Assessment of insecticide resistance in diamondback moth

A report prepared for

Vegfed Fresh Market Sector

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1 EXECUTIVE SUMMARY

Apparent failures to control diamondback moth (DBM) with insecticide applications prompted studies to determine if insecticide resistance was responsible. Populations of DBM were sampled in three main vegetable brassica growing regions, Pukekohe, Hawke's Bay and Gisborne, and standard leaf dip assays conducted to determine the susceptibility of these populations to four insecticides by comparison with New Zealand and USA standards.

The levels of resistance compared to the USA standard were 41 to 565 fold for the synthetic pyrethroid Karate, 12 to 31 for the organophosphate Tamaron, 7 to 13 fold for the carbamate Lannate, and 1.3 to 2.7 fold for the *Bacillus thuringiensis* product Dipel 2X.

Compared with insecticide resistance of DBM in the USA, the levels of resistance recorded for Karate and Tamaron are likely to cause control failures. Resistance to both Lannate and Dipel 2X was comparatively low and these products are still likely to be effective.

The high levels of resistance to the synthetic pyrethroid corresponded to high use of these products in a region. Correspondingly, low resistance to *Bacillus thuringiensis* in all regions was related to the minor or recent use of these products.

The levels of insecticide resistance recorded in this study are considered to be currently reducing the sustainablity of vegetable brassica growing. The continuation of this research to provide the basis of a resistance management strategy is recommended.

2 INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella* (L.), is the key pest of vegetable brassicas worldwide. Insecticide resistance in this insect has been reported in south-east Asia, Japan, the USA, Central America, and Australia (Sun 1992). The species has developed resistance to all major insecticide classes including *Bacillus thuringiensis* (Shelton et al. 1993) and insect growth regulators (Sun 1992). Recent reports from Australia show that DBM has developed high levels of resistance to permethrin in Victoria (Endersby & Ridland 1994) and Queensland (Heisswolf 1994), and to several insecticides in South Australia (Baker 1994). Identification of this resistance and associated control failures has led to the development of resistance management strategies.

In New Zealand, although DBM is often stated by growers to be the most difficult pest to control using insecticides, it is unclear if insecticide resistance is responsible for reported control failures. Such failures are commonly a result of inadequate application of insecticides and rapid re-infestation when weather conditions favour DBM. Bell & Fenemore (1990) documented significantly greater survival in assays of a Pukekohe population compared with a susceptible population, but these survival rates were not associated with control problems. This result demonstrated the difficulty of relating laboratory assays to field efficacy in New Zealand. In a recent series of studies, Cameron et al. (in press) related resistance levels from Pukekohe to those in the USA, and determined what resistance levels were associated with control failure. The present study determines if resistance of DBM in New Zealand to four insecticide classes is sufficient to cause control failures.

3 METHODS

3.1 Sites

Key brassica growers in the Pukekohe, Hawke's Bay and Gisborne vegetable growing regions were identified with the assistance of the Fresh Vegetable Research & Development Committee. These growers were interviewed to assess the current methods of control, the status of control problems and the efficacy of current control approaches.

Diamondback moth larvae and pupae were collected from three to five sites in each region. Each region was visited at least twice, and collections were made in February or March. Sites with a history of control difficulties were emphasised. The growers were asked to provide details of insecticide applications including a diary of past applications, rates, application methods and infestation levels.

3.2 Assays

The collections of DBM and the standard reference culture of DBM (designated Pukekohe 1) were reared in the laboratory using methods already developed for the standard culture (Cameron et al. in press). The larvae and pupae from each collection were reared separately to produce adults. The progeny of these adults (the F1 generation) were reared to the 2nd or 3rd instar larval stage for assays. Approximately 1000 larvae were required for one comparison, and if fewer larvae were present the population was reared through another generation for a further month to obtain sufficient larvae. Although the proposal was based on the minimal rearing option, all but one site required additional rearing effort.

The susceptibility of DBM larvae from each collection, to each insecticide, was compared with that of the standard DBM population. The insecticides, one from each insecticide class, were an organophosphate, Tamaron; a carbamate, Lannate; a synthetic pyrethroid, Karate; a *Bacillus thuringiensis*, Dipel 2X (Table 1). The measurements of susceptibility were based on leaf dip bioassays. Small DBM larvae (0.2-0.4 mg) were placed on disks of cabbage leaf dipped in a range of five to six doses of each test insecticide. Five larvae were placed on each of six disks at each dose (30 larvae at each dose). The standard was included in each experiment to minimise uncontrolled variables. Estimates of the LC_{50} for each population and insecticide were based on probit analysis of the log(concentration) and the population response using POLO (Robertson & Preisler 1992). To determine the degree of resistance for each insecticide group in each region, the LC_{50} of different populations was compared with the standard New Zealand population (Pukekohe 1).

