the 48 h exposure are given.

TABLE B.10 : LD_{50} values (± 95% C.L.) obtained for the contact application of malathion to the larvae of *M. brassicae* after 24 h exposure.

Larval instar	Mean weight ± S.E. (mg)	LD_{50} (µg/substrate)	Exact 95% C.L.		Regression equation	Slope B ± S.E.	Change in susceptibility
			Lower	Upper			
I	0.49 ± 0.04	0.4	0.01	18.0	Y = 7.205 + 0.5023X	0.5023 ± 0.066	1.0
II	3.45 ± 1.00	4.0	1.0	15.0	Y = 7.170 + 0.6381X	0.6381 ± 0.058	0.1
III	6.77 ± 1.05	0.9	0.1	21.0	Y = 7.862 + 0.7073X	0.7073 ± 0.106	4.4
IV	27.99 ± 2.09	2,925.0	735.4	11,633.0	Y = 5.369 + 0.6916X	0.6916 ± 0.069	0.0003
V	130.88 ± 4.38	43,390.0	23,884.0	78.832.0	Y = 3.689 + 2.0570X	2.0570 ± 0.344	0.07
VI	304.61 ± 8.41	144,800.0	77,625.0	270,396.0	Y = 3.680 + 1.1370X	1.1370 ± 0.254	0.30

TABLE B.11 : LD_{50} values (± 95% C.L.) obtained for the contact application of malathion to the larvae of *M. brassicae* after 36 h and 48 h exposure.

Exposure h	Larval instar*	Mean weight ± S.E. (mg)	LD_{50} (µg/substrate)	Exact 95% C.L. Lower	Exact 95% C.L. Upper	Regression equation	Slope B ± S.E.	Change in susceptibility
36	IV	27.99 ± 2.09	788.0	108.4	5,716	Y = 5.718 + 0.6506X	0.6506 ± 0.058	1.00
	V	130.88 ± 4.38	25,480.0	16,975.0	43,251	Y = 4.294 + 1.7390X	1.7390 ± 0.259	0.03
	VI	304.61 ± 8.41	57,530.0	35,132.0	94,211	Y = 4.098 + 1.1870X	1.1870 ± 0.218	0.44
48	IV	27.99 ± 2.09	320.0	37.4	2,735.0	Y = 5.923 + 0.6178X	0.6178 ± 0.052	1.00
	V	130.88 ± 4.38	15,730.0	9,993.0	24,746.0	Y = 4.693 + 1.5600X	1.5600 ± 0.227	0.02
	VI	304.61 ± 8.41	33,130.0	21,527.0	50,982.0	Y = 4.234 + 1.4720X	1.4720 ± 0.232	0.47

* Only larval instars with results obtainable at 36 h exposure are given.

values (Table B.10). The IV and VI instars showed a decrease in susceptibility of several orders of magnitude with a progressively decreasing susceptibility from the IV to the VI instars. The Probit-regression lines were reasonably parallel between the II, III and IV instars. The results for the 36 h and 48 h exposures (Table B.11) showed a similar trend from the IV to VI instars. However, the slopes of the Probit-regression lines were only parallel for the V and VI instars after 48 h exposure.

B.4.b. Determination of LT_{50} values for the contact application of DDT and malathion to the larval instars of *M. brassicae*

Besides the LD_{50} determinations made for the contact method of application LT_{50} values were estimated for some of the test concentrations. The LT_{50} value represents the time required to kill 50% of a population at a certain dose or concentration of pesticide (Matsumura, 1975).

Tables B.12 and B.13 show the time (h) required for 0.5 and 2.0% (w/v) DDT to kill 50% of the insect population. The LT_{50} values for 0.5% varied considerably for the different instars. Rather unexpectedly, the V instar took the longest time followed in order by the VI, IV, III, I and finally the II instar (Table B.12). In contrast, the LT_{50} values for 2.0% (w/v) DDT showed the VI instar to have longest LT_{50}, followed by the V, IV, II, III and finally the I instar (Table B.13).

Rather clearer results were obtained with the malathion treatments (Tables B.14 and B.15). At both 10% (v/v) and 20% (v/v) malathion the LT_{50} value increased progressively with increasing instar number. The 95% confidence limits showed that the first three instars responded in considerably less time than the later instars.

For both insecticides, there were generally much greater differences in the LT_{50} values between IV to VI instars than between I to III

TABLE B.12 : LT_{50} values (± 95% C.L.) obtained for the contact application of 0.5% (w/v) DDT to the larvae of *M. brassicae*.

Larval stage	Mean weight ± S.E. (mg)	LT_{50} (h)	Exact 95% C.L. (h)		Regression equation	Slope B ± S.E.
			Lower	Upper		
I	0.49 ± 0.04	11.76	7.20	19.21	Y = 3.344 + 1.547X	1.547 ± 0.18
II	3.45 ± 1.00	10.93	9.87	12.13	Y = 0.322 + 4.503X	4.503 ± 0.48
III	6.77 ± 1.05	19.11	14.11	25.85	Y = 2.749 + 1.757X	1.757 ± 0.17
IV	27.99 ± 2.09	19.35	17.45	21.50	Y = 0.009 + 3.879X	3.879 ± 0.33
V	130.88 ± 4.38	39.14	35.45	43.25	Y = 6.259 + 7.069X	7.069 ± 1.39
VI	304.61 ± 8.41	31.06	26.67	36.14	Y = 0.700 + 3.820X	3.820 ± 0.58

TABLE B.13 : LT_{50} values (± 95% C.L.) obtained for the contact application of 2.0% (w/v) DDT to the larvae of *M. brassicae*.

Larval stage	Mean weight ± S.E. (mg)	LT_{50} (h)	Exact 95% C.L. (h)		Regression equation	Slope B ± S.E.
			Lower	Upper		
I	0.49 ± 0.04	3.89	2.77	5.46	Y = 3.373 + 2.759X	2.759 ± 0.24
II	3.45 ± 1.00	7.73	5.80	10.29	Y = -0.218 + 5.876X	5.876 ± 0.67
III	6.77 ± 1.05	6.37	5.38	7.55	Y = 1.637 + 4.183X	4.183 ± 0.39
IV	27.99 ± 2.09	10.29	8.28	12.77	Y = 2.473 + 2.496X	2.496 ± 0.19
V	130.88 ± 4.38	21.23	18.89	23.84	Y = -4.452 + 7.122X	7.122 ± 1.08
VI	304.61 ± 8.41	22.71	20.04	25.71	Y = -2.252 + 5.347X	5.347 ± 0.69

TABLE B.14 : LT_{50} values (± 95% C.L.) obtained for the contact application of 10% (v/v) malathion to the larvae of *M. brassicae*.

Larval stage*	Mean weight ± S.E. (mg)	LT_{50} (h)	Exact 95% C.L. (h)		Regression equation	Slope B ± S.E.
			Lower	Upper		
I	0.49 ± 0.04	2.23	1.76	2.80	Y = 4.058 + 2.712X	2.712 ± 0.22
II	3.45 ± 1.00	2.59	2.02	3.34	Y = 3.968 + 2.488X	2.488 ± 0.19
III	6.77 ± 1.05	3.08	2.64	3.59	Y = 3.772 + 2.512X	2.512 ± 0.22
IV	27.99 ± 2.09	8.71	7.59	9.97	Y = 1.504 + 2.843X	2.843 ± 0.28
V	130.88 ± 4.38	12.25	9.55	15.70	Y = 2.940 + 1.894X	1.894 ± 0.29

* Only larval instars with results obtainable at the 10% (v/v) malathion are given.

TABLE B.15 : LT_{50} values (± 95% C.L.) obtained for the contact application of 20% (v/v) malathion to the larvae of *M. brassicae*.

Larval stage	Mean weight ± S.E. (mg)	LT_{50} (h)	Exact 95% C.L. (h)		Regression equation	Slope B ± S.E.
			Lower	Upper		
I	0.49 ± 0.04	0.89	0.73	1.05	Y = 5.140 + 2.453X	2.453 ± 0.26
II	3.45 ± 1.00	1.21	1.03	1.42	Y = 4.789 + 2.566X	2.566 ± 0.24
III	6.77 ± 1.05	1.55	1.27	1.88	Y = 4.527 + 2.470X	2.470 ± 0.27
IV	27.99 ± 2.09	4.14	3.69	4.62	Y = 2.228 + 4.496X	4.496 ± 0.45
V	130.88 ± 4.38	7.65	6.32	9.26	Y = 2.165 + 3.208X	3.208 ± 0.44
VI	304.61 ± 8.41	12.14	9.29	15.83	Y = 3.243 + 1.620X	1.620 ± 0.22

instars (e.g. Table B.15).

C. FEEDING EXPERIMENTS

Where appropriate data in this section was subjected to ANOVA (see appendices) using a MINITAB computer program. For comparisons within treatments or instars the computed 95% confidence limits were used. For comparison of feeding rates, data was standardised to 4 h periods before ANOVA.

C.1. Effects of DDT and malathion on the rate of feeding by larval instars of *M. brassicae* on cabbage leaf discs

C.1.a. Number of larvae (out of 50) which completely consumed insecticide treated leaf discs

The results are presented as the cumulative numbers of larvae which completely consumed leaf discs within different time periods.

With DDT, as the concentration of insecticide increased the number of insects completely consuming the leaf disc declined for all instars examined (Fig. C.1). The time taken for the first individual to complete a leaf disc also increased with increasing concentrations of DDT. In both cases, the effects were most marked in the IV instar, least in the VI instar. Significantly, fewer IV instar larvae completed the discs compared to the V and VI instars up to 0.5% (w/v) DDT (Appendix Table C.1, $P < 0.01$). Above this concentration no IV instar larvae completely consumed a disc within 24 h. Similarly, no V instar completed the 2.0% (w/v) DDT-treated discs. However, in the VI instars over 50% of larvae consumed the leaf discs treated with 2.0% (w/v) DDT (Fig. C.1).

Within each instar, the number completing leaf discs increased

Fig. C.1 : Cumulative number of IV, V and VI instar larvae of *M. brassicae* which completely consumed a DDT-treated cabbage "Leaf-sandwich" at different time intervals.

% (w/v) DDT

(1) R.17 oil control

(2) 0.025

(3) 0.050

(4) 0.100

(5) 0.250

(6) 0.500

(7) 1.000

(8) 2.000

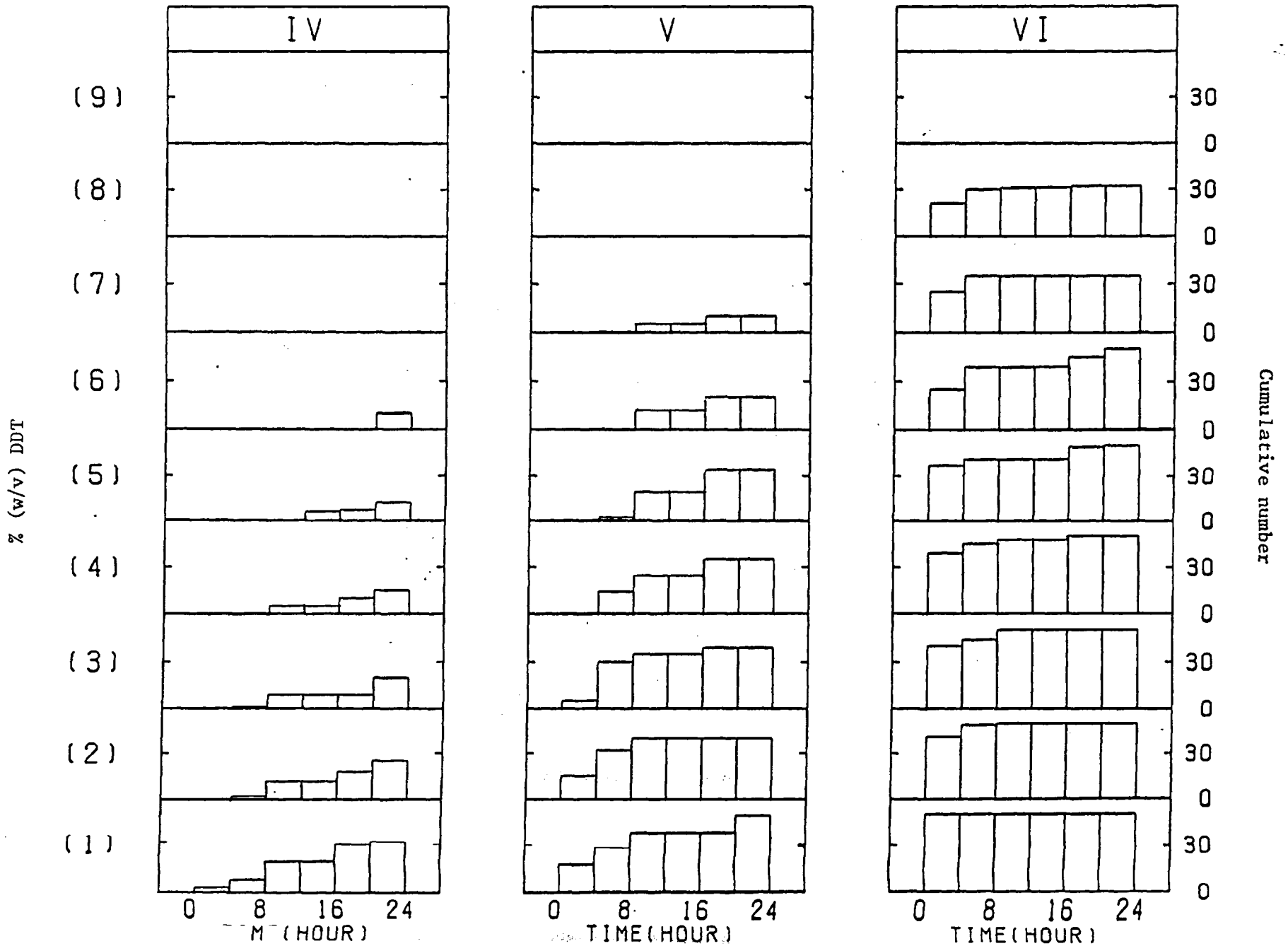

IV
V
VI
(9)
(8)
(7)
(6)
(5)
(4)
(3)
(2)
(1)
% (w/v) DDT
0
8
16
24
TIME(HOUR)
30
0
Cumulative number

Fig. C.2 : Cumulative number of IV, V and VI instar larvae of *M. brassicae* which completely consumed a malathion-treated cabbage "Leaf-sandwich" at different time intervals.

	% (v/v) Malathion
(1)	Olive oil control
(2)	0.00125
(3)	0.0125
(4)	0.025
(5)	0.25
(6)	1.25
(7)	2.5
(8)	5.0
(9)	10.0

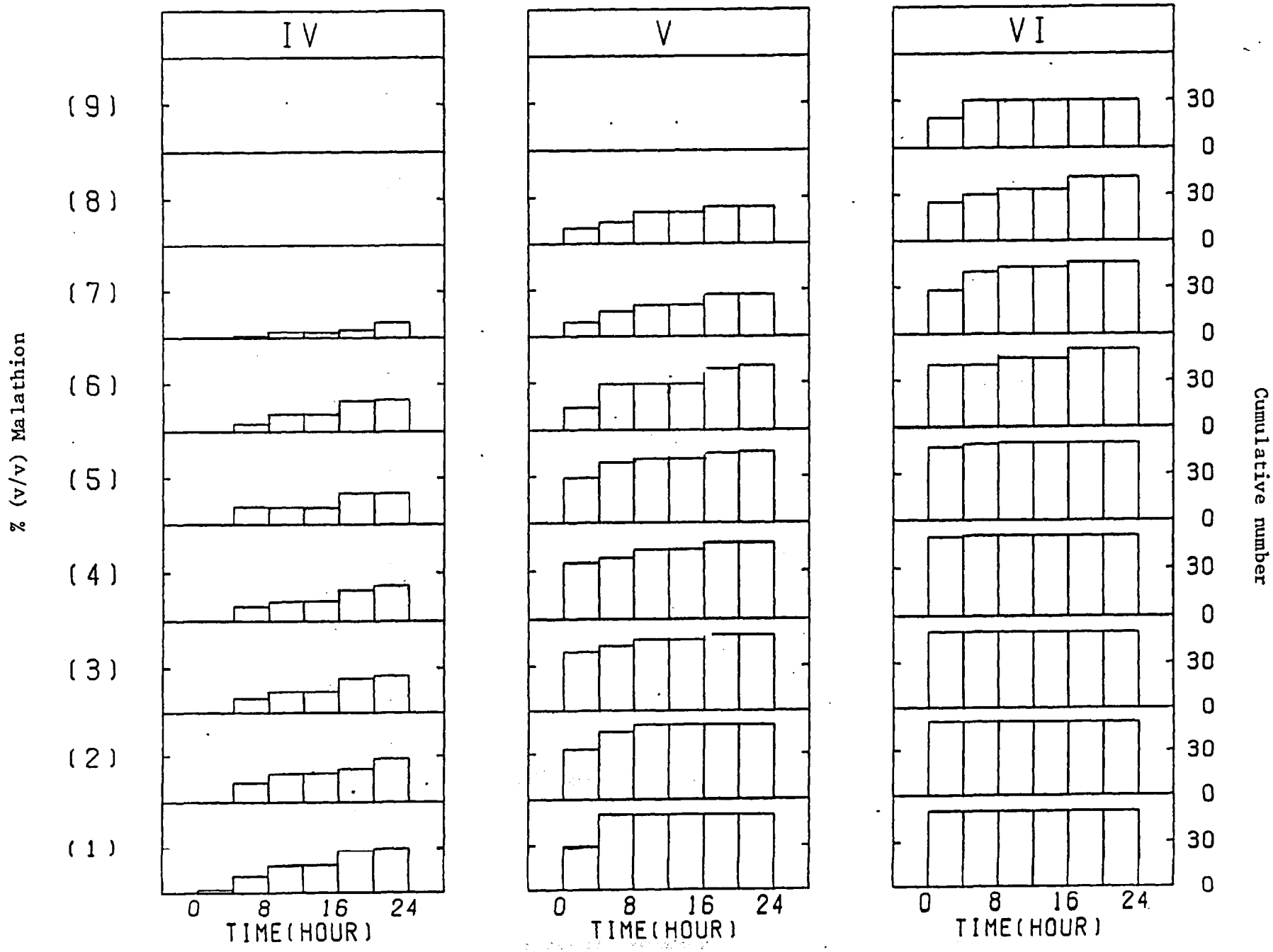

IV
V
VI
(9)
(8)
(7)
(6)
(5)
(4)
(3)
(2)
(1)
% (v/v) Malathion
0
8
16
24
TIME(HOUR)
30
0
Cumulative number

TABLE C.1 : LD_{50} values (± 95% C.L.) for larvae of *M. brassicae* on insecticide-treated leaf discs after 24 h in the rate of feeding experiments (leaf sandwich method).

Insecticide	Larval instar	LD_{50} (%)	Exact 95% C.L.		Regression equation
			Lower	Upper	
DDT (w/v)	IV	0.2102	0.1679	0.2632	Y = 6.086 + 1.603X
	V	0.4360	0.3355	0.5664	Y = 5.497 + 1.379X
	VI	0.8119	0.5991	1.1003	Y = 5.124 + 1.366X
Malathion (v/v)	IV	0.3606	0.2519	0.5159	Y = 5.533 + 1.204X
	V	3.239	2.4865	4.2141	Y = 4.143 + 1.679X
	VI	11.080	9.3090	13.2160	Y = 1.964 + 2.907X

with time and significantly fewer larvae completed the disc at the higher concentrations of DDT in a given time (Appendix Table C.1, $P < 0.01$).

Larval paralysis and mortality were observed after 24 h for all three instars (Appendix Table C.3). The LD_{50} values show a decrease in susceptibility with increasing larval age (Table C.1).

A similar pattern was observed with malathion-treated leaf discs (Fig. C.2); significant differences being observed both between the instars and the malathion treatments (Appendix Table C.3; $P < 0.01$), In the IV and V instars the majority of individuals completed the disc by 16-24 h; the VI instar larvae completed the disc in 4-8 h (Appendix Table C.2). Increasing the malathion concentration reduced the number completely consuming the disc in each instar (Appendix Table C.2). Larval paralysis and mortality were observed over 24 h (Appendix Table C.3b). The LD_{50} values for malathion clearly showed a decrease in susceptibility with increasing instar number (Table C.1).

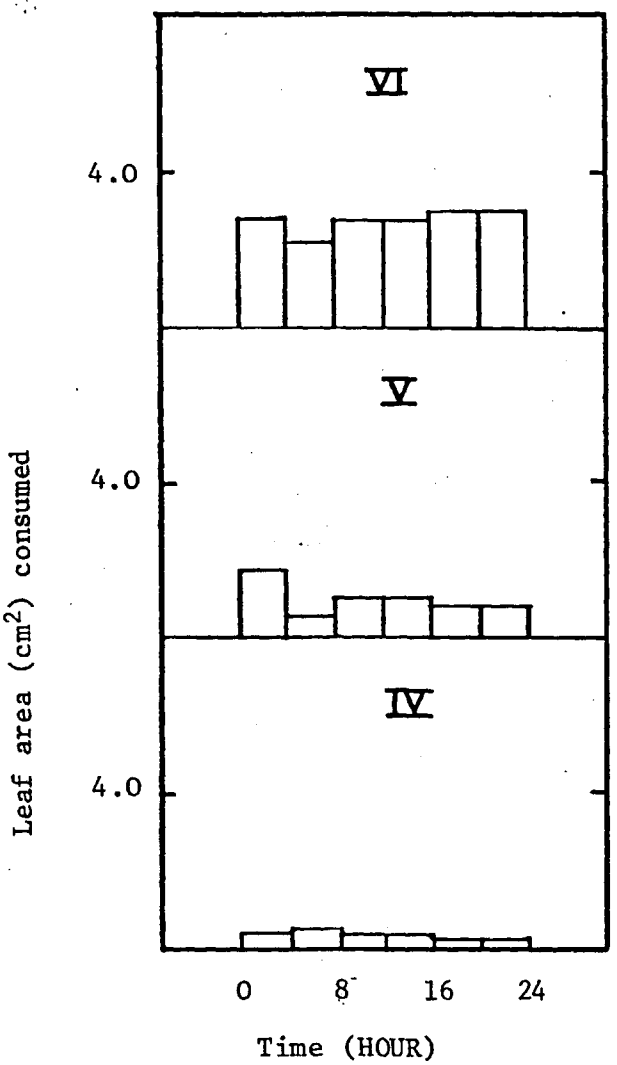
C.1.b. Area (cm^2) of cabbage leaf disc consumed in 24 h by individual larvae of *M. brassicae*

The results obtained by the gravimetric method are presented as the mean cabbage leaf area consumed within each time interval.

C.1.b.i. Area (cm^2) of untreated cabbage leaf discs consumed in 24 h by larval instars of *M. brassicae*

A progressive increase in leaf area consumed was observed from the IV to the VI instar, with the VI instar consuming significantly greater amounts at all times (Fig. C.3; Appendix Table C.4, $P < 0.01$). Within each instar however, the pattern of feeding differed. In the IV instar over 50% of the total amount eaten was in the initial 8 h period

Fig. C.3 : MEAN AREA (cm^2) OF AN UNTREATED CABBAGE LEAF DISC CONSUMED BY IV, V AND VI INSTAR LARVAE OF *M. BRASSICAE* AT VARIOUS TIME INTERVALS.



(Appendix Table C.5). The V instar was more variable, but showed the greatest consumption in the first 4 h, while the VI instar showed a relatively constant rate of feeding over the 24 h period (Fig. C.3; Appendix Table C.5).

C.1.b.ii. Area (cm^2) of insecticide-treated cabbage leaf consumed in 24 h by larval instars of *M. brassicae*

The results for DDT are shown in Fig. C.4. The presence of R.17 oil alone did not appear to greatly affect the pattern of leaf consumption by IV or V instars compared to that of untreated leaves (Appendix Tables C.6 and C.7). However, in the VI instar R.17 appeared to result in a significant decline (Appendix Table C.6; $P < 0.01$) in consumption after the initial 0-4 h period. The presence of DDT generally enhanced this decline in the VI instar and there was an overall trend for the rate of consumption to progressively decline with increasing concentration of DDT for all instars (Fig. C.4; Appendix Table C.7).

The concentration of DDT at which a 50% reduction in the total area consumed by the larvae occurred compared to the control appeared to be relatively constant between instars at 0.05-0.5% (w/v) DDT (Appendix Table C.7).

At all concentrations of DDT the VI instar ate at a significantly greater rate than the IV or V instars over the 24 h period (Appendix Tables C.6 and C.7; $P < 0.05$) although the results for different concentrations of DDT showed considerable variation in the pattern of feeding between different time intervals (Fig. C.4).

The LD_{50} values for DDT indicate that although an increase in value occurs with increasing instar number, the overlap in the 95% C.L.

Fig. C.4 : Mean area (cm^2) of a DDT-treated cabbage leaf disc consumed by IV, V and VI instar larvae of *M. brassicae* at various time intervals.

