FACTORS INFLUENCING DIFFERENTIAL TOXICITY OF AVERMECTIN B_1 IN NOCTUID MOTH LARVAE (LEPIDOPTERA)

BY

PAUL TIMOTHY CHRISTIE

M.Sc. (Lond.), B.Sc. (Lond.), D.I.C.

Thesis submitted for the Degree of Doctor of Philosophy of the University of London

Imperial College of Science, Technology and Medicine

Department of Biology

Silwood Park

Ascot

Berkshire SL5 7PY

February 1990

This thesis is dedicated to
my parents and Mark

The caterpillar on the leaf,
Repeats to theethy mother's grief.
Kill not the moth or butterfly,
For the Last Judgement draweth nigh.

William Blake, 1757-1827

ABSTRACT

The toxicological effects of the neuroactive pesticide avermectin $B_{1a/1b}$ (80:20) (AVMB1 or abamectin) have been studied in the noctuid moths, Spodoptera littoralis (Boisduval) and Heliothis (Helicoverpa) armigera (Hübner), along with radiochemical and electrophysiological studies.

Topical application of AVMB1 against second to sixth instar S. littoralis larvae showed differential toxicity; the fourth and fifth instars being very insensitive, but the sixth instar very sensitive to AVMB1. An enhanced sensitivity of the sixth compared with the fifth instar was not found in H. armigera. Fifth instar H. armigera were more sensitive than fifth instar S. littoralis, but the sixth instar larvae were less sensitive to AVMB1. Topical application of lambda cyhalothrin and malathion did not show the same trend in fifth or sixth instar S. littoralis larvae.

Application of the microsomal oxidase inhibitor piperonyl butoxide (PB) significantly increased the toxicity of AVMB1 to second to fifth instar S. littoralis but not fifth instar H. armigera or sixth instar S. littoralis larvae. Injection of AVMB1 increased the toxicity of AVMB1 to fifth but not sixth instar S. littoralis nor to fifth instar H. armigera larvae.

The distribution and uptake of ^{3}H-AVMB1 in both species

was investigated. Comparisons were made between the amount of AVMB1, metabolites and the total amount of radioactivity in tissue samples of larvae showing either symptoms or no symptoms of poisoning with significantly more radioactivity in ventral nerve cord and fat body samples of larvae showing symptoms of poisoning. Fifth instar H. armigera larvae had significantly more radioactivity in the ventral nerve cord and fat body samples compared with sixth instar H. armigera and fifth and sixth instar S. littoralis larvae. Fifth instar S. littoralis larvae had a significantly higher proportion of radioactivity as metabolites in the ventral nerve cord compared with sixth instar S. littoralis or H. armigera larvae but this was significantly reduced when fifth instar S. littoralis larvae were treated previously with PB.

The action of topically-applied AVMB1 on an in vivo nerve preparation of fifth and sixth instar larvae was also investigated. Spontaneous activity was recorded extracellularly and significant correlations found between the total amount of radioactivity (and AVMB1) present in the ventral nerve cord and the action potential (A.P.) frequency in both species and both instars. There were significant differences between larvae showing different degrees of poisoning and A.P. frequency. A.P. amplitudes were also reduced after treatment with AVMB1.

The distribution and uptake of AVMB1 are discussed in relation to symptoms of poisoning and toxicological results.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my supervisor Dr Denis Wright for his encouragement, suggestions, advice and supervision of this project and criticism of the manuscript. I hope I have not put him off Cl^- channels for too long!

I also wish to thank Mark Broderick, Ray Crichton, Dr Lucy Leake and Dr Martyn Ford of Portsmouth Polytechnic for their considerable advice and assistance with the electrophysiology equipment used. My thanks also to Dr Stephen Young for his statistical advice; to Dr John Galley for help with the liquid scintillation counting; to Dr Mustaffa Djamgoz and Golly Righton for electrophysiological advice. My thanks also to Drs Geoff Le Patourel, Jim Hardie, Steve Dakin, Terry Corbitt, John Brady and Professors Keith Rudduck and Tony Lees for their advice and encouragement. My thanks also to Tim Carty, Sandra Smith and Sally Hill for supplying the insects, and to Merck, Sharp and Dohme Ltd for supply of the avermectins.

I am obliged to the Science and Engineering Research Council for their Research Studentship that enabled me to carry out this work.

Finally, my sincere thanks to all my friends and collegues at Silwood, past and present, especially Tanya Wright, Chris Addison, Ken de Souza, Fauziah (Ziee) Ismail, Marcel Holyoak, and John Watterson.

CONTENTS

		PAGE
TITLE PAGE		1
DEDICATION		2
ABSTRACT		3
ACKNOWLEDGEMENTS		5
CONTENTS		6
SECTION 1.	INTRODUCTION	
1.1.	The Avermectins-Isolation and chemistry.	17
1.2.	Biological activity.	22
1.2.1.	Nematodes.	22
1.2.2.	Lethal action on arthropods.	23
1.2.3.	Systemic, translaminar and residual toxicity against arthropods.	25
1.2.4.	Sublethal effects on arthropods.	28
1.2.4.1.	Reduction in feeding.	28
1.2.4.2.	Developmental abnormalities and effects on metamorphosis.	29
1.2.4.3.	Effects on reproduction and related processes.	32
1.3.	Cellular level.	33
1.3.1.	GABA systems.	34
1.3.1.1.	Early electrophysiological work.	34
1.3.1.2.	GABA receptors and binding sites.	39

1.3.1.2.1. Vertebrates. 39

1.3.1.2.2. Work on nematodes. 42

1.3.1.2.3. Arthropods. 44

1.3.1.3. Chloride ion conductance studies. 50

1.3.2. Non-GABA chloride channels. 50

1.4. The present study. 54

1.4.1. The experimental insects. 54

1.4.1.1. Spodoptera littoralis. 54

1.4.1.2. Heliothis armigera. 55

1.4.2. The aims of the project. 57

SECTION 2. GENERAL MATERIALS AND METHODS.

2.1. Insect culture. 59

2.2. Selection of larval instars. 60

2.3. Chemicals. 61

2.4. Topical bioassays. 61

2.5. Calibration of Arnold microapplicator and Agla syringe. 62

2.6. Analysis of bioassay data. 64

SECTION 3. TOXICOLOGICAL STUDIES.

3.1.	Introduction.	66
3.2.	Materials and methods.	66
3.2.1.	Toxicity of topically-applied AVMB1 to II, to VI instar larvae of S. littoralis.	66
3.2.2.	Toxicity of topically-applied AVMB1 with PB to III, IV, V and VI instar larvae of S. littoralis.	67
3.2.3.	Toxicity of topically-applied AVMB1 with DEF to V instar larvae of S. littoralis.	68
3.2.4a.	Injection of AVMB1 into V and VI instar S. littoralis and VI instar H. armigera.	68
3.2.4b.	Injection of PB into V instar S. littoralis followed by topical application of AVMB1.	68
3.2.5.	Toxicity of topically-applied malathion to V(±PB) and VI instar larvae of S. littoralis.	69
3.2.6.	Toxicity of topically-applied lambda cyhalothrin (Karate) to V(±PB) and VI instar larvae of S. littoralis.	70
3.2.7.	Toxicity of topically-applied AVMB1 to V instar(±PB) and VI instar larvae of H. armigera.	70
3.2.8.	Toxicity of topically-applied AVMB1 to VI instar larvae of S. littoralis (Egyptian	

field strain; F_0). 71

3.3. Results. 72

3.3.1. Toxicity of topically-applied AVMB1 to II, III, IV, V and VI instar larvae of S. littoralis. 72

3.3.2. Toxicity of topically-applied AVMB1 with topically-applied or injected PB to III, IV, V and VI instar larvae of S. littoralis. 74

3.3.3. Toxicity of topically-applied AVMB1 with DEF to V instar S. littoralis larvae. 74

3.3.4. Injection of AVMB1 into V and VI instar larvae of S. littoralis. 75

3.3.5. Toxicity of topically-applied malathion to V (±PB) and VI instar larvae of S. littoralis. 75

3.3.6. Toxicity of topically-applied lambda cyhalothrin (Karate) to V (±PB) and VI instar larvae of S. littoralis. 77

3.3.7. Toxicity of topically-applied AVMB1 to V instar(±PB) and VI instar and of injected AVMB1 to V instar H. armigera larvae. 77

3.3.8. Toxicity of topically-applied AVMB1 to Egyptian field strain (F_0) VI instar of S. littoralis. 79

3.4. Discussion. 80

3.4.1. Symptoms of poisoning with AVMB1. 80

3.4.2. Toxicity of topically-applied AVMB1 to larval instars of S. littoralis and H. armigera. 81

3.4.3. Comparative toxicity. 87

SECTION 4. UPTAKE OF ^{3}H-AVMB1 BY S. LITTORALIS AND H. ARMIGERA.

4.1. Introduction. 89

4.2. Materials and Methods. 89

4.2.1. Treatment of insects. 89

4.2.2. Isolation of radioactivity from S. littoralis and H. armigera. 91

4.2.2.1. Extraction of ^{3}H-AVMB1. 92

4.2.2.2. Thin layer chromatography (TLC) of ^{3}H-AVMB1. 93

4.2.2.3. TLC of chloroform extracts from ventral nerve cord and fat body tissues. 94

4.2.3. Toxicity of topically-applied AVMB1 (E.C.) to larvae of S. littoralis and H. armigera. 97

4.2.4. Radioactivity recovered from gut membrane and gut lumen of S. littoralis and H. armigera. 97

4.2.5. Calculation of the concentration of AVMB1 in tissue samples (as pMoles/mg wet weight). 98

4.2.6. Uptake and penetration of ^{3}H-AVMB1 into S. littoralis V and VI instar larvae over 24h. 98

4.3.	Results.	100
4.3.1.	Treatment of insects.	100
4.3.2.	Proportion of radioactivity present as $AVMB_1$ and its metabolites in tissue samples.	100
4.2.3.	The effect of piperonyl butoxide (PB).	110
4.3.4.	Radioactivity recovered from gut membrane and gut lumen contents from $AVMB_1$-treated S. littoralis and H. armigera larvae.	110
4.3.5.	Uptake and penetration of ^3H-$AVMB_1$ into S. littoralis V and VI instar larvae over 24 h.	112
4.4.	Discussion.	117

SECTION 5. ELECTROPHYSIOLOGY

5.1.	Introduction.	125
5.2.	Materials and Methods.	125
5.2.1.	The recording system.	125
5.2.2.	The experimental set-up.	127
5.2.3.	Data analysis.	129
5.3.	Results	131
5.4.	Discussion	143

SECTION 6. GENERAL CONCLUSIONS. 151

REFERENCES 160

APPENDICES 173

1.1. A comparison of doses of AVMB1 causing mortality in H. virescens, H. zea, S. eridania, S. frugiperda, H. armigera and S. littoralis 48, 72 and 96 h after treatment. 173

2.2. Artificial diet for S. littoralis and H. armigera. 174

3.1.1. Toxicity of topically-applied AVMB1, to larval instars of S. littoralis reared on artificial diet. 175

3.1.2. Toxicity of topically-applied AVMB1, with PB (5000 µg ml^{-1}) topically-applied 1 h before to larval instars of S. littoralis. 176

3.1.3. Toxicity of topically-applied AVMB1, with DEF to V instar S. littoralis. 177

3.1.4. Toxicity of AVMB1 following injection into V and VI instar of S. littoralis and V instar H. armigera larvae and toxicity of AVMB1 topically-applied following injection of 1µl 5000µg ml^{-1} PB into V

instar S. littoralis. 177

3.3.5. Toxicity of topically-applied malathion to V(±PB) and VI instar S. littoralis. 178

3.3.6. Toxicity of topically-applied lambda cyhalothrin (Karate) to V(±PB) and VI instar S. littoralis. 179

3.3.7. Toxicity of topically-applied AVMB1 to V (+PB) and VI instar H. armigera larvae. 179

3.3.8. Toxicity of topically-applied AVMB1 to VI instar S. littoralis larvae (Egyptian field strain; F_0). 180

4.1. Level of radioactivity recovered from internal standard-treated extract of V instar S. littoralis larvae and after direct addition to vial. 181

4.2. Level of ^{3}H-AVMB1 determined by TLC method from internal standard-treated nerve extract of V instar S. littoralis. 181

4.3. Proportion of radioactivity as AVMB1 following TLC of chloroform extracts from randomly-selected nerve cord samples. 182

4.4. Proportion of radioactivity as AVMB1 following TLC of chloroform extracts from randomly-selected nerve cord samples from S. littoralis and H. armigera, at 48 and 24 h respectively after application of ^{3}H-AVMB1. 183

4.5. Proportion of AVMB1 and metabolites extracted

from randomly-selected S. littoralis and H. armigera nerve cord samples 48 and 24 h respectively after application of ^{3}H-AVMB$_1$. 184

4.6. Radioactivity in randomly-selected S. littoralis and H. armigera nerve cords 48 and 24 h respectively after topical application of ^{3}H-AVMB$_1$. 185

4.7. Proportion of radioactivity as AVMB$_1$ following TLC of chloroform extracts of nerve cords from paralysed and non-paralysed larvae. 186

4.8. Proportion of radioactivity as AVMB$_1$ following TLC of chloroform extracts from S. littoralis and H. armigera nerve cord samples from paralysed and non-paralysed larvae, at 48 and 24 h respectively after application of ^{3}H-AVMB$_1$. 187

4.9. Proportion of AVMB$_1$ and metabolites extracted from nerve cord samples from paralysed and non-paralysed larvae 48 and 24 h respectively after application of ^{3}H-AVMB$_1$. 188

4.10. Radioactivity in randomly-selected S. littoralis and H. armigera nerve cords 48 and 24 h respectively after topical application of ^{3}H-AVMB$_1$. 189

4.11. Proportion of radioactivity as AVMB$_1$ following TLC of chloroform extract of fat body from paralysed and non-paralysed larvae. 190

4.12. The proportion of radioactivity as AVMB1 following TLC of chloroform extracts of fat body samples from paralysed (P) and non-paralysed (NP) S. littoralis and H. armigera at 48 and 24 h respectively after application of ^{3}H-AVMB1. 191

4.13. Proportion of AVMB1 and metabolites extracted from fat body samples from paralysed and non-paralysed larvae 48 and 24 h respectively after application of ^{3}H-AVMB1. 192

4.14. Radioactivity in randomly-selected S. littoralis and H. armigera fat body 48 and 24 h respectively after topical application of ^{3}H-AVMB1. 193

4.15. Radioactivity recovered from gut membrane and gut lumen of S. littoralis and H. armigera 48 and 24 h respectively following topical application with ^{3}H-AVMB1. 194

4.16. Calculation of AVMB1 concentration (pMoles/mg). 195

4.17. Wash-off samples from S. littoralis V and VI instar larvae over 24 h treated with 1 µl (uncalibrated volume) of ^{3}H-AVMB1. 196

4.18. Haemolymph samples from S. littoralis V and VI instar larvae over 24 h treated with 1 µl (uncalibrated volume) of ^{3}H-AVMB1. 197

4.19. Fat body samples from S. littoralis V and VI

instar larvae over 24 h treated with 1 µl (uncalibrated volume) of ^{3}H-AVMB$_{1}$. 198

5.1. Radioactivity extracted from nerve-cord samples of paralysed and non-paralysed larvae (24 or 48 h after application of ^{3}H-AVMB$_{1}$, following electrophysiology experiments. 199

5.2. Spike frequency from untreated, treated-non -paralysed and treated-paralysed S. littoralis and H. armigera fifth and sixth instar larvae. 200

5.3. Action potentials ($\bar{X}$ Hz ±S.E.) over 24 h. 202

5.4. Arrangement of recording and datalogging equipment. 204

1. INTRODUCTION

1.1. The Avermectins-Isolation and Chemistry.

The avermectins (AVM) are a complex of chemically related compounds which were first isolated from a novel species of actinomycete, Streptomyces avermitilis MA-4680 (NRRL 8165), during an anthelmintic screening programme for Merck, Sharp and Dohme in 1976 (Burg et al., 1979).

Structurally, AVM consist of a rigid sixteen membered lactone ring system, with a spiroacetal system (C-17 to C-25) attached so as to form two-six membered rings. There is also present either a cyclohexendiol or methoxy-cyclohexenediol group, which is cis-fused to a five membered cyclic ether. Another characteristic of these compounds is the presence of a dissacharide substituent, consisting of two identical a-l-oleandrose monomers coupled to C-13 through an oxygen bond (Fisher, 1984).

A closely related group of sixteen membered macrocyclic lactones are the milbemycins which are also derived from a Streptomyces strain. The principle differences between the AVM and the milbemycins are the absence of the C-13 hydroxy disaccharide substituent and the saturation at the 22-23 position in the latter. The AVM also have isopropyl and

sec-butyl substituents at C-25 whereas the milbemycins have methyl or ethyl substituents (Fisher, 1984; Fisher and Mrozik, 1984). Structurally, the AVM are also similar to macrolide antibiotics but do not exhibit antibacterial or antifungal properties (Onishi and Miller, 1985; Wang and Pong, 1982).

The naturally-occurring AVM can be separated into four major (A_{1a}, A_{2a}, B_{1a}, B_{2a}) and four minor (A_{1b}, A_{2b}, B_{1b}, B_{2b}) homologous components (Fig. 1.1.). Mixtures of homologous pairs containing at least 80% of the 'a' component and not more than 20% of the 'b' component are referred to as AVM A_1, A_2, B_1, B_2, (Campbell et al., 1983; Fisher, 1984). The 'B' series are generally the more biologically active; the relative toxicity of the B_1 and B_2 groups varying according to the target species (Campbell et al., 1983).

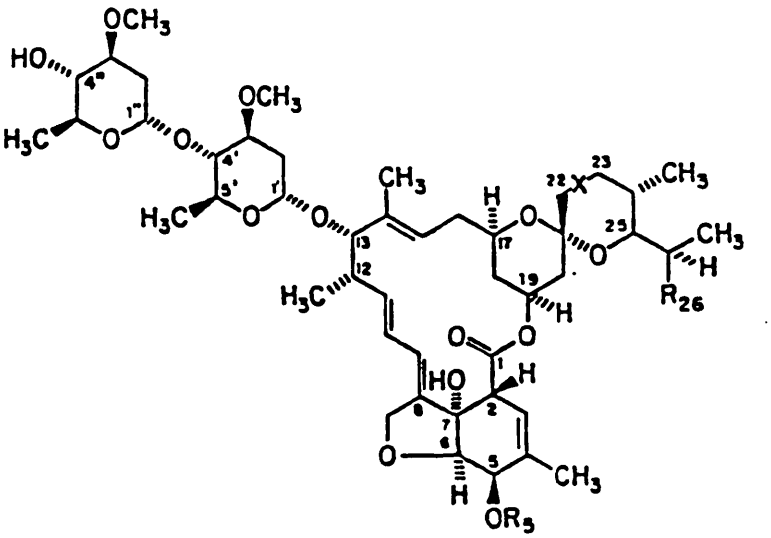
AVM have been shown to be active against nematodes, acarines and many insect species, including members of the orders Lepidoptera, Coleoptera, Hemiptera and Diptera, but have been found to be inactive against cestodes, trematodes, and protozoa (Putter et al., 1981; Campbell et al., 1981; Wright, 1986; Strong and Brown, 1987).

A synthetic derivative of $AVMB_1$, 22-23-dihydro-avermectin ($DHAVMB_1$, MK933 or ivermectin) is available as a

veterinary product for use against nematodes and arthropods (Campbell et al., 1983). Avermectin B_1 ($AVMB_1$, MK936 or abamectin) has been developed as an agricultural insecticide/acaricide (Dybas and Green, 1984; Strong and Brown, 1987) and can control pests which have become resistant to conventional acaricides and insecticides (Roush and Wright, 1986; Hoy and Conley, 1987; Dybas and Babu, 1988).

A semi-synthetic AVM derivative, 4"-deoxy-4"-methylamino-4"-epiavermectin B_1 hydrochloride (MK243, Fig. 1.2) which has a particularly good activity against lepidopterous larvae is currently undergoing trials (Trumble et al., 1987; Dybas and Babu, 1988; Dybas et al. 1989).

Fig. 1.1. Avermectin structures (after Fisher, 1984).



components A : $R_5 = CH_3$

components B : $R_5 = H$

components a : $R_{26} = C_2H_5$

components b : $R_{26} = CH_3$

components 1: X = -CH=CH-

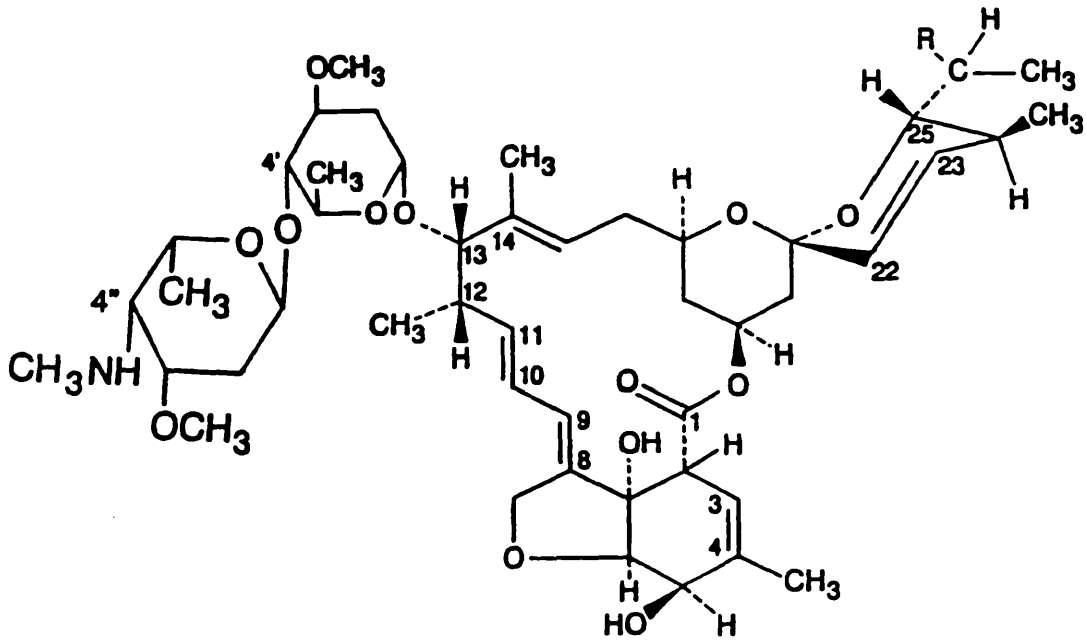
components 2: X = -CH_2-CH(OH)-

Avermectin B_{1a} (Williams and Yarbrough, 1979):(2aE,4E,5'S,6S,6'R,7S,8E,11R,13R,15S,17aR,20R,20aR, 20bS)-6'-[(R)-sec butyl]-7-[[2,6 dideoxy-4-O-(2,6-dideoxy-3-O-methyl-α-L-aribinohexopyranosyl)-3-O-methyl-α-L-aribino-hexopyranosyl] oxy] -5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy 5',6,8,19-tetramethyl-spiro-[11,15-methano-2H,13H,17H-furo-[4,3,2-pq] [2,3] benzodioxyacyclo-octadecin-13,2'-[2H] pyran]-17-ane.

Molecular weight: 823 (Mellin et al., 1983).

Fig. 1.2. MK-243 structure (after Dybas and Babu, 1988).

A mixture containing a minimum of 80% 4"-deoxy-4"-methylamino-4"-epiavermectin B1a hydrochloride and a maximum of 20% 4"-deoxy-4"-methylamino-4"-epiavermectin B1b hydrochloride



a-Component R = C_2H_5 ≥ 80%
b-Component R = CH_3 ≤ 20%

Empirical formulae (molecular weight):

B_{1a} $C_{49}H_{75}N_1O_{13}$.HCl (922.5) B_{1b} $C_{48}H_{73}N_1O_{13}$.HCl (908.53)

(Dybas and Babu, 1988).

1.2. Biological activity.

1.2.1. Nematodes

Most studies on nematodes have examined the inhibitory action of AVM on movement although some work has suggested that AVM can also inhibit the embryonic development of the microfilarial stage of the human filarial worm, Onchocerca volvulus (Campbell et al., 1983) and the dog heartworm, Dirofilaria immitis (Anantaphruti et al., 1982).

$AVMB_{1a}$ (1.5µg), injected into adult Ascaris suum was found to immobilize the worms without producing either hypercontraction or flaccid paralysis; the worms remaining capable of movement when stimulated mechanically (Kass et al., 1980). The sensitivity of nematodes to AVM is illustrated by studies on two animal parasitic species belonging to the Metastrongylidae, where complete paralysis occurred when intact worms were bathed in 3.6aM (3.6×10^{-15} M) $AVMB_{1a}$, although other parasitic species examined required at least 3.6nM (Sano et al., 1981). Likewise the plant parasitic nematode, Meloidogyne incognita (J2 stage) showed a marked triphasic response to concentrations of AVM >1 nM, which consisted of an initial loss of locomotor activity (1-10 min.) during which the nematodes were still sensitive to touch, a recovery phase (10-30 min.) and a final loss of activity during which the nematodes were insensitive to stimulation, (Wright et al., 1984). AVM have

been found to give very good control of plant nematodes in soils of low organic content at rates 10-30 fold lower than carbamate and organophosphate nematicides, but activity is lost in organic soils due to adsorption, (Putter et al., 1981; Sasser et al., 1982).

Wright et al. (1984) provided some circumstantial evidence for the involvement of γ-aminobutyric acid (GABA) in the action of AVM on nematodes by showing that the GABA antagonist bicuculine could completely prevent the triphasic response to AVM in M. incognita.

1.2.2. Lethal action on arthropods

In contrast to nematodes, AVM kill insects and mites directly although rather less rapidly than other neuroactive pesticides. Wright (1986) stated that it was uncertain whether the relative speed of kill with AVM was due to their mode of action (eg. because of the lack of hyperactive symptoms compared with other compounds) or to the relatively slow accumulation of AVM at critical target sites. Early symptoms of AVM poisoning, often observed a few h after application, such as paralysis of the hind legs of lepidopteran larvae (Putter et al., 1981), may therefore reflect an initial action at relatively exposed peripheral target sites. At high doses, treated insects are progressively immobilized; some showing a disturbed water

balance becoming distended with fluid, while in others disruption of moulting and metamorphosis have been observed (Strong and Brown, 1987).

Marked differences in sensitivity between various arthropod species have been reported, particularly in noctuid moth larvae. For example, Anderson et al. (1986) found Spodoptera eridania to be 150 times more tolerant of topically applied AVMB1 compared with Heliothis virescens and 415 times as tolerant in foliar-residue assays. Bull (1986) used radiolabelled AVMB1 to investigate differential toxicity in H. virescens and Heliothis zea compared with Spodoptera frugiperda (96h LD_{50} values 0.032, 0.026 and >10.0 ug AVMB1/insect respectively) and found that absorption of pesticide from the gut and its accumulation in the head were similar for all three species. He considered, therefore, that differential rates of cuticular penetration were unlikely to explain the tolerence shown by S. frugiperda and suggested that differential toxicity was related to target site sensitivity. A summary of recent reports, which together show differences in sensitivity against the larvae of various noctuid moths to AVMB1 is given in Appendix 1.1.

1.1.2.3. Systemic, translaminar and residual toxicity against arthopods.

Ostlind et al., (1979) first reported that a crude fermentation broth extract of AVM possessed insecticidal activity against the confused flour beetle, Tribolium confusum and against larvae of the robust bot fly, Cuterbrid sp. in mice. In the latter case where AVM extracts were applied to infected mice, a systemic action for AVM was also demonstrated.

In another early study, James et al. (1980) found a LC_{50} of 0.12µg/ml (ppm) with a crude AVM extract against first instar larvae of the blowfly, Lucilia cuprina. Purified components showed even greater activities, which compared favourably with the organophosphorus insecticide diazinon. In addition, it was found that up to 31 weeks protection was given against L. cuprina larvae (insecticide susceptible strain) when a patch of wool was saturated with a crude mixture of AVM, whereas diazinon at a comparable concentration gave only 10 weeks protection.

The systemic activity of $AVMB_1$ was also shown by Standfast et al. (1984), working on Culicoides brevitarsis - a vector of cattle arboviruses; flies feeding on cattle at 10 and 18 days after subcutaneous injection of $AVMB_1$ at 200ug per kg treatment showing 48h mortalities of 99% and

40% respectively. No mortality occurred in flies that alighted on treated cattle but did not feed. Similar activity has been shown with other dipteran species, including the Tsetse fly, Glossina palpalis palpalis feeding on guineapigs and goats treated with DHAVMB1 (Distelmans et al., 1983).

AVMB1 has been shown to possess considerable intrinsic contact/ingestion toxicity towards T. urticae (72h LD_{50} of 0.02-0.03 ppm) when applied directly to adults or nymphs and was reported to have residual acaricidal activity on plants against T. urticae for up to one month (Putter et al., 1981).

The translaminar activity of AVMB1 against the red spider mite, Tetranychus urticae and two aphid species, Aphis fabae and Aphis gossypii was demonstrated by Wright et al. (1985a). Translaminar acaricidal activity was shown to vary between cotton, French bean and chrysanthemum leaves and it was suggested that this may be due in part to quantitative and/or qualitative differences in cuticular waxes affecting penetration of AVMB1. The addition of safflower oil or Sunspray 6E oil was found to increase the translaminar activity of AVMB1. Translaminar activity of AVMB1 was greater against T. urticae when compared with the aphids tested, which may have been due partly to differences in feeding. Thus while aphids feed selectively within the

phloem, T. urticae feed destructively on parenchyma cells and may come into greater contact with the AVMB1. Another factor likely to be involved in the differences in toxicity of AVMB1 against these species was body size (Wright et al., 1985a).

Grafton-Cardwell and Hoy (1983) studied the comparative toxicity of AVMB1 against the predatory mite Metaseiulus occidentalis and two species of spider mites, T. urticae and Panonychus ulmi. They demonstrated a differential toxicity of AVMB1 to the spider mites and to the predatory mites. This could be of practical importance in the field especially if resistant predator strains are developed or if refuges exist. Similarly, Trumble (1985) found that AVMB1 supressed populations of the leafminer, Liriomyza trifolii but did not adversely affect seasonal percent parasitism, adult parasite mortality or emergence of immature parasites in six parasitoid species on treated foliage. Pelag (1987) found similar differential toxicity to the Californian red scale (Aonidella aurantii) when compared with its parasite Aphytis holoxanthus.

The observed differential toxicity of AVM between beneficial and phytophagus species of arthropods may be associated with the distribution and persistence of AVM on foliage as Bull et al. (1984) have reported the half life of toxic AVM residues on the surface of foliage to be less than

one day, whereas Wright et al. (1985b) found significant activity in leaves against T.urticae for 14 days post spraying

In the field, AVMB1 has been tested successfully against a variety of arthropod pests of agriculture (Strong and Brown, 1987). For example, cotton sprayed with 28.3 g a.i./ha produced 75% more cotton than untreated plants infested with H. virescens (Wright, J.E. et al., 1985) and Wolfenbarger et al. (1985) found reduced numbers of H. virescens larvae on tobacco plants sprayed with 11-33 g a.i./ha over two years.

1.1.2.4. Sublethal effects on arthropods.

1.1.2.4.1. Reduction in feeding

A number of previous studies have shown that a reduction in insect feeding can be caused by low doses of several classes of insecticides such as formamidines and pyrethroids (Davenport and Wright, 1987).

Pienkowski and Mehring (1983) were the first to report that AVMB1 had an "antifeedant" action, in studies on the weevil Hypera postica where they found that the feeding of second instar larvae was significantly reduced by 10 and 100 ppm AVMB1 (sublethal doses), whilst that of fourth instars

was significantly reduced by 100ppm AVMB1. An "antifeedant" activity was also noted for AVMB1 with the soybean looper Pseudoplusa includens (Beach and Todd, 1985) while Corbett et al. (1985) showed that AVMB1 greatly reduced the extent and rate of feeding in third instar larvae of S. littoralis.

An antifeedant substance produces a localized reduction in feeding damage by imparting a noxious taste or masking phagostimulants in the food. But repellency is the active avoidance of a volatile substance (Davenport and Wright, 1987). Tedders et al. (1984) studied three species of pecan aphids, Monelliopsis pecanis, Melanocallis caryfoliae and Monellia caryella on foliage sprayed with 3 ppm AVMB1. They showed that AVMB1 residues provided protection against all three species migrating onto treated foliage and since dead insects were not seen, indicated that the pesticide acted as a repellent. In contrast, Cochran (1985) found that AVMB1, applied in the diet to nymphal Blatella germanica did not appear to cause repellancy or unpalatability.

1.1.2.4.2. Developmental abnormalities and effects on metamorphosis

There are several reports of AVM affecting developmental processes in insects. For example, Parrish and Roberts (1984) reported that pupation was completely inhibited in yellowjacket larvae, Vespula maculifrons

treated with 10 ppm AVMB1, although this was thought to be due to the paralysing action of AVMB1. Similarly AVMB1 was found to block metamorphosis in the dipteran species Chrysomya bezziana and Calliphora vomitoria, probably due to inhibition of head eversion inside the puparium (Spradbery et al., 1985; Strong, 1986a, b). Wright (1984) also found that AVMB1 interfered with the development of pupae of the boll weevil Anthonomus grandis into adults.

Among lepidoptera studies on S. frugiperda, H. virescens and H. zea showed that following topical application of AVMB1 to larvae, some subsequently developed into larval-pupal intermediate forms that either died or remained at the final larval stage beyond the normal time for pupation (Bull, 1986). Jyothi et al., (1986) found the application of AVMB1 to fourth instar larvae of the castor semilooper, Achaea janata resulted in moult disturbances and treated larvae were totally or partly unable to shed their old larval exuviae, often dying one or two days later and at some doses formed larval-pupal intermediates and malformed pupae. Reed et al., (1985) also found that AVMB1 has a number of harmful effects on development in the moth Cydia pomonella; the pupal stage being greatly extended and this may have been due to reduced feeding. Reduced feeding may be a factor in many of the observed effects of AVMB1.

