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INSECTICIDE RESISTANCE IN DIAMONDBACK MOTH

(*Plutella xylostella*) (Lepidoptera: Plutellidae)

A thesis presented in partial fulfilment
of the requirements
for the degree of Master of Philosophy
in Plant Health at Massey University.

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ABSTRACT

Diamondback moth is a cosmopolitan species of considerable importance as a pest of cruciferous plants. It is capable of rapid growth in numbers and has a high potential for the development of insecticide resistance. By 1986 resistance had been confirmed to 23 insecticides in 16 countries.

A susceptible population was identified from a forage brassica crop on a Massey University farm. Insects collected were used to establish a susceptible laboratory colony. Full dose mortality curves for a range of insecticides were constructed for this colony using leaf dip bioassays. The LD_{95} values were determined for each of the 7 insecticides tested and used as diagnostic doses to screen field populations for resistance.

Field populations regularly exposed to insecticides were sampled at five locations in intensive market garden areas in the North Island. Larvae and pupae collected from these sites were reared to the F1 generation in the laboratory. Third instar larvae were then tested for resistance using leaf dip bioassays treated with the diagnostic doses. Some insecticide resistance was detected at each of the five sites. Insect survival for the site showing the highest resistance levels ranged from 82% to 16% when exposed to the diagnostic dose (LD_{95} for the

susceptible population). Only one site showed resistance to all of the insecticides screened and there seemed to be no pattern to the cross resistance spectra encountered on each site. All five sites had different histories of pesticide usage. Two of the five sites were in close proximity but they were geographically isolated from the remaining 3 sites which were isolated from each other.

High levels of parasitism by *Diadegma semiclausum* was evident in all of the field populations tested. The impact that this is having on the development of resistance is unknown but warrants further study.

Even though resistant insects were found, their numbers were low and the crop loss too small to be of concern to the growers. However in the light of experience in South East Asia it would be prudent to formulate resistance management tactics for New Zealand conditions.

I suggest that a number of reccomendations should be made to growers with respect to their diamondback moth control programmes. Pyrethroid use should be restricted to one application per brassica crop. The use of control action threasholds should be encouraged as should the use of less persistent insecticides such as dichlorvos and mevinphos. Urgent attention should be given to the development of an efficient grower operated monitoring programme. The feasibility of operating an integrated pest management programme should also be investigated.

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TABLE OF CONTENTS.

page

Abstract ii

Acknowledgements iv

Chapter 1: Insecticide Resistance 1

Chapter 2: The Diamondback Moth 7

2.1 The Adult 8

2.2 The Egg 8

2.3 The Larva 9

2.4 The Pupa 12

2.5 Confusion with *Plutella antiphona* 13

Chapter 3: Measuring Resistance 15

3.1 Resistance ratios 15

3.2 Single dose tests 17

3.3 Biochemical assays 23

3.4 Identification of suspected resistant populations 26

3.5 Population sampling 27

3.6 Establishing a laboratory colony 28

3.7 Using bioassays 28

3.8 Insect handling methods 34

Chapter 4: Experimental Techniques For Determining

Resistance in Diamondback Moth 35
4.1 Kalra and Chawla (1977) 35
4.2 Sudderudin and Kok Pooi-Fong (1978) 37
4.3 Busvine (1978) F.A.O. method 21 38
4.4 Liu, Chen and Sun (1984) 39
4.5 Tabashnik and Cushing (1987) 40
4.6 Tabashnik et al (1987) 41
4.7 Iman et al (1986) 42
4.8 Cheng (1986) 45

Chapter 5: Insect Handling Methods 48
5.1 Field collection of diamondback moth larvae and pupae 48
5.2 Egg numbers 49
5.3 Insect removal for testing 49

Chapter 6: Experimental methods with insecticides 57
6.1 Identification of a susceptible population 57
6.2 Sampling field populations of diamondback moth 63
6.3 Resistance testing 66
6.4 Stability of resistance in diamondback moth 69

Chapter 7: Results and Discussion 70
7.1 Identification of a susceptible

population 70

7.2 Sampling field populations of diamondback moth 79

7.3 Pesticide usage at each site 82

7.4 Resistance testing 83

7.5 Stability of resistance in diamondback moth 94

Chapter 8: Managing Insecticide Resistance In Diamondback Moth 98

8.1 Resistance mechanisms in diamondback moth 106

8.2 Managing resistance in diamondback moth 110

8.3 Resistance management case studies 118

8.4 Insecticide resistance management programme for diamondback moth 122

References 124

LIST OF FIGURES

		page
Figure 1:	The development of insecticide resistance	4
Figure 2:	Frequency distribution of head capsule widths of 150 larvae from the Massey laboratory colony.	11
Figure 3:	Dose mortality curves for permethrin for *Culex quefasciatus*.	19
Figure 4:	Probit mortality lines for diazinon, brush verse vibration removal.	52
Figure 5:	Probit mortality lines for permethrin, brush verse vibration removal.	53
Figure 6:	Probit mortality lines for esfenvalerate, brush verse vibration removal.	54
Figure 7:	Probit mortality lines for methomyl, brush verse vibration removal.	55
Figure 8:	Map of the North Island of New Zealand showing the collection sites for diamondback moth resistance testing.	65
Figure 9:	Pupal structures showing antennal and leg cases of *P. antiphona* and *P. xylostella*.	67
Figure 10:	Dose mortality lines using leaf dip bioassays.	75
Figure 11:	Diagnostic dose test Pukekohe 1 colony	85
Figure 12:	Diagnostic dose test Pukekohe 2 colony	86
Figure 13:	Diagnostic dose test Hawkes Bay colony	86

Figure 14: Diagnostic dose test Palmerston North colony. 87

Figure 15: Diagnostic dose test Levin colony 87

Figure 16: Diagnostic dose test Massey colony (control). 88

Figure 17: A comparison of the resistance levels and insecticide selection for each site. 89

Figure 18: The resistance ratios of two strains of diamondback moth selected with phenthoate 92

Figure 19: Changes in susceptibility to 4 pyrethroids of a mixed strain of diamondback moth upon relaxation of selection pressure. 96

Figure 20: Changes in susceptibility to 4 organophosphates of a mixed strain of diamondback moth upon relaxation of selection pressure. 97

LIST OF TABLES

page

Table 1: Mean head capsule widths of diamondback moth larvae. 11

Table 2: Susceptibility of diamondback moth larvae to diazinon by different bioassay procedures. 30

Table 3: A comparison of different treatment methods for *Epiphyas postvittana* larvae. 31

Table 4: Effectiveness of various insecticides against a resistant strain of diamondback moth in laboratory and field trials. 45

Table 5: Summary of data comparing two different insect removal methods for each of 4 insecticides. 51

Table 6: Comparison of LD_{50} values for topically applied insecticide for the Massey colony and other susceptible colonies. 71

Table 7: Comparison of LC_{50} values for leaf dip bioassays for the Massey colony and other susceptible colonies. 71

Table 8: LC_{95} values for the Massey University diamondback moth colony. 73

Table 9: Conformation of the Massey dose mortality curves for the Massey population to the

curves for the Massey population to the probit model. 78

Table 10: Insecticide collection. 80

Table 11: Laboratory selection studies of cross resistance in diamondback moth. 94

Table 12: Stability of resistance in the Pukekohe 2 colony after 5 generations. 95

Table 13: Comparison of leaf dip, vial and disposable cup bioassays treated with the diagnostic dose. 102

Table 14: Comparison of laboratory bioassays and field test results. 103

CHAPTER ONE

INSECTICIDE RESISTANCE.

There is considerable complexity and even controversy over the concept of insecticide resistance. However in practical crop protection, resistance is inevitably connected to the expected result of a pesticide application and manifests itself as insect control failures (Brattsten 1989). Insecticide resistance was first recorded between 1911 and 1916 when San Jose scale resistance to lime sulphur and Californian red scale resistance to hydrogen cyanide was detected (Long 1982). To date, insects have developed resistance to nearly every type of insecticide in common usage (Brown and Payne 1988) with over 447 economically important insect species showing resistance to one or more insecticides (Wilson 1988, Brattsten 1989).

Resistant strains develop through the survival and reproduction of individuals carrying a genome that allows them to withstand exposure to doses of an insecticide that would have otherwise proven fatal (Brattsten et al 1986). The process by which insect populations become resistant is one of evolutionary change, where the organism is changing to meet changes in its environment. In this case the changes are the addition of toxic chemicals to their habitat by man. However this type of selection pressure is

not new to phytophagous insects, as many plants produce toxic compounds as part of their defence system against insects. Some insects, in response to such selection pressure imposed on them by plants. have evolved methods of escaping the effects of these natural toxins. The host range of any given phytophagous insect species is in fact limited not so much by the nutritive value of the plants available as food but rather by its ability to tolerate the allelochemics of the potential host plants.

In those cases where the methods of escape from these plant toxins have been elucidated it appears that the insect has not one defence against the toxic plant chemicals but several or even many. Tobbacco hornworm for example appears to have six or seven built in mechanisms which enable it to avoid poisoning by nicotine (Brattsten 1989). If such multiple defence systems are the end point of the development of insect resistance to allelochemics then perhaps the same will prove true of the development of resistance to synthetic insecticides. Thus for example Sun et al (1986) observed that both metabolic and non metabolic mechanisms could be responsible for pyrethroid resistance in DBM. Liu et al (1981) claim that enhanced microsomal oxidation is a major factor in fenvalerate and permethrin resistance in DBM and that enhanced esterase hydrolysis was an additional minor contributor to permethrin resistance. The main mechanism for organophosphate and carbamate resistance in DBM seems to

be acetylcholinesterase insensitivity (Liu et al 1981, Chen and Sun 1986, Miyata et al 1986, Sun etal 1986).

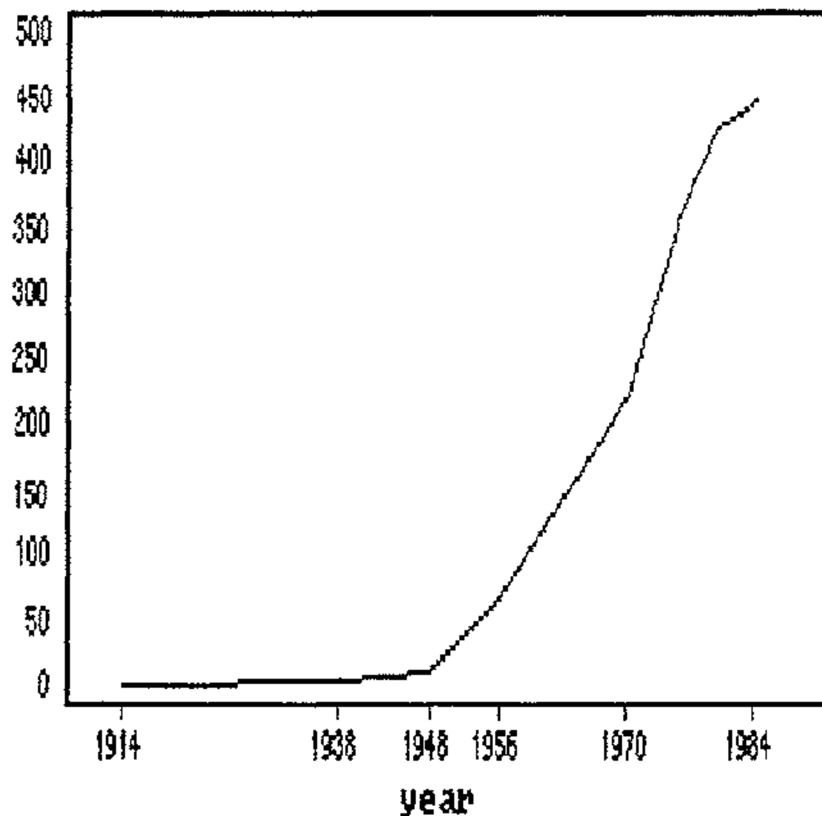
Insecticide resistance seems to be an inevitable and unavoidable consequence of persistent selection pressure. The only uncertainty is the time taken for the resistant strain to become predominant.

In contrast to the presumably slow evolution of resistance to natural toxins, resistance to synthetic insecticides has developed extremely rapidly. Brattsten et al (1986) suggest that this is probably due in part to some of the mechanisms that have evolved as a defence to plant allelochemicals being also an appropriate defence against synthetic insecticides. Figure 1. shows that the number of insect species showing resistance to one or more insecticides has increased dramatically in the last two decades. This is a phenomenon which in retrospect should not have been entirely unexpected and further development of resistance may be expected to continue at an increasing rate, unless we change the way in which we manipulate the insect's environment.

Brattsten (1989) claims that the concept of resistance as commonly used is a little artificial. For many years we have considered resistance as if it were a unique problem that happens to interfere with the chemical control of harmful insect species. However, in reality resistance is a case of biotype evolution. This is a natural process that occurs in response to any form of

selection pressure. Modern agricultural and horticultural practices with their heavy reliance on pesticides have increased the selection pressure on insect pests.

Fig 1: The Development of Insecticide Resistance.
(Metcalf 1980)



This may mean that field populations in areas regularly treated with pesticides are likely to have acquired some degree of resistance, inferring that the genetic constitution has been changed in practically all insects that inhabit areas exposed to pesticides. This has a number of important implications when it comes to

measuring insecticide resistance.

The diamondback moth is a cosmopolitan insect species of considerable importance as a pest of cruciferous plants. Diamondback moth (*Plutella xylostella* Lepidoptera: Plutellidae) has 14-28 generations per year in Malaysia, 15-20 in Taiwan, 5-12 in Japan and 6-7 in New Zealand, with overlapping of all development stages (Miyata et al 1986; Valentine 1975). This means that the species is capable of rapid growth in numbers and has a high potential for the development of insecticide resistance. Since 1953 numerous cases of insecticide resistance by diamondback moth to various types of insecticide have been reported from around the globe. By 1986 resistance to 23 insecticides in 16 countries had been confirmed (Georghiou 1981; Sun et al 1986). The highest levels of resistance have been found in the Ban Chau strain in Taiwan where the resistance ratios (LD_{50} of the resistant population/LD_{50} of a susceptible population) range from 2 for *Bacillus thuringiensis* to 50,000 for cyanofenphos, with intermediate values for eleven other insecticides (Sun et al 1986). In 1987 the newly formed New Zealand Committee on Pesticide Resistance identified diamondback moth as a potential resistance problem in this country (Elliot et al 1987).

The aim of this work was to establish whether or not diamondback moth was developing resistance to insecticides in New Zealand and to propose a set of management

strategies that would either minimise the impact of resistance or reduce the likelihood of its development.

CHAPTER 2

THE DIAMONDBACK MOTH.

Diamondback moth (*Plutella xylostella*) is one of the most widely distributed insects in the world. It has been reported from more than 80 countries and is probably found wherever crucifers are grown. Large numbers of adult moths have even been caught as far as 1600 kilometres out in the Atlantic and Pacific Oceans (Tabashnik et al 1987). Robertson (1939) reports that in 1939 diamondback moth was known to have been present in New Zealand for at least 50 years.

The life history of diamondback moth (DBM) has been studied by many researchers around the world. Robertson (1939) summarised much of the early work on DBM. She found that the life cycle varied considerably in the different environments in which it occured. In England and Northern Europe there are two to four generations a year and the insect appears to overwinter in the pupal stage. In the lower Volga region of Russia there are four complete and overlapping generations a year. In Colorado, United States of America, there are seven generations a year but the insects are only active during the summer months and spend the winter hibernating as adults. In the Southern States of the United States of America diamondback moth is widespread and is active throughout the whole year. DBM is also a wide spread pest of cruciferous crops in South

Africa, where there are as many as ten generations per year without any hibernation period. In tropical countries the moth is active during the whole year but is more abundant during the cooler months although this difference seems to be due to rainfall rather than temperature. Robertson (1939) reported that in New Zealand there are six or possibly seven generations per year and the insect remains active during the whole year. It is evident from this data that DBM has the capacity to modify its life cycle to suit almost any climate.

Robertson (1939) and Valentine (1975) have described the life cycle of *Plutella xylostella* in New Zealand.

2.1 The Adult

The adult moths are small, greyish and with a wing span of about 7mm. They are usually inactive during the day and are frequently found resting on the underside of leaves during daylight hours. The adult stage probably lives more than 10 to 14 days in the field. Robertson (1939) kept moths alive under laboratory conditions for 25 to 30 days.

2.2 The Egg.

The egg is oval in outline and measures approximately 0.49mm by 0.26mm. It is light yellow in colour and has a crinkly sculptured surface. The eggs may be laid singly, in groups of 3 or 4, or in batches of 10 to 20. In the

field the eggs are usually laid on the underside of host plant leaves around the leaf margins and along the veins. However in enclosed cages in the laboratory, the eggs may be laid on both surfaces of the leaves and on petioles. Robertson (1939) reported wide variation in the number of eggs laid by a single female ranging from 60 to 450 per female. There was no correlation between longevity of the insect and the number of eggs laid. Robertson (1939) concluded that the number of eggs per female is dependant largely on environmental factors and that the number laid under artificial conditions gives little indication of the number normally laid in the field. Valentine (1975) claimed that each female lays up to 100 eggs in natural conditions. Harcourt (1986) found that the fecundity of DBM was correlated to the crude protein content of cabbage leaves (r^2 - 0.954).

2.3 The Larva.

Robertson (1939) claimed that DBM larvae pass through four instars. However, Valentine (1975) says that there are five larval instars. This apparent disparity between the two authors is of considerable concern because the F.A.O. standard method for resistance testing in DBM states that there are five instars and that the fourth instar should be used for testing (Busvine 1978).

Therefore in the present work an investigation was undertaken to determine the number of instars and to

produce a set of reference specimens of each instar to assist in distingishing the different instars later in the experiments. Robertson (1939) claims that the measurement of the head capsule width is the only reliable method of distinguishing the different instars of diamondback moth.

Approximately 150 larvae were randomly selected from the Massey University laboratory colony. The larvae were killed and placed dorsal surface uppermost, on microscope slides that had been lightly smeared with petroleum jelly. The head capsule width of each larva was measured using a microscope fitted with an ocular micrometer and the larva placed in the appropriate size class (intervals 0.01mm). Figure 2 shows the frequency distribution of the head capsule widths. This graph clearly indicates four larval instars.

Robertson (1939) also measured the head capsule width of DBM larvae and her results and those of Harcourt (1986) together with my data are shown in table 1. The close similarity of these three sets of data indicate four larval instars in DBM. However, although a review of the literature revealed that most authors consider that there are four instars, opinion is divided. Tabashnik and Cushing (1987) used third instar larvae in their tests as did Kalra and Chawla (1977), under the belief that there are a total of four instars, while Busvine (1978) and

Figure 2: Frequency distribution of head capsule widths of 150 diamondback moth larvae from the Massey laboratory colony.

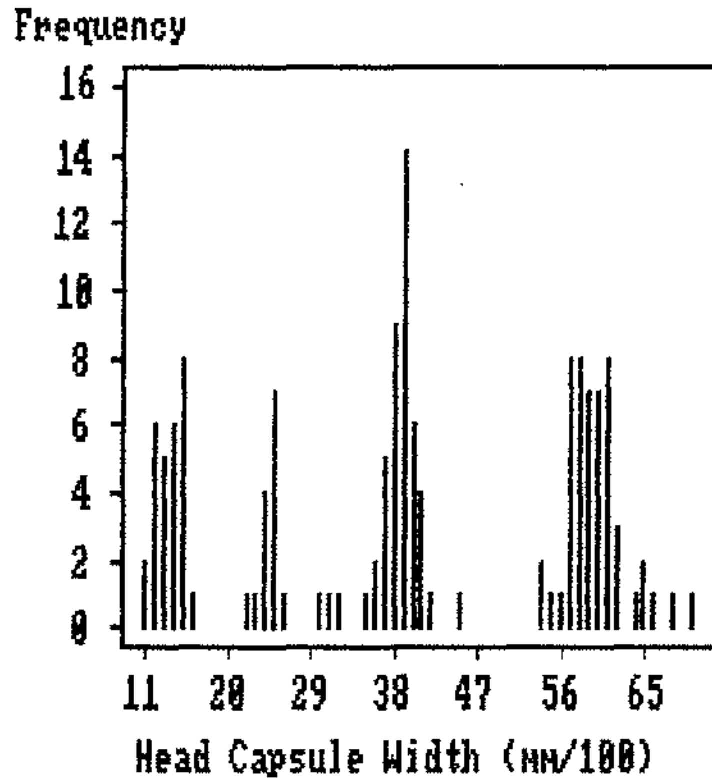


Table One: Mean head capsule widths of diamondback moth larvae.

Population	Larval Instar			
	1st	2nd	3rd	4th
Robertson	0.163mm	0.264mm	0.378mm	0.628mm
Harcourt	0.156mm	0.240mm	0.379mm	0.597mm
Massey Un.	0.135mm	0.244mm	0.387mm	0.597mm

Sudderuddin and Kok Pooi-Fong (1978) used fourth instar in

their trials on the understanding that there are five larval instars. Salinas (1986) studied the life cycle of DBM in Venezula and found four larval instars. Ooi (1986) reports that the life cycle of DBM in Malaysia varies considerably depending on the environment under which it develops. In the lowlands of Malaysia the life cycle can be completed in as little as 13 days while in the highlands it can take as long as 27 days, but in both cases the number of larval instars recorded was four. Bhalla and Dubey (1986) found four larval instars in populations of DBM collected from the Northwestern Himalayas. Chelliah and Srinivasan (1986) report that different workers in India have found four and five instars in DBM. It would seem that in most situations this insect has four larval instars but that this may not always be the case. While it seems that both four and five instars may have been found in New Zealand four is probably the most common.

2.4 The Pupa.

Towards the end of the fourth larval instar the larva ceases feeding and frequently migrates from the host plant to a dead leaf lying on the ground or to some other crucifer near by. When a protected position suitable for pupation has been found the larva commences to spin a cocoon. This is usually completed within 24 hours and is followed by a period of quiescence marking the prepupal

stage. Under laboratory conditions it has been found that between one and a half to two days elapse between completion of the cocoon and pupation. The pupa is of the obtect type characteristic of the more specialised groups of lepidoptera.

2.5 Confusion with *Plutella antiphona*.

Dugdale (1973) found that the New Zealand collections of *P. xylostella* contained two species superficially indistinguishable but having distinctly different larval pupal and genitalic characters. The two species were *P. xylostella* and *P. antiphona*. Dugdale (1973) reported that these two species are sympatric over most of New Zealand and that there are a number of ways of telling them apart.