% (w/v) DDT

(1) R.17 oil control

(2) 0.0005

(3) 0.05

(4) 0.5

(5) 2.0

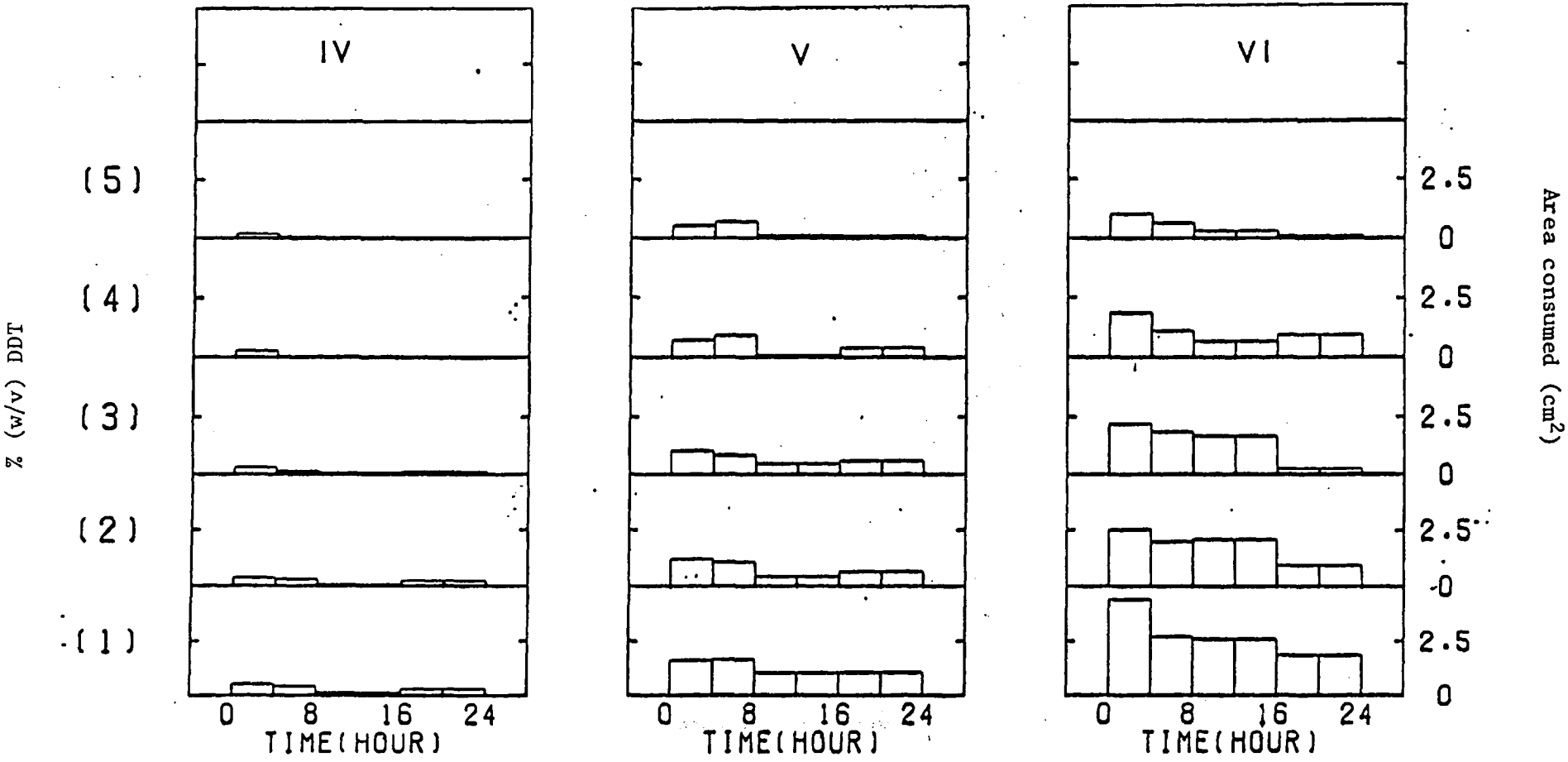
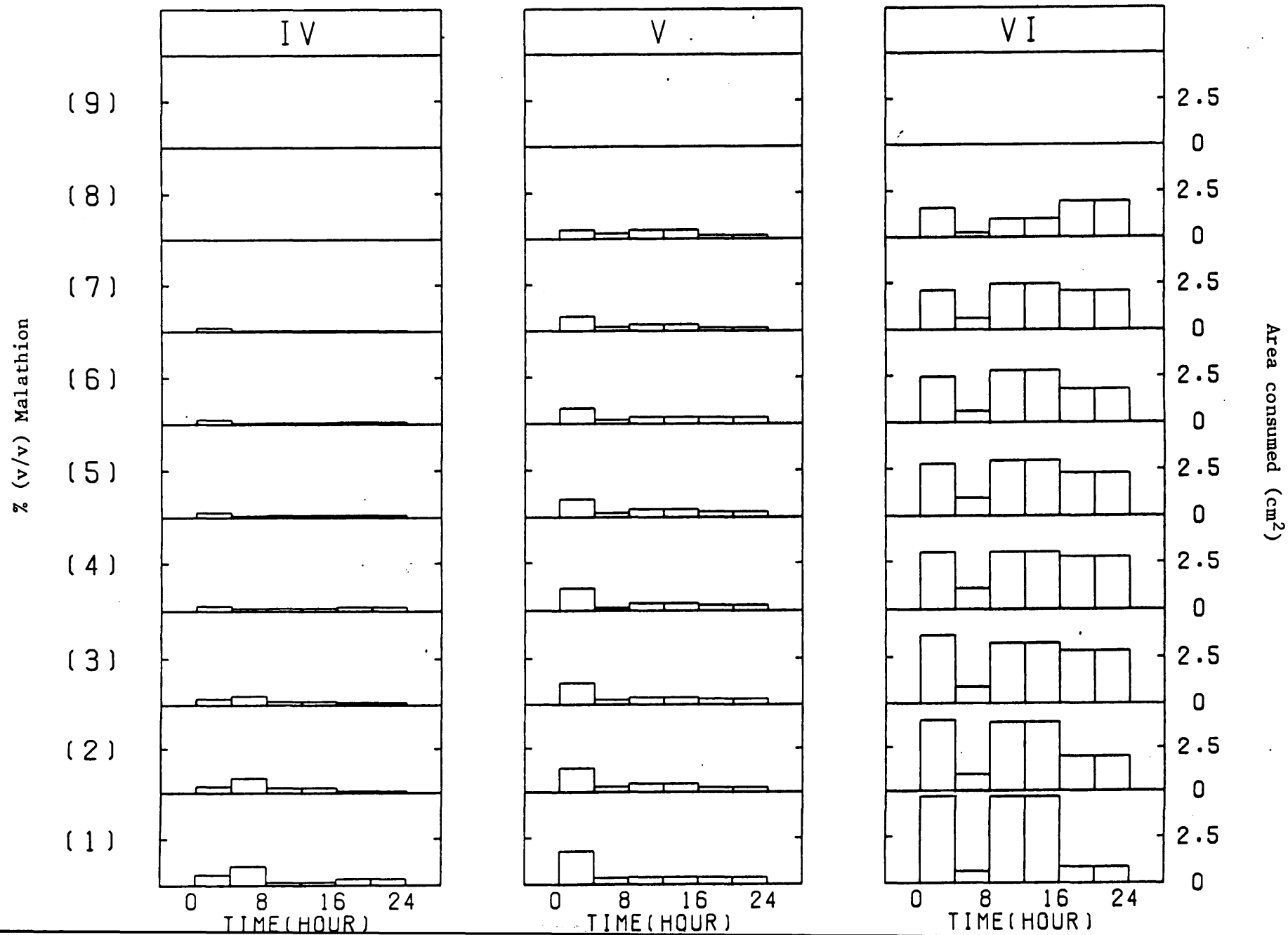

IV
V
VI
(5)
(4)
(3)
(2)
(1)
0
8
16
24
TIME(HOUR)
2.5
0
Area consumed (cm²)
DDT (v/w) %

Fig. C.5 : Mean area (cm^2) of a malathion-treated cabbage leaf disc consumed by IV, V and VI instar larvae of *M. brassicae* at various time intervals.

	% (v/v) Malathion
(1)	Olive oil control
(2)	0.00125
(3)	0.0125
(4)	0.025
(5)	0.25
(6)	1.25
(7)	2.50
(8)	5.0
(9)	10.0


IV
V
VI
(9)
(8)
(7)
(6)
(5)
(4)
(3)
(2)
(1)
% (v/v) Malathion
0
8
16
24
TIME(HOUR)
2.5
0
Area consumed (cm²)

implies similar susceptibility levels for the IV and V, and V and VI instars respectively; the VI instar being 480X more tolerant to DDT than the IV (Table C.2).

The effect of malathion on the area of cabbage leaf consumed differed somewhat from that of DDT (Fig. C.4 and C.5). With olive oil alone there was no apparent difference in the pattern of consumption by the IV and V instars compared to that of untreated leaves (Fig. C.3 and C.5). However, olive oil appeared to cause considerable fluctuation in the rate of feeding by VI instar larvae. The most noticeable feature being the reduction in feeding between 4-8 h, which was found consistently in control and malathion-treated leaf discs. The decline in feeding after 16 h in the controls (Fig. C.5) was due to lack of leaf material.

In the IV instar, 0.0125% (v/v) malathion significantly reduced consumption over 24 h by 40% (Fig. C.5; Appendix Tables C.9; $P < 0.01$); increasing the concentration of malathion further reduced the rate of consumption (Appendix Table C.8; $P < 0.01$). For the V and VI instars a different trend was observed. There was little decrease in feeding at increasing concentrations of malathion for the V, and in the VI instar there appeared to be an increase in leaf consumption after 16 h at the highest concentration of malathion compared to the olive oil control (Fig. C.5; Appendix C.8; $P < 0.01$).

The LD_{50} values increased with larval instar number, but considerable overlap of their individual 95% C.L. suggests a relatively constant susceptibility level for these three instars (Table C.2; Appendix Table C.10).

TABLE C.2 : LD_{50} values (± 95% C.L.) for larvae of *M. brassicae* on insecticide-treated leaf discs after 24 h in the rate of feeding experiments (Gravimetric method).

Insecticide	Larval instar	LD_{50} (%)	Exact 95% C.L.		Regression equation
			Lower	Upper	
DDT (w/v)	IV	0.0008	0.00001	0.0486	Y = 6.920 + 0.6205X
	V	0.1049	0.0116	0.9479	Y = 5.7520 + 0.7685X
	VI	0.3842	0.0553	2.6705	Y = 5.3680 + 0.8865X
Malathion (v/v)	IV	0.3962	0.0746	2.1047	Y = 5.3320 + 0.8251X
	V	6.0920	2.0012	18.5663	Y = 3.7760 + 1.5590X
	VI	9.7060	2.6090	36.1077	Y = 3.184 + 1.839 X

C.2. Feeding behaviour of the early instar larvae of *M. brassicae*

C.2.a. Preliminary behaviour experiments

C.2.a.i. Choice of feeding site on cabbage leaves by I instar larvae of *M. brassicae*

It was found that 93% of the larvae present preferred the lower epidermis of isolated cabbage leaves, only 2% the upper epidermis (Appendix Table C.11.a).

In a similar experiment with potted cabbage plants 96% of the I instar larvae present chose the lower epidermis (Appendix Table C.11.b).

Under the stereomicroscope, it was observed that the larvae first wandered over the surface, then selected a potential site and with their prolegs firmly attached, probed the leaf with their head. If no suitable spot was found, the larvae moved away and started probing again. Normally, feeding continued for up to 3 min, after which the larvae began to wander over the leaf, or the tissue paper lining the container.

While probing for a suitable feeding site larvae appeared antagonistic to each other but it was found that larvae would feed close together in groups and even from the same lesion. In most instances, feeding by I instar larvae on the lower epidermis did not penetrate to the upper epidermis.

C.2.a.ii. Factors influencing the choice of feeding sites by I and II instar larvae of *M. brassicae* on cabbage leaves

When the leaf was turned upside down so that the true lower

epidermis faced upwards towards the incident light the majority of I and II instar larvae (87% and 85% respectively) chose to feed or wander on the surface facing away from the light (Appendix Table C.12).

C.2.a.iii. Confirmation of the influence of light on the choice of feeding sites by early instar larvae of *M. brassicae*

In the dark; I and II instar larvae did not discriminate clearly between the lower and upper epidermis (Appendix Table C.13). In fact, in both instars significantly more larvae preferred the upper epidermis which was uppermost, to the lower epidermis ($P < 0.05$ I instar; $P < 0.05$ II instar), and the greatest proportion of larvae were found wandering elsewhere in the container.

C.2.a.iv. Feeding behaviour of early instar larvae of *M. brassicae* in the presence of insecticides

The results indicated that DDT had little or no effect on site selection by I instar larvae, 95% larvae being found on leaves treated with 2.0% (w/v) DDT compared to 93% on R.17 oil-treated leaves (Appendix Table C.14.a). In the II instars, DDT appeared to have a greater effect with significantly fewer larvae choosing the lower epidermis of leaves treated with higher concentrations of insecticides (0.2 and 2.0% (w/v) DDT; Appendix Table C.14.a; $P < 0.05$). At the 2.0% (w/v) DDT, 52% of larvae were found on the lower epidermis compared to 100% in R.17 controls. Risella 17 oil had no apparent effect on site selection by I and II instar larvae.

Malathion had a rather different effect on the two instars. At 0.00125 and 0.25% (v/v) malathion, most I instar larvae selected the lower surface but at 2.5% (v/v), no larvae were found on this surface (Appendix

Table C.14.b). In contrast, although all concentrations of malathion reduced the number of II instar larvae on the lower epidermis (Appendix Table C.14.b; $P < 0.05$) increasing the concentration had no apparent effect and 72.5% were still found on this surface in the presence of 2.5% (v/v) malathion compared to 87.5% in the olive oil control. Olive oil had no apparent effect on site selection by either I or II instar larvae.

The presence of R.17 oil appeared to slightly delay the amount of feeding by both I and II instar larvae on the leaf surface (Appendix Table C.15.a). In the I instar the addition of DDT had little further effect except at the highest concentration (2.0% w/v) where no feeding was observed. The effect of DDT on the II instar was less clear and there seemed to be no increased delay with increasing concentration of DDT.

Olive oil and malathion had similar effects to R.17 oil and DDT (Appendix Table C.15.b).

DDT and malathion both caused significant reductions in the amount of cabbage leaf area consumed by I and II instar larvae; feeding being reduced by the greatest amount at the highest concentration of insecticide (Appendix Table C.16; $P < 0.05$). Risella 17 oil had no significant effect on leaf consumption by I and II instars (Table C.3.a and b; Appendix Table C.16; $P < 0.01$). Olive oil had no effect on I instar larvae but appeared to slightly stimulate feeding by II instar larvae although there was an overlap between the 95% C.L. and the untreated controls (Table C.3.b).

The mortality of I and II instar larvae on insecticide treated leaf surfaces after 24 h is shown in Appendix Table C.17.a and b. The LD_{50} values appear to increase from the I instar to the II for both

TABLE C.3 : Effect of insecticides on the amount of cabbage leaf consumed after 24 h, by early instar larvae of *M. brassicae*.

a) DDT-treated cabbage leaf

Larval instars	Mean leaf area (cm^2) ± 95% C.L. consumed				
	Untreated control	Risella 17 oil-treated control	% (w/v) DDT		
			0.025	0.2	2.0
I	8.5 ± 1.7	8.3 ± 2.0	5.3 ± 2.8	2.8 ± 0.8	0.01 ± 0.02
II	16.4 ± 2.1	15.1 ± 0.8	11.5 ± 1.2	12.6 ± 1.6	1.3 ± 0.9

b) Malathion-treated cabbage leaf

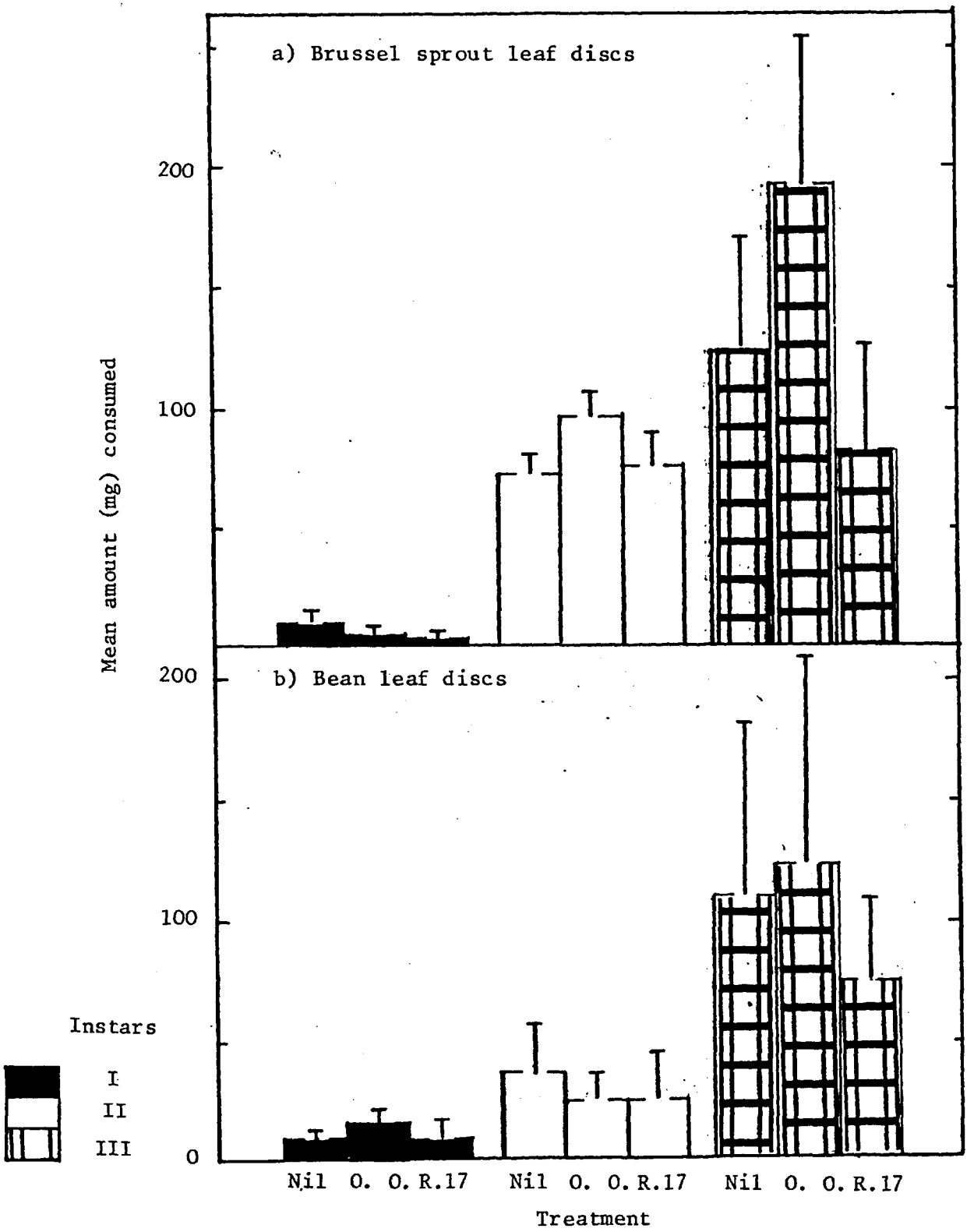
Larval instars	Mean leaf area (cm^2) ± 95% C.L. consumed				
	Untreated control	Olive oil-treated control	% v/v Malathion		
			0.00125	0.25	2.5
I	6.8 ± 1.4	6.5 ± 0.8	3.7 ± 1.4	2.1 ± 1.2	0.2 ± 0.1
II	10.0 ± 2.7	14.1 ± 2.2	11.7 ± 1.3	8.1 ± 1.2	1.7 ± 0.9

TABLE C.4 : LD_{50} values (± 95% C.L.) for the I and II instar larvae of *M. brassicae* exposed to insecticide-treated cabbage leaf for 24 h.

Insecticide	Larval instar	LD_{50} (%)	Exact 95% C.L.		Regression equation
			Lower	Upper	
DDT (w/v)	I	0.2 - 0.4*	-	-	-
	II	0.6	0.40	3.00	Y = 5.6 + 2.3X
Malathion (v/v)	I	0.03	0.01	0.10	Y = 5.9 + 0.6X
	II	0.70	0.33	1.67	Y = 5.1 + 0.9X

* Rough estimate (see Appendix C.16.a)

Fig. C.6 : MEAN AMOUNT (± 95% C.L.) OF LEAF DISCS CONSUMED BY 15 I, II OR III INSTAR LARVAE OF *M. BRASSICAE* AFTER 24 h IN A "THREE-WAY" CHOICE CHAMBER CONTAINING AN UNTREATED LEAF DISC (Nil), A LEAF DISC TREATED WITH OLIVE OIL (O. O.) AND A LEAF DISC TREATED WITH R.17 OIL (R.17).



insecticides (Table C.4).

C.2.b. Feeding of early instar larvae of *M. brassicae* in different choice chambers

The units of insecticide concentration were changed from % to ppm ($\mu g\ cm^{-3}$) in the figures. These units are therefore used interchangeably in this section.

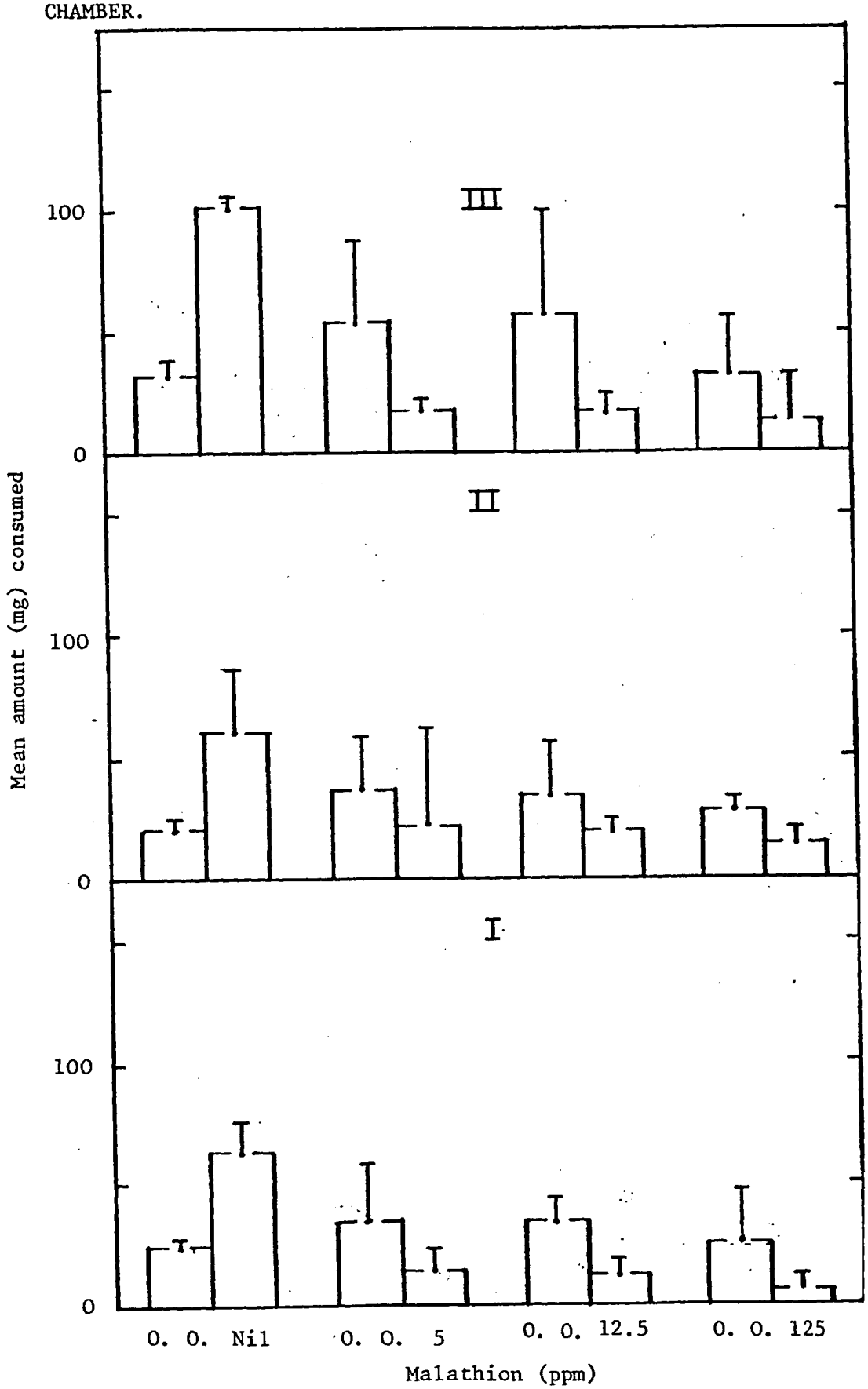
C.2.b.i. Feeding of the early instar larvae of *M. brassicae* in a "Three-way" choice chamber

The results for I, II and III instar larvae for Brussel sprout and Bean leaves are presented in Figs. C.6.a, b. For both crops, the total amount consumed within each choice chamber increased significantly with instar (Appendix Table C.18.a, b; $P < 0.05$). The effect of the treatment on the choice of each instar was different for the two crops.

In the Brussel sprout experiments, the I instar seemed to prefer the untreated control (NIL) leaf discs and consumed the least of R.17 oil treated discs although the differences were not significant ($P < 0.05$). The olive oil-treated disc was significantly more palatable than the untreated disc to the II instar (Appendix Table C.18; $P < 0.05$) while virtually the same amounts of the R.17-treated and untreated leaf discs were consumed. In the III instar the Olive oil-treated leaf disc appear to be the most palatable (Fig. C.6.a).

The effect of the different treatments on the amount of Bean leaf discs consumed was more variable (Fig. C.6.b). In the I instar larvae, the olive oil-treated disc appeared to be more palatable than either the untreated or R.17-treated leaf discs; but in the II and III instars there were no clear trends (Fig. C.6.b).

Fig. C.7 : MEAN AMOUNT (± 95% C.L.) OF OLIVE OIL (O. O.) AND MALATHION-TREATED BRUSSEL SPROUT LEAF DISCS CONSUMED BY 20 I, II OR III INSTAR LARVAE OF *M. BRASSICAE* AFTER 24 h IN A "TWO-WAY" CHOICE CHAMBER.



C.2.b.ii. Feeding of the early instar larvae of *M. brassicae* in a "Two-way" choice chamber

The results for chambers containing a malathion-treated leaf disc are shown in Fig. C.7. The total amount consumed for each treatment combination was greater for the III instar (Appendix Table C.19) than for the II and I instars in which similar quantities were consumed. In each instar a clear-cut preference was shown for the untreated leaf disc compared to the olive oil treatment (Appendix Table C.19; $P < 0.05$). In the chambers with insecticides however, the olive oil-treated leaf appeared to be preferred by all three instars (Appendix Table C.19).

There appeared to be an overall trend for the total amount of leaf disc eaten to decline from the control chambers to the chambers with the greatest concentration of malathion (Fig. C.7). In a similar experiment, where DDT-treated cabbage or Bean leaf discs were offered to the larvae the total amount consumed was greatest for the III instar, and least for the I (Appendix Table C.20.a, b; $P < 0.05$).

No obvious preference was shown for the untreated control over the R.17-treated cabbage leaf disc in the I instar; in the II and III instars the former appeared to be preferred (Fig. C.8.a). For cabbage, in cases where DDT was present the mean amount consumed from the R.17 controls was greater than that from DDT-treated leaves, and with 2 and 25 ppm (0.0002 and 0.0025%) DDT the amounts were significantly greater for the I and II, and the II and III instars respectively (Appendix Table C.20; $P < 0.05$).