1.1.2.4.3. Effects on reproduction and related processes

In most insects which have been examined, sub-lethal doses of AVMB1 have shown deleterious effects on reproduction. For example, a reduction in the number and size of oocytes and in the number of oothecae produced by female Blattella germanicae, fed on AVMB1 was reported by Cochran, (1985). These appeared to be permanent changes following ingestion of AVMB1 by adults but were not found in females which had survived treatment as nymphs.

Lofgren and Williams (1982) investigated the delayed toxicity of AVMB1 against the red imported fire ant, Solenopsis invicta. AVMB1 was found to be a good control agent since workers distributed the food containing the toxicant via trophylaxis to other members of the colony including the queen. AVMB1 was found to affect the reproductive capacity of the queen leading to the slow elimination of the colony. The effects of AVMB1 on the ovaries were direct and included hypertrophy of the squamous epithelium, fewer eggs which were smaller in size and with reduced yolks (Glancey et al., 1982).

AVMB1 has also been shown to cause reduced egg hatch, egg laying and stippling by females of the serpentine leafminer, Liriomyza trifoli (Schuster and Everett, 1983). It was suggested that AVMB1 caused irreversible damage to

the ovaries as was reported for the red imported fire ant Solenopsis invicta (Glancey et al. 1982). A more likely explanation is that muscles associated with the ovipositor might have been paralysed so that the use of the ovipositor was restricted. Reduced stippling by the flies "paralysed" ovipositor may have caused a reduction in food intake and therefore starvation and increased adult mortality. Early embryonic development was not inhibited and it is possible that the larvae may have been killed by contact with residues in or on the chorions or in the leaf mesophyll.

Finally, AVMB1 has been reported to reduce pheromone production in 6 and 12 day old adult females of the boll weevil, Anthonomus grandis. AVMB1 was found to reduce feeding and cause paralysis and the reduction in pheromone production was thought to be an indirect effect (Wright, 1984). Glossina morsitans females feeding on blood containing DHAVMB1 were found to abort larvae or eggs, but re-established normal cycles when fed on untreated blood (Langley and Roe, 1984).

1.3. Cellular Level

The literature on the mode of action of AVM at the cellular action is large and difficult to interpret; encompassing a wide range of studies on vertebrates and invertebrates. For example, while early electrophysiology studies were carried out on invertebrates, early binding studies were on vertebrates. However there have been several reviews that include work on the cellular action of AVM which have helped to clarify the situation (Wright, 1986; Wann, 1987; Wright, 1987). More recently, receptor/membrane binding studies and electrophysiology have both been carried out on invertebrates and these will be considered in some detail.

Studies on AVM at the cellular level can be divided into three main areas: electrophysiology, pharmacology and receptor/membrane binding studies. Many studies have supported the idea that AVM acts by the potentiation of GABA-mediated Cl^- channel conductance which could be due to one or more of the following effects: 1) AVM acting as a postsynaptic agonist of GABA, either at the GABA receptor or at an adjacent site, 2) The potentiation of GABA-receptor binding and 3) The stimulation of presynaptic GABA release (Wang and Pong, 1982). There is also some evidence that non-GABAergic receptors may be affected including, at higher concentrations of AVM, glutaminergic (excitatory) responses

in insects (Duce and Scott, 1985b).

However as mentioned above, while electrophysiological experiments which link AVM with Cl^- channel conductance have mostly used AVM-sensitive organisms, many of the ligand binding studies have used rat brain membranes and cannot necessarily be related to the mode of action against invertebrate target species due to pharmacological differences between phyla (Wright, 1986; Wann, 1987).

For these reasons the discussion has been divided into

1) GABA systems - early electrophysiological work.
2) GABA receptors and binding sites in a) vertebrates b) nematodes and c) arthropods.
3) GABA-Cl^- conductance studies.
4) Non-GABA system work on Cl^- channels.

1.3.1. GABA systems

1.3.1.1. Early Electrophysiological Work

The first electrophysiological investigation into the mode of action of $AVMB_{1a}$ was carried out by Fritz et al. (1979), working on the lobster neuromuscular junction. It was shown that $AVMB_{1a}$ irreversibly eliminated both inhibitory post synaptic [IPSP] and excitatory post synaptic [EPSP] potentials; the former being reduced faster than the latter. The amplitudes of the evoked potentials were greatly

affected but those of the membrane potentials were not. However, $AVMB_{1a}$ did reduce the muscle membrane resistance which led to a reduction in the EPSP or IPSP. This work suggested that $AVMB_{1a}$ acts to increase membrane permeability to those ions whose equilibrium potentials are close to the resting potential, such as Cl^- and K^+. When $AVMB_{1a}$ was applied in standard lobster Ringer's solution the muscle membrane was hyperpolarised by up to 5mV within a few minutes, and the IPSP became much reduced, indicating that the drug increased the membrane permeability to ions whose equilibrium potential is more negative than the control resting potential. It was found that $AVMB_{1a}$ affected Cl^- and not K^+ by bathing muscle in K^+-free Ringer. This resulted in the IPSP being depolarised, due to the resting potential being more negative than the IPSP equilibrium potential. When $AVMB_{1a}$ was added, the muscle membrane was depolarised by several millivolts and the IPSPs again became greatly reduced (Fritz et al. 1979).

Since $AVMB_{1a}$ causes hyperpolarisation in standard Ringer and a depolarisation in K^+-free Ringer, it was suggested that $AVMB_{1a}$ increased the permeability of the membrane to Cl^- and not K^+ (Fritz et al., 1979). The rapid reduction in the IPSP and the slow reduction in the EPSP by $AVMB_{1a}$ could be explained by the shift in membrane potential towards the IPSP equilibrium potential. The IPSP was reduced due to the decreased ionic driving force and the decreased

muscle input resistance. The slower reduction in the EPSP was caused by a decreased muscle input resistance. Application of picrotoxin (a GABA antagonist) after $AVMB_{1a}$ treatment of muscle resulted in an increase in the EPSP. When the picrotoxin was removed, the EPSP became reduced again. However, picrotoxin is not completely specific to GABA synapses, acting rather on Cl^- ion channels and this implicates these channels with the action of $AVMB_{1a}$ (Fritz et al., 1979).

In another early electrophysiological study, Kass et al. (1980) also suggested that AVM acted as a postsynaptic agonist of GABA in the nematode, Ascaris suum ; potentiating GABA Cl^- channel conductance. They showed AVM reversibly blocked transmission between interneurone(s) and excitatory motorneurones in the ventral nerve cord of A. suum, reducing depolarisation of muscle cells. It also irreversibly inhibited transmission between inhibitory motorneurones and muscle cells and thereby reducing hyperpolarization of the latter, but had little effect on excitory neuromuscular transmission.

Mellin et al. (1983) confirmed that $AVMB_{1a}$ blocked postsynaptic transmission in the lobster opener and stretcher muscles. Both GABA and $AVMB_{1a}$ decreased the slope of the current-voltage curves in stretcher muscle, reflecting an increase in membrane conductance. These

postsynaptic effects were found to be inhibited by picrotoxin and bicuculline [a vertebrate GABA-A receptor antagonist (Bowery et al., 1980)]. These observations suggested AVMB$_{1a}$ may act postsynaptically on the Cl^- channels (Mellin et al., 1983; Fritz et al., 1979) or act presynaptically on GABA receptors to cause a sustained and potent release of GABA (Pong et al., 1980).Fritz et al. (1979) observed that at the lobster neuromuscular junction, AVMB$_{1a}$ reduced the input resistance of muscle fibres in control Ringer's solution as well as in Ringer's solution in which Co^{2+} was substituted for Ca^{2+}. This was in accord with the Ca^{2+}-independent stimulation of GABA release seen with synaptosomes (Pong et al., 1980). Replacement of Ca^{2+} by Co^{2+} in the lobster saline bathing the stretcher muscle preparation also did not affect membrane resistance in the post-synaptic muscle fibres but did block nerve-evoked IPSPs which are known to be caused by Ca^{2+}-dependent presynaptic GABA release.

If the effects of AVM are caused by increased GABA release, then the fact that AVM also reduced muscle membrane resistance in Co^{2+}-saline suggests AVM could be capable of stimulating GABA release in the absence of Ca^{2+} (Pong et al., 1980). This is in accord with the Ca^{2+}-independent stimulation of GABA release seen with rat brain synaptosomes; a marked, sustained increase in the release of GABA being found from pre-loaded rat brain synaptosomes in

the presence of $AVMB_{1a}$ (Pong et al., 1980). The apparently irreversible action of $AVMB_{1a}$ could be related to sustained AVM stimulation of GABA release and the existence of a GABA re-uptake system in the inhibitory nerve endings helping to replenish the GABA supply. A good correlation was found between antiparasitic activity and GABA-releasing activity among various $AVMB_{1a}$ derivatives, which suggested that the ability of $AVMB_{1a}$ to release this neurotransmitter may be the basis of its antiparasitic action (Pong et al., 1980).

The black widow spider toxin α-latrotoxin is known to stimulate indiscriminately the release of several neurotransmitters including GABA and Tzeng et al. (1978) have shown that the effect of α-latrotoxin on GABA release was independent of Ca^{2+}. Misler and Hurlburt (1979) showed that the stimulation of GABA release from frog cutaneous pectoris nerve was also independent of calcium but was dependent on the presence of at least one other divalent cation such as Mg^{2+}, Co^{2+} or Mn^{2+}. Pong et al. (1980) observed that in their experiments, the synaptosomal GABA-releasing activity of AVM was always tested in the presense of Mg^{2+}, so it was not known whether there was a common mechanism in the action of α-latrotoxin and $AVMB_{1a}$ on the nervous system.

1.3.1.2. GABA Receptors and Binding Sites

1.3.1.2.1. Vertebrates

Pong and Wang (1980) reported that $AVMB_{1a}$ had a presynaptic action on GABA neurones. Radiolabelled $AVMB_{1a}$ was found to bind specifically to brain synaptosomes in both dogs and rats. This high affinity binding was saturable but was not found to be competitive with GABA, its agonists or antagonists, which suggested that the $AVMB_1$ receptors were not associated with GABA post-synaptic receptors.

Pong *et al*. (1981) showed that GABA and AVM act in a similar way to modulate benzodiazepine receptor binding in rat brain membranes. It was shown that there were different binding sites for GABA, $AVMB_{1a}$ and benzodiazepines which were thought to be at the GABA post synaptic receptor-Cl^- channel complex. Subsequently, the recognition sites for $AVMB_{1a}$, benzodiazepines, pentobarbital, tracazolate and picrotoxin were all found to be allosterically coupled to the GABA receptor-Cl^- channel complex in rat brain, but in different ways (Pong *et al*., 1982). $AVMB_{1a}$, pentobarbital and tracazolate all potentiated ligand binding to the GABA and benzodiazepine receptors and could ultimately lead to a prolonged opening of the Cl^- channel, allowing an increase in the Cl^- influx to the post synaptic nerve terminal and inhibit neurotransmission. It was found that picrotoxin blocked $AVMB_{1a}$-enhanced GABA binding by 80 to 85%, but only

9 to 12% of GABA binding due to tracazolate was blocked. This suggested that $AVMB_{1a}$ and picrotoxin sites may interact to some extent. $AVMB_{1a}$ plus pentobarbitol or tracazolate did not increase GABA binding more than $AVMB_{1a}$ alone, which indicated that the binding site for $AVMB_{1a}$ may be shared with pentobarbitol and tracozolate. It was suggested that conformational changes of the GABA receptor complex may occur when the receptor binds different ligands under different experimental conditions and this might affect interaction of picrotoxin with the GABA receptor complex (Pong et al., 1982).

Wang and Pong (1982) stated that AVM had specific, high affinity binding sites in the rat brain synaptic membrane, closely associated with the GABA-benzodiazepine receptor-Cl^- channel complex. The density of available postsynaptic GABA receptors was increased by AVM in a process which required Cl^- and was inhibited by picrotoxin. AVM appeared to increase the number of GABA binding sites without significantly changing the apparent dissociation constant. Nipecotic acid, an inhibitor of GABA uptake (Johnston et al., 1976), had no effect on AVM-induced GABA binding. The AVM-induced GABA binding sites were apparently not involved in the presynaptic uptake of GABA. Also, since GABA uptake requires the presence of Na^+, and none was present in these experiments, it would appear that AVM-induced GABA receptors are located postsynaptically (Wang and Pong, 1982). This

observation was further supported by the finding that picrotoxin only inhibited AVM-induced GABA binding and did not affect control GABA binding (Pong and Wang, 1982).

Drexler and Sieghart (1984) found that specific, high affinity reversible binding of ^{3}H-AVMB$_{1a}$ occurred in membranes from several rat brain regions, confirming earlier work by Pong and Wang (1980). This was in apparent contrast to studies by Fritz et al. (1979) and Paul et al. (1980) who reported an irreversible action of AVM in vivo and in vitro respectively. This discrepancy may be explained by the hydrophobicity of AVM. It is possible that ^{3}H-AVM can only dissociate from it's binding site in exchange with cold AVM, when cold AVM is present in aqueous solution in micellar form. Without AVM micelles, ^{3}H-AVM dissociating from it's specific binding sites might immediately and non-specifically precipitate on the membrane in aqueous solution (Drexler and Sieghart, 1984).

The high affinity ^{3}H-AVM binding site in rat brain membranes was found to be partly inhibited by GABA agonists; this effect being blocked by Cl^{-} (Drexler and Sieghart, 1984). The presence of Cl^{-} in the incubation medium may explain why Pong and Wang (1980) found GABA had no effect on ^{3}H-AVM binding. The action of GABA agonists on ^{3}H-AVM was blocked by selective GABA antagonists such as bicuculline, indicating that at least part of the specific high affinity

binding of ^{3}H-AVM was at a GABA receptor. This was also supported by the observed small inhibitory effect of $AVMB_{1a}$ on ^{3}H-muscimol binding which may have been caused by AVM being incorporated into the lipid phase of membranes causing the exposure of new GABA binding sites (Drexler and Sieghart, 1984). This idea was supported by the observations by Wang and Pong (1982) and Pong et al. (1981) that there was an increase in density of GABA receptors with increasing AVM concentrations, who also reported that $AVMB_{1a}$ increased the number of benzodiazepine binding sites. However, GABA receptor agonists did not completely displace ^{3}H-muscimol binding to the GABA receptor, suggesting that the specific high affinity binding sites for ^{3}H-AVM although associated with GABA receptors were not identical with these receptors (Wang and Pong, 1982 and Pong et al., 1981).

Thus, the work of Drexler and Sieghart (1984) indicated a connection between the pharmacological action of AVM in vivo, the specific, high affinity ^{3}H-AVM binding site, and the GABA-benzodiazepine receptor-Cl^{-} channel complex and supported earlier work of Pong et al. (1982) and Wang and Pong (1982).

1.3.1.2.2 Work on Nematodes

Kass et al. (1984) showed that $AVMB_{1a}$ blocks interneuronal stimulation of excitory motorneurones in

A. suum but had no effect on excitatory neuromuscular transmission. Two GABAergic agonists, muscimol and pipereazine, were found to mimic the effects of AVM which suggested that the action of AVM was related to a GABAergic mechanism. Ventral inhibitory neuromuscular transmission was also blocked by AVM. These studies suggested the principal action of AVM appeared to be in the ventral nerve cord of nematodes (Kass et al., 1984).

In nematodes, few receptor-ligand binding studies have been published and the electrophysiological evidence suggests that the main action of AVM is at synapses believed to be mediated by GABA (Wright, 1986). However, Martin (1987) has reported that DHAVMB$_{1a}$ (10^{-7}M) acts as a GABA agonist at extrajunctional GABA receptor on somatic muscle cells of A. suum .

Schaeffer et al. (1988) studied the binding of ^{3}H-DHAVMB$_{1a}$ to membranes isolated from the free living nematode, Caenorhabditis elegans and demonstrated a specific DHAVMB$_{1a}$ binding site. Initially, a rapidly reversible complex was formed which was then transformed to a much more slowly reversible complex. The in vivo effect of DHAVMB$_{1a}$ and its derivatives on the nematode motility was found to be concentration dependent and correlated well with their relative binding affinities. Putative neurotransmitters, including GABA, glutamate and dopamine had no effect on

DHAVMB$_{1a}$ binding. Specific DHAVMB$_{1a}$ binding sites were found by Schaeffer et al., (1988) in rat brain but had a lower affinity than that observed in C. elegans and stereospecificity studies showed significant structural differences in the two binding sites.

The pharmacology of A. suum muscle GABA receptor using GABA agonists and antagonists was studied by Holden-Dye et al. (1988). AVM was found to inhibit GABA-evoked increase in the membrane conductance and membrane hyperpolarisation on quiescent muscle cells. When applied to spontaneously active (spiking) muscle cells, AVM irreversibly inhibited action potentials after a delay of about 25 min. The apparent inability of a range of agonists to block the effect of GABA at this site suggested that it could not be classified as either a GABA$_A$ or a GABA$_B$ receptor. Picrotoxin and TBPS (piperidine-4-sulphonic acid) which were believed to be active at this GABA site were without effect on A. suum muscle cells, suggesting the recognition site at the chloride ionophore was different from that in other invertebrate and vertebrate systems (Holden-Dye et al., 1988).

1.3.1.2.3. Arthropods

Application of AVMB$_1$ at a low concentration (10^{-7}M) to the leg muscles of P. americana resulted in the failure of

the muscles to respond to external stimuli within 30 min. without affecting the magnitude of contraction (Tanaka and Matsumura, 1985). This suggested that AVMB1 blocked the transmission of the stimuli from the central nervous system (CNS) to the muscles without affecting the muscle tension strength. This agreed with Fritz et al. (1979) and Mellin et al. (1983) who had found that AVMB1 blocked postsynaptic transmission in crustacean opener and stretcher muscles (Section 1.3.1.1.).

The binding of ^{3}H-muscimol to P. americana muscle and brain membranes was not found to be increased by AVMB1 but was inhibited by bicuculline methiodide (10^{-4}M) (Tanaka and Matsumura, 1985). In contrast, Pong et al. (1982) found that AVMB1 (10^{-4}M) stimulated ^{3}H-muscimol binding to rat membranes. In addition, AVMB1a (10^{-5}M or 10^{-7}M) did not increase ^{3}H-benzodiazepine binding to P. americana brain membranes in contrast to its action on rat brain preparations. It was thus suggested that benzodiazepine receptors have little involvement with the action of AVMB1 in P. americana (Tanaka and Matsumura, 1985), in contrast to the findings of Paul et al. (1980), Pong et al. (1981) and Pong et al. (1982) on mammalian tissues. ^{3}H-Dihydro-picrotoxinin binding to P. americana membranes was not affected by AVMB1 except at the relatively high concentration of 10^{-5}M where binding was slightly inhibited. Hence AVMB1 does not have an effect on the picrotoxinin (the

a.i. of picrotoxin) receptor (an integral part of the GABA complex but independent from GABA and benzodiazepine receptors in mammalian brains; Tanaka and Matsumura, 1985).

Lummis and Sattelle (1985) showed that in nerve cord extracts taken from P. americana, specific binding sites for ^{3}H-GABA were present. The vertebrate $GABA_B$ receptor agonist, baclofen (Bowery et al., 1980) and the vertebrate $GABA_A$ receptor antagonist, bicuculline did not inhibit this binding, indicating the binding component possessed a different pharmacological profile from that of vertebrates. These authors also showed a preparation from the CNS of P. americana could specifically bind ^{3}H-flunitrazepam; binding being enhanced by low ^{3}H-GABA concentrations. This indicated that the benzodiazepine binding component was linked to a GABA binding site.

Studies on cultured neurones taken from the CNS of P. americana showed that their GABA receptors appeared to be similar to $GABA_A$ receptors which have a widespread distribution in vertebrates (Lees and Beadle, 1986). Thus, they had inhibitory effects which were mediated by a rapid, transient increase in Cl^- permeability and which could be blocked by picrotoxin. Vertebrate $GABA_A$ receptors were blocked by bicuculline but insect cells were insensitive to this compound (Lees and Beadle, 1986). These findings appear to support the observations of Pong et al. (1980) that GABA

receptors differ subtly in their pharmacological specificity across phylogenetic boundaries.

Abalis and Eldefrawi (1986) identified a putative GABA receptor in the honeybee (Apis mellifera) brain using ^{3}H-muscimol which was found to show high and low affinity binding. High affinity binding in A. mellifera was most sensitive to GABA and the GABA agonist muscimol, but was insensitive to the $GABA_A$ antagonist bicuculline and to the $GABA_B$ agonist baclofen or to flunitrazepam, picrotoxinin and pentobarbital which bind to allosteric sites in mammalian GABA receptors). Low affinity ^{3}H-muscimol binding was inhibited by various GABA agonists including imidazole acetic acid. These observations suggested the honey-bee brain receptor was more like a $GABA_A$ than a $GABA_B$ receptor. Differential binding was said to represent either binding to two sites on the same GABA receptor or to two kinds of GABA receptor (Abalis and Eldefrawi, 1986). $AVMB_{1a}$ was the most potent inhibitor of high affinity ^{3}H-muscimol binding (IC_{50}=10pM) and also inhibited low-affinity ^{3}H-muscimol binding. GABA uptake inhibitors [DL-nipocotic acid and l-pipecolic acid] were not found to inhibit ^{3}H-muscimol binding which suggested that the high affinity site was not an uptake site but was on a GABA receptor. Abalis and Eldefrawi (1986) referred to unpublished work which showed that $AVMB_1$ inhibited binding of ^{3}H-muscimol to mammalian brain tissues. This was in contrast to the potentiation of

^{3}H-GABA binding to mammalian GABA receptors reported by Pong et al. (1982).

Inhibition of ^{3}H-muscimol binding to the bee brain by AVMB$_{1a}$ may result from its action as an agonist or antagonist of GABA. The finding that AVMB$_{1a}$ potentiated the binding of benzodiazepines to mammalian GABA receptors and inhibited the binding of ^{35}S-t-butylbicyclophosphorothionate to the Cl^{-} channel site of the mammalian GABA receptor indicated that when bound to the ^{3}H-muscimol binding site on the GABA receptor AVMB$_{1a}$ was probably acting as an agonist (Abalis et al., 1986).

1.3.1.3. Chloride ion conductance studies

Tanaka and Matsumura (1985) showed that AVMB$_1$ significantly increased the influx of $^{36}Cl^-$ into P. americana leg muscle tissues when compared with control muscle. This enhancement of $^{36}Cl^-$ influx was found to be concentration dependent. AVMB$_1$ (10 μM) was found to be more potent than GABA in enhancing $^{36}Cl^-$ influx. GABA in combination with AVMB$_1$ could not change the $^{36}Cl^-$ influx induced by AVMB$_1$, which suggested an identical site of their action. Milbemycin, increased $^{36}Cl^-$ influx to the same extent as AVMB$_1$.

Picrotoxinin and bicuculline methiodide (10μM) blocked AVMB$_1$-enhanced $^{36}Cl^-$ influx by 50% and 20% respectively. When all Na^+, except for sodium phosphate, were replaced by cholinium cation, it was found that the AVMB$_1$-enhanced $^{36}Cl^-$ influx was slightly less than that observed in normal saline (Tanaka and Matsumura, 1985).

When AVMB$_1$-treated muscles were exposed to K^+-free saline containing $^{36}Cl^-$, the AVMB$_1$-induced $^{36}Cl^-$ influx was suppressed to 60% of the control (Tanaka and Matsumura, 1985). The inhibition of $^{36}Cl^-$ influx into AVMB$_1$-treated muscle bathed in K^+ free saline was explained by the work of Fritz et al. (1979) where the efflux of intracellular Cl^- increased sufficiently, for a short time in the in K^+ free

saline to cause depolarisation to occur. Tanaka and Matsumura (1985) suggested that AVMB1 was selectively increasing Cl^- permeability across the muscle membrane; AVMB1 acting either as a presynaptic inhibitor by opening Cl^- channels at the GABA synapse in such a way that the presynaptic membrane is unable to release an excitatory transmitter, or as a postsynaptic inhibitor by opening the Cl^- channel in the postsynapic region.

Drexler and Sieghart (1984), suggested that in rat brains picrotoxin could interact with its binding sites in the GABA-benzodiazepine receptor ionophore complex causing conformational changes which would inhibit Cl^- fluxes and stimulate ^3H-AVM binding. GABA agonists (eg. pentobarbital) by binding to their sites could facilitate Cl^- fluxes but partly inhibit binding of ^3H-AVM.

1.3.2. Non-GABA chloride channels

Duce and Scott (1985a) suggested AVM had a dual action in insects. Using the locust Schistocerca gregaria they found that the microperfusion of DHAVMB1 onto the GABA-sensitive extensor tibiae muscle bundle 33 induced a reversible, dose-dependent increase in the input conductance which was abolished in Cl^--free saline. It also partly blocked GABA-induced increases in input conductance.

The reversible actions of DHAVMB1 on locust muscle have been reported to involve the GABA receptor-Cl^- complex, but the finding that DHAVMB1 also induced an irreversible increase in Cl^- permeability (conductance) suggested another site of action independent of the GABA receptor-Cl^- channel complex in locust muscle (Duce and Scott, 1985a).

Ibotenic acid (1μM to 1mM) has been found to induce dose-dependent increases in conductance in both GABA-sensitive and insensitive bundles (Duce and Scott, 1985b). Cull-Candy (1976) also found ibotenic acid selectively increased the permeability of locust muscle bundles to Cl^- by activating extrajunctional glutamate H receptors. Changes in input conductance induced by ibotenic acid in GABA-sensitive and insensitive bundles were reduced in a dose dependent manner by 12nM-1.2μM and 0.6-1.2μM of DHAVMB1 respectively; concentrations of DHAVMB1 in the same range as those found causing irreversible effects on GABA-insensitive and sensitive muscle bundles (Duce and Scott, 1985b).

Glutamate H receptors were found to be desensitised by ibotenic acid, although characteristic irreversible increases in input conductance could still be induced. Given that the responses to DHAVMB1 were induced despite desensitisation of the H receptor by ibotenic acid, this suggested that the action of DHAVMB1 was at the Cl^- channel

of extrajunctional glutamate H receptor-Cl^- channel complex, (Duce and Scott, 1985b; Scott and Duce, 1985). The irreversible changes in input conductance caused DHAVMB1 were not affected by penicillin G or bicuculline, but picrotoxin and zinc chloride reduced DHAVMB1 responses. It was concluded that DHAVMB1 has a number of sites of action on locust muscle that include effects on the glutamate H receptor-Cl^- channel complex as well as effects on the GABA receptor-Cl^- channel (Scott and Duce, 1985).

Scott and Duce (1986) suggested that the DHAVMB1 induced irreversible increase in conductance involve an ion channel with a greater selectivity for Cl^- in S. gregaria. However, DHAVMB1-induced changes in the fitering properties of a single type of Cl^- channel are not ruled out. This is consistent with DHAVMB1 action not being dependent upon GABA sensitivity of the locust muscle fibres.

Application of DHAVMB1 (0.1-1.0μM) to cultured neurones from P. americana brain resulted in slow conductance increases which were accompanied by a membrane time constant (Lees and Beadle, 1986). However, the conductance response to the application of DHAVMB1 was not restricted to neurones which responded to GABA. Direct effects of DHAVMB1 on muscle fibres and neuronal membranes with no inhibitory innervation indicated that the Cl^- channels which were activated were not necessarily be associated with GABA receptor complexes

(Duce and Scott, 1985; Lees and Beadle, 1986).

Additional evidence for non-GABAergic effects of AVM includes studies by Bokish and Walker (1986) and Zufall et al. (1988). The application of $AVMB_1$ to identified neurones in the suboesophageal ganglionic mass of the snail, Helix aspersa resulted in an increase in Cl^- conductance (induced an outward current) in certain neurones. This direct effect of $AVMB_1$ occurred irrespective of whether the neurones were sensitive to GABA or not and was generally irreversible at concentrations of 0.01-1.0μM. It was concluded that $AVMB_1$ can interact with Cl^- channels to induce an outward current and can reduce Cl^- mediated responses associated with GABA and acetylcholine (Bokisch and Walker, 1986).

Recently, Zufall et al. (1988, 1989) showed that AVM opened multitransmitter-gated Cl^- channels in crayfish muscle. The bursting behaviour of these channels, which was normally seen by activation with glutamate, acetylcholine or GABA, was strongly reduced with AVM. They concluded that there was an irreversible binding of AVM to the Cl^- channel receptor complex.

1.4. The present study

1.4.1. The experimental insects

The insects used in this study were the larval instars of two noctuid moths (Lepidoptera: Noctuidae)- the Egyptian Cotton Leafworm, Spodoptera littoralis (Boisduval), formerly called Prodenia litura (F.) and the Cotton Bollworm Heliothis armigera (Hübner) [now referred to by some authors as Helicoverpa armigera (Hübner)]. Both can be serious pests of cotton (Gubbins and Campion, 1982; Hosny et al., 1980; Van Den Berg et al., 1988.

1.4.1.1. Spodoptera littoralis

This insect (Plate 1.1.) is found throughout much of Africa, the Mediterranean, Madagascar and the Near East. It is a leaf-eater but can behave like a cutworm. It is polyphagous, in Egypt, attacking Berseem (Trifolium alexandronum L.) during the winter, cotton in the summer and vegetables in the autumn. Other host plants include lucerne, potatoes, peppers, legumes, tomatoes and okra (Salama, 1983). In Egypt there can be seven or eight generations a year (Salama, 1983). It can be reared in the laboratory on an artificial diet (Appendix 2.1.). Control practices include using children to collect egg masses, regulation of crop planting and the use of insecticides such as synthetic pyrethroids and mixtures of organophosphates and acylureas

such as chlorpyrifos and diflubenzuron (Salama, 1983). Resistance to pyrethroids has recently been reported to be becoming a problem in Egypt. (El Guindy et al., 1982; Sawicki, 1989).

1.4.1.2. Heliothis (Helicoverpa) armigera

This insect (Plate 1.2.) is found throughout much of Africa, the Near East, India, Pakistan and much of Asia. It is a bollworm, burrowing into young cotton bolls and doing great damage to the developing cotton bolls. It is polyphagous, also attacking legumes, lucerne and many other crops. There can be several generations each year (Van Den Berg et al., 1988). Like S. littoralis it can be reared in the laboratory on artificial diet (see Appendix 2.1.). Control practices involve the use of synthetic pyrethroid and organophosphate insecticides such as chlorpyrifos. Resistance to pyrethroids and organophosphates has been reported in several parts of the world (Gledhill, 1982; Collins, 1986).

Plate 1.1. Sixth instar S. littoralis larva



Plate 1.2. Fifth instar H. armigera larva



1.4.2. The aims of the project

The AVM have been shown to have differential toxicity to noctuid moth species (section 1.2.2.). Most of this work has been on New World (American) noctuid species including Heliothis zea and Spodoptera frugiperda (Bull, 1986; Dybas and Babu, 1988) and apart from Corbett (1987) and Corbett et al. (1989) little work has been done on Old World species.

The aims of the present project were as follows:

A) To confirm and expand upon reports of inter- and intra-specific variations in the toxicity of AVMB1 to larval instars of S. littoralis and H. armigera.

B) To investigate and determine the factors involved in the differential toxicity of AVMB1 to the above noctuid moth species.

The work can be divided into three main sections:

1) Toxicology. To determine the levels of differential toxicity using topical application of insecticides and to investigate, using synergists and injection bioassays, the involvement of metabolism and penetration respectively.

2) Metabolism. Radiotracer studies with ^{3}H-AVMB$_1$ are used to determine the extent of metabolism in the fifth and sixth instars of both species and the level of AVMB$_1$ in fat body tissue and ventral nerve cord tissue in relation to symptoms of poisoning.

3) Neurophysiology. Using <u>in vivo</u> recording methods, the frequency of nerve impulses in the ventral nerve cord is compared in untreated insects and in insects treated previously with ^{3}H-AVMB$_1$, showing no or marked symptoms of paralysis. In this section the levels of radioalabelled AVMB$_1$ in the ventral nerve cord are also related to nervous activity and symptoms of poisoning.

2. GENERAL MATERIALS AND METHODS

2.1. Insect culture

All the insects were cultured and tested at 26(±1)°C under a 16:8 L:D cycle.

Spodoptera littoralis (Boisduval)

Freshly laid batches of *Spodoptera littoralis* eggs (Canary Island strain) were obtained from ODNRI (formerly TDRI), Porton, Wiltshire. The eggs were sterilised using 0.2% (w/v) sodium hypochlorite solution for 30 min. followed by 4% (w/v) formaldehyde for 30 min. They were then placed in covered plastic containers (27x15x10cm) until they hatched, whereupon artificial diet (Appendix 2.1.) was provided as a food source. At the second instar stage, the larvae were transferred to waxed paper cones (50ml) containing solidified artificial diet (20ml) and the cones covered with clingfilm. The cones were held (in groups of 30) in plastic racks and enclosed in plastic bags to maintain a high level of humidity. Five to seven larvae were put in each waxed paper cone. The larvae were not touched again until either they were needed for experiments or after pupation. Pupation occurred about 15 days after hatching. On emergence, some 14 days later, the adults were placed in plastic cylinder cages (30cm dia. x 50cm high) lined with

nappy liners (cellulose wadding) onto which eggs could be laid after pairing. The adults were provided with a honey solution with added vitamins as a food source (Appendix 2.1.).

Heliothis armigera (Hubner)

Heliothis armigera eggs were obtained from the Institute of Virology, Oxford. Eggs were sterilised with 0.2% (w/v) sodium hypochlorite for 30 min. and the larvae treated as for S. littoralis except that the second instar larvae were placed in individual 5cm dia. plastic Petri dishes. Upon pupation, the pupae were sterilised in 4% (w/v) formaldehyde for 30 min. The adults were provided with cotton nappy liners and cotton plants as oviposition sites and with adult diet (Appendix 2.1.).

2.2. Selection of larval instars

Larval instars were separated by means of their body weight and head capsule width. Since body weight between instars was found to overlap, only larvae of body weights falling within the ranges detailed in Table 2.1. were used in bioassays.