(A) The adult

In New Zealand the most dependable characters that can be used to distingush adults of these two species are genitalic: the needle like aedeagus, the string brush of setae at the valval base, and the presence of an ostiolar papilla.

(B) The pupa

In the pupa of *P. antiphona* the apices of the middle tarsal sheaths extend beyond the haustellum sheath apex. However in the pupa of *P. xylostella* the haustellum sheath is longer than the middle tarsal sheaths. These

characteristics can be readily seen on old pupal cases.

(c) The larva

The larva of *P antiphona* has incrassate strongly capitate setae. These are more pronounced on the prothoracic sheild.

Dugdale (1973) found both *P. xylostella* and *P. antiphona* to be spread throughout New Zealand and an examination of collections of *Plutella* revealed that they may have been considered as a single species in the past.

CHAPTER 3

MEASURING RESISTANCE.

The first signs of insecticide resistance in a wild insect population are usually manifest as control failures. However such control failures may be caused by a host of factors. If the problem is due to insecticide resistance it is usually confirmed using one or more of three types of laboratory tests.

3.1 Resistance Ratios:

This test method uses a range of insecticide doses to produce a dose mortality curve. Resistance is then expressed as the ratio of the LD_{50} or the LD_{95} of the resistant strain to that of the susceptible strain (Halliday and Burnham 1990). The resistance ratios based on LD_{50}s for the Ban Chau strain of DBM in Taiwan, one of the most resistant strains known, range from 50,000 for cyanofenphos to 2 for *Bacillus thuringiensis*, (Sun et al 1986).

There are a number of problems associated with this method. First, the reference (ie susceptible) population may also have some degree of tolerance to the pesticide. This can be alleviated to some extent by comparing the data for the reference population to that of other researchers. If the LD_{50} of the test population is significantly greater than that reported for others then

its rejection should be considered. If a suitable reference population cannot be found then it may be possible to use data from other populations for comparison provided that the test method is identical. Sudderuddin and Kok Pooi-Fong (1978) tested a strain of DBM from the Cameron Highlands in Malaysia for insecticide resistance. This is an area where the insect is a serious pest, and if cabbage crops are not treated regularly with insecticides then most will be lost to DBM attack. In this situation it is unlikely that a susceptible strain could be identified with confidence in the field. In this case the authors obtained susceptible insects from a colony in France.

Roush and Millar (1986) claim that computation of the resistance ratio is a very inefficient method of testing for resistance as the frequency of resistant individuals must be high, say greater than 20%, before the LD_{50} is appreciably changed. This implies that the method is relatively insensitive.

Furthermore the development of a complete dose mortality curve is time consuming and requires large numbers of individuals for testing. If the level of resistance in the wild population under test is such that few individuals can be isolated to establish a laboratory colony then this could give rise to two major problems. Firstly, the insects may have to be reared through several generations to produce sufficient offspring to enable the

test to proceed. This could lead to a reduction in the level of resistance in the colony. The second problem is that if only a few insects are used to establish the colony then it may not fully represent the resistant genotype occurring in the field.

3.2 Single Dose Tests.

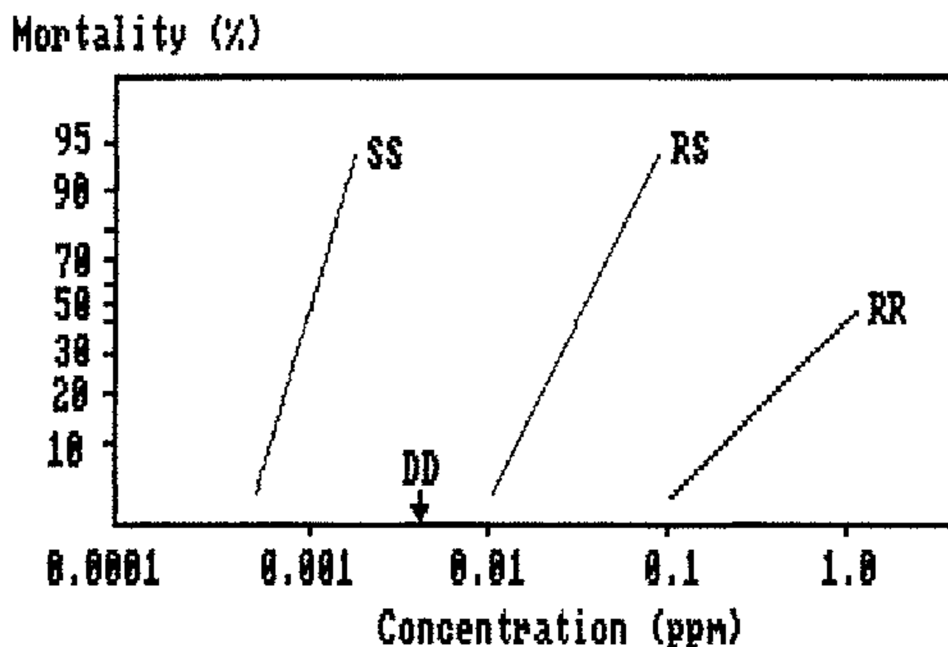
These tests involve the use of a single test dose and a comparison of the mortalities of the susceptible and the test strains at that dose. These tests have been used by various authors and referred to as either discriminating or diagnostic dose tests. Halliday and Burnham (1990) explain the usage of this terminology. "The term discriminating dose is properly used when enough genetic and toxicological evidence has accumulated that shows that a dose causes a different response between genotypes. Diagnostic dose is a less rigorous term and is used when one wants to monitor resistance but is less certain that the dose does separate out genotypes." The major advantage of a single dose test is speed. Fewer individuals need be tested, the test can be conducted more quickly and less time is spent raising insects for testing. It may also be possible to test the F1 generation of the test population rather than raise it through several generations to obtain the numbers necessary to produce a complete dose mortality curve.

However, there are also problems associated with this

test method. A discriminating dose can only be accurately determined once resistance has developed to the point where a dose mortality line can be constructed for a population known to contain both resistant and susceptible individuals. Figure 3 shows the dose mortality lines for the larvae of *Culex quinquefasciatus*, susceptible (SS), heterozygous (RS), and homozygous resistant (RR) strains tested with permethrin by Georghiou and Taylor (1986). For this population an insecticide dose (DD) can be selected that is likely to kill all of the susceptible insects without causing significant mortality of those carrying the resistance gene.

Where there is insufficient data to establish a discriminating dose, Busvine (1980) states that the discriminating dose can be based on the dose mortality regression line established for a susceptible population by selecting a dose with a high probability of killing all of a sample of normally susceptible insects. If we use the terminology of Halliday and Burnham (1990) then this dose should be referred to as diagnostic rather than discriminating. Busvine (1980) suggests that this, the diagnostic dose, should be set at the LD_{99} level for a susceptible population and that resistance should be suspected if more than 1% of the test insects survive the treatment. While the $LD_{99.9}$ would be less likely to give a false indication of resistance the use of this dose may mean that low levels of resistance are not detected.

Figure 3: Dose mortality curves for permethrin for *Culex quinquefasciatus* (Georghiou and Taylor 1986).



Roush and Millar (1986) point out that, where the test technique is not perfectly diagnostic, as could well be the case when the selection of the test dose is based on data obtained from a susceptible population, one cannot rely on detecting at least one resistant individual. The use of an LD_{99} value as a diagnostic dose, as recommended by Busvine (1980), assumes that 1% of the treated susceptible individuals will survive. This means that a statistical test must be used to determine if the observed fraction of survivors is significantly greater that the 1% expected. Roush and Millar (1986) claim that the most appropriate test would be a single sided Z test.

Daly and Murray (1988) claim that using a diagnostic

dose to estimate the frequency of resistance may be inaccurate because we do not know if applying a diagnostic dose reliably distinguishes between susceptible and resistant individuals. A truly discriminating dose should kill all susceptible but no heterozygous or homozygous resistant insects. Daly and Murray (1988) found that this is unlikely when the LD_{99} for a susceptible population is used as the discriminating dose. In a mixed strain of resistant and susceptible *Heliothis armigera* in Australia 5 - 60% of the heterozygotes were killed at the diagnostic dose when it was set at the LD_{99} for susceptible insects. This is not unexpected where resistance is weak (low level) or incompletely recessive.

Guided by the experience of Daly and Murray (1988) McCutchen et al (1989) used the LD_{80-90} rather than the LD_{99} as a diagnostic dose to test for resistance in a population of *Heliothis virescens*. When they plotted percent mortality against log-dose it was evident that had they used the LD_{99}, between 50% and 80% of the resistant insects in their test population would have died. They concluded that the use of the LD_{80-90} as the diagnostic dose allowed them to detect resistance at a relatively low frequency. They confirmed this by using their diagnostic dose to select out the resistant individuals from a population of neonate larvae. The survivors were raised on artificial diet until they reached the third instar and

then exposed to the insecticide in a simulated field situation at field rates of chemical; only 13% of them died.

Halliday and Burnham (1990) developed a model to investigate the power of diagnostic dose tests to monitor insecticide resistance when the dose response curves of susceptible and heterozygous insects overlap. They found that seven independent factors contributed to the accuracy of this test method. Of these seven factors (slope, resistance factor, frequency of resistance, inheritance of resistance, dose, number of susceptible strain insects, and the number of test strain insects) only the dose and the number of insects treated can be altered to improve the accuracy of the test. They found that the optimal dose increased as the slope, resistance level and dominance of resistance increased. The optimal dose decreased as the resistance frequency increased and was unaffected by sample size. Their findings indicate that the use of single dose tests might not always be appropriate. If for example, the slope of the dose mortality line is low it will severely limit the accuracy of the test. The best results are obtained when the slope is greater than 5. Halliday and Burnham (1990) state that this does not mean that all diagnostic dose experiments will produce incorrect results. It is still possible to detect differences in mortality between susceptible and field strains but the degree of accuracy may not always be as

high as one would like and it is likely to vary considerably between insecticides and insect strains. This means that low levels of resistance may not always be detected using this method. Halliday and Burnham (1990) report that there is a trend to conduct diagnostic dose tests in which the dose is chosen rather arbitrarily by multiplying the LD_{99} or $LD_{99.9}$ by a factor of two or three. They claim that this will result in failure to detect resistance in some circumstances. Their work indicates that the most effective dose ranged from $LD_{89.0}$ to $LD_{98.1}$

Savin et al (1977) found the estimates of LD_{50}'s and LD_{90}'s obtained from probit and logit analysis to be very similar to each other. However, they found that for LD_{99}'s logit estimates were consistently higher. Savin etal (1977) state that present toxicological theory provides no firm basis for preferring probit to logit analysis or vice versa. Hence there appears to be no reasonable basis for comparing the effectiveness of insecticides at such an extreme dosage as the LD_{99}. This also presumably means that the susceptibility of insect populations to a particular insecticide should not be compared at such extreme doses either.

Testing for the build up of insecticide resistance in a field population is difficult. The recommended field application rate bears little relation to the doses used in laboratory tests to measure resistance. The only

relationship between the field rate and its laboratory equivalent, the discriminating dose, is that both should kill all or nearly all of the susceptible insects (Sawicki, 1987). A major difference between a laboratory discriminating dose and field rates is that each is derived from genetically different populations. The recommended field rate is set to give a level of control at least as good as that of competing products, at a price that the user can afford, using what often amounts to dubious application techniques and poor spray coverage. The recommended rate is usually based on field trials done in many parts of the world on populations with different treatment histories and cross resistance patterns. In contrast to this the susceptible laboratory colonies are much more uniform in their genetic composition and each individual is uniformly exposed to the pesticide under test.

3.3 Biochemical Assays.

The third method of detecting resistance is to use one of the biochemical assays that have been developed to detect specific resistance mechanisms in insect populations (Halliday and Burnham 1990). Brown and Brogdon (1987) state that for decades we have relied on susceptibility tests for detecting insecticide resistance and that there are a number of practical difficulties inherent in these tests.

(1) Only one insecticide can be tested per insect.

(2) Without a known discriminating dose, large numbers of insects are needed to produce dose/mortality lines. Low levels of resistance are not easily detected using this method.

(3) The use of a discriminating dose establishes the presence or absence of resistance using smaller sample sizes but gives no indication of the level of resistance. Variation between populations means that the discriminating dose determined for one population may not be applicable to another.

Brown and Brogdon (1987) claim that biochemical tests would overcome these problems provided they had the following attributes.

(1) The method should detect resistance and provide information on the mechanism likely to be involved.

(2) The method must permit the analysis of single insects or small groups of insects.

(3) The method must permit multiple assays from a single insect.

(4) The method must be fast and accurate.

(5) The equipment should be simple, inexpensive and easy to carry and operate.

(6) The equipment should be adaptable for most or all insect species and should provide all needed assessment information in the field.

This set of rather rigorous criteria can be met by either

biochemical or immunologic methods. A number of techniques have been developed that can be used in the field to detect resistance. A filter paper technique has been developed for rapid determination of phenotypes with high esterase activity in organo-phosphate resistant *Culex quinquefasciatus*. In this method insects are crushed in distilled water and blotted onto filter paper. After 10 - 20 mosquito homogenates have been placed on the filter paper it is sequentially immersed in enzyme substrate, stain and fixing solution and then dried. (Brown and Brogdon 1987). The advantage of this method is that it is simple, portable and provides a permanent record of the results. Brogdon and Dickinson (1983) developed a hydrolase microassay for use in microtiter plates. Their method is sufficiently sensitive that up to 30 microassays can be performed from a single mosquito. They have developed microassays for acetylcholinesterase, malathion carboxylester hydrolase, general hydrolase and nonspecific esterase. Brown and Brogdon (1987) also report that monoclonal antibodies are being produced for resistance enzymes such as glutathione S-transferase and to receptor sites such as those responsible for altered acetylcholinesterase and DDT resistance due to the *kdr* gene. Brown and Brogdon (1987) state that recent work in Haiti and Guatemala has centred around the introduction of biochemical detection methods into the field. It was possible to identify homozygous susceptible and resistant

individuals as well as heterozygotes.

While there is considerable potential for the use of biochemical methods for the detection of insecticide resistance a full range of tests has not yet been developed.

It can be seen that there are a number of problems associated with each of the main methods for detecting resistance. There exists a further set of problems that are associated with the collection and preparation of insects for testing. These problem are less significant where bioassay techniques have been developed to test for resistance in the field as opposed to the laborartory.

3.4 Identification of suspected resistant populations.

As pointed out in the beginning of this chapter the insect population in question may not have been controlled by the pesticide for any one of a number of reasons other than the development of resistance. The most common reasons are under application of the pesticide or poor spray coverage. In most situations these are more likely than insecticide resistance. This makes it difficult to quickly identify those populations that are becoming resistant to an insecticide as control failures are usually considered to be due to other factors and only when repeated failures have occurred and all other possibilities have been exhausted is resistance considered.

3.5 Population Sampling

In resistance testing we are looking for the presence of genetic material that confers insecticide resistance on individuals in the population. Therefore ideally our sample should represent all the genotypes present so that we can be sure that the resistance gene or genes, if present in the population, will be included. If we can't be sure that our sample will include all of the genotypes present then we can never be sure that a negative result (no resistance found) is a true reflection of the state of the wild population.

Sample sizes at any given location must often be large (in the order of hundreds of individuals) to reliably detect resistant individuals when they are present at frequencies of 10% or less. For example when the frequency is 1% and a 95% probability of detection is required then a sample size of approximately 1500 individuals would be needed (Roush et al 1986). In populations where the frequency of resistant insects has increased to much higher levels the sample could be much smaller. However, in those situations where resistance has not yet led to significant control failures, and the pest population is being held at low levels by the current pest control strategies, then it is likely to be difficult to collect the large numbers of individuals required to detect resistance. This is most likely to be the situation

with DBM in New Zealand.

Collection of the insects can impose a number of difficulties in its own right. A poor collection method can give rise to high mortalities thereby increasing the likelihood of losing the resistance gene(s), especially if the incidence of resistance is low.

3.6 Establishing a Laboratory Colony.

"Establishing a laboratory colony is necessary when field collections alone do not yield enough animals to characterise the full toxicological response of the population." (Riedl et al 1985). However a lab. colony may not have the same genetic diversity as the wild population that it purports to represent because there is a high possibility that sporadic genotypes may not be represented in the original collection, or that they may be lost during the establishment phase of the colony. This may be particularly important for those genotypes that are less fit in these conditions. Thus in field collected samples many individuals may fail to reproduce so that the genetic range actually raised for testing will often be much less than that sampled from the field. Even if the resistance gene(s) is present in the laboratory colony it may be lost during subsequent generations in the absence of insecticide selection pressure (Riedl et al 1985).

3.7 Using Bioassays.

There are a number of problems associated with using bioassays to test for the development of insecticide resistance in a population. Both the age and size of the insect within each instar can have a profound effect on the dose that is required to kill it. Savin et al (1982) found that for *Choristoneura occidentalis* the quantity of insecticide required per mg of body weight changed as the body weight of the larvae changed within instars. Therefore different dose rates were required per mg of body weight depending on the stage of development of the larvae and on their body weight. This means that if a valid comparison is to be made between two populations then the larvae must be at the same stage of development and of the same body weight. Failure to ensure this could give rise to a misleading result.

A further problem is that it is difficult to devise a laboratory pesticide application technique that meets experimental requirements yet still reproduces the pesticide distribution patterns found in the field. If the aim is to measure the levels of insecticide resistance in a population then the requirement is to determine the dose of insecticide to kill a predetermined proportion of the population.

Kalra and Chawla (1977) tested three bioassay techniques on DBM with diazinon. These were; direct spraying of the insects using a commercial formulation, topical application of technical grade diazinon in

acetone, and impregnation of filter paper. Their results are summarised in Table 2. The LC_{50} values ranged from 0.016% for the direct spray treatment to 0.098% for the impregnated filter paper. Such differences are not unexpected as they probably reflect efficiency by which the insecticide is absorbed by the insect. It should be noted that only in the case of topical application can the exact dose received by the test insect be assured.

Table 2: Susceptibility of DBM Larvae to Diazinon by Different Bioassay Procedures (Kalra and Chawla 1977)

Bioassay Method	χ^2	LC_{50} in %	Slope	% Mortality untreated
Topical	3.48 (4df)	0.027	1.58 ± 0.19	0 - 5
Filter paper Impregnation	2.20 (2df)	0.098	1.59 ± 0.27	10 - 25
Direct Spray	2.05 (2df)	0.016	3.12 ± 0.31	5 - 20

Kalra and Chawla (1977) found that the most sensitive test method, as depicted by the slope of the dose mortality line, was direct spraying of the insects with the Potter Tower. However, they point out that considerable handling of the test insects is involved in the direct spray method and this can lead to unacceptably high control mortalities. The topical application and

impregnated filter paper methods gave similar results, but the authors considered that the topical method was the better of the two since it produced lower control mortalities.

Suckling et al (1984) compared three methods of determining the resistance ratio in *Epiphyas postvittana*. They compared topical application and direct spraying of larvae with a Potter Tower to exposure to treated leaf discs. Table 3 sets out their results and shows that there was considerable difference between the methods.

Table 3: A Comparison of Different Treatment Methods for *Epiphyas postvittana* Larvae (Suckling et al 1984).

Application Method	Larval Instar	Resistance Ratio
Topical	3rd and 4th	140
Leaf Disc	3rd and 4th	13
Potter Tower	1st	20
Leaf Disc	1st	5

Direct application of the insecticide to *E. postvittana* whether by the Potter Tower or by topical application, accentuated the difference between the resistant and susceptible strains. Again such results should not be unexpected as they reflect the method by which the pesticide was absorbed by the insect. It is likely that the sensitivity of a test method will vary with the insect

species tested and the pesticide used. For *E. postvittana* the most sensitive method for azinphos methyl was topical application. However Hinkle et al (1985) state that, topical application does not reflect the mode by which residual insecticides are acquired by insects in practical usage. Application of insecticides to a substrate with subsequent exposure of the test insects allows them to pick up the toxicant in a manner analogous to field situations. When Hinkle et al (1985) compared a number of different application techniques they found that the most sensitive method for testing for pyrethroid resistance was the dry film technique. However, when testing for DDT resistance the most sensitive method was topical application.

While many alternative methods are available to expose insects to pesticides in the laboratory so that dose mortality curves can be constructed, none of the currently available methods comes close to approximating the field situation. The different methods not only give rise to different dose mortality curves but also to different resistance ratios.

Thus any attempt to predict field performance from laboratory results is difficult because of the many variables involved. Even slight modifications in bioassay techniques (eg using different solvents for topical application, using different instar larvae) can alter the result of the tests (Taylor 1982).

Because of such variations it is important that internationally recognised standard tests are established. The FAO method for the detection of insecticide resistance in DBM (FAO method 21) requires topical application of insecticide to larvae (Busvine, 1980). Although this method is useful (Noppun et al 1983) it does have a number of serious draw backs. While topical application appears to be a sensitive test method for DBM resistance it is labour intensive and probably does not realistically mimic the mode of application of insecticide in the field (Tabashnik and Cushing 1987). Larvae tend to feed on the underside of the leaves in the field so the effects of ingesting pesticide and coming into contact with spray deposits are likely to be important as well as direct contact with spray droplets. Tabashnik and Cushing (1987) compared leaf residues and topical application for assessing insecticide resistance in DBM. The results from the two methods were similar but the leaf residue test required less labour, more closely resembled field exposure and gave lower control mortalities. It should be remembered that the ultimate aim of laboratory resistance testing is to apply the results in the field so the ideal test method is one that is sensitive yet mimics field conditions.

Savin et al (1977) tested the basic hypothesis that the dose response curve for a given chemical on a particular target organism is fixed. They collected

insects from a range of sites that to the best of their knowledge had not been treated with insecticide and reared them in the laboratory through successive generations. Dose mortality curves were constructed for each generation. Their findings on the test organism, western spruce bud worm, *Choristoneura occidentalis*, cast doubt on the validity of this hypothesis. They found that the LD_{50} values for the three chemicals used in their tests shifted between generations. For DDT the LD_{50} varied by a factor of up to three in a cyclic fashion, while for the pyrethrins and mexacarbate it varied by up to two times. In the light of these findings it would be prudent to treat resistance ratio figures with some caution if they are very low (say less than 10), as it may be due to shifts in the dose mortality curve rather than the development of insecticide resistance.

3.8 Insect Handling Methods.

All of the laboratory methods require that the insects be handled, and this can induce high levels of control mortality. For example topical application usually demands that the insects be anaesthetised so that a very small quantity of the test material can be applied to the chosen place. This is usually done with CO_2 or by chilling. If the control mortalities are high and the differences between the populations small, then it will be difficult to obtain a statistically significant result.