The total amount consumed by I and II instars did not appear to be greatly affected by the presence of DDT for cabbage leaves but in the III instar, the total amount consumed was significantly reduced

Fig. C.8 : MEAN AMOUNT (± 95% C.L.) OF R.17 OIL (R.17) AND DDT-TREATED LEAF DISCS CONSUMED BY 20 I, II OR III INSTAR LARVAE OF *M. BRASSICAE* AFTER 24 h IN A "TWO-WAY" CHOICE CHAMBER.

a) Cabbage leaf discs

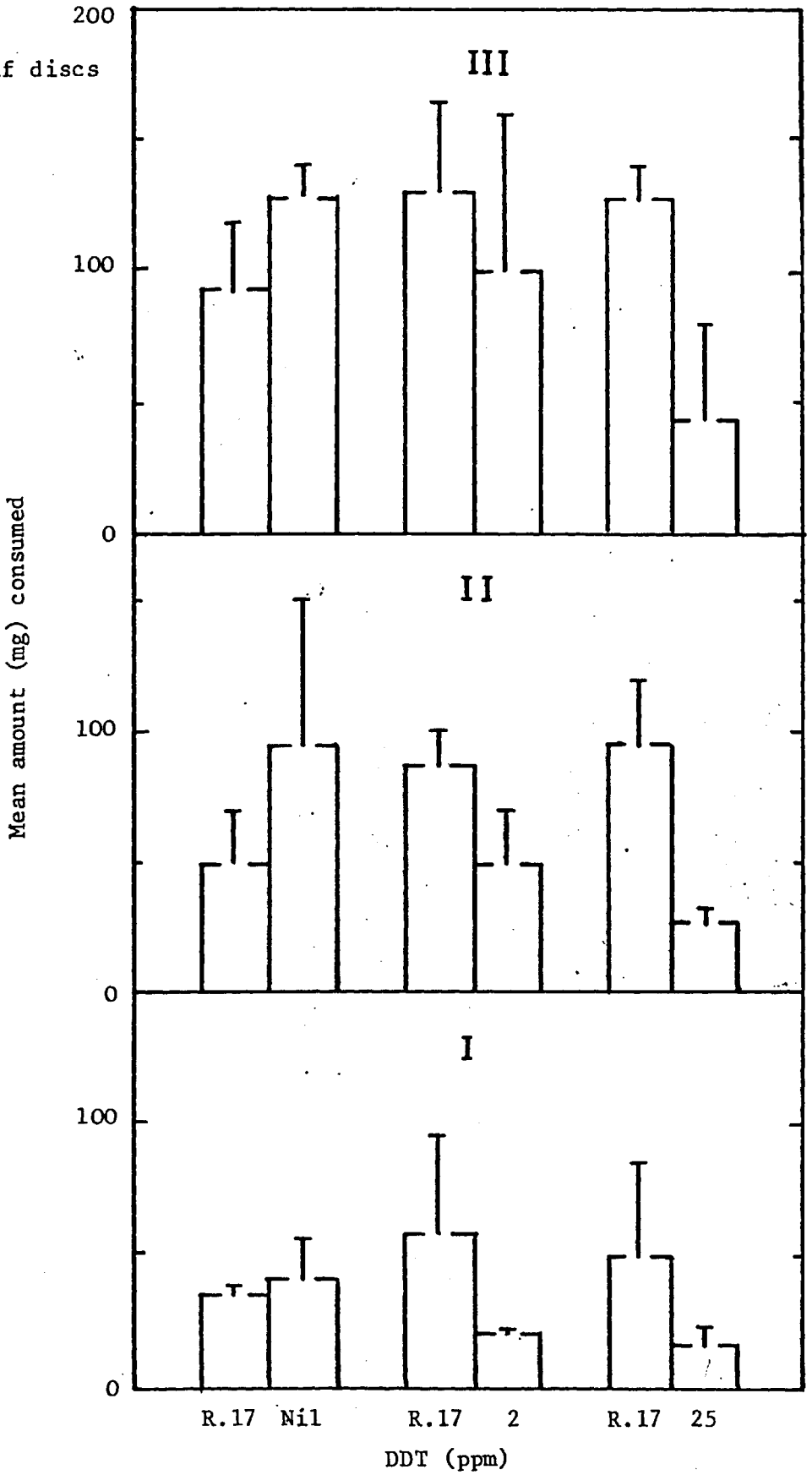


Fig. C.8 : Continued

b) Bean leaf discs

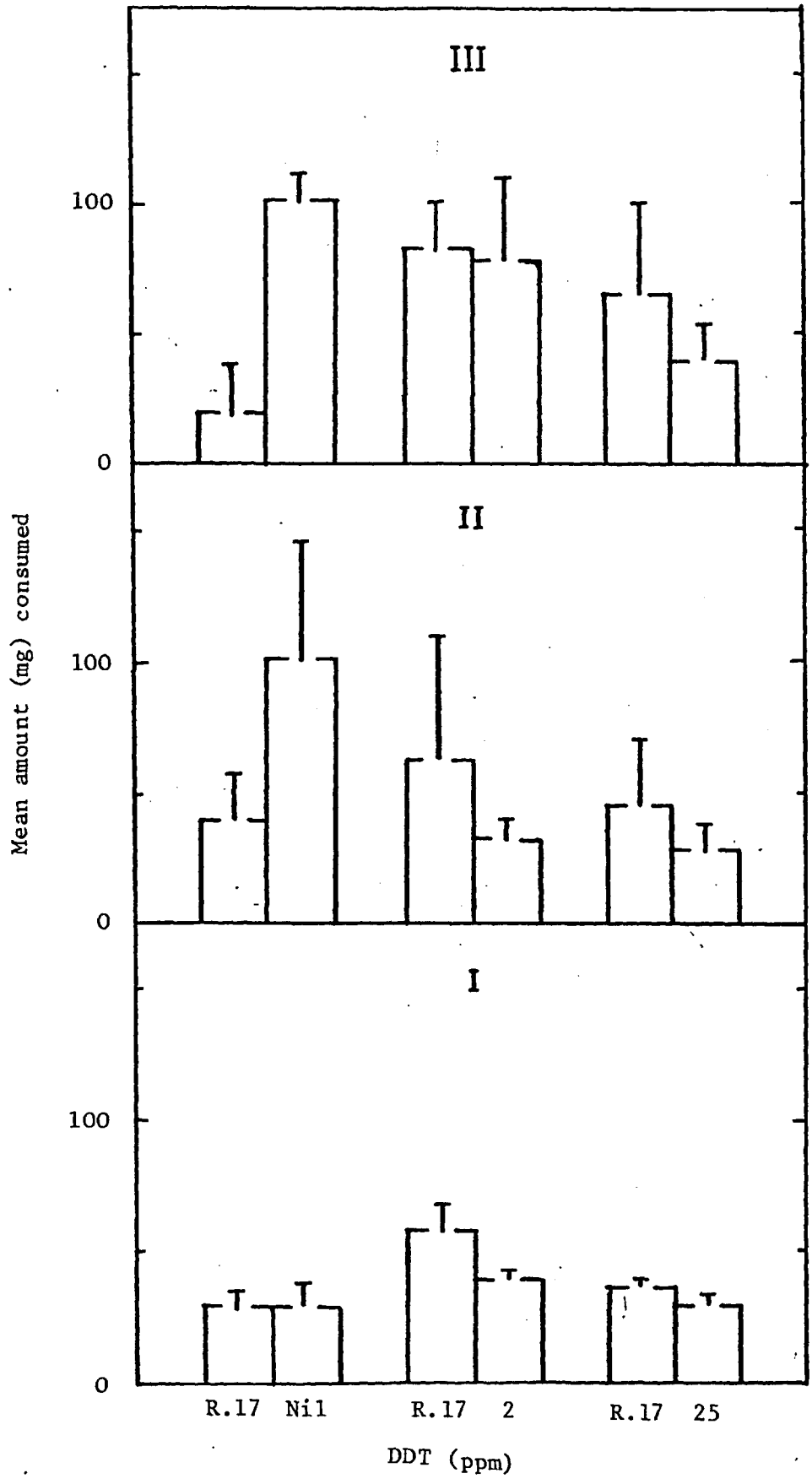
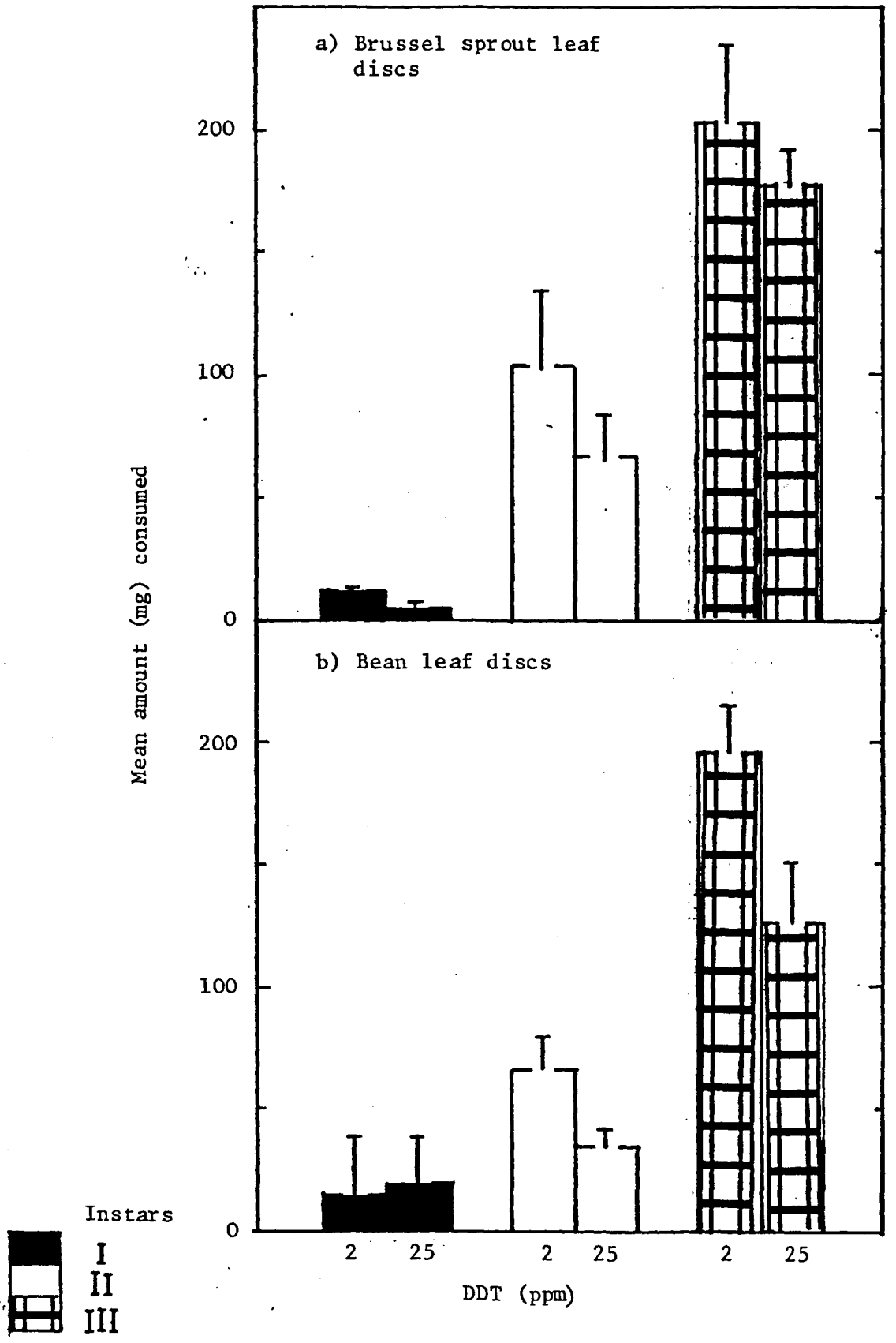


Fig. C.9 : MEAN AMOUNT (± 95% C.L.) OF DDT-TREATED LEAF DISCS CONSUMED BY 20 I, II OR III INSTAR LARVAE OF *M. BRASSICAE* AFTER 24 h IN A "TWO-WAY" CHOICE CHAMBER.



at 25 ppm (w/v) DDT (Appendix Table C.20; $P < 0.05$).

The difference between treatments for beans was less apparent (Fig. C.8.b). The I instar showed no preference towards the untreated control compared to the R.17 treatment, but the II and particularly the III ($P < 0.05$) showed a clear preference for the untreated controls. The total amount consumed by the II instar generally fell with increasing DDT concentration (Appendix Table C.20.b), but in I and III instars the total consumed was greatest when 2 ppm (0.0002%) DDT was present. A similar amount was consumed in the presence of 25 ppm DDT as in the control chambers (Fig. C.8.b).

Where the two DDT treatments were compared directly in a "Two-way" choice chamber, the amount of Brussel sprout or Bean leaf consumed by successive instars significantly increased (Figs. C.9.a, b; Appendix Table C.21; $P < 0.05$). With Brussel sprout leaf discs, significantly greater amounts of the 2 ppm treated disc were consumed by the I instar than the 25 ppm disc (Appendix Table C.21.a; $P < 0.05$), no significant differences were found for the II and III instars (Fig. C.9.a; Appendix C.21.a; $P > 0.05$).

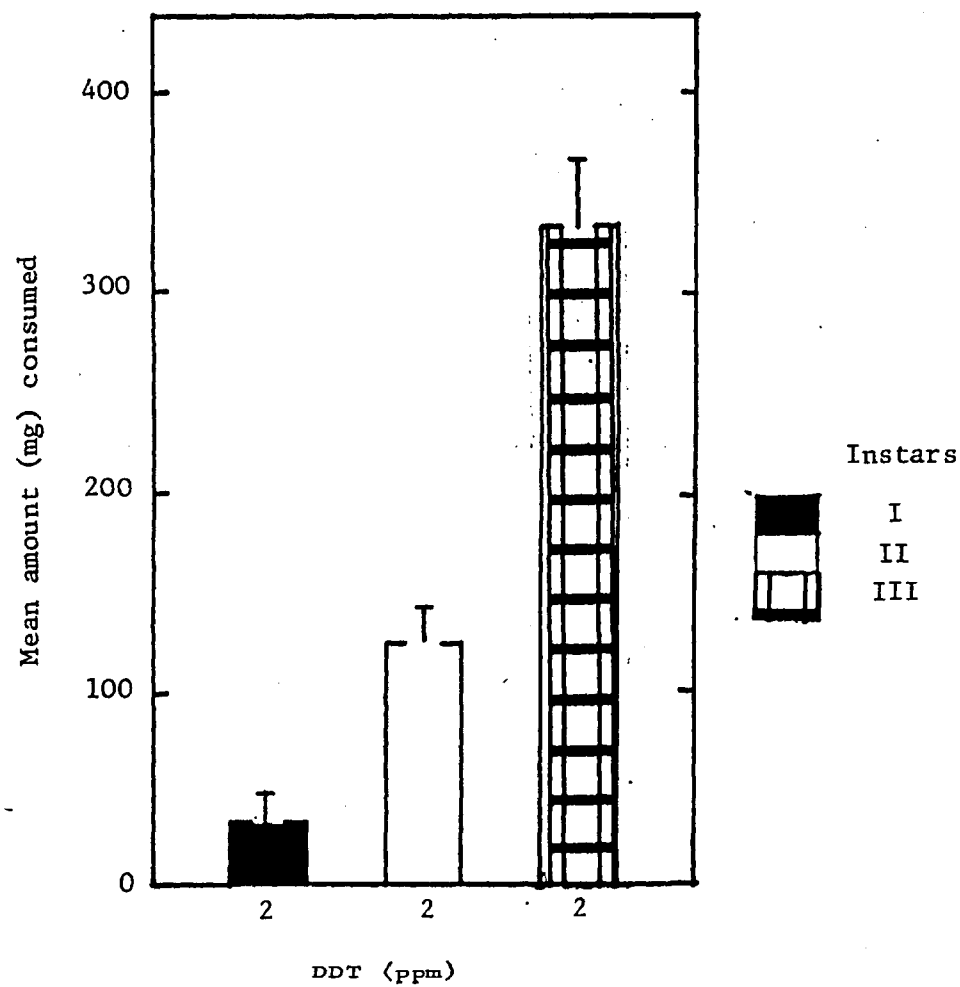
In contrast, for Bean leaves while the I instar larvae showed no significant difference in amounts consumed between the 2 and 25 ppm treated discs ($P > 0.05$) significantly less of the 25 ppm treated Bean leaf discs were consumed by the II and III instars (Fig. C.9.b; Appendix Table C.21.b; $P < 0.05$).

C.2.b.iii. Feeding of the early instar larvae of *M. brassicae* in a "One-way" choice chamber

When the larvae were given no choice, there was no signi-

Fig. C.10 : MEAN AMOUNT (± 95% C.L.) OF DDT-TREATED LEAF DISCS CONSUMED BY 20 I, II OR III INSTAR LARVAE OF *M. BRASSICAE* AFTER 24 h IN A "ONE-WAY" CHOICE CHAMBER.

a) Brussel sprout leaf discs



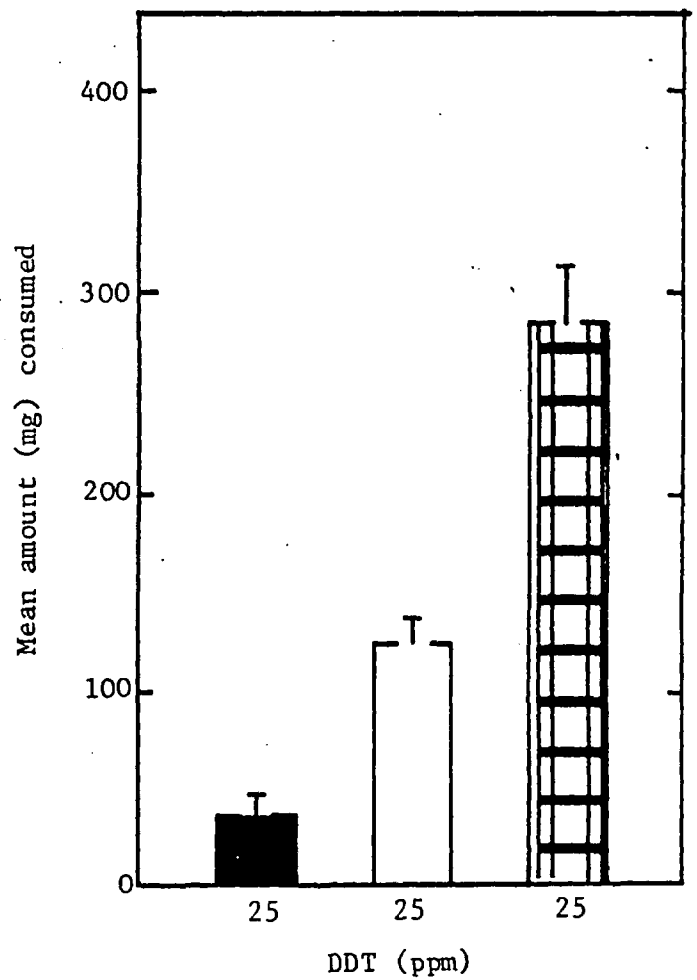
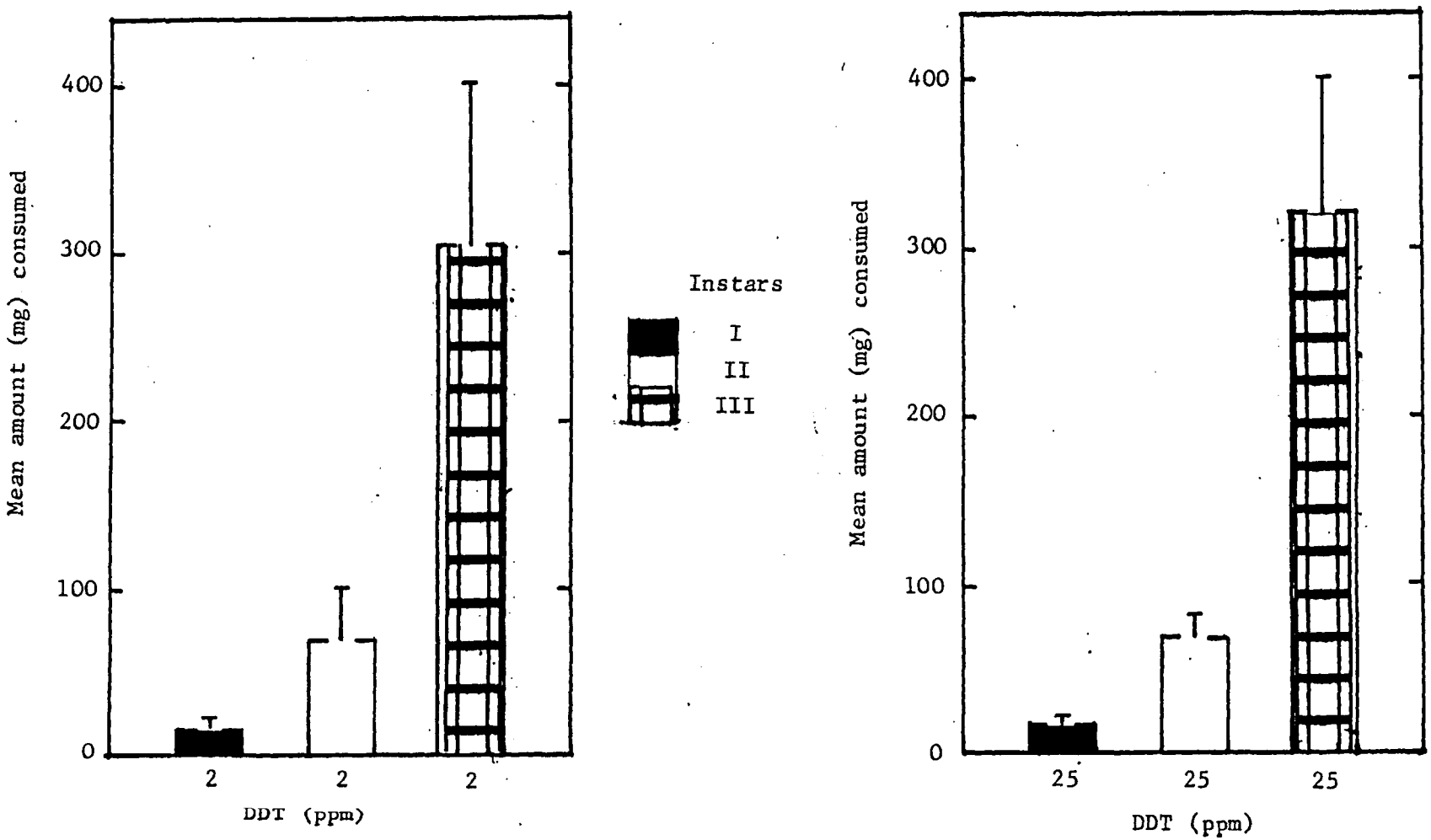


Fig. C.10 : Continued

b) Bean leaf discs



ficant difference in the amount of 2 ppm DDT-treated leaf consumed compared to the 25 ppm treated disc for both Brussel sprout and Bean leaves (Fig. C.10.a, b; Appendix Table C.22; $P > 0.05$).

D. BIOCHEMICAL STUDIES

D.1. pH measurements of the body fluids of larval instars of *M. brassicae*

For all the instars the haemolymph fluid was slightly acidic at pH 6.5 whilst both the regurgitated and mid-gut fluids were alkaline (Table D.1). Little variation was obtained for the two gut fluids apart from in the VI instar.

TABLE D.1 : pH values (mean ± S.E.) of some body fluids from the IV, V and VI instar larvae of *M. brassicae*.

Larval instar	Mean pH ± S.E.*		
	Regurgitated fluid	Mid-gut fluid	Haemolymph
IV	8.8 ± 0.1	8.8 ± 0.1	6.5 ± 0.0
V	8.3 ± 0.1	8.6 ± 0.1	6.5 ± 0.0
VI	7.9 ± 0.1	8.3 ± 0.2	6.5 ± 0.0

* n = 40

D.2. Hydrolysis of malathion-^{14}C at alkaline pH values

The results showed that malathion-^{14}C was hydrolysed more rapidly in alkaline media than in slightly acidic media (Table D.2). However, when hydrolysis is expressed as a proportion of the total malathion-^{14}C present the rate of hydrolysis can be seen to be relatively slow even at pH 8.8 (Table D.2).

TABLE D.2 : Hydrolysis of malathion-^{14}C in 3 h at different pH values.

pH value*	Mean activity of hydrolysis products ± S.E. d.p.m.	Hydrolysis as % of d.p.m. added
6.5 (P)	375 ± 15	0.003 ± 0.0001
7.9 (P)	1,242 ± 61	0.009 ± 0.0004
7.9 (T)	1,612 ± 146	0.011 ± 0.0010
8.3 (T)	2,993 ± 196	0.020 ± 0.0010
8.8 (T)	5,626 ± 600	0.040 ± 0.0040

* Phosphate (P) or Tris (T) buffers used.

The results obtained from the less accurate bioassay experiment agreed with a generally low rate of hydrolysis. Hence, mortality of IV instar *A. aegypti* did not decrease appreciably in media of increasing alkalinity.

D.3. Hydrolysis of malathion-^{14}C by gut homogenates of V and VI instar larvae of *M. brassicae*

The rate of hydrolysis of malathion-^{14}C by VI instar larvae was found to be significantly greater ($P < 0.03$) than that by V instar larvae (Table D.3), the former hydrolysing malathion-^{14}C 39.5% faster after correction for control hydrolysis. In the VI instar this accounted for 30.5% of the total malathion-^{14}C added (Table D.3).

In both instars the rate of conversion of malathion to malaoxon was three orders of magnitude less than the rate of hydrolysis (Table D.4). The rate of oxidation to malaoxon was approximately two-fold greater in the gut extracts for the VI instar than the V instar.

The alternative methods for estimating rate of hydrolysis (detoxication) of malathion by gut extracts of *M. brassicae* larvae using bioassay techniques were abandoned as being less accurate. Preliminary experiments with more concentrated gut extracts of VI instar larvae over longer time periods than in the malathion-^{14}C experiments indicated the virtually complete detoxication of malathion (Appendix Tables D.7 and D.10).

D.4. Effect of 10^{-4} M T.P.P. on hydrolysis of malathion-^{14}C by gut homogenates of VI instar larvae of *M. brassicae*

The addition of 10^{-4} M T.P.P., a specific inhibitor of carboxylesterase activity (Welling *et al.*, 1974) resulted in 84% inhibition in the rate of malathion-^{14}C hydrolysis after correction for control hydrolysis (Table D.5).

TABLE D.3 : Hydrolysis of malathion-^{14}C by gut homogenates of V and VI instar larvae of *M. brassicae*.

Larval instar	Mean (± S.E.)* malathion-^{14}C hydrolysed in 15 min at 25°C			Hydrolysis as % of total d.p.m. added
	µg	n moles	µ moles g^{-1} gut	
Control	0.03	0.1	0.1	-
V	1.42	4.4 ± 0.5	4.4 ± 0.5	21.9
VI	1.99	6.1 ± 0.6	6.1 ± 0.6	30.5

* Mean d.p.m. added = 294,000. For calculations see Appendix Table D.5.

TABLE D.4 : Metabolism of malathion-^{14}C to malaoxon by gut homogenates of V and VI instar larvae of *M. brassicae*.

