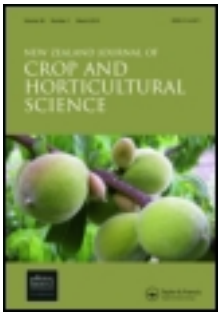

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P. J. Cameron [a] , A. M. Shelton [b] , G. P. Walker [a] & J. D. Tang [b]

[a] New Zealand Institute for Crop & Food Research Limited, Private Bag 92 169, Auckland, New Zealand

[b] New York State Agricultural Experiment Station, Department of Entomology, Cornell University, Geneva, NY, 14456, United States

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Comparative insecticide resistance of New Zealand and North American populations of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

P. J. CAMERON[1]
A. M. SHELTON[2]
G. P. WALKER[1]
J. D. TANG[2]

[1]New Zealand Institute for Crop & Food Research Limited
Private Bag 92 169
Auckland, New Zealand

[2]New York State Agricultural Experiment Station
Department of Entomology
Cornell University, Geneva
NY 14456, United States

Abstract The susceptibility of two New Zealand populations of diamondback moth, *Plutella xylostella*, to insecticides from three classes was compared with the susceptibility of a standard North American population (Geneva 88) in laboratory assays at the New York Experiment Station during 1993. Leaf dip assays showed that the New Zealand populations had developed moderate resistance to permethrin compared with the Geneva 88 population, but were still susceptible to methamidophos, *Bacillus thuringiensis* subsp. *kurstaki*, and *B. thuringiensis* subsp. *aizawai*. One of the New Zealand populations, Pukekohe 1, was 10 times more resistant to permethrin at the LC_{50} compared to the Geneva 88 population. This level of resistance was consistent with the greater use of synthetic pyrethroids, particularly permethrin, compared with other insecticides on vegetable brassicas at Pukekohe. Use of the Pukekohe 1 population as a standard for resistance assays in New Zealand indicated that diamondback moth from a reported control failure in Pukekohe were 4.9 times more resistant to lambdacyhalothrin, and may be as resistant to synthetic pyrethroids as the most resistant North American populations reported in Shelton et al. (1993b).

Keywords *Plutella xylostella*; insecticide resistance; bioassays; synthetic pyrethroid; *Bacillus thuringiensis*

INTRODUCTION

Diamondback moth, *Plutella xylostella* (L.), is the key pest of vegetable brassicas worldwide, including New Zealand. Insecticide resistance in this insect has been reported in south-east Asia, Japan, the United States, Central America, and Australia (Sun 1992). The species has developed resistance to all major insecticide classes including *Bacillus thuringiensis* (Shelton et al. 1993a) and insect growth regulators (Sun 1992). Recent reports from Australia show that diamondback moth 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 diamondback moth 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 reinfestation when weather conditions favour diamondback moth. Observations in the Pukekohe, South Auckland area of New Zealand (Beck 1991), showed that the pest was more abundant in dry, warm periods and populations rapidly declined in cool or wet weather. Parasitoids produce high rates of mortality early in the season, but increasing pest

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populations are often not controlled by the existing parasitoids, *Diadegma semiclausum* (Hellen) and *Diadromus collaris* (Gravenhorst). Bell & Fenemore (1990) documented significantly lower mortality in a Pukekohe population compared with a susceptible population exposed