CHAPTER 4.

EXPERIMENTAL TECHNIQUES FOR DETERMINING RESISTANCE IN DIAMONDBACK MOTH.

Diamondback moth is an insect which has had a major impact on the world's food production. It is not surprising that a considerable amount of time and money has been devoted to developing new control methods and attempting to forestall the development of insecticide resistance in this cosmopolitan insect pest. When working with resistance in an insect like DBM it is desirable that there be some degree of uniformity in the experimental techniques that are used, so that results can be compared. This chapter is a review of techniques that have been used to investigate insecticide resistance in DBM.

4.1 Kalra and Chawla 1977.

These authors compared three methods of exposing DBM larvae to pesticides. Larvae, collected from infested cauliflowers, were reared to the F1 generation on cauliflower leaves. Third instar larvae 0.5 ± 0.1 cm in length and having a mean weight of 2mg, were used in the bioassays.

Method one: Direct spray application

Batches of ten larvae were placed in petri dishes and sprayed with 1ml of pesticide mix in a Potter Tower.

Different concentrations of a commercial formulation of diazinon (Basudin 25%ec) were prepared by diluting the product with tap water. The insects were allowed to dry for five minutes and then transferred to glass jars and provided with fresh unsprayed cauliflower leaves. Mortality was recorded after 24 hours.

Method two: Topical application.

1 µl of insecticidal solution (technical grade in acetone) was applied to the dorsal surface of each larva with a micro-syringe. The larvae were then transferred to glass jars containing fresh cauliflower leaves. Mortality was recorded after 24 hours.

Method three: Impregnated Filter Paper.

Whatman No. 1 filter papers were treated with 2 ml of an insecticidal solution in a solvent mixture of 1 part refined white oil, 3 parts petroleum ether and 1 part acetone. The filter papers were allowed to dry for one hour and then placed in glass jars. Twenty larvae were exposed to the treated paper in each jar for 24 hours, at which time mortality was assessed.

Results.

The most sensitive test method was direct application with the Potter tower. However the lowest control mortality was obtained from the topical application

treatment and the authors concluded that the topical application method was the most satisfactory of the three because of this.

4.2 Sudderuddin and Kok Pooi-Fong 1978.

This work examined the spectrum and level of insecticide resistance in a strain of DBM from the Cameron Highlands of Malaysia. A susceptible strain was obtained from France and the resistant strain was collected from cabbage plants on a single property. Both strains were reared in muslin cages at 28 ° ± 2°C and 86 ± 6% relative humidity with a 16 hour light and 8 hour dark regime. The larvae were fed daily on fresh *Brassica chinensis* leaves. Fourth instar larvae were used for all assays. The larvae were larger than those used by Kalra and Chawla (1977) with a mean weight of 6.17mg compared to 2mg. The caterpillars were treated topically with 1µl droplets of acetone solution of insecticide. At least 25 larvae were used per treatment and four to six concentrations per insecticide. The treated larvae were held at 28°C and mortality was recorded after 24, 48 and 72 hours. The resistance ratio was calculated by dividing the LD_{50} of the R strain by that of the S strain.

Results:

The wild strain showed resistance to all of the insecticides tested. The resistance ratios ranged from 5

for resmethrin to 2096 for malathion. The authors did not compare the mortalities after the different exposure times so I presume that they were not significantly different.

4.3 Busvine 1978. (F.A.O. Method 21)

This method was proposed by Busvine (1978) as an international standard recommended by the F.A.O. The paper describes his proposed standard bioassay and colony establishment conditions.

Part 1. Maintaining a reference colony.

Busvine (1978) held his colony at 23°C and the life cycle was completed in 21 - 24 days. However he suggests that colonies could be kept at 25 - 30°C if shorter life cycles were required. Pupae can be stored at 5°C until they are needed. This technique can be used to synchronise the development of the population. When the pupae are removed from cool storage adults will begin to emerge after 3 days at which time potted brassica plants can be placed in the enclosure for oviposition. After oviposition the plants are removed from the adult enclosure and larvae allowed to develop on them.

Part 2. Testing For Resistance.

Fourth instar larvae are removed from the plants with a camel hair paint brush and placed in a petri dish lined with filter paper. The larvae are then anaesthetized with carbon dioxide and droplets (0.5 µl) of technical grade insecticide dissolved in acetone are applied to the dorsal

surface of each larva. After treatment the larvae are transferred to clean dry glass jars and supplied with cabbage or cauliflower leaves. The larvae are kept at 25°C for four days before mortality counts are made. A susceptible population is used to establish a dose mortality curve from which a diagnostic dose is selected. Busvine suggests that the LD_{99} be used as the diagnostic dose. Once the diagnostic dose has been established then field populations can be tested for resistance using single dose tests.

Liu , Chen and Sun 1984

Liu Chen and Sun investigated the effect of synergism of several pyrethroids on susceptible and resistant strains of DBM. Acetone solutions of technical grade pesticide and synergist were applied directly to fourth instar larvae using a Shandon spraygun. Thirty to 40 larva were treated with each concentration and each treatment was replicated twice. Mortality was assessed 24 hours after treatment. Each bioassay consisted of four treatments and a control. Temperatures were not held constant throughout the trial but ranged from 22 to 28°C. Where the results of two or more bioassays were to be compared then these tests were conducted simultaneously to minimise the influence of fluctuating temperature.

Results.

Only permethrin was synergised by the esterase

inhibitors but all four pyrethroids tested (permethrin, fenvalerate, deltamethrin and cypermethrin) were synergised in both the susceptible and resistant strain by a microsomal oxidase inhibitor.

4.5 Tabashnik and Cushing 1987.

This work compared two methods of exposing the larvae to the insecticide, leaf residues and topical application.

Topical Application.

Third instar larvae were used and their size standardized by rejecting any group of larvae whose mean weight was outside the range of 1.5 to 2.5 mg. Droplets (0.5 µl) of acetone solutions of technical grade insecticide were applied to the dorsal region of each larva. After treatment the insects were transferred to clean petri dishes, supplied with cabbage leaves and kept at 28°C for 24 hours, at which time mortality was assessed.

Leaf Residue Tests.

Third instar larvae were used but their weights were not standardized as for the topical tests. Discs (6cm diameter) were cut from fresh fully expanded cabbage leaves. The leaf disc were dipped for 5 seconds in distilled water solutions of formulated commercial insecticides and hung to air dry in a fume cupboard at

23°C for 2 hours. Once dry each disc was placed in a petri dish on top of two pieces of polystyrene. Eight to twelve larvae were placed on each leaf disc and held at 28°C for 24 hours at which time mortality was assessed.

Results:

The authors concluded that the methods gave similar results but the leaf residue tests required less labour, more closely resembled field exposure and gave lower control mortalities than did the topical method. If the control mortality for any particular replicate exceeded 20% the authors excluded it from the analysis. This occurred for 16 of 40 replicates with topical treatment but for none of the 29 replicates for the residue test. The overall control mortalities (after those over 20% had been rejected) were 4.6% for the topical treatment and 0.3% for the residue test.

4.6 Tabashnik et al 1987.

DBM larvae were collected from a number of field sites in Hawaii. Between 50 and 300 larvae were collected from each site and reared in the laboratory on cabbage plants at 28°C with a photoperiod of 14hours light and 10hours dark. With the exception of the susceptible colony, larvae used in the bioassays were either the F1 or F2 generation. The susceptible colony was maintained in the laboratory without insecticide treatments for more than 20

generations. The bioassay was performed using the leaf residue method of Tabashnik and Cushing (1987). Eight to 13 larvae were placed on each leaf disc and held at 28°C for 24 hours before they were checked for mortality.

Results.

The mean control mortality was less than 0.2% with a range of 0 - 1.7%. Resistance ratios were calculated by dividing the LC_{50} of each field population by the LC_{50} of the susceptible population. The resistance ratios ranged from 130 for DDT to 5 for diazinon, and some populations separated by less than 10 kilometers differed in their susceptibility.

4.7 Iman et al 1986.

These workers assessed the susceptibility of four field strains of DBM from Indonesia to pyrethroids using both laboratory and field trials. Two bioassay techniques were used in the laboratory tests.

Method one: Topical application.

Insecticide in 0.05 µl acetone was applied to the dorsal thoracic surface of third instar larvae. After treatment the larvae were transferred to 9cm diameter petri dishes containing cabbage leaf discs and maintained at 25°C and 70% RH for 24 hours on a 12:12 L:D photoperiod. A minimum of 6 insecticide concentrations

plus a control were used for each bioassay and three replicates of 10 larvae for each concentration.

Method two: Dry film technique.

Nine centimetre petri dishes were treated with acetone solutions of insecticide and the acetone evaporated off. Twenty 2nd or 3rd instar larvae were exposed to the dry insecticide residues inside the petri dishes for one hour. After exposure the larvae were transferred to untreated dishes containing fresh cabbage leaf discs. Mortality was assessed 24 hours later.

Field trials.

Six treatments, each replicated four times were arranged in a randomised block design. Each plot was planted with 100 cabbage plants at 80 x 50 cm spacings. The insecticides were applied using a knapsack sprayer in water volumes of 500 to 1100 l/ha, depending on the age of the crop, when the DBM population had reached the estimated economic injury level (0.1 larvae/plant). The first application was required approximately 14 days after planting and repeat applications once a week until harvest. The plant damage levels were assessed by systematically selecting 10 plants per plot and calculating the percentage injury as follows.

$$P = \frac{\Sigma(n \times v)}{5N} \times 100$$

Where P = percent injury level; n = total number of

leaves in an infestation class; v = numerical value of infestation class (0 to 5); where 0 = no leaf damage, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80% and 5 = 100% of the leaf area damaged, and N = the total number of leaves observed.

Results.

Only the Lembang strain was represented in both the laboratory and field trials. In the first laboratory test (topical application) the resistance ratio, calculated from the LD_{50}s, for permethrin was 11 for the Lembang strain and 3 for the Kopeng strain. In a second laboratory test (dry film technique) conducted one year after the first test the resistance ratios for permethrin were 3.8 in the Lembang strain and 7.5 in the Kopeng strain. The authors considered that the change in the resistance ratios reflected changing use patterns in the field during the intervening 12 months. Table 4 is a comparison of the LD_{50}s from the dry film technique and the results of the field trial. There is no significant difference in the damage levels for each of the three pyrethroids in the field trial but the resistance ratio for fenvalerate is higher in the laboratory study, although the authors give no indication of the confidence intervals for the resistance ratio figures. Therefore while there appeared to be a difference in the levels of resistance determined in the laboratory there was no difference in the

effectivness of insect control in the field.

Table 4: Effectiveness of various insecticides against a resistant strain of DBM in laboratory and field trials. (Iman et al 1986).

Insecticide	Resistance ratio (Laboratory)	Injury level (P) (Field)
Fenvalerate	18.1	45.00
Permethrin	3.8	39.76
Cypermethrin	2.5	39.82
Control		84.19

It would seem that small differences in resistance ratios determined in laboratory trials using the dry film technique are not always reflected in control differences in typical field use situations.

Cheng 1986.

One of the aims for this programme was to develop a sampling method for large vegetable growing districts. The assumption was that if the resistance was homogeneous over a large area then the sampling intensity could be lowered but if the resistance levels varied widely from one field to another then a general survey of resistance would be of little practical significance.

The test area was divided into four regions with a distance of 4 - 5 km between adjacent regions. Four sampling points were clustered on independent cruciferous crops at the centre of each region. At each sampling point 200 - 300 larvae or pupae were collected and returned to

the laboratory for rearing. The insects were reared on insecticide free cabbage plants to the adult stage and the adults feed on a honey and water mixture. After mating the insects were held in screen cages with potted cabbage plants for oviposition. Third instar larvae from the F1 generation were used for a LC_{50} test using cartap and permethrin as the test insecticides.

Results.

Three of the four regions were homogeneous in both insecticide susceptibility and the rate of parasitism. The fourth region was surrounded by rice paddy and was disparate in terms of cropping pattern. The test results confirmed that DBM from the fourth region were more susceptible to permethrin than those collected from the other three areas.

The highest levels of resistance were found in those districts where brassicas are intensively cropped all year round. The author found that DBM populations found in crops which were scattered amongst rice paddy fields or upland crops had lower levels of resistance than those in intensive monoculture production areas. A survey of insecticide resistance in DBM showed that almost all populations in Taiwan had developed insecticide resistance. The only exception was a strain isolated by mountains. This strain has remained susceptible to all of the registered insecticides.

Therefore it would seem that areas with different cropping histories and geographically isolated areas should be sampled for resistance separately.

It can be seen that a number of diverse techniques have been used to estimate the level of insecticide resistance in DBM populations, even since the publication of the F.A.O. standard method. This is because to date no one method has been devised that seems to meet all the requirements of an effective testing program.

Chapter 5

Insect Handling Methods.

While there is a wealth of information in the literature on methods of rearing DBM there is very little available on suitable handling techniques for this insect. A series of preliminary subjective trials were undertaken to determine the most appropriate methods of handling the insect as it was evident in some of the papers (Kalra and Chawla 1977, Tabashnik and Cushing 1987), that high control mortalities could be a problem.

5.1 Field Collection of DBM. Larvae & Pupae.

DBM larvae (2nd, 3rd and 4th instar) and pupae were collected from field grown plants by excising the portion of the plant supporting the insect and placing it in a plastic bag. A number of such pieces were placed in each bag. The bags were placed in an insulated container and transferred to cool storage as soon as practicable. On return to the laboratory the excised plant parts with larvae attached were pinned to the leaves of potted cabbage plants. After a time they moved onto the new plant and began to feed once more. The pupae that had been collected were placed in plastic bags in cool storage at ca 4°C. As each of the larvae on the potted cabbage plants pupated they too were removed and held in cool storage until all of the field collected insects had pupated. At this time the

pupae were removed from the cool room and held at room temperature to await adult emergence.

The insect survival rates were very high and this method was adopted for all future collections.

5.2 Egg Numbers

When the adults emerged from the pupa, potted cabbage plants were placed in the fine mesh enclosure for oviposition. There seemed to be considerable variation in the fecundity of different batches of adult insects. When the egg load on the potted plants was high, larval development was slow, there was a wide range in the size and stage of development of the larvae and the ensuing adults seemed to have reduced fecundity. The numbers of eggs on each plant could be regulated to some degree by altering the length of time that the plants remained in the adult enclosure for oviposition. Despite this many plants often carried too many eggs. When this was the case it was found that if the leaves were removed from the overburdened plant when the larvae had reached the second instar (emerged from the leaf mines), cut up and pinned to a number of new plants a more suitable insect density could be achieved. If the insects were redistributed before they had reached the second instar then high mortality levels resulted.

5.3 Insect Removal For Testing

A number of different methods were tested for removing

the 3rd instar larvae from cabbage plant leaves.

The first method tried. was to gently sweep the caterpillars from the leaf surface using a fine camel hair brush. In most cases the insects where easily removed using this method, but often, particularly if the population level on the leaf was high and little green tissue remained, the caterpillars were firmly webbed to the leaf surface and they could not be removed in this manner. When preliminary tests were made using topical applications of insecticide to the dorsal surface of the insect very high control mortalities were observed when this method of insect removal had been used. A number of other types of brush were used but the control mortalities remained similarly high.

The second method was to try and use the brush to lift the caterpillars off the leaf by sliding the brush under the caterpillar and lifting it clear of the leaf. Again those caterpillars that were firmly webbed to the leaf surface were difficult to remove using this method. Preliminary trial results from topical applications of insecticide on larvae using this method gave control mortalities even higher than the first method. When examined through a dissecting microscope considerable wounding was evident on the sides of the caterpillars.

During this preliminary trial program it was noticed that some of the larvae would descend from the cabbage plant leaf on a thread in response to vibration. When whole

leaves were removed from the plant and the leaf petiole held against a test tube shaker many of the larvae would thus descend from the leaf and could be easily collected with little physical damage.

Since the bulk of the trial program was to be conducted using leaf dip assays it was decided to run a preliminary trial as to the most suitable removal method using leaf dip assays to compare the two different removal methods. The two methods, sweeping with a fine brush and vibration were compared using four different insecticides, diazinon (organophosphate), permethrin (synthetic pyrethroid), esfenvalerate (fourth generation pyrethroid) and methomyl (carbamate). The results were analysed using the probit transformation after correction for control mortality using Abbott's formula.

The results of this work are presented in figs 4 to 7 and are summarised in table 5.

Table 5: Summary of data comparing two different insect removal methods for each of four insecticides.

Insecticide	χ^2 goodness of fit		LD_{50}		g (0.99) index of sig	
	Brush	Vibrat	Brush	Vibrat	Brush	Vibrat
Permethrin	6.37	5.23	0.01	0.01	3.05	1.14
Esfenvalerate	17.4	5.44	0.01	0.01	1.08	0.20
Methomyl	53.8	0.56	0.78	1.48	6.97	0.14
Diazinon	5.86	4.01	0.16	0.03	0.23	0.21

Control mortality: Brushed = 10.0%, Vibration = 1.3%

Figure 4: Probit Mortality Lines for Diazinon, Brush verses Vibration Removal.

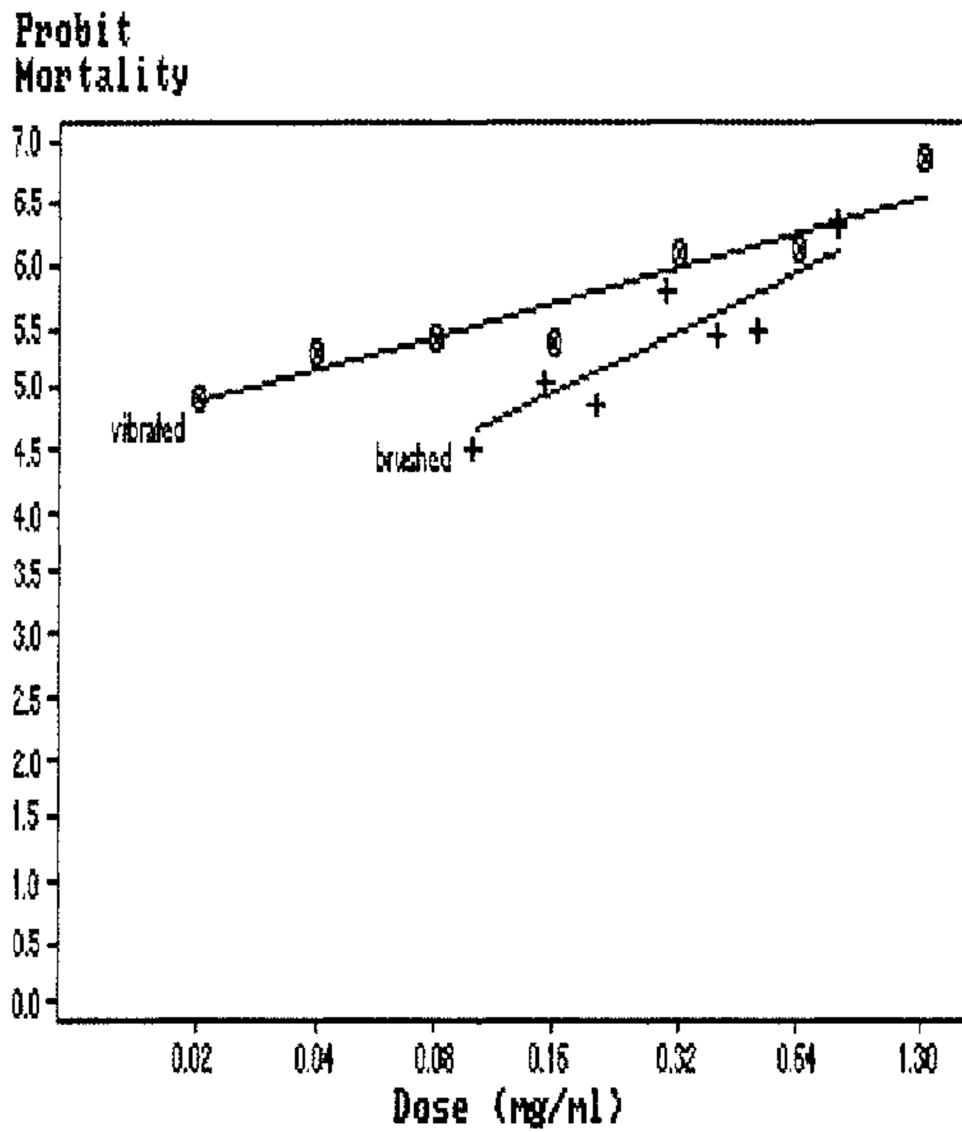


Figure 5: Probit Mortality Lines for Permethrin, Brush verses Vibration Removal.