Larval instar	Mean (± S.E.)* malaoxon-^{14}C produced in 15 min at 25°C		
	ng	p moles (10^{-12})	n moles g^{-1} gut
V	1.0 ± 0.3	3.1 ± 0.9	3.1 ± 0.9
VI	2.3 ± 0.3	7.4 ± 0.9	7.4 ± 0.9

* Corrected for control oxidation. Mean d.p.m. added = 294,000. For calculations see Appendix Table D.6.

TABLE D.5 : Effect of 10^{-4} M Triphenyl phosphate (T.P.P.) on hydrolysis of malathion-^{14}C using gut homogenates of VI instar larvae of *M. brassicae*.

Enzyme system	Mean (± S.E.)* malathion hydrolysed in 15 min at 25°C		% inhibition
	µg	n moles	
Control	0.03 ± 0.01	0.09 ± 0.03	-
Gut homogenate	2.07 ± 0.4	6.31 ± 1.1	-
Gut homogenate + T.P.P.	0.37 ± 0.1	1.01 ± 0.2	83.9

* As for Table D.3.

D.5. Comparative inhibition of ChE from head extract of VI instar larvae of *M. brassicae*

The relative inhibition of head ChE activity by malathion and malaoxon is shown in Table D.6. The I_{50} values were estimated graphically (Fig. D.1).

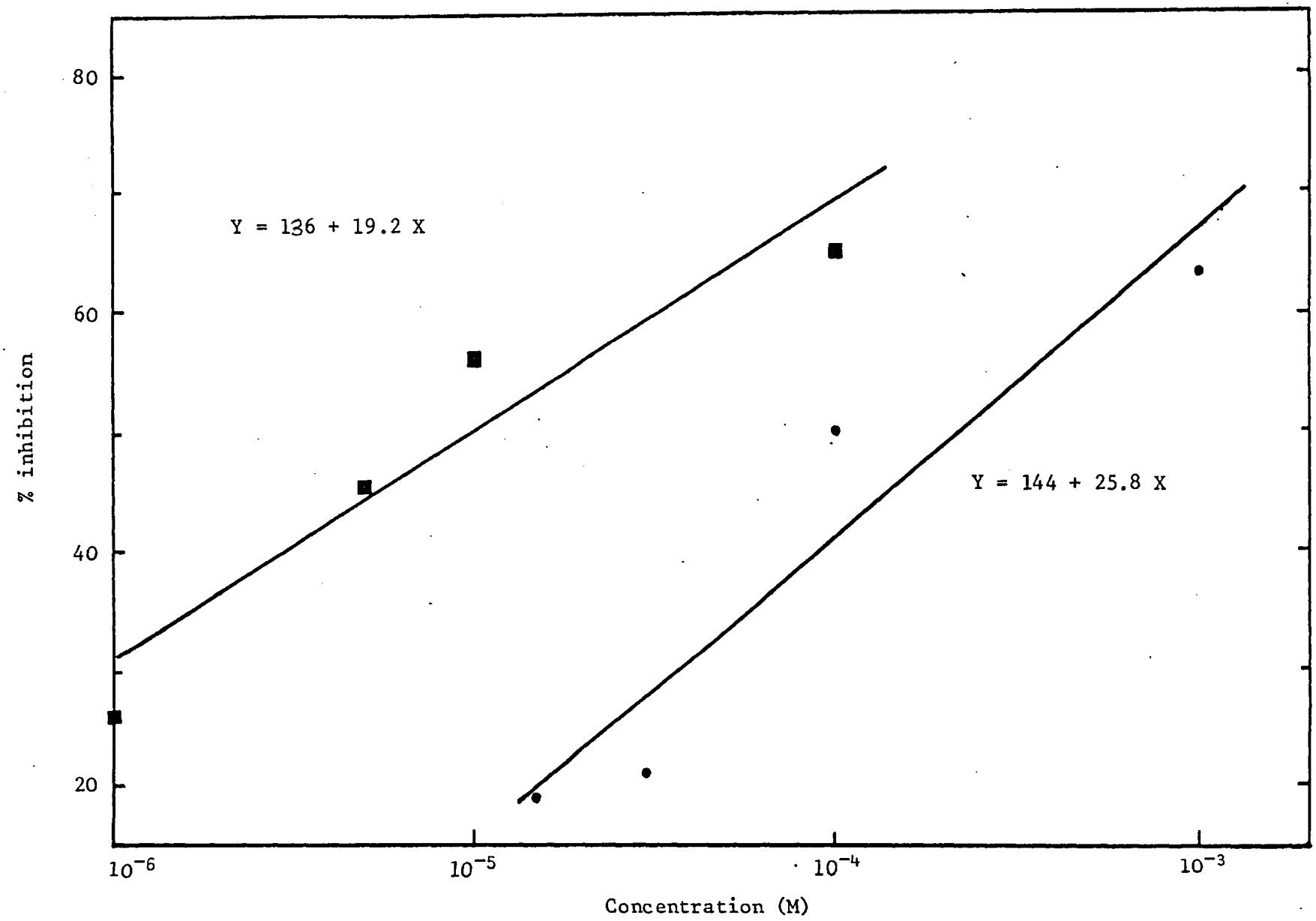
Malaoxon was shown to be more than 10X as potent an inhibitor than malathion (I_{50} values of 1×10^{-5} M and 2.2×10^{-4} M respectively). The slopes of the calculated regression lines (Fig. D.1) also suggest that malaoxon may be an increasingly more potent ChE inhibitor relative to malathion at low concentrations of pesticide.

TABLE D.6 : Inhibition of ChE activity from head extracts of VI instar larvae of *M. brassicae* by malathion and malaoxon.

Concentration of pesticide (M)	% inhibition of ChE activity*	
	Malaoxon	Malathion
1×10^{-6}	26	-
5×10^{-6}	45.5	-
1×10^{-5}	56	-
1.5×10^{-5}	-	19
3×10^{-5}	-	21
1×10^{-4}	65	50
1×10^{-3}	-	63

* n = 2

Fig. D.1 : COMPARATIVE INHIBITION OF ChE BY MALAOXON (■) AND MALATHION (•).



DISCUSSION

The toxicity of an insecticide applied to an insect is determined by the rate of its uptake and penetration, metabolism, elimination and/or storage as well as by the sensitivity of the target site(s) to the insecticide or its metabolites (Corbett, 1974).

With regards to penetration, the insect cuticle is of primary importance for contact insecticides. The insect body covering - the integument is made up of the cuticle and the underlying epidermis. The cuticle of an insect, which is up to 200 μ thick, comprises the epicuticle, exocuticle and endocuticle (Chapman, 1971; Matsumura, 1975). The epicuticle (1-4 μ thick) is made up of a thin layer of tanned protein, impregnated on its outer surface with lipoid or wax, overlaying the cuticuline layer which is comprised of protein mixed with polymerized phenols to form a hard crust. The endocuticle is 70% water and contains chitin arranged parallel to the cuticle surface, but fully rotatable in that plane, embedded in a matrix of arthropodin (water-soluble protein). The exocuticle is sclerotized and is dark, hard, dry and rigid. In most insects, the cuticle is perforated by pore canals which reach from the hypodermal cells to the exocuticle (Chapman, 1971; Matsumura, 1975).

There are two schools of thought to explain the mode of insecticide penetration in insects. In one, the haemolymph is believed to be the principal carrier of contact or topically applied insecticides after their penetration through the insect cuticle (Ahmed & Gardiner, 1970; Burt, 1970; Kurihara *et al.*, 1970; Benezet & Forgash, 1972; Welling, 1977). The other believes that contact insecticides such as the organochlorine DDT appear to follow a lateral route through the integument and tracheae direct to the target organ (Lewis, 1965; Gerolt, 1970).

In both cases, however, penetration of substances through the integument of a living insect is restricted by the structure of the cuticle and the absorptive capacity of the underlying tissues. The rate of penetration has also been shown to be dose dependent for DDT; greater penetration rates resulting from the application of low concentrations of a given insecticide (Poonawalla & Quraishi, 1970).

In the present work, topical application of DDT resulted in the II, III and V instar larvae of *M. brassicae* having a significantly greater degree of susceptibility than the IV and VI instars (Table B.2). However, no differences in susceptibility were found following topical application of malathion (Table B.3). In these experiments, the volume of insecticide applied varied with each instar being roughly in accord with larval size. However, it is unlikely that the volume applied remained constant per unit area of cuticle for each instar and thus factors such as the relative difference in the rate of insecticide penetration between instars cannot be easily assessed.

However, it has been shown that later instars (IV, V and VI) of lepidopteran larvae have a greater proportion of adipose tissues than the early instars (Chapman, 1971). The differences in toxicity of topically applied DDT to larvae of *M. brassicae* may therefore be due principally to differential storage of DDT - a non polar compound in the adipose tissues, including parietal layers of fat beneath the cuticle. Differential storage in the cuticle may also be important but this is most likely to be due to quantitative rather than qualitative changes in the cuticular structure. While cuticular structures are known to change with age in many groups of insects, the larval cuticle of holometabolous insects such as Lepidoptera remains relatively undifferentiated to facilitate growth

and associated moulting (Chapman, 1971).

In the tobacco budworm, *Heliothis virescens* it has been demonstrated that the larger larvae (late instars) were naturally resistant to the chlorinated insecticides DDT, TDE, Toxaphene and endrin but were only slightly more resistant to organophosphates such as mevinphos and trichlorfon (Gast, 1959). Sun (1968) has pointed out that although the larger fat bodies of later instars of *M. domestica* could increase resistance to organochlorine insecticides, with organophosphate insecticides the increase would be minimal because the latter partition to a greater extent into the aqueous phase. The observed toxicity of topically applied DDT and malathion to the larval instars of *M. brassicae* agrees reasonably well with these observations.

The toxicity of injected DDT and malathion showed no variation between the IV, V and VI instars although injected materials were still subjected to the processes of detoxication, elimination, storage or binding before reaching their site(s) of action. The fact that the toxicity of malathion remained constant between instars following its injection supports the corresponding topical application data. For DDT, the lack of variation in susceptibility following its injection suggests that differential storage in the outer regions of the body tissues were principally responsible for the variation obtained following topical application.

It is perhaps surprising that the LD_{50} values for injected DDT or malathion relative to body weight remain constant between instars, although the normal physiological changes that occur, for example, in the haemolymph of insect larval stages are associated particularly with moulting (Lee, 1961; Chapman, 1971). Thus, for a given insect, the

conditions in the haemolymph would be expected to be fairly constant between successive instars. For instance, the present investigations showed that the pH of the haemolymph of the IV, V and VI instar larvae of *M. brassicae* was constant and slightly acidic, as found in most other Lepidopteran insects (Waterhouse, 1949).

The toxicity of both DDT and malathion were clearly affected by the mode of application; injected doses being more toxic to the IV, V and VI instars than topical application (Table 2). This would be expected since by injection, the insecticides have avoided the cuticular barrier. When insecticides are applied topically losses occur due to evaporation and possible degradation on the cuticle surface as well as to dissolution into the epicuticular wax, and binding to the lipids and protein components after diffusion into the more polar exocuticular and endocuticular layers (Sun & Johnson, 1969; Winteringham, 1969; Matsumura, 1975). Another source of loss of insecticide encountered in the current study was to the Petri dishes in which larvae were confined after treatment. Unfortunately this loss could not be quantified for the methods used, but it was considered to be of potential importance for both the topical and contact methods of application.

If it is assumed that the haemolymph is the major route for insecticide transport then an insecticide should behave similarly in the insect haemocoel, irrespective of its mode of application. The increased potency of injected DDT and malathion may thus be largely due to their greater immediate availability as relatively "free" insecticides in the haemolymph. However, in some cases this may be true only over relatively short time intervals, as in the housefly *M. domestica* application of Dieldrin both by injection and topical application resulted in the accumulation of equal

TABLE 2 : LD_{50} values for the topical and injection methods of insecticide application to the larvae of *M. brassicae* after 24 h exposure.

a) DDT

Larval instar	Methods	
	Topical µg mg^{-1}	Injection µg mg^{-1}
I	-	-
II	0.0087	-
III	0.0070	-
IV	0.0138	0.0036
V	0.0074	0.0051
VI	0.0152	0.0069

b) Malathion

Larval instar	Methods	
	Topical µg mg^{-1}	Injection µg mg^{-1}
I	-	-
II	0.0037	-
III	0.0073	-
IV	0.0113	0.0033
V	0.0102	0.0050
VI	0.0127	0.0059

quantities of the insecticide in the central nervous system after 4 h (Sun *et al.*, 1967), and the LD_{50} values obtained for the topical and injection methods were similar. This was not the case over the 24 h test period in the present work. The substantial differences observed in the toxicity of DDT and malathion to IV, V and VI instars of *M. brassicae* when the pesticides were applied topically as compared to by injection would appear to be due to "penetration factors" reducing the initial rate of accumulation of pesticide in the insect (Busvine & Iwuala, 1975). This presumably enabled existing degradative and elimination mechanisms to cope more effectively with the pesticide.

The relationship between the toxicity of DDT and malathion to the larval instars of *M. brassicae* and their mode of application was similar to that found in other insects. For example, the organochlorines DDT and dieldrin, the carbamate, carbaryl and the organophosphates parathion and monocrotophos were more toxic to *M. domestica* by injection than by topical application (Sun *et al.*, 1967; Sun & Johnson, 1969). Similarly, DDT, malathion and the natural insecticide rotenone were more toxic by injection than by topical application to *P. americana* (Winteringham, 1969) and injection of DDT, malathion, bioresmethrin (a pyrethroid) and carbaryl was the most potent method of application compared to feeding and contact methods to the Dipteran larvae *Lucilia sericata, Chrysomya putoria* and *M. domestica* (Busvine & Iwuala, 1975).

The greatest changes in toxicity with increasing instar number were demonstrated by the contact method of insecticide application (Table 3). In general, the early instar larvae of *M. brassicae* were more susceptible than the later instars, and their LT_{50} values showed that the early instar larvae also responded within a shorter time than the later instars; an

TABLE 3 : LD_{50} ratios* obtained for the contact, topical and injection methods of insecticide application to the larvae of *M. brassicae* after 24 h exposure.

a) DDT

Larval instar	Weight ratios	Methods		
		Contact	Topical	Injection
I	1.0	1.00 (Ref)	-	-
II	7.0	0.15	1.0 (Ref)	-
III	13.8	7.10	1.6	-
IV	57.1	12.70	12.8	1.0 (Ref)
V	267.1	-	32.1	6.6
VI	621.6	-	154.0	20.9

b) Malathion

Larval instar	Weight ratios	Methods		
		Contact	Topical	Injection
I	1.0	1.0	-	-
II	7.0	10.0	1.0 (Ref)	-
III	13.8	2.2	3.9	-
IV	57.1	7,312.5	24.9	1.0 (Ref)
V	267.1	108,475.0	105.6	7.0
VI	621.6	362,000.0	304.1	19.4

* Calculated from µg insecticide/larva.

observation similar to that of Sato and Suwanai (1976). In the latter's work, different instars of *M. brassicae* were dipped into γ BHC, dichlorvos and malathion for various times; the VI instar being found to require the longest LT_{50} values for all three insecticides. This might be expected as in a given time the early instars are more likely to pick up effective doses than the later instars due to the greater surface area: volume ratio and activity of the former.

A comparison of the LD_{50} ratios between instars for the contact, topical and injection methods (Table 3) showed that between the II and IV instars the LD_{50} ratio for DDT increased 6-7 X more for the contact method than for topical application. While for malathion, the ratio for the contact method increased 100 X more than for the topical method between the II and VI instars. Differences in LD_{50} ratios for the contact and topical methods between instars are probably due to the behaviour of the larvae. Throughout the contact exposure period, larvae particularly the early instars were found to move away from the treated substrates (Appendix Tables B.10-B.11); a factor not applicable to the other three methods. It might be expected that the insecticide penetrated at a slower rate in the contact method and therefore degradative enzymes and other processes were better able to reduce the level of insecticide reaching the target sites.

When the insecticides were introduced into the diet, the V and VI instars of *M. brassicae* were of similar susceptibility to DDT, but the VI was 2.5 X less susceptible than the V to malathion. As for cuticular penetration, the rate of insecticide uptake through the gut is dose dependent. The amount of toxicant finally arriving at the site of action is dependent on a number of factors, for example, on whether the presence

of the insecticide in the diet has a stimulatory or antifeedant effect on the insect.

The present investigations into the effect of DDT and malathion on the rate of feeding of IV, V and VI instar larvae showed the rate to be age dependent. The VI instar was the most voracious feeder at all insecticide concentrations tested followed by the V and lastly the IV instar. Generally, all instars showed a progressive decline in the rate of feeding over 24 h at all concentrations of DDT (Fig. C.4). Similarly, malathion reduced the amount of cabbage leaf consumed by the IV and V instars over 24 h (Fig. C.5). In the VI instar, however, a sharp reduction in feeding occurred within 4-8 h for all concentrations of malathion and in the olive oil control. This tendency was also seen in the V instars at relatively higher concentrations of malathion. It is possible that this is a normal feeding rhythm which was augmented by the presence of olive oil, since it was just apparent in the V and VI instar larvae feeding on the untreated cabbage leaf discs. However, there was also some evidence that olive oil was an overall stimulant to feeding for the VI instar larvae (Fig. C.5). The IV and V instars did not demonstrate such an effect.

The results of this work on the rate of feeding do not appear to agree with the corresponding toxicological findings nor with work on other insects (Ho, 1978; Temple, 1980) in which the insect with the greater feeding rate was associated with increased susceptibility to insecticides.

If the rate of feeding by instars of *M. brassicae* is expressed as the area eaten per unit body weight a different pattern emerges for each of the two insecticides. In this case, the unit rate of feeding shows a decline with increasing instar number for the DDT treatments although there was little difference between the V and VI instars (Table 4a). In the malathion

TABLE 4 : Mean area (cm^2) of insecticide-treated cabbage leaf disc consumed/100 mg body wt. of the IV, V and VI instar of *M. brassicae* after 24 h*.

a) DDT-treated leaf discs

Larval instar	% (w/v) DDT				
	R.17 oil control	0.0005	0.05	0.5	2.0
IV	7.0	5.4	4.5	2.0	2.6
V	5.7	3.9	3.2	2.0	1.4
VI	5.4	3.2	2.6	2.1	0.9

b) Malathion-treated leaf discs

Larval instar	% (v/v) Malathion							
	Olive oil control	0.00125	0.0125	0.25	1.25	2.5	5.0	10.0
IV	9.9	7.7	6.0	4.8	4.3	3.5	2.7	-
V	2.9	2.5	2.4	2.3	2.1	2.2	2.0	2.2
VI	5.6	5.6	5.7	5.4	4.8	4.1	3.8	3.4

* Data from Appendix Tables C.7 and C.9 respectively.

experiments, the IV instar had the greatest unit rate of feeding in control and at low concentrations of pesticide, but at higher concentrations of malathion the VI instar had the greatest rate of feeding. In all malathion treatments the V instar fed at the slowest rate (Table 4b).

While the amount of toxicant required to kill an organism would not be necessarily expected to increase in direct proportion to increasing body weight, greater amounts of toxicant would be required to cause the same effect in a much larger organism. In fact, the LD_{50} values for malathion and DDT with *M. brassicae* remained constant per unit body weight for each instar when the insecticide was injected, suggesting a direct relationship between the toxic dose and body weight from the IV to the VI instars.

The fact that the unit rate of feeding was reasonably constant for V and VI instar larvae in the presence of DDT is in agreement with the similar toxicity of DDT to these instars when fed in the diet. The approximately two-fold increase in the rate of feeding from the V to the VI instar larvae in the presence of malathion is less easy to accommodate with the greater sensitivity of the V instar to this pesticide when fed. Obviously other factors are more important in determining the toxicity of malathion in these instars.

It is possible for example that an increased rate of feeding by an individual insect induces increased activity in the degradative enzymes of the midgut, with the overall result that the amount of toxicant actually accumulating at the site of action is lower than expected. Differences in the intrinsic rate of degradation of malathion in the gut of these two instars may also be important as is discussed later.

The effect of DDT and malathion on the feeding behaviour of the I-III instar larvae of *M. brassicae* when confronted with a choice between differently treated leaves was also examined. This approach is particularly important since under field conditions it is impossible to completely cover crops with insecticides; instead "patchy" distribution of pesticide occurs (Matthews, 1979). The early instars were used in these experiments because it is economical to control insect pests at an early stage as possible and because of the convenience of handling smaller sized insects in large numbers in such behaviour experiments.

Normally the feeding behaviour of insects have been divided into four components: 1) orientation to dietary substrates; 2) initiation of feeding; 3) maintenance of feeding and 4) cessation of feeding; (Thorpe *et al.*, 1947; Thorsteinson, 1960; Feir & Beck, 1963). Essentially, different stimuli are involved for each component.

Preliminary experiments on the feeding behaviour of I and II instar larvae of *M. brassicae* on cabbage have shown that these early instars feed on the leaf surface away from incident light. The selection of feeding sites on the lower leaf surface was unaffected by the presence of low concentrations of DDT or malathion (Appendix Table C.14a, b). At the higher concentrations of insecticide the I instar appeared to be repelled and no feeding occurred; high mortalities were found at such concentrations. The observation on feeding site selection by *M. brassicae* larvae showed that the I and II instars have a negative phototactic and probably positive thigmotactic response. This has also been observed for other lepidopteran larvae and according to Bonnemaison (1965), this behaviour affords the early larval instars protection against predators and harsh environmental factors. It was also observed that the I instar larvae could not penetrate

through to the upper leaf surface while feeding, and thus the preference of the larvae for the lower leaf surface further reduces the possibility of good target coverage by insecticide sprays.

In the presence of insecticides, the second component of the normal sequence of insect feeding was affected, especially in the I instar. With increasing concentrations of DDT and malathion the onset of feeding by the I instar was progressively delayed until at the highest concentrations tested no feeding was observed (Appendix Table C.15a, b) due to the paralysis and eventual death of the larvae. However, in the II instar the concentrations of DDT and malathion tested had no apparent effect on the time taken to start feeding.

Maintenance of feeding by *M. brassicae* was affected by both DDT and malathion. Thus, in the I and II instars smaller amounts of the leaves treated with high concentrations of insecticides were consumed compared to controls and the larvae were often observed to "wander" away from the treated surfaces. "Wandering" is possibly the main behavioural response to unfavourable conditions. Beck (1956) observed that when not feeding the earlier instars of the European corn borer, *Pyrausta nubilalis* (Hübn) "wander" to a much greater extent than the later instars. This also seems to be the case for *M. brassicae*, and was certainly more pronounced in the presence of DDT and malathion.

Both insecticides exerted deleterious effect on the feeding of the I and II instars. However, the concentrations tested were so high that mortality interferred with the interpretation of results.

In the experiments designed to establish the possible antifeeding effects of DDT and malathion there was considerable variation in the total

amount of leaf disc consumed by I-III instars in the different types of choice chambers and thus direct comparisons were not attempted. However, for a given choice chamber the I instar generally consumed the least leaf material, the III instar the most.

It is generally assumed that in a given choice chamber an insect will select the most suitable diet meeting its nutritional requirements (Thorsteinson, 1960; Mulkern, 1967; House, 1970). The I, II and III instar larvae showed slightly different preferences to both Brussel sprout and Bean leaf discs treated with either olive oil or Risella 17 oil and presented together (Fig. C.6a, b). In general olive oil appeared stimulatory to II and III instar larvae while the opposite effect was shown by R.17 oil in the I and III instars (Fig. C.6a). On Bean leaf discs the effect of these two solvents on all three instars was inconclusive (Fig. C.6b). Thus, in general R.17 oil and olive oil can be described as having antifeeding and stimulating properties respectively R.17 is a mineral oil while olive oil is of plant origin and thus differences in their effects on feeding might be expected.

In the choice chamber containing malathion-treated Brussel sprout leaf discs (Fig. C.7) the larvae appeared to prefer the olive oil-treated controls. Only at 12.5 ppm malathion was there a significant reduction in the amount consumed by I instar and due to some degree of mortality the antifeedant effects of malathion are not proven. However, these results do suggest that cholinergic sense organs may be functional on the mouthparts of *M. brassicae* even in the I instar larvae. Compared to the I instar it seems likely that greater concentrations of malathion are required to exert a marked antifeeding effect on the II and III instar larvae (Fig. C.7), which is in fair agreement with Chapman (1971) that for

any stimulus the threshold level required to evoke a response is age dependent. Young and MacMillan (1979) have reported that another anti-ChE inhibitor, the carbamate insecticide carbaryl, has an antifeedant effect on the lepidopteran *Spodoptera frugipeda*.

Rather more interesting results were obtained from similar experiments with DDT. For the Brussel sprout and Bean leaf discs the I instar was unable to discriminate between the R.17 and untreated controls. The total amount consumed of the paired discs in the R.17 oil v. 2 ppm DDT-treated chamber was greater than in the control chamber for the I and III instar larvae (Fig. C.8). In addition, in all three instars a fairly constant proportion of the R.17 oil-treated control Brussel sprout leaf discs was consumed in the presence of DDT. This may suggest that, the concentrations of DDT tested showed some antifeedant properties. This effect was noticeable in the R.17 oil v. 2 ppm DDT choice chamber for the I instar and at R.17 oil v. 25 ppm DDT chamber for the II and III instars (Fig. C.8a). The inability to clearly establish the same effect in Bean leaf disc experiments may be a reflection of nutritional differences in host plants.

Why the I instar larvae alone did not react to R.17 oil in control chambers is intriguing. This could imply that the underlying sensory mechanism(s) are not fully functional in the I instar. Where DDT concentrations were compared directly, there seemed to be some reduction in the total amount consumed by the I, II and III instar larvae (Fig. C.9), although only I instar larvae ate significantly less of the 25 ppm DDT-treated Brussel sprout leaf disc (Fig. C.9a).

The fact that all three instars ate equal quantities of the 2 ppm and 25 ppm DDT-treated Brussel sprout or Bean leaf discs where no choice was

given ("One-way" choice chamber, Fig. C.10a, b) is puzzling. From the previous observations, it may be that DDT is an antifeedant over a certain concentration range, and that within this range its effect is reasonably constant. This suggests that DDT under field conditions would protect crops mostly through its toxic effect as a contact or residual insecticide, since given the choice the larvae of *M. brassicae* would feed reasonably well on untreated leaves. Similar effects for DDT have been reported by Young and Hamm (1966). They found that DDT showed marked antifeeding effects to *Heliothis zea* on maize leaf discs, although in the field the chemical presumably exerted much of its action through residual and contact effects. In the present work, larval mortality was caused both by uptake of pesticide on contact with the leaf disc as well as through ingestion of the disc.

With reference to DDT previous experiments have shown that during its penetration through the gut tissues, some degradation into water-soluble products occurred in *M. domestica, P. americana* and *B. germanica* (Shah & Guthrie, 1970). It is likely that this occurs in *M. brassicae* although this was not examined. Also, being a non-polar insecticide, binding of DDT to gut tissue (Shah *et al.*, 1972) and storage in fat would occur and should be higher for the VI instar of *M. brassicae* since it has a higher adipose tissue content than the V instar larvae.