2.3. Chemicals

Test solutions of technical grade avermectin B_{1a}/B_{1b} (80:20) (91% w/v) (abamectin, $AVMB_1$), malathion (93% w/v), lambda cyhalothrin (71% w/v) (Karate), piperonyl butoxide (PB; 90% w/v) and S,S,S-tributyl phosphorothioate (DEF; 99% w/v) were prepared in ethyl methyl ketone (EMK) and stored at -20°C. For radiochemical studies, an emulsifiable concentrate (EC) formulation of ^{3}H-$AVMB_1$ (MK936; 1.8% v/v EC; [5-^{3}H]; 9.67mCi, 358MBq in 2.5ml) was prepared in distilled water. For neurophysiological studies, aqueous solutions of $AVMB_1$ (MK936, 113M; 1.8% w/v EC) were prepared in distilled water.

2.4. Topical bioassay

One µl of test solution (uncalibrated, marked volume) was applied to the mid-dorsal thoracic region of each larva using an Arnold microapplicator fitted with an Aglar glass syringe, the needle of which was bent at an angle of 90°. However, with second instar larvae only 0.5 µl (uncalibrated, marked volume) of test solution was applied. The larvae were allowed to dry before being placed in suitable containers. With second and third instars, 10 larvae were placed in covered plastic dishes (10x5cm) containing larval diet. Fourth, fifth and sixth instar larvae (of both species) were placed individually into

sections of Dufaylite Impregnated Kraft Paper Honeycomb, containing diet with a piece of plastic sheeting placed on top (Appendix 2.1.). Mortality was assessed after 96 h; any larva that responded when prodded was recorded as alive.

Table 2.1.

S. littoralis and H. armigera larval head capsule and body weight range

		$\bar{X}$ (95% C.L.)	
Species	Instar	Head Capsule (mm)	Weight(mg)
S. littoralis	II	0.34 (0.23-0.47)	1.6 (1.2-2.2)
	III	0.74 (0.66-1.00)	16.4 (12.3-20.4)
	IV	1.13 (1.04-1.23)	23.3 (18.9-29.9)
	V	1.74 (1.65-1.88)	187.5 (134.4-231.2)
	IV	2.45 (2.35-2.58)	863.0 (704.1-974.5)
H. armigera	V	1.06 (0.97-1.21)	103.0 (85.2-124.0)
	IV	1.64 (1.54-1.73	259.5 (192.1-322.8)

n=40

2.5. Calibration of Arnold microapplicator and Aglar syringe

A solution of ^{14}C-hexadecane in EMK containing 577 dpm per µl was dispensed (1µl and 0.5µl) with an Arnold microapplicator onto pieces of aluminium foil. These were placed in scintillation vials with 10ml of Cocktail T scintillation fluid (BDH Ltd) and the radioactivity determined by using a Beckman LS-250 liquid scintillation countercalibrated against ^{14}C-hexadecane. Counting was for 100 minutes or until a 2 sigma error of 0.5% was achieved. For injection experiments, 1µl of ^{14}C-hexadecane was injected into larvae and the same procedure as above was carried out after the larvae had been digested using 1ml of NCS tissue digestant solution and kept in the dark for 24h to reduce chemiluminescence. The volumes actually dispensed by the application equipment are given in Table 2.2. and all subsequent dose calculations were ammended accordingly. There were ten replicates for each calibration experiment.

Table 2.2.

Calibration of Arnold Microapplicator and Aglar Glass Syringe

Marked volume (µl) of Solution	$\bar{X}$ ± SE volume Delivered (µl) Ethyl methyl ketone	Aqueous
Topical Application		
1.0	0.80±0.02	0.52±0.03
0.5	0.42±0.03	-
Injection		
1.0	0.52±0.03	-

n=10

2.6. Analysis of bioassay data

Probit log dose regression lines were fitted to bioassay data using a computer program based on Finney (1971) and written by Statistical Research Service, Canada Department of Agriculture 1968 (called S103 Probit Single Line And Parallel Line Analysis). The LD_{50} and LD_{95} values, that is the doses expected to produce mortality in 50% and 95% of the population respectively, were calculated together

with their 95% confidence limits. The slope ±S.E. and chi-square values were also obtained for each set of data. A value of chi-square more than the tabulated value indicated heterogeneity while a value less than that tabulated indicated homogeneity. If the chi-square value indicated heterogeneity then the data was discarded.

Data was also tested for parallelism. If they are parallel the value of the slope for each of the single lines will not be significantly different from the joint analysis estimated value ($P>0.05$). The potency of each test solution relative to a pre-indicated standard were deduced together with LD values and 95% confidence limits. Due to the inherent variability of bioassay data individual LD_{50} and LD_{95} values were compared at the 5% significance level using 95% confidence limits for each value.

3. TOXICOLOGICAL STUDIES

3.1. Introduction

In this section, the main aim was to confirm and expand upon earlier reports of differential toxicity of AVMB1 to larvae of noctuid moth species (Corbett et al., 1985). The effects of penetration and metabolism were examined using injection bioassays and synergists (piperonyl butoxide (PB) and s,s,s-tributyl phosphorothioate (DEF)) respectively.

3.2. Materials and methods

For all of the following experiments about 50 larvae were used per treatment with the exception of sections 3.2.4a. and 3.2.8. (see below), each experiment was duplicated. Provided the individual Probit-log dose regression data was homogeneous and parallel, each replicate was combined for joint Probit analysis (see Appendix 3.1).

3.2.1. Toxicity of topically-applied AVMB1 to second, third, fourth, fifth and sixth instar larvae of S. littoralis.

Second, third, fourth, fifth and sixth instar larvae of S. littoralis reared on artificial diet (Appendix 2.1.), were treated topically (Section 2.4.) with the following

concentrations of $AVMB_1$:

Instar	$AVMB_1$ ($\mu g\ ml^{-1}$)
II	35, 186, 394, 511, 1871
III	186, 394, 511, 1871, 2285
IV	186, 511, 2285, 4571, 10573
V	511, 2285, 4571, 4697, 9143, 51800
VI	35, 186, 394, 511, 1871, 2285, 4571

3.2.2. Toxicity of topically-applied $AVMB_1$ with PB to third, fourth, fifth and sixth instar larvae of S. littoralis

All the larvae tested, were treated with 1µl of 50000 µg PB ml^{-1}, topically applied to the dorsal, posterior abdomen. After 1h, S. littoralis larvae were treated topically (Section 2.4.) with the following concentrations of $AVMB_1$:

Instar	$AVMB_1$ ($\mu g\ ml^{-1}$)
III	35, 186, 394, 511, 1871
IV	186, 511, 2285, 4571, 10573
V	186, 511, 2285, 4571, 9143
VI	35, 186, 394, 511, 2285

Fifth instar larvae were also treated with 1µl of 5000ug PB ml^{-1}, topically applied as above. After 1h, larvae were treated topically with the following concentrations of $AVMB_1$: 613, 993, 1587, 2285 and 4697 µg ml^{-1}.

3.2.3. Toxicity of topically-applied $AVMB_1$ with DEF to fifth instar larvae of S. littoralis

Fifth instar larvae were treated topically on the dorsal posterior abdomen (Section 2.4.) with 1µl of 10000 µg DEF ml^{-1}. After 1h, the larvae were treated topically on the mid-dorsal thorax with the following concentrations of $AVMB_1$: 511, 2285, 4697, 10573 and 51800 µg ml^{-1}.

3.2.4.a) Injection of $AVMB_1$ into fifth and sixth instar larvae of S. littoralis and fifth instar H. armigera.

b) Injection of PB into fifth instar S. littoralis followed by topical application of $AVMB_1$.

Larvae were injected with 1µl (marked, uncalibrated volume) of test solution using a straight 30 gauge needle fitted to a glass syringe (Section 2.4.). The following concentrations of $AVMB_1$ were used:

Species	Instar	AVMB1 (µg ml^{-1})
S. littoralis	V	186, 511, 1871, 4571
	VI	186, 394, 511, 2285, 4571
H. armigera	V	663, 993, 1587, 2285, 4697

A minimum of 35 larvae were treated per concentration. Mortality was assessed after 96h.

In a separate experiment, 1µl PB (5000 µg ml^{-1}) was injected into the abdominal region of fifth instar S. littoralis larvae 1h before topical application of the following concentrations of AVMB1 : 613, 993, 1587, 2285 and 4697 µg ml^{-1}.

3.2.5. Toxicity of topically-applied malathion to fifth (±PB) and sixth instar larvae of S. littoralis

Fifth and sixth instar larvae of S. littoralis were treated topically (Section 2.4.) with the following concentrations of malathion:

Instar	Malathion (µg ml^{-1})
V	500, 1000, 2500, 5000, 10000
VI	100, 250, 500, 1000, 2500, 5000

Fifth instar larvae were also treated with 1µl PB (50000 µg ml^{-1}) 1h prior to treatment with the following concentrations of malathion: 100, 250, 500, 1000 and 2500 µg ml^{-1}

3.2.6. Toxicity of topically-applied lambda cyhalothrin (Karate) to fifth (±PB) and sixth instar larvae of S. littoralis

Fifth and sixth instar larvae of S. littoralis were treated topically (Section 2.4.) with the following concentrations of lambda cyhalothrin:

Instar	Lambda cyhalothrin (µg ml^{-1})
V	1, 10, 15, 25, 50
VI	10, 15, 20, 25, 50, 100

Fifth instar larvae were also treated topically with 1ul PB (50000 µg ml^{-1}) 1h prior to treatment with the following concentrations of lambda cyhalothrin: 0.5, 1, 10, 15, 25 and 50 µg ml^{-1}.

3.2.7. Toxicity of topically-applied AVMB1 to fifth (± PB) and sixth instar larvae of H. armigera

Fifth and sixth instar larvae of H. armigera were treated topically (Section 2.4.) with the following

concentrations of $AVMB_1$:

Instar	$AVMB_1$ ($\mu g\ ml^{-1}$)
V	186, 394, 511, 1871, 2285, 4571
VI	35, 186, 511, 1871, 4571

Fifth instar larvae were also treated topically with 1ul PB (50000 $\mu g\ ml^{-1}$) (Section 2.4.) 1h prior to treatment with the following concentrations of $AVMB_1$: 35, 394, 511, 2285 and 4571 $\mu g\ ml^{-1}$.

3.2.8. Toxicity of topically-applied $AVMB_1$ to sixth instar S. littoralis (Egyptian field strain; Fo).

Thirty five sixth instar larvae were treated topically (Section 2.4.) for each of the following concentrations of $AVMB_1$: 35, 186, 511, 1871 and 4571 $\mu g\ ml^{-1}$.

3.3. Results

3.3.1. Toxicity of topically-applied AVMB1 to second, to sixth instar larvae of S. littoralis

The relative LD_{50} and LD_{95} values (ng mg^{-1}) of topically-applied AVMB1 were not significantly different ($P>0.05$) between the second to third larval instars of S. littoralis, but there was a marked decrease in toxicity against the fourth instar at the LD_{50} and LD_{95} levels (6.5 and 46 fold respectively). The slope of the Probit-log dose regression line was significantly reduced ($P<0.05$; Table 3.1.).

AVMB1 seemed to show increased toxicity against fifth instar S. littoralis although the LD_{50} and LD_{95} values were not significantly different ($P>0.05$) from the fourth instar. However, AVMB1 was 519 and 1623-fold more toxic at the LD_{50} and LD_{95} levels respectively against the sixth compared with the fifth instar ($P<0.05$; Table 3.1.).

The above differences in the relative toxicity of AVMB1 were also shown when the values for AVMB1 were calculated as ugs a.i. per insect, but in this case the fifth instar was the least sensitive instar (estimated LD_{50} values were 0.09, 1.17, 10.6, 41.7 and 0.36 µg a.i. per insect for the second, third, fourth, fifth and sixth instars respectively).

Table 3.1.

Comparative toxicity of AVMB$_1$ applied topically to the dorsal thorax of S. littoralis and H. armigera larvae and effect of synergists.[1].

Instar	ng mg^{-1} body wt (95% C.L.) 96 h LD_{50}		96 h LD_{95}		Slope±S.E.[2].
S. littoralis					
II	56.3	(44.8-67.9)	410	(303-634)	1.91±0.20a
III	71.7	(57.2-92.1)	995	(612-1940)	1.44±0.16b
III + PB	17.5	(13.9-22.0)	243	(159-434)	1.44±0.16b
IV	471	(294 -1002)	45569	(10505-533809)	0.84±0.13c
IV + PB	92.8	(71.6-120)	1552	(886-3647)	1.34±0.15b
V	223	(136 -392)	16722	(6850-54559)	0.88±0.07c
V + PB	27.7	(19.6-41.7)	2074	(921-6198)	0.88±0.07c
V + PB*	35.0	(21.0-62.6)	2624	(1063-8668)	0.88±0.07c
V + DEF	69.0	(47.6-104)	5175	(2383-14583)	0.88±0.07c
V + PB[3].	13.7	(10.0-24.8)	168	(64.2-1688)	1.51±0.20ab
VI	0.43	(0.31-0.56)	10.3	(6.5-19.1)	1.19±0.10bc
VI + PB	0.34	(0.26-0.44)	8.2	(4.9-16.2)	1.19±0.10bc
VI Field[4].	0.77	(0.36-1.91)	228	(32.2-36069)	0.66±0.16c
H. armigera					
V	12.1	(8.6-17.2)	632	(317-1626)	0.96±0.08c
V +PB	14.7	(9.5-18.3)	683	(338-1796)	0.96±0.08c
VI	15.8	(10.4-25.8)	825	(157-2651)	0.96±0.08c

1. AVMB$_1$, piperonyl butoxide (PB; 50000 and 5000* µg ml^{-1}) and s,s,s-tributyl phosphorothioate (DEF; 10000 µg ml^{-1}) were topically applied in ethyl methyl ketone.
2. For each species, values with a common letter are not significantly different (P>0.05); slope for joint probit analysis not significantly (P>0.05) different from the single line slopes for each treatment.
3. PB (0.52 µl of 5000 µg ml^{-1}) injected into the abdominal region of larvae 1 h before topical application of AVMB$_1$.
4. S. littoralis Egyptian field strain; F_0.

3.3.2. Toxicity of topically-applied AVMB$_1$ with topically-applied or injected PB to third, fourth, fifth and sixth instar larvae of S. littoralis.

The topical application of PB at 50000 µg ml^{-1} significantly increased ($P<0.05$) the relative toxicity of AVMB$_1$ at the LD_{50} and LD_{95} levels against third (4-fold), fourth (5 and 29-fold) and fifth (8-fold) instar larvae compared with topical application of AVMB$_1$ alone (Table 3.1.). Topical application of PB at 5000 µg ml to fifth instar larvae significantly ($P<0.05$) increased the relative toxicity of AVMB$_1$ at LD_{50} and LD_{95} levels against fifth instar S. littoralis by 6-fold while injection of PB at 50000 µg ml^{-1} into fifth instar S. littoralis increased the toxicity of AVMB$_1$, against this instar by 16 and 99-fold at the LD_{50} and LD_{95} levels respectively ($P<0.05$; Table 3.1.).

3.3.3. Toxicity of topically-applied AVMB$_1$ with DEF to fifth instar S. littoralis larvae.

Topical application of DEF (10000 µg ml^{-1}) significantly ($P<0.05$) increased the toxicity of AVMB$_1$ against fifth instar larvae at the LD_{50} level (3-fold) but had no significant ($P>0.05$) effect at the LD_{95} level (Table 3.1.).

3.3.4. Injection of $AVMB_1$ into fifth and sixth instar larvae of S. littoralis

Injection of $AVMB_1$ significantly ($P<0.05$) reduced the differential toxicity between the fifth and sixth instars to 12-fold for the LD_{50} and LD_{95} levels, (Table 3.3.) compared with 519 and 1623-fold respectively for topical application (Table 3.1.).

In these injection experiments control mortality was 16-18%, which was higher than in the other experiments and was a reflection of the difficulty of the injection method. High control mortality prevented this method being used with second, third or fourth instar larvae.

3.3.5. Toxicity of topically-applied malathion to fifth (+PB) and sixth(-PB) instar larvae of S. littoralis.

The relative toxicity of malathion at the LD_{50} level (ng mg^{-1}) was significantly ($P<0.05$) greater against the sixth compared with fifth instar S. littoralis (4-fold) while malathion was significantly ($P<0.05$) more toxic against fifth but significantly ($P<0.05$) less toxic against sixth instar S. littoralis larvae compared with $AVMB_1$ at LD_{50} levels (Tables 3.1. and 3.2.). PB had no significant ($P>0.05$) effect on the toxicity of malathion against fifth instar S. littoralis (Table 3.2.).

Table 3.2.

Toxicity of malathion and lambda-cyhalathrin applied topically to the dorsal thorax of S. littoralis larvae and effect of PB.[1].

Instar	ng mg^{-1} body wt (95% C.L.) 96h LD_{50}	96h LD_{95}	Slope±S.E.[2]
Malathion			
V	9.5 (7.7-11.6)	117 (73.4-234)	1.51±0.16a
V + PB	7.0 (4.6-14.1)	605 (143-10849)	0.85±0.15b
VI	2.4 (1.6- 4.0)	110 (34.7-1021)	0.99±0.16b
Lambda-cyhalathrin			
V	0.05 (0.04-0.06)	0.28 (0.21-0.46)	2.27±0.31a
V + PB	0.04 (0.03-0.05)	0.38 (0.27-0.58)	1.61±0.10b
VI	0.02 (0.01-0.03)	0.17 (0.10-0.41)	1.59±0.23b

1. Applied in ethyl methyl ketone; PB (50000 µg ml^{-1}) applied to ventral thorax.
2. For each pesticide, values with common letter are not significantly (P>0.05) different.

3.3.6. Toxicity of topically-applied lambda cyhalothrin (Karate) to fifth(±PB) and sixth instar larvae of S. littoralis.

The relative toxicity of lambda cyhalothrin at the LD_{50} level (ng mg^{-1}) was significantly ($P<0.05$) greater against fifth compared with sixth instar S. littoralis larvae (2.5-fold; Table 3.2.). Compared with topically-applied $AVMB_1$ and malathion, lambda cyhalothrin was significantly ($P<0.05$) more toxic against fifth and sixth instar S. littoralis compared with $AVMB_1$ at the LD_{50} levels (ng mg^{-1}) (Tables 3.1. and 3.2.). PB had no significant ($P>0.05$) effect on the toxicity of lambda cyhalothrin against fifth instar larvae (Table 3.2.).

3.3.7. Toxicity of topically-applied $AVMB_1$ to fifth(±PB) and sixth instar and of injected $AVMB_1$ to fifth instar H. armigera larvae.

The toxicity (LD_{50} and LD_{95} ng/mg) of topically-applied $AVMB_1$ did not differ significantly ($P>0.05$) between the fifth and sixth instars. PB (50000 µg ml^{-1}) had no significant ($P>0.05$) effect on the toxicity of $AVMB_1$ against fifth instar H. armigera (Table 3.1.).

$AVMB_1$ was significantly ($P<0.05$) less toxic to fifth instar S. littoralis compared with fifth instar H. armigera,

Table 3.3

Toxicity of injected AVMB$_1$ against S. littoralis and H. armigera larvae.[1]

Instar	ng mg^{-1} body wt (95% C.L.) 96h LD_{50}		96h LD_{95}		Slope±S.E.[2]
S. littoralis					
V	11.2	(7.0-20.5)	961	(291-7240)	0.85±0.12a
VI	0.95	(0.63-1.6)	81.7	(26.7-544)	0.85±0.12a
H. armigera					
V	9.4	(7.3-13.0)	91.4	(44.3-445)	1.66±0.32b

1. Applied in ethyl methyl ketone.

2. Values with common letter are not significantly different ($P>0.05$); slope for joint probit analysis not significantly ($P>0.05$) different from single line slopes for each treatment.

but was significantly ($P<0.05$) more toxic to sixth instar S. littoralis compared with sixth instar H. armigera at the LD_{50} and LD_{95} levels (ng mg^{-1}) (Table 3.1.).

Injection of $AVMB_1$ into fifth instar H. armigera (Table 3.3.) had no significant ($P>0.05$) effect on toxicity when compared with topical application (Table 3.1.).

3.3.8 Toxicity of topically applied $AVMB_1$ to Egyptian field strain (F_0) sixth instar S. littoralis.

The toxicity of $AVMB_1$ to field strain S. littoralis sixth instar larvae was not significantly ($P>0.05$) different from the data obtained with the Canary Island Strain of S. littoralis reared on a haricot bean diet (Table 3.1.).

3.4. Discussion

3.4.1. Symptoms of poisoning with $AVMB_1$

Larval instars of H. armigera and S. littoralis showed signs of paralysis of the abdominal legs and lack of movement within one and two days of $AVMB_1$-treatment respectively; death occurring up to four days post-treatment. Similar symptoms of poisoning by $AVMB_1$ in insects were observed by Putter et al. (1981) in lepidopteran larvae and Botham and Nicholson (1985) in the housefly, Musca domestica. Corbett (1987) observed that S. littoralis larvae showed signs of paralysis within two days of $AVMB_1$-treatment while adult male P. americana responded within six hours of injection with $AVMB_1$.

Wright (1986) stated that it is uncertain whether the relative speed of kill with AVM is due to their mode of action, because of the lack of hyperactive symptoms compared with other compounds, or to a relatively slow accumulation of AVM at critical target sites. The first symptoms of AVM poisoning, occurring a few hours after application, such as paralysis of the hind legs in lepidopterous larvae (Putter et al., 1981) may reflect an initial action at relatively exposed peripheral target sites (Wright, 1987).

Some $AVMB_1$-treated larvae of S. littoralis and

H. armigera larvae failed to pupate properly, developing into larval-pupal intermediates. This has also been observed in H. virescens, S. frugiperda and H. zea larvae (Bull, 1986) and larval V. maculifrons (Parrish and Roberts, 1984). These intermediates may have been produced by partially paralysed larvae being unable to completely shed the last larval cuticle.

Jyothi et al. (1986) found that larvae of the caster semilooper A. janata treated with AVMB1 were totally or partly unable to moult their old larval exuviae. When the cuticle of an apolysed larva was removed the new cuticle was found underneath. This suggested AVMB1 does not affect the physiological processes involved in metamorphosis but causes paralysis.

3.4.2. Toxicity of topically-applied AVMB1 to larval instars of S. littoralis and H. armigera.

Topically-applied AVMB1 was found to be markedly more toxic to sixth instar S. littoralis than to earlier instars (Table 3.1.) confirming a previous report (Corbitt et al., 1989). AVMB1 did not differ appreciably in activity against fifth and sixth instar H. armigera (Table 3.1.).

The greatly increased toxicity of AVMB1 to sixth instar S. littoralis is unusual, since as mentioned earlier, the

most common trend observed with insecticides is one of decreasing relative toxicity with increasing larval weight and instar (Lund et al., 1979; Abro, 1985 ; Aikins and Wright, 1985). Even where increasing sensitivity to an insecticide has been reported, as in the case of diflubenzuron against larval stages of the Eastern Budworm, Choristoneura fumiferana (Granett and Retnakaran, 1977), the changes in toxicity are relatively small. The increased toxicity of AVMB1 to sixth instar S. littoralis larvae in this study was also shown when the LD_{50} values for AVMB1 were calculated as µg a.i. per insect. Such increasing relative toxicity with increasing larval weight and instar did not occur with the chemically unrelated insecticides cypermethrin (Corbett et al., 1989) nor with malathion and lambda-cyhalothrin (Table 3.2.) against fifth and sixth instar S. littoralis nor with AVMB1 against fifth and sixth H. armigera in this study.

Differential toxicity of insecticides has been related to a number of factors such as enhanced metabolic degradation, altered target site and reduced penetration (Oppenoorth and Welling, 1976). The possible role of enhanced metabolic degradation in the differential toxicity of AVMB1 to S. littoralis larvae was examined using the specific metabolic inhibitors PB (Casida, 1970) -a microsomal or mixed function oxidase (MFO) inhibitor, and DEF -an esterase inhibitor (Hemingway and Georgiou, 1984).

Synergism of AVMB1 with PB against third to fifth, instar S. littoralis but the absence of synergism with sixth instar S. littoralis and fifth instar H. armigera, suggests that oxidative mechanisms are involved in the differential toxicity of AVMB1. These mechanisms seem to be relatively selective since there was a lack of synergism of malathion and lambda cyhalothrin by PB in the fifth instar S. littoralis. Changes in microsomal activity with age have also been correlated with the differential toxicity of various insecticides against larvae of the fall armyworm, Spodoptera frugiperda (Yu, 1983). The lack of effect of PB with malathion (an organophosphate) may be due to the fact that OPs need oxidation to convert them to the actual toxicant (malaxon) and the initial action of PB on toxicity is to antagonise (Dyte and Rowlands, 1967), but PB also interferes with the dealkylation and with the "leaving group" scission (hydrolytic and oxidative). Both these actions are synergistic and the overall effect of PB with insecticides needing oxidative activation is a balance of synergism and antagonism, which sometimes tends to be antagonistic but often tends to no effect. This may explain why PB had no synergistic effect with malathion.

The esterase inhibitor DEF appeared to have some synergistic action on AVMB1 against fifth instar S. littoralis at the LD_{50} level although its effect was significantly less compared with the action of injected or

topically applied PB. This suggests hydrolytic mechanisms may also be involved in the degradation of $AVMB_1$ in S. littoralis.

Anderson et al. (1986), using topical application, found that PB potentiated the toxicity of $AVMB_1$ against third instar S. eridania larvae; potentiation being greater when PB and $AVMB_1$ were applied together than when PB was applied 2h before $AVMB_1$. They suggested that the potentiation by PB was mainly due to improved cuticular penetration of $AVMB_1$, since substitution of an emulsified oil for PB had a similar result. However, their data for PB and emulsified oil did not show potentiation for $AVMB_1$ against third instar H. virescens larvae and it was suggested the differences between the species were due to reduced cuticular penetration in S. eridania (Anderson et al., 1986).

However, while it is possible that PB may enhance penetration of $AVMB_1$ under certain conditions, and the rate of penetration of $AVMB_1$ is important in determining its differential toxicity, differential metabolism of $AVMB_1$ and inhibition of microsomal oxidase activity by PB could be important in noctuids. In vertebrates, microsomal detoxification of AVM to their C-25, methyl alcohol and its monosaccharide has been reported by Miwa et al., 1983 and Chiu et al., 1984.

There are several reasons to reject a significant action of PB on cuticular penetration of AVMB1 :

a) When insects were treated topically with PB and AVMB1 , these compounds were applied at different times and to different regions of the body, thereby reducing the possibility of a direct interaction. Potentiation of AVMB1 also occurred when the dose of topically applied PB was reduced 10-fold, and when this lower dose (5000 $\mu g\ ml^{-1}$) was injected rather than topically applied.

b) Topically applied PB had no significant effect on the toxicity of AVMB1 against sixth instar S. littoralis or fifth instar H. armigera, or on the toxicity of lamda-cyhalothrin and malathion against fifth instar S. littoralis. Taken together, these results are difficult to reconcile with a non-specific action on pesticide penetration. Further supportive evidence for the synergistic action of PB is discussed in Section 4.4.

However oxidative and hydrolytic degradation alone cannot account for the observed difference in toxicity of AVMB1 between fifth and sixth instar S. littoralis . The involvement of a penetration factor was implied by the finding that injection bioassays with AVMB1 increased its relative toxicity significantly at the LD_{50} level; injection of AVMB1 resulting in an 20-fold increase and a 2-fold decrease in toxicity against fifth and sixth instar

S. littoralis respectively, when compared with topical application. Whether these intraspecific differences can be related to changes in cuticular composition is unknown (Patil and Guthrie, 1979a,b).

The mechanism by which the cuticle inhibits the penetration of insecticides is not fully understood, but it has been related to increased lipid and protein content (Weismann, 1957; Vinson and Law, 1971), increased cuticular thickness (Weissmann, 1947) and to altered lipid composition (Patil and Guthrie, 1979,a,b). It is likely that the increasing tolerance of the instars to AVMB1 up to the fifth instar S. littoralis was at least partly due to reduced cuticular penetration. The fall in tolerance during the sixth instar may imply a reduction in the thickness of the cuticle or a change in its chemistry.

The insensitivity of fourth and fifth instar S. littoralis to AVMB1 compared with the other instars may also be partly due to increased storage of AVMB1 in the former. Chapman (1969) showed that later lepidopteran instars possess more adipose tissue than earlier instars, but this does not explain why the sixth instar was so much more sensitive.

3.4.3. Comparative toxicity

In the present study AVMB1 was significantly less toxic to fifth instar S. littoralis compared with fifth instar H. armigera, but was significantly more toxic to sixth instar S. littoralis compared with sixth instar H. armigera at the LD_{50} and LD_{95} levels. PB had no significant effect on the toxicity of AVMB1 against fifth instar H. armigera. These results suggest that a penetration factor is not involved in H. armigera since injection of AVMB1 did not increase its relative toxicity against fifth instars of this species. In addition the lack of synergism of AVMB1 by PB suggests that oxidative metabolic degradation is not an important factor in determining the toxicity of this compound in H. armigera, unlike the results in S. littoralis.

The above interspecific differences seem to involve differences in metabolic degradation of and penetration of the insecticide into the larvae. Interspecific differences have been observed several times previously between AVMB1 and new world noctuid moth larvae (Wolfenbarger, 1985; Anderson et al., 1986; Bull, 1986; Strong and Brown, 1987). Bull, (1986) for example found differential toxicity of AVMB1 to third and last instar larvae of Heliothis virescens, Heliothis zea and Spodoptera frugiperda. Short-term mortality effects and delayed disruption of metamorphosis were most severe in H. virescens and least

severe in S. frugiperda.

Studies on the fate of topically-applied ^{3}H-AVMB$_{1a}$ by Bull (1986) indicated that similar concentrations (1.1-3.5% of applied dose) were absorbed by all three species examined. Orally-administered ^{3}H-AVMB$_{1a}$ was readily absorbed from the midgut, metabolised and excreted along with its metabolites by last-stage larvae, the rates being slowest in H. virescens. At 4 and 8 h after application of ^{3}H-AVMB$_{1a}$ to last instar larvae, significantly more radiolabelled material was recovered from the heads of H. virescens than the other two species. Bull (1986) concluded these differences were not sufficient to explain the substantial differences in susceptibility to this compound among the test species and suggested neurophysiological studies might show differences in the affinity of the AVMB$_1$ for, and interaction with GABA receptors. Bull (1986) considered the rate of cuticular penetration unlikely to explain the tolerance shown by S. frugiperda, an explanation favoured by Anderson et al. (1986). Differences in penetration and metabolism found between the fifth and sixth instar S. littoralis and fifth instar H. armigera larvae in the present study although important, appear to be insufficient to account for all the intra and inter-specific differences found in the toxicity of AVMB$_1$. This is in agreement with the findings of Bull (1986).

4. UPTAKE OF ^{3}H-AVMB$_{1}$ BY S. LITTORALIS AND H. ARMIGERA.

4.1. Introduction

In this section, the main aims were to determine the amount of ^{3}H-AVMB$_{1}$ in the ventral nerve cord of randomly-selected, paralysed and non-paralysed insects, and in fat body samples from paralysed and non-paralysed insects treated with sufficient AVMB$_{1}$ to produce symptoms of paralysis in 40-60% of the insects in a given time. The effects of PB were also examined on AVMB$_{1}$ in randomly-selected treated insects. The degree to which AVMB$_{1}$ was metabolised was also examined; the proportion of radioactivity present as the parent compound or as metabolites being determined by the method of Burg et al. (1979).

4.2. Materials and methods

4.2.1. Treatment of insects

Fifth and sixth instar S. littoralis and H. armigera larvae were treated topically (Section 2.4.) with sufficient ^{3}H-AVMB$_{1}$ (E.C.) to produce toxic symptoms (paralysis) in 40-60% of the population. The concentrations of ^{3}H-AVMB$_{1}$ used were as follows:

Species	Instar	µg/ml	µg/insect	dpm/mg
H. armigera	V	3000	3.0	910042
	VI	1500	1.5	463861
S. littoralis	V	4500	4.5	1370410
	VI	4500	4.5	1370410

The insects were left until symptoms of poisoning - twitching, paralysis of hind legs and lack of eating were shown by 40-60% of the insects (at 24 and 48 h for H. armigera and S. littoralis respectively) and then dissected. The ventral nerve cord up to and including the metathoracic ganglion was removed together with separate samples of fat body. Each batch of tissue (from 6-10 insects) was placed into individual pre-counted, pre-weighed glass scintillation vials and the vials re-weighed. Each treatment was replicated four times. To each vial NCS tissue solubiliser (0.4ml; Amersham International) was added to digest the tissue sample. The vials were stored at 30°C for 48 h. Cocktail T scintillant (10ml; BDH Ltd) was then added to each vial and left in the dark for 48h to reduce spontaneous chemiluminescent reactions. The vials were counted in a Beckman-250 liquid scintillation counter for 50 minutes or until a 2 sigma error value of 0.2% was obtained. Disintegrations per minute (dpm) were calculated using a standard quench curve for ^{3}H in Cocktail T and the dpm

values converted into pM (10^{-12}M) equivalents of ^{3}H-AVMB$_1$ per mg wet weight tissue. Control tissues were taken from untreated adults and subjected to the same digestion and counting procedures.

4.2.2. Isolation of radioactivity from S. littoralis and H. armigera tissues

Fifth and sixth instar S.littoralis and H. armigera larvae were treated with ^{3}H-AVMB$_1$ as in Section 4.2.1., left for 48 and 24 h respectively and then dissected. Tissue samples were taken as in Section 4.2.1. This experiment was repeated (for nerve-cord samples only) with randomly-selected fifth and sixth instar S. littoralis which had been treated with 1ul PB (5000 ppm) applied topically to the dorsal posterior abdomen (Section 2.4.). After 1h the larvae were treated with ^{3}H-AVMB$_1$ as in Section 4.2.1.

Three main sets of data were obtained:

a) Nerve-cord samples from randomly selected larvae.

b) Nerve-cord samples from paralysed and non-paralysed larvae.

c) Fat body samples from paralysed and non-paralysed larvae.