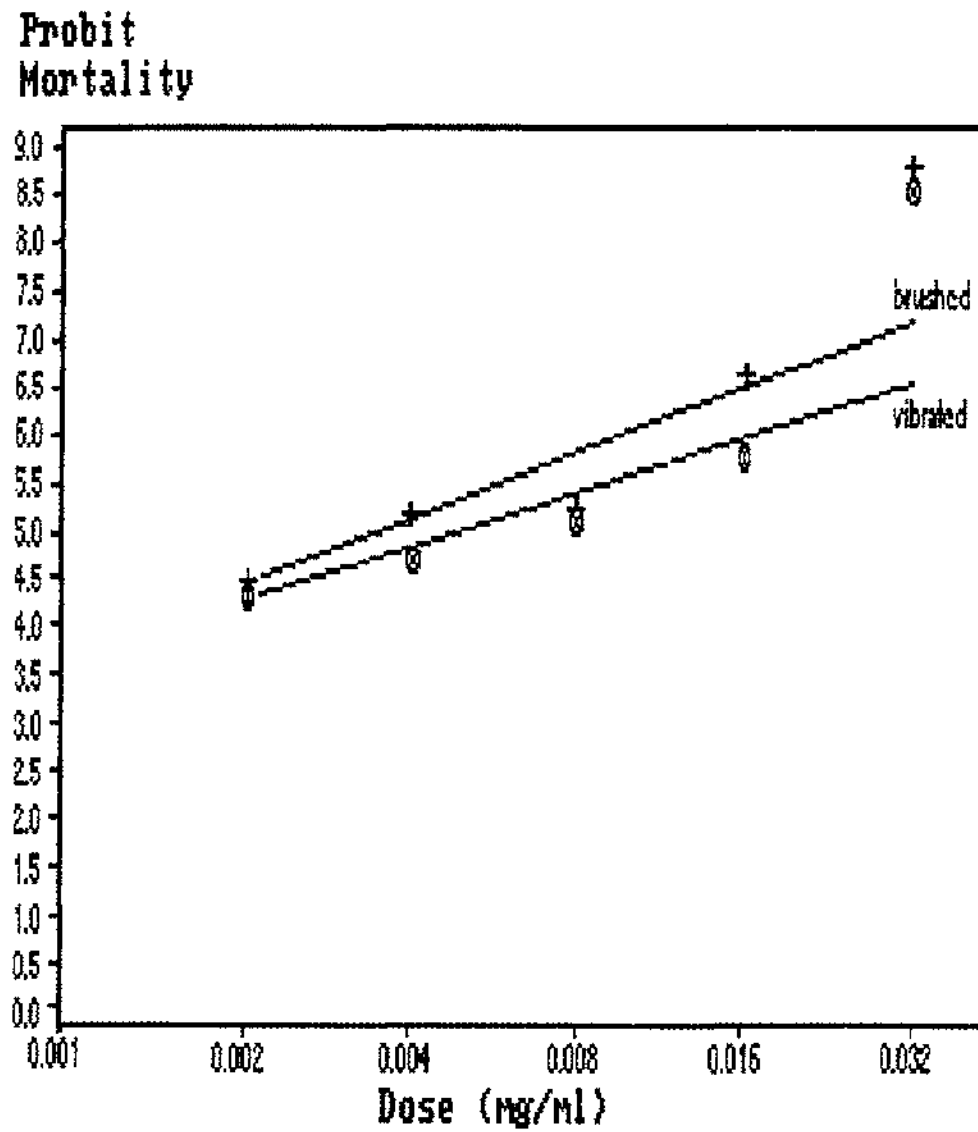


Figure 6: Probit Mortality Lines for Esfenvalerate, Brush verse Vibration Removal.

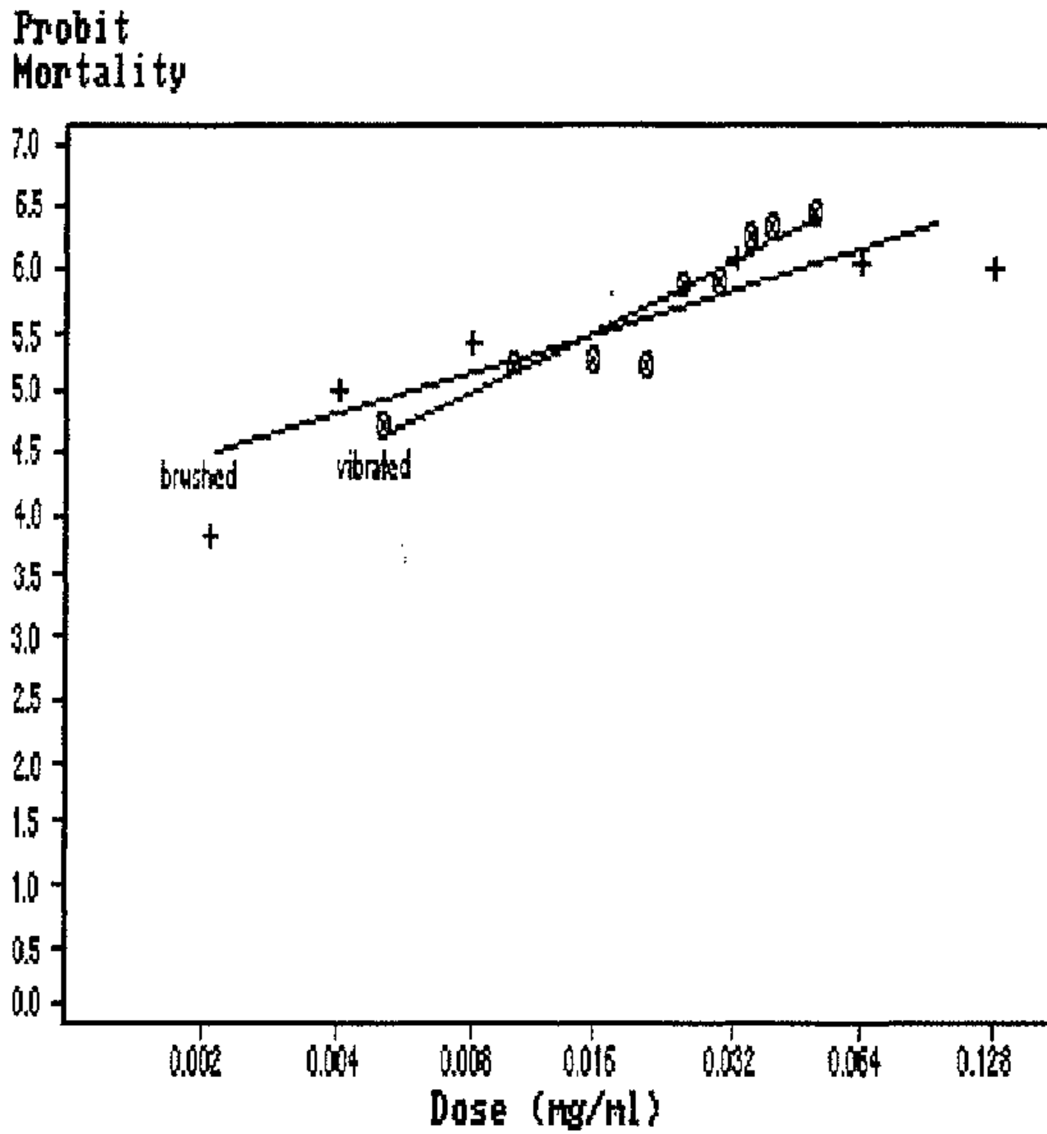
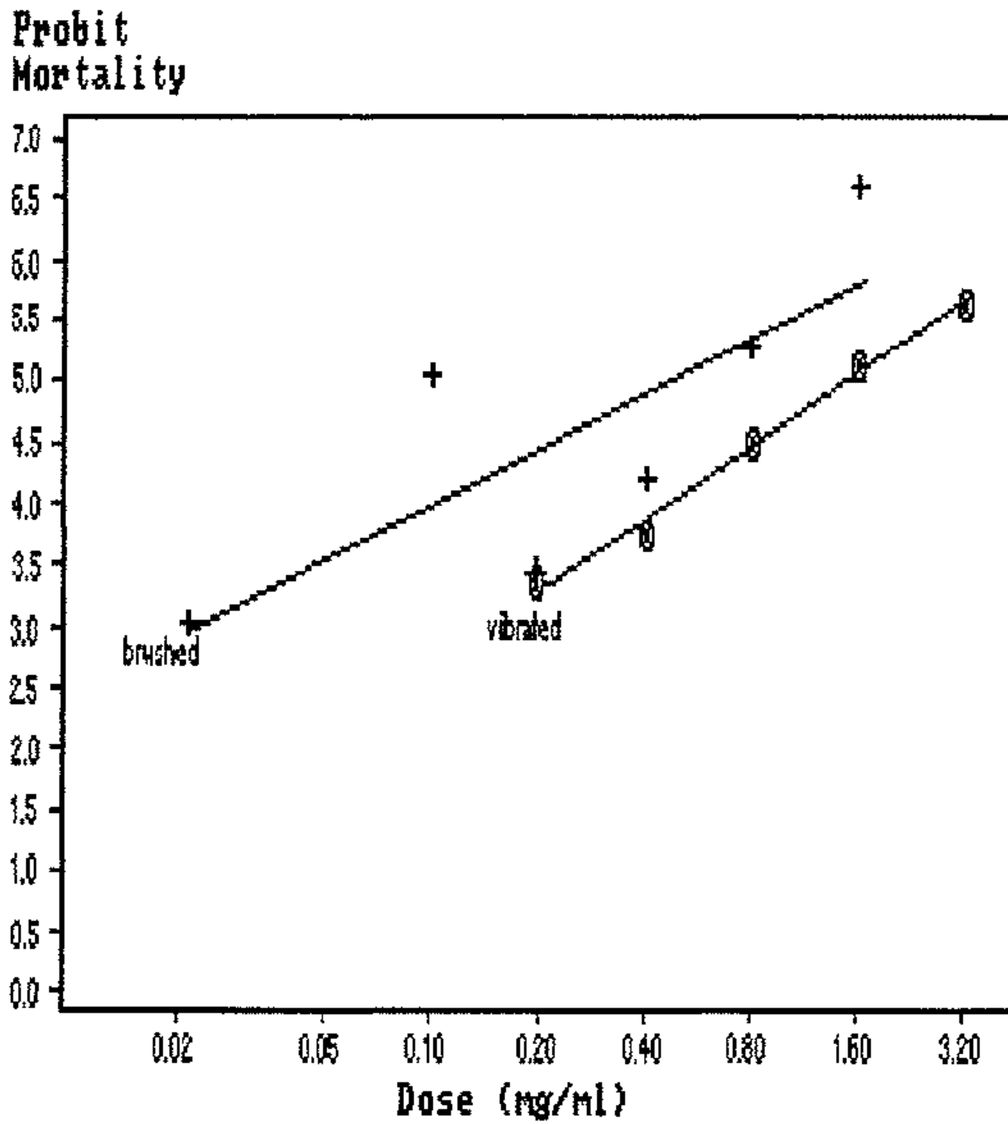


Figure 7: Probit Mortality Lines for Methomyl, Brush verse Vibration Removal.



Figures 4 to 7 show plots of the dose mortality curves for the four insecticides and the two removal methods. While the plots of the dose mortality lines seem to be quite different the LD_{50}, LD_{90} and the LD_{95} values for the two removal methods are not significantly different for any of the four insecticides. However it will be noted from table 5 that the χ^2 goodness of fit values are generally lower for the vibrated than the brushed removal method and that the g values (index of significance for potency estimation are also consistently lower for vibration removal than brushed. Finney (1972) states that "with almost all good sets of data, g will be substantially smaller than 1.0, and seldom greater than 0.4". It is therefore most likely that the confidence intervals for the dose mortality curve will be narrower for vibration removal than for the brush method. This means that low levels of resistance may be more readily detected if the test larvae are shaken from the plant rather than brushed.

Given that the vibration removal method gave results that conformed better to the probit model than brush removal it was decided to use this method for all further DBM larvae removal from plant leaves.

CHAPTER 6

EXPERIMENTAL METHODS WTH INSECTICIDES

A number of experimental techniques have been developed to measure insecticide resistance in DBM. The method that I have chosen is essentially that of Tabashnik and Cushing (1987). However, I examined each step involved in the process of the identification of insecticide resistant populations of DBM and have adopted some of the ideas used by other workers in the field.

My trial programme has been conducted in four parts. The first was the identification and testing of a susceptible population in order to identify a diagnostic dose. The second part was the sampling of DBM populations from regularly sprayed crops and rearing them for testing. The third part was to test the F1 generation of these field populations for insecticide resistance. The final part of the programme was to select a resistant population, if one was detected, and to rear it through five generations without insecticide selection pressure and then to test for any reversion in the levels of resistance.

6.1 Identification of a susceptible population.

New Zealand is a little more fortunate than other countries because large areas of forage brassicas are

planted each year by arable farmers as supplementary feed for their livestock. In most situations the use of insecticides against aphids, white butterfly and DBM in these crops is perceived as being uneconomic so they remain untreated even when infestation levels are high. It is therefore conceivable that in such areas remote from market gardens DBM populations that have remained free of selection pressure from insecticides may be found.

The first step in identifying and testing for a susceptible population was to collect DBM larvae from an unsprayed forage brassica crop in an area with little or no market gardening activity. Larvae were collected from a forage brassica crop on a Massey University farm. This property had a history of no insecticide use and was situated well away from any market gardening activity. Third and fourth instar larvae and pupae were collected from the field during the summer months and insect development was synchronised. This process was complicated somewhat by high levels of parasitism of the field collected caterpillars by the larval parasite *Diadegma semiclausum*. The colony was cleared of the parasite by removing the adult wasps from the cage as they emerged. When most of the adult DBM had emerged, insecticide free potted cabbage plants cv. "flower of spring" were placed in the cage for oviposition. After one to two days these plants were removed from the oviposition enclosure and placed in a clean cage. The developing caterpillars were

supplied with clean fresh potted cabbage plants as required.

The first batch of larvae raised in this manner were used to count the number of larval instars and to learn how to recognise each instar. Samples of caterpillars of each instar were placed in alcohol and used as a comparative reference to ensure that caterpillars selected for resistance testing were not only third instar but had not recently moulted or were not about to enter the fourth instar.

The aim of testing the Massey University population was to establish a diagnostic dose that could be used to screen other populations for the development of insecticide resistance. This meant that it was also important to establish that the Massey population was truly susceptible. I elected to do this by comparing the LD_{50}'s for the Massey population with those of other "susceptible" populations in the literature. Since there has been an array of different experimental techniques used in the past it was necessary to use both topical application of the pesticide in acetone and leaf residue tests to obtain sufficient data to make a valid comparison.

Test Methods

1 Topical application.

The method of topical application used was essentially

that of Busvine (1978).

(i) Third instar larvae were removed from the cabbage leaves using the vibration technique and collected in clean glass vials, approximately ten insects per vial.

(ii) Petri dishes were chilled at a temperature of ca 3°C for one hour.

(iii) A dilution series was prepared using technical grade pesticide, where it was available, or formulated product if it was unavailable, in analytical grade acetone.

(iv) Larvae from the glass vials were placed in chilled petri dishes and the insecticide solution was applied to the dorsal surface of each insect using a micrometer operated syringe. Each larva received a 0.5 µl droplet of the solution. It was found that 1.0 µl droplets as used by Kalra and Chawla (1977) were too large and much of the material would run off onto the petri dish. This meant that the quantity of insecticide retained by the larvae would not be accurately known and comparisons with other trial data would not be valid.

(v) Leaf discs 7 cm in diameter were cut from fresh insecticide free cabbage leaves cv. "flower of spring". These leaf discs were placed in clean dry petri dishes right side up on top of three small polystyrene blocks. The blocks were used to allow

access to both sides of the leaf by the larvae.

(vi) Once the treated larvae had resumed movement (ca 30 seconds after treatment) they were placed on the centre of a leaf disc and moved to an incubator where they were held at 25°C and 60% relative humidity for 24 hours on a 12 hour light, 12 hour dark regime.

(vii) After 24 hours the number of larvae affected by the treatment was determined. Caterpillars were considered dead if they failed to respond when prodded gently with a dissecting needle.

(viii) The results were analysed by probit analysis using the computer programme POLO (Robertson et al 1980).

2 Leaf Dip Method

(i) Third instar larvae were collected in clean glass vials as for the topical application method.

(ii) Leaf discs 7 cm in diameter were cut from fresh insecticide free cabbage leaves.

(iii) A range of concentrations of commercial formulations of the pesticides under test were prepared with distilled water. Tween 20 was added to each at 0.5%. A mixture of distilled water and tween 20 was used for the controls.

(iv) The leaf discs were dipped in the pesticide water

mixture until the the leaf had become completely wet. The length of time required to achieve this varied between pesticides and with the water / pesticide ratio. Presumably this was due to differing amounts of wetting agent in the formulations. Lannate (methomyl) in particular seemed to have a reduced ability to wet the leaves compared to the other products.

(v) Once dipped the leaves were hung in a fume cupboard to dry. Tabashnik and Cushing (1987) hung their leaf discs in a fume cupboard to dry for 2 hours. This was not appropriate for the experiments reported here. If the discs were left for two hours then unacceptable wilting could occur. In addition some of the insecticides were more volatile than others and time in the fume cupboard could cause an unknown reduction in the quantity of pesticide left on the leaf disc. For these reasons it was decided to minimise the time that the leaf discs were left in the fume cupboard by removing them as soon as there was no free water visible on either surface of the disc. The actual amount of time involved ranged from about 45 minutes on hot dry days to 1 hour 20 minutes on cooler dull days.

(vi) Once dry the leaf discs were placed in petri dishes on three polystyrene blocks as for the topical treatment. Approximately 10 larvae were removed from

a glass vial using the vibration technique onto the centre of the dry treated leaf disc. Four discs were used for each treatment.

(vii) The petri dishes were held in an incubator at 25°C and 60% RH on a 12 : 12 light dark cycle for 24 hours, after which time mortality counts were conducted. The data was analysed in the same manner as for the topical application.

6.2 Sampling Field Populations of Diamondback Moth.

Tabashnik et al (1987) collected DBM larvae from ten different field populations in the Hawaiian islands and tested their susceptibility to a number of insecticides. They found that populations on the same island separated by less than ten kilometers differed in their susceptibility to the insecticides used in the trial programme. They felt that this variation was probably due to local variation in insecticide use. Cheng (1986) found that in those areas of Taiwan with diverse cropping patterns insecticide resistance in DBM was also variable. Because of the possibility of this type of variation occurring in New Zealand I decided to collect larvae from a number of widely separated sites where vegetable brassicas are a major crop.

Collection Methods.

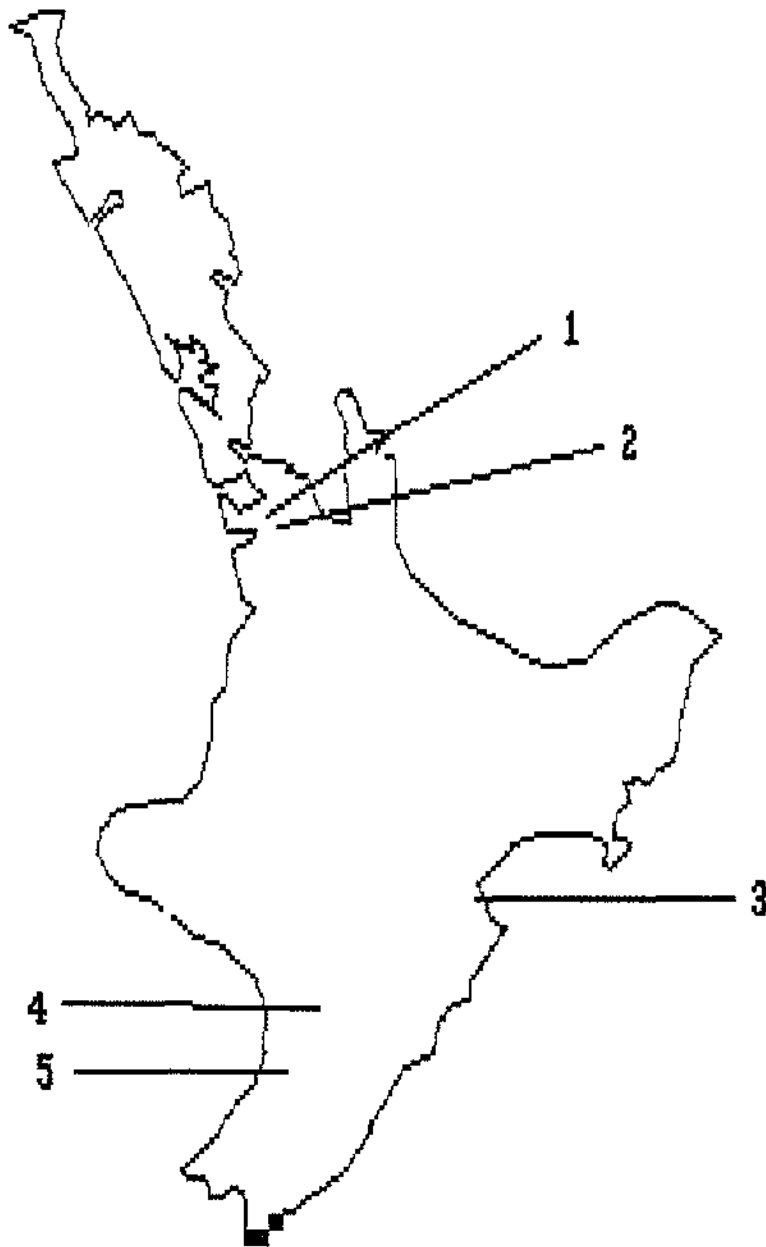
Larvae and pupae were collected from cabbage plants at each site. The collection sites were located on commercial market gardens, treated frequently with insecticides to prevent damage from DBM, white butterfly, aphids and fungal infections. In all cases the level of crop damage was low, with only about 10% of the plants showing what was deemed to be economically significant insect damage (feeding damage in the developing heart or on three layers of wrapper leaves).

Diamondback moth seem to prefer to feed on the young leaves of the developing heart of a cabbage plant. For this reason I chose to collect caterpillars from cabbages that were just starting to form hearts. The sample was selected by searching for damaged plants on a transect across the paddock. Each time a damaged plant was found the centre portion was excised and examined for the presence of live larvae or pupae. When found they were placed in a polythene bag and held in an insulated container until they could be placed in cool storage. While I intended to collect at least 50 insects from each site this was not always possible. A large proportion of cabbage plants showing damage were found to contain live caterpillars, but the incidence of damage in the crops was low and the parasitism rate of larvae was often high.

Diamondback moth larvae for resistance testing were collected from market gardens in Pukekohe (sites 1 & 2)

Hawkes Bay (site 3) Palmerston North (site 4) and Levin (site 5). Figure 8 shows the location of the insect collection sites.

Figure 8: Map of the North Island of New Zealand Showing The Collection Sites For DBM Resistance Testing.



Once the insects reached the laboratory the colony was

synchronised and cleared of parasites.