Of the physiological/biochemical factors influencing the greater susceptibility of V than VI instar larvae of *M. brassicae* to malathion in the diet pH was not considered to be important. Little variation in the pH of the gut fluids from different instars was found and the rate of hydrolysis of malathion-^{14}C although shown to increase with increasing alkalinity was insignificant compared to enzymatic hydrolysis of malathion

by gut extracts (Tables D.2 and D.3).

Resistance to organophosphate insecticides is mainly correlated with increased carboxylesterase (aliesterase) activity in insects (e.g. Matsumura & Brown, 1961; Devonshire, 1977). The present work has shown that malathion is liable to rapid hydrolysis by mid-gut enzymes of *M. brassicae* larvae and that the largest proportion of this activity in the VI instar is due to carboxylesterase (Table D.5). It has also been shown that degradation of pesticides occurs to an appreciable degree in the mid-gut of other polyphagous insects such as *Manduca sexta* (L.) and *Blatella discoidalis* (O'Brien, 1957; Hopkins, 1961; Jarczyk, 1966; Benke & Wilkinson, 1970; Shah & Guthrie, 1970; Krieger *et al.*, 1971).

In the present study hydrolysis of malathion-^{14}C to non-toxic metabolites was found to be significantly greater in mid-gut extracts of the VI instar compared to the V instar (Table D.3); the rate being approximately 40% greater in the former (Table D.3). Since most of the hydrolytic activity in the VI instar was found to be due to carboxylesterase activity it can be inferred that such enzymes increase in quantity and/or activity with increasing larval age. A small increase in the rate of degradation of a pesticide by the gut tissues (e.g. 40%) would be particularly important if the rate of uptake and transport through the gut tissues was only slightly in excess of the rate of degradation.

The mid-gut extracts of the VI instar larvae were also found to convert malathion to its more potent toxic metabolite malaoxon at a greater rate than in the V instar (Table D.4). This activity is most likely to be due to Mixed-Function-Oxidase (M.F.O.) systems (Nakatsugawa & Morelli, 1976). Increases in M.F.O. activity with larval age have been shown in *M. domestica* (Schonbrod *et al.*, 1965); and *Periplaneta americana*

(Turnguist & Brindley, 1975).

The relative potency of malaoxon and malathion to ChE activity in head extracts of VI instar larvae of *M. brassicae* agreed with previous findings of an increased toxicity for the oxygen analogues of organophosphate insecticides (March *et al.*, 1956; Heath, 1961; Matsumura, 1975; Oppenoorth & Welling, 1976). Malaoxon was found to be approximately 10 X as potent an inhibitor than malathion of ChE activity from *M. brassicae* (Fig. D.1). This result seems to contradict the observed decrease in susceptibility to malathion from the V to the VI instars. However, the amounts of malaoxon formed in both instars was three orders of magnitude less than the corresponding rates of detoxication (hydrolysis) by the mid-gut enzymes. Thus, despite the increased potency of malaoxon, the amounts produced are likely to be insignificant in the overall pharmacokinetics of malathion in the gut of *M. brassicae*.

The sensitivity of ChE activity from the V and VI instars was not compared and this may differ gradually with age. However, from the present study it seems possible to explain the selective toxicity of oral application of malathion to the V and VI instars on the basis of differences in hydrolytic enzyme (carboxylesterase) activity in the two instars as well as to differential storage in adipose tissues associated with the digestive tract.

SUMMARY

When DDT was applied topically the LD_{50} values obtained for the II, III and V instar larvae of *M. brassicae* were lower than those for IV and VI instars. However, the toxicity of topically applied malathion was fairly constant for all instars. Neither DDT nor malathion was found to have differential toxicities to the IV, V and VI instar larvae when applied by injection. These observations suggested that differential storage of DDT in the parietal adipose tissues of the larvae was responsible for the observed differences in sensitivity when DDT was applied topically.

An increase in larval instar number was found to result generally in a considerable decrease in susceptibility to DDT and malathion when the insecticides were applied to the substrate (i.e. by contact). Of the factors contributing to this trend, the decreasing surface area: volume ratio associated with successive instars was identified. Compared to the topical method, the LD_{50} values for the contact method of application of DDT and malathion were greater particularly for the malathion treatments. The greater activity of the early instar larvae may also partly account for the higher LD_{50} values obtained with the contact method of application.

On feeding insecticide-treated leaf discs to the V and VI instars, the toxicity of DDT was similar for both instars. However, the V instar was more susceptible (X 2.5) to malathion applied in the diet than the VI instar. A comparison of the rate of feeding on DDT and malathion-treated cabbage leaf discs by these two instars, showed the VI instar to have a greater unit rate of feeding than the V instar on malathion-treated discs. In contrast, the unit rate of feeding on DDT-treated leaf discs was similar for both V and VI instars. Thus, differences in the susceptibility of V and VI instar larvae to malathion in the diet were not easily explicable in terms of variations in the rate of feeding.

It was also found that low concentrations of DDT on Brussel sprout leaves had an anti-feedant effect. When given the choice between a Risella 17 oil and a DDT-treated leaf disc the I, II and III instar larvae of *M. brassicae* would feed preferentially on the control leaf disc. Preliminary experiments showed that the early instar larvae would select the lower surface of the leaf, and this preference was not affected by low concentrations of DDT and malathion. Also, in the I instar larvae, feeding from the lower epidermis was not deep enough to penetrate the leaf to the upper epidermis. From these observations it is concluded that although DDT may protect crops from insects through its anti-feedant effects, under field conditions where insecticide coverage can never be complete (especially on the lower surface of foliage) its effects as a contact insecticide are more important.

Investigations into the physiological basis underlying the reduced sensitivity of the VI instar compared to the V when malathion was applied in the diet showed that hydrolysis of malathion-^{14}C by gut extracts from the VI instars was 40% greater than that by the V instars. It was also found that in the VI instars hydrolysis of malathion was largely due to carboxylesterase activity. The rate of conversion of malathion-^{14}C to malaoxon in the VI instar was over two fold greater than in the V instar, and malaoxon was found to be 10 X as potent an inhibitor of VI instar head cholinesterase activity than was malathion. However, in both instars the rates at which malaoxon was produced were three orders of magnitude less than the corresponding rates of detoxication. Thus, in the present study the differences in the toxicity of malathion in the diet to the V and VI instars can best be explained by the increased hydrolytic activity of carboxylesterases in the gut of VI instar larvae.

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APPENDIX A

TABLE A.1 : Duration (days) of the developmental stages of *M. brassicae*.

Generation		Egg	Larval instars						Prepupal	Larval duration	Pupa	Adult	Total lifespan
			I	II	III	IV	V	VI					
1	Mean*	4.82	3.06	2.96	3.14	2.92	4.28	5.90	4.24	26.52	18.22	18.66	63.40
	S.D. ±	0.39	0.51	0.35	0.50	0.67	0.83	0.74	0.52	1.20	2.68	1.67	4.52
2	Mean	4.02	3.44	3.28	3.10	2.88	4.60	5.94	4.40	27.80	18.56	15.40	61.72
	S.D. ±	0.14	0.58	0.61	0.46	0.63	0.76	0.74	0.61	1.50	1.92	2.62	4.62
3	Mean	4.06	3.30	3.14	3.08	2.82	4.70	5.90	4.24	27.18	17.72	16.48	61.48
	S.D. ±	0.24	0.61	0.57	0.53	0.63	0.81	0.74	0.52	1.60	1.47	2.84	4.40
4	Mean	4.00	3.34	3.10	3.12	2.90	4.60	5.90	4.50	27.46	19.32	17.52	64.30
	S.D. ±	-	0.72	0.61	0.52	0.76	0.78	0.74	0.70	1.94	1.33	2.29	4.47
5	Mean	4.00	3.28	2.86	3.16	2.92	4.56	5.88	4.42	27.22	19.20	17.00	63.42
	S.D. ±	-	0.50	0.67	0.51	0.72	0.81	0.72	0.61	1.56	1.12	2.54	3.73
Grand	Mean	4.18	3.28	3.07	3.12	2.90	4.55	5.90	4.36	27.24	18.63	17.01	62.66
	S.D. ±	0.96	1.23	1.21	1.14	2.75	1.46	1.32	1.21	0.49	1.00	9.60	7.56

* n = 50

Table A.2 Cast head capsule measurements for the larval stages of *M. brassicae*

Rep	Width (mm) of cast head capsules					
	Larval instars					
	I	II	III	IV	V	VI
1	1.224	2.142	3.060	4.743	8.262	12.240
2	1.224	1.836	3.060	5.202	8.262	12.240
3	1.224	2.142	3.366	4.896	7.650	13.158
4	1.224	2.142	3.060	4.896	7.650	13.158
5	1.224	1.836	3.366	4.590	7.650	12.852
6	1.224	2.142	3.060	4.284	7.650	13.158
7	1.224	2.142	3.060	5.049	7.650	12.852
8	1.224	2.142	3.672	5.202	7.650	12.852
9	1.224	2.142	3.366	4.896	6.120	12.240
10	1.224	2.142	3.366	4.896	8.262	12.852
11	1.224	2.142	3.060	4.896	7.650	12.852
12	1.224	2.142	3.366	4.896	7.650	12.852
13	1.224	2.142	3.060	4.896	7.650	12.852
14	1.224	2.142	3.366	4.743	8.262	12.852
15	1.224	1.836	3.060	4.896	7.650	13.158
16	1.224	2.142	3.366	4.590	7.650	13.158
17	1.224	2.142	3.060	4.590	7.650	13.464
18	1.224	2.142	3.060	4.896	7.650	12.240
19	1.224	2.142	3.060	4.590	8.262	12.240
20	1.224	2.142	3.060	4.896	7.650	12.546
21	1.224	2.142	3.366	4.896	7.650	13.770
22	1.224	2.142	3.366	4.896	7.650	12.240
23	1.224	2.142	3.366	4.896	7.650	13.158
24	1.224	2.142	3.366	4.896	7.650	12.852
25	1.224	2.142	3.060	4.896	7.650	12.852
26	1.224	2.295	3.060	4.896	7.650	12.852
27	1.224	1.836	3.366	4.284	8.262	13.158
28	1.224	2.142	3.672	4.590	8.262	12.240
29	1.224	2.142	3.060	4.590	6.120	12.240
30	1.224	2.142	3.060	4.896	7.650	12.852

Table A.2 cont'd

Rep	I	II	III	IV	V	VI
31	1.224	2.295	3.366	4.896	7.650	13.158
32	1.224	2.142	3.366	4.896	7.650	12.852
33	1.224	2.142	3.060	4.896	7.650	12.852
34	1.224	2.142	3.060	4.896	7.650	12.852
35	1.224	1.836	3.060	4.284	7.650	12.852
36	1.224	2.142	3.060	5.202	8.262	13.464
37	1.224	2.295	3.366	4.590	6.120	13.464
38	1.224	2.142	3.366	5.202	8.262	12.852
39	1.224	2.142	3.366	4.590	7.650	12.240
40	1.224	2.142	3.672	4.896	7.650	13.770
41	1.224	2.142	3.060	4.473	7.650	13.770
42	1.224	1.836	3.060	5.049	7.650	13.158
43	1.224	1.836	3.366	4.896	8.262	13.158
44	1.224	2.142	3.060	4.590	7.650	12.240
45	1.224	2.142	3.366	4.590	7.650	12.240
46	1.224	2.142	3.366	5.049	7.650	12.240
47	1.224	2.142	3.060	4.896	7.650	12.546
48	1.224	2.142	3.060	4.590	7.650	12.546
49	1.224	2.142	3.366	4.590	8.262	13.770
50	1.224	2.142	3.366	4.284	8.262	13.158
51	1.224	2.295	3.060	4.896	8.262	13.464
52	1.224	2.142	3.366	4.896	7.650	13.158
53	1.224	2.142	3.060	4.896	7.650	12.852
54	1.224	1.836	3.366	4.896	7.650	13.158
55	1.224	2.142	3.672	4.896	7.650	13.158
56	1.224	2.142	3.060	4.896	7.650	12.240
57	1.224	2.142	3.060	5.049	7.650	12.240
58	1.224	2.142	3.366	4.896	7.650	12.240
59	1.224	2.142	3.366	4.896	7.650	12.240
60	1.224	2.142	3.060	4.743	6.120	12.546
Total	61.200	126.684	194.004	288.864	460.836	770.508
Mean	1.224	2.111	3.233	4.814	7.681	12.842
S.D.$\pm$	0	0.115	0.189	0.217	0.490	0.461
S.E.$\pm$	0	0.021	0.035	0.039	0.089	0.084

Footnote to Table A.2.

Calibration of micrometer

Eye-piece micrometer 36 units
Stage micrometer = 11 mm

$\therefore$ 1 unit of eye-piece micrometer; $\frac{11}{36}$ = 0.306 mm
at a magnification of 10 x 1.25

Table A.3 Body weight (mg) of the larval instars of M. brassicae

Rep	I	II	III	IV	V	VI
1	0.44	3.57	10.06	24.41	123.14	311.04
2	0.48	3.43	9.30	25.87	125.51	289.61
3	0.23	3.61	8.61	25.77	126.72	338.63
4	0.37	3.70	7.34	28.87	134.33	325.31
5	0.34	3.33	6.12	28.91	128.88	316.28
6	0.42	2.86	6.68	25.75	129.36	281.22
7	0.36	2.81	6.38	27.68	124.40	288.31
8	0.34	2.80	6.40	28.66	125.48	307.38
9	0.39	2.94	5.86	24.16	130.23	306.13
10	0.34	4.00	7.52	35.25	141.12	307.21
11	0.31	2.77	8.18	34.78	126.34	308.15
12	0.37	3.24	8.00	30.80	128.46	300.22
13	0.33	2.98	6.45	26.76	125.66	300.30
14	0.35	2.64	6.78	22.37	124.71	321.51
15	0.43	2.94	6.50	25.85	127.30	311.19
16	0.28	3.55	5.52	27.76	127.34	323.02
17	0.34	4.03	5.78	29.70	128.99	324.12
18	0.31	2.96	6.62	28.84	140.84	320.25
19	0.41	4.00	5.60	26.99	140.61	320.18
20	0.34	4.20	5.62	21.35	135.55	278.34
21	0.34	4.10	5.33	26.55	136.37	296.81
22	0.39	3.78	8.05	25.64	135.44	284.83
23	0.47	4.08	6.14	29.32	130.52	294.55
24	0.30	4.06	6.31	27.81	136.66	300.56
25	0.39	4.17	6.14	29.11	134.69	312.17
26	0.56	4.00	6.23	25.55	130.77	308.22
27	0.44	3.81	6.50	29.23	129.33	297.63
28	0.32	4.11	6.43	27.42	129.55	291.91
29	0.44	4.20	6.66	26.74	128.48	286.61
30	0.43	4.12	6.52	30.42	129.86	281.45
31	0.38	3.50	6.86	31.51	129.86	300.39

Table A.3 cont'd

Rep	I	II	III	IV	V	VI
32	0.38	3.46	6.82	30.23	128.65	319.31
33	0.63	3.39	6.18	29.98	129.73	307.42
34	0.64	2.98	6.11	29.35	130.67	299.11
35	0.73	2.88	6.23	28.99	121.11	301.50
36	0.77	3.72	6.59	29.00	132.41	300.37
37	0.65	2.99	6.03	25.49	130.00	299.08
38	0.57	2.97	6.28	26.70	140.07	289.12
39	0.89	3.83	6.91	27.78	133.70	314.20
40	0.64	3.19	6.48	28.80	127.81	302.38
41	0.76	4.00	6.50	29.89	130.56	316.54
42	0.64	2.78	7.41	29.40	137.81	290.49
43	0.63	3.80	7.21	30.20	139.00	289.81
44	0.67	3.49	7.46	28.09	134.55	288.32
45	0.64	3.23	7.85	26.43	129.81	302.24
46	0.65	3.44	7.51	20.41	129.69	302.41
47	0.64	2.88	6.19	29.11	134.42	322.81
48	0.59	3.69	6.22	28.89	131.09	330.78
49	0.55	3.11	6.60	26.78	128.89	309.85
50	0.54	3.19	8.40	29.99	129.91	308.71
51	0.58	3.24	6.91	26.74	132.81	305.16
52	0.63	3.48	8.14	30.70	129.90	305.36
53	0.63	3.77	6.39	29.41	140.12	310.61
54	0.50	3.71	7.30	28.28	129.64	302.58
55	0.44	2.92	6.18	26.67	125.59	304.30
56	0.56	4.11	8.14	28.98	127.81	303.93
57	0.58	4.03	6.33	29.22	132.14	323.68
58	0.63	2.98	6.44	25.52	130.30	288.28
59	0.33	2.86	6.41	29.90	128.89	295.55
60	0.63	2.59	6.28	28.64	129.90	308.58
Total	29.36	207.00	407.20	1,679.40	7,852.80	18,276.01
Mean	0.49	3.45	6.77	27.99	130.88	304.60
S.D.±	0.15	0.48	0.91	2.61	4.58	13.11
S.E.±	0.02	0.06	0.12	0.34	0.59	1.69

APPENDIX B

Table B.1 Calibration of the Arnold electric microapplicator

Test volume (ul)	Replicates	Background Counts c.p.m.	S-values	Efficiency (%)	d.p.m.	Actual volume (µl)
1.0	1	32	541	88.8	2432	0.76
	2	32	535	88.7	2460	0.76
	3	32	533	88.7	2400	0.74
	4	32	542	88.8	2360	0.73
	Mean	-	-	88.75	2413	0.75
	S.D. ±	-	-	-	-	0.02
0.5	1	32	539	88.8	1197	0.37
	2	32	535	88.7	936	0.29
	3	32	533	88.7	1062	0.33
	4	32	540	88.8	1160	0.36
	5	32	531	88.5	1034	0.32
	Mean	-	-	88.7	1078	0.34
	S.D. ±	-	-	-	-	0.03
0.3	1	32	532	88.5	481	0.15
	2	32	532	88.5	687	0.21
	3	32	529	88.5	633	0.20
	4	32	539	88.8	655	0.20
	5	32	542	88.8	571	0.18
	Mean	-	-	88.6	605	0.19
	S.D. ±	-	-	-	-	0.02

Table B.1 cont'd

Test volume (µl)	Replicates	Background Counts c.p.m.	S-values	Efficiency (%)	d.p.m.	Actual volume (µl)
0.2	1	32	536	88.7	320	0.10
	2	32	536	88.7	481	0.15
	3	32	531	88.5	342	0.11
	4	32	540	88.8	340	0.11
	5	32	531	88.5	331	0.10
	Mean	-	-	88.6	363	0.11
	S.D. ±	-	-	-	-	0.02
0.1	1	32	538	88.8	130	0.04
	2	32	542	88.8	143	0.04
	3	32	527	88.3	119	0.04
	4	32	536	88.7	188	0.06
	5	32	535	88.7	153	0.05
	Mean	-	-	88.7	147	0.05
	S.D. ±	-	-	-	-	0.01

* 1 µl of malathion-^{14}C in Risella 17 oil contained 3210 d.p.m.

Table B.2 Larval mortality of M. brassicae (out of 20) 24h after topical application of DDT

Larval stage	Rep	% DDT ($^{w}/_{v}$) 0.0	0.025	0.05	0.1	0.25	0.5	1.0	2.0
II	1	0	4	8	18	18	20	20	20
	2	1	4	8	12	19	20	20	20
	3	0	5	8	12	19	20	20	20
	Total	1	13	24	42	56	60	60	60
III	1	0	5	12	17	19	20	20	20
	2	0	7	11	16	19	20	20	20
	3	0	6	12	15	18	19	20	20
	Total	0	18	35	48	56	59	60	60
IV	1	0	1	5	5	10	15	19	19
	2	0	3	3	5	12	15	18	20
	3	1	3	4	4	12	13	15	20
	Total	1	7	12	14	34	43	52	59
V	1	0	1	4	6	9	10	16	19
	2	0	2	4	5	8	9	16	20
	3	0	2	5	6	7	10	15	19
	Total	0	5	13	17	24	29	47	58
VI	1	0	0	0	0	5	9	15	16
	2	0	0	0	1	5	10	15	17
	3	0	0	0	0	4	8	14	15
	Total	0	0	0	1	14	27	44	48

Table B.3a Larval mortality of M. brassicae (out of 10) 24h after the injection of DDT

Larval stage	Rep	% DDT (w/v) 0.0	0.00025	0.0025	0.025	0.5	2.0
IV	1	0	3	0	2	6	10
	2	0	0	2	3	6	10
	3	0	1	2	3	7	10
	4	1	0	3	2	6	10
	Total	1	4	7	10	25	40

Table B.3b Larval mortality of M. brassicae (out of 20) 24h after the injection of DDT

Larval stage	Rep	% DDT (w/v) 0.0	0.025	0.05	0.1	0.25	0.5	1.0	2.0
V	1	0	0	5	5	13	14	17	20
	2	0	0	5	6	14	14	17	20
	3	0	1	5	7	13	13	16	18
	Total	0	1	15	18	40	41	50	58
VI	1	0	3	3	5	6	12	17	20
	2	0	2	4	5	5	12	16	20
	3	0	2	5	6	7	12	15	17
	Total	0	7	12	16	18	36	48	57

Table B.4 Larval mortality of M. brassicae (out of 20) 24h after feeding with DDT

Larval stage	Rep	% DDT (w/v) 0.0	0.025	0.05	0.1	0.25	0.5	1.0	2.0
V	1	0	1	3	5	7	8	13	17
	2	0	2	2	3	7	8	15	18
	3	0	1	1	3	5	8	14	15
	Total	0	4	6	11	19	24	42	50
VI	1	0	2	2	4	4	7	20	20
	2	0	1	4	6	7	9	19	20
	3	1	2	2	6	5	9	18	20
	Total	1	5	8	16	16	25	57	60

Table B.5 Larval mortality of M. brassicae (out of 20) 24h after topical application of malathion

Larval stage	Rep	% malathion (v/v) 0.0	0.00125	0.0125	0.025	0.25	1.25	2.5	5.0	10.0	20.0
II	1	0	6	7	8	12	17	19	20	20	20
	2	0	5	7	8	15	19	20	20	20	20
	3	0	5	6	9	15	18	20	20	20	20
	Total	0	16	20	25	42	54	59	60	60	60
III	1	0	2	5	7	13	18	20	20	20	20
	2	1	3	6	8	14	20	20	20	20	20
	3	0	3	6	8	13	19	19	20	20	20
	Total	1	8	17	23	40	57	59	60	60	60
IV	1	0	1	0	6	11	16	18	20	20	20
	2	0	0	1	7	11	18	19	20	20	20
	3	0	0	0	6	12	17	18	20	20	20
	Total	0	1	1	19	34	41	55	60	60	60
V	1	1	2	3	4	5	12	14	18	20	20
	2	0	2	1	4	7	9	15	20	20	20
	3	0	2	3	4	5	10	14	19	20	20
	Total	1	6	7	12	17	31	43	57	60	60
VI	1	0	1	2	4	5	9	12	14	19	20
	2	0	3	3	5	4	11	11	15	17	20
	3	0	1	1	5	5	10	12	13	20	20
	Total	0	5	6	14	14	30	35	42	56	60

Table B.6 Larval mortality of M. brassicae (out of 20) 24h after the injection of malathion

Larval stage	Rep	% malathion (v/v) 0.0	0.00125	0.0125	0.025	0.25	1.25	2.5	5.0	10.0	20.0
IV	1	0	3	7	10	12	16	17	19	20	20
	2	0	4	8	8	14	16	16	20	20	20
	3	0	2	7	8	12	15	15	18	20	20
	Total	0	9	22	26	38	47	48	57	60	60
V	1	0	2	4	6	7	11	14	20	20	20
	2	0	3	3	7	8	12	15	20	20	20
	3	1	3	5	6	8	11	15	20	20	20
	Total	1	8	12	19	23	34	44	60	60	60
VI	1	0	2	2	6	7	11	15	18	19	20
	2	0	1	5	6	7	10	16	19	18	20
	3	0	2	3	5	8	10	16	18	18	20
	Total	0	5	10	17	22	31	47	55	55	60

Table B.7 Larval mortality of M. brassicae (out of 20) 24h after feeding with malathion

Larval stage	Rep	% malathion (v/v) 0.0	0.00125	0.0125	0.025	0.25	1.25	2.5	5.0	10.0	20.0
V	1	0	0	0	1	0	3	5	10	18	19
	2	0	0	0	1	0	4	6	10	17	20
	3	0	0	0	0	0	3	7	9	19	20
	Total	0	0	0	2	0	10	18	29	54	59
VI	1	0	1	0	0	2	3	4	7	11	16
	2	0	0	0	0	2	2	2	6	13	15
	3	0	0	0	0	2	2	2	6	13	15
	Total	0	1	0	0	6	7	8	19	37	46

Table B.8a Mortality of the first instar larvae of M. brassicae (out of 20) after different contact times (h) with DDT.

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	1	2	2	
2	0	0	0	0	0	3	3	1
3	0	0	0	0	1	2	3	
Total	0	0	0	0	2	7	8	
1	0	0	0	0	1	2	2	
2	0	0	0	0	2	3	4	2
3	0	0	0	1	2	2	5	
Total	0	0	0	1	5	7	11	
1	0	0	0	0	1	3	4	
2	0	0	0	0	3	3	6	4
3	0	0	0	1	2	3	8	
Total	0	0	0	1	6	9	18	
1	0	0	3	5	6	6	19	
2	0	1	3	3	3	4	17	8
3	0	0	3	3	5	7	17	
Total	0	1	9	11	14	17	53	
1	0	0	3	5	7	11	19	
2	0	1	3	5	10	10	18	12
3	0	0	3	3	7	9	19	
Total	0	1	9	13	24	30	56	
1	0	0	3	4	7	15	20	
2	0	1	3	4	10	16	20	24
3	0	1	5	5	12	17	20	
Total	0	2	11	13	29	48	60	

Table B.8b Mortality of the second instar larvae of M. brassicae (out of 30) after different contact times (h) with DDT.