4.2.2.1. Extraction of ^{3}H-AVMB$_{1}$.

^{3}H-AVMB$_{1}$ was extracted from nerve and fat body samples using the method of Burg et al. (1979). Known weights of tissue were shaken for 5 min. in an acetone:hydrochloric acid (1M) solution (10ml). Five ml of chloroform was then added to each sample which was shaken for a further 5 min. The samples were then centrifuged at 1500g and the pellet discarded. Two ml of distilled water was added to the supernatent and the above extraction procedures repeated. The upper aqueous layer was then pipetted off and its volume determined by weighing and three 200µl aliquots per sample were taken for scintillation counting (Section 4.2.1.). The lower, chloroform layer was then evaporated to dryness under nitrogen and the residue taken up in 1ml of chloroform. Three separate 50µl aliquots were counted per sample (Section 4.2.1.).

The extraction efficiency of the above procedure was assessed using the following internal standards:

1) One ul of ^{3}H-AVMB$_{1}$ (1289463 dpm) was added to a known weight of tissue in a vial. The tissue was then allowed to dry before extraction procedures were carried out (Section 4.2.1.) and the percentage recovery determined (n=4)

2) Untreated insects were dissected and nerve samples taken,

extraction procedures were carried out as previously described (Section 4.2.1.) (n=4).

3) One µl of ^{3}H-AVMB$_1$ (1289463 dpm) was added to an empty vial (n=4), extraction procedures carried out as above (Section 4.2.1.) and the percentage recovery determined.

4.2.2.2. Thin layer chromatography (TLC) of ^{3}H-AVMB$_1$.

Aliquots (50µl) of the chloroform layer from the tissue and control extracts, and from the internal standard (Section 4.2.2.1.) together with 50µl of unlabelled AVMB$_1$ (2285ppm) were spotted separately onto silica gel (0.2mm) aluminium-backed TLC plates (Merck). These plates had been pre-heated at 100°C for 24h and scored into sections using a scalpel to prevent cross-contamination. A mixture of chloroform: methanol: dichloromethane and ethyl-acetate (9:1:2:9) was used as the solvent phase (Burg et al., 1979). Following chromatography, the unlabelled standards were sprayed with 1% (w/v) ceric sulphate (cerium IV sulphate) in 10% (v/v) sulphuric acid and heated to 100°C to visualise AVMB$_1$. The silica in the area corresponding to the AVMB$_1$ position (referred to as band 3) was put into pre-counted scintillation vials containing 10ml of Cocktail T scintillant and counted. The silica bands below and above band 3 were scraped into separate vials and counted.

4.2.2.3. TLC of chloroform extracts from ventral nerve cord and fat body tissues.

Visualisation of unlabelled AVMB1 (2µg) on a TLC plate revealed that AVMB1 was located in a band with a Rf value of 0.38+0.02, (n=10). TLC of the ^{3}H-AVMB1 stock solution (1.8% E.C. formulation) revealed that 92.9% of the activity was located in this band (Rf = 0.38), which was the third band on the TLC plate (Table 4.2.) and that there was a loss of only 3.4% activity by TLC compared with direct measurement of radioactivity (Table 4.1.).

Internal standards (Table 4.1.) revealed that there was relatively little loss of radioactivity during the extraction and separation procedures (only 8.6%). The % activity in this band on the TLC plates was used to estimate the dpm/mg due to AVMB1 in the chloroform sample (Tables 4.4., 4.7., 4.10.). Radioactivity in the aqueous samples was taken to be polar metabolites and not AVMB1.

Table 4.1.

Radioactivity recovered following topical application of internal standard (^{3}H-AVMB$_1$) to nerve cord extracts of V instar S. littoralis larvae or after direct addition to vial (n=4).

Treatment (Tissue)	Extract	$\bar{X}$ Dpm/mg Initial	$\bar{X}$ Dpm Recovered (%)	%Dpm/mg Of Initial Recovered	$\bar{X}$ Dpm/mg[1] Metab. (%)	$\bar{X}$ Dpm/mg AVMB$_1$ (%)
Nerve[2]· cord	Total	136960	125128	91.4		
	Chloroform		124745 (99.7)		8894	115851
	Aqueous		383 (0.3)		383	-
					9277 (7.4)	115851 (92.6)
Direct[3]· into vial	Total	136960	129743	94.7		
	Chloroform		129267 (99.6)		9579	119688
	Aqueous		476 (0.4)		476	-
					10055 (7.8)	119688 (92.2)

1· Calculated from TLC values (Table 4.2)

2· 4.5 µg AVMB$_1$ added to known weight of tissue in vial. Tissue allowed to dry prior to extraction.

3· 4.5 µg AVMB$_1$ added directly to vial and extraction procedure carried out as before.

Original data given in Appendix 4.1.

Table 4.2.

Amounts of ^{3}H-AVMB$_1$ determined by TLC following topical application of internal standard to nerve cords of V instar S. littoralis (n=4).[1]

$\bar{X}$ total Dpm recovered	$\bar{X}$ Dpm in chloroform sample (bands 1-7)	$\bar{X}$ Dpm in band 3 of chloroform sample	Band 3 Dpm as % of solvent sample
125128	124745	115851	92.9

[1] 4.5ug AVMB$_1$ added to known weight of tissue in vial. Tissue allowed to dry prior to extraction. Original data given in Appendix 4.2.

4.2.3. Toxicity of topically-applied AVMB1 (E.C.) to larvae of S. littoralis and H. armigera.

Fifth and sixth instar larvae of S. littoralis and H. armigera were treated topically (Section 2.4.) with the following concentrations of AVMB1 (E.C. formulation):

Species	Instar	µg/ml
H. armigera	V	3000
	VI	1500
S. littoralis	V	4500
	VI	4500

4.2.4. Radioactivity recovered from gut membrane and gut lumen of S. littoralis and H. armigera following topical application of ^{3}H-AVMB1.

Larvae (V and VI instars) of both species were treated with the same doses of ^{3}H-AVMB1 and for the same length of time as used in Section 4.2.3. Insects showing a) paralysis and b) no signs of paralysis were selected and dissected. The gut was removed and the contents squeezed and washed out with distilled water into empty scintillation vials. The gut membranes were placed in separate vials. Each sample was digested and counted as in Section 4.2.1.

4.2.5. Calculation of the concentration of AVMB1 in tissue samples (as pmoles/mg wet weight)

It was possible, knowing the specific activity of the original compound (mCi/ml), the dose applied, the half life of tritium and the molecular weight of AVMB1 to calculate the pmoles of AVMB1 present in the tissues analysed. An example of such a calculation is shown in Appendix 4.16. It was not possible to identify the metabolites and standards are not available, so it was impossible to determine the concentrations of these substances. Metabolites have been expressed in terms of the radioactivity (dpm/mg) and as a proportion of the total radioactivity present (see Tables 4.5., 4.6. and 4.8.). The concentrations of AVMB1 (as pmoles/mg wet weight) are given in Tables 4.4., 4.7. and 4.9.

4.2.6. Uptake and penetration of ^{3}H-AVMB1 into S. littoralis V and VI instar larvae over 24h

^{3}H-AVMB1 (726622 dpm; E.C.) was administered topically (Section 2.4.) to the thorax of S. littoralis V and VI instar larvae. At 0, 1, 3, 6 and 24 h after treatment, the insects were rinsed with 5ml of a solvent mixture containing chloroform, methanol, dichloromethane and ethyl acetate (9:1:2:9) in a scintillation vial (this mixture had given

the best wash-off results in preliminary experiments using various solvent systems and was the same as used in Section 4.2.2.2.). The vials were shaken vigorously for 5 min and the larvae removed. Five larvae were used at each time interval.

A sample of haemolymph (30 and 50 ul from V and VI instars respectively) was taken from the base of a severed proleg using a Drummond microcap. The larvae were dissected and fat body samples were removed and weighed. All samples were digested and counted as in Section 4.3.1.

For each wash-off sample, an internal standard (^{3}H-hexadecane) was added after the first count to compensate for substantial quenching by the solvent mixture.

4.3. Results

4.3.1. Treatment of insects

The doses used in the experiments were sufficient to ensure ca. 40-60% of the treated larvae showed symptoms of poisoning (Table 4.3.), including paralysis of the hind legs, lethargy, lack of feeding, twitching and production of strings of faecal pellets at the time required. On the basis of these results, the larvae were dissected 24 and 48 h after application of $AVMB_1$ in *H. armigera* and *S. littoralis* respectively.

4.3.2. Proportion of radioactivity present as $AVMB_1$ and its metabolites in tissue samples.

Using the values for the % $AVMB_1$ from the TLC plates and knowing the amount of radioactivity in the aqueous samples (metabolites only), the proportion of the total radioactivity which was metabolite or $AVMB_1$ (Tables 4.5., 4.6. and 4.8.) was calculated.

The level of total radioactivity present in ventral nerve cord tissue from randomly selected fifth and sixth instar larvae is given in Table 4.4. Fifth instar

Table 4.3.

The response of V and VI instar larvae of S.littoralis and H. armigera 24-96 h after topical application of AVMB1 (E.C.).

Species/ instar	Dose[1]	% Paralysed(P) and % Dead(D) 24h P	24h D	48h P	48h D	72h P	72h D	96h P	96h D
Spodoptera									
V	4.5	20	5	42	5	48	12	52	15
VI	4.5	22	2	44	10	46	25	20	67
Heliothis									
V	3.0	42	0	50	13	78	18	73	27
VI	1.5	52	0	67	3	83	12	68	32

[1] Dose of AVMB1 used given as µg in 1 µl (uncalibrated volume) per insect (Section 2.5.).

No control mortality was recorded in any treatment.

N=60.

Table 4.4.

Radioactivity in randomly-selected S. littoralis and H. armigera nerve cords at 48 and 24 h respectively after topical application of ^{3}H-AVMB$_1$ (n=4).

			$\bar{X} \pm SE$	
Instar	Dose	Time(h)	Total Dpm/mg[1]	pM AVMB$_1$/mg[2]
Spodoptera				
V	4.5	48	328.9±39.0 a	0.59±0.07
V+PB	4.5	48	297.9±20.4 a	0.76±0.05
VI	4.5	48	109.4±13.5 b	0.30±0.04
VI+PB	4.5	48	148.8±19.1 b	0.39±0.05
Heliothis				
V	3.0	24	1129.4±361.0 c	2.93±0.94
VI	1.5	24	353.0±48.0 a	0.83±0.11

[1] Values sharing a common letter not significantly different at P>0.05 level (Mann-Whitney Test). Total dpm is all the radioactivity extracted from tissue samples including aqueous and chloroform extracts.

[2] Calculated using data in Tables 4.5. and Appendix 4.4. Data given in Appendix 4.6.

Table 4.5.

Proportion of AVMB$_1$ and its metabolites extracted from randomly-selected S. littoralis and H. armigera nerve cord samples at 48 and 24 h respectively after topical application of ^{3}H-AVMB$_1$ (n=4).

Instar/ dose[1]		$\bar{X}$ Dpm/mg±S.E.		$\bar{X}$%±S.E.[4]		
		AVMB$_1$[2]	Metabolites[3]	AVMB$_1$		Metabolites
Spodoptera						
V	4.5	189.4±28.3	189.6±25.0	49.7±2.0	a	50.3±2.0
V+PB	4.5	199.6±7.5	80.7±8.4	71.4±1.6	bc	28.6±1.6
VI	4.5	116.3±4.7	35.1±5.1	77.0±2.9	b	23.0±2.9
VI+PB	4.5	149.4±12.4	56.6±10.9	72.9±3.6	bc	27.1±3.6
Heliothis						
V	3.0	1256.1±335.7	487.5±108.1	71.3±1.0	bc	28.7±1.0
VI	1.5	209.5±32.5	109.4±7.4	64.8±2.5	c	35.2±2.5

[1] See Table 4.3.

[2] Calculated from chloroform sample values in Appendix 4.4.

[3] Assumes all radioactivity in aqueous sample is from metabolites, along with bands 1-2 and 4-7 of TLC plate.

[4] S.E. calculated following Arc Sine Transformation of % data. Values sharing a common letter not significantly different (P>0.05) (Mann-Whitney Test).

Data given in Appendix 4.5.

H. armigera larvae had significantly more ($P<0.05$) radioactivity in the ventral nerve cord than in the other species/instars, although they were only treated with 3.0µg $AVMB_1$ per insect compared with 4.5µg per insect for S. littoralis fifth and sixth instar.

There was significantly less ($P<0.05$) total radioactivity present as $AVMB_1$ in nerve cords from randomly-selected fifth instar S. littoralis larvae (49.7%) than from sixth instar S. littoralis (77.0%), fifth (71.3%) and sixth instar (64.8%) H. armigera larvae (Table 4.5.). Sixth instar H. armigera larvae also had a significantly ($P<0.05$) lower level of radioactivity as $AVMB_1$ than in sixth instar S. littoralis.

There were no significant differences ($P>0.05$) in the proportion of total radioactivity in nerve cords present as $AVMB_1$ between larvae which were paralysed or non-paralysed in either species or instar except for sixth instar H. armigera larvae where there was a significant reduction ($P<0.05$, Table 4.6.). Fifth instar S. littoralis larvae had a significantly lower proportion ($P<0.05$) of the total radioactivity as $AVMB_1$ (both in paralysed and non-paralysed larvae) than in the other species and instars, (Table 4.6.).

When ventral nerve cord and tissues from ^3H-$AVMB_1$-

Table 4.6.

Proportion of $AVMB_1$ and metabolites extracted from S. littoralis and H. armigera nerve cord samples from paralysed (P) and non-paralysed (NP) larvae at 48 and 24h respectively after topical application of ^3H-$AVMB_1$ (n=4).

Instar/Symptoms /Dose/Time(h)[1]	$\bar{X}$ Dpm/mg±SE		$\bar{X}$%±SE[5]	
	$AVMB_1$[2]	Metabolite[3]	$AVMB_1$[4]	metabolite[6]
Spodoptera				
V NP 4.5 48	131.7±9.1	137.1±6.9	49.0±1.0ns	51.0±1.1a
P 4.5 48	171.2±6.0	159.8±6.1	51.7±0.6	48.3±0.6a
VI NP 4.5 48	95.4±5.2	41.0±4.2	70.1±1.8ns	29.9±1.8bc
P 4.5 48	110.4±3.3	38.5±2.1	74.1±1.1	25.9±1.1b
Heliothis				
V NP 3.0 24	1246.7±55.3	426.3±39.6	72.9±1.4ns	27.1±1.4b
P 3.0 24	1141.7±19.5	391.1±21.5	74.4±1.4	25.6±1.4b
VI NP 1.5 24	228.4±4.4	100.6±2.5	69.4±0.5*	30.6±0.5c
P 1.5 24	216.3±7.0	114.3±3.5	65.4±0.8	34.6±0.8d

[1] See Table 4.3.

[2] Calculated from values obtained in Appendices 4.7. and 4.8.

[3] Assumes all radioactivity in aqueous sample is from metabolites together with bands 1-2 and 4-7 of TLC plate.

[4] Significance of difference between P and NP samples: $P<0.05$ (*), $P>0.05$ (ns) (Mann-Whitney test).

[5] SE calculated following Arc Sine Transformation of % data.

[6] Values sharing a common letter not significantly different ($P>0.05$ level) (Mann-Whitney Test).

Table 4.7.

Radioactivity in S. littoralis and H. armigera nerve cord samples from paralysed and non-paralysed larvae at 48 and 24 h respectively following topical application with ^{3}H-AVMB$_{1}$ (n=4).

				$\bar{X}\pm$S.E.[3]	
Instar	Symptoms	Dose[1]	Time(h)	Total Dpm/mg[2]	pM AVMB$_{1}$/mg[4]
Spodoptera					
V	NP	4.5	48	166.7±22.2 *	0.30±0.04 *
	P	4.5	48	495.2±111.9	0.97±0.22
VI	NP	4.5	48	107.4±8.3 *	0.28±0.02 *
	P	4.5	48	242.5±28.8	0.68±0.08
Heliothis					
V	NP	3.0	24	317.7±43.2 *	0.88±0.11 *
	P	3.0	24	1585.6±238.0	4.45±0.64
VI	NP	1.5	24	272.7±24.7 ns	0.72±0.06 ns
	P	1.5	24	630.0±146.0	1.57±0.35

[1] See Table 4.3.

[2] Total dpm is all radioactivity in aqueous and chloroform samples extracted from tissue samples.

[3] Difference between non-paralysed (NP) and paralysed (P) samples: $P<0.05$ (*), $P>0.05$(NS) (Mann-Whitney test).

[4] Calculated using values obtained from TLC extraction in Table 4.6. and Appendices 4.8. and 4.9.
Original data given in Appendix 4.10.

treated fifth and sixth instar larvae showing either symptoms or no-symptoms of poisoning were compared, it was found that larvae showing signs of poisoning (paralysis) had significantly ($P<0.05$) greater levels of radioactivity in nerve-cord samples from fifth and sixth instar S. littoralis and fifth instar H. armigera. However there was no significant ($P>0.05$) difference between paralysed and non-paralysed sixth instar H. armigera larvae (Table 4.7.).

There were no significant differences ($P>0.05$) in the proportion of radioactivity which was $AVMB_1$ in fat body tissues from paralysed and non-paralysed larvae from fifth and sixth instar S. littoralis. However, there were significant differences ($P<0.05$) with fifth and sixth instar H. armigera larvae (Table 4.8.). The fat body samples consistently contained proportionally more $AVMB_1$ than the equivalent ventral nerve cord samples (Tables 4.6. and 4.8.).

When fat body samples were compared, significantly greater ($P<0.05$) levels of radioactivity were also found in samples from insects showing symptoms of paralysis of both species and both instars except for fifth instar S. littoralis larvae where $P>0.05$ (Table 4.9.).

Table 4.8.

Proportion of $AVMB_1$ and its metabolites extracted from S. littoralis and H. armigera fat body samples from paralysed and non-paralysed larvae at 48 and 24h respectively after topical application of ^3H-$AVMB_1$ (n=3).

Instar/Symptoms				$\bar{X}$ Dpm/mg±SE		$\bar{X}$%±SE[5]	
Dose/Time(h)[1]				$AVMB_1$[2]	Metabolite[3]	$AVMB_1$[4]	Metabolite[6]
Spodoptera							
V	NP	4.5	48	230.7±20.7	57.3±12.2	80.6±1.9*	19.4±1.9b
	P	4.5	48	243.3±13.4	80.3±5.9	75.2±1.2	24.8±1.2a
VI	NP	4.5	48	43.8±2.5	15.3±1.7	75.1±1.1ns	24.9±1.1a
	P	4.5	48	94.4±6.3	27.7±2.9	77.3±0.8	22.7±0.8ab
Heliothis							
V	NP	3.0	24	300.2±9.8	89.6±6.2	77.0±0.9*	23.0±0.9ab
	P	3.0	24	1715.6±20.1	411.3±25.7	82.8±1.4	17.2±1.4c
VI	NP	1.5	24	110.5±1.2	30.7±0.3	78.3±0.1*	21.7±0.1b
	P	1.5	24	424.6±1.0	88.2±1.9	82.8±0.3	17.2±0.3c

[1] See Table 4.3.

[2] Calculated using data from Appendices 4.11. and 4.12.

[3] Assumes all radioactivity in aqueous sample is from metabolites together with bands 1-2 and 4-7 of TLC plate.

[4] Significance of difference between P and NP samples: $P<0.05$ (*), $P>0.05$ (ns) (Mann-Whitney test).

[5] SE calculated following Arc Sine Transformation of % data.

[6] Values sharing a common letter not significantly different ($P>0.05$ level) (Mann-Whitney Test).

Table 4.9.

Radioactivity in S. littoralis and H. armigera fat body samples from paralysed and non-paralysed larvae at 48 and 24h respectively following topical application with ^{3}H-AVMB$_{1}$ (n=4).

				$\bar{X}\pm$S.E.[3]			
Instar	Symptoms	Dose[1]	Time(h)	Total Dpm/mg[2]		pM AVMB$_{1}$/mg[4]	
Spodoptera							
V	NP	4.5	48	248±85.0	ns	0.72±0.25	ns
	P	4.5	48	466±66.3		1.27±0.18	
VI	NP	4.5	48	67±9.0	*	0.18±0.02	*
	P	4.5	48	133±11.9		0.37±0.03	
Heliothis							
V	NP	3.0	24	401±25.3	*	1.11±0.07	*
	P	3.0	24	2370±258.0		7.08±0.77	
V1	NP	1.5	24	169±32.9	*	0.48±0.09	*
	P	1.5	24	520±40.1		1.55±0.12	

[1] See Table 4.3.

[2] Total dpm is all the radioactivity extracted from tissue samples including aqueous and chloroform extracts.

[3] Difference between non-paralysed (NP) and paralysed (P) samples: $P<0.05$ (*), $P>0.05$ (ns) (Mann-Whitney test).

[4] Calculated using values obtained following TLC extraction in Appendices 4.12. and 4.13.

Original data given in Appendix 4.14.

4.3.3. The effect of piperonyl butoxide (PB)

The application of PB 1h before $AVMB_1$ to fifth and sixth instar S. littoralis, from randomly selected nerve cords, did not significantly increase ($P>0.05$) the total amount of radioactivity (dpm/mg) recovered from fifth or sixth instar larvae, (Table 4.4.). The application of PB significantly increased ($P<0.05$) the proportion of the total radioactivity which was $AVMB_1$ in fifth instar S. littoralis larvae from 49.7% to 71.4%, but caused a significant ($P<0.05$) decrease in the proportion of radioactivity as $AVMB_1$ in sixth instar larvae, (72.9% compared with 77.0%) (Table 4.5.).

4.3.4. Radioactivity recovered from gut membrane and gut lumen contents from $AVMB_1$-treated S. littoralis and H. armigera larvae.

Significantly more radioactivity was recovered from the gut membranes of larvae showing signs of paralysis than from larvae not showing signs of paralysis, in S. littoralis fifth ($P<0.01$) and sixth ($P<0.05$) instar and H. armigera fifth ($P<0.01$) instar larvae (Table 4.10.). In H. armigera sixth instar larvae, the difference in radioactivity between paralysed and non-paralysed insects was not significant ($P>0.05$). The differences were most marked in H. armigera fifth instar larvae.

Table 4.10.

Radioactivity recovered from gut membrane and gut lumen of S. littoralis and H. armigera 48 and 24 h respectively following topical application with ^{3}H-AVMB$_{1}$ (n=10).

		$\bar{X}$ Dpm/mg±S.E.[2]					
Instar/		Gut membrane			Gut lumen		
Dose[1]		Paralysed	Non Paralysed		Paralysed	Non Paralysed	
Spodoptera							
V	4.5	120±21.1	46±6.6	**	510±59.8	272±36.2	**
VI	4.5	61±6.3	39±4.8	*	153±16.2	86±8.9	**
Heliothis							
V	3.0	134±15.5	39±2.3	**	1368±174	259±20.2	**
VI	1.5	17±3.2	16±1.1	ns	59±6.6	56±6.6	ns

[1] See Table 4.3.

[2] Significance of difference between paralysed and non-paralysed samples: P<0.05 (*), P<0.01 (**), P>0.05 (ns) (2 sample non-related t-test).

Original data given in Appendix 4.15.

Significantly more ($P<0.01$) radioactivity was recovered from the gut lumen of paralysed larvae than larvae showing no signs of paralysis, (S. littoralis fifth and sixth and H. armigera fifth instar larvae), but with H. armigera sixth instar larvae, the differences were not significant ($P>0.05$) (Table 4.10).

4.3.5. Uptake and penetration of ^{3}H-AVMB$_1$ into S. littoralis V and VI instar larvae over 24h.

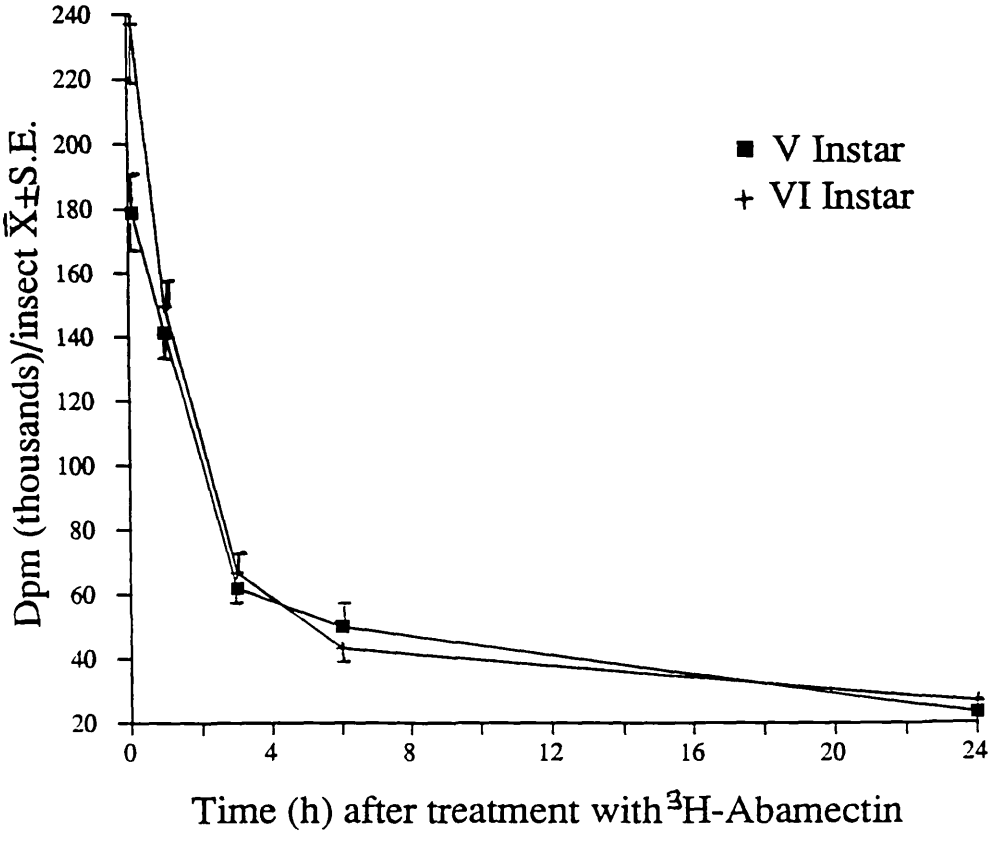
The mean dpm recovered at time 0.1h was 24.6% and 32.6% of the applied dose for S. littoralis fifth and sixth instar larvae respectively. The differences in the dpm recovered from both instars were not significant ($P>0.05$; Mann-Whitney test) at any of the times tested. Over the 24h, there was a significant reduction ($P<0.05$) in the dpm washed off (recovered) with both instars. The data is shown in Figure 4.1. The original data is given in Appendix 4.17.

The dpm recovered from haemolymph samples (at the same times as in the wash-off experiment) were significantly different ($P<0.05$; Mann-Whitney Test) at T= 3 and 6h but were not significantly different ($P>0.05$) at T= 0.1, 1 and 24h after application of ^{3}H-AVMB$_1$. The data is shown in Figure 4.2. The original data is given in Appendix 4.18.

The dpm recovered from fat body samples (at the same

times as in the wash-off experiment) were significantly different ($P<0.05$; Mann-Whitney Test) only at T= 3 h but were not significantly different ($P>0.05$) at T= 0.1, 1, 6 and 24 h after application of ^{3}H-AVMB$_1$. The data is shown in Figure 4.3. The original data is given in Appendix 4.19.

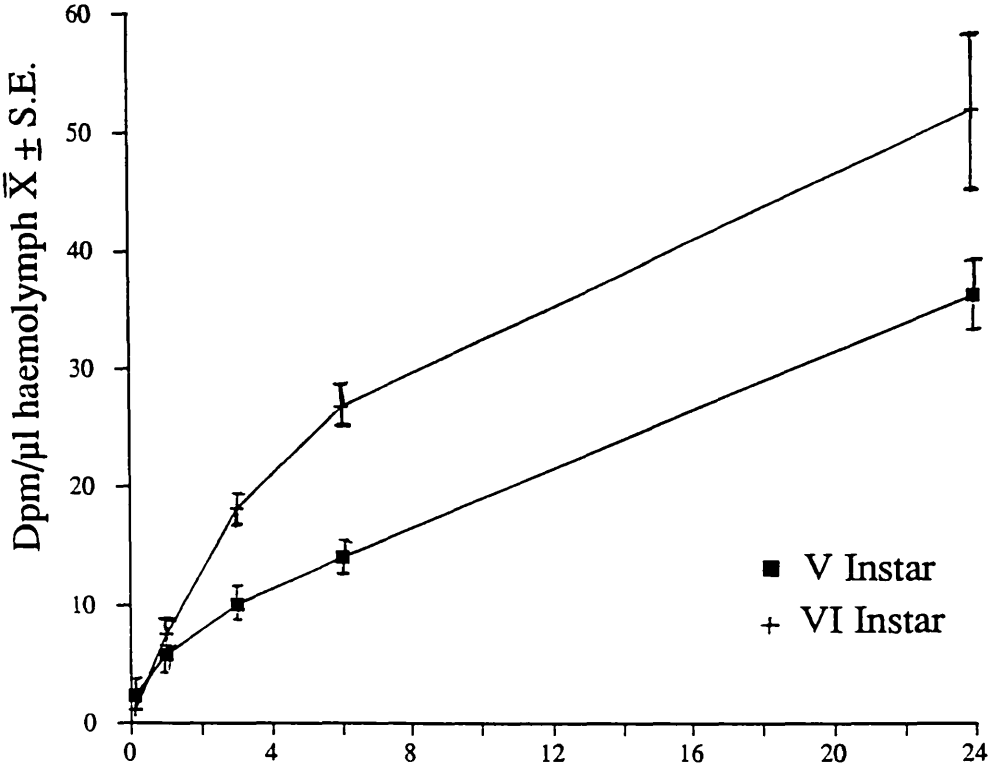
Figure 4.1. Wash-off samples from S. littoralis V and VI instar larvae over 24 h after treatment with 1 µl (uncalibrated volume) of ^{3}H-Abamectin



n = 5

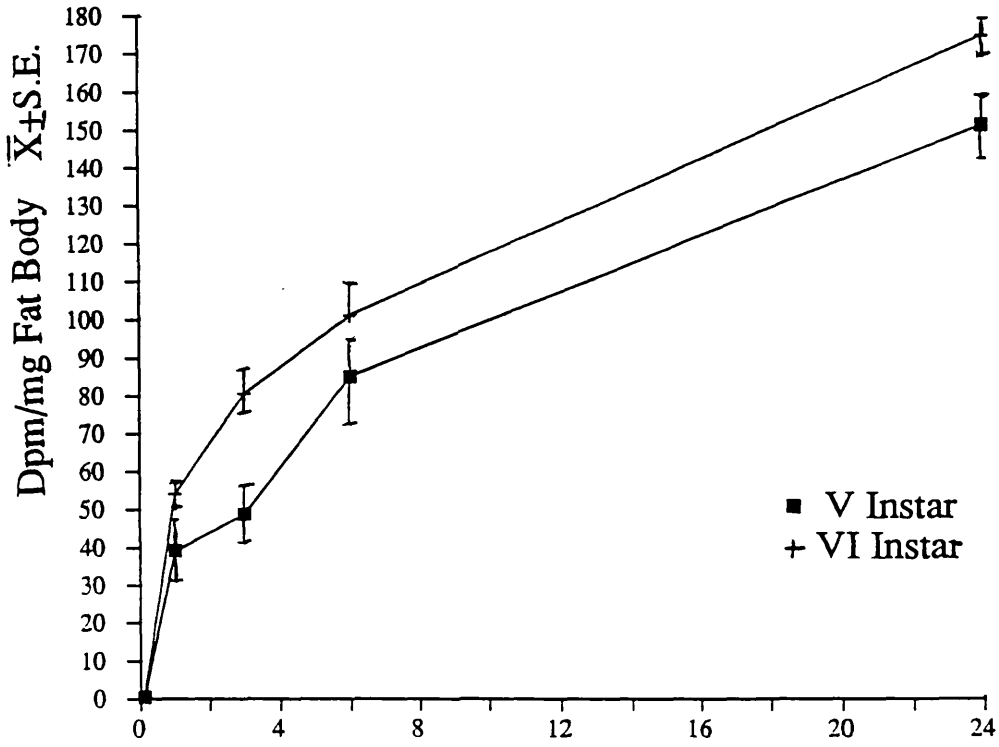
Insects treated with 1 µl (uncalibrated volume) of ^{3}H-Abamectin (726622 Dpm)

Figure 4.2.
Haemolymph samples from S. littoralis V and VI instar larvae over 24 h treated with 1 µl (uncalibrated volume) of ^{3}H-Abamectin



n = 5
Insects treated with 1 µl (uncalibrated volume) of ^{3}H-Abamectin (726622 Dpm)

Figure 4.3. Fat body samples from S. littoralis larvae over 24 h treated with ^{3}H-Abamectin



n = 5
Insects treated with 1 µl (uncalibrated volume) of ^{3}H-Abamectin (726622 Dpm)

4.4. Discussion

In the present study, topical application of ^{3}H-AVMB$_{1}$ onto fifth and sixth instars of S. littoralis and H. armigera resulted in the uptake of radioactivity by all the tissues investigated including the ventral nerve cord and fat body.

It was found that paralysed S. littoralis of both instars and fifth but not sixth instar H. armigera had significantly greater levels of total radioactivity (and AVMB$_{1}$) compared with treated non-paralysed insects or unaffected insects, pointing to a relationship between the level of radioactivity and the stage of paralysis reached. The apparently different result for sixth instar H. armigera may have been due to the small number of replicates [n=4].

The data for fifth and sixth S. littoralis and fifth instar H. armigera show that radioactivity can accumulate in the nerve cord (a possible major site of action) during the normal time course of poisoning, although there was no significant difference in the proportion of total radioactivity as AVMB$_{1}$ between paralysed and non-paralysed insects except for H. armigera sixth instar larvae.

When the radiotracer experiment was repeated in conjunction with topical application of PB, there was a

significant increase in the proportion of radioactivity present as AVMB$_1$ (rather than total radioactivity) in the nerve cord of fifth but not sixth instar S. littoralis larvae. This supports the bioassay results discussed in Section 3, where topically-applied PB had no significant effect on the toxicity of AVMB$_1$ against sixth instar S. littoralis or fifth instar H. armigera but did increase the toxicity of AVMB$_1$ against fifth instar S. littoralis larvae. Taken together, these experiments suggest that selective oxidative mechanisms contribute towards the differential toxicity of AVMB$_1$.