6.3 Resistance Testing.

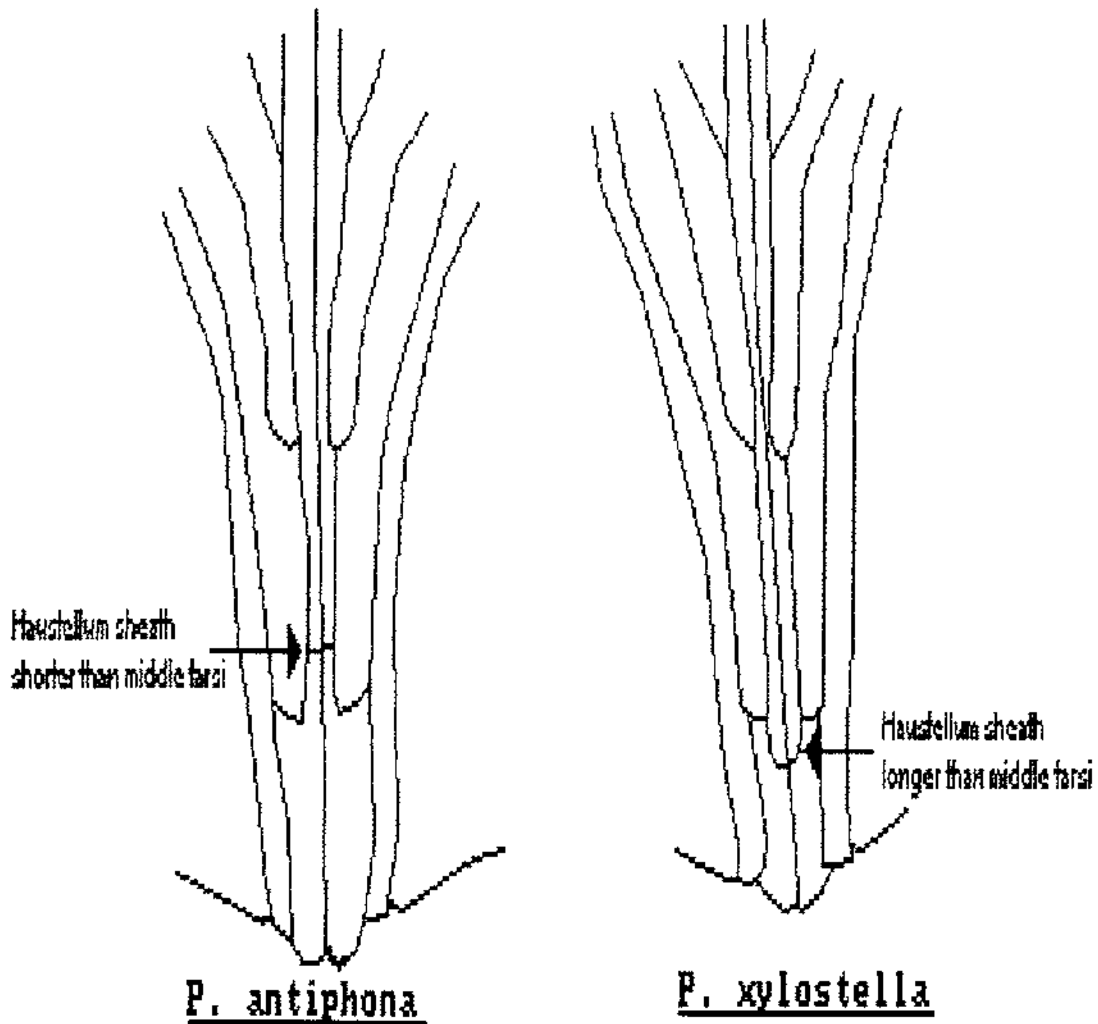
1. Insect Identification.

Dugdale (1973) found that *P. xylostella and P. antiphona* occur together throughout most of New Zealand. This means that although *P. xylostella* would appear to be the most abundant of the two species it is important that a positive identification of each insect collected from the field be made. The identifications were made by examining the pupal cases after adult emergence. In *P.xylostella* the haustellum sheath is longer than the middle tarsi while in *P. antiphona* the apices of the middle tarsal sheaths extend beyond the haustellum sheath apex. Fig 9 (from Dugdale 1973) illustrates the differences in the pupal cases for these two species.

2. Diagnostic Dose.

While DBM has been identified as a potential resistance problem in New Zealand (Elliot et al), no control failures had been attributed to insecticide resistance at the time of initiating this project. It therefore seemed likely that the number of insects surviving in frequently sprayed crops would be low. For this reason it was decided that single dose testing would be the most appropriate method to use as it requires fewer individuals than resistance ratios.

Figure 9: Pupal structures showing antennal and leg cases of *P. antiphona* and *P. xylostella*. From Dugdale (1973).



No appropriate bio-chemical tests for this species have been published. All proponents of single dose testing seem to agree that when a diagnostic dose is used to verify

insecticide resistance, selection of the correct dose is paramount. (Busvine 1978, Roush & Miller 1986, Georghiou & Taylor 1986, Tabashnik & Cushing 1987, Daly & Murry 1988 McCutchen 1989, Halliday & Burnham 1990,). However, not all agree as to just what level the insecticide dose should be set if it is to reliably discriminate between homozygous susceptible and heterozygous insects. Halliday and Burnham (1990) have modelled the effect of seven different parameters (both operational and genetic) on the accuracy of the diagnostic dose test (see chapter 3). Guided by the outcomes of their model I arbitrarily set the diagnostic dose at the LD_{95} level of the susceptible population. If this dose fails to detect the presence of insecticide resistance then a range of doses could be used between the LD_{50} and the LD_{95} in an attempt to improve sensitivity.

3. Insect treatment method.

Three methods of exposing larvae to insecticide have been used to test DBM larvae for the development of insecticide resistance (see discussion chapter 4). Of the three methods (dry film, topical and treated leaf discs), I chose to use the treated leaf disc as it is a simple technique that is a closer approximation to the field situation than are the other two methods and has been used successfully by a number of authors. (Tabashnik & Cushing 1987, Tabashnik et al 1987).

The larvae were exposed to treated leaf discs in the same manner as the susceptible Massey University population.

6.4 Stability of Resistance in DBM.

Chen and Sun (1986) found a rapid regression of organophosphate resistance compared to pyrethroid resistance in DBM. Since the rate at which resistance is lost in the absence of selection pressure (fitness of the resistance strain) is an important factor in designing resistance management programmes a small additional study was undertaken to examine the rate of regression of one of the resistant New Zealand strains. This resistant strain was selected and held in the laboratory without insecticide selection pressure for five generations. The fifth generation was tested for resistance using the leaf dip bioassay.

CHAPTER 7

RESULTS AND DISCUSSION.

7.1 Identification of a susceptible population.

Larvae and pupae collected from forage brassicas on a Massey University farm were reared in the laboratory through ca 4 generations. The pupal cases from the first generation were used to check the identification of the insects (Dugdale 1973). All specimens were identified as *Plutella xylostella*. *P. antiphona*, with which it may be confused, was not present.

Tables 6 and 7 compare LD_{50}s and LC_{50}s for a range of insecticides for the Massey colony with susceptible strains used in other DBM resistance studies. While these data are from trials using similar experimental techniques, methods were not always identical. The most important difference was in the larval instar used in the bioassay. Savin et al (1982) found that for *Choristoneura occidentalis* the quantity of insecticide required per mg of body weight changed as the body weight of the larvae changed within and between instars. Therefore it is likely that the different instars used in the above tests would influence LD_{50}s and LC_{50}s making precise comparisons impossible.

Table 6: Comparison of LD_{50} values for topically applied insecticides for the Massey colony and other susceptible colonies.

Insecticide	Colony/Author	LD_{50} (µg/larva)	Instar treated
Diazinon	1	0.13	4th
	2	2.6	3rd
	3	1.6	4th
	4	0.27	3rd
	Massey	0.92	3rd
Dichlorvos	1	0.66	4th
	3	0.73	4th
	5	0.32	4th
	Massey	0.08	3rd
Carbaryl	3	10.0	4th
	5	2.39	4th
	Massey	2.47	3rd
Methomyl	5	0.19	4th
	Massey	0.11	3rd

Table 7: Comparison of LC_{50} values for leaf dip bioassays for the Massey colony and other susceptible colonies.

Insecticide	Colony/Author	LC_{50} mg/ml	Instar treated
Diazinon	2	0.75	3rd
	6	0.19	3rd
	Massey	0.03	3rd
Carbaryl	7	3.2	3rd
	Massey	5.75	3rd
Permethrin	6	0.017	3rd
	7	0.02	3rd
	Massey	0.006	3rd

The insect colonies/authors referred to in tables 6 and 7 are as follows:

1. Busvine (1980). This was a reference colony held at the Rothamsted Experimental Station in England.

2. Tabashnik and Cushing (1987). This colony was collected from the field at Pulehu on Maui Island in Hawaii.

3. Miyata, Saito and Noppun (1986). This colony was collected from the field in Osaka, Japan.

4. Kalra and Chawla (1977). These authors collected their colony from Ludhiana in the Punjab.

5. Sudderudin and Kok (1978). This colony was obtained from a laboratory in France as the authors felt they would be unable to find a susceptible strain in the Cameron Highlands, Malaysia.

6. Tabashnik, Cushing and Johnson (1987). This work was completed using the same colony of susceptible insects as Tabashnik and Cushing (1987).

7. Kumar and Chapman (1983). These authors collected eggs larvae and pupae from a local field population at Lincoln University, New Zealand.

Despite the difficulties in making comparisons it is evident that that the Massey University colony is as susceptible to organophosphates, carbamates and synthetic pyrethroids as any of the reference colonies in the literature and therefore can be confidently used to estimate the levels of insecticide resistance in populations regularly exposed to pesticides.

Having established that the Massey colony is susceptible with topical and leaf dip application it was used to generate dose mortality curves for each of the insecticides under test, using the leaf dip method. Figures 10(a - g) are the dose mortality curves for 7 insecticides. Table 8 gives the LC_{95} values for each of the insecticides. The LC_{95}s were used as diagnostic doses to evaluate the resistance status of a number of field populations regularly exposed to insecticides.

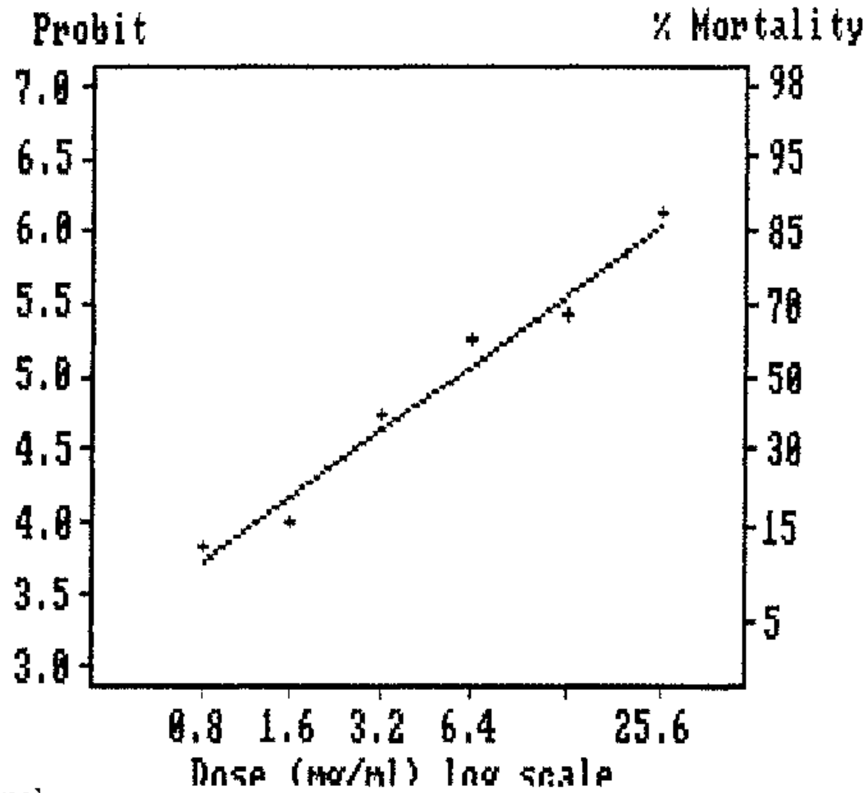
Table 8: LC_{95} values for the Massey University DBM colony using the leaf dip method.

Insecticide	% control mortality	LC_{95} mg/ml	95% C.L.
Carbaryl	2.38	60.96	35.40-148.41
Methomyl	0	9.75	5.69- 24.51
Diazinon	0	1.68	0.75 - 8.07
Mevinphos	0	0.38	0.23 - 1.02
Dichlorvos	0	0.88	0.41 - 4.85
Permethrin	5.40	0.04	0.02 - 0.40
Esfenvalerate	3.70	0.07	0.05 - 0.16

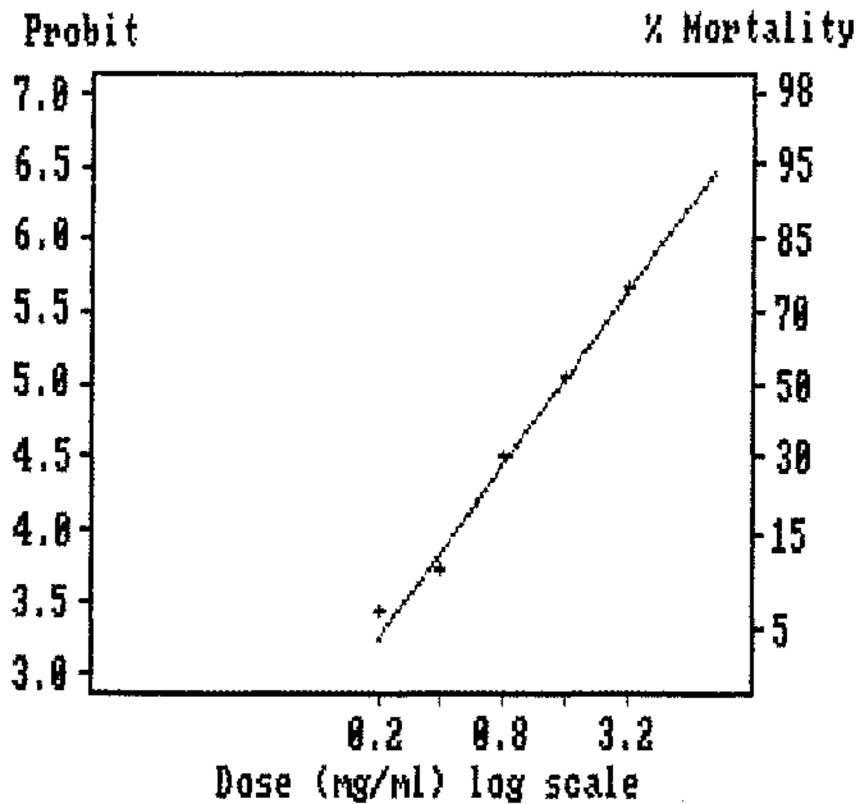
It can be seen from Table 8 that untreated control mortalities ranged from 0% to 5.4% with a mean of 1.6% for the leaf dip treatments. Abbott's correction was used where necessary in the calculation of the LC_{95} values (Abbott 1925). Control mortalities for the topical applications (acetone applied as a control) were higher, and ranged from 0% to 20% with a mean of 3.8%. This indicates that the leaf dip bioassay method is preferable to topical application. The high LC_{95} values for carbaryl and methomyl show that these carbamates are not particularly effective against DBM. At the highest concentrations continuous agitation was required to maintain the carbaryl suspensions while the leaf dips were being made. Once dry the leaf discs appeared glaucous with a coating of the commercial preparation of carbaryl. It may have been more appropriate to have used distilled water plus Tween 20 and talcum as a control rather distilled water and Tween 20 alone as it is possible that the large amounts of talcum on the leaf discs could have influenced the results.

While it did not appear to be very effective, even on the susceptible Massey strain, methomyl has been widely used in South East Asia for DBM control (Sakai 1986, Rushtapakornchai and Vattanatangum 1986, Sun et al 1978). The carbamate insecticides are not commonly used as DBM control agents in New Zealand and none of the growers involved in this study used carbamates as part of their programme.

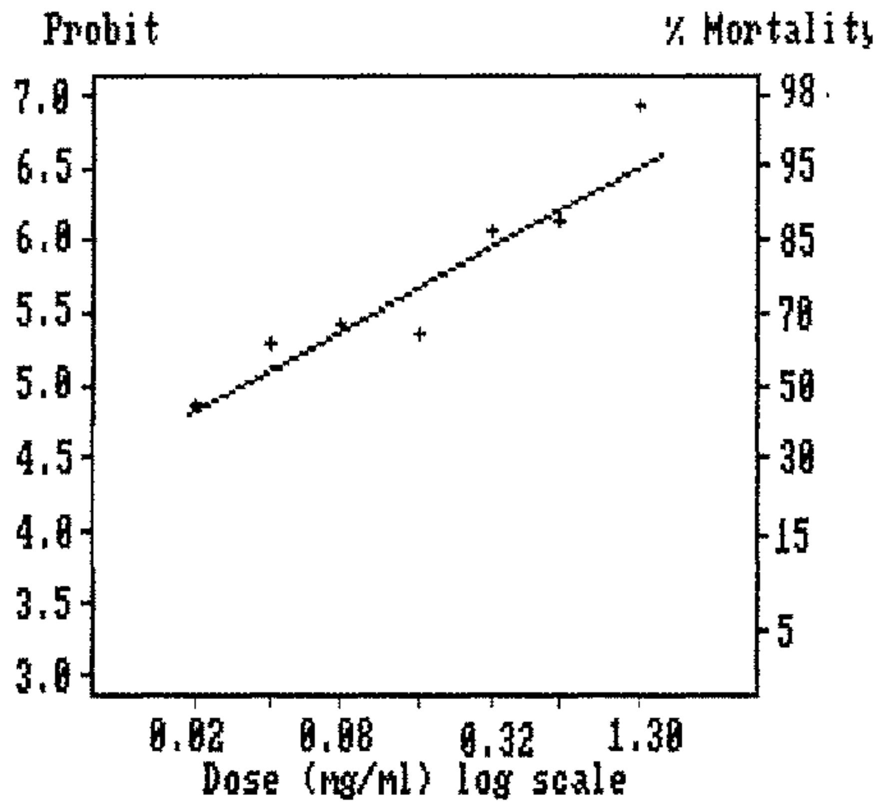
Figure 10: Dose mortality lines using leaf dip bioassays.
(a) carbaryl.