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	0	0	0	1
2	0	0	0	0	0	0	0	
Total	0	0	0	0	0	0	0	
1	0	0	0	0	0	0	0	2
2	0	0	0	0	0	0	0	
Total	0	0	0	0	0	0	0	
1	0	0	0	0	0	1	2	4
2	0	0	0	0	0	2	5	
Total	0	0	0	0	0	3	7	
1	0	0	0	2	2	4	8	8
2	0	0	0	0	4	6	9	
Total	0	0	0	2	6	10	17	
1	0	0	0	7	14	21	30	12
2	0	0	0	10	13	18	30	
Total	0	0	0	17	27	39	60	
1	0	2	2	18	24	27	30	24
2	0	1	3	20	25	29	30	
Total	0	3	5	38	49	56	60	

Table B.8c Mortality of the third instar larvae of M. brassicae (out of 10) after different contact times (h) with DDT

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	0	0	0	
2	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	2.0
4	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	
6	0	0	0	0	0	0	0	
Total	0	0	0	0	0	0	0	
1	0	0	0	0	0	1	4	
2	0	0	0	0	0	1	4	
3	0	0	0	0	0	3	3	4.0
4	0	0	0	0	0	2	2	
5	0	0	0	0	0	1	4	
6	0	0	0	0	0	3	1	
Total	0	0	0	0	0	11	18	
1	0	0	0	0	2	4	6	
2	0	0	0	0	1	3	5	
3	0	0	0	0	1	3	6	8.0
4	0	0	0	0	1	3	4	
5	0	0	0	0	0	2	4	
6	0	0	0	0	0	3	6	
Total	0	0	0	0	5	18	31	

Table B.8c cont'd

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	2	2	5	10	
2	0	0	0	0	2	4	8	
3	0	0	0	1	1	3	10	12
4	0	0	0	0	3	4	9	
5	0	0	0	2	3	3	8	
6	0	0	0	2	3	3	10	
Total	0	0	0	7	14	22	55	
1	0	0	0	2	4	5	10	
2	0	0	0	1	3	4	10	
3	0	0	0	2	4	4	10	24
4	0	0	0	1	4	4	10	
5	0	0	0	2	3	4	10	
6	0	0	0	2	4	4	10	
Total	0	0	0	10	22	25	60	
1	0	0	0	2	4	7	10	
2	0	0	0	3	4	6	10	
3	0	0	0	2	6	6	10	36
4	0	0	0	2	5	6	10	
5	0	0	0	3	4	8	10	
6	0	0	0	3	6	8	10	
Total	0	0	0	15	29	41	60	
1	0	0	0	2	5	7	10	
2	0	0	1	3	6	9	10	
3	0	0	1	3	8	7	10	48
4	0	0	0	3	6	8	10	
5	0	0	0	4	6	9	10	
6	0	0	0	3	7	10	10	
Total	0	0	2	18	38	50	60	

Table B.8d Mortality of the fourth instar larvae of M. brassicae (out of 10) after different contact times (h) with DDT

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	0	0	1	
2	0	0	0	0	0	0	2	
3	0	0	0	0	0	0	3	4
4	0	0	0	0	0	0	2	
5	0	0	0	0	0	0	3	
6	0	0	0	0	0	0	4	
Total	0	0	0	0	0	0	15	
1	0	0	0	0	0	0	1	
2	0	0	0	0	0	2	4	
3	0	0	0	0	0	0	5	8
4	0	0	0	0	0	1	3	
5	0	0	0	0	0	0	3	
6	0	0	0	0	0	0	5	
Total	0	0	0	0	0	3	21	
1	0	0	0	0	0	2	5	
2	0	0	0	0	1	3	6	
3	0	0	0	0	0	4	5	12
4	0	0	0	0	2	3	6	
5	0	0	0	0	1	1	6	
6	0	0	0	0	0	3	7	
Total	0	0	0	0	4	16	35	

Table B.8d cont'd

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	2	4	5	
2	0	0	0	0	1	5	8	
3	0	0	0	0	2	7	10	24
4	0	0	1	2	3	7	7	
5	0	0	2	2	3	6	8	
6	0	0	1	2	2	8	8	
Total	0	0	4	6	13	37	46	
1	0	0	1	2	4	7	10	
2	0	1	2	4	8	9	9	
3	0	0	2	4	5	8	10	36
4	0	1	3	1	6	8	8	
5	0	0	1	2	4	9	8	
6	0	0	1	3	5	9	8	
Total	0	2	10	16	32	50	53	
1	0	0	1	4	6	10	10	
2	0	1	2	4	10	10	10	
3	0	1	2	5	6	8	10	48
4	0	1	4	2	8	9	10	
5	0	0	3	3	8	10	10	
6	0	0	3	4	6	10	10	
Total	0	3	15	22	44	57	60	

Table B.8e Mortality of the fifth instar larvae of M. brassicae (out of 3) after different contact times (h) with DDT

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	0	1	2	
2	0	0	0	0	0	0	2	
3	0	0	0	0	0	0	2	
4	0	0	0	0	0	1	3	
5	0	0	0	0	0	0	3	24
6	0	0	0	0	0	0	2	
7	0	0	0	0	0	0	2	
8	0	0	0	0	0	0	3	
9	0	0	0	0	0	0	3	
10	0	0	0	0	0	0	2	
Total	0	0	0	0	0	2	24	
1	0	0	0	0	0	1	2	
2	0	0	0	0	0	1	2	
3	0	0	0	0	1	2	3	
4	0	0	0	0	0	2	3	
5	0	0	0	0	1	1	3	36
6	0	0	0	0	0	0	2	
7	0	0	0	0	0	1	2	
8	0	0	0	0	0	1	3	
9	0	0	0	0	0	2	3	
10	0	0	0	0	0	1	3	
Total	0	0	0	0	2	12	26	
1	0	0	0	0	1	2	3	
2	0	0	0	0	1	2	3	
3	0	0	0	1	2	2	3	
4	0	0	0	0	1	2	3	
5	0	0	0	1	2	3	3	48
6	0	0	0	0	2	2	3	
7	0	0	0	1	2	3	3	
8	0	0	0	0	1	1	3	
9	0	0	0	0	0	3	3	
10	0	0	0	0	1	2	3	
Total	0	0	0	3	13	22	30	

Table B.8f Mortality of the sixth instar larvae of M. brassicae (out of 3) after different contact times (h) with DDT

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	0	1	1	
2	0	0	0	0	0	1	1	
3	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	12
6	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	
10	0	0	0	1	1	0	0	
Total	0	0	0	1	1	2	2	
1	0	0	0	0	0	1	1	
2	0	0	0	0	1	1	1	
3	0	0	0	0	0	1	2	
4	0	0	0	0	0	1	1	
5	0	0	0	0	0	2	2	24
6	0	0	0	0	0	1	1	
7	0	0	0	0	0	1	2	
8	0	0	0	0	0	1	2	
9	0	0	0	0	0	2	2	
10	0	0	0	1	1	1	2	
Total	0	0	0	1	2	12	16	

Table B.8f cont'd

Rep	No treatment	% (w/v) DDT 0.0	0.00025	0.0025	0.025	0.5	2.0	Time (h)
1	0	0	0	0	0	2	2	
2	0	0	0	0	1	1	2	
3	0	0	0	1	1	1	2	
4	0	0	0	0	1	2	2	
5	0	0	0	0	0	2	3	36
6	0	0	0	0	0	2	3	
7	0	0	0	0	0	1	2	
8	0	0	0	0	1	1	3	
9	0	0	0	0	1	2	3	
10	0	0	0	1	1	1	2	
Total	0	0	0	2	6	15	24	
1	0	0	0	0	0	2	3	
2	0	0	0	0	2	2	3	
3	0	0	0	1	1	2	3	
4	0	0	0	0	1	3	3	
5	0	0	0	1	2	2	3	48
6	0	0	0	0	2	2	3	
7	0	0	0	0	1	2	3	
8	0	0	0	0	3	3	3	
9	0	0	0	0	1	3	3	
10	0	0	0	1	3	3	3	
Total	0	0	0	3	16	24	30	

Table B.9a Mortality of the first instar larvae of M. brassicae (out of 30) after different contact times (h) with malathion

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	3	3	3	10	10	0.5
2	0	2	2	4	4	8	9	
Total	0	2	5	7	7	18	19	
1	0	1	3	3	3	13	17	1.0
2	0	3	4	5	5	11	14	
Total	0	4	7	8	8	24	31	
1	2	3	4	3	5	20	24	2.0
2	0	2	4	5	7	21	23	
Total	2	5	8	8	12	41	47	
1	2	3	6	7	10	27	27	4.0
2	1	4	5	8	12	29	30	
Total	3	7	11	15	22	56	57	
1	2	3	6	8	14	30	30	8.0
2	1	5	7	9	24	30	30	
Total	3	8	13	17	38	60	60	
1	2	6	11	19	25	30	30	12.0
2	1	5	11	14	27	30	30	
Total	3	11	22	33	52	60	60	
1	2	7	27	27	30	30	30	24.0
2	3	7	26	28	30	30	30	
Total	5	14	53	55	60	60	60	

Table B.9b Mortality of the second instar larvae of M. brassicae (out of 30) after different contact times (h) with malathion

Rep	% malathion (v/v) No treatment	0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	1	1	6	0.0
2	0	0	0	0	0	1	4	
Total	0	0	0	0	1	2	10	
1	0	0	0	0	1	3	12	1.0
2	0	0	0	1	2	1	9	
Total	0	0	0	1	3	4	21	
1	1	1	2	3	10	12	25	2.0
2	0	1	1	5	8	17	23	
Total	1	2	3	8	18	29	48	
1	1	1	2	4	17	21	27	4.0
2	0	2	2	5	16	25	27	
Total	1	3	4	9	33	46	54	
1	1	1	2	5	18	25	30	8.0
2	0	3	3	7	16	28	29	
Total	1	4	5	12	34	53	59	
1	1	1	2	15	18	25	30	12.0
2	0	3	4	11	16	28	29	
Total	1	4	6	26	34	53	59	
1	1	1	14	28	29	30	30	24.0
2	0	3	17	29	30	30	30	
Total	1	4	31	57	59	60	60	

Table B.9c Mortality of the third instar larvae of M. brassicae (out of 10) after different contact times (h) with malathion

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	0	1	3	
2	0	0	0	0	0	0	3	
3	0	0	0	0	3	2	4	1
4	0	0	0	0	1	3	2	
5	0	0	0	0	1	1	3	
6	0	0	0	0	0	1	3	
Total	0	0	0	0	5	8	18	
1	0	0	0	0	2	3	9	
2	0	0	1	0	1	1	7	
3	0	0	0	0	4	3	6	2
4	0	0	0	0	2	5	6	
5	0	0	0	0	2	2	5	
6	0	0	0	0	3	4	6	
Total	0	0	1	0	14	18	39	
1	0	0	0	1	2	7	9	
2	0	0	1	0	2	4	9	
3	0	0	0	0	4	8	7	4
4	0	0	0	0	3	6	8	
5	0	0	0	0	2	4	9	
6	0	0	0	0	3	7	8	
Total	0	0	1	1	16	36	50	

Table B.9c cont'd

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	1	2	8	8	10	
2	0	0	2	3	6	7	10	
3	0	0	1	4	5	9	9	8
4	0	0	1	4	7	9	10	
5	0	0	2	2	7	9	9	
6	0	0	2	2	8	8	8	
Total	0	0	9	17	41	50	56	
1	0	0	3	4	9	9	10	
2	0	0	2	3	7	8	10	
3	0	0	5	6	7	10	10	12
4	0	0	2	3	8	9	10	
5	0	0	3	4	7	10	10	
6	0	0	4	5	8	10	10	
Total	0	0	19	25	46	56	60	
1	0	0	8	9	9	10	10	
2	0	0	8	10	10	10	10	
3	0	0	8	10	10	10	10	24
4	0	0	9	10	10	10	10	
5	0	0	10	9	10	10	10	
6	0	0	10	10	10	10	10	
Total	0	0	53	58	59	60	60	

Table B.9d Mortality of the fourth instar larvae of M. brassicae (out of 10) after different contact times (h) with malathion

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	0	0	1	
2	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	2
4	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	1	
6	0	0	0	0	0	0	1	
Total	0	0	0	0	0	0	3	
1	0	0	0	0	1	3	5	
2	0	0	0	0	1	2	5	
3	0	0	0	0	1	1	4	4
4	0	0	0	0	0	2	6	
5	0	0	0	0	1	2	7	
6	0	0	0	0	1	2	5	
Total	0	0	0	0	5	12	32	
1	0	0	0	0	1	3	8	
2	0	0	0	0	1	4	10	
3	0	0	0	0	3	5	10	8
4	0	0	0	0	2	5	8	
5	0	0	0	0	1	4	9	
6	0	0	0	0	1	5	9	
Total	0	0	0	0	9	26	54	

Table B.9d cont'd

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	4	6	9	
2	0	0	0	0	2	6	10	
3	0	0	0	0	3	7	10	12
4	0	0	0	0	2	6	10	
5	0	0	0	0	2	6	10	
6	0	0	0	0	2	6	9	
Total	0	0	0	0	15	37	58	
1	0	0	1	4	6	9	10	
2	0	0	0	5	7	7	10	
3	0	0	2	3	8	8	10	24
4	0	0	2	3	7	9	10	
5	0	0	1	2	7	9	10	
6	0	0	1	2	6	8	10	
Total	0	0	7	19	41	52	60	
1	0	0	1	4	7	10	10	
2	0	0	2	4	9	10	10	
3	0	1	2	5	10	10	10	36
4	0	0	2	4	7	10	10	
5	0	0	1	2	8	10	10	
6	0	1	1	4	8	10	10	
Total	0	2	9	23	49	60	60	
1	0	0	2	5	9	10	10	
2	0	1	3	3	10	10	10	
3	0	1	2	5	10	10	10	48
4	0	0	2	5	9	10	10	
5	0	0	2	3	9	10	10	
6	0	1	2	5	9	10	10	
Total	0	3	13	26	56	60	60	

Table B.9e Mortality of the fifth instar larvae of M. brassicae (out of 3) after different contact times (h) with malathion

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	0	1	1	
2	0	0	0	0	0	1	1	
3	0	0	0	0	0	1	1	
4	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	1	4
6	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	1	
8	0	0	0	0	0	0	1	
9	0	0	0	0	0	1	1	
10	0	0	0	0	0	0	1	
Total	0	0	0	0	0	4	8	
1	0	0	0	0	0	1	2	
2	0	0	0	0	0	1	2	
3	0	0	0	0	0	1	1	
4	0	0	0	0	0	2	2	
5	0	0	0	0	0	1	1	8
6	0	0	0	0	0	2	2	
7	0	0	0	0	0	1	1	
8	0	0	0	0	0	2	1	
9	0	0	0	0	0	1	1	
10	0	0	0	0	0	1	2	
Total	0	0	0	0	0	13	15	
1	0	0	0	0	0	2	2	
2	0	0	0	0	0	2	2	
3	0	0	0	0	0	1	2	
4	0	0	0	0	1	1	2	
5	0	0	0	0	0	1	1	12
6	0	0	0	0	1	1	1	
7	0	0	0	0	0	2	2	
8	0	0	0	0	1	2	2	
9	0	0	0	0	1	1	1	
10	0	0	0	0	1	2	2	
Total	0	0	0	0	5	15	17	

Table B.9c cont'd

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	1	0	2	3	
2	0	0	0	0	1	2	3	
3	0	0	0	0	1	2	3	
4	0	0	0	0	1	2	3	
5	0	0	0	0	1	2	3	24
6	0	0	0	0	1	3	3	
7	0	0	0	0	1	2	3	
8	0	0	0	0	0	3	3	
9	0	0	0	0	1	1	3	
10	0	0	0	0	0	2	3	
Total	0	0	0	1	7	21	30	
1	0	0	0	1	1	3	3	
2	0	0	0	0	2	2	3	
3	0	0	0	0	1	2	3	
4	0	0	0	0	1	2	3	
5	0	0	0	1	1	3	3	36
6	0	0	0	0	2	3	3	
7	0	0	0	0	2	2	3	
8	0	0	0	0	1	3	3	
9	0	0	0	0	1	2	3	
10	0	0	0	0	1	2	3	
Total	0	0	0	2	13	24	30	
1	0	0	0	1	2	3	3	
2	0	0	0	0	2	2	3	
3	0	0	0	1	2	2	3	
4	0	0	0	1	1	3	3	
5	0	0	0	1	2	3	3	48
6	0	0	0	0	1	3	3	
7	0	0	0	0	2	3	3	
8	0	0	0	0	2	2	3	
9	0	0	0	0	1	2	3	
10	0	0	0	0	2	3	3	
Total	0	0	0	4	17	26	30	

Table B.9f Mortality of the sixth instar larvae of M. brassicae (out of 3) after different contact times (h) with malathion

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	0	0	0	
2	0	0	0	0	0	0	1	
3	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	
5	0	0	0	0	0	1	1	2
6	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	1	
8	0	0	0	0	0	0	1	
9	0	0	0	0	0	0	1	
10	0	0	0	0	0	0	1	
Total	0	0	0	0	0	1	6	
1	0	0	0	0	0	0	0	
2	0	0	0	0	0	1	1	
3	0	0	0	0	0	0	1	
4	0	0	0	0	0	0	0	
5	0	0	0	0	1	1	1	4
6	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	1	
8	0	0	0	0	0	0	1	
9	0	0	0	0	0	0	0	
10	0	0	0	0	1	1	1	
Total	0	0	0	0	2	3	6	

Table B.9f cont'd

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	1	1	0	
2	0	0	0	0	0	0	1	
3	0	0	0	0	0	1	1	
4	0	0	0	0	1	1	1	
5	0	0	0	0	1	1	1	8
6	0	0	0	0	0	0	1	
7	0	0	0	0	0	0	0	
8	0	0	0	0	1	1	1	
9	0	0	0	0	0	0	0	
10	0	0	0	0	0	0	0	
Total	0	0	0	0	4	5	7	
1	0	0	0	0	1	1	2	
2	0	0	0	0	0	0	1	
3	0	0	0	0	0	1	2	
4	0	0	0	0	1	1	1	
5	0	0	0	0	1	1	2	12
6	0	0	0	0	0	1	2	
7	0	0	0	0	0	0	0	
8	0	0	0	0	1	0	1	
9	0	0	0	0	0	1	2	
10	0	0	0	0	0	0	1	
Total	0	0	0	0	4	6	14	
1	0	0	0	1	1	1	2	
2	0	0	0	0	0	1	2	
3	0	0	0	0	1	1	3	
4	0	0	0	0	1	1	2	
5	0	0	0	0	1	1	2	24
6	0	0	0	0	0	1	2	
7	0	0	0	0	0	0	2	
8	0	0	0	0	1	1	1	
9	0	0	0	0	1	2	2	
10	0	0	0	0	0	0	2	
Total	0	0	0	1	6	9	20	

Table B.9f cont'd

Rep	No treatment	% malathion (v/v) 0.0	0.00125	0.25	2.5	10.0	20.0	Time (h)
1	0	0	0	0	1	2	2	
2	0	0	0	1	1	2	2	
3	0	0	0	0	1	1	3	
4	0	0	0	0	1	1	2	
5	0	0	0	0	1	2	2	36
6	0	0	0	0	1	2	3	
7	0	0	0	0	1	1	2	
8	0	0	0	1	1	2	2	
9	0	0	0	0	1	3	3	
10	0	0	0	0	0	2	2	
Total	0	0	0	2	8	19	23	
1	0	0	0	0	1	2	3	
2	0	0	0	1	2	3	3	
3	0	0	0	0	1	2	3	
4	0	0	0	0	1	1	3	
5	0	0	0	0	1	2	3	48
6	0	0	0	0	1	2	3	
7	0	0	0	0	2	2	2	
8	0	0	0	1	1	2	2	
9	0	0	0	0	1	3	3	
10	0	0	0	0	1	2	3	
Total	0	0	0	2	12	21	28	

Table B.10 The number of larvae of M. brassicae (out of 60) which had moved away[+] from a DDT-treated substrate at different times (h).

a) I INSTAR

Time (h)	% (w/v) DDT					
	Control	Control with Risella 17 oil	0.00025	0.025	0.5	2.0
2	56	45	39	55	44	28
4	50	35	34	47	47	21
8	48	34	29	27	39	6
12	33	37	26	32	24	1
24	29	29	21	23	7	0

b) II INSTAR

Time (h)	% (w/v) DDT						
	Control	Control with Risella 17 oil	0.00025	0.0025	0.025	0.5	2.0
2	22	33	29	28	23	25	9
4	23	25	12	27	22	24	0
8	19	24	31	19	24	20	1
12	11	10	9	5	6	0	0
24	4	7	0	0	0	0	0

[+]i.e. Larvae on walls and lid of Petri dish

Table B.10 cont'd

c) III INSTAR

Time (h)	% (w/v) DDT						
	Control	Control with Risella 17 oil	0.00025	0.0025	0.025	0.5	2.0
2	24	27	28	25	19	16	0
4	22	13	15	29	18	24	0
8	39	21	18	21	13	17	5
12	27	17	20	17	10	7	4
24	7	9	11	9	7	0	0

d) IV INSTAR

Time (h)	% (w/v) DDT						
	Control	Control with Risella 17 oil	0.00025	0.0025	0.025	0.5	2.0
2	1	8	17	6	16	20	7
4	7	13	10	9	10	14	3
8	10	10	19	8	10	7	4
12	8	15	13	9	14	5	2
24	5	12	7	3	4	1	0

Table B.10e The number of V instar larvae of M. brassicae (out of 30) which had moved away+ from a DDT-treated substrate at different times (h).

Time (h)	% (w/v) DDT						
	Control	Control with Risella 17 oil	0.00025	0.0025	0.025	0.5	2.0
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
8	0	0	1	0	0	0	0
12	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0

+i.e. Larvae on walls and lid of Petri dish

Table B.10f The number of VI instar larvae of M. brassicae (out of 30) which had moved away+ from a DDT-treated substrate at different times (h).

Time (h)	% (w/v) DDT						
	Control	Control with Risella 17 oil	0.00025	0.0025	0.025	0.5	2.0
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0

+i.e. Larvae on walls and lid of Petri dish

Table B.11 The number of larvae of M. brassicae (out of 60) which had moved away[+] from a malathion-treated substrate at different times (h)

a) I INSTAR

Time (h)	% (v/v) Malathion						
	Control	Control with olive oil	0.000125	0.25	2.5	10	20
2	38	34	27	23	42	8	1
4	36	20	17	18	38	4	2
8	30	26	17	27	18	1	2
12	15	14	12	11	4	0	0
24	9	6	4	5	0	0	0

b) II INSTAR

Time (h)	% (v/v) Malathion						
	Control	Control with olive oil	0.000125	0.25	2.5	10	20
2	19	33	25	24	23	28	11
4	21	29	20	25	20	19	6
8	24	33	23	27	16	12	3
12	19	34	27	18	7	8	0
24	21	22	13	1	2	3	0

[+]i.e. larvae on walls and lid of Petri dish

Table B.11 cont'd

c) III INSTAR

Time (h)	% (v/v) Malathion						
	Control	Control with olive oil	0.000125	0.25	2.5	10	20
2	16	6	14	28	31	32	17
4	19	9	21	25	37	19	9
8	12	15	23	31	15	16	7
12	20	17	21	24	11	9	0
24	11	3	0	1	7	0	0

d) IV INSTAR

Time (h)	% (v/v) Malathion						
	Control	Control with olive oil	0.000125	0.25	2.5	10	20
2	21	18	9	21	20	18	27
4	24	6	18	12	33	36	12
8	15	21	15	27	21	27	9
12	27	12	18	24	15	19	1
24	21	5	9	12	12	3	0

Table B.11e The number of V instar larvae of M. brassicae (out of 30) which had moved away+ from a malathion-treated substrate at different times (h)

Time (h)	% (v/v) Malathion						
	Control	Control with olive oil	0.000125	0.25	2.5	10	20
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0

+i.e. Larvae on walls and lid of Petri dish

Table B.11f The number of VI instar larvae of M. brassicae (out of 30) which had moved away+ from a malathion-treated substrate at different times (h)

Time (h)	% (v/v) Malathion						
	Control	Control with olive oil	0.000125	0.25	2.5	10	20
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0

+i.e. Larvae on walls and lid of Petri dish

APPENDIX C

TABLE C.1 : Effect of DDT on the rate of feeding of larvae of *M. brassicae* on cabbage leaf discs. Cumulative number of larvae out of 50 which completely consumed discs at given times.

a) IV instar

Time (h)	% (w/v) DDT							
	0.0*	0.025	0.05	0.1	0.25	0.5	1.0	2.0
0	-	-	-	-	-	-	-	-
4	3	-	-	-	-	-	-	-
8	8	2	1	-	-	-	-	-
16	19	14	9	7	6	-	-	-
20	29	18	9	10	7	-	-	-
24	32	25	20	15	12	10		

b) V instar

Time (h)	% (w/v) DDT							
	0.0	0.025	0.05	0.1	0.25	0.5	1.0	2.0
0	-	-	-	-	-	-	-	-
4	17	15	5	-	-	-	-	-
8	28	32	30	14	2	-	-	-
16	38	40	35	35	34	18	10	-
20	38	40	39	35	34	20	10	-
24	49	40	39	35	34	20	10	-

* Risella 17 oil control.

- Denotes none of the larvae had completely consumed the leaf discs.