Anderson et al. (1986) found that topically-applied PB potentiated the toxicity of AVMB$_1$ against third instar S. eridania larvae and these authors suggested that this potentiation was due primarily to improved cuticular penetration by AVMB$_1$ (see Section 3). However, while penetration may be an important factor determining the differential toxicity of AVMB$_1$ and PB may improve penetration of AVMB$_1$ under certain circumstances, the present work and the earlier toxicology experiments (Section 3) support the idea that differential metabolism of AVMB$_1$ is also important in noctuid moth larvae and that PB appears to potentiate AVMB$_1$ by inhibition of microsomal oxidase (mixed function oxidase) activity. Certainly, microsomal detoxification of AVM to their C-25, methyl alcohols and monosaccharides has been reported in vertebrates by Miwa et

al. (1983) and Chiu et al. (1984).

More recently, Scott (1989) has reported cross-resistance to AVMB1 in two pyrethroid resistant strains of the housefly (Musca domestica). Cross-resistance in a laboratory-selected strain was found to be polygenic, associated with genes on chromosomes 2 and 3 and could be partially suppressed by PB. Based on genetic and toxicological data, the mechanisms associated with pyrethroid-induced cross-resistance to AVMB1 appeared to be microsomal oxidase-mediated metabolism and decreased penetration (Scott, 1989). Abro et al. (1988) have reported that PB has a synergistic effect (two-fold) with AVMB1 on a field strain of P. xylostella and suggested that this may reflect an activated microsomal oxidase detoxification system. They also suggested that low level cross-resistance to AVMB1 was present in this field strain (Abro et al., 1988).

The involvement of microsomal oxidases may have important implications for resistance to other AVM in the future. Resistance to DHAVMB1 has been found in a species of sheep bot fly, on sheep treated regularly with this compound (J. Schaeffer and M. Turner, personal communication).

The apparent selectivity of microsomal oxidase activity may be related to age and development. For example,

Yu (1983) correlated changes in microsomal oxidase activity with age with the differential toxicity of various insecticides against larvae of the fall armyworm, S. frugiperda. While organophosphate resistance in a strain of S. littoralis was shown by Dittrich et al. (1980) to be due mainly to a very efficient microsomal oxidation system. Jødsudason et al. (1988) showed that the cytochrome P-450-dependent microsomal oxidase systems were present at higher levels in the abdominal microsomes of feeding-stage larvae than from prepupae of the Mexican Bean Beetle, Epilachna varivestis. Pimprikar and Georgiou (1979 and 1982) also showed resistance to diflubenzuron in housefly larvae was due mainly to efficient oxidation by microsomal oxidases.

From the present study, it would seem possible that microsomal oxidases were most active in the fourth and fifth instar larvae of S. littoralis but are reduced or turned off in the sixth instar just prior to pupation. This may account for the apparent lack of activity in the last instar. In fifth instar H. armigera larvae, microsomal oxidases appear to be less active than in fifth instar S. littoralis larvae (see Table 3.1.).

In mice, Lindberg and Negishi (1989) have recently shown that a single amino acid mutation is sufficient to reverse the substrate specificity of cytochrome P-450.

Subtle changes in the substrate specificity of cytochrome P-450 may thus exist between the two noctuid species and instars studied and these could help to account for the apparent differences in microsomal oxidases suggested by the current studies using PB.

There were no significant differences in the proportion of radioactivity present as AVMB$_1$ in fat body samples from paralysed and non-paralysed larvae. This, together with the fact that the total amount of radioactivity present as AVMB$_1$ was proportionally more than in the equivalent nerve cord samples, suggested that metabolism occurred to a lesser extent in the fat body than in the nerve cord.

The fat body in insects may act therefore as a storage organ for AVMB$_1$, as has been shown for other insecticides (Oppenoorth and Welling, 1976), leading to reduced bioavailability and reduced metabolism by microsomal oxidases. Nakatsugawa and Morelli (1976) and Wilkinson and Brattsten (1972) have stated that microsomal oxidases are localized mainly in those tissues that represent the major "portals" of entry of foreign compounds into the insect, for example, the gut lumen. Differences in tissue sensitivity and in the activity of tissues in hydrolysing trans-permethrin were found by Dowd and Sparks (1987), and hydrolysis of different pyrethroids by the same tissues has been reported to vary within a given insect species

(Abdel-Aal and Soderlund, 1980). These studies of differential tissue sensitivity and the activity of microsomal oxidases suggest that there may be little or no metabolism of AVMB$_1$ in fat body compared with the nerve cord.

The radioactivity recovered from the gut membrane and gut lumen was significantly greater in paralysed compared with non-paralysed larvae in all but sixth instar H. armigera, while more AVMB$_1$ was recovered from the gut of fifth than sixth instar larvae of S. littoralis (Table 4.10.). In both sets of comparisons this could indicate excretion is a factor in the differential toxicity of AVMB$_1$. However, very little radioactivity was found in the faeces (less than 5% of the total radioactivity applied, data not shown) and this was considered negligible during the course of these experiments. Over a longer period, excretion may be more important, but a majority of the treated insects were already showing symptoms of poisoning by the end of the experiments. Once in the gut lumen, AVMB$_1$ may not be as effective even if it is not rapidly removed from the insect.

Bull (1986) stated that ^{3}H-AVMB$_1$ was readily absorbed from the midgut, metabolised and excreted together with its metabolites from three species of noctuid moth larvae. However, the magnitude of the observed differences in the post-treatment fate of AVMB$_1$ was not thought sufficient to

explain the substantial differences in susceptibility to the compound among the test species (Bull, 1986). This is in agreement with the results found in the present study, although Bull (1986) fed his insects on the AVMB1 whereas in the present study AVMB1 was applied topically.

The wash-off experiments showed that despite the use of a number of different solvent mixtures, a maximum of 33% of the applied dose was recovered. This might have been expected, as the extreme hydrophobicity of AVM has been noted by Pong and Wang (1980), although over the course of the present experiments the amount washed off both species did decrease and the amounts recovered from both fat body and haemolymph samples did increase (see Figures 4.1., 4.2. and 4.3.). That there were no significant differences in the amounts washed off between the two instars of S. littoralis tested was unexpected, since as described in Section 3 (see Table 3.3.), injection had a much greater effect on fifth than sixth instar S. littoralis, suggesting penetration was an important factor. Sixth instar larvae did have significantly more radioactivity in the haemolymph at 3 and 6 h after application, although by the end of the experiment (24 h) these differences were not significant. With the fat body samples, there was only a significant difference 3 h after application.

The above wash-off experiment results suggest that

penetration and metabolism alone cannot account for the observed differences and differences in the sensitivity of the ventral nerve cord to AVMB$_1$ between the two instars may also be involved. However, the proportion of insects showing symptoms of paralysis was small up to 24 h (Table 4.3.). It was between 24 and 48 h after application that the symptoms of poisoning were observed in a large proportion of the treated insects and differences in the fat body samples were most pronounced after 48 h (Table 4.9.).

Due to the complexity of the AVM molecule, very few of the many transformation products observed in studies with rat and steer liver microsomal preparations (Miwa et al., 1982), or with noctuid moth larvae (Bull et al., 1984), have so far been identified (Bull, 1986). In the present work, there appeared to be at least seven different metabolites in the chloroform phase following separation by TLC and there were presumably other, more polar metabolites in the aqueous samples. Like Bull's (1986) data, the data in the present study was simplified to give the proportion of the total radioactivity present as the parent compound (AVMB$_1$) and as total metabolites (both polar and non polar).

5. ELECTROPHYSIOLOGY

5.1. Introduction

The aim of these *in vivo* experiments was to see if there were differences in the responses of and sensitivity of the CNS to topically-applied AVMB$_1$. The use of a computer to log and analyse data made it possible to summarise the results of long experiments and facilitate the analysis of trends in the data.

5.2. Materials and Methods

The recording and data analysis system used was based on one used at Portsmouth Polytechnic (M. Broderick, R. Crichton, L. Leake and M. Ford, personal communication). It consisted of a computer-based datalogging system developed for use on an Acorn BBC series microcomputer (Crichton *et al.*, 1988) and an electrophysiological system consisting of preamplifier and filter, a dual beam storage oscilloscope and stereo cassette deck. The system used a Spike Capture Programme, copyright R. Crichton, 1988 and RC Datalogger made by R. Crichton.

5.2.1. The recording system

The insects (fifth and sixth instar larvae of

S. littoralis and H. armigera) were anaesthetized with carbon dioxide, then attached with Loctite Superglue 3 to a block of Sylgard silicone rubber (B.D.H. Ltd.) 25mm long X 6mm wide X 4mm deep.

The two electrodes used were both stainless-steel headless entomology pins (A1 size, Watkins and Doncaster Ltd.) which were dipped in 1M orthophosphoric acid for one min., washed in distilled water and dried, then soldered onto the screened leads used. The electrodes with miniature coaxial leads were each held by crocodile clips on retort stands in a Faraday cage made of angle-iron and copper gauze on an isolated slate base. The electrical equipment was held on a rack adjacent to the Faraday cage. The signals were passed from the electrodes to a CFP preamplifier 8121 with a low frequency cut at 200Hz and a high frequency cut at 1KHz and a gain setting (AC) of X1000. The preamplifier was connected to an AC single-ended (SE) filter (A103 design by Isleworth Electronics Ltd.) set at low frequency cut at 200Hz and high frequency cut at 1KHz with a fixed gain of 3.5. The output lead was terminated in a BNC plug which interfaced with a Tektronix 2220 60MHz digital storage oscilloscope (via a T-piece) to allow observation of the signal. The signal was then passed to a Technics RS-T80R stereo cassette deck and recorded onto Sony HF-S90 type 1 (Normal) position crystal gamma cassette tapes. The input level controls on the cassette deck were fixed at their

maxima and the noise-reduction switches were turned off. The equipment is shown in a block diagram (Appendix 5.4.).

All the electrical equipment was run off a power supply from an isolating transformer to reduce extraneous electrical signals.

5.2.2. The experimental set-up.

In the first experiment, fifth and sixth instar larvae of both species were pretreated with ^{3}H-AVMB$_1$ (S. littoralis fifth and sixth instars, 4.5 µg a.i. per insect; H. armigera fifth instar, 1.5 µg a.i. per insect; H. armigera sixth instar, 3.0 µg a.i. per insect). At 24 and 48 h after treatment, paralysed and non-paralysed insects were selected. For H. armigera, insects were used after 24 h only and separated by symptoms displayed as above.

The two electrodes were placed centrally, along the longitudinal axis (2mm apart) midway between the second and third posterior prolegs (through the underside of the Sylgard silicone rubber block). In the first experiment, for each species and instar, recordings were made from the following:

10 untreated insects

10 treated- non-paralysed insects

10 treated- paralysed insects

Each insect was left for 30 min. after being prepared then recordings were made for 5 min. After each recording the insects were dissected to check the electrodes were both positioned on or within 0.5mm of the CNS. If either of the electrodes were not correctly positioned then the recording was discarded.

For ^{3}H-AVMB$_{1}$-treated insects, the nerve cords were then removed and pairs of nerve cords placed in preweighed scintillation vials and the level of radioactivity determined as in Section 4.1. Where possible, nerve cords from insects which gave similar action potential frequencies were paired together.

The proportion of radioactivity present as AVMB$_{1}$ was estimated for S. littoralis 24 h samples as in Section 4.1. The data in Section 4.2. for S. littoralis and H. armigera was also used to calculate the amount of AVMB$_{1}$ present in the nerve cords, (see Table 5.1.).

In the second experiment, insects (S. littoralis fifth and sixth instar larvae) were set up as above and recordings taken from each insect after 30 min. The 1 µl (marked, uncalibrated volume) of 4500ppm AVMB$_{1}$ (E.C. formulation) was then applied (Section 2.4.) to each insect in situ. and recordings (5 min.) made after 1, 2, 4, 8, 12 and 24 h.

Three insects with implanted electrodes were tested concurrently with recordings staggered for each recording time. The insect preparation itself was not touched after the start of the recording. The control insects were prepared as above but no $AVMB_1$ was applied.

5.2.3. Data analysis

The data from the cassette tapes was played into a RC datalogger linked to the microcomputer running a Spike Capture programme. This programme considered time as a series of discrete intervals (bins); in these experiments, the bins were set at 0.5 sec. duration. The number of action potentials (APs) which exceeded a user defined threshold voltage (this was set sufficiently above the background noise level so that the background noise and baseline did not cross the threshold) in each bin were counted and the amplitudes, rise and fall times of APs were summed within each bin. The variation in background noise was not considered to be an important factor in determining AP frequency. The total equipment gain was calculated by multiplying the gains of the individual pieces of equipment:

preamplifier = 933
filter = 3.5
cassette deck= 5.64

This gave a total gain of 18400.

From the above data, the programme calculated the average amplitudes for APs in each bin. The rise and fall times were not used as these are more relevant for intracellular than extracellular recordings. The data was recorded onto 5.25 inch floppy disks. This data could then be tabulated, plotted or made into data files for statistical analysis. From each recording, the mean A.P. frequency from 120 x 0.5sec. bins was calculated and expressed as A.P.s sec^{-1} (Hz). The amplitude data from the second experiment was expressed as relative values, the mean values at each measuring time expressed as a proportion of the original T = 0 h value, (since there were initial uncertainties over the actual values obtained due to problems with calibration of the datalogger and with the stage gain).

5.3. Results

The action potential (AP) or spike frequencies from $AVMB_1$-treated insects which were not showing signs of paralysis were significantly ($P<0.05$) less compared with untreated insects for both instars of *H. armigera* 24 h and both instars of *S. littoralis* 24 and 48 h after treatment (Table 5.1.). There were also significantly less ($P<0.05$) APs recorded from $AVMB_1$-treated insects which were showing signs of paralysis compared with treated insects which were not showing signs of paralysis for both instars of *H. armigera* 24 h and both instars of *S. littoralis* 24 and 48 h after treatment (Table 5.1.).

The levels of radioactivity recovered (as $AVMB_1$) in the ventral nerve cords from which recordings were made are given in Table 5.1., using data from Table 5.2. and Appendix 5.1. In each case, significantly ($P<0.05$) more $AVMB_1$ was recovered from insects showing symptoms of paralysis compared with insects not showing symptoms of paralysis. The values for the proportion of radioactivity present as $AVMB_1$ obtained (Table 5.2.), agreed well with the values obtained in an earlier experiment (Table 4.7.).

There were significant ($P<0.001$) correlations between the levels of radioactivity (as log Dpm) recovered from

Table 5.1.

Action potential (AP) frequency (Hz) from untreated and $AVMB_1$-treated S. littoralis and H. armigera larvae and the concentration of $AVMB_1$ recovered from ventral nerve cords of treated larvae after topical application of ^3H-$AVMB_1$ (n=10).

			$\bar{X}\pm$S.E. Hz and (pM $AVMB_1$/mg nerve cord)[2],[3]		
Species/ Instar	Time[1] (h)		Untreated	Treated-[1] non-paralysed	Treated-[1] paralysed
Heliothis	V	24	35.5±4.4	12.3±2.6 (1.03)	2.7±0.4 (4.76)
	VI	24	49.8±4.3	29.0±2.2 (0.73)	11.1±1.6 (2.24)
Spodoptera	V	24	32.0±2.5	11.8±2.2 (0.34)	2.2±0.7 (0.86)
	V	48	32.0±2.5	7.2±0.8 (0.34)	1.7±0.5 (0.92)
	VI	24	39.5±3.3	14.8±1.8 (0.32)	4.0±0.8 (0.62)
	VI	48	39.5±3.3	15.5±1.0 (0.36)	8.8±1.0 (0.66)

1 Insects treated with 4.5µg (S. littoralis V and VI instars), 3.0µg (H. armigera VI) and 1.5µg ^3H-$AVMB_1$ (H. armigera V). Time = h after application of ^3H-$AVMB_1$.

2 Mean pM $AVMB_1$/mg nerve cord calculated from Appendix 5.1.

3 In each case, the differences in A.P. frequencies between untreated and treated-non-paralysed and between treated-non-paralysed and treated-paralysed insects were significant ($P<0.05$) (t-test).

Table 5.2.

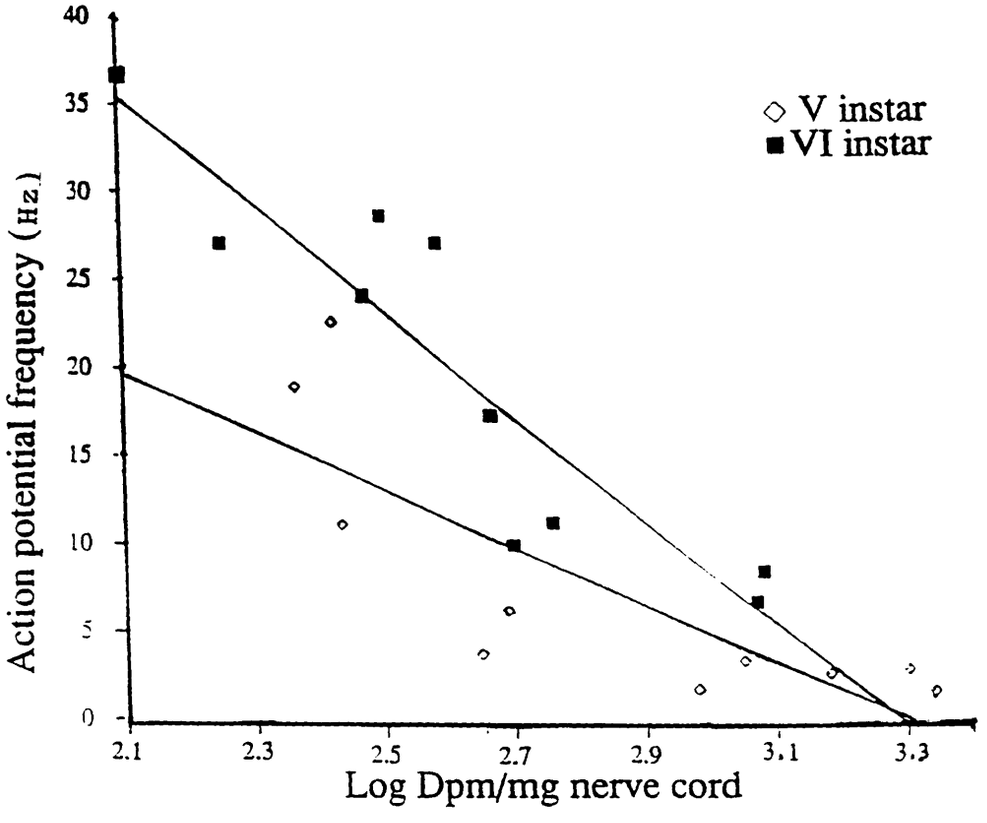
Proportion of radioactivity as AVMB1 in chloroform extracts and as AVMB1 in total sample extracted from paralysed and non-paralysed nerve cord samples from S. littoralis and H. armigera larvae (See Table 5.1.) n=4.

Species/symptoms[1] instar/h		%AVMB1 in chloroform extract±S.E.[2]	%AVMB1 in total sample±S.E.[2]
Heliothis			
V 24	NP	82.1±0.2	74.4±1.4
	P	81.6±0.7	72.9±1.4
VI 24	NP	83.2±1.2	65.4±0.8
	P	82.8±0.3	69.4±0.5
Spodoptera			
V 24	NP	85.0±0.8	56.2±0.8
	P	84.3±0.6	55.1±0.9
V 48	NP	74.4±1.8	51.7±0.6
	P	71.6±2.1	49.7±0.6
VI 24	NP	83.9±1.1	75.2±0.9
	P	81.7±0.8	74.6±0.8
VI 48	NP	83.4±0.2	74.1±1.1
	P	80.7±0.6	70.1±1.8

1 NP = non-paralysed, P = paralysed.

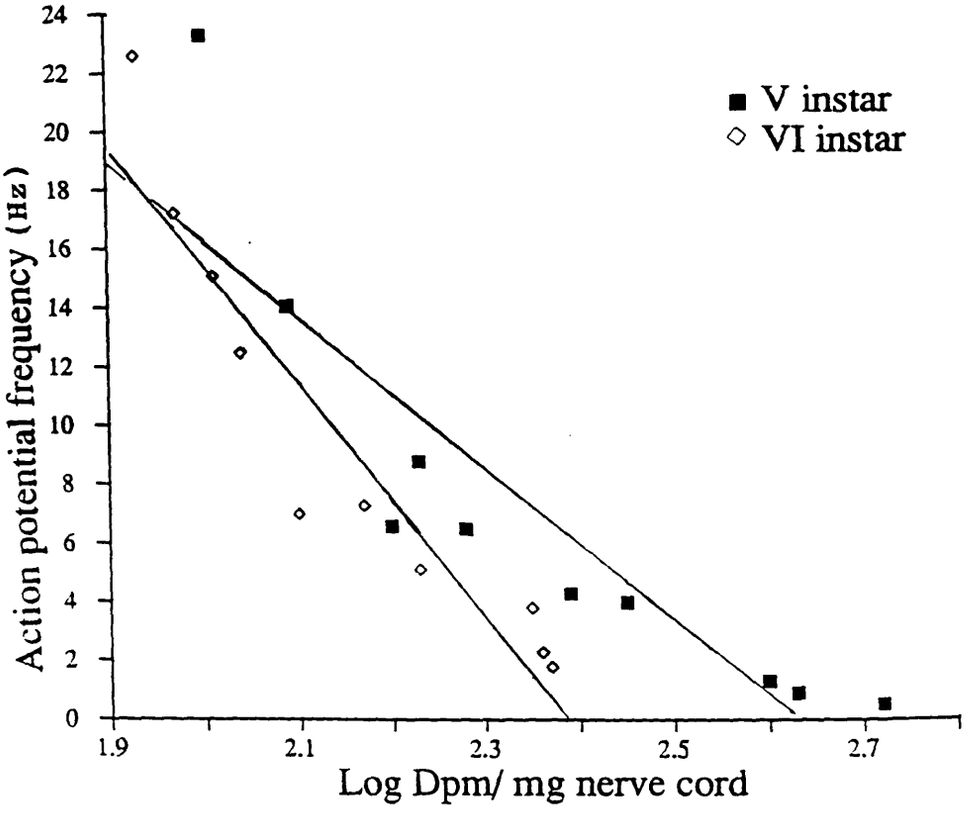
2 S.E. calculated following arcsine transformation. Data used in Table 5.1. (includes some data from Table 4.4. and Appendix 4.5.).

Figure 5.1. Radioactivity in ventral nerve cord and action potential frequency in H. armigera larvae 24 h after application of ^{3}H-abamectin



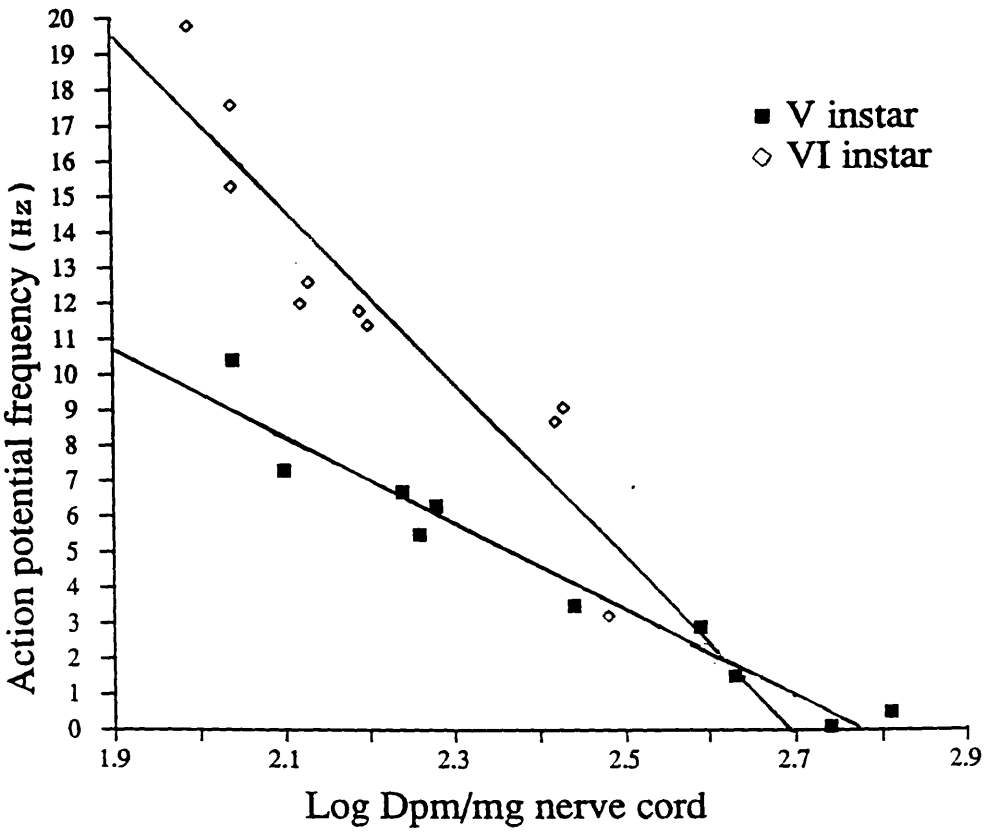
V instar correlation coefficient (r) = -0.814
Equation of line: Y = -16.395X + 54.342
VI instar correlation coefficient (r) = -0.908
Equation of line: Y = -29.886X + 98.341

Figure 5.2. Radioactivity in ventral nerve cord and action potential frequency in S. littoralis larvae 24 h after application of ^{3}H-abamectin



V instar correlation coefficient (r) = -0.887
Equation of line: Y = -26.213X + 68.894
VI instar correlation coefficient (r) = -0.940
Equation of line: Y = -39.330X + 94.148

Figure 5.3. Radioactivity in ventral nerve cord and action potential frequency in S. littoralis larvae 48 h after application of ^{3}H-abamectin



V instar correlation coefficient (r) = -0.973
Equation of line: Y = -12.025X + 33.487
VI instar correlation coefficient (r) = -0.920
Equation of line: Y = -24.333X + 65.779

Table 5.3.

Summary of the correlation coefficients obtained between radioactivity extracted from ventral nerve cords and the action potential frequencies from corresponding larvae following topical application of ^{3}H-abamectin and their level of significance.

Sample	Correlation Coefficient(r)	Number of pairs of data points[1]	Level of significance (P)
Heliothis 24h			
V	-0.814	10	P<0.001
VI	-0.908	10	P<0.001
Spodoptera 24h			
V	-0.887	10	P<0.001
VI	-0.940	10	P<0.001
Spodoptera 48h			
V	-0.973	10	P<0.001
VI	-0.920	10	P<0.001

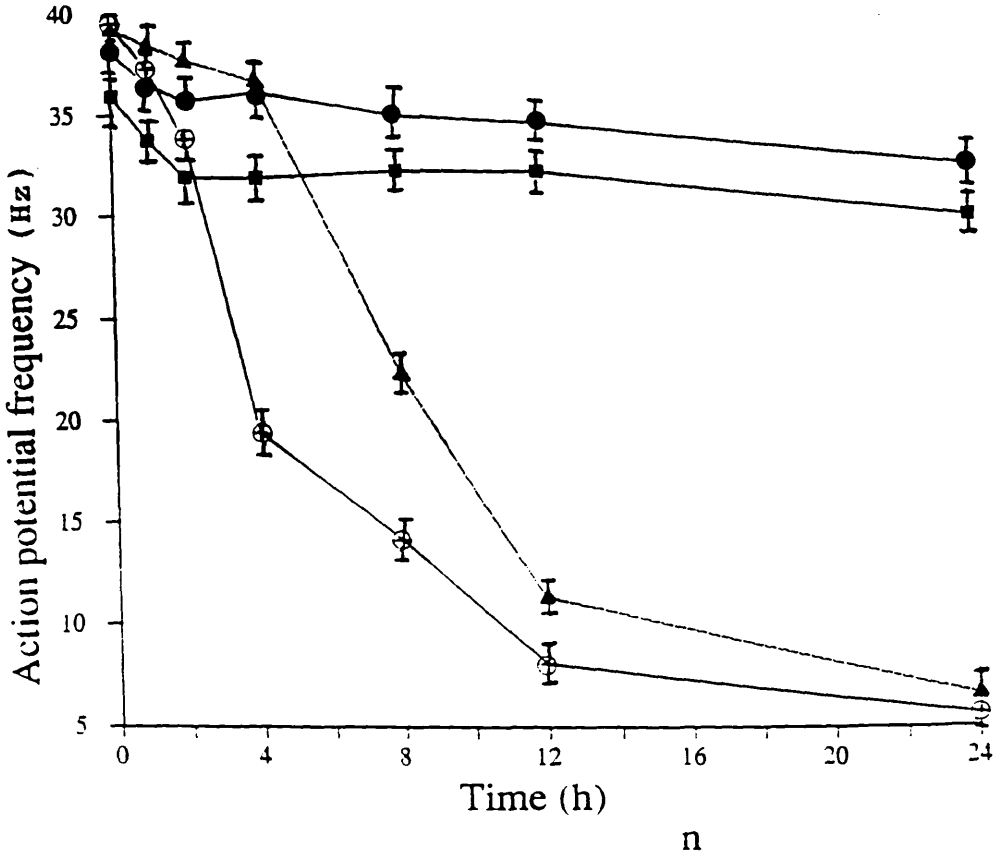
[1] Each X and Y value used was the mean of 2 samples. This data is given in Figures 5.1., 5.2. and 5.3.

the nerve cord and the A.P. frequency for fifth and sixth instar H. armigera larvae 24 h after treatment with ^{3}H-AVMB$_{1}$ (Figure 5.1.) and for fifth and sixth instar S. littoralis larvae both 24 and 48 h after treatment with ^{3}H-AVMB$_{1}$ (Figures 5.2. and 5.3. respectively). The correlation coefficients ranged from r= -0.814 for fifth instar H. armigera larvae to r= -0.973 for fifth instar S. littoralis larvae (48 h), (see Table 5.3.).

The action potential frequencies (Hz) over a 24 h period for AVMB$_{1}$-treated and untreated fifth and sixth instar S. littoralis larvae are shown in Figure 5.4. Comparing the AP frequency at a given recording time with that obtained at time T= 0 h, there were no significant differences ($P>0.05$; Mann-Whitney Test) for both fifth and sixth instar control larvae except at 24 h, when $P<0.05$. With AVMB$_{1}$-treated fifth instar S. littoralis larvae there was no significant difference ($P>0.05$) until T= 8 h, when ($P<0.05$). With AVMB$_{1}$-treated sixth instar S. littoralis larvae there was no significant difference ($P>0.05$) until T= 2 h, when $P<0.05$, (data given in Appendix 5.3.).

The amplitudes of the APs also to decreased with time in AVMB$_{1}$-treated fifth and sixth instar S. littoralis larvae (see Plates 5.1. and 5.2.). In the treated insects (top traces), the amplitudes were greatly reduced when compared with the control traces (bottom traces). The same trend was

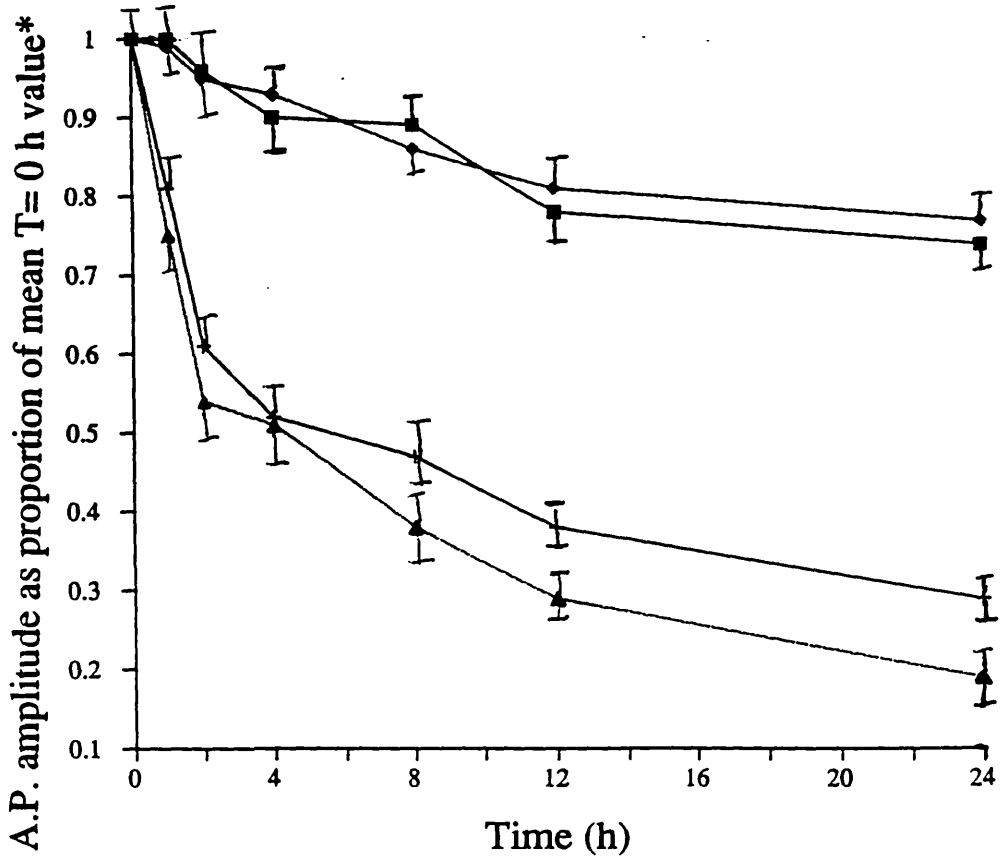
Figure 5.4. Action potential frequency ($\bar{X}$ Hz ± S.E.) from abamectin-treated (1µl uncalibrated volume of 4500 µg/ml) and untreated fifth and sixth instar S. littoralis larvae over 24 h.



	n
● V instar control	10
▲ V instar abamectin-treated	11
■ VI instar control	10
⊕ VI instar abamectin-treated	12

Data given in Appendix 5.3.

Figure 5.5. Action potential (A.P.) amplitudes as a proportion of the mean original (T= 0 h) values against time.