Table 1: Insecticides used in resistance assays.

Insecticide class	Common name	Trade name	Similar registered products
Organophosphate (OP)	methamidophos	Tamaron	Monitor
Synthetic pyrethroid (SP)	lambdacyhalothrin	Karate	
Carbamate	methomyl	Lannate	
Bacillus thuringiensis (Bt)	*Bacillus thuringiensis* (Bt)	Dipel 2X	Delphin, Agree

The relationship of these results to resistance levels at which control failures may occur was derived from previous comparisons of New Zealand and USA standards (Cameron et al. in press). As the New Zealand standard (Pukekohe 1) was more resistant than the USA standard population, the New Zealand results were multiplied by the Pukekohe:USA resistance ratio. This allowed comparison with the resistance ratios at which each of the insecticides were expected to fail in the USA (see Table 3).

4 RESULTS AND DISCUSSION

4.1 Achievement of proposed work

The full schedule of assays proposed for this project was not completed because cool wet weather, including cyclones on the East Coast interfered with collection and subsequent rearing. This situation was notified to Vegfed in a letter on 27 March 1997 and the interim report on 23 April 1997. The collection and rearing effort was more than doubled compared with that proposed. Assays were carried out for four sites but the number of larvae limited results to one from each region rather than two per region.

4.2 Site information

4.2.1 Pukekohe

Six sites were visited (Howe Young, Frank Wai Shing, Sam Das, John van Lieshout, Hira Bhana, Alan Fong) and five sampled. Two of these sites produced sufficient DBM to rear for assays:

- Site 1: Collected 590 DBM on 6 February. These were assayed on 24-27 February. The results have been reported to the grower and a copy sent to Vegfed. This site is designated in Tables 2 and 3 as "Pukekohe 3", and

- Site 2: Collected 425 DBM on 6-7 March. These are still being reared but there have been insufficient numbers for assays.

By comparison with surveys in 1993/94 (Cameron et al. in press) synthetic pyrethroid (SP) use appeared to have declined at sites in the region where growers consider that it had reduced efficacy. However, SPs were still a major component of pest control in Pukekohe. Organophosphates (OP) were generally reported as still effective, but there were some reports of poor control. There was much more wide use of Bts than recorded in previous surveys in 1993/94. Although the use of Bt may have improved control in 1997, the wetter season also reduced pest pressure. Most growers practice some form of alternation of insecticide applications, but this may be ineffective because it is not coordinated throughout the region. Spray frequency was sometimes very high, reaching a minimum interval of three days. This approach is likely to encourage rapid increases in resistance.

4.2.2 *Hawke's Bay*

Nine growers were contacted, and four sites visited and sampled (Martin James, Robert Joe, Scott Lawson, Alan Young). Two crops were sufficiently infested to allow DBM to be collected for assays:

- Site 1: Collected 550 DBM on 17 February. These were reared through two generations and tested in April. This site was designated in Tables 2 and 3 as "Hawke's Bay 2", and

- Site 2: Collections from a second site in March were insufficient to start a colony.

Conventional brassica growers were using OPs, mainly Tamaron, to control DBM because they suspected resistance to SPs. SPs were considered to be effective up until the last season or two when growers had difficulty controlling DBM. Growers also reported hearing about problems with SP resistance and therefore returned to using OPs. There was little use of Bt in the region except by organic growers.

4.2.3 *Gisborne*

Of the six growers contacted, three were visited and three sites sampled (Higgins and two Leaderbrand sites). Two crops were sufficiently infested to allow collections. DBM numbers were low because of wet weather from the cyclones:

- Site 1: Collected 260 larvae on 4 February. There were insufficient DBM to rear for assays so they were reared through one generation and were assayed in March, and

- Site 2: Collections from 17-21 March were reared but wet conditions from the cyclones encouraged fungal disease which destroyed the culture.

Growers who were previously using SPs reported that they had changed to using OPs and Bts in an alternation strategy. Reported control failures included the failure of an application of Attack (mixture of OP and SP) and failures with separate applications of SPs and OPs. Bts are now being used more widely in Gisborne, and one grower reported the use of plant scouting to assist in timing of applications.

4.3 Resistance assays

Assays were successfully performed for three sites, one from each region. Assays were commenced for two other sites but insufficient larvae were obtained and the assays were not completed. The LC_{50} values for DBM from each site are presented in Tables 2a, b and c. These compare the 1997 test populations with the standard 1993 test population.