TABLE C.1 : Continued

c) VI instar

Time (h)	% (w/v) DDT							
	0.0	0.025	0.05	0.1	0.25	0.5	1.0	2.0
0	-	-	-	-	-	-	-	-
4	50	41	40	39	37	25	25	21
8	50	49	44	45	41	39	35	30
16	50	50	50	50	41	41	35	32
20	50	50	50	50	49	45	35	32
24	50	50	50	50	50	50	35	32

Two-way ANOVA

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	4015.8	2007.9	55.16 **
Treatments	7	2758.0	394.0	10.82 **
Error	14	510.3	36.4	
Total	23	7284.0		

** $P < 0.01$

TABLE C.1 : Continued

Two-way ANOVA on differences in numbers consuming each treatment (DDT) at the different times for each instar.

a) IV instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	2	763.1	381.6	20.516**
Treatments	6	780.5	130.1	6.995**
Error	12	223.5	18.6	
Total	20	1767.1		

** P < 0.01

b) V instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	4	3520.8	880.2	19.517**
Treatments	7	5746.4	820.9	18.202**
Error	28	1262.3	45.1	
Total	39	10529.6		

** P < 0.01

TABLE C.1 : Continued

c) VI instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	4	639.4	159.85	17.72 **
Treatments	7	1960.4	280.06	31.05 **
Error	28	252.6	9.02	
Total	39	2852.4		

** P < 0.01

TABLE C.2 : Effect of malathion on the rate of feeding of larvae of *M. brassicae* on cabbage leaf discs. Number of larvae out of 50 which completely consumed leaf discs at given times.

a) IV instar

Time (h)	% (v/v) Malathion							
	0.0*	0.00125	0.0125	0.025	0.25	1.25	5.0	10.0
0	-	-	-	-	-	-	-	-
4	2	-	-	-	-	-	-	-
8	11	12	10	9	11	5	1	-
16	23	21	19	17	18	14	5	-
20	28	21	23	19	20	19	5	-
24	30	28	25	22	20	20	10	-

* Olive oil control

b) V instar

Time (h)	% (v/v) Malathion								
	0.0	0.00125	0.0125	0.025	0.25	1.25	5.0	10.0	20.0
0	-	-	-	-	-	-	-	-	-
4	29	33	39	35	29	14	9	10	-
8	50	45	43	38	39	29	16	14	-
16	50	50	50	47	45	38	27	24	-
20	50	50	50	47	45	39	27	24	-
24	50	50	50	47	46	41	27	24	-

TABLE C.2 : Continued

c) VI instar

Time (h)	% (v/v) Malathion								
	0.0	0.00125	0.0125	0.025	0.25	1.25	5.0	10.0	20.0
0	-	-	-	-	-	-	-	-	-
4	50	50	50	49	47	40	28	25	19
8	50	50	50	50	49	40	40	30	30
16	50	50	50	50	50	49	45	38	30
20	50	50	50	50	50	50	46	41	30
24	50	50	50	50	50	50	46	41	30

Two-way ANOVA

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	3991.4	1995.7	54.23 **
Treatments	8	3151.9	394.0	10.70 **
Error	16	589.3	36.8	
Total	26	7732.5		

** $P < 0.01$

TABLE C.2 : Continued

Two-way ANOVA on differences in numbers consuming each treatment (Malathion) at the different times for each instar.

a) IV instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	2	584.33	292.17	31.862**
Treatments	7	1173.29	167.61	18.278**
Error	14	128.33	9.17	
Total	23	1885.95		

** P < 0.01

b) V instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	4	1567.8	391.9	30.858**
Treatments	8	10386.8	1298.4	102.235**
Error	32	405.4	12.7	
Total	44	12360.0		

** P < 0.01

TABLE C.2 : Continued

c) VI instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	4	288.6	72.1	6.216**
Treatments	8	2618.0	327.3	28.216**
Error	32	370.6	11.6	
Total	44	3277.2		

** P < 0.01

TABLE C.3 : Mortality (out of 50) of larvae of *M. brassicae* after 24 h exposure to insecticides in the "leaf-sandwich" method of assessing rate of feeding.

a) DDT-treated leaf discs

Larval instar	% (w/v) DDT							
	Risella 17 oil treated control	0.025	0.05	0.1	0.25	0.5	1.0	2.0
IV	0	3	9	16	28	34	40	50
V	0	1	3	11	24	25	36	38
VI	0	1	1	7	13	19	27	35

b) Malathion-treated leaf discs

Larval instar	% (v/v) Malathion								
	Olive oil treated control	0.00125	0.0125	0.025	0.25	1.25	5.0	10.0	20.0
IV	0	0	1	8	17	35	47	49	-
V	0	0	0	0	2	11	30	43	44
VI	0	0	0	0	0	0	9	21	39

TABLE C.4 : Mean amount* (± 95% C.L.) of untreated cabbage leaf discs consumed by larvae of *M. brassicae* between various time intervals expressed as mg of graph paper traced from the disc.

Larval instar	mg graph paper	Time (h)			
		0-4	4-8	8-16	16-24
IV	Mean	2.5	3.8	3.1	2.6
	95% C.L. ±	2.3	4.5	2.3	2.3
V	Mean	13.3	5.0	16.3	14.0
	95% C.L. ±	2.3	8.6	13.6	2.7
VI	Mean	23.1	18.0	47.0	47.6
	95% C.L. ±	2.3	11.3	31.7	18.1

* n = 4

One-way ANOVA on instars

IV instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Time	5	19.40	3.88	2.45
Error	18	28.55	1.59	
Total	23	47.95		

TABLE C.4 : Continued

V instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	154.4	30.9	1.63
Error	18	340.4	18.9	
Total	23	494.8		

VI instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	103.6	20.7	0.76
Error	18	492.8	27.4	
Total	23	596.4		

Two-way ANOVA on amounts consumed by all instars at various time intervals

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	97.3	19.5	1.2
Instars	2	5394.5	2697.3	168.6 **
Interaction	10	180.1	18.0	1.1
Error	54	861.8	16.0	
Total	71	6533.7		

** $P < 0.01$

Fig. 1 : THE RELATIONSHIP BETWEEN LEAF WEIGHT (g) AND LEAF AREA (cm^2) ESTIMATED BY THE GRAVIMETRIC METHOD USING A4 GRAPH PAPER.

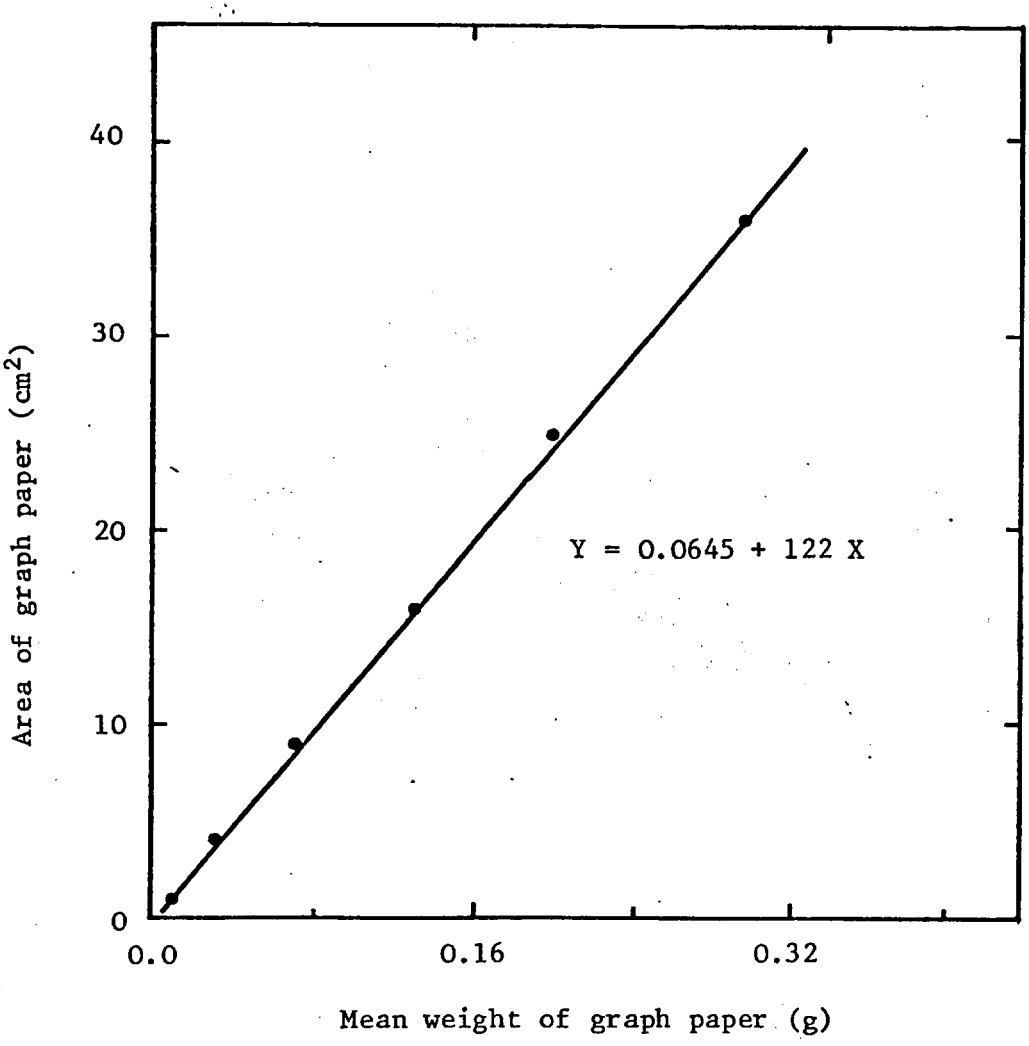


TABLE C.5 : Mean area* (cm^2) of untreated cabbage leaf discs consumed by larvae of *M. brassicae* between various time intervals.

Larval instar	Time intervals (h)				
	0-4	4-8	8-16	16-24	Total
IV	0.395	0.5281	0.4427	0.3817	1.722
V	1.6871	0.6745	2.0531	1.7725	6.187
VI	2.8827	2.2605	5.7985	5.8717	16.813

* Areas were calculated from data in Table C.4 as:

$Y = 0.0645 + 122X$ where

X = weight consumed in grams,

Y = area in cm^2

TABLE C.6 : Mean amount* (± 95% C.L.) of DDT-treated cabbage leaf discs consumed by larvae of *M. brassicae* between time intervals expressed as mg of graph paper traced from the discs.

Larval instar	Time (h)	% (w/v) DDT				
		Control R.17 oil	0.0005	0.05	0.5	2.0
IV	0-4	3.7 ± 2.3	2.5 ± 2.3	2.1 ± 2.3	1.7 ± 2.3	0.9 ± 0.5
	4-8	3.5 ± 2.3	2.4 ± 2.3	1.4 ± 2.3	0.3 ± 0.5	0.6 ± 2.3
	8-16	2.0 ± 0.9	1.8 ± 0.5	3.0 ± 0.9	1.3 ± 0.5	0.3 ± 0.2
	16-24	4.8 ± 4.5	3.7 ± 0.9	1.7 ± 0.5	0	0
V	0-4	11.9 ± 2.3	9.3 ± 2.3	7.9 ± 2.3	5.5 ± 2.3	3.9 ± 2.3
	4-8	13.3 ± 4.5	8.6 ± 4.5	6.9 ± 2.3	7.7 ± 2.3	5.7 ± 2.3
	8-16	16.7 ± 6.8	6.8 ± 2.3	7.7 ± 2.3	1.2 ± 2.3	1.8 ± 0.5
	16-24	17.2 ± 6.8	15.5 ± 6.8	9.8 ± 6.8	8.4 ± 2.3	1.8 ± 0.9
VI	0-4	35.5 ± 27.2	20.1 ± 2.3	17.4 ± 2.3	14.8 ± 4.5	7.7 ± 2.3
	4-8	22.4 ± 13.6	16.2 ± 2.3	15.1 ± 9.0	9.1 ± 4.5	5.1 ± 2.3
	8-16	42.5 ± 22.7	34.1 ± 2.3	27.4 ± 2.3	11.0 ± 5.6	4.7 ± 2.3
	16-24	32.3 ± 8.6	15.0 ± 6.1	4.0 ± 2.3	15.5 ± 9.0	2.1 ± 2.3

* n = 4

Two-way ANOVA on instars

IV instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	28.85	5.77	14.79**
Treatments	4	67.79	16.95	43.35**
Interaction	20	22.77	1.13	2.91**
Error	90	35.18	0.39	
Total	119	154.61		

** P < 0.01

TABLE C.6 : Continued

V instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	440.81	88.16	65.19**
Treatments	4	820.36	205.09	153.05**
Interaction	20	86.21	4.31	3.22**
Error	90	120.36	1.34	
Total	119	1467.73		

** P < 0.01

VI instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	2109.7	421.9	31.02**
Treatments	4	5161.5	1290.4	94.88**
Interaction	20	795.4	39.8	2.93**
Error	90	1227.6	13.6	
Total	119	9294.3		

** P < 0.01

TABLE C.7 : Mean area* (cm^2) of DDT-treated cabbage leaf discs consumed by larvae of *M. brassicae* between various time intervals.

Larval instar	Time (h)	% (w/v) DDT				
		Control R.17 oil	0.0005	0.05	0.5	2.0
IV	0-4	0.5159	0.3695	0.3207	0.2719	0.1743
	4-8	0.4915	0.3573	0.2353	0.1011	0.1377
	8-16	0.3085	0.2841	0.4305	0.2231	0.4305
	16-24	0.6501	0.5159	0.2719	0	0
	Total	1.9660	1.5268	1.2584	0.5961	0.7425
V	0-4	1.5163	1.1991	1.0283	0.7355	0.5403
	4-8	1.6871	1.1137	0.9063	1.0040	0.7599
	8-16	2.1019	0.8941	1.0039	0.2109	0.2841
	16-24	2.1629	1.9555	1.2601	1.0893	0.2841
	Total	7.4674	5.1624	4.1986	3.0397	1.8684
VI	0-4	4.3955	2.5167	2.1873	1.8701	1.0039
	4-8	2.7973	2.0409	1.9067	1.1747	0.6867
	8-16	5.2495	3.3463	3.4073	1.4065	0.6379
	16-24	4.0051	1.8945	0.5525	1.9555	0.3207
	Total	16.4474	9.7984	8.0538	6.4068	2.6492

* Calculated for data in Table C.6.

TABLE C.7 : Continued

Two-way ANOVA

a) 0-4 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	11.043	5.521	15.956**
Treatments	4	4.240	1.060	3.063
Error	8	2.770	0.346	
Total	14	18.053		

** P < 0.01

b) 4-8 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	5.419	2.709	21.164**
Treatments	4	2.239	0.560	4.375
Error	8	1.022	0.128	
Total	14	8.679		

** P < 0.01

TABLE C.7 : Continued

c) 8-16 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	21.227	10.613	11.42 **
Treatments	4	9.749	2.437	2.623
Error	8	7.432	0.929	
Total	14	38.408		

** P < 0.01

d) 16-24 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	4.763	2.381	5.765*
Treatments	4	6.565	1.641	3.973*
Error	8	3.306	0.413	
Total	14	14.633		

* P < 0.05

TABLE C.7 : Continued

e) 0-24 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatment	4	84.37	21.09	3.93 *
Instars	2	151.53	75.77	14.11 **
Error	8	42.99	5.37	
Total	14	278.89		

* $P < 0.05$

** $P < 0.01$

TABLE C.8 : Mean amount* (± 95% C.L.) of malathion-treated cabbage leaf discs consumed by larvae of *M. brassicae* between various time intervals expressed as mg. of graph paper traced from the discs.

Larval instar	Time (h)	% (v/v) Malathion							
		Control olive oil	0.00125	0.0125	0.25	1.25	2.5	5.0	10.0
IV	0-4	4.2 ± 2.6	2.4 ± 2.6	2.1 ± 2.6	1.7 ± 1.8	1.7 ± 2.6	1.5 ± 2.6	1.2 ± 0.9	-
	4-8	8.4 ± 4.5	6.8 ± 2.6	4.0 ± 4.5	1.2 ± 0.2	1.0 ± 0.9	0.9 ± 0.9	0.6 ± 0.5	-
	8-16	2.7 ± 2.6	4.6 ± 2.6	3.0 ± 2.6	2.5 ± 2.6	2.8 ± 2.6	1.5 ± 1.3	1.1 ± 0.4	-
	16-24	5.4 ± 2.6	1.8 ± 1.3	2.6 ± 0.9	3.5 ± 2.6	2.3 ± 2.6	2.0 ± 2.6	1.2 ± 1.3	-
V	0-4	14.1 ± 6.8	10.5 ± 6.8	9.3 ± 2.6	8.8 ± 2.6	7.3 ± 2.6	6.5 ± 2.6	5.9 ± 2.6	3.6 ± 2.6
	4-8	2.9 ± 2.6	2.5 ± 1.6	2.1 ± 1.6	1.7 ± 0.9	1.8 ± 0.9	2.0 ± 0.9	1.9 ± 1.3	2.5 ± 0.9
	8-16	5.8 ± 2.6	7.7 ± 4.5	6.5 ± 2.6	6.8 ± 2.6	6.7 ± 2.6	6.8 ± 4.5	6.3 ± 2.6	9.2 ± 2.6
	16-24	6.2 ± 4.5	4.3 ± 2.6	5.3 ± 2.6	5.2 ± 4.5	4.9 ± 2.6	6.7 ± 2.6	5.1 ± 2.6	5.7 ± 2.6
VI	0-4	41.0 ± 2.6	32.6 ± 6.8	32.0 ± 11.4	24.5 ± 4.5	22.2 ± 4.5	19.5 ± 4.5	16.7 ± 4.5	12.4 ± 4.5
	4-8	5.2 ± 6.8	7.6 ± 2.6	7.5 ± 2.6	8.8 ± 2.5	7.7 ± 4.5	5.1 ± 4.5	5.0 ± 4.5	5.1 ± 4.5
	8-16	76.7 ± 34.0	64.6 ± 15.8	52.7 ± 13.6	50.1 ± 4.5	48.4 ± 9.0	45.6 ± 4.5	40.1 ± 11.4	23.6 ± 9.0
	16-24	14.3 ± 22.7	32.4 ± 13.6	47.9 ± 13.6	45.6 ± 15.8	39.7 ± 11.4	30.0 ± 9.0	30.5 ± 11.4	41.7 ± 30.5

* n = 4

TABLE C.8 : Continued

Two-way ANOVA

IV instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	71.275	14.255	29.33**
Treatments	7	223.376	31.911	65.66**
Interaction	35	185.259	5.293	10.89**
Error	144	69.977	0.486	
Total	191	549.887		

** P < 0.01

V instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	795.579	159.116	212.44**
Treatment	7	40.077	5.725	7.64**
Interaction	35	271.399	7.754	10.35**
Error	144	107.880	0.749	
Total	191	1214.935		

** P < 0.01

TABLE C.8 : Continued

VI instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Times	5	8695.04	1739.01	180.02**
Treatments	7	2715.04	387.86	40.15**
Interaction	35	4344.27	124.12	12.85**
Error	144	1391.35	9.66	
Total	191	17145.70		

** $P < 0.01$

TABLE C.9 : Mean area* (cm^2) of malathion-treated cabbage leaf discs consumed by larvae of *M. brassicae* between various time intervals.

Larval instar	Time (h)	% (v/v) Malathion							
		Control olive oil	0.00125	0.0125	0.25	1.25	2.5	5.0	10.0
IV	0-4	0.5769	0.3573	0.3207	0.2719	0.2719	0.2475	0.2109	-
	4-8	1.0893	0.8941	0.5525	0.2109	0.1865	0.1743	0.1377	-
	8-16	0.3939	0.6257	0.4305	0.3695	0.4061	0.2475	0.1987	-
	16-24	0.7233	0.2841	0.3817	0.4915	0.3451	0.3085	0.2109	-
	Total	2.7834	2.1612	1.6854	1.3438	1.2096	0.9778	0.7582	
V	0-4	1.7847	1.3455	1.1991	1.1381	0.9551	0.8575	0.7843	0.5037
	4-8	0.4183	0.3695	0.3207	0.2719	0.2841	0.3085	0.2963	0.3695
	8-16	0.7721	1.0039	0.8575	0.8941	0.8819	0.8941	0.8331	1.1869
	16-24	0.8209	0.5891	0.7111	0.6989	0.6623	0.8819	0.6867	0.7599
	Total	3.7960	3.3080	3.0884	3.0030	2.7834	2.9420	2.6004	2.8200
VI	0-4	5.0665	4.0407	3.9685	3.0535	2.7729	2.4435	2.1019	1.5773
	4-8	0.6989	0.9900	0.9795	1.1381	1.0039	0.6867	0.6745	0.6867
	8-16	9.4219	7.9437	6.438	6.1767	5.9693	5.6277	4.9567	2.9437
	16-24	1.8091	4.0123	5.9083	5.6277	4.9078	3.7245	3.7855	5.1519
	Total	16.9964	16.9867	17.3501	16.4351	14.6539	12.4824	11.5186	10.3596

* Calculated for data in Table C.8.

TABLE C.9 : Continued

Two-way ANOVA

a) 0-4 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	33.438	16.719	64.057**
Treatments	7	6.496	0.928	3.555*
Error	14	3.654	0.261	
Total	23	43.587		

* $P < 0.05$

** $P < 0.01$

b) 4-8 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	1.0993	0.5496	8.327**
Treatments	7	0.7077	0.1011	1.531
Error	14	0.9234	0.0660	
Total	23	2.7303		

** $P < 0.01$

TABLE C.9 : Continued

c) 8-16 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	160.31	80.15	54.155**
Treatments	7	12.04	1.72	1.162
Error	14	20.67	1.48	
Total	23	193.01		

** P < 0.01

d) 16-24 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	73.828	36.914	65.80 *
Treatments	7	3.150	0.450	0.802
Error	14	7.856	0.561	
Total	23	84.834		

** P < 0.01

TABLE C.9 : Continued

e) 0-24 h

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatment	6	34.54	5.76	2.42
Instars	2	700.52	350.26	147.17 **
Error	12	28.56	2.38	
Total	20	763.62		

** $P < 0.01$

TABLE C.10 : Mortality (out of 4) of the larvae of *M. brassicae* between 0-24 h exposure to insecticides in the "Gravimetric method" of assessing rate of feeding.

a) DDT-treated leaf discs

Larval instar	% (w/v) DDT				
	Risella 17 oil-treated control	0.0005	0.05	0.5	2.0
IV	0	2	3	4	4
V	0	0	2	3	3
VI	0	0	1	2	3

b) Malathion-treated leaf discs

Larval instar	% (v/v) Malathion							
	Olive oil-treated control	0.00125	0.0125	0.25	1.25	2.5	5.0	10.0
IV	0	0	1	1	2	3	4	-
V	0	0	0	0	1	1	1	3
VI	0	0	0	0	0	1	1	2

TABLE C.11 : Choice of feeding site by newly hatched I instar larvae of *M. brassicae*.

a) Cabbage leaves

Position of larvae	Mean* % larvae	
	Mean	95% C.L. ±
Lower epidermis	93	10
Upper epidermis	2	3
Elsewhere in container	5	7

* Five batches used

b) Potted cabbage plants

Position of larvae	Mean* (%) larvae (± 95% C.L.)	Extent of feeding
Upper epidermis	5 ± 4	0
Lower epidermis	96 ± 4	Considerable†

* Five batches on either the lower or upper epidermis.

† When the eggs were attached to the lower epidermis the lesions merged together due to extensive group feeding.

TABLE C.12 : Feeding site selection by early instar larvae of *M. brassicae* when the true lower epidermis was placed upwards facing the light (called "upper" epidermis).

Larval instar		% Larvae feeding or wandering	
		"Upper" epidermis	"Lower" epidermis*
I	Mean	3	87
	95% C.L. ±	6	18
II	Mean	8	85
	95% C.L. ±	7	14

* A few larvae were found wandering either on the tissue paper lining or on the lid of the experimental chamber.

TABLE C.13 : Feeding site selection by early instar larvae in total darkness compared to control.

Larval instar		% Larvae		
		Upper epidermis	Lower epidermis	Elsewhere
I	Control	0	93	7
	Dark			
	Mean	34	19	47
	95% C.L. ±	13	13	23
II	Control	3	63	34
	Dark			
	Mean	32	20	48
	95% C.L. ±	13	22	32

Two way ANOVA

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Distribution of larvae	2	208.33	104.17	17.69**
Instars	1	0.00	0.00	0.00
Interaction	2	1.00	0.50	0.09
Error	12	70.67	5.89	
Total	17	280.00		

** $P < 0.01$

TABLE C.13 : Continued

One-way ANOVA on the distribution of I instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Distribution of larvae	2	104.67	52.33	13.46**
Error	6	23.33	3.89	
Total	8	128.00		

** P < 0.01

One way ANOVA on the distribution of II instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Distribution of larvae	2	104.67	52.33	6.63*
Error	6	47.33	7.89	
Total	8	152.00		

* P < 0.05

TABLE C.14 : Selection of feeding site by early instar larvae of *M. brassicae* on insecticide-treated cabbage leaves after 24 h.

a) DDT-treated cabbage leaves

Larval instar		% Larvae on lower epidermis				
		Untreated control	Risella 17 oil treated control	% (w/v) DDT		
				0.025	0.2	2.0
I	Mean*	98	93	97	90	95
	95% C.L. ±	5	5	8	31	15
II	Mean	95	100	82	59	52
	95% C.L. ±	16	-	25	10	9

* n = 4 batches (30 larvae per batch)

One-way ANOVA on II instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Distribution of larvae	4	155.2	38.80	6.54**
Error	15	89.0	5.93	
Total	19	244.2		

** P < 0.01

TABLE C.14 : Continued

b) Malathion-treated cabbage leaves

Larval instar		% Larvae on lower epidermis				
		Untreated control	Olive oil-treated control	% (v/v) Malathion		
				0.00125	0.25	2.5
I	Mean	97.5	99.3	97.5	87.5	0
	95% C.L. ±	4.8	2.5	2.8	14.0	0
II	Mean	85.8	87.5	65.8	66.8	72.5
	95% C.L. ±	17.8	20.7	5.4	4.5	7.9

One-way ANOVA on I instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Distribution of larvae	4	2652.8	663.20	397.92**
Error	15	25.0	1.67	
Total	19	2677.8		

** $P < 0.01$

TABLE C.14 : Continued

One-way ANOVA on II instar

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Distribution of larvae	4	155.2	38.80	6.54**
Error	15	89.0	5.93	
Total	19	244.2		

** P < 0.01

TABLE C.15 : Effect of insecticides on the initiation of feeding by the early instar larvae of *M. brassicae*.

a) DDT-treated cabbage leaf

Larval instar	Rep.	Time (min) taken to start feeding by 50% larvae				
		Untreated control	Risella 17 oil treated control	% (w/v) DDT		
				0.025	0.2	2.0
I	1	0 - 10	10 - 20	10 - 20	10 - 20	>120
	2	0 - 10	0 - 20	10 - 20	10 - 20	>120
	3	0 - 10	0 - 20	0 - 10	10 - 20	>120
	4	0 - 10	0 - 20	0 - 10	20 - 30	>120
II	1	0 - 10	0 - 10	20 - 30	10 - 20	10 - 20
	2	0 - 10	10 - 20	10 - 20	10 - 20	10 - 20
	3	0 - 10	10 - 20	10 - 20	10 - 20	10 - 20
	4	0 - 10	10 - 20	10 - 20	10 - 20	10 - 20

TABLE C.15 : Continued

b) Malathion-treated cabbage leaf

Larval instar	Rep.	Time (min) taken to start feeding by 50% larvae				
		Untreated control	Olive oil treated control	% (v/v) Malathion		
				0.00125	0.25	2.5
I	1	0 - 10	10 - 20	10 - 20	30 - 40	>120
	2	0 - 10	10 - 20	20 - 30	40 - 50	>120
	3	0 - 10	10 - 20	10 - 20	50 - 60	>120
	4	0 - 10	10 - 20	10 - 20	40 - 50	>120
II	1	0 - 10	0 - 10	10 - 20	10 - 20	10 - 20
	2	0 - 10	10 - 20	10 - 20	10 - 20	10 - 20
	3	0 - 10	10 - 20	10 - 20	10 - 20	10 - 20
	4	0 - 10	10 - 20	10 - 20	10 - 20	10 - 20

TABLE C.16 : Two-way ANOVA on area of insecticide-treated leaf consumed by I and II instar larvae of *M. brassicae*.

a) DDT-treated cabbage leaf

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatments	4	701.72	175.43	175.43**
Instars	1	409.98	409.98	409.98**
Interaction	4	79.94	19.98	19.98**
Error	30	30.09	1.00	
Total	39	1221.73		

** P < 0.01

a.i. One-way ANOVA on area consumed by I instar within 24 h.