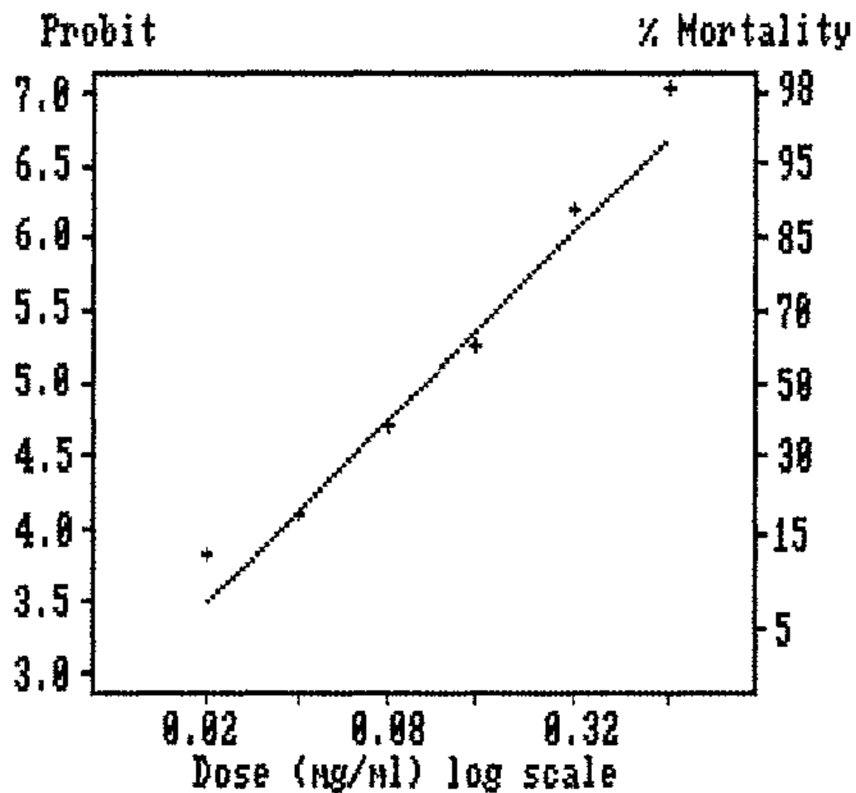
(b) methomyl



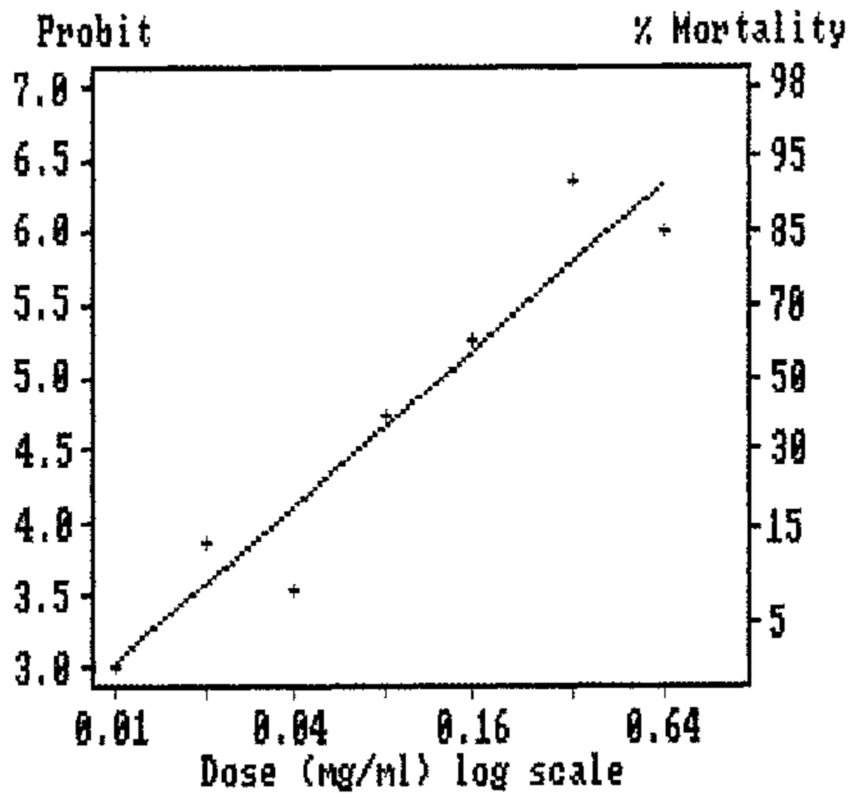
(c) diazinon



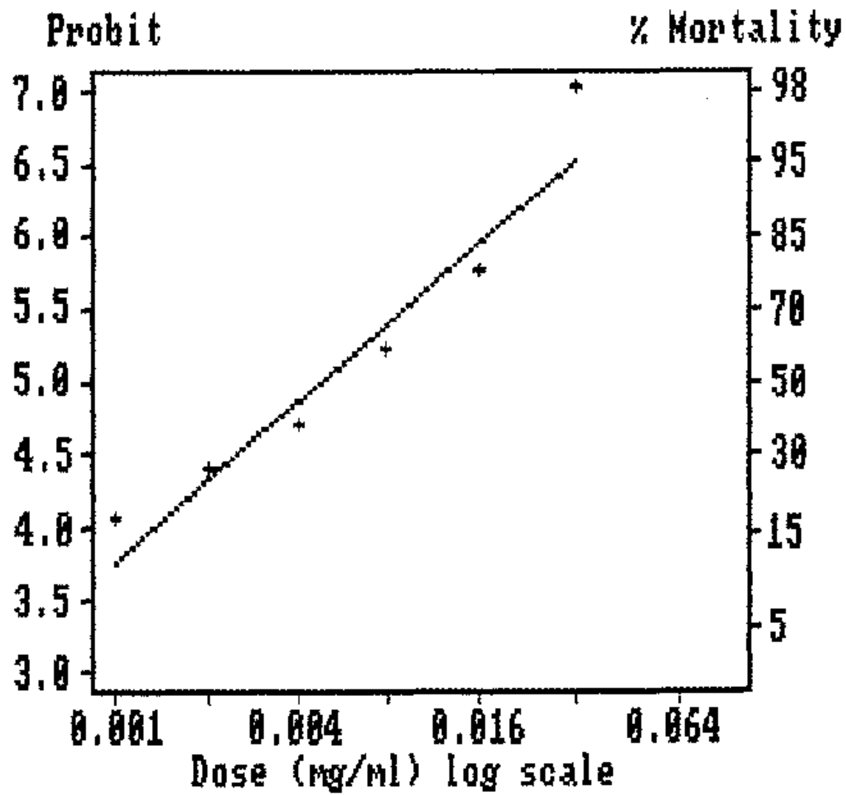
(d) mevinphos



(e) dichlorvos



(f) permethrin



(g) esfenvalerate

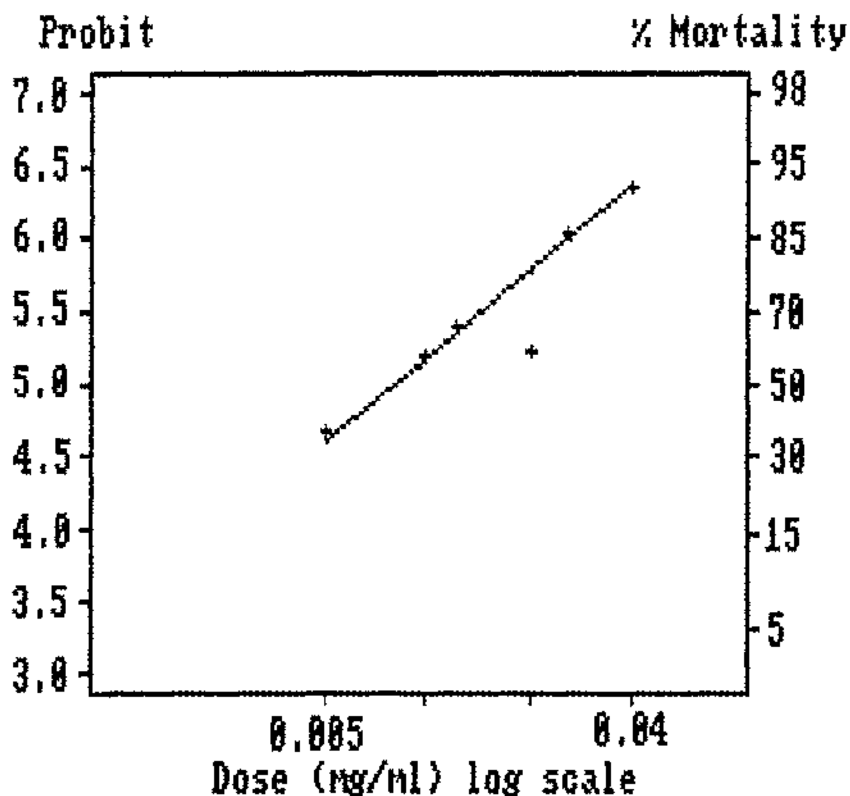


Table 9: Conformation of the dose mortality curves for the Massey Population to the Probit Model.

Insecticide	df	χ^2	Heterogeneity	g (0.95)
Carbaryl	4	3.581	0.895	0.071
Methomyl	3	0.560	0.187	0.078
Diazinon	5	4.001	0.800	0.123
Mevinphos	4	5.968	1.492	0.133
Dichlorvos	5	14.83	2.966	0.179
Permethrin	4	5.234	1.309	0.414
Esfenvalerate	7	5.442	0.777	0.115

Table 9 illustrates the goodness of fit of each of the seven dose mortality curves for the susceptible Massey

University colony to the probit model. The heterogeneity factor equals the chi-squared value divided by the degrees of freedom. Values greater than 1.0 mean that the model fits the data poorly. A poor fit may be due to either random variation (experimental error) or systematic variation from linear regression. If the variation is systematic the use of a different mathematical function to analyse the data may be more appropriate (Finney 1971). The heterogeneity factor exceeds 1.0 for the dose mortality curves for mevinphos, dichlorvos and permethrin. In all three cases the variation appeared to be random rather than systematic. The "index of significance for potency estimation" is the statistic "g" in table 9. This figure is used in the calculation of the confidence limits for the lethal dose estimates. With almost all good sets of data "g" will be substantially less than 1.0 and seldom greater than 0.4 (Finney 1971). This indicates that the dose mortality data derived from the Massey colony conforms to the probit model and thus the lethal dose estimates can be used to test the susceptibility of other populations to these insecticides.

7.2 Sampling Field Populations of Diamondback Moth.

Diamondback moth pupae and larvae were relatively easily found at the Pukekohe sites (sites 1 & 2), despite rigorous spray programs. Site 1 had been treated with insecticide 2 hours prior to collecting the insects and site 2 five days prior to collection. Insects were

slightly more difficult to find on the Palmerston North site (site 4), and more scarce on site 3 (Hawkes Bay). At the Levin site (site 5) surviving insects in treated crops were extremely difficult to find. Table 10 lists the numbers of insects collected from each site and the level of parasitism found in the insects collected.

Table 10: Insect collection.

Site	Collection Date	No. Insects Collected	% Parasitism
1	19/12/89	62	71
2	19/12/89	67	73
3	24/3/90	30	97
	17/4/90	36	72
4	23/5/90	77	78
5	17/2/90	2*	-
	24/2/90	18	72

* Both insects died before pupating

Two collections had to be made at site 3 because of the high incidence of parasitism found in the insects collected on 24/3/90. Most of the DBM collected at this time were in the pupal stage. The pupae were not abundant but a high proportion of those collected were found in exposed positions on the upper surface on the outer leaves of cabbage plants. During the second collection these exposed pupae were avoided in favour of larvae feeding in the developing heart of the plant and pupae in less exposed positions. The second collection provided sufficient insects to establish a laboratory colony. This could indicate that, due to a behavioural response by the parasitized insects, they pupate in more exposed positions

on the plant than healthy ones. Therefore the sampling method used to collect the insects for evaluation could lead to a disproportionate number of parasitized pupae being collected. Surviving insects were very difficult to find on site 5. Approximately 20 man hours were spent searching for DBM on this property to yield only 20 larvae and pupae. Large numbers of white butterfly (*Pieris rapae*) were evident however. While very few insects were used to establish the laboratory colonies from sites 3 and 5 it is felt that the colonies represented the genotypes present in the field populations because almost all of the plants in the crop showing damage symptoms were dissected in the search for larvae and pupae.

The high levels of parasitism encountered caused a considerable reduction in the numbers of DBM adults that emerged. While the true level of parasitism is difficult to determine because of the collection technique used it is likely to be high enough to be having an impact on the development of insecticide resistance. Beck and Cameron (1990) found that parasitism of DBM pupae ranged from 69% to 93% in insecticide free brassicas at the DSIR Research Stations at Pukekohe and Mt. Albert. A sample of DBM was collected from their trial at the Pukekohe Research Station and taken to the laboratory at Massey University with a view to establishing a colony and testing it for insecticide resistance because, even though the crop had not been sprayed with insecticides, the research station lies in the centre of an intensive market garden area

regularly treated with pesticides. However this was not successful as very high levels of parasitism prevented the establishment of the colony.

7.3 Pesticide Usage At Each Site:

Site 1:

(1) Lepidoptera - permethrin on a 10 - 14 day schedule.

(2) Aphids - demeton-s-methyl as required

(3) Fungi - mixture of benomyl and maneb as required

(4) Last application of insecticide before collection, 19/12/89 (2 hours prior to collection).

Site 2 :

(1) Lepidoptera - lambda cyhalothrin (most frequently used) or deltamethrin on a 7 - 14 day schedule.

(2) Aphids - demeton-s-methyl or dimethoate as required

(3) Fungi - mixture of benomyl, captafol and maneb as required.

(4) Last application of insecticide before collection, 14/12/89 (5 days prior to collection).

Site 3:

(1) Lepidoptera - permethrin or lambda cyhalothrin on a 21 day schedule with mevinphos close to harvest.

(2) Aphids - demeton-s-methyl as required with

mevinphos close to harvest.

(3) Fungi - Bravo and captafol as alternating treatments as required.

(4) Last insecticide treatment before the second collection, 9/4/90 (8 days prior to collection).

Site 4:

(1) Lepidoptera - methamidophos on a 10 - 14 schedule with mevinphos close to harvest.

(2) Aphids - demeton-s-methyl as required.

(3) Fungi - Bravo on a 10 to 14 day schedule.

(4) Last application of insecticide before collection, 15/5/90 (8 days prior to collection).

Site 5:

(1) Lepidoptera - permethrin and dichlorvos as alternating sprays on a 14 - 21 day schedule.

(2) Aphids - additional applications of dichlorvos if required.

(3) Fungi - benomyl or Bravo as required.

(4) Last application of insecticide before collection, 7/2/90 (17 days prior to collection).

7.4 Resistance Testing.

A. Insect Identification.

The identification of the field collected insects used to establish the laboratory colonies was checked by examination of the pupal cases after adult emergence

(Dugdale 1973). It was found that only *P. xylostella* had been included in the collections.

2. Diagnostic Dose Tests.

Figures 11 - 16 are bar graphs showing the percentage of third instar DBM larvae from the F1 generation of each of the five field populations tested that survived exposure to the diagnostic dose (LD_{95} for the Massey colony). A single sided Z test was used to test the significance of each result by calculating the number of survivors that would indicate a significant (95% C.L.) departure from the expected survival rate of 5%. The number of survivors necessary to achieve significance was calculated as;

$s = Z * sqrt(ng(1-g)) + ng + 1/2$ (Roush and Millar 1986, Snedecor and Cochran 1967). Where s = number of survivors necessary to achieve significance, n is the sample size, and g is the fraction of survivors expected in a susceptible strain. The percentage of survivors required to achieve significance is plotted alongside the the total survivors for each of the insecticides used. This means that when the bar for the colony is longer than the Z test bar, significant (95% CL) resistance to the pesticide is indicated.

It is evident from these results that some resistance has developed on each of the five sites tested. However the number of resistant individuals remains small and none of the growers involved considered it to be an economic

problem. In fact none of the growers were aware of the presence of insecticide resistant DBM in their crops. Further work is required to determine how the resistance levels are likely to change in the future if the selection pressure remains. It is possible that on each of these sites the high levels of parasitism by *Diadegma semiclausum* is a factor in controlling the development of insecticide resistance. If this is the case then the development of an integrated pest control programme would offer considerable advantages. However if the numbers of resistant DBM are not being effectively regulated by *D. semiclausum* then it is possible that the number of resistant insects will increase to the point where control failures occur.

Figure 11: Diagnostic Dose Test Pukekohe 1 Colony.

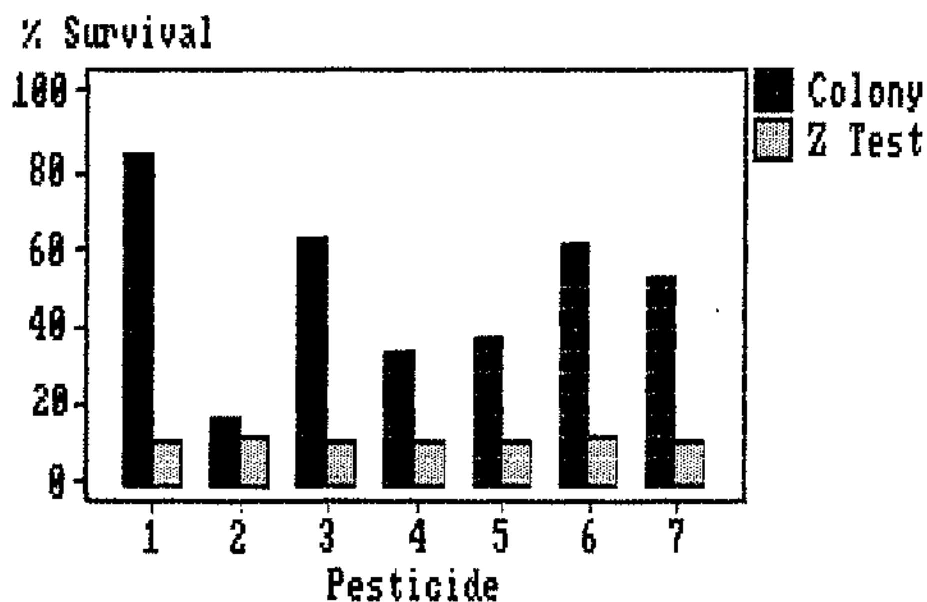


Figure 12: Diagnostic Dose Test Pukekohe 2 Colony (Site 2).

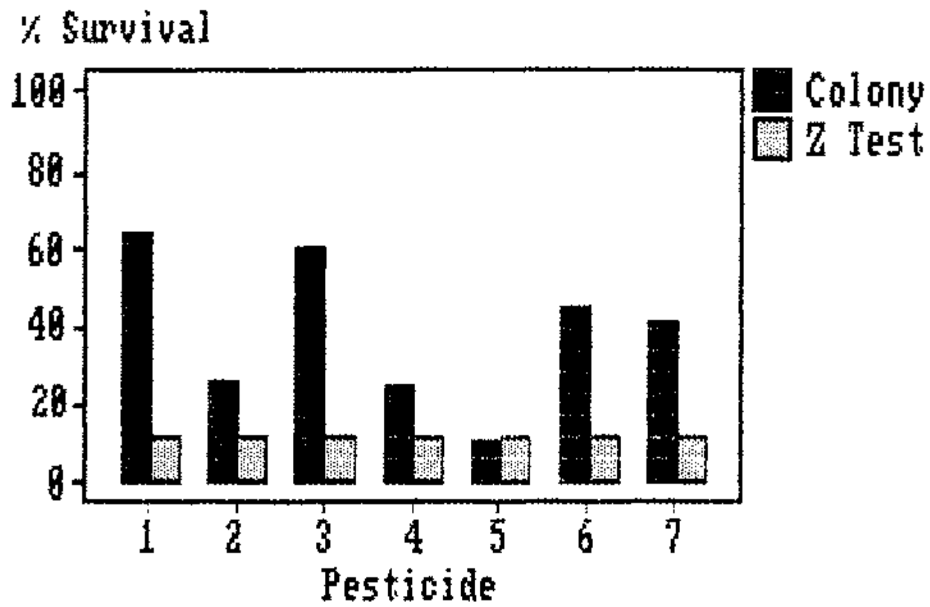


Figure 13: Diagnostic Dose Test Hawkes Bay Colony (Site 3).

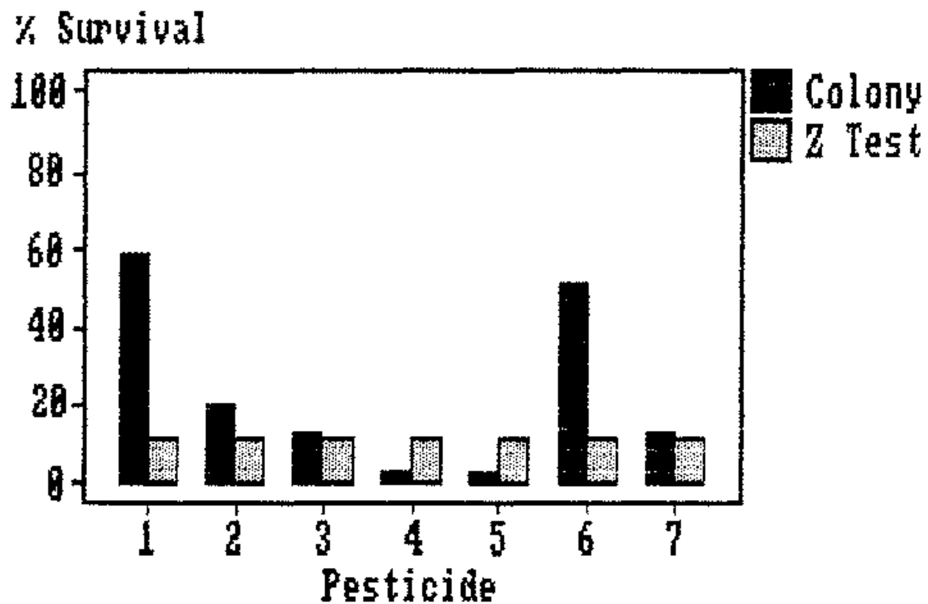


Figure 14: Diagnostic Dose Test Palmerston North Colony (Site 4).

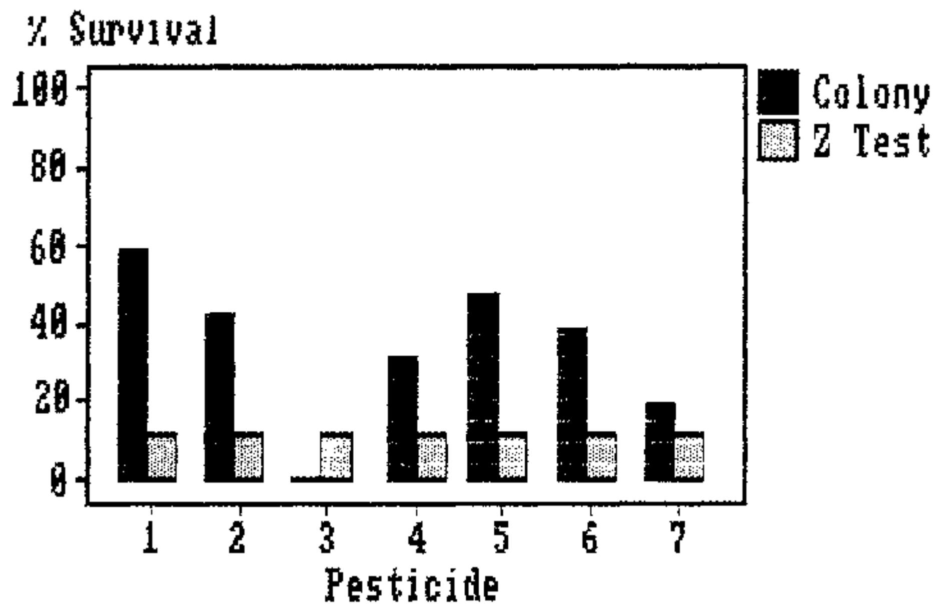


Figure 15: Diagnostic Dose Test Levin Colony (Site 5).

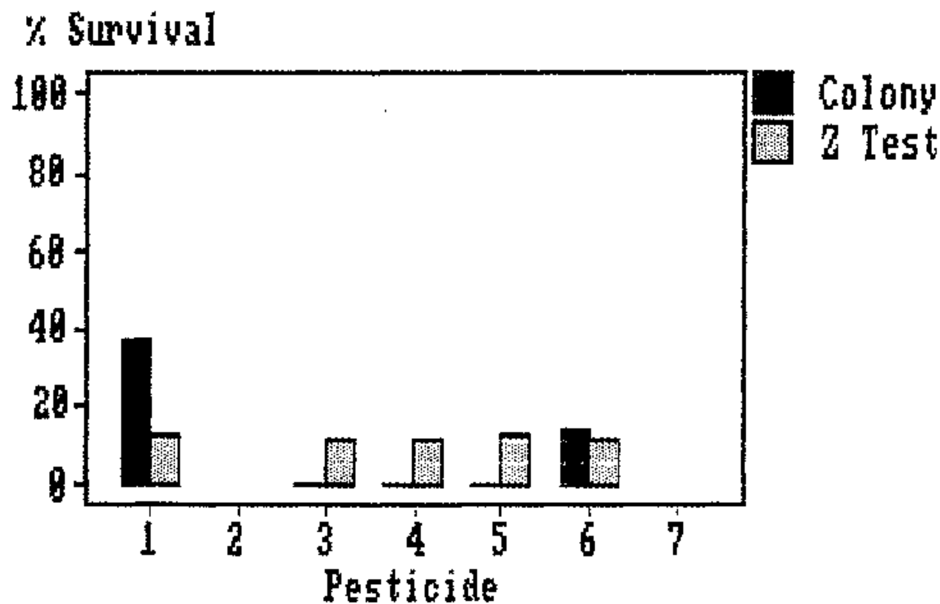
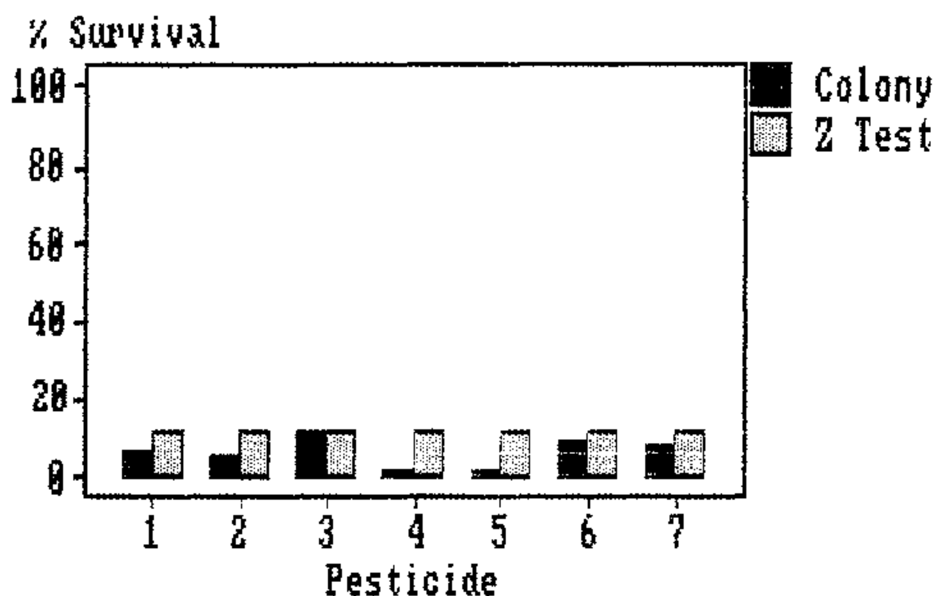


Figure 16: Diagnostic Dose Test Massey (susceptible) Colony.