4.3.1 *Pukekohe*

Minor changes in the LC_{50} and resistance ratio for Dipel 2X at the Pukekohe site (Table 2a) show there was no significant change in DBM susceptibility to *Bacillus thuringiensis* from 1993 to 1997. This probably reflects the minor use of Bt products in the region. Significantly increased LC_{50}s for DBM for the other three insecticides at Pukekohe indicate decreases in the susceptibility of DBM to these products. The resistance ratios show that the changes in resistance to Lannate and Tamaron were minor, but there were large increases in resistance to Karate. This result is consistent with the predominant use of synthetic pyrethroids (SPs) on brassicas at Pukekohe. Comparison of this data with the standard USA population (Table 3) indicates that the resistance ratios for both Dipel 2X and Lannate are well below levels likely to give control failures. Tamaron may not give control, and it is unlikely that Karate would maintain control.

Table 2a: Comparative resistance of diamondback moth (DBM) from test and standard populations. Test population from Pukekohe, February 1997.
* = significant ratio ($P < 0.05$)

Insecticide (Class)	Toxicity (LC_{50} g ai/*l*) Test DBM 1997	Standard DBM 1993	Comparison resistance ratio[1] 1997/1993
Dipel 2X (*Bacillus thuringiensis*)	12.4	14.2	1.1
Lannate (Carbamate)	1.5	0.41	3.6*
Tamaron (Organophosphate)	0.32	0.09	3.5*
Karate (Synthetic pyrethroid)	0.17	0.003	56.5*

[1] = comparison of LC_{50}s.

4.3.2 *Hawke's Bay*

The resistance ratios from the Hawke's Bay site (Table 2b) showed significant but relatively minor increases in resistance to all four insecticides. The greatest increase was for Lannate. It was notable that the lowest increase in SP resistance was recorded in the Hawke's Bay region where growers indicated that they had moved away from use of this insecticide class. Although resistance levels in Tamaron and Karate were both above levels for expected control failure (Table 3), the resistance levels were less than those recorded from the Pukekohe site. Lannate and Dipel 2X remained effective.

Table 2b: Comparative resistance of diamondback moth (DBM) from test and standard populations. Test population from Hawke's Bay, February 1997.
* = significant ratio (P < 0.05)

	Toxicity (LC_{50} g ai/*l*)		
Insecticide (Class)	Test DBM 1997	Standard DBM 1993	Comparison resistance ratio[1] 1997/1993
Dipel 2X (*Bacillus thuringiensis*)	3.24	1.46	2.2*
Lannate (Carbamate)	2.75	0.43	6.4*
Tamaron (Organophosphate)	0.116	0.048	2.4*
Karate (Synthetic pyrethroid)	0.0055	0.0013	4.1*

[1] = comparison of LC_{50}s.

4.3.3 Gisborne

At the Gisborne site (Table 2c), significant differences in the resistance ratios for both Tamaron and Karate showed increases in resistance by comparison with the 1993 standard. The minor increase for Tamaron by comparison with Karate reflected insecticide usage patterns in this region. Comparison with the level for expected control failures (Table 3) suggest that Tamaron resistance may be reaching levels that will compromise control, and Karate is unlikely to give control.

Table 2c: Comparative resistance of diamondback moth (DBM) from test and standard populations. Test population from Gisborne, February 1997.
* = significant ratio (P < 0.05)

	Toxicity (LC_{50} g ai/*l*)		
Insecticide (Class)	Test DBM 1997	Standard DBM 1993	Comparison resistance ratio[1] 1997/1993
Tamaron (Organophosphate)	0.206	0.151	1.36*
Karate (Synthetic pyrethroid)	0.0102	0.00149	6.85*

[1] = comparison of LC_{50}s.

The levels of resistance to Karate (compared to the Pukekohe 1 standard) in this study are similar to those recorded in Australia for other SPs. For example, in Victoria resistance ratios of 20 to 49 fold for permethrin were associated with control failures (Endersby & Ridland 1994). In South Australia (Baker 1994), the pattern of resistance to different insecticide groups was similar to that in New Zealand; i.e. permethrin (SP) 102 fold resistance, Tamaron (OP) 15 fold, Lannate (carbamate) 47 fold. The recommendations from the Australian National Workshop on Managing Insecticide Resistance in DBM (Ridland & Endersby 1994) emphasised management strategies including resistance monitoring, development of alternating insecticide strategies, use of new insecticide chemistries, crop monitoring and use of biological control. Although Crop & Food Research has researched all of these areas, the key to their implementation is grower awareness of the risks to sustainability posed by insecticide resistance.

Table 3: Resistance ratios for each region compared to USA populations. Results for the 1997 test sites have been multiplied by the ratio for the New Zealand standard to allow comparison with USA populations.

Insecticide	1993 Pukekohe 1[1] (NZ Standard)	1997 Pukekohe 3	1997 Gisborne 1	1997 Hawke's Bay 2	Level for expected control failure in USA[2]
Dipel 2X	1.2	1.3	-	2.67	15
Lannate	2.0	7.2	-	12.8	100
Tamaron	8.7	30.5	11.8	20.9	10
Karate	10.0	565.0	68.5	41.0	20

[1] Comparison of New Zealand standard population with the USA standard (Cameron et al. in press).
[2] Derived from Shelton et al. (1993).