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatment	4	213.98	53.49	43.97**
Error	15	18.25	1.22	
Total	19	232.23		

** P < 0.01

TABLE C.16 : Continued

a.ii. One-way ANOVA on area consumed by II instar within 24 h.

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatment	4	567.67	141.92	179.73**
Error	15	11.85	0.79	
Total	19	579.52		

** P < 0.01

b) Malathion-treated cabbage leaf

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatments	4	416.313	104.078	120.18**
Instars	1	277.202	277.202	320.10**
Interaction	4	63.337	15.834	18.28**
Error	30	25.980	0.866	
Total	39	782.832		

** P < 0.01

TABLE C.16 : Continued

b.i. One-way ANOVA on area consumed by I instar within 24 h.

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatment	4	126.698	31.674	70.14**
Error	15	6.773	0.452	
Total	19	133.472		

** P < 0.01

b.ii. One-way ANOVA on area consumed by II instar within 24 h.

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Treatment	4	352.95	88.24	68.91**
Error	15	19.21	1.28	
Total	19	372.16		

** P < 0.01

TABLE C.17 : Mean mortality (± S.D.) of early instar larvae of *M. brassicae* (out of 30) after 24 h exposure to insecticide-treated cabbage leaf.

a) DDT-treated cabbage leaf

Larval instar	Untreated control	Risella 17 oil treated control	% (w/v) DDT		
			0.025	0.2	2.0
I	0	1 ± 1	3 ± 1	10 ± 1	30 ± 0
II	0	1 ± 1	3 ± 1	5 ± 1	27 ± 2

b) Malathion-treated cabbage leaf

Larval instar	Untreated control	Olive oil treated control	% (v/v) Malathion		
			0.00125	0.25	2.5
I	0	0	8 ± 1	17 ± 2	29 ± 1
II	0	0	1 ± 1	5 ± 1	24 ± 2

TABLE C.18 : Mean amount (mg)† (± 95% C.L.) of oil-treated leaf disc consumed by fifteen early instar larvae of *M. brassicae* in a "Three-way" choice chamber.

a) Brussel sprout leaves

Larval instar		Amount (mg)		
		Untreated control	Olive oil	R.17 oil
I	Mean	9.9	4.3	2.5
	95% C.L. ±	4.4	2.5	2.2
II	Mean	70.2	94.9	73.6
	95% C.L. ±	8.1	9.9	14.4
III	Mean	121.5	203.9	80.2
	95% C.L. ±	49.5	55.0	75.0

† n = 5

Two-way ANOVA on the total amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.120125	0.060063	69.68**
Treatments	2	0.021722	0.010861	12.60**
Interaction	4	0.020077	0.005019	5.82**
Error	36	0.031022	0.000862	
Total	44	0.192946		

** $P < 0.01$

TABLE C.18 : Continued

b) Bean leaves

Larval instar		Amount (mg)		
		Untreated control	Olive oil	R.17 oil
I	Mean	8.1	14.7	8.1
	95% C.L. ±	3.9	6.2	8.6
II	Mean	35.1	24.3	25.5
	95% C.L. ±	21.1	11.0	18.0
III	Mean	109.4	121.8	74.0
	95% C.L. ±	72.7	86.6	31.9

Two-way ANOVA on the total amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.07038	0.03519	33.84**
Treatments	2	0.00273	0.00137	1.32
Interaction	4	0.00392	0.00098	0.92
Error	36	0.03744	0.00104	
Total	44	0.11448		

** $P < 0.01$

TABLE C.19 : Mean amount (mg)† (± 95% C.L.) of malathion-treated Brussel sprout leaf disc consumed by the early instar larvae of *M. brassicae* after 24 h in a "Two-way" choice chamber.

Larval instar		% (v/v) Malathion							
		Untreated control	Control olive oil	Control olive oil	0.0005	Control olive oil	0.00125	Control olive oil	0.0125
I	Mean	61.8	26.9	36.0	16.7	34.5	11.6	25.4	6.0
	95% C.L. ±	17.2	12.9	26.0	8.0	8.0	11.0	28.0	8.0
II	Mean	61.1	21.5	37.5	23.2	34.6	20.7	27.2	15.0
	95% C.L. ±	28.0	4.0	23.0	4.0	23.0	4.0	4.0	8.0
III	Mean	101.9	31.9	55.5	19.3	57.7	15.7	33.0	12.2
	95% C.L. ±	40.0	4.0	40.0	17.0	40.0	4.0	26.0	2.0

† n = 3

TABLE C.19 : Continued

Two-way ANOVA on the total amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.003852	0.001926	10.94**
Treatments	3	0.015646	0.005215	29.80**
Interaction	6	0.002887	0.000481	2.73*
Error	24	0.004233	0.000176	
Total	35	0.026617		

* $P < 0.05$

** $P < 0.01$

Two-way ANOVA on the differences in amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.00033	0.000165	1.0
Treatments	3	0.032732	0.010911	66.13**
Interaction	6	0.004539	0.000757	4.59**
Error	24	0.003965	0.000165	
Total	35	0.041567		

** $P < 0.01$

TABLE C.20 : Mean amount† (mg) (± 95% C.L.) of DDT-treated leaf disc consumed by twenty early instar larvae of *M. brassicae* after 24 h in a "Two-way" choice chamber.

a) Cabbage leaves

Larval instar		% (w/v) DDT					
		Untreated control	Control R.17 oil	Control R.17 oil	0.0002	Control R.17 oil	0.0025
I	Mean	40.0	35.1	57.5	20.8	48.2	15.6
	95% C.L. ±	16.0	5.0	50.0	2.0	40.0	9.0
II	Mean	94.5	48.8	86.6	48.9	94.9	25.3
	95% C.L. ±	60.0	20.0	20.0	30.0	36.0	5.0
III	Mean	127.9	93.5	134.3	99.9	127.3	43.9
	95% C.L. ±	20.0	30.0	36.0	70.0	17.0	50.0

† n = 3

Two-way ANOVA on total amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.084206	0.042103	235.2 **
Treatments	2	0.005380	0.00264	14.7 **
Interaction	4	0.002561	0.00064	3.6
Error	18	0.003226	0.000179	
Total	26	0.095272		

** $P < 0.01$

TABLE C.20 : Continued

Two-way ANOVA on the differences in amounts consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.000281	0.000141	0.226
Treatments	2	0.038906	0.019453	31.27 **
Interaction	4	0.006545	0.001637	2.63
Error	18	0.011199	0.000622	
Total	26	0.056932		

** $P < 0.01$

b) Bean leaves

Larval instar		% (w/v) DDT					
		Untreated control	Control R.17 oil	Control R.17 oil	0.0002	Control R.17 oil	0.0025
I	Mean	31.7	31.9	59.5	40.6	36.9	29.2
	95% C.L. ±	27.0	20.0	43.0	3.0	7.0	7.0
II	Mean	105.0	40.4	62.6	37.4	44.7	27.2
	95% C.L. ±	49.0	20.0	56.0	5.0	32.0	9.0
III	Mean	105.1	20.8	82.8	77.5	64.6	39.6
	95% C.L. ±	10.0	24.0	30.0	40.0	44.0	20.0

TABLE C.20 : Continued

Two-way ANOVA on total amounts consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.012954	0.006477	49.1 **
Treatments	2	0.00776	0.00388	29.4 **
Interaction	4	0.0078	0.00195	14.8 **
Error	18	0.002368	0.000132	
Total	26	0.030883		

Two-way ANOVA on differences in amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.003293	0.001647	3.98 *
Treatments	2	0.026311	0.013155	31.78 **
Interaction	4	0.00948	0.00237	5.72 **
Error	18	0.007459	0.000414	
Total	26	0.046543		

* $P < 0.05$

** $P < 0.01$

TABLE C.21 : Mean amount† (mg) (± 95% C.L.) of DDT-treated leaf disc consumed by the early instar larvae of *M. brassicae* after 24 h in a "Two-way" choice chamber.

a) Brussel sprout leaves

Larval instar		% (w/v) DDT	
		0.0002	0.0025
I	Mean	11.4	4.4
	95% C.L. ±	3.3	0.8
II	Mean	103.3	66.2
	95% C.L. ±	30.8	16.1
III	Mean	202.9	177.8
	95% C.L. ±	31.5	13.9

† n = 5

Two-way ANOVA on total amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.335529	0.167765	354.72**
Error	12	0.005675	0.000473	
Total	14	0.341205		

** $P < 0.01$

TABLE C.21 : Continued

Two-way ANOVA on differences in amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.002291	0.001145	2.07
Error	12	0.006654	0.000555	
Total	14	0.008946		

b) Bean leaves

Larval instar		% (w/v) DDT	
		0.0002	0.0025
I	Mean	15.1	17.9
	95% C.L. ±	23.0	20.0
II	Mean	65.7	34.6
	95% C.L. ±	13.2	7.0
III	Mean	195.1	125.5
	95% C.L. ±	19.6	26.0

TABLE C.21 : Continued

Two-way ANOVA on the total amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.226286	0.113143	903.58**
Error	12	0.001503	0.000125	
Total	14	0.227788		

Two-way ANOVA on the differences in amount consumed within a choice chamber

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.013118	0.006559	13.13**
Error	12	0.005994	0.000499	
Total	14	0.019112		

** $P < 0.01$

TABLE C.22 : Mean amount† (mg) (± 95% C.L.) of DDT-treated leaf disc consumed by early instar larvae of *M. brassicae* after 24 h in a "One-way" choice chamber.

a) Brussel sprout leaves

1)

Larval instar		% (w/v) DDT
		0.0002
I	Mean	32.2
	95% C.L. ±	11.4
II	Mean	123.6
	95% C.L. ±	18.0
III	Mean	338.3
	95% C.L. ±	37.0

2)

Larval instar		% (w/v) DDT
		0.0025
I	Mean	35.9
	95% C.L. ±	10.0
II	Mean	122.5
	95% C.L. ±	14.0
III	Mean	284.0
	95% C.L. ±	31.0

† n = 5

One-way ANOVA on the total amount of DDT-treated leaf disc consumed within a choice chamber

a) 0.0002% (w/v)

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.091668	0.045834	112.09**
Error	12	0.004907	0.000409	
Total	14	0.096575		

** P < 0.01

TABLE C.22 : Continued

b) 0.0025% (w/v)

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.077242	0.038621	368.37**
Error	12	0.001258	0.000105	
Total	14	0.0785		

** P < 0.01

b) Bean leaves

1)

Larval instar		% (w/v) DDT
		0.0002
I	Mean	16.1
	95% C.L. ±	5.3
II	Mean	70.7
	95% C.L. ±	29.7
III	Mean	355.6
	95% C.L. ±	62.0

2)

Larval instar		% (w/v) DDT
		0.0025
I	Mean	17.0
	95% C.L. ±	2.2
II	Mean	67.6
	95% C.L. ±	15.0
III	Mean	321.4
	95% C.L. ±	93.0

TABLE C.22 : Continued

One-way ANOVA on the total amount of DDT-treated leaf disc consumed within a choice chamber

a) 0.0002% (w/v)

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.332206	0.166103	167.04**
Error	12	0.011933	0.000994	
Total	14	0.344138		

b) 0.0025% (w/v)

Due to	D.F.	S.S.	MS = S.S./D.F.	F
Instars	2	0.26609	0.13304	71.06**
Error	12	0.02247	0.00187	
Total	14	0.28855		

** $P < 0.01$

APPENDIX D

Table D.1. Hydrolysis of malathion-^{14}C at different pH values

pH* at 25 C	Rep	c.p.m.	Background counts c.p.m.	S-Values	Efficiency %	d.p.m.	Total d p m in aqueous phase**	Hydrolysis as % of Malathion-^{14}C added ***
6.5(P)	1	168	37	487	86.5	151	377	
	2	172	37	483	86.5	156	390	
	3	156	37	494	87.0	137	343	
	4	181	37	472	85.7	168	420	
	5	158	38	511	87.6	137	343	
	Mean						375	0.003
	S.E.$_1^+$						15	0.0001
7.9(P)	1	462	37	472	85.7	496	1240	
	2	425	37	480	86.2	450	1125	
	3	457	37	481	86.2	487	1217	
	4	438	37	486	86.5	463	1157	
	5	555	38	514	87.9	588	1470	
	Mean						1242	0.009
	S.E.$^+$						61	0.0004
7.9(T)	1	650	38	493	87.0	703	1757	
	2	554	38	515	87.9	587	1467	
	Mean						1612	0.011
	S.E.$^+$						146	0.001

Table D.1. cont'd

pH* at 25 C	Rep	c.p.m.	Background counts c.p.m.	S-Values	Efficiency+ %	d.p.m.	Total d.p.m in aqueous phase**	Hydrolysis as % of Malathion-^{14}C added***
8.3(T)	1	942	38	511	87.6	1032	2580	
	2	1208	38	507	87.4	1339	3347	
	3	994	38	506	87.4	1094	2735	
	4	1195	38	506	87.4	1324	3310	
	Mean						2993	0.020
	S.E.±						196	0.001
8.8(T)	1	1630	38	504	87.4	1824	4559	
	2	1659	38	504	87.4	1855	4639	
	3	2437	38	500	87.2	2751	6877	
	4	2286	38	505	87.4	2572	6430	
	Mean						5626	0.040
	S.E.±						600	0.004

+ Calculated from standard curve with ^{14}C-hexadecane in Bray's scintillant.

* Letters in parentheses indicate buffer used.
P = Phosphate buffer (Na_2HPO_4 - NaH_2PO_4) (0.1M).
T = Tris (hydroxymethyl) aminomethane buffer (0.05M).

** Total volume of aqueous fraction = 2.5 cm^3.
Thus, d.p.m. values (of 1.0 cm^3 aliquots) were multiplied by 2.5 to obtain the total d.p.m.

*** Total d.p.m. added 147,000 d.p.m.

T-tests between 6.5 and 7.9(p), 8.3 and 8.8 were significant at all levels.

T-test between 8.3 and 8.8 was significant at $P < 0.0059$.

Table D.2. Mortality of IV instar larvae of A. aegypti (out of 30) in malathion-treated medium after 24h

Replicate	Final concentration of malathion (ppm)					
	0.0	0.015	0.03	0.045	0.06	0.075
1	1	8	11	18	19	27
2	2	6	12	18	20	27
Total	3	14	23	36	39	54

LD50 estimated to be 0.043 ppm.

Table D.3. Mean mortality (± S.E.) with time, of IV instar larvae of A. aegypti (out of 30) in media of varying pH containing malathion

Time (h)	pH values					
	Control	7.0	7.7	8.3	8.6	8.8
4	0 (2)	1.5 ± 0.5 (2)	1.5 ± 0.5 (2)	1.0 ± 0.0 (2)	1.5 ± 0.5 (2)	1.5 ± 0.5 (2)
12	0 (2)	8.0 ± 1.0 (2)	3.0 ± 1.0 (2)	3.5 ± 0.5 (2)	5.5 ± 0.5 (2)	6.5 ± 0.5 (2)
24	1 (2)	16.0 ± 0.0 (2)	17.5 ± 0.5 (2)	16.5 ± 1.5 (2)	15.5 ± 0.5 (2)	15.0 ± 1.0 (2)

Table D.4. Percent mortality[+] with time of IV instar larvae of A. aegypti (out of 30) in media of varying pH containing malathion

Time (h)	pH value				
	7.0	7.7	8.3	8.6	8.8
4	5.0	5.0	3.3	5.0	5.0
12	26.7	10.0	11.7	18.3	21.7
24	51.7	56.9	53.5	50.1	48.3

[+] The percentage mortality was calculated after correction had been made for control mortality.

Table D.5. Hydrolysis of malathion-^{14}C by gut homogenates+ of V and VI instar larvae of M. brassicae

Larval Instar	Rep	c.p.m. in 1.0 cm^3	Background counts c.p.m.	S-values	Efficiency %	d.p.m. in 1.0 cm^3	Total d.p.m in aqueous phase*	Hydrolysis as % of Total d p m added**
V	1	17,928	36	471	85.7	20,877	52,194	
	2	11,845	36	457	84.8	13,926	34,814	
	3	24,099	36	475	86.0	27,980	69,951	
	4	10,659	36	474	86.0	12,352	30,881	
	5	15,638	36	470	85.7	18,205	45,513	
	6	19,161	36	480	86.2	22,187	55,467	
	7	29,657	36	458	85.1	34,807	87,018	
	8	23,284	36	471	85.7	27,127	67,818	
	9	31,788	36	462	85.1	37,311	93,278	
	10	36,867	36	463	85.4	43,128	107,819	
	Mean						64,475	0.22
	S.E.±						7,972	0.03
VI	11	25,808	36	522	88.1	29,253	73,133	
	2	39.867	36	526	88.3	45,109	112,772	
	3	18,387	36	512	87.6	20,949	52,372	
	4	34,139	36	517	87.9	38,797	96,994	
	5	26,562	36	459	85.1	31,170	77,926	
	6	14,097	36	455	84.8	16,581	41,453	
	7	35,365	36	453	84.8	41,662	104,154	
	8	37,977	36	460	85.1	44,584	111,460	
	9	35,040	36	466	85.4	40,988	102,470	
	10	42,306	36	462	85.1	49,671	124,177	
	Mean						89,691	0.31
	S.E.±						8,561	0.06

Table D.5. cont'd

*Total d.p.m. were calculated by multiplying by 2.5

**Total d.p.m. added 294,000

T-test significant at $P < 0.03$

+0.003g gut tissue was homogenized in $1.5cm^3$ of 0.1M pH 8.0 (Na_2HPO_4 - NaH_2PO_4) Phosphate buffer

Sp. act. = 20 uCi malathion-^{14}C mg^{-1}

i.e. $20 \times 2.22 \times 10^6$ d.p.m. mg^{-1}

$= 4.44 \times 10^6$ d.p.m. mg^{-1}

or 44.4×10^3 d.p.m. μg^{-1}

μg malathion / assay $= \frac{294,000}{44,400} = \underline{\underline{6.62}}$

$= 13.24$ ppm or $\frac{13.24}{330}$ mg^{-1}

$= \underline{4.0 \times 10^{-5} M}$

The mean amount of malathion-^{14}C hydrolysed have been obtained by dividing the mean d.p.m. by the specific activity of 20 uCi malathion-^{14}C mg^{-1}.

Table D.6. T L C analysis of the organic (Chloroform) fraction from Malathion-^{14}C experiment with larval gut homogenate

Larval instar	Rep	Total d.p.m in organic fraction	Malaoxon-^{14}C activity as % of total activity in organic fraction	Malaoxon-^{14}C % corrected for control Malaoxon-^{14}C	Malaoxon-^{14}C due to metabolism		
					d.p.m.	ng	p moles (10^{-12})
V	1	207,707	0.0735	0.0304	63	1.4	4.4
	2	239,497	0.0512	0.0081	19	0.4	1.3
	3	216,172	0.0660	0.0229	50	1.1	3.5
	Mean					1.0	3.1
	S.E.$\pm$					0.3	0.9
VI	1	209,555	0.0836	0.0405	85	1.8	5.7
	2	208,836	0.1002	0.0571	119	2.6	8.2
	3	220,367	0.0993	0.0562	124	2.7	8.5
	Mean					2.3	7.4
	S.E.$\pm$					0.3	0.9

Mean control % malaoxon 0.0431 (n = 2)

Specific activity = 6.6 uCi / m mol (20 uCi/mg malathion or 20.886 uCi/mg malaoxon)

$20.886 \times 2.22 \times 10^6 = 46.367 \times 10^6$ d.p.m. malaoxon mg^{-1}

$\therefore$ 1 ng malaoxon $= \frac{46.367 \times 10^6}{10^6} = \frac{46.367 \text{ d.p.m.}}{}$

Table D.7. Inhibition of ChE activity in gut/head extract mixtures of VI instar larvae of M. brassicae by malathion

Treatment	Mean[+] increase in absorbance in 10 min
Gut/head mixture untreated with malathion	0.250
Gut/head mixture treated with malathion for 10 min before assay	0.120
Gut pre-incubated for 1h with malathion + 10 min with gut/head mixture before assay	0.237

[+] n = 2

Table D.8. ChE activity of heated gut homogenates from VI instar larvae of M. brassicae

Pre-assay temperature (°C)	Increase in absorbance in 10 min	
	Heating duration (min)	
	5	10
Control (25)	0.165	0.180
35	0.165	0.168
40	0.160	0.155
50	0.120	0.105
60	0.065	0.050
70	0.060	0.060
80	0.045	0.065
90	0.025	0.020

Table D.9. Mortality (out of 30) of malathion-treated IV instar larvae of A. aegypti after 24h

Rep	Final concentration of malathion (ppm) (v/v)						
	Control	0.01	0.02	0.04	0.06	0.08	0.1
1	0	0	4	27	28	29	30
2	0	1	6	23	25	30	30
3	0	1	5	25	28	30	30
Total	0	2	15	75	81	89	90

LD50 value estimated to be 0.03 ppm

Table D.10. Mortality of IV instar larvae of A. aegypti (out of 30), exposed to malathion-treated gut extracts, after 24h

Rep	Control (15 ppm (v/v) malathion)	Gut extract + 90 ppm (v/v) malathion 2h incubation	Gut extract + 90 ppm (v/v) malathion 24h incubation
1	16	0	0
2	14	0	0
3	15	0	0
4	16	0	0
Total	61	0	0

Table D.11. Effect of 10^{-4} M Triphenyl phosphate (T.P.P.) on hydrolysis of Malathion-^{14}C by gut homogenates^{+} of VI instar larvae of M. brassicae

Substrate	Rep	c.p.m in 1.0 cm^3	Background counts c.p.m.	S-values	Efficiency %	d.p.m in 1.0 cm^3	Total d.p.m in aqueous phase*
Gut homogenate	1	24,692	36	021	61.0	40,420	101,049
	2	12,119	36	009	60.0	20,138	50,346
	3	43,531	36	483	86.5	50,283	125,708
	4	34,256	36	491	86.7	39,469	98,674
	Mean						93,944
	S.E.±						15,765
Gut homogenate with T.P.P.	1	3,136	36	486	86.5	3,584	8,960
	2	5,465	36	473	86.0	6,313	15,782
	3	7,471	36	477	86.0	8,645	21,613
	4	6,281	36	471	85.7	7,287	18,218
	Mean						16,143
	S.E.±						2,676
Control without homogenate	1	560	36	474	86.0	609	1,523
	2	393	36	487	86.5	413	1,032
	Mean						1,277
	S.E.±						248

*Total d.p.m. values obtained by multiplying by 2.5

T-test significant at P 0.0028

+0.003g gut tissue homogenized in 1.5 cm^3 pH 8.0 (Na_2HPO_4 - NaH_2PO_4) phosphate buffer.

Table D.12. Absorbance readings (at 412 nm) for cholinesterase (ChE) activity from head homogenates of VI instar larvae of M. brassicae in the presence of malathion

Time (h)	Concentration of malathion (M)+									
	0.0		1.5×10^{-5}		3×10^{-5}		1×10^{-4}		1×10^{-3}	
	1	2	1	2	1	2	1	2	1	2
0	0.115	0.160	0.120	0.192	0.162	0.195	0.084	0.185	0.118	0.135
1	0.124	0.175	0.125	0.205	0.165	0.208	0.086	0.190	0.120	0.138
2	0.132	0.183	0.130	0.212	0.176	0.215	0.092	0.196	0.124	0.140
3	0.138	0.190	0.138	0.218	0.184	0.218	0.094	0.205	0.125	0.146
4	0.145	0.197	0.143	0.223	0.195	0.223	0.098	0.208	0.128	0.146
5	0.153	0.205	0.148	0.228	0.202	0.230	0.102	0.210	0.133	0.148
6	0.162	0.210	0.155	0.233	0.206	0.237	0.106	0.215	0.138	0.152
7	0.169	0.218	0.162	0.238	0.210	0.245	0.109	0.218	0.140	0.155
8	0.175	0.225	0.168	0.242	0.212	0.252	0.112	0.220	0.142	0.158
9	0.182	0.230	0.173	0.244	0.215	0.256	0.115	0.225	0.142	0.162
10	0.190	0.238	0.178	0.248	0.218	0.260	0.118	0.228	0.145	0.165
Activity	0.075	0.078	0.058	0.056	0.056	0.065	0.034	0.043	0.027	0.030
% Inhibition	Ref.		23	15	25	17	55	45	64	62

+Enzyme extracts were incubated at these concentrations of pesticide for 15 min at 25°C before assay.

Table D.13. Absorbance readings (at 412 nm) for ChE activity from head homogenates of VI instar larvae of M. brassicae in the presence of malaoxon

Time (h)	Concentration of malaoxon (M)+									
	0.0		1×10^{-6}		5×10^{-6}		1×10^{-5}		1×10^{-4}	
	1	2	1	2	1	2	1	2	1	2
0	0.117	0.152	0.126	0.115	0.155	0.118	0.105	0.153	0.108	0.128
1	0.120	0.165	0.138	0.120	0.160	0.123	0.114	0.158	0.110	0.132
2	0.128	0.175	0.143	0.126	0.168	0.125	0.117	0.160	0.115	0.135
3	0.136	0.186	0.146	0.132	0.173	0.130	0.120	0.165	0.117	0.137
4	0.145	0.194	0.150	0.138	0.176	0.135	0.124	0.169	0.122	0.143
5	0.153	0.202	0.155	0.144	0.180	0.140	0.128	0.173	0.124	0.146
6	0.164	0.210	0.163	0.149	0.185	0.143	0.133	0.175	0.126	0.148
7	0.173	0.216	0.168	0.155	0.192	0.148	0.134	0.178	0.128	0.150
8	0.180	0.220	0.174	0.160	0.194	0.153	0.135	0.184	0.130	0.154
9	0.188	0.225	0.178	0.166	0.198	0.156	0.137	0.188	0.133	0.157
10	0.196	0.232	0.188	0.170	0.200	0.160	0.137	0.191	0.135	0.157
Activity	0.079	0.080	0.062	0.055	0.045	0.042	0.032	0.038	0.027	0.029
% Inhibition	Ref.		21	31	43	48	59	53	66	64

+ As for Malathion experiment.

Inhibition of head ChE of VI instar larvae of M. brassicae by malaoxon and malathion

Analysis of variance for:-

i. Regression equation $Y = 146 + 19.2X$

where Y = % inhibition

X = $\log_{10}$ concentration of malaoxon (M)

Due to	D.F.	S.S.	M.S. = S.S./D.F.	F.
Regression	1	765.6	765.6	19.73*
Residual	2	77.6	38.8	
Total	3	843.2		

*Significant at 5%

ii. Regression equation $Y = 144 + 25.8X$

where Y = % inhibition

X = $\log_{10}$ concentration of malathion (M)

Due to	D.F.	S.S.	M.S. = S.S./D.F.	F.
Regression	1	1281.4	1281.4	18.65
Residual	2	137.3	68.7	
Total	3	1418.8		