* At T= 0 h, mean original values = 1.00 for each set of data.
n = 10, except for S. littoralis VI instar where n = 8

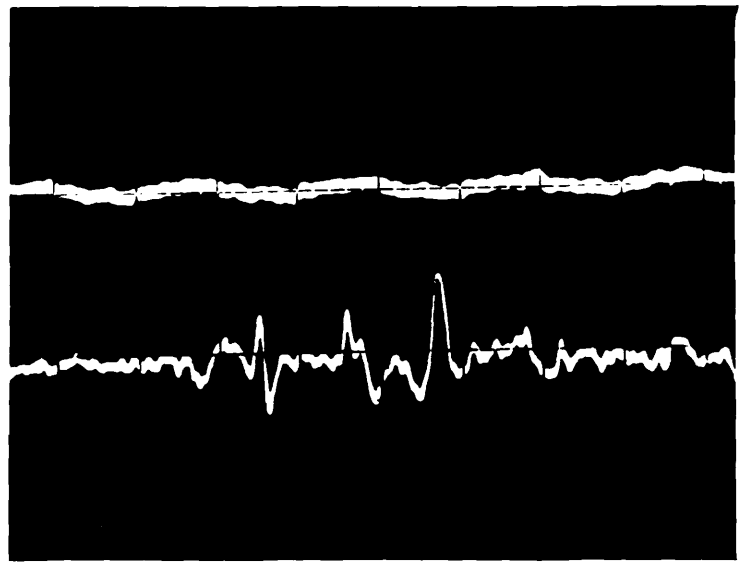
■ V instar control
+ V instar $AVMB_1$-treated
◆ VI instar control
▲ VI instar $AVMB_1$-treated

shown when the amplitudes were expressed as a proportion of the original (T= 0 h) values and plotted against time (Figure 5.5.). Although the control insect amplitudes were significantly ($P<0.05$) reduced over 24h, the $AVMB_1$-treated insect amplitudes were also significantly ($P<0.05$) reduced, but significantly ($P<0.05$) more so than in the control insects at all recording times. The reduction in amplitudes was significantly ($P<0.05$) greater in sixth than in fifth instar $AVMB_1$-treated larvae at T= 12 and 24 h.

Plate 5.1. Fifth instar S. littoralis larvae A.P.s from control (lower trace) and AVMB1-treated (upper trace 24 h after application of $AVMB_1$ to larvae.



Plate 5.2. Sixth instar S. littoralis larvae A.P.s from control (lower trace) and AVMB1-treated (upper trace 24 h after application of $AVMB_1$ to larvae.



Scale Vertical- bar= 1uV. Horizontal- bar= 1ms

5.4. Discussion

Comparatively little work has still been published on the effects of AVM on the insect compared with the vertebrate nervous system (Section 1.3.). Electrophysiological experiments which link AVM with Cl^- channel conductance have mostly used AVM-sensitive organisms such as crustaceans and insects, but as mentioned in Section 1.3., most of the ligand binding studies have used mammalian brain membranes and cannot necessarily be related to the mode of action against invertebrate target species due to pharmacological differences between phyla (Wright, 1986; Wann, 1987).

In the present, *in vivo* experiments on fifth and sixth instar larvae of *S. littoralis* and *H. armigera* treated topically with ^{3}H-AVMB$_1$, the level of spontaneous activity within the ventral nerve cord decreased significantly in each instar and species compared with control insects. Treated-paralysed larvae of each species and instar tested also had significantly lower spontaneous activity than treated larvae not showing signs of paralysis (Table 5.1.).

As in Section 4.3., treated-paralysed larvae were found to possess significantly greater amounts of total radioactivity (and ^{3}H-AVMB$_1$) in their ventral nerve cords compared with treated non-paralysed larvae. This suggests

that there is an inverse relationship between the level of total radioactivity within the nerve cord and spontaneous neural activity (Figures 5.1., 5.2., 5.3.). This work agrees with the findings of Corbett (1987) who found an inverse relationship between the level of ^{3}H-AVMB$_{1}$ (plus any toxic metabolites) within the ventral nerve cord and the level of spontaneous neural activity in the American cockroach, P. americana.

The amount of ^{3}H-AVMB$_{1}$ used in the present experiments was sufficient to produce symptoms of poisoning in 40-60% of the treated insects 24-48 h after topical application. It was noticeable that fifth instar H. armigera larvae treated topically with 1.5 µg AVMB$_{1}$ per insect had 3 and 5.5-fold as much AVMB$_{1}$ in the ventral nerve cords of treated-non-paralysed and treated-paralysed insects respectively compared with equivalent fifth instar S. littoralis larvae treated with 4.5 µg AVMB$_{1}$ per insect. However, these doses all had similar effects on the larvae, as can be seen from the decrease in action potential frequencies (Table 5.1.). These observations would initially seem to indicate that the H. armigera CNS is less sensitive to AVMB$_{1}$, compared with that of S. littoralis but while this is compatible with the initial symptoms of poisoning observed, it does not agree with the final mortality data (Table 3.1.).

Matsumura (1985) proposed that although there were no detailed studies on the relative importance of penetration through the insect cuticle versus the nerve surroundings, some predictions may be made based upon the known pharmacological behaviour of insecticides. For example, Matsumura and Hayashi (1969) showed that the cuticle and intersegmental membranes were not the only possible barriers to insecticides reaching the target site in resistant and susceptible German cockroaches (Blattella germanica). In a comparison of the amount of dieldrin taken up by the nerve cord of over an identical treatment period they showed that susceptible insects accumulated more dieldrin than their resistant counterparts. This difference existed even at the same stage of poisoning as measured by the LT_{50} time but no inter-strain differences were found in the rate of cuticular penetration of the pesticide.

There are a number of factors (eg. equilibrium through partition) working in the insect to reduce the body burden in a relatively long time span so that the initial surge in the insecticide level in the CNS following acute exposure could be partially avoided (Matsumura, 1985). The rate of accumulation of toxicants in the nervous system could be reduced in a number of ways, including reduced penetration through the nerve sheath, reduced binding properties at the target site and changes in partitioning behaviour of the

toxicant because of an increase in the affinity of other tissues, such as fat body and haemolymph (Matsumura, 1985). Thus, it is possible that differences in the nerve sheath and other structures around the ventral nerve cord could affect the amount of ^{3}H-AVMB$_{1}$ entering the nerve cord and this may help to explain the differences in the amounts of AVMB$_{1}$ recovered from the nerve cords of H. armigera and S. littoralis.

The fact that the onset of poisoning in both species was first noticeable as paralysis of the hind legs, the production of strings of faecal pellets (suggesting a problem with posterior musculature), and a general lethargy (Section 4.2.1.) could imply an action on the musculature, either directly or via the neuromuscular junction. Putter et al. (1981) and Botham and Nicholson (1985) have also reported that AVMB$_{1}$ has a paralysing effect on insect appendages.

Tanaka and Matsumura (1985) found AVMB$_{1}$ caused the coxal leg muscles of P. americana to fail to respond to stimuli within 30 min. of application, but did not affect the magnitude of contractions and they suggested that AVMB$_{1}$ blocked the transmission of stimuli passing from the CNS to the muscle by opening Cl^{-} channels in the postsynaptic muscle membrane, leading to hyperpolarisation of the postsynaptic region. Fritz et al. (1979), Kass et al. (1980)

and Mellin et al. (1983) have also suggested that the action of AVMB1 was on postsynaptic Cl^- channels (See Section 1.3.1.1.).

In H. zea, Agee (1985) found that moths treated with AVMB1 and showing symptoms of poisoning such as uncoordinated leg and/or wing movement and blindness still generated A.P.s in the CNS. A.P.s (motor spikes) were also detected in the ganglia of torpid moths. These findings and the present results (comparing symptoms with A.P. frequency) are compatible with the conclusion that AVMB1 is functioning as an impulse blocking agent at the neuromuscular junction Fritz et al.(1979) although a direct action at the axon or synapse cannot be discounted.

When the A.P. frequency was examined over 24 h (Figure 5.4.) there was no significant difference between the control and AVMB1-treated insects (S. littoralis) until 8 h (fifth instar) or 2 h (sixth instar). Thus, AVMB1 appeared to reduce the A.P. frequency significantly faster in sixth instar than in the fifth instar (see Table 3.1.) of S. littoralis. However, since signs of poisoning were only readily noticeable in both instars after 12-24 h in these initial stages of poisoning it was not possible to relate A.P. frequency with symptoms.

Another effect of AVMB1 was a reduction in the

amplitudes of the A.P.s. This can be seen in Table 5.5. and Plates 5.1. and 5.2. Although the mean control amplitudes for fifth and sixth instar larvae of S. littoralis also decreased, this was at a much slower rate (74 and 77% of their original values respectively after 24 h) compared with $AVMB_1$-treated larvae, where mean amplitudes declined to 29 and 19% of their original (T = 0h) values for fifth and sixth instar larvae respectively. The reduction in amplitude was significantly greater in sixth than in fifth instar S. littoralis larvae after 12 and 24 h. However, interpretation of the amplitude data can be difficult and relative values for amplitudes were used (the mean values at each measuring time being expressed as a proportion of the original T = 0h value).

Amplitudes may be affected by extraneous factors, including positioning of the electrodes in relation to the ventral nerve cord (the further away from the nerve cord the electrodes are placed, the smaller the amplitudes obtained [data not given]) and movement of the insect while recording. It is also possible that $AVMB_1$ in opening Cl^- channels may alter the ion balance across nerve membranes, thus affecting the amplitudes of the A.P.s. However, the data obtained in the present study cannot substantiate this.

Other authors have noted reductions in amplitudes of A.P.s but again have expressed caution in analysing the

reasons behind this effect. Thus, Pickens et al. (1988) found that feeding $AVMB_1$ to S. frugiperda resulted in smaller electroretinograms at all wavelengths of light. While Fritz et al. (1979), working on the lobster neuromuscular junction, found $AVMB_{1a}$ irreversibly "eliminated" both inhibitory post synaptic (IPSP) and excitatory post synaptic (EPSP) potentials; the amplitudes of evoked but not resting potentials being greatly reduced. The latter authors suggested $AVMB_{1a}$ acts to increase membrane permeability to those ions whose equilibrium potentials are close to the resting potential, such as Cl^- and K^+.

As mentioned earlier, while it is possible to correlate the amounts of $AVMB_1$ in the ventral nerve cord with symptoms of poisoning, it is more difficult to relate this to the mortality data. Corbett (1987) found that less radioactivity (and $AVMB_1$) was found in the nerve cords of paralysed P. americana after 96 h compared with 24 h and suggested $AVMB_1$ caused a toxic lesion which remained when the level of $AVMB_1$ declined.

The same problem also applies to correlating electrophysiological observations with toxicity data. For while A.P. frequencies and amplitudes were significantly reduced in $AVMB_1$-treated insects within a few h, symptoms of poisoning were as discussed earlier only readily noticeable

after 12-24 h with mortality occurring 2-4 days after treatment. Thus, although A.P.s declined to a very low level within 24 h in all cases, they were not totally extinguished for several days (data obtained at 24 and 48 h only). Broderick and Leake (1989) have suggested death to be when all activity in the insect nervous system has ceased, which seems sensible. However, in a bioassay this is not practical and if the insect does not respond to mechanical stimulation (as used in Section 3,) it can in practice be considered dead since once paralysed insects do not recover from AVM treatments (Wright, 1986).

In conclusion, the in vivo system used in the present study was suitable for long-term studies on which simultaneous observations can be made of both toxicological and neurophysical effects (Broderick and Leake, 1989). The main disadvantage is that there is no way of identifying exactly what is being recorded. The signals obtained are a mixture of single and compound, ascending and descending A.P.s which could be central and/or peripheral in origin. Signals from individual neurones cannot at present be identified. Intracellular recordings might overcome this problem and enable the order in which signals are affected to be determined.

6. GENERAL DISCUSSION AND CONCLUSIONS

The present work has confirmed an earlier report (Corbett, 1987 and Corbett et al., 1989) that abamectin ($AVMB_1$) shows marked differential toxicity between larval instars of the noctuid moth, S. littoralis. The greatly increased toxicity of $AVMB_1$ to the sixth instar larvae compared with earlier instars is unusual as the most common trend observed with insecticides is one of decreasing relative toxicity with increasing larval weight and instar (Aikins and Wright, 1985). Such changes did not occur when S. littoralis was treated with the chemically unrelated insecticides, malathion and lambda-cyhalothrin nor with $AVMB_1$ against fifth and sixth instar H. armigera. This suggests that a relatively selective mechanism is involved in determining the toxicity of $AVMB_1$ against the former species and evidence presented in the first part of this work suggests a metabolic role in differential toxicity, possibly involving both oxidative (MFO) and hydrolytic enzymes.

Recently, Scott (1989) has suggested resistance to $AVMB_1$ in a laboratory-selected M. domestica was due to MFO-mediated metabolism and to decreased penetration. MFOs have been shown to be active against a number of different insecticide groups in many insect species (eg. Jedsudasun et al., 1988; Pimprikar and Georgiou, 1979, 1982). It is

possible that these enzymes are active in fourth and fifth instar larvae of S. littoralis but are reduced or turned off in the sixth instar, and are also less active in fifth instar H. armigera. Alternatively, MFO's with different substrate specificities may be expressed in the sixth instar. Differences in tissue sensitivity and activity of micosomal oxidases has been shown within a given insect species (Wilkinson and Brattsten, 1972; Abdel-Aal and Soderlund, 1980; Dowd and Sparks, 1987).

The involvement of a penetration factor in the differential toxicity of AVMB1 against S. littoralis larvae was implied by a comparison of injection and topical bioassays. However, while reduced penetration of AVMB1 in fifth compared with sixth instar larvae may be important, nothing is known of structural differences in the cuticle or intersegmental membranes between these two instars. The mechanisms by which the cuticle inhibits the penetration of insecticides are not fully understood, although this has been related to increased lipid and protein content (Weismann, 1957; Vinson and Law, 1971), altered lipid composition (Patil and Guthrie, 1979a, b) and increased cuticular thickness (Weismann, 1947). Differences in tolerance to an insecticide have also been related to changes in the cuticle (Busvine, 1971).

The wash-off experiments clearly illustrated the

lipophilicity of the AVM molecule, which appeared to rapidly partition into the cuticular wax layers. These experiments also showed, rather surprisingly, that although the radioactivity washed-off decreased and radioactivity in the haemolymph and fat body increased with time in both fifth and sixth instar S. littoralis, after 24 h the differences between the two instars were not significant.

The injection bioassays and wash-off experiments both had disadvantages. With the injection method (Section 3.2.4.) it was difficult to ensure the insecticide was injected in the same place and to the same depth each time. There was also some loss of haemolymph (and AVMB1 ?) in some injected larvae. In the wash-off experiments, while the solvent mixture washed off some radioactivity and more was recovered from the tissue samples, an unknown quantity was left unextracted from the cuticular layers. Gerolt (1972) emphasised that rinsing houseflies, M. domestica with methanol only removed part of an oximecarbamate (that had been topically-applied to the houseflies) present in the body wall. The addition of an internal standard to the wash-off samples after the first count to compensate for substantial quenching by the solvents used also probably led to some loss of accuracy in the present study.

AVMB1 may be stored preferentially in the fat body of S.littoralis and H. armigera larvae, since the % of total

radioactivity as AVMB1 was greater in fat body samples than in equivalent nerve cord samples, although this could reflect differences in metabolism as well as in partition coefficients. Storage within the fat body has been demonstrated for a wide range of insecticides (Metcalf, 1955; Oppenoorth and Welling, 1976). Chapman (1969) showed that later instars of lepidopteran larvae possessed more adipose tissue than in earlier instars. Thus, the relative insensitivity of the fourth and fifth instars of S. littoralis to AVMB1 compared with the third instar could be partly due to increased storage of AVMB1 in the former. However, this cannot explain why the sixth instar of S. littoralis was more sensitive to AVMB1 than the earlier instars.

Paralysed fifth and sixth instar larvae of S. littoralis and of fifth instar H. armigera had significantly more radioactivity in the gut membrane and gut lumen than non-paralysed larvae which could indicate that excretion is an important factor. Although excretion of lipophilic pesticides in their unchanged form is not generally found in insects (Perry and Agosin, 1974). In fact, very little radioactivity was found in the faeces during the course of these experiments. Like Bull (1986), the magnitude of observed differences in the post-treatment fate of AVMB1 was not thought sufficient to explain the differences in susceptibility to AVMB1 among the test

species. Perry and Agosin (1974) state that α-chlordane penetrates the housefly cuticle readily and accumulates unchanged in tissues to fairly high levels within 24 h of topical application.

The radiotracer experiments showed that radioactivity (and AVMB$_1$) can accumulate in the nerve cord (a potentially major site of action) during the normal time course of poisoning. Paralysed fifth and sixth instar S. littoralis and fifth instar H. armigera larvae also had significantly greater levels of total radioactivity and AVMB$_1$ compared with treated non-paralysed insects. This suggests a relationship between the level of radioactivity within the CNS and the stage of paralysis reached.

In addition, neurophysiological studies on the action of AVMB$_1$ on spontaneous neural activities within the ventral nerve cord of S. littoralis and H. armigera showed that activity was related to the degree of paralysis exhibited by the insect and to the level of radioactivity taken up by the ventral nerve cord. This agrees with the findings of Corbett (1987), who found that in in vivo preparations of P. americana injected with AVMB$_1$ the level of spontaneous activity within the CNS was progressively reduced in partially and fully paralysed cockroaches.

Considering the doses of ^{3}H-AVMB$_1$ used and their

effects on the neural activity of S. littoralis and H. armigera, it would seem that the H. armigera CNS is less sensitive to AVMB1 when compared with S. littoralis. However, while this does agree with the initial symptoms of poisoning observed, it does not relate as well to the final mortality data. For while it is possible to correlate the amounts of AVMB1 in the ventral nerve cord and A.P. frequency with symptoms of poisoning, it is more difficult to relate the amounts of AVMB1 in the ventral nerve cord to the mortality data. The same problem applies to correlating electrophysiological with toxicological data, for while A.P. frequency and amplitude were reduced within a few h of treatment, symptoms of poisoning were only noticeable after 12-24 h and mortality occurring 2-4 days after treatment.

Thus, further work is required to determine whether target site insensitivity (in addition to the rate of metabolism and possibly penetration) contribute to differential toxicity of AVMB1 in noctuid larvae. Of interest is the work of Argentine and Clark (1990) who generated two AVMB1-resistant strains of the Colorado potato beetle (Leptinotarsa decemlineata) through the use of the mutagen, ethyl methanesulfonate, and intensive selection with AVMB1 in a field strain. Both resistance factors were determined to be autosomal and incompletely recessive via reciprocal F_1 crosses to the susceptible laboratory strain. It was suggested that target-site insensitivity may be

involved as a resistance mechanism.

The difficulty in relating symptoms and end-point mortalities with the various parameters investigated in the present study could be considered partly analogous to the problems encountered with pyrethrins/pyrethroids in relating knockdown with death of the insect. These insecticides are well known for their rapid knockdown action and many pyrethroids have been shown to cause repetitive firing in the nervous system (Hassall, 1983). However, this action does not necessarily relate to their lethal toxicity and the amount needed to kill the insect is usually much greater than that for knockdown (Matsumura, 1985). The negative correlation between temperature and pyrethroid efficacy is also not necessarily consistent with increased frequency of repetitive discharges being a direct cause of death (Hassall, 1983). In apparent contrast with AVM, insects intoxicated with pyrethroids generally recover from knockdown because of the functioning of biotransformation systems (Ford and Pert, 1974; Nishimura et al., 1982 and Nishimura et al., 1988).

The first symptoms of poisoning with AVM, such as paralysis of the hind legs of insects would suggest an action on the musculature, either directly or via the neuromuscular junction or on the nerve cord itself. Various authors including Fritz et al. (1979), Kass et al. (1980)

and Tanaka and Matsumura (1985) have suggested that the action of $AVMB_1$ is on postsynaptic Cl^- channels. While Duce and Scott (1985 a, b) reported that $DHAVMB_{1a}$ had an action on extrajunctional glutamate H receptor Cl^- channels present in the tibiae muscle bundles of the locust S. gregaria, thus pointing to $AVMB_1$ also having a non-synaptic action. However, the role of extrajunctional receptor/ion channels in insects is still unclear and the relative importance of synaptic and non-synaptic target sites for AVM remains uncertain (Section 1.3.2.).

The in vivo electrophysiological system used in the present study proved to be suitable for long-term studies where simultaneous observations could be made of both toxicological and neurophysiological effects. However, its major disadvantage was that there was no way of identifying exactly what was being recorded; the signals obtained being a mixture of ascending and descending, single and compound A.P.s which could be central and/or peripheral in origin. Thus, it was not possible in this study to determine whether the effects of AVM on the ventral nerve cord were central and/or peripheral in nature.

Rather more convincing evidence for a central action of AVM on insects in vivo was reported by Corbett (1987) who found that the voltage required to evoke compound A.P.s in the ventral nerve cord when an electrical stimulus was

applied to the cercus of P. americana progressively increased with symptoms of poisoning. With the insect species used in the current study, a preparation to which an electrical stimulus can be reproduceably applied has yet to be developed.

The present in vivo studies need to be followed by intracellular methods, such as patch clamp (single ion channel) recordings, ideally with concurrent binding studies on membrane receptors. Such studies might enable different effects within the peripheral and CNS to be isolated and to ascertain in which order these occur.

REFERENCES

Abalis, I.M. and Eldefrawi, A.T. (1986). ^{3}H-Muscimol binding to a putative GABA receptor in honey bee brain and its interaction with avermectin B_{1a}. Pestic. Biochem. Physiol. 25: 279-287.

Abdel-Aal, Y.A.I. and Soderlund, D.M. (1980). Pyrethroid hydrolysing esterases in southern armyworm larvae: Tissue distribution, kinetic properties and selective inhibition. Pestic. Biochem. Physiol. 14: 282-289.

Abro, G.H. (1985). Varietal resistance and insecticide efficacy against the diamondback moth, Plutella xylostella L. Ph.D. thesis, University of London. 159 pp.

Abro, G.H., Dybas, R.A., Green,A.St.J. and Wright, D.J. (1988). Toxicity of Avermectin B_1 against a susceptible laboratory strain and an insecticide-resistant strain of Plutella xylostella (Lepidoptera: Plutellidae). J. Econ. Entomol. 81(6): 1575-1580.

Agee, H.R. (1985). Neurobiology of the bollworm moth: effects of abamectin on neural activity and on sensory systems. J. Agric. Entomol. 2(4): 325-336.

Aikins, J.A. and Wright, D.J. (1985). Toxicity of DDT and Malathion to various larval stages of Mamestra brassicae L. Pestic. Sci. 16: 73-80.

Anantaphruti, M., Kino, H., Terada, M., Ishii, A.I., Sano, M. (1982). Studies on chemotherapy of parasitic helminths (XIII). Efficacy of ivermectin on the circulating microfilia and embryonic development in the female worm of Dirofilaria immitis. Japanese Journal of Parasitology 31: 517-529.

Anderson, T.E., Babu, J.R., Dybas, R.A. and Mehta, H. (1986). Avermectin B_1: Ingestion and contact toxicity against Spodoptera eridania and Heliothis virescens (Lepidoptera: Noctuidae) and potentiation by oil and piperonyl butoxide. J. Econ. Entomol. 79: 197-201.

Argentine, J.A. and Clark, J.M. (1990). Selection for abamectin resistance in Colarado potato beetle (Coleoptera: Chrysomelidae). Pestic. Sci. 28: 17-24.

Beach, R.M. and Todd, J.W. (1985). Toxicity of avermectin to larva and adult soybean looper (Lepidoptera: Noctuidae) and adult fertility and fecundity. J. Econ. Entomol. 78: 1125-1128.

Bokish, A.J. and Walker, R.J. (1986) The action of avermectin (MK 936) on identified central neurones from Helix and interactions with acetylcholine and gamma-aminobutyric acid (GABA) responses. Comp. Biochem. Physiol. 84C(1): 119-125.

Botham, R.P. and Nicolson, R.A. (1985). Biochemical and ultrastructural studies on avermectin in the insect : evidence for central and peripheral sites of action. In: Neurotox '85', 2nd International Symposium on Insect Neurobiology and Pesticide Action, Bath, 31 March-4 April, (abstract).

Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemass, D.N., Shaw, J. and Turnbull, M. (1980). (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature (Lond.) 283: 92-94.

Brattsten, L.B. (1979a). Biochemical defence mechanisms in herbivores against plant allelochemicals. In: Herbivores: Their interactions with secondary plant metabolites (ed. Rosenthal, G.A. and Jansen, D.H.) p 199-270, Academic Press, New York.

Brattsten, L.B. (1979b). Ecological significance of mixed-function oxidations. Drug Metab. Rev. 10: 35-52.

Broderick, M.P. and Leake, L.D. (1989). What is death? British Crop Protection Council Monograph No. 43 Progress and Prospects in Insect Control. Reading, September 1989, p273 (Abstract).

Bull, D.L., Ivie, G.W., MacConnell, J.G., Gruger, V.F., Ku, C.C., Arison, B.H., Stevenson, J.M. and VandenHeuval, W.J.A. (1984). Fate of avermectin B_{1a} in soil and plants. J. Agric. Food Chem. 32: 94-102.

Bull, D.L. (1986). Toxicity and pharmacodynamics of avermectin in the Tobacco budworm, Corn earworm and Fall armyworm (Noctuidae). J. Agric. Food Chem. 34: 74-78.

Burg, R.W., Miller, B.M., Baker, E.E., Birnbaum, J., Currie, J.A., Hartman, R., Kong, Y-L., Monaghan, R.L., Olsen, G., Putter, I., Tunac, J.P., Wallick, H., Stapley, E.O., Oiwa, R. and Omura, S. (1979). Avermectins, new family of potent antihelmintic agents: producing organism and fermentation. Antimicrob. Agents Chemother. 15: 361-367.

Busvine, J.R. (1971). In: A critical review of the techniques for testing insecticides (2nd edn.) Commonwealth Agricultural Bureaux, London.

Campbell, W.C. (1981). An introduction to the avermectins. N. Z. vet. J. 29: 174-178.

Campbell, W.C., Fisher, M.H., Stapley, E.O., Albers-Schonburg, G. and Jacob, T.A. (1983). Ivermectin: A potent new antiparasitic agent. Science 221: 823-828.

Chapman, R.F. (1969). The Insects: Structure and Function. Hodder and Stoughton, London. 565pp.

Chiu, S.H.L., Sestokas, E., Taub, R., Smith, J.L., Arison, B., Lu, A.Y.H. (1984). The metabolism of avermectin-H_2B_{1a} and -H_2B_{1b} by pig liver microsomes. Drug Metab. dispos. 12(4): 464-469.

Cochran, D.G. (1985). Mortality and reproductive effects of $AVMB_1$ fed to German cockroaches. Entomol. Exp. Appl. 37: 83-88.

Collins, M.D. (1986). Pyrethroid resistance in the cotton bollworm, Heliothis armigera: a case study for Thialand. 1986 British Crop Protection Conference, Brighton, p 583-590.

Corbitt, T.S., Wright, D.J. and Green, A.St.J. (1985). The Toxicity of abamectin (MK-936) on cabbage to first and third instars of Spodoptera littoralis (Boisd). Meded. Fac. Landbouw. Rijk. Gent. 50: 639-642.

Corbitt, T.S. (1987). The toxicity and mode of action of avermectin B_1 against insects. Ph.D. thesis, University of London 148pp.

Corbitt, T.S., Green, A.St.J. and Wright, D.J. (1989). Relative potency of abamectin against larval stages of Spodoptera littoralis (Boisd.), Heliothis armigera (Hubn.) and Heliothis virescens (F.) (Lepidoptera: Noctuidae). Crop Protection 8: 127-132.

Crichton, R., Greenwood, R., Ford, M.G. and Salt,D. (1988). The use of microcomputer based data logging and analysis systems in neurotoxicological studies of pesticide action. In: Neurotox 88 Molecular Basis of Drug and Pesticide Action. 10-15 April, Nottingham. p216-218 (abstract).

Cull-Candy, S.G. (1976). Two types of extrajunctional L-glutamate receptors in locust muscle fibres. J. Physiol. 255 (2): 449-464.

Davenport, A.P. and Wright, D.J. (1987). Effect of the formamidine insecticide chordimeform on feeding and

reproduction in the Noctuid moth, Spodoptera littoralis (Boisduval). Crop Protection 6(1): 3-6.

Distelmans, W., D'Haeseleer, F. and Mortelmans, J. (1983). Efficacy of systemic administration of ivermectin against tsetse flies. Annls. Soc. Belge. Med. Trop. 63: 119-125.

Dittrich, V. Leutkemeier, N. and Voss, G. (1980). OP-resistance in Spodoptera littoralis: Inheritance, larval and imaginal expression, and consequences for control. J. Econ. Entomol. 73: 356-359.

Dowd, P.F. and Sparks, T.C. (1987). Comparisons of midgut, fat body, and cuticular enzymes from Pseudoplusia includens (Walker) and Heliothis virescens (F.) responsible for the hydrolysis of permethrin and fenvalerate. Pestic. Biochem. Physiol. 27: 309-317.

Drexler, G. and Sieghart, W. (1984). Properties of a high affinity binding site for ^{3}H-AVMB$_{1a}$. European Journal of Pharmacology 99: 269-277.

Duce, I.R. and Scott, R.H. (1985a). Action of dihydroavermectin B$_{1a}$ on insect muscle. Br. J. Pharmacol. 85: 395-401.

Duce, I.R. and Scott, R.H. (1985b). Interactions of avermectin, GABA and ibotinic acid on imput conductance of locust (Schistocerca gregaria) muscle. In: Neurotox '85', 2nd International Symposium, On Insect Neurobiology and Pesticide Action, Bath, 31 March-4 April, 1985 (abstract).

Dybas, R.A. and Green, A.St.J. (1984). Avermectins: Their chemistry and pesticidal activities. In: Proceedings 1984 British Crop Protection Conference, Brighton, 1984, 3: 947-953.

Dybas, R.A. and Babu, J.R. (1988). 4"-deoxy-4"-methylamino-4"-epiavermectin B$_1$ hydrochloride (MK-243): A novel avermectin insecticide for crop protection. In: Proceedings Brighton Crop Protection Conference-Pests and Diseases -1988, 1: 57-64.

Dybas, R.A., Hilton, N.J., Babu, J.R., Preiser, F.A. and Dolce, G.J. (1989). Novel second-generation avermectin insecticides and miticides for crop protection. In: Novel Microbial Products for Medicine and Agriculture (Pap. Int. Conf. Biotechnical. Microb. Prod.), 1st 1988. (ed. Demain, A.L., Somkuti, G.A., Hunter-Cevera, J.C., Rossmoore, H.W.) p203-212 Elsevier, Amsterdam.

Dyte, C.E. and Rowlands, D.G. (1967). Some aspects of the specificity of insecticide synergists. Proceedings 4th British Insecticide and Fungicide Conference, 1: 344-354.

El-Guindy, M.A., Mardi, S.M., Keddis, M.E., Issa, Y.H. and Abdel-Sattar, M.M. (1982). Development of resistance to pyrethroids in field populations of the Egyptian cotton leafworm Spodoptera littoralis (Boisd.). International Pest Control. 124: 6-17.

Finney, D.J. (1971). Probit Analysis, Cambridge University Press, Cambridge.

Fisher, M.H. (1984). The avermectins. In: The Royal Society of Chemistry. Proceedings of symposia of the Royal Society of Chemistry and Pesticides Group of the Society of Chemical Industry. Cambridge 25-27 Sept. Special publication number 53 -Recent Advances in the Chemistry of Insect Control. 53-72.

Fisher, M.H. and Mrozik, H. (1984). The avermectin family of macrolide-like antibiotics. In: Macrolide Antibiotics (ed. Omura, S.), p553-606, Academic Press, New York.

Ford, M.G. and Pert, D.R. (1974). Time-dose-response relationships of pyrethroid insecticides with special reference to knockdown. Pestic. Sci. 5: 635-641.

Fritz, L.C., Wang, C.C. and Gorio, A. (1979). Avermectin B_{1a} irreversibly blocks postsynaptic potentials at the lobster neuromuscular junction by reducing muscle membrane resistance. Proc. Natl. Acad. Sci. USA. 76: 2062-2066.

Gerolt, Ph. (1972). Mode of entry of oxime carbamates into insects. Pestic. Sci. 3: 43-55.

Glancey, M.B., Lofgren, C.S. and Williams, D.F. (1982). $AVMB_{1a}$: Effects on the ovaries of red imported fire ant queens (Hymenoptera; Formicidae). J. Med. Entomol. 19: 743-747.

Gledhill, J.A. (1982). Progress and problems in Heliothis management in tropical Southern Africa. In: Proceedings of the International workshop on Heliothis Management. IRCISAT, Patancheru, India, 15-20 November 1981, p 375-384.

Grafton-Cardwell, E.E. and Hoy, M.A. (1983). Comparative toxicity of avermectin B_1 to the predator Metaseilus occidentalis (Nesbitt) (Acari; Phytoseiidae) and the spider mites, Tetranychus urticae (Koch) and Panonychus ulmi (Koch) (Acari; Tetranychidae). J. Econ. Entomol.

76: 1216-1220.

Granett, J. and Retnakaran, A. (1977). Stadial susceptibility of eastern Spruce Budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae), to the insect growth regulator Dimilin. Can. Entomol. 106: 893-894.

Gubbins, K.E. and Campion, D.G. (1982). Economic aspects of pheremone trapping techniques for the control of Egyptian Cotton Leafworm. Outlook on Agriculture. 11(2): 62-68.

Hassall, K.A. (1983). The chemistry of pesticides-their metabolism, mode of action and uses in crop protection. Macmillan Press Ltd. London, 372pp.

Hemmingway, J. and Georgiou, G.P. (1984). Differential suppression of organophosphate resistance in Culex quinquefasciatus by the synergists IBP, DEF and TPP. Pestic. Biochem. Physiol. 21: 1-9.