5 CONCLUSIONS

Comparisons with field results from the USA provide an indication of the likely effectiveness of insecticides. The levels of resistance recorded for Karate and Tamaron against DBM from the sample sites are likely to cause control failures. Resistance to both Lannate and Dipel 2X was low and these products retained their effectiveness.

Interpretation of these comparisons should only be applied to their use at recommended rates with correct application procedures. It should also be noted that resistance will only appear as a problem when DBM populations are dense enough to cause damage. Therefore, partial control may be sufficient when populations are only slightly above damage thresholds.

The data indicate some variability between regions, particularly in the extent of resistance to Karate. However, the variability between sites within regions is unknown, therefore the results may be specific to sites rather than regions. Information from further sites is required to clarify this issue.

The relevance of these results to insecticides in the same classes is variable. Although DBM that are resistant to one insecticide may respond similarly to other insecticides in the same class, resistance to organophosphates appears to be less cross-linked. Therefore, resistance to Tamaron does not imply resistance to other organophosphates. The presence of cross resistance by DBM to SPs suggests that the resistance measured to Karate in this study may affect other SPs. The results indicate that there is little resistance to *Bacillus thuringiensis* and if these products are applied correctly they should give control.

The failure of key products to give control is a threat to the sustainability of vegetable brassica growing. Some growers already report that they do not grow brassicas over the summer because of pest problems. The further development of a resistance management strategy is essential to preserve the efficacy of present insecticides and maintain the viability of brassica growing in these regions. Recent research reports from the Australia (Ridland & Endersby 1994) and the Third International Workshop on Management of Diamondback Moth in Malaysia in 1996 support an integrated approach.

6 RECOMMENDATIONS

Recommendations for future work were outlined in our unsuccessful 1997 application to Vegfed for funds for the project "Management of Diamondback Moth". As the results in this report were available and presented prior to the application, the recommendations below are essentially unchanged.

- Further insecticide resistance assays are required to determine how widespread resistance is within each region and to determine if these levels are changing in response to insecticide use. It is recommended that this work is focused on the Pukekohe region where resistance appears to be the most severe. These assays would use existing methods and extend the data for the same four insecticides.

- An effective survey of insecticide use and efficacy is required to relate any control failures to particular insecticides. This is also proposed for the Pukekohe area.

- Information from the above projects could be used to revise the existing insecticide resistance management strategy (Cameron 1996; see excerpt in Appendix I). This strategy currently recommends a region-wide insecticide alternation strategy that would be developed in consultation with the industry. It also encourages the use of integrated pest management approaches such as crop scouting and reduced spray programmes (Beck et al. 1992), and these will in turn encourage the activity of natural enemies including newly established parasitoids (Cameron et al. 1995).

7 ACKNOWLEDGEMENTS

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9 APPENDIX

Appendix I Diamondback moth resistance management strategy (Excerpt from Cameron 1996)

General strategy

The general strategy is to maximise non-insecticidal controls and reduce selection pressure by applying insecticides only when necessary, and by rotating insecticide use between different chemical groups in a planned programme.

The following guidelines are recommended.

- Use clean transplants to avoid early infestations. Spray in the nursery if necessary. Grow seedlings away from brassica field plantings.

- Destroy cruciferous weeds such as wild radish and mustard to remove sources of infestation. Do not spray these weeds with insecticide.

- Monitor plants at least weekly to detect infestations. Insecticide treatments are necessary when 12-15% of plants are infested (Beck et al. 1992).

- Comply with label rates and use correct application procedures. Calibrate sprayers at least once a season.

- Do not use insecticides from one chemical group all season. Consider using the biological insecticide *Bacillus thuringiensis* early in the season and then alternate among other groups. The most effective alternation is a co-ordinated area-wide programme to ensure mobile populations are not continually subjected to the same insecticide group.

- Plough in crop residues to bury larvae and pupae remaining immediately after harvest.

- Research stategies should include the following:
  - Continue efforts to find more efficient parasitoids.
  - The efficacy of alternative insecticide groups including *Bacillus thuringiensis* should be confirmed.
  - Resistance monitoring should be extended.

Implementation

- Growers should monitor plants to ensure insecticides are applied only when necessary.

- Regional grower organisations should develop planned rotation schedules for the co-ordinated use of insecticide groups.

- Insecticides with label claims for use on brassicas should carry the following label statement:

> IMPORTANT - RESISTANCE MANAGEMENT
>
> Diamondback moth resistance to this insecticide could develop from excessive use. To minimise this risk use strictly in accordance with label instructions. Avoid using this insecticide exclusively all season and avoid unnecessary spraying. Maintain good cultural practices.