For figures 11 to 16.

1 = carbaryl

2 = methomyl

3 = diazinon

4 = mevinphos

5 = dichlorvos

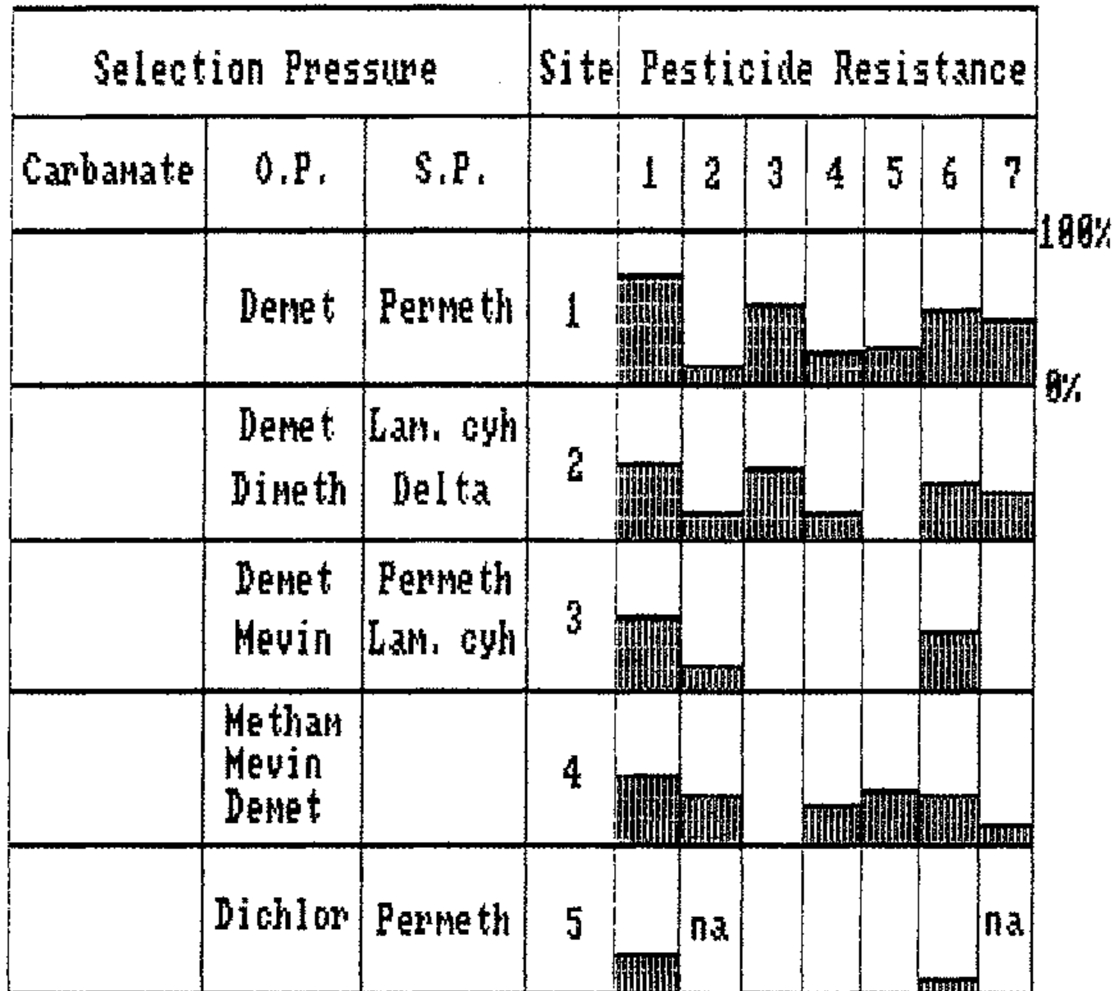
6 = permethrin

7 = esfenvalerate

The Levin colony (site 5) was not tested against the full range of insecticides as insufficient larvae were available because of the limited number of adult moths from field collection.

Figure 17 compares the insecticide resistance levels and the selection pressure (insecticides used against the field populations) for each of the 5 sites to establish any cross resistance patterns that may exist between the 7 insecticides.

Figure 17: A comparison of the resistance levels and insecticide selection for each site.



Demet = demeton-s-methyl

Dimeth = dimethoate

Metham = methamidophos

Delta = deltamethrin

Permeth = permethrin

Lam. cyh = lambda cyhalothrin

na = not assessed

1 = carbaryl

2 = methomyl

3 = diazinon

4 = mevinphos

5 = dichlorvos

6 = permethrin

7 = esfenvalerate

The left hand columns of figure 17 show the insecticides that were included in the spray programmes used on each site (selection pressure) to control insect pests. On the right hand side of the diagram is a graphical representation of the level of insecticide resistance present in the F1 generation from each site to the 7 insecticides tested. The bar graphs where derived by plotting the percentage survival at the diagnostic dose minus the percentage of survivors necessary to indicate a significant result at the 95% confidence limit.

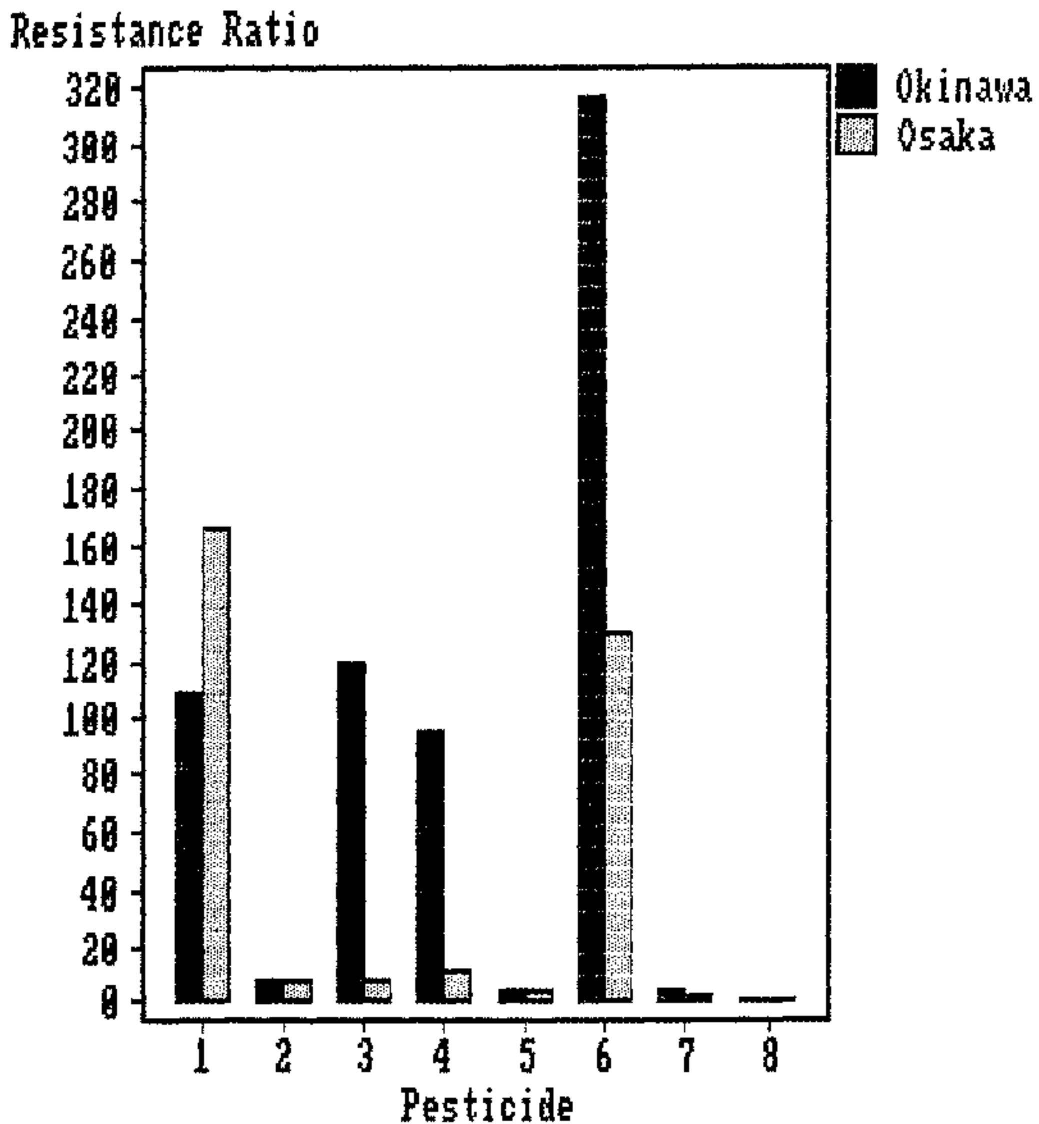
It is evident that the pattern of resistance is different on each of the five sites. Site 1 shows resistance to all of the insecticides included in the testing programme with the highest level of resistance being to carbaryl and the lowest to methomyl, both carbamates. Insects collected from site 2 show a similar pattern but with lower levels of resistance compared to site 1. They were resistant to some extent to all of the pesticides used except dichlorvos. The resistance pattern was quite different on site 3. This strain appeared to be resistant to carbaryl and permethrin and only slightly resistant to methomyl. The strain from site 4 was resistant to all of the pesticides used except diazinon. Site 5 showed the lowest levels of resistance with only carbaryl and permethrin being affected. The susceptible Massey colony was used as a control comparison and showed no insecticide resistance when it was screened with the diagnostic dose of each of the 7 pesticides.

Cheng (1986) induced resistance to a range of insecticides in a susceptible colony from Taiwan. He found that the cross resistance patterns were so complicated that only a few conclusions could be drawn. Cheng concluded that there is a common mechanism for synthetic pyrethroid resistance and that synthetic pyrethroid selected DBM also become less sensitive to organophosphate insecticides. In addition cross resistance within the organophosphate group is common and diverse. My data confirms the findings of Cheng (1986).

Insects from each of the 5 sites showed resistance to carbaryl yet carbaryl was not included in any of the current spray programmes.

Cheng (1988) reports that most carbamates are not very effective against even susceptible strains of DBM. He suggested that susceptible strains of DBM, like houseflies, may be inherently tolerant to carbaryl and possibly even methomyl or propoxur, because methomyl was ineffective against DBM when it was introduced into Thailand in 1972. Methomyl used at commercially competitive rates in New Zealand has never given more than 70% control of larvae in efficacy trials (Cornwell pers com). This means that the low resistance levels for methomyl may be due to the reduced efficacy of this compound against DBM but this does not explain why all of the field populations show highest resistance to carbaryl.

Figure 18: The resistance ratios of two strains of DBM selected with phenthoate (data of Miyata et al 1986).



1 = phenthoate

2 = dichlorvos

3 = prothiophos

4 = cyanophos

5 = acephate

6 = methomyl

7 = cartap

8 = fenvalerate

Miyata et al (1986) collected susceptible strains of DBM from Okinawa and Osaka in Japan and selected them for resistance with phenthoate. The selection was repeated 8 times during 9 generations. Figure 18 shows the resistance ratios of these selected strains for a range of insecticides. Miyata et al (1986) concluded from this study that when different strains are selected with the same insecticide they will not necessarily show the same cross resistance spectra.

The five sites I tested were both widely separated and selected with different insecticides so it is not surprising that they show disparate cross resistance patterns. However one trend is apparent in the data. Selection with either the synthetic pyrethroids or organophosphates gives cross resistance to carbaryl and to a lesser extent methomyl. Tabashnik et al (1987) collated the work of a number of authors on cross resistance in DBM. These trials were on regressed field strains subsequently re-selected with the chosen insecticide. Table 11 shows the cross resistance patterns found. Most pairings of conventional insecticides show some positive cross resistance. While there appear to be few patterns in the development of resistance it is evident that high selection pressure can give rise to insecticide resistance with extensive but unpredictable cross resistance patterns.

Table 11: Laboratory selection studies of cross resistance in DBM (data of Tabashnik et al 1987).

Insecticide	R.R	Cross resistance to other chemicals					
		Per	Fen	Mev	Pro	Car	Met
Diazinon	14	26	9	-	-	-	-
Methomyl	15	0.3	1.7	-	-	-	-
Carbofuran	36	3	1.8	3.3	0.3	-	0.8
Fenvalerate	14	3.3	-	3.7	2.2	6.4	2.1
Mevinphos	7	2.7	1.8	-	4.4	-	-
Profenophos	33	5.1	10	4.8	-	-	-
Permethrin	7.2	-	27	3.9	7.5	-	-

R.R = resistance ratio

Per = permethrin Fen = fenvalerate

Mev = mevinphos Pro = profenophos

Car = carbofuran Met = methomyl

7.5 Stability of Resistance in DBM.

The Pukekohe 2 colony was reared through five generations in the laboratory in the absence of insecticide treatment. The F5 generation was then tested for resistance using the same diagnostic doses as the previous tests. The percentage survival of the F1 and the F5 generations are compared in Table 12. It is evident that the level of resistance to all of the insecticides regressed in the absence of selection pressure. The Z test statistic in Table 12 is the percentage of survivors that

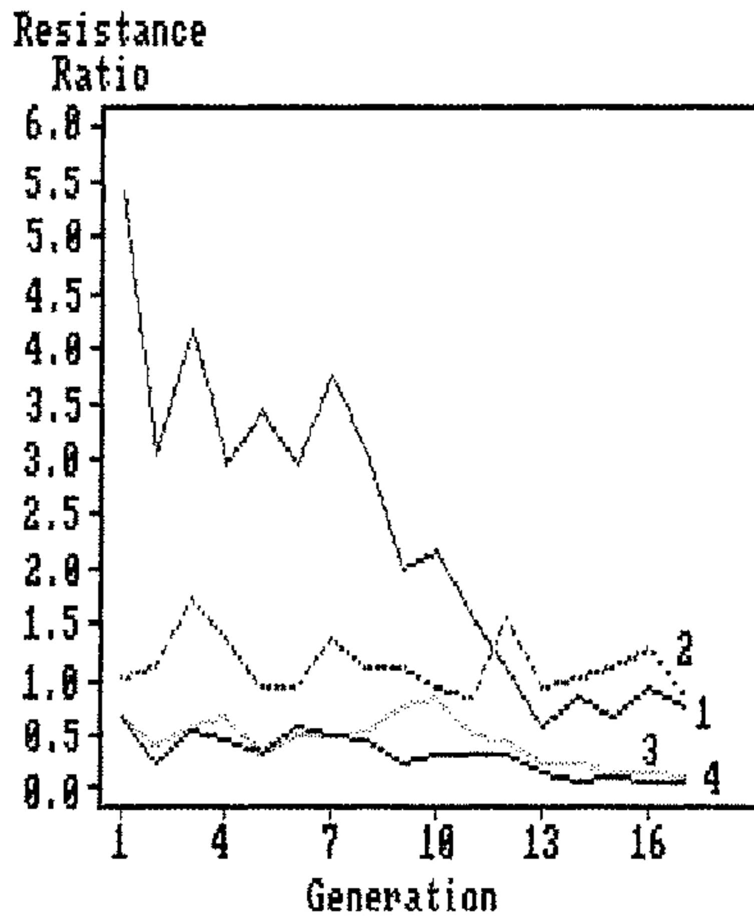
Table 12: Stability of resistance in the Pukekohe 2 Colony after 5 generations.

Insecticide	% survival at diagnostic dose		Z test
	F1 generation	F5 generation	
Carbaryl	63.4	39.6	11.2
Methomyl	26.3	6.7	11.5
Diazinon	59.5	2.5	11.9
Mevinphos	25.0	7.3	11.8
Dichlorvos	10.0	2.6	12.1
Permethrin	44.7	29.3	11.8
Esfenvalerate	41.0	15.0	11.9

are required in the F5 generation to indicate significant resistance at the 95% confidence level. This means that the F5 generation was still slightly resistant to carbaryl permethrin and esfenvalerate while resistance to methomyl, diazinon and mevinphos has become insignificant.

Under New Zealand conditions there are 6 or possibly 7 generations of DBM per year so this regression is particularly significant to resistance management because it could occur in one growing season. Chen and Sun (1986) measured the change in susceptibility to pyrethroids and organophosphates of a mixed field strain of DBM upon relaxation of insecticide selection pressure. A comparison of figures 19 and 20 shows that the DBM resistance to synthetic pyrethroids is slower to regress in the absence of selection pressure than is resistance to the organophosphate insecticides.

Figure 19: Changes in susceptibility to 4 pyrethroids of a mixed field strain of DBM upon relaxation of insecticide selection pressure (data of Chen and Sun 1986).



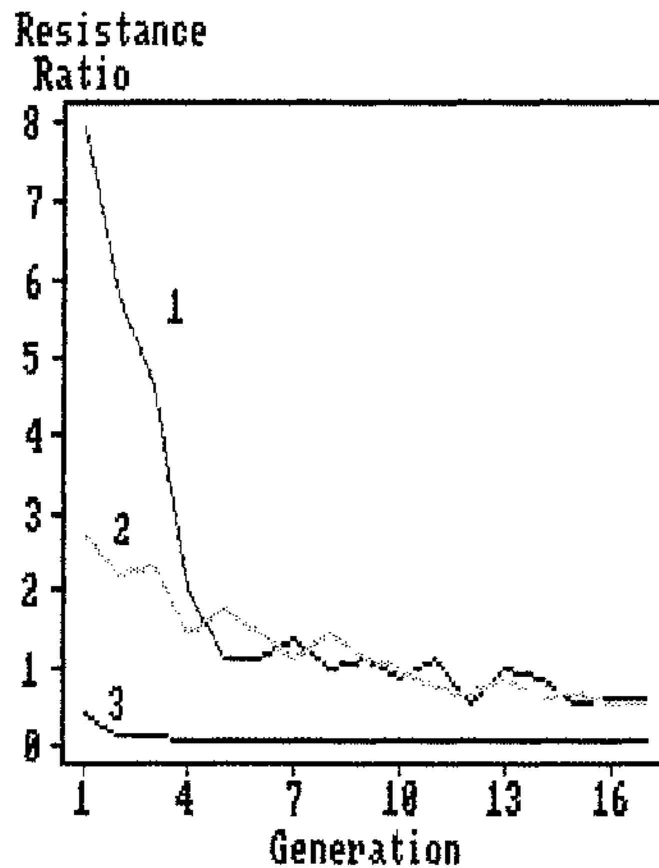
1 = fenvalerate

2 = deltamethrin

3 = cypermethrin

4 = permethrin

Figure 20: Changes in susceptibility to 4 organophosphates of a mixed field strain of DBM upon relaxation of insecticide selection pressure (data of Chen and Sun 1986).



1 = prothiophos   3 = mevinphos

2 = profenofos

The quicker regression of resistance to the organophosphates means that they may be a useful tool in resistance management programmes, especially those with a short residual life such as mevinphos and dichlorvos.

CHAPTER 8

MANAGING RESISTANCE IN DIAMONDBACK MOTH.

Diamondback moth is a serious pest of cruciferous vegetable crops in many parts of the world. It is an insect that has not only survived, but prospered through its ability to become resistant to insecticides (Georghiou 1981, Cheng 1988). In tropical countries, where DBM may produce 21 generations per year and field populations may become resistant to new pesticides after 2-5 years exposure, researchers have come to realise that the continued introduction of new pesticides is not a long term solution to this resistance problem (Cheng 1988).

From work undertaken for this thesis it is evident that insecticide resistant DBM can now be found in New Zealand. While the frequency of resistant individuals is still low enough to avert control failures these strains could cause problems in the future. However, two major considerations remain unexplored. First, further studies are required to determine the effect that the larval parasite *Diadegma semiclausum* is having on the development and spread of insecticide resistance in DBM. Second, further work is required to determine what effect the seemingly diverse range of pesticides employed in DBM control within relatively small areas is having. While the long term status of insecticide resistance in DBM in New Zealand is unknown it would be prudent, based on overseas

experience, to formulate resistance control strategies.

When planning measures to deal with an insecticide resistance problem there are a number of courses of action that can be considered, but the foremost requirement is for a good resistance monitoring programme which measures changes in the frequency or degree of resistance in time and space (Cheng 1988). Resistance monitoring is most useful when undertaken well before the advent of control failures. To be effective a monitoring programme must fulfil seven requirements (Brent 1986):

(1) Check for the frequency of occurrence of resistance individuals in the population.

(2) Provide an early warning that the frequency of such resistance is rising.

(3) Determine the effectiveness of management strategies introduced to avoid or delay resistance problems.

(4) Determine if control failures are due to resistance or some other problem.

(5) If resistance has been confirmed, determine subsequent changes in its incidence, distribution and severity.

(6) Give practical guidance on pesticide selection.

(7) Provide information on the behaviour of resistant forms in the field in relation to genetic, epidemiological and management factors.

Resistance monitoring must embrace a representative insect sampling method, test methods that are not only objective but also approximate the manner in which the pesticides are applied in the field, and an effective method of interpreting the results of the testing programme. It is evident from the diagnostic dose tests presented here that the levels of resistance and the cross resistance patterns in DBM in New Zealand may be quite diverse and therefore an intensive monitoring programme would be required. This diversity is probably due to different cropping patterns, geographic isolation of populations and different pesticide usage. It is conceivable that this diversity of resistance in DBM in New Zealand is limiting the spread of the resistant genotypes and resistance management must not jeopardise this.

Insect samples for resistance monitoring taken from the field have to be large, or represent a large proportion of surviving insects to reliably detect changes in the level of tolerance to an insecticide at frequencies low enough to allow action to forestall the build up of

resistance. Roush et al (1986) claim that if resistance is present at a phenotypic frequency or only 1% then control could be lost in as little as 6 generations, ie just 1 year under New Zealand conditions.

A resistance monitoring scheme to help forestall the development of pyrethroid resistance in *Heliothis armigera* in Australia failed to detect the build up of resistance until growers started to observe control failures. (Gunning et al 1984). Prior to the advent of these failures, resistance levels were monitored by looking for changes in the LD_{50} and slopes of full probit mortality lines (Daly and Murray 1988). Roush and Miller (1986) demonstrated the inability of this technique to reliably detect resistance when it is present at low levels. Coincident with the advent of control failures Gunning et al (1984) developed a discriminating dose test as a more reliable method of monitoring resistance levels in *H. armigera*. A voluntary resistance management programme has been put in place that is having a high level of grower compliance and considerable success in halting the intensification of insecticide resistance in *H. armigera* in Australia (Forrester and Cahill 1987). This programme is based on a strategy of restricting pyrethroid usage to three applications over a 42 day period, which corresponds to the least generation time of one of the 4-5 generations per year of *H. armigera*. Alternative pesticides to which there is no cross resistance are used before and after the

42 day pyrethroid period. The effectiveness of this strategy is closely monitored using discriminating dose tests (Sawicki and Denholm 1987).