Holden-Dye, L., Hewitt, G.M., Wann, K.T., Krogsgaard-Larsen, P. and Walker, R.J. (1988). Studies involving avermectin and the 4-aminobutyric acid (GABA) receptor of Ascaris suum muscle. Pestic Sci. 24(3): 231-246.

Hosny, M.M. (1980). The control of cotton pests in Egypt. Outlook on Agriculture. 10(4): 204-205.

Hoy, M.A. and Conley, J. (1987). Selection for Abamectin Resistance in Tetranychus urticae and T. pacifus (Acari: Tetranychidae). J. Econ. Entomol. 80(1): 221-225.

Hue, B., and Callec, J.J. (1983). Presynaptic inhibition in the cercal-afferent giant-interneurone synapses of the cockroach, Periplanata americana L. J. Insect Physiol. 29: 741-748.

James, P.S., Picton, J. and Riek, R.K. (1980). insecticidal activity of the avermectins. Vet. Rec. 106: 59.

Johnson, C.D. and Stretton, A.O.W. (1980). Neural control of locomotion in Ascaris: Analogy, electrophysiology and biochemistry. In: B.M. Zuckerman, Ed. Nematodes as Biological Models, vol. 1, New York, Academic Press, p 159-195.

Johnston, G.A.R., Krogsgaard-Larsen, P., Stephenson, A. and Twitchia, B. (1976). Inhibition of the uptake of GABA and related amino acids in brain slices by the optical isomers of nipecotic acid. J. Neurochem. 26: 1029-1032.

Judsudason, P., Levi, P.E., Weiden, M. and Roe, R.M. (1988). Developmental changes in the microsomal monooxygenase system and the in vivo metabolism of aldrin in larvae of the Mexican Bean Beetle (Coleoptera: Coccinellidae). J. Econ. Entomol. 81(6): 1598-1605.

Jyothi, K.N.; Prasuna, A.L., Osmani, Z. and Kaiser Jamil (1986). Moult inhibitory activity of L-676- an avermectin on castor semilooper, Achaea janata L. (Lepidoptera Noctuidae). International Pest Control, 28(6): 144-145.

Kass, I.S., Wang, C.C., Walrond, J.P. and Stretton, A.O.W. (1980). AVMB1, a paralysing antihelmintic that affects interneurons and inhibitory motorneurons in Ascaris. Proc. Natl. Acad. Sci. U.S.A. 77: 6211-6215.

Kass, I.S., Stretton, A.O.W. and Wang, C.C. (1984). The effects of avermectin and drugs related to acetylcholine and 4-aminobutyric acid on neurotransmission in Ascaris suum. Molec. Biochem. Parasit. 13: 213-225.

Langley, P.A. and Roe, J.M. (1984). Ivermectin as a possible control agent for the Tsetse fly, Glossina morsitans. Entomol. Exp. Appl. 36: 137-143.

Lees, G. and Beadle, D.J. (1986). Dihydroavermectin B_1 : actions on cultured neurones from the insect central nervous system. Brain Res. 366: 369-372.

Lindberg, R.L.P. and Negishi, M. (1989). Alteration of mouse cytochrome $P\text{-}450_{coh}$ substrate specificity by mutation of a single amino-acid residue. Nature 339: 632-634.

Lofgren, C.S. and Williams, D.F. (1982). $AVMB_{1a}$: Highly potent inhibitor of reproduction by queens of the red imported fire ant. J. Econ. Entomol. 75: 798-803.

Lummis S.C.R. and Sattelle, D.B. (1985). Binding sites for 4-aminobutyric acid and benzodiazepines in the central nervous system of insects. In: Neurotox '85', 2nd International Symposium of Insect Neurobiology and Pesticide Action, Bath, 31 March-4 April, 1985 (Abstract).

Lund, A.E., Hollingsworth, R.M. and Shankland, D.L. (1979). Chlordiform: plant protection by a sublethal, noncholergic action on the central nervous system. Pestic Biochem. Physiol. 11: 117-128.

Martin, R.J. (1987). The α-aminobutyric acid receptor of Ascaris as a target for antihelmintics. Biochem. Soc. Trans. 15: 61-65.

Matsumura, F. (ed) (1985). In Toxicology of insecticides. 2nd edition. Plenum Press, New York and London. 598pp.

Matsumura and Hayashi (1969). Residue Rev. 25:256 quoted in Toxicology of insecticides. 2nd edition (Matsumura, F.

editor, 1985). Plenum Press, New York and London. 598pp.

Mellin, T.N., Busch, R.D. and Wang, C.C. (1983). Postsynaptic inhibition of invertebrate neuromuscular transmission by avermectin B_{1a}. Neuropharmacology, 22: 89-96.

Misler, S. and Hurlburt, W.P. (1979). Action of black widow spider venom on quantized release of acetylcholine at the frog neuromuscular junction: Dependence upon external Mg^{+}. Proc. Natl. Acad. Sci. USA. 76: 991-995.

Miwa, G.T., Walsh, J.S., VandenHeuvel, W.J.A., Arison, B., Sestokas, E., Buhs, R., Rosegay, A., Avermitilis, S., Lu, A.Y.H., Walsh, M.A.R., Walker, R.W., Taub, R. and Jacob, T.A. (1983). The metabolism of avermectins B_{1a}, H_2B_{1a} and H_2B_{1b} by liver microsomes. Drug Metabolism and Disposition. 10(3): 268-274.

Nakatsugawa, T. and Morelli, M.A. (1976). Microsomal oxidation and insecticide metabolism. In: Insect Biochemistry and Physiology (ed. Wilkinson, C.F.) 64-104, Plenum Press, New York. 786 pp.

Nishimura, K., Kitahaba, T., Ikemoto, Y. and Fujita, T. (1988). Quantitative structure-activity studies of pyrethroids. 14. Physiochemical structural effects of tetramethrin and its related compounds on knockdown activity against house flies. Pestic Biochem. Physiol. 31: 155-165.

Nishimura, K., Okajima, N. Fujita, T. and Nakajimi, M. (1982). Quantitative structure-activity studies of substituted benzyl chrysanthemates. 4. Physiochemical properties and the rate of progress of knockdown symptoms induced in houseflies. Pestic. Biochem. Physiol. 18: 341-350.

Onishi, J.C. and Miller, T.W. (1985). The effect of avermectin B_1 on fungi. J. Antibiot. 38: 1568-1573.

Oppenorth, D.A. and Welling, W. (1976). Biochemistry and Physiology of Resistance. In: Insect Biochemistry and Physiology (ed. Wilkinson, C.F.) 507-551, Plenum Press, New York. 768pp.

Ostlind, D.A., Cifelli, S., and Lang, R. (1979). Insecticidal activity of the anti-parasitic avermectins. Vet. Rec. 105: 168.

Parrish, M.D. and Roberts, R.B. (1984). Toxicity of avermectin B_1 to larval yellowjackets, Vespula maculifrons (Hymenoptera: Vespidae). J. Econ. Entomol.

77: 769-772.

Patil, V.L. and Guthrie, F.E. (1979a). Effect of anomalous cuticular phospholipids on penetration of insecticides in susceptible and resistant houseflies. Pestic. Biochem. Physiol. 11: 13-19.

Patil, V.L. and Guthrie, F.E. (1979b). Cuticular lipids of two resistant and a susceptible strain of houseflies. Pestic. Sci. 10: 399-406.

Paul, S.M., Skolniek, P. and Zatz, M. (1980). AVMB1a : An irreversible activator of the GABA-benzodiazepine-chloride-ionophore receptor complex. Biochem. Biophys. Res. Com. 96: 632-638.

Pelag, B.A. (1987). Effect of avermectin B1 on the development and fecundity of the California red scale, Aonidiella aurantii and on two of its natural enemies: Chilocorus bipustulatus and Aphytis holoxanthus. Phytoparasitica 15(2): 115-123.

Perry, A.S. and Agosin, M. (1974) The physiology of insectide resistance by insects. In Physiology of Insecta 6. Rockstein, M. (Editor). Academic Press, New York and London. 548pp.

Pickens, L.G., Redfern, R.E. and Hayes, D.K. (1988). Narcotic compounds affect electroretinograms of some adult moths. Bulletin of the Entomological Society of America. 3(2): 70-71.

Pienkowski, R.L. and Mehring, P.R. (1983). Influence of avermectin B1 and carbofuran on feeding by alfalfa weevil larvae (Coleoptera: Curculionidae). J. Econ. Entomol. 76: 1167-1169.

Pimprikar, G.D. and Georgiou, G.P. (1979). Mechanisms of resistance to diflubenzuron in the housefly Musca domestica (L.). Pestic. Biochem. Physiol. 12: 10-22.

Pimprikar, G.D. and Georgiou, G.P. (1982). Effect of sesemex on the in vivo metabolism of diflubenzuron in larvae of susceptible and resistant strains of the housefly, Musca domestica L.. Agric. Food Chem. 30: 615-618.

Pong, S.-S., Wang, C.-C. and Fritz, L.C. (1980). Studies on the mechanism of AVMB1a: stimulation of release of GABA from brain synaptosomes. J. Neurochem. 34: 351-358.

Pong, S.-S. and Wang, C.-C. (1980). The specificity of high affinity binding of AVMB1a to mammalian brain. Neuropharmacology. 19: 311-317.

Pong, S.-S. and Wang, C.-C. (1982). Avermectin B_{1a} modulation of gamma-aminobutyric acid receptors in rat brain membranes. Journal of Neurochemistry 38(2): 375-379.

Pong, S.-S., Dehaven, R. and Wang, C.-C. (1981). Stimulation of benzodiazepine binding to rat brain membranes and solubilized receptor complex by $AVMB_{1a}$ and GABA. Biochem. Biophys. Acta. 646: 143-150.

Pong, S.-S., Dehaven, R. and Wang, C.-C. (1982). A comparative study of avermectin B_{1a} and other modulators of the GABA-receptor-chloride ion channel complex. J. Neurosci. 2: 966-971.

Putter, I., MacConnell, J.G., Preiser, F.A., Haidri, A.A., Ristich, S.A. and Dybas, R.A. (1981). Avermectins: Novel insecticides, acaricides and nematicides from a soil microorganism. Experientia. 37: 963-964.

Reed, D.K., Tromley, N.J. and Reed, G.L. (1985). Activity of avermectin B_1 against Codling Moth (Lepidoptera: Olethreutidae). J. Econ. Entomol. 78(5): 1067-1071.

Roush, R.T. and Wright, J.E. (1986). Abamectin: Toxicity to House Flies (Diptera: Muscidae) resistant to synthetic organic insecticides. J. Econ. Entomol. 79: 562-564.

Salama, H.S. (1983). Cotton pest management in Egypt. Crop Protection. 2(2): 183-191.

Sano, M., Treada, M., Ishii, A.I. and Kino, H. (1981) Effects of Avermectin B_{1a} on the motility of various parasitic helminths. Experientia 37: 844-846.

Sasser, J.N., Kirkpatrick, T.L. and Dybas, R.A. (1982). Efficacy of avermectins for root-knot control on tobacco. Plant Dis. 66: 691-693.

Sawicki, R.M. (1989). Current insecticide management practices in cotton around the world - short-term successes or templates for the future? Pestic. Sci. 26(4): 401-411.

Schaeffer, J.M., Haines, H.W. and Turner, M.J. (1988). Avermectin binding in Caenorhabditis elegans: A two-state model for the avermectin binding site. In: Neurotox 88 Molecular Basis of Drug and Pesticide Action. 10-15 April, Nottingham. p107-108 (abstract).

Schuster, D. and Everett, P.H. (1983). Response of Liriomyza trifolii (Diptera : Agromyzidae) to insecticides on tomato. J. Econ. Entomol. 76: 1170-1174.

Scott, J.G. (1989). Cross resistance to the biological insecticide abamectin in pyrethroid-resistant houseflies. Pestic. Biochem. Physiol. 34: 27-31.

Scott, R.H. and Duce, I.R. (1985). Effects of dihydroavermectin B_{1a} on locust (Schistocerca gregaria) muscles may involve several sites of action. Pestic. Sci. 16: 599-604.

Scott, R.H. and Duce, I.R. (1986). Anion selectivity of GABA and DHAVM induced conductance changes on locust muscle. Neurosci. Lett. 68: 197-201.

Spradbery, J.P., Tozer, R.S., Drewett, N. and Lindsey, M.J. (1985). The efficacy of ivermectin against larvae of the screw-worm fly (Chrysoma bessiana). Aust. vet. J. 62: 311-314.

Standfast, H.A., Muller, M.J. and Wilson, D.D. (1984). Mortality of Culicoides brevitarsis (Diptera: Ceratopogonidae) fed on cattle treated with ivermectin. J. Econ. Entomol. 77: 419-421.

Strong, L. (1986a). Inhibition of pupariation and adult development in Calliphora vomitoria treated with ivermectin. Entomologia exp. appl. 41: 157-164.

Strong, L. (1986b). Ivermectin prevents head eversion in the blowfly Calliphora vomitoria (L.). Experientia. 42: 1295-1296.

Strong, L. and Brown, T.A. (1987). Avermectins in insect control and biology: a review. Bull. Ent. Res. 77: 357-389.

Tanaka, K. and Matsumura, F. (1985). Action of avermectin B_1 on the leg muscles and nervous system of the American cockroach. Pestic. Biochem. Physiol. 24: 124-135.

Tedders, W.L., Payne, J.A. and Taylor, J.L. (1984). Laboratory evaluation of avermectin B_1 against the foliar aphids of pecan. J. Georgia entomol. Soc. 19: 344-350.

Trumble, J.T. (1985). Integrated pest management of Liriomyza trifolii: Influence of avermectin, cyromazine and methomyl on leafminer ecology in celery. Agriculture, Ecosystem and Environment. 12: 181-188.

Trumble, J.T., Moar, W.J., Babu, J.R. and Dybas, R.A. (1987). Laboratory bioassays of the acute and antifeedant effects of avermectin B_1 and a related analogue of Spodoptera exigua (Hubner). J. Agric. Entomol. 4: 21-28.

Tzeng, M.C. and Siekevitz, P. (1978). The effect of the purified major protein factor (a-latrotoxin) of the black widow spider venom on the release of acetycholine and norepinephrine from mouse cerebral cortex slices. Brain Res. 139(1): 190-196.

Brain Res. 139(1): 190-196.

Van Den Berg, H., Waage, J.K., Cock, M.J.W. (1988). Natural enemies of Helicoverpa armigera in Africa - A review. p 5-8, C.A.B. International, Chameleon Press Ltd., London.

Vinson, S.B. and Law, P.K. (1971). Cuticular composition and DDT resistance in the tobacco budworm. J. Econ. Entomol. 64: 1387-1390.

Wang, C.-C. and Pong S.-S. (1982). Action of avermectin B_1 on GABA nerves. Progress in Clinical and Biological Research. 97: 373-395.

Wann, K.T. (1987). The cellular actions of the avermectins. Phytotherapy Research. 1(4): 143-150.

Weismann, R. (1947). unterschungen uber die physiolgiste verhalten von Musca domestica L. vorschiender provenienzen. Mitt. Entomol. Ges. Basel. 20: 484-504.

Weismann, R. (1957). vergleichend histologische untersuchungen an normasensiblen und gegen DDT-subs-tanz resistenten stammen von Musca domestica L. J. Insect Physiol. 1: 187-197.

Wilkinson, C.F. and Brattsten, L.B. (1972). Microsomal drug metabolising enzymes in insects. Drug Metab. Rev. 1: 153-228.

Williams, M. and Yarbrough, G.G. (1979). Enhancement of in vitro binding and some of the pharmacological properties of diazepam by a novel antihelmintic agent, avermectin B_{1a}. European Journal of Pharmacology. 56: 273-276.

Wolfenbarger, D.A., Johnson, A.W., Herzog, G.A. and Tappen, W.B. (1985). Activity of avermectin in the laboratory and the field against the boll weevil and Heliothis Spp. on cotton and flue-cured tobacco. The Southwestern Entomologist. 7: 17-26.

Wright, D.J., Birtle, A.J. and Roberts, I.T.J. (1984). triphasis locomotor response of a plant-parasitic nematode to avermectin: inhibition by the GABA antagonists bicuculline and picrotoxin. Parasitology, 88: 375-382.

Wright, D.J., Loy, A., Green, A.St.J. and Dybas, R.A. (1985a). The translaminar activity of abamectin (MK-936) against mites and aphids. Meded. Fac. Landbouw. Rijk. Gent. 50: 595-601.

Wright, D.J., Roberts, I.T.J., Androher, A., Green, A.St.J. and Dybas, R.A. (1985b). The residual activity of abamectin (MK-936) against Tetranychus urticae (Koch) on cotton. Meded. Fac. Landbouw. Rijk. Gent. 50: 633-637.

Wright, D.J. (1986). Biological activity and mode of action of avermectins. In: Neuropharmacology and Pesticide Action. (Edited by Ford, M.G., Lunt, G.G., Reay, R.C. and Usherwood, P.N.R.). p 174-202. Ellis Horwood, Chichester, England.

Wright, D.J. (1987). Avermectins: action on target pest species. Biochem. Soc. Trans. 15: 65-67.

Wright, J.E. (1984). Biological activity of avermectin B_1 against the boll weevil (Coleoptera, Curculionidae). J. Econ. Entomol. 77: 1029-1032.

Wright, J.E., Jenkins, J.N. and Villavaso, E.J. (1985). Evaluation of avermectin B_1 (MK936) against Heliothis spp. in the laboratory and in field plots and against the boll weevil in field plots. The Southwestern entomologist suppl. no. 7: 11-16.

Yu, S.J. (1983). Age variation in insecticide susceptibility and detoxification capacity of Fall Armyworm (Lepidoptera: Noctuidae) Larvae. J. Econ. Entomol. 76: 219-222.

Zufall, F., Franke, C. and Hatt, H. (1988). Avermectin B_{1a} directly opens the multi-transmitter gated chloride channel in crayfish muscle. In: Neurotox 88 Molecular Basis of Drug and Pesticide Action. 10-15 April, Nottingham. p118-119 (abstract).

Zufall, F., Franke, C. and Hatt, H. (1989). The insecticide Avermectin B_{1a} activates a chloride channel in crayfish muscle membrane. J. Exp. Biol. 142: 192-205.

Appendix 1.1.

A comparison of doses of AVMB1 causing mortality in H. virescens, H. zea, S. eridania, S. frugiperda, H. armigera and S. littoralis 48, 72 and 96 h after treatment.

Species	Method of application	48 h LD_{50}	48 h LD_{95}	72 h LD_{50}	96 h LD_{50}
H. virescens[a]	Topical	0.015	0.158	-	-
H. virescens[a]	Topical	0.0024[1]	0.172[1]	-	-
H. virescens[b]	Topical	0.015	-	-	0.0053
H. virescens[c]	Topical	-	-	-	0.032
H. virescens[d]	Topical	-	-	0.01-0.02[2]	-
H. zea[b]	Topical	0.43	-	-	0.31
H. zea[c]	Topical	-	-	-	0.026
S. eridania[d]	Topical	-	-	3.81-5.45[2]	-
S. frugiperda[c]	Topical	-	-	-	>10.0
S. littoralis V[e]	Topical	-	-	-	> 7.2
S. littoralis VI[e]	Topical	-	-	-	0.127
S. frugiperda[c]	Oral	-	-	-	>10.0
H. virescens[c]	Oral	-	-	-	0.501
H. zea[c]	Oral	-	-	-	15.038
S. littoralis(I)[e]	Oral	-	-	-	1.23*
H. armigera (I)[e]	Oral	-	-	-	0.15*
H. virescens (I)[e]	Oral	-	-	-	0.033*
S. littoralis(III)[e]	Oral	-	-	-	10.96*
H. armigera (III)[e]	Oral	-	-	-	1.29*

All doses are in µg/insect except for those marked * which are given as ug a.i./ml.

[1] AVMB1 as emulsifiable concentrate

[2] Doses calculated on basis of larval weights

This appendix modified from Strong and Brown (1987), including data from: [a] Wright et al. (1985); [b] Wolfenbarger et al. (1985); [c] Bull (1986); [d] Anderson et al. (1986); [e] Corbett et al. (1989).

APPENDIX 2.1
Artificial diet for S. littoralis and H. armigera

Larval diet

a)	Haricot bean seed (finely ground) dried wt.	1000.0g
b)	Ascorbic acid	15.7g
c)	Methyl-4-hydroxybenzoate	10.0g
d)	Sorbic acid	5.0g
e)	Tetracycline	0.75g
f)	Linseed oil	40.0ml
g)	Vitamin stock solution (see below)	33.0ml
h)	Water	1670.0ml
i)	Yeast	160.0g
j)	Agar	58.0g
k)	Water (for yeast and agar)	2400.0ml

Adult diet

Sucrose	50.0g
Methyl-4-hydroxybenzoate	1.0g
Vitamin stock solution (see below)	10.0ml
Water	500.0ml

Vitamin stock solution (kept at $5^{o}C$)

Nicotinic acid	1.526g
Calcium pantothenate	1.526g
Riboflavin	0.764g
Aneurine hydrochloride	0.382g
Pyridoxine hydrochloride	0.382g
Folic acid	0.382g
D-biotin	0.306g
Cyanocobalamine	0.003g
Water	500.0ml

Preparation of larval diet

Haricot bean seeds (a) were soaked overnight in water, then finely ground. Water (h) was added to ingredients (a-g) and stirred. The water (k) for the yeast/agar was brought to boiling, agar and yeast were then added while stirring and combined with ingrediants (a-h). The mixture was brought to the boil, allowed to cool slightly and poured either into waxed paper cones or into a plastic tray and allowed to set. Diet was stored at 5 C. For bioassays, liquid diet was poured into plastic trays lined with aluminium foil and a Dufaylite impregnated paper honeycomb was pressed onto the trays so that the diet was in the bottom of each individual piece of honeycomb.

Preparation of adult diet

The ingredients were added to a 500ml flask and mixed. The diet was put into plastic pots containing cottonwool.

APPENDIX 3.1.1.

Toxicity of topically applied AVMB1 to larval instars of Spodoptera littoralis reared on artificial diet.

Instar	Concentration($\mu g\ ml^{-1}$)	n	Number Responding
Second	0	100	9
	35	100	14
	186	100	53
	394	100	71
	511	100	83
	1871	100	96
	Mean weight of larvae =1.52 mg		
Third	0	98	8
	186	99	16
	394	99	24
	511	100	37
	1871	94	50
	2285	99	71
	Mean weight of larvae =15.70 mg		
Fourth	0	104	3
	186	97	9
	511	98	15
	2285	105	28
	4571	104	35
	10573	109	56
	Mean weight of larvae =22.42 mg		
Fifth	0	163	23
	511	85	11
	2285	129	28
	4571	88	24
	4697	45	14
	9143	85	29
	51800	100	61
	Mean weight of larvae =188 mg		
Sixth	0	84	10
	35	44	8
	186	49	16
	394	102	55
	511	100	64
	1871	50	42
	2285	90	72
	4571	40	37
	Mean weight of larvae =804 mg		

APPENDIX 3.1.2.

Toxicity of topically applied AVMB$_1$ with PB (50000 μg ml^{-1}) topically applied 1 h before, to larval instars of Spodoptera littoralis.

Instar	Concentration(μg ml^{-1})	n	Number Responding
Third	0	80	6
	35	94	8
	186	89	42
	394	92	51
	511	87	54
	1871	88	72
	Mean weight of larvae=16.7 mg		
Fourth	0	101	9
	186	100	17
	511	93	20
	2285	95	45
	4571	95	62
	10573	94	76
	Mean weight of larvae=24.5 mg		
Fifth	0	103	0
	186	104	9
	511	96	16
	2285	100	30
	4571	103	41
	9143	106	57
	Mean weight of larvae=224.4 mg		
Fifth (5000 μg ml^{-1})			
	0	50	3
	613	50	10
	993	50	14
	1587	50	16
	2285	50	18
	4697	50	24
	Mean weight of larvae=170mg		
Sixth	0	100	8
	35	95	16
	186	96	44
	394	85	50
	511	89	58
	2285	99	80
	Mean weight of larvae=798 mg		

APPENDIX 3.1.3.

Toxicity of topically-applied AVMB1 with DEF to V instar S. littoralis.

Instar	Concentration ($\mu g\ ml^{-1}$)	n	Number responding
Fifth +DEF	0	102	11
	511	104	23
	2285	102	31
	4697	106	40
	10573	102	50
	51800	100	68

Mean weight of larvae =200mg

APPENDIX 3.1.4.

Toxicity of AVMB1 following injection into V and VI instar S. littoralis and V instar H. armigera larvae and toxicity of AVMB1 topically-applied following injection of 1 µl 5000 $\mu g\ ml^{-1}$ PB into V instar S. littoralis.

Instar	Concentration($\mu g\ ml^{-1}$)	n	Number responding
S. littoralis			
Fifth	0	78	12
	186	87	24
	511	86	36
	1871	87	44
	4571	95	56

Mean weight of larvae = 147mg

Sixth	0	95	13
	186	95	24
	394	74	31
	511	95	43
	2285	90	48
	4571	75	53

Mean weight of larvae = 953mg

H. armigera

Fifth	0	40	3
	663	50	10
	997	50	19
	1587	50	23
	2285	50	29
	4697	50	35

Mean weight of larvae = 122mg

Fifth instar S. littoralis injection 1µl PB.

0	40	2
613	50	9
993	50	12
1587	50	15
2285	50	21
4697	50	30

Mean weight of larvae = 210mg

APPENDIX 3.1.5.

Toxicity of topically-applied malathion to V(±)PB and VI (-)PB instar S. littoralis.

Treatment (+/-PB)	Instar	Concentration (µg ml^{-1})	n	Number responding
-PB	Fifth	0	80	5
		500	100	16
		1000	100	34
		2500	100	54
		5000	98	65
		10000	93	76
	Mean weight of larvae =223mg			
+PB	Fifth	0	102	7
		100	100	17
		250	100	31
		500	95	36
		1000	98	44
		2500	98	55
	Mean weight of larvae =210mg			
-PB	Sixth	0	85	12
		100	51	11
		250	101	27
		500	50	17
		1000	100	41
		2500	99	57
		5000	84	54
	Mean weight of larvae =947mg			

APPENDIX 3.1.6.

Toxicity of topically-applied lambda cyhalothrin (Karate) to V(±)PB and VI(-)PB instar S. littoralis.

Treatment (+/-PB)	Instar	Concentration ($\mu g\ mg^{-1}$)	n	Number Responding
+PB	Fifth	0	104	10
		1	99	11
		10	100	48
		15	50	31
		25	100	81
		50	105	97
	Mean weight of larvae =177mg			
-PB	Fifth	0	104	3
		0.5	53	3
		1	102	14
		10	100	47
		15	54	36
		25	108	88
		50	113	105
	Mean weight of larvae =182mg			
-PB	Sixth	0	97	12
		10	102	42
		15	50	27
		20	48	28
		25	97	59
		50	98	81
		100	50	43
	Mean weight of larvae =926mg			

APPENDIX 3.1.7.

Toxicity of topically-applied AVMB1 to V(±)PB and VI(-)PB instar H. armigera.

Treatment (+/-PB)	Instar	Concentration ($\mu g\ ml^{-1}$)	n	Number Responding
+PB	Fifth	0	100	3
		186	46	8
		394	50	12
		511	100	33
		1871	100	50
		2287	25	14
		4571	100	68
	Mean weight of larvae=115mg			

-PB	Fifth	0	95	8
		35	96	13
		394	95	30
		511	95	43
		2285	95	55
		4571	97	72
	Mean weight of larvae=91.9 mg			
-PB	Sixth	0	73	5
		35	46	7
		186	97	16
		511	75	18
		1871	97	34
		4571	96	50
	Mean weight of larvae=248.3mg			

APPENDIX 3.1.8.

Toxicity of topically-applied AVMB$_1$ to VI instar S littoralis larvae (Egyptian field strain; F$_0$)

Concentration ($\mu g\ ml^{-1}$)	n	Number Responding
0	40	3
35	37	10
186	35	12
511	35	18
1871	35	21
4571	35	26
Mean weight of larvae=812mg		

Appendix 4.1.

Level of radioactivity recovered from internal standard-treated nerve cord extract of fifth instar S. littoralis larvae and after direct addition of internal standard to vial.

Treatment (tissue)	Total Dpm recovered	Chloroform Dpm recovered	Aqueous Dpm recovered
Nerve cord[1]	125462	125049	413
	125926	125506	420
	124901	124514	387
	124223	123911	312
Mean Dpm	125128	124745	383
Direct into vial[2]	130456	129941	515
	129274	129024	250
	129542	129327	215
	129700	128776	924
Mean Dpm	129743	129267	476

[1] 4.5 µg AVMB1 added to known weight of tissue in vial. Tissue allowed to dry prior to extraction.
[2] 4.5 µg AVMB1 added directly to vial and extraction procedure carried out as before (see Section 4.2.2.1.). This data used in Table 4.1.

Appendix 4.2.

Level of ^{3}H-AVMB1 determined by TLC method from internal standard-treated nerve extract of fifth instar S. littoralis.[1]

Total Dpm recovered	Chloroform Dpm recovered	Dpm in band 3 of chloroform sample	Band 3 as % of solvent sample
125462	125049	116439	93.11
125926	125506	116306	92.67
124901	124514	115702	92.92
124223	123911	114957	92.77
X 125128	124745	115851	92.87

[1] 4.5 µg AVMB1 added to known weight of tissue in vial. Tissue allowed to dry prior to extraction. This data used in Table 4.2.

Appendix 4.3.

Proportion of radioactivity as abamectin following TLC of chloroform extracts from randomly-selected nerve cord samples.

% $AVMB_1$

Species/instar		Replicate 1	2	3	4	Mean
Spodoptera	V	76.4	74.8	68.7	75.0	73.7
	V+PB	84.6	80.0	87.6	81.7	83.5
	VI	86.5	83.5	85.5	85.7	85.3
	VI+PB	86.8	82.5	88.4	77.5	83.8
Heliothis	V	85.5	79.5	84.4	85.5	83.7
	VI	78.1	84.0	82.6	83.3	82.0

% Metabolites

Species/instar		Replicate 1	2	3	4	Mean
Spodoptera	V	23.6	25.2	31.3	25.0	26.3
	V+PB	15.4	20.0	12.4	18.3	16.5
	VI	13.5	16.5	14.5	14.3	14.7
	VI+PB	13.2	17.5	11.4	22.5	16.2
Heliothis	V	14.5	20.5	15.6	14.5	16.3
	VI	21.9	16.0	17.4	16.7	18.0

Data used in Appendix 4.4.

Appendix 4.4.

Proportion of radioactivity as AVMB1 following TLC of chloroform extracts of randomly-selected nerve cord samples from S. littoralis and H. armigera, at 48 and 24h respectively after application of ^{3}H-AVMB1 (n=4).

			$\bar{X}$%±S.E.[3]	
Instar	Dose[1]	Time (h)	AVMB1 [2]	Metabolites
Spodoptera				
V	4.5	48	73.73±1.7	26.27±1.7
V+PB[3]	4.5	48	83.48±1.7	16.52±1.7
VI	4.5	48	85.30±0.6	14.70±0.6
VI+PB	4.5	48	83.80±2.4	16.20±2.4
Heliothis				
V	3.0	24	83.73±1.4	16.27±1.4
VI	1.5	24	82.00±1.4	18.00±1.4

[1] See Table 4.3.
[2] These figures were used to calculate AVMB1 in chloroform samples in Table 4.5.
[3] Standard error calculated following Arc Sine Transformation of % data. Original data given in Appendix 4.3.

Appendix 4.5.

Proportion of AVMB$_1$ and metabolites extracted from randomly-selected S. littoralis and H. armigera nerve cord samples 48 and 24 h respectively after application of ^{3}H-AVMB$_1$.

Species/ instar			Metabolite replicates[1] 1	2	3	4	Mean
Spodoptera	V	Dpm	130.3	220.6	167.7	239.8	189.6
		%	53.8	53.1	45.2	49.2	50.3
	V+PB	Dpm	94.1	86.2	56.0	86.5	80.7
		%	30.5	30.4	23.8	29.5	28.6
	VI	Dpm	41.4	42.0	37.0	20.1	35.1
		%	24.2	27.6	25.3	14.7	23.0
	VI+PB	Dpm	38.3	48.3	51.7	88.2	56.6
		%	18.0	26.0	29.8	34.8	27.1
Heliothis	V	Dpm	429.0	807.6	350.5	362.7	487.5
		%	34.6	26.5	28.8	24.8	28.7
	VI	Dpm	90.6	125.2	105.6	116.1	109.4
		%	42.3	30.8	32.4	35.3	35.2

Species/ instar			AVMB$_1$ replicates 1	2	3	4	Mean
Spodoptera	V	Dpm	112.2	194.5	203.1	247.8	189.4
		%	46.2	46.9	54.8	50.8	49.7
	V+PB	Dpm	214.4	197.4	179.7	206.9	199.6
		%	69.5	69.6	76.2	70.5	71.4
	VI	Dpm	129.4	110.2	109.1	116.6	116.3
		%	75.8	72.4	74.7	85.3	77.0
	VI+PB	Dpm	174.8	137.2	121.6	166.1	149.9
		%	82.0	74.0	70.2	65.2	72.9
Heliothis	V	Dpm	811.9	2245.4	865.5	1101.4	1256.1
		%	65.4	73.5	71.2	75.2	71.3
	VI	Dpm	123.4	281.2	221.0	212.4	209.5
		%	57.7	69.2	67.6	64.7	64.8

[1] Assumes all radioactivity in aqueous sample is from metabolites, not AVMB$_1$.

[2] Calculated from chloroform sample using data obtained in Appendices 4.3. and 4.4.

PB = Piperonyl Butoxide

Data used in Table 4.5.

Appendix 4.6.

Radioactivity in randomly-selected S. littoralis and H. armigera nerve cords 48 and 24 h respectively after topical application of ^{3}H-AVMB$_1$.

Species/instar		Dpm/mg Replicates 1	2	3	4	Mean
Spodoptera	V	436.3	321.4	249.9	307.8	328.9
	V+PB	342.1	298.6	307.4	243.6	297.9
	VI	104.6	77.4	142.9	112.7	109.4
	VI+PB	141.6	192.1	160.5	100.8	148.8
Heliothis	V	1847.6	522.6	492.9	1654.3	1129.4
	VI	470.6	326.4	240.7	374.6	353.0

Data used in Table 4.5.

Appendix 4.7.

Proportion of radioactivity as AVMB1 following TLC of chloroform extracts of nerve cords from paralysed and non-paralysed larvae.

% AVMB1

Species/instar	Symptoms	Replicates 1	2	3	Mean
Spodoptera V	Non-paralysed	71.6	68.0	75.1	71.6
	Paralysed	71.0	77.0	75.1	74.4
VI	Non-paralysed	79.6	81.6	80.8	80.7
	Paralysed	83.4	83.1	83.7	83.4
Heliothis V	Non-paralysed	80.3	82.4	82.1	81.6
	Paralysed	82.0	81.8	82.5	82.1
VI	Non-paralysed	82.9	82.1	83.4	82.8
	Paralysed	83.3	81.1	85.2	83.2

% Metabolites

Species/instar	Symptoms	Replicates 1	2	3	Mean
Spodoptera V	Non-paralysed	28.4	24.9	32.0	28.4
	Paralysed	29.0	23.0	24.9	25.6
VI	Non-paralysed	20.4	18.4	19.1	19.3
	Paralysed	16.6	16.3	16.9	16.6
Heliothis V	Non-paralysed	19.7	17.6	17.9	18.4
	Paralysed	18.0	18.2	17.5	17.9
VI	Non-paralysed	17.1	17.9	16.6	17.2
	Paralysed	16.7	18.9	14.8	16.8

Data used in Table 4.6.

Appendix 4.8.

The proportion of radioactivity as AVMB1 following TLC of chloroform extracts from S. littoralis and H. armigera nerve cord samples from paralysed and non-paralysed larvae, at 48 and 24 h respectively after application of ^{3}H-AVMB1. (n=3)

Instar	Symptoms[1]	Dose[2]	Time(h)	$\bar{X}$%± SE[4] AVMB1[3]	Metabolites
Spodoptera					
V	NP	4.5	48	71.6±2.1	28.4±2.1
	P	4.5	48	74.4±1.8	25.6±1.8
VI	NP	4.5	48	80.7±0.6	19.3±0.6
	P	4.5	48	83.4±0.2	16.6±0.2
Heliothis					
V	NP	3.0	24	81.6±0.7	18.4±0.7
	P	3.0	24	82.1±0.2	17.9±0.2
VI	NP	1.5	24	82.8±0.3	17.2±0.3
	P	1.5	24	83.2±1.2	16.8±1.2

1 NP = non-paralysed P = paralysed
2 See Table 4.3.
3 These figures used to calculate AVMB1 values in chloroform samples in Table 4.6.
4 S.E. calculated following Arc Sine Transformation of data Original data given in Appendix 4.7.

Appendix 4.9.
Proportion of AVMB$_1$ and metabolites extracted from nerve cord samples from paralysed and non-paralysed larvae 48 and 24 h respectively after application of ^{3}H-AVMB$_1$.

Species/ instar	Symptoms		Metabolite replicates [1] 1	2	3	4	Mean
Spodoptera V							
	N	Dpm	142.9	130.6	121.7	153.2	137.1
		%	48.2	52.2	51.6	52.4	51.1
	P	Dpm	173.6	147.4	166.3	151.9	159.8
		%	48.2	48.3	49.9	46.7	48.3
Spodoptera VI							
	N	Dpm	46.3	36.4	49.6	31.7	41.0
		%	32.9	25.8	32.8	28.1	29.9
	P	Dpm	36.4	39.9	43.6	34.2	38.5
		%	24.6	25.1	29.1	24.6	25.8
Heliothis V							
	N	Dpm	501.4	472.6	323.6	407.6	426.3
		%	28.6	29.8	24.8	26.2	27.3
	P	Dpm	374.6	452.9	396.2	352.7	394.1
		%	24.4	29.2	26.1	22.9	25.7
Heliothis VI							
	N	Dpm	94.6	99.8	101.3	106.9	100.7
		%	30.3	30.5	29.8	31.7	30.6
	P	Dpm	122.1	111.3	117.4	106.2	114.3
		%	34.0	35.0	35.9	33.4	34.6

Species/ instar	Symptoms		AVMB$_1$ replicates[1] 1	2	3	4	Mean
Spodoptera V							
	N	Dpm	153.6	119.6	114.3	139.1	131.7
		%	51.8	47.8	48.4	47.6	48.9
	P	Dpm	186.4	157.8	167.2	173.4	171.2
		%	51.8	51.7	50.1	53.3	51.7
Spodoptera VI							
	N	Dpm	94.3	104.6	101.5	81.1	95.4
		%	67.1	74.2	67.2	71.9	70.1
	P	Dpm	111.4	119.2	106.1	104.9	110.4
		%	75.4	74.9	70.9	75.4	74.2
Heliothis V							
	N	Dpm	1250.7	1111.4	982.7	1146.7	1122.9
		%	71.4	70.2	75.2	73.8	72.7
	P	Dpm	1159.6	1099.4	1121.0	1186.9	1141.7
		%	75.6	70.8	73.9	77.1	74.4
Heliothis VI							
	N	Dpm	217.6	227.0	239.1	230.4	228.5
		%	69.7	69.5	70.2	68.3	69.4
	P	Dpm	237.1	206.8	209.7	211.4	216.3
		%	66.0	65.0	64.1	66.6	65.4

[1] Assumes all radioactivity in aqueous sample is metabolite.
[2] Calculated from chloroform sample using Table 4.6. Data used in Table 4.7.

Appendix 4.10.

Radioactivity in randomly-selected S. littoralis and H. armigera nerve cords 48 and 24 h respectively after topical application of ^{3}H-AVMB$_{1}$.

Species/ instar	Symptoms	Dpm/mg Replicates 1	2	3	4	Mean
Spodoptera						
V	non-paralysed	207.4	104.2	185.2	170.1	166.7
	paralysed	622.4	229.1	728.6	400.5	495.2
VI	non-paralysed	93.8	121.8	92.2	121.9	107.4
	paralysed	254.2	168.1	239.4	308.1	242.5
Heliothis						
V	non-paralysed	224.5	308.2	433.8	304.4	317.7
	paralysed	2188.3	1272.3	1737.9	1143.7	1585.6
VI	non-paralysed	329.0	220.6	297.3	244.0	272.7
	paralysed	1062.4	551.6	455.2	450.2	630.0

Data used in Table 4.7.

Appendix 4.11.

Proportion of radioactivity as AVMB1 following TLC of chloroform extract of fat body from paralysed and non-paralysed larvae.

% AVMB1

Species/ instar		Symptoms	Replicates 1	2	3	Mean
Spodoptera	V	non-paralysed	85.2	87.9	90.4	87.9
		paralysed	86.5	87.2	87.3	87.0
	VI	non-paralysed	82.3	84.1	85.3	83.9
		paralysed	85.9	87.4	88.3	87.2
Heliothis	V	non-paralysed	84.2	85.7	86.6	85.5
		paralysed	87.2	88.9	90.0	88.7
	VI	non-paralysed	85.7	85.9	86.7	86.1
		paralysed	84.9	87.3	85.8	86.0

% Metabolites

Species/ instar		Symptoms	Replicates 1	2	3	Mean
Spodoptera	V	non-paralysed	14.7	12.1	9.6	12.1
		paralysed	13.5	12.8	12.7	13.0
	VI	non-paralysed	17.7	15.9	14.7	16.1
		paralysed	14.1	12.6	11.7	12.8
Heliothis	V	non-paralysed	15.8	14.3	13.4	14.5
		paralysed	12.8	11.1	10.0	11.3
	VI	non-paralysed	14.3	14.1	13.3	13.9
		paralysed	15.1	12.7	14.2	14.0

Data used in Table 4.8.

Appendix 4.12.

The proportion of radioactivity as AVMB$_1$ following TLC of chloroform extracts of fat body samples from paralysed (P) and non-paralysed (NP) S. littoralis and H. armigera, 48 and 24h respectively after topical application of ^{3}H-AVMB$_1$ (n=3).

Instar	Symptoms	Dose[1]	Time(h)	X%+S.E.[3] AVMB$_1$ [2]	Metabolites
Spodoptera					
V	NP	4.5	48	87.9±1.5	12.1±1.5
	P	4.5	48	87.0±0.2	13.0±0.2
VI	NP	4.5	48	83.9±0.9	16.1±0.9
	P	4.5	48	87.2±0.7	12.8±0.7
Heliothis					
V	NP	3.0	24	85.5±0.7	14.5±0.7
	P	3.0	24	88.7±0.8	11.3±0.8
VI	NP	1.5	24	86.1±0.3	13.9±0.3
	P	1.5	24	86.0±0.7	14.0±0.7

[1] See Table 4.3.
[2] These figures used to calculate AVMB$_1$ values in chloroform samples in Table 4.8.
[3] S.E. calculated following Arc Sine Transformation of % data. Original data given in Appendix 4.11.

Appendix 4.13.
Proportion of AVMB1 and metabolites extracted from fat body samples of paralysed and non-paralysed larvae 48 and 24 h respectively after application of ^{3}H-AVMB1.

Species/ instar	Symptoms		Metabolite Replicates 1	2	3	Mean
Spodoptera V						
	non-paralysed	Dpm	36.2	78.4	57.3	57.3
		%	15.9	22.5	19.9	19.4
	paralysed	Dpm	90.5	80.3	70.1	80.3
		%	27.2	23.1	24.1	24.8
Spodoptera VI						
	non-paralysed	Dpm	12.6	18.3	15.0	15.3
		%	22.8	26.5	25.5	24.9
	paralysed	Dpm	22.0	30.4	30.7	27.7
		%	21.3	22.7	23.9	22.6
Heliothis V						
	non-paralysed	Dpm	78.6	90.2	100.0	89.6
		%	21.8	22.3	24.7	22.9
	paralysed	Dpm	366.7	411.3	455.9	411.3
		%	18.0	19.1	14.4	17.2
Heliothis VI						
	non-paralysed	Dpm	30.2	31.0	30.9	30.7
		%	21.9	21.7	21.8	21.8
	paralysed	Dpm	85.2	91.7	87.7	88.2
		%	16.7	17.7	17.2	17.2

Species/ instar	Symptoms		AVMB1 Replicates 1	2	3	Mean
Spodoptera V						
	non-paralysed	Dpm	191.4	269.9	230.7	230.7
		%	84.1	77.5	80.1	80.6
	paralysed	Dpm	242.6	266.9	220.4	243.3
		%	72.8	76.9	75.9	75.2
Spodoptera VI						
	non-paralysed	Dpm	42.7	50.8	43.9	45.8
		%	77.2	73.5	74.5	75.1
	paralysed	Dpm	82.2	103.5	97.5	94.4
		%	78.7	77.3	76.1	77.4
Heliothis V						
	non-paralysed	Dpm	281.6	314.7	304.3	300.2
		%	78.2	77.7	75.3	77.1
	paralysed	Dpm	1675.6	1739.0	1732.2	1715.6
		%	82.0	80.9	85.6	82.8
Heliothis VI						
	non-paralysed	Dpm	108.1	112.0	111.4	110.5
		%	78.1	78.3	78.2	78.2
	paralysed	Dpm	425.2	426.0	422.6	424.6
		%	83.3	82.3	82.8	82.8

1 Assumes all radioactivity in aqueous sample is metabolites.
2 Calculated from chloroform sample using Appendices 4.11. and 4.12. Data used in Table 4.9.

Appendix 4.14.

Radioactivity in randomly-selected S, littoralis and H. armigera fat body 48 and 24 h respectively after topical application of ^{3}H-AVMB$_{1}$.

Species/ instar	Symptoms	Dpm/mg Replicates 1	2	3	4	Mean
Spodoptera						
V	non-paralysed	497.2	118.9	167.6	207.7	247.9
	paralysed	542.0	273.1	562.1	487.5	466.2
VI	non-paralysed	43.5	87.1	69.4	68.3	67.1
	paralysed	98.9	135.7	152.7	145.4	133.2
Heliothis						
V	non-paralysed	337.7	459.6	413.7	392.9	401.0
	paralysed	2737.9	2888.8	1902.5	1951.9	2370.3
VI	non-paralysed	72.2	183.0	210.6	210.1	169.0
	paralysed	631.1	452.1	472.8	524.7	520.2

Data used in Table 4.9.

Appendix 4.15.

Radioactivity recovered from gut membrane and gut lumen of S. littoralis and H. armigera 48 and 24 h respectively following topical application with ^{3}H-AVMB$_{1}$.

Gut Membrane

	Replicates (Dpm/mg)									
Sample	1	2	3	4	5	6	7	8	9	10
Spodoptera										
V P	48	35	107	221	191	66	75	109	141	203
V NP	47	74	25	64	18	70	22	60	32	46
VI P	65	26	77	57	82	36	81	44	59	79
VI NP	29	40	71	32	25	30	44	60	34	29
Heliothis										
V P	129	212	70	82	151	140	86	200	105	165
V NP	49	29	32	39	41	30	47	36	48	40
VI P	18	39	9	9	8	30	12	15	17	12
VI NP	21	15	15	19	12	21	18	17	14	12

Gut lumen

	Replicates (Dpm/mg)									
Sample	1	2	3	4	5	6	7	8	9	10
Spodoptera										
V P	303	325	437	818	669	747	393	531	578	299
V NP	439	253	233	313	124	152	438	374	176	219
VI P	241	85	99	172	17	208	136	153	174	90
VI NP	85	134	67	96	47	48	120	90	79	98
Heliothis										
V P	1003	2139	2055	551	1829	774	1536	1217	936	1646
V NP	391	197	204	209	296	287	244	287	304	184
VI P	84	47	85	26	55	34	73	59	44	78
VI NP	41	44	66	94	34	36	80	40	73	56

Data used in Table 4.10.
P = Paralysed larvae NP = Non-paralysed larvae.

Appendix 4.16.

Calculation of $AVMB_1$ concentration (pMoles/mg).

In this example, data from a S. littoralis fifth instar non-paralysed nerve cord has been used.

Original concentration (radioactivity) = 9.37 mCi in 2.5ml
= 3.748 mCi in 1ml

Original concentration = 1.8% E.C. formulation
= 18000 ppm
Concentration of $AVMB_1$ used = 4500 ppm (µg/ml)
= 4.5 µg/µl
So 4.5 µg = 9.37×10^{-4} mCi
But 1 mCi = 2.22×10^{9} Dpm
So 4.5 µg = 2080140 Dpm/ml
Activity = 70% of original (calculated from half life of tritium [12.26 yrs])*

So activity (4.5 µg) = 2080140x0.7
= 1456098 Dpm/ml
Therefore 1.0 µg = 323577 Dpm/µg

Taking S. littoralis V N.P. nerve cord sample
= 167 Dpm/mg
= 167000 Dpm/g

To convert to ug = $\frac{167000}{323577}$
= 0.516 µg/g

1 Mole $AVMB_1$ = 820.2g/l (assuming an 80:20 mixture $AVMB_{1a}$:$AVMB_{1b}$ and molecular wt. of $AVMB_{1a}$ = 823 [Mellin et al., 1983]).
1 uMole = 820.2 µg/l
1 uMole = 0.8202 µg/g
Therefore 0.516 µg = 0.629 µMolar/kg
= 0.629 nMoles/g
= 0.629 pMoles/mg
(assuming all radioactivity is from $AVMB_1$)
However from TLC the % $AVMB_1$ was found to be 48.9% for this sample, therefore the concentration of $AVMB_1$
= 0.629x0.489
= 0.307 pMoles/mg

* In Chapter 4 the activity was calculated as 70% of the original, but in Chapter 5, carried out at a later date, the activity was calculated as 64% of the original.

Appendix 4.17.

Wash-off samples from S. littoralis V and VI instar larvae over 24 h treated with 1 µl (uncalibrated volume) of ^{3}H-AVMB$_{1}$.

Time (h)	Dpm per insect[2]	
	V instar	VI instar
0.1[1]	136676	164535
	162412	254707
	192726	184856
	213677	285057
	188859	296732
1.0	142742	164004
	143657	146603
	135159	143642
	122922	119689
	162262	175325
3.0	58388	49015
	67392	68919
	56722	83465
	66375	55814
	51693	77003
6.0	52887	46215
	31102	31585
	61888	51044
	51791	43052
	52176	43612
24.0	15474	30141
	34525	25492
	12164	21402
	15522	25350
	37708	30954

[1] It took 6 mins. to collect first set of samples (=0.1h).
[2] For dose see Section 4.2.6.
Data used in Figure 4.1.

Appendix 4.18.

Haemolymph samples from S. littoralis V and VI instar larvae over 24 h treated with 1 µl (uncalibrated volume) of ^{3}H-AVMB$_{1}$.

Time (h)		Dpm/ul haemolymph[2]	
		V instar	VI instar
0.1[1]		4.3	0.0
		0.1	3.2
		3.0	2.4
		4.0	0.0
		0.0	0.1
	$\bar{X}\pm$S.E.	2.3±0.9	1.1±0.7
1.0		3.9	9.0
		5.4	6.8
		3.7	6.5
		8.4	7.8
		7.7	8.1
	$\bar{X}\pm$S.E.	5.8±1.0	7.6±0.5
3.0		10.2	16.7
		13.8	17.9
		8.5	20.9
		7.7	16.1
		10.1	19.5
	$\bar{X}\pm$S.E.	10.1±1.1	18.2±0.9
6.0		14.5	23.6
		13.5	26.0
		10.7	25.6
		19.2	33.6
		12.5	25.3
	$\bar{X}\pm$S.E.	14.1±1.4	26.8±1.7
24.0		43.9	34.3
		30.1	43.1
		29.8	46.5
		42.5	76.7
		35.8	60.1
	$\bar{X}\pm$S.E.	36.4±3.0	52.1±7.4

[1] It took 6 mins to collect first set of samples (=0.1h).
[2] For dose used see Section 4.2.6.
Data used in Figure 4.2.

Appendix 4.19.

Fat body samples from S. littoralis V and VI instar larvae over 24 h treated with 1 µl (uncalibrated volume) of ^{3}H-AVMB$_{1}$.

Time (h)		Dpm/mg fat body[2]	
		V instar	VI instar
0.1[1]		0.0	0.9
		0.0	0.9
		1.3	0.5
		0.0	0.0
		0.5	0.1
	$\bar{X}\pm$S.E.	0.4±0.3	0.5±0.2
1.0		36.5	58.2
		64.1	48.6
		32.6	59.9
		23.1	56.4
		-	47.6
	$\bar{X}\pm$S.E.	39.1±8.8	54.1±2.5
3.0		29.9	73.9
		65.9	89.5
		46.0	64.7
		64.7	80.8
		37.6	91.7
	$\bar{X}\pm$S.E.	48.8±7.2	80.5±4.7
6.0		51.8	82.8
		89.7	99.2
		75.2	127.8
		118.5	104.3
		89.9	91.5
	$\bar{X}\pm$S.E.	85.0±10.9	101.1±7.6
24.0		152.9	157.9
		170.4	170.2
		126.1	181.2
		159.2	175.3
		149.4	190.2
	$\bar{X}\pm$S.E.	151.6±7.3	175.0±5.4

[1] It took 6 mins to collect first set of samples (=0.1h).
[2] For dose used see Section 4.2.6.
Data used in Figure 4.3.

Appendix 5.1.

Radioactivity extracted from nerve-cord samples of paralysed and non-paralysed larvae (24 or 48 h after application of ^{3}H-AVMB$_{1}$) following electrophysiology experiments.

Instar/h		Symptoms	Replicate (dpm/mg) [1] 1	2	3	4	5	Mean
Heliothis								
V	24	NP	490.2	442.1	262.5	226.6	266.6	337.6
		P	944.5	1130.3	2150.0	1498.4	2203.6	1585.4
VI	24	NP	294.3	392.5	124.5	318.1	179.5	261.8
		P	1172.1	573.3	464.5	1201.9	505.3	783.4
Spodoptera								
V	24	NP	169.6	122.1	160.3	189.7	101.4	148.6
		P	243.6	430.6	284.2	399.8	530.2	377.7
V	48	NP	174.7	192.1	182.9	126.3	110.3	157.3
		P	390.7	277.1	640.1	547.2	430.3	457.1
VI	24	NP	109.1	127.2	102.3	93.4	84.3	103.3
		P	169.7	230.3	224.4	148.6	236.7	201.9
VI	48	NP	97.3	108.6	131.3	110.1	134.7	116.4
		P	301.6	270.2	264.1	159.6	156.1	230.3

[1] Values are mean of two samples.
Data used in Table 5.1. and in Figures 5.1., 5.2. and 5.3.

Appendix 5.2

Spike frequency from untreated, treated-non-paralysed and treated-paralysed S. littoralis and H. armigera fifth and sixth instar larvae.

Replicate	Heliothis V 24h $\bar{X}$ Hz±S.E.		
	Untreated	Treated-non-paralysed	Treated-paralysed
1	63.3±2.3	7.1±0.6	2.3±0.6
2	26.3±0.5	6.1±1.5	1.8±0.2
3	35.6±2.5	4.7±0.7	2.9±0.5
4	27.4±0.5	3.5±0.3	4.5±1.2
5	28.1±0.5	22.8±1.6	3.6±0.3
6	30.9±0.8	22.9±3.6	2.6±0.2
7	57.8±2.4	24.1±0.7	4.4±1.0
8	24.4±1.2	14.2±1.5	1.3±0.3
9	25.2±3.1	13.9±0.5	1.7±0.7
10	35.9±2.1	8.9±1.7	1.9±0.2

Replicate	Heliothis VI 24h $\bar{X}$ Hz±S.E.		
	Untreated	Treated-non-paralysed	Treated-paralysed
1	30.1±1.1	27.3±0.3	9.8±0.7
2	41.8±1.5	21.5±0.3	4.4±0.3
3	34.7±0.7	20.2±0.4	14.5±0.4
4	75.7±0.8	34.8±0.7	8.6±0.6
5	39.6±0.6	30.0±1.1	14.4±0.4
6	66.5±0.8	44.0±0.6	21.0±0.3
7	44.3±0.6	29.9±0.6	12.1±0.6
8	38.5±1.0	28.1±0.5	5.6±0.6
9	20.2±0.4	30.6±0.5	7.4±0.4
10	33.0±0.5	24.1±0.8	13.3±0.5

Replicate	Spodoptera V 24h $\bar{X}$ Hz±S.E.		
	Untreated	Treated-non-paralysed	Treated-paralysed
1	29.8±0.9	9.3±1.1	6.5±0.7
2	29.3±1.2	8.2±0.2	2.1±0.6
3	25.4±2.2	15.7±0.9	0.7±0.1
4	25.2±2.5	12.5±2.1	1.0±0.2
5	48.0±3.7	6.0±0.2	4.7±1.0
6	25.9±2.1	7.2±0.9	3.2±1.1
7	33.2±3.1	4.1±0.3	2.1±0.2
8	31.6±0.6	8.9±0.8	0.4±0.2
9	28.8±0.5	20.6±1.9	0.4±0.1
10	43.6±0.8	25.9±1.5	0.6±0.1

Replicate	Spodoptera V 48h $\bar{X}$ Hz±S.E.		
	Untreated	Treated-non-paralysed	Treated-paralysed
1	29.8±0.9	8.7±1.1	3.1±0.4
2	29.3±1.2	4.7±0.1	2.7±0.4
3	25.4±2.2	5.1±0.3	2.4±0.2
4	25.2±2.5	7.5±0.3	4.5±0.3
5	48.0±3.7	6.4±0.7	0.2±0.05
6	25.9±2.1	4.5±0.4	0.7±0.2
7	33.2±3.1	5.2±0.2	0.1±0.02
8	31.6±0.6	9.4±1.2	0.1±0.01
9	28.8±0.5	11.3±0.6	0.8±0.1
10	43.6±0.8	9.4±1.5	2.1±0.6

Replicate	Spodoptera VI 24h $\bar{X}$ Hz±S.E.		
	Untreated	Treated-non-paralysed	Treated-paralysed
1	44.5±0.9	12.0±0.8	2.5±0.6
2	59.1±1.2	12.9±0.3	7.6±1.5
3	57.9±0.7	9.0±0.2	1.5+0.1
4	56.9±0.8	4.9±0.2	3.0±0.1
5	23.6±0.5	15.9±0.4	2.2±0.2
6	25.4±0.5	14.2±0.5	5.4±0.2
7	26.1±0.8	19.3±0.7	7.0±0.3
8	25.6±0.5	15.1±1.5	7.6±0.3
9	38.7±0.8	25.8±0.5	2.1±0.2
10	28.8±0.6	19.4±0.4	1.5±0.1

Replicate	Spodoptera VI 48h $\bar{X}$ Hz±S.E.		
	Untreated	Treated-non-paralysed	Treated-paralysed
1	44.5±0.9	20.6±0.4	2.8±0.3
2	59.1±1.2	19.0±0.4	3.5±0.3
3	57.9±0.7	18.5±0.4	8.5±0.6
4	56.9±0.8	16.7±0.4	9.6±0.3
5	23.6±0.5	12.8±1.4	8.3±0.8
6	25.4±0.5	11.2±0.2	9.1±1.0
7	26.1±0.8	15.0±1.0	11.4±0.3
8	25.6±0.5	15.5±0.7	11.4±0.3
9	38.7±0.8	13.6±0.5	11.9±0.2
10	28.8±0.6	11.6±0.4	11.7±0.3

The values given are the means of 120 0.5second bins of data.
Data used in Table 5.1. and in Figures 5.1., 5.2. and 5.3.

Appendix 5.3.
Action Potentials ($\bar{X}$ Hz±S.E.) over 24 h

Spodoptera 6th instar control - untreated

	0h	1h	2h	4h	8h	12h	24h
1	33.0±0.9	29.2±1.4	27.0±0.6	26.6±0.8	26.8±0.8	26.3±0.8	27.6±0.7
2	32.8±1.0	27.7±1.1	27.1±0.9	27.3±0.7	28.2±0.7	29.7±0.9	25.1±1.0
3	28.9±1.1	26.3±1.0	25.1±1.0	27.2±0.8	27.6±0.9	28.8±0.9	26.6±1.0
4	36.3±0.7	34.9±1.4	29.6±1.0	29.6±1.2	29.1±1.1	29.8±1.3	29.7±1.0
5	34.5±0.9	34.2±1.0	29.8±1.1	27.3±0.9	30.3±1.2	29.2±1.7	24.9±0.9
6	40.7±0.7	38.9±0.8	38.7±0.9	39.2±1.0	37.3±0.8	36.9±0.7	32.8±0.6
7	41.8±0.8	40.9±0.6	39.7±0.7	39.4±0.8	38.8±1.0	40.0±0.8	36.4±0.7
8	38.7±0.7	38.0±0.8	38.2±0.7	37.8±0.9	37.9±0.8	36.4±0.9	35.8±0.7
9	40.7±0.8	38.8±0.9	35.4±0.8	35.2±0.7	37.1±0.9	36.3±0.8	34.6±0.8
10	32.1±0.8	29.2±0.6	29.1±0.8	29.9±0.6	30.2±0.7	29.9±0.7	27.8±0.8

Appendix 5.3.
Action Potentials ($\bar{X}$ Hz±S.E.) over 24 h

Spodoptera 6th instar treated with 1µl AVMB1

	0h	1h	2h	4h	8h	12h	24h
1	43.2±1.2	39.4±1.6	29.6±0.8	15.8±0.6	7.6±0.4	2.9±0.3	2.3±0.3
2	43.5±1.3	35.8±1.5	29.4±1.5	18.2±0.6	13.9±0.5	1.5±0.2	1.4±0.2
3	42.0±1.3	37.9±1.4	31.2±1.5	16.3±0.5	13.7±0.2	4.1±0.4	3.7±0.7
4	37.4±1.2	36.2±1.2	32.4±0.4	14.8±0.9	13.5±0.8	12.1±0.6	8.3±1.0
5	31.6±2.1	29.6±0.8	24.2±1.1	11.3±0.7	10.0±0.8	8.9±1.2	4.9±0.6
6	37.9±1.2	37.7±1.0	37.3±1.0	17.3±0.8	15.1±0.9	10.3±0.8	9.9±0.7
7	35.8±1.0	34.8±1.2	32.7±1.1	20.1±1.0	14.3±0.9	9.2±0.9	4.0±0.7
8	39.6±1.1	38.6±1.1	36.9±1.2	23.6±1.0	19.4±0.6	12.2±0.5	8.7±0.5
9	44.9±1.3	42.8±1.2	39.7±1.4	24.7±1.1	17.5±0.6	11.6±0.6	9.3±0.6
10	36.8±1.2	35.4±0.9	34.5±0.7	21.4±0.8	12.1±0.5	5.7±0.4	3.1±0.4
11	40.3±1.4	39.7±1.3	38.4±1.3	26.1±1.0	18.3±0.5	8.7±0.7	3.7±0.4
12	41.5±1.0	40.2±1.2	40.4±1.4	23.7±0.9	14.6±0.7	9.7±0.4	8.3±0.6

Appendix 5.3.
Action Potentials ($\bar{X}$ Hz±S.E.) over 24 h

Spodoptera 5th instar control - untreated

	0h	1h	2h	4h	8h	12h	24h
1	34.1±2.6	30.8±1.1	29.9±0.9	31.7±0.8	30.1±0.8	31.6±0.9	30.1±1.1
2	35.6±0.6	31.4±0.9	29.9±1.0	32.5±1.4	31.5±1.1	29.2±1.0	28.7±0.9
3	33.8±1.1	30.8±1.2	30.2±1.6	33.5±1.4	28.0±0.8	29.1±0.7	26.1±0.7
4	39.4±0.9	38.6±0.8	37.9±0.9	37.8±0.7	38.4±0.8	38.6±0.7	32.4±0.7
5	42.3±0.8	41.6±1.0	40.3±0.9	39.4±0.8	39.6±0.7	38.3±0.8	37.4±0.7
6	40.9±1.2	39.8±1.0	39.7±0.9	38.4±1.0	38.2±0.8	36.3±0.7	33.7±0.8
7	39.9±1.0	39.4±0.9	38.7±0.7	38.8±0.9	37.1±0.8	36.5±0.8	34.7±0.7
8	38.2±1.1	37.1±0.8	36.4±1.0	36.0±0.9	35.2±0.8	35.6±0.7	36.1±0.9
9	39.7±0.9	39.3±0.8	39.1±0.8	37.8±0.9	36.9±0.7	37.3±0.9	34.7±0.7
10	37.7±1.0	36.8±0.9	35.3±0.7	36.2±0.9	35.9±0.7	35.2±0.9	32.8±0.8

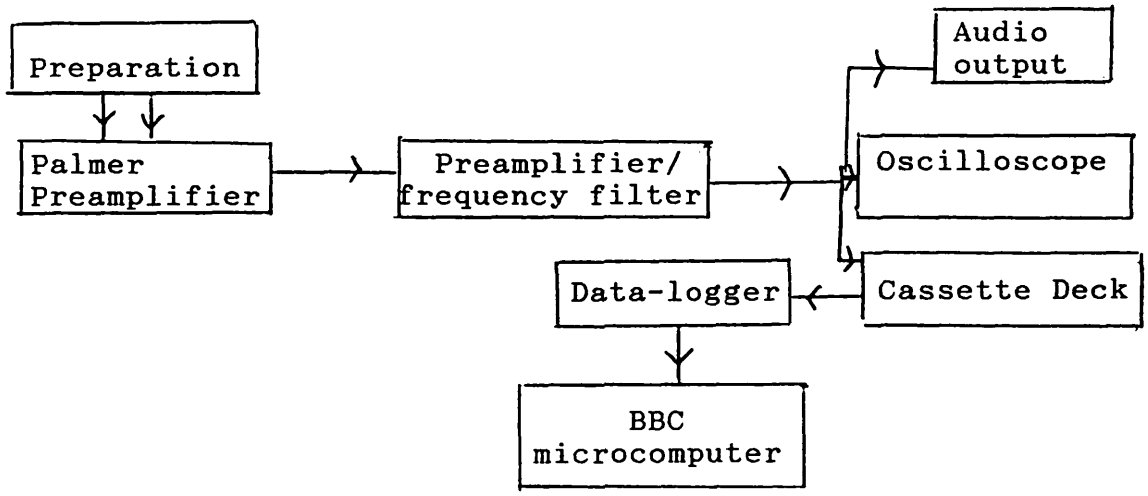
Appendix 5.3.
Action Potentials ($\bar{X}$ Hz±S.E.) over 24 h

Spodoptera 5th instar treated with 1µl $AVMB_1$

	0h	1h	2h	4h	8h	12h	24h
1	43.4±0.7	42.7±1.1	40.7±0.7	39.9±0.9	24.1±0.9	9.8±1.0	5.2±0.8
2	38.3±0.6	36.9±1.0	35.9±0.6	36.0±0.8	18.6±1.2	7.2±0.6	4.1±0.5
3	39.1±0.8	37.9±0.9	37.2±0.8	35.1±0.6	23.6±1.3	10.1±0.7	3.6±0.6
4	39.3±0.7	38.7±0.8	38.9±0.9	35.9±0.8	24.8±0.9	12.3±0.9	8.7±0.8
5	38.3±0.6	37.4±0.9	36.4±0.8	37.3±0.7	20.7±0.9	11.4±0.8	4.2±0.8
6	42.8±0.8	41.5±0.6	41.6±0.7	40.1±0.8	25.7±1.1	13.2±0.7	10.2±0.8
7	36.9±0.6	37.0±0.8	35.8±0.7	35.4±0.7	21.6±0.6	10.9±0.4	3.9±0.5
8	41.8±1.1	40.8±0.9	39.7±0.8	38.6±0.9	20.9±0.8	12.2±0.6	9.2±0.4
9	35.6±0.8	34.8±0.9	34.9±0.7	33.7±0.7	18.5±0.6	10.0±0.6	8.4±0.3
10	36.7±1.2	36.9±1.0	35.9±0.9	33.7±0.5	23.9±0.7	14.7±0.5	10.3±0.6
11	39.9±1.1	38.9±1.0	38.4±0.6	38.9±0.7	24.2±0.8	13.2±0.7	4.7±0.4

Appendix 5.4.

A). Arrangement of recording equipment.



B). Set up of equipment for datalogging.