Magaro and Edelson (1990) developed a technique for monitoring insecticide resistance in DBM in field populations. Disposable 29.6 ml plastic cups were treated with diagnostic concentrations of each insecticide, dissolved in 97% pure denatured ethanol. The diagnostic concentration was the LC_{90} estimated from a leaf dip bioassay using a susceptible population. One ml of insecticide solution was pipetted into a cup and it was rolled for approximately 1 min to ensure that all surfaces received treatment. The remaining solution was then drained from the cup and it was air dried in a fume cupboard at 25°C for 24 hours. Approximately 10 larvae are placed in each cup and the mortality assessed after 4 hours.

Table 13: Comparison of leaf dip, vial and disposable cup bioassays, treated with the diagnostic dose. (Magaro and Edelson 1990).

Treatment	Mean % Mortality		
	Leaf Dip	Vial	Cup
Methamidophos	82.5 a	95.0 a	95.0 a
Methomyl	82.9 a	100.0 a	100.0 a
Permethrin	95.0 a	100.0 a	95.0 a
Endosulfan	84.7 a	100.0 a	100.0 a
Control	9.8 b	0.0 b	0.0 b

In table 13 means in a column or a row followed by the same letter are not significantly different (P > 0.05, Duncan's multiple range test). Thus means that there was no significant difference between any of the treatments. Magaro and Edelson (1990) further evaluated this technique by testing a field population of DBM using disposable cups treated with the diagnostic concentration and comparing the results with those of a small plot trial using the recommended rates of insecticides on the original population.

Table 14: Comparison of laboratory bioassays and field test results (Magaro and Edelson 1990).

Insecticide	Plastic cup % mortality	Field plots	
		mean no. surviving / plant	estimated % mortality
Permethrin	7	1.3	0
Methamidophos	14	1.4	0
Endosulfan	23	1.2	0
Methomyl	30	0.3	71
Control	0	1.2	0

Table 14 shows the percentage mortality after 4 hours exposure to insecticide in the disposable plastic cups, the mean number of surviving DBM larvae per plant 24 hours after treatment in the field and an estimate of % mortality in the field plots. From this data it is evident

that only methomyl gave any measure of DBM control in the field . The purpose of the trial was to determine if there was any correlation between mortality in the field plots and the predicted level of control from the treated plastic cups. The authors claim that their plastic cup system works because the highest mortalities occurred in the methomyl treated cups and the field plots with the highest mortalities had also been treated with methomyl. However I do not agree with their conclusion as there was no significant difference between the percent mortality in the methomyl or the endosulfan treated cups, yet in the field endosulfan gave no control of DBM while methomyl produced 71% mortality. Furthermore the methomyl diagnostic dose gave only 30% mortality compared to 71% in the field . In my opinion further testing is required before this technique can be recommended with confidence. The major advantage of this monitoring technique is that pre treated plastic cups can be held frozen for later use.

For a DBM resistance monitoring programme in New Zealand to be effective, I envisage that the testing would have to be conducted on a grower by grower basis. Under these circumstances a biochemical test may be more appropriate that bioassays. However, as a stop gap measure until a suitable biochemical test becomes available, it may be possible to supply interested growers with pre-prepared leaf discs treated with a diagnostic dose of

insecticide, placed in a petri dish, then frozen to minimise chemical breakdown. The grower could test for resistance by thawing the disc in the unopened petri dish (to avoid condensation on the leaf disc) then count 10 third instar larvae into the dish and check their survival after 24 hours. Preliminary experiments indicate that the outer leaves of cabbage plants may resist cell damage due to freezing sufficiently to be employed in this technique. However further work would be needed to verify the viability of this system and the palatability of the thawed leaf discs. If effective this would be a quick and comparatively simple method of testing for resistance. By conducting this test using a range of insecticides the grower could establish which of them gave the best results and develop a control programme based on alternating pesticide treatments.

Resistance management strategies are usually aimed at reducing selection pressure to a minimum so that resistant individuals do not have a fitness advantage over susceptible insects. Time is at a premium in this exercise, as the longer a resistant population remains under selection pressure then the less likely it is that it will regress to its susceptible state when the pressure is removed. Frequent applications of pesticide favour resistant individuals. Pesticide applications should be spaced to allow periods of time when there is no selection pressure. During these times it is likely that

spaced to allow periods of time when there is no selection susceptible individuals will have a fitness advantage and the proportion of resistance insects in the population will decrease, provided that resistance has not developed to the point where the resistant insects have adapted to the extent that the deleterious effects of resistance in the absence of pesticides have been ameliorated. If this process, called co-adaption by Roush and McKenzie (1987), should occur, then repeated use of an insecticide on a population that has already built up a tolerance to the compound will tend to stabilize the resistance so that in the absence of selection pressure the resistance level will not reduce as fast as it might have. This means that resistant management strategies are easier if implemented before control failures occur. It would therefore be prudent to consider the implementation of strategies to reduce the selection pressure on New Zealand DBM populations as soon as possible to reduce the likelihood of control failures.

8.1 Resistance Mechanisms in DBM.

(A) DDT.

Liu et al (1982) tested susceptible and multi-resistant strains of DBM from Taiwan with a microsomal oxidation inhibitor, piperonyl butoxide (PB), and a DDT dehydrochlorinase inhibitor (DMC), and subsequently exposed them to DDT. There was no apparent synergism by PB

or DMC, raising the possibility of a non metabolic mechanism for DDT resistance. This mechanism may also play an important role in DBM resistance to the pyrethroids and may be similar to previously observed non metabolic mechanisms for DDT resistance in house flies and mosquitoes (Liu et al 1982).

(B) Pyrethroids

High levels of resistance in DBM to four major synthetic pyrethroids were found only three to four years after their introduction to Taiwan (Liu et al 1981). Synergist studies have revealed that esterase hydrolysis contributes only to a moderate extent to permethrin resistance in DBM. When PB was used to suppress the oxidative degradation of pyrethroids the residual resistance was still quite substantial (Sun et al 1986). This means that both metabolic and non metabolic mechanisms could be responsible for pyrethroid resistance in DBM. Variations in the degree of cross resistance between different strains of DBM to the same pesticide could be due to differences in the level of resistance that can be attributed to the two different types of mechanism. Liu et al (1981) claim that enhanced microsomal oxidation is a major factor for fenvalerate and permethrin resistance, and that enhanced esterase hydrolysis is a minor factor for permethrin resistance. Liu et al (1982) claim that insensitivity of the nervous system may also

contribute to pyrethroid resistance in DBM. Repeated synergist studies indicate that only permethrin could be synergised consistently and effectively by the esterase inhibitors triphenyl phosphate (TPP) and S,S,S-tributyl phosphorotrithioate (TBPT), while PB synergised all four major pyrethroids to varying degrees (Sun et al 1986). When strains of DBM were selected with fenvalerate plus PB they quickly became resistant to the mixture of insecticide and synergist. This resistance could be due to reduced penetration, accelerated metabolism or qualitative or quantitative changes that might occur to render the microsomal oxidases less sensitive to the inhibitory action of PB. However, PB resistance is quite unstable and regresses to the original levels after about five generations (Chen and Sun 1986).

(C) Organophosphates and Carbamates

Trials with a number of synergists that inhibit microsomal oxidases and esterases associated with the metabolism of organophosphate and carbamate insecticides showed that they had little effect on DBM (Sun et al 1986). A number of researchers indicate that one of the main mechanisms for organophosphate and carbamate resistance is probably acetylcholinesterase insensitivity (Liu et al 1981, Chen and Sun 1986, Miyata et al 1986, Sun et al 1986). However, Sun et al (1986) claim that this mechanism still could not account for the extremely high

levels of resistance to methyl parathion and malathion.

Sun et al (1978) selected laboratory strains of DBM with methomyl for 19 generations and diazinon for 14, and as a result, colonies with resistance ratios of 17.5 and 14.4 were produced. Electrophoretic studies revealed qualitative differences in esterase and phosphatase zymograms between these colonies.

(D) Chitin synthesis inhibitors.

Perng et al (1988) selected two strains of DBM for 20 and 29 generations with teflubenzuron. These strains developed a 12 fold and 7.5 fold resistance to the selecting agent. There was no apparent cross resistance with other benzoylphenylureas and a selection of conventional insecticides. While it appears from these results that this resistance developed rather more slowly than for other types of insecticide it would be foolish to conclude that this will be the case in the field, as the speed of development is dependent on the frequency of the resistance gene(s), which could be different in different field populations. Piperonyl butoxide, restored susceptibility to teflubenzuron indicating that microsomal oxidation was the major resistance mechanism. The lack of cross resistance between teflubenzuron, chlorfluazuron and conventional insecticides indicates that different microsomal oxidases may have been involved.

(E) *Bacillus thuringiensis.*

Tabashnik et al (1990) showed that the LC_{50} and LC_{95} for a field strain of DBM treated repeatedly with *B. thuringiensis* were 25 to 33 times greater than for two susceptible laboratory colonies. They conclude that the potential for resistance development in pest populations to *B. thuringiensis* endotoxin is an important consideration when contemplating the deployment of the toxin genes in genetically engineered crop plants.

8.2 Managing Resistance in DBM

While many resistance management practices have been proposed over the last 40 years few have been put into practice. (Leeper et al 1986). Five different management tactics have some potential for use in a resistance management programme for DBM in New Zealand.

(A) Reducing the fitness advantage of resistant genotypes.

Most cases of insecticide resistance in field strains are caused by a single gene. For this reason single gene models are usually more appropriate for evaluating resistance management tactics (Roush 1989). Pesticide resistance develops in an insect population because those individuals carrying the resistance gene(s) have a fitness advantage in the presence of insecticides, over those that

do not. Selection for resistance will occur most rapidly when the pesticide discriminates between heterozygotes and susceptible homozygotes. In this situation resistance is effectively dominant. Selection will be slower when resistance is effectively recessive, that is when heterozygotes die from field applications (Taylor and Georghiou 1979, Roush 1989). Heterozygotes are the most common carriers of the resistance gene while the resistance alleles are still rare. This makes them the most important genotype in terms of resistance management. The fact that resistance is slow to evolve when it is recessive shows that the key for managing resistance is managing heterozygotes (Roush 1989). Cheng (1988) tested the susceptibility of the F1 and F2 generations of crosses between susceptible and resistant DBM to five insecticides. The LC_{50} of the crosses (R♀ X S♂ and R♂ X S♀) were both intermediate between the LC_{50} values for the resistant and the susceptible populations for each of the insecticides. Pesticide doses which remove only the homozygous susceptible insects will allow a rapid build up of resistance. Higher doses that will kill all susceptible homozygous insects and the heterozygotes may still lead to build up in resistance even if only a few resistant homozygotes survive. On the other hand if at least a small percentage of susceptible homozygotes escapes exposure and interbreeds with the resistant homozygotes, resistance can be delayed substantially (Roush 1989). This high kill

approach is most useful in those situations where the resistance alleles are rare and it is likely that some fraction of the population will either escape exposure or immigrate from another location. The pesticide must not be so persistent that it prevents mating between susceptible and resistant individuals. High doses of a transient insecticide would be a better strategy.

Reducing the quantity of pesticide applied may not significantly delay the onset of resistance either. Tabashnik and Croft (1982) report that the mortality of susceptible homozygotes would have to be reduced to about 70% before a significant delay in the onset of resistance would be achieved. This would probably be an unacceptable level of control in most cases unless the increased pest density improved the effectiveness of other mortality factors (eg predation and parasitism). It would seem that in most cases it is difficult to manage resistance by changing the dose rate.

Frequent applications of pesticide will favour resistant individuals. If pesticide applications were spaced to allow periods of time when there was no selection pressure, then during these times it is likely that the susceptible individuals will have the fitness advantage.

Many growers, encouraged by the chemical companies and their distributors, apply pesticides on a calendar schedule rather than on the presence or absence of the

pest species concerned. Their philosophy is one of protecting the crop from attack rather than allowing a problem to develop before treating it. Therefore their pesticide application dates are based on renewing the "protective cover" before it has completely decayed, rather than on insect life cycles or even economic considerations. This practice means that the selection pressure is usually much higher than it need be. A change in thinking towards basing insect control programmes on economic thresholds rather than calendar schedules would reduce selection pressure by spacing out the applications and allowing some time for recovery of the population and/or re-invasion of susceptible individuals.

The use of less persistent compounds will have the same effect as spacing out application dates assuming that growers resist the temptation to compensate by applying the pesticide more frequently.

(B) Maintaining the frequency of the S gene(s).

Insecticide resistance will develop quickly if large areas are treated at any one time, eliminating almost all of the susceptible genotypes and leaving only resistant individuals to breed with each other producing resistant offspring, especially if the adults are moderately mobile and have an efficient means of locating a mate. If on the other hand many susceptible individuals are left behind as well as the resistant ones then it is likely that some of

the resistant individuals will mate with susceptible ones and resistance will be lost if the resistance gene(s) is recessive.

Control of a pest in single fields rather than over a large area can leave populations of the pest in surrounding areas to migrate to the previously treated area diluting the frequency of the resistance gene(s) and slowing the development of resistance. This tactic will only work satisfactorily if the insect species concerned is sufficiently mobile to be able to effectively recolonize the treated area before the resistant population has had time to establish itself.

Encouraging the immigration of susceptible pest genotypes into a treated area may be an effective way of dealing with a small insect population with a high proportion of resistant individuals. However this re-introduction must be sufficiently large to swamp the endemic resistant population if it is to be effective in reducing the build up of resistance. Tabashnik et al (1987) found that in some cases in Hawaii gene flow was insufficient to overcome differences in insecticide susceptibility between populations of DBM separated by less than 10 kilometres.

(C) Avoiding resistance.

The simultaneous use of two or more pesticides with different modes of action could be a useful tactic for

preventing the development of insecticide resistance. The use of mixtures to retard resistance is based on the idea that if resistance to each of the compounds is independent and initially rare, then cases of resistance to both compounds will be extremely rare (Tabashnik 1989). If an insect pest is resistant to one of the components of the mixture then the other component will ensure its mortality. A mixture can only be effective as a resistance management tool when individuals resistant to both insecticides are rare and when some fraction of the population escapes exposure diluting this resistance, otherwise resistance will occur quickly to both pesticides (Roush 1989). It is likely therefore that such mixtures will not prevent the development of resistance but merely delay it, and when it arrives it will be to two compounds rather than one. If the use of such mixtures is delayed until after resistance has developed to one of the compounds then it holds no advantage over using the other compound alone. Another potential disadvantage of mixtures is increased cost. Of equal concern is the potential effects of the mixture on non target pests and beneficial organisms. Biological control of pest species may be disrupted causing a rapid resurgence in the target organism after treatment or the elevation of a non pest species to pest status.

An alternative to using mixtures of insecticides is to use a number of different compounds in sequence or

rotation. The use of rotations is based on the assumption that the frequency of individuals resistant to one pesticide (A) will decline during application of an alternative pesticide with no cross resistance (B). In this situation there is a greater time interval between applications of a particular insecticide than there would be if the compound were used alone or in a mixture. If the time between repeat applications is great enough, then any resistance that may have started to build up will have time to regress before the next application of the offending material. Again there is a problem with this approach. There is sometimes considerable cross resistance between compounds within a chemical group and even between insecticides in different chemical groups. If this system is to be effective in preventing the occurrence of control failures through the development of resistance then the pesticides used in rotation will have to be free from cross resistance.

Tabashnik (1989) states that multiple pesticide use tactics have many potential pitfalls and are unlikely to provide long term solutions to pesticide resistance problems. Emphasis on reducing pesticide use and developing alternative controls is more likely to be productive.

There are a number of synergists available that block the insect's resistance mechanism so that the insecticide can still be used to good effect because the insect is no

longer able to detoxify it. However these synergists are expensive and difficult to formulate in mixture with pesticides.

The most common approach used to deal with insecticide resistance so far has been to change to a new chemical with a different mode of action. The result is a few years respite while resistance to the new compound develops to the point of causing control failures. Given the present slow down in the synthesis of new compounds this strategy may not always be available. However cycling of a number of insecticides from different chemical groups may slow down the development of resistance.

(D) Integrated pest management.

Integrated pest management relies on the preservation and even encouragement of an insect pest's natural enemies. This provides an additional mortality factor that does not discriminate between resistant and susceptible insects. It is therefore an effective way of removing resistant individuals following their selection by a pesticide.

(E) Different levels of resistance.

This tactic involves targeting those life stages of an insect pest that are less likely to develop resistance to levels that will cause control failures. For example the early larval stages of lepidoptera are usually more

susceptible to insecticides than are the later instars, and are therefore less likely to develop resistance to the point where control failures will occur. In the case of *Spodoptera littoralis* resistance to many compounds is much greater in larvae than in either adults or eggs because the larvae have better metabolic defences. Larvae of many holometabolous insects feed on plants that contain toxic compounds, whereas the adults feed on nectar and pollen and do not require detoxifying enzymes and thus lack any metabolic capability that could be modified to produce resistance (Roush 1989).

8.3 Resistance Management Case Studies.

(1) Management of insecticide resistance in *Heliothis armigera* in Australia (Forrester and Cahill 1987).

This management strategy was formulated following the field failures of pyrethroids against *H. armigera* on cotton crops in 1983. The aim of the strategy was to preserve the viability of the pyrethroids as a control agent for this pest (already showing resistance to a number of organophosphates). *H. armigera* has 4-5 generations per year in Australia and the central feature of the resistance control strategy is limiting the populations exposure to pyrethroids to one generation per year. Pyrethroid use is restricted to three applications

over a 42 day period each year. Outside this time alternative pesticides with no cross resistance to the pyrethroids must be used to control all pests on cotton crops. Regular checks on the frequency of resistant individuals has revealed that so far less than 10% of the larvae are resistant at the beginning of each season and the programme has been in operation for four seasons. While the incidence of resistant individuals increased during the populations' exposure to pyrethroids it decreased during the intervening 11 months when alternative pesticides were used. This drop signifies the success of the strategy. Sawicki and Denholm (1987) report that similar strategies limiting the use of pyrethroids to one generation per year are working successfully on this pest in Zimbabwe and Egypt.

(2) Resistance management strategies for horn flies (Byford et al 1987, Byford and Sparks 1987).

The predominant method for horn fly (*Haematobia irritans)* control on cattle in the USA is the use of pyrethroid impregnated ear tags. This approach is now threatened by the development of pyrethroid resistance in populations of horn fly over vast areas of the country. There is strong cross resistance to all of the pyrethroids but none is evident to the carbamates or organophosphates. There is also some evidence of modified behaviour on the

part of the resistant horn flies. They tend to congregate on the underside of the host, avoiding the insecticide treatment. Ear tags containing carbamate or organophosphate insecticides may give a temporary respite, but given the continual selection pressure that this delivery system imposes on the population, it is inevitable that resistance to these materials will also develop quickly. Ear tags containing pyrethroid plus a synergist have given some control of the resistant strain, but the horn fly infestation level remained unacceptably high. New ear tags where the insecticide is delivered intermittently have been developed and they may help to solve the problem.

Adult horn flies have been known to move between cattle herds separated by up to 1.7 km. Thus applying different insecticides in different sectors of a grid pattern may help to stall the development of resistance. If insect movements occur between sectors within a generation the mosaic functions as a mixture, that is individuals not killed by one component in the mixture/mosaic will be killed by another because the probability that any one individual will be resistant to all of the compounds is remote. Where exchange between the sectors occurs across generations the mosaic would function in much the same way as a rotation of pesticides.

(3) Insecticide Resistance in the light brown apple moth (Suckling et al 1984).

In this situation in the Nelson district there were a few apple orchards where the resident population of light brown apple moth (*Epiphyas postvittana*) (LBAM) contained a high proportion of individuals resistant to azinphos methyl. These orchards were surrounded by a area containing a mixed population of susceptible and resistant LBAM. Outside this area only susceptible individuals could be found. The choice of insecticides was limited as they had to be compatible with the integrated mite control programme practised in this area. A number of resistance management tactics have been put into place or recommended.

(a) Close monitoring of the level and distribution of resistance using pheromone collected males.

(b) Autumn 1984:chlorpyrifos applied post harvest to minimise the occurrence of overwintering larvae.

(c) Winter 1984: Alternate hosts inside those areas containing the resistant and the mixed populations destroyed.

(d) Spring 1985: Pyrethroids applied to any remnants of the alternate hosts and simultaneously chlorpyrifos applied in the orchards.

(e) Conserve the reservoirs of azinphos methyl

susceptible LBAM.

(f) Encourage the immigration of susceptible males into the resistant area by using the female sex pheromone.

(g) Continue to seek non organophosphate chemicals with high efficacy for leaf roller control but with a low toxicity to predatory mites.

While there is some evidence that the resistant population has been present for about 10 years without spreading to other orchards, the management tactics have been successful in as far as they have alleviated the control problems without jeopardizing the IMC programme used in the area.

8.4 Insecticide Resistance Management Programme For DBM.

As a result of this study I recommend that the following steps be taken to try and forestall the development on insecticide resistance in DBM in New Zealand.

(1) Treatment with synthetic pyrethroids should be restricted to one application per crop of brassicas.

(2) Pyrethroid treatments should be followed with either dichlorvos or mevinphos as cross resistance appears to be less than for other

organophosphates or carbamates with the possible exception of methomyl. However, the level of insect control provided by methomyl may not be adequate.

(3) The use of control action thresholds as developed by Beck and Cameron (1990) should be encouraged as they will ensure that small numbers of susceptible insects will be available to mate with resistant individuals, reducing the likelihood of control failures.

(4) The present diversity of treatments used for DBM control should be maintained. While it is evident that gene flow between populations is limited, the informal pesticide mosaic currently in effect may be helping to prevent the spread of resistance.

(5) The use of less persistant pesticides such as dichlorvos and mevinphos should be encouraged.

(6) Urgent attention should be given to developing an efficient resistance monitoring programme.

(7) The feasibility of operating an integrated pest management programme using the larval parasite *Diadegma semiclausum* should be considered as soon as possible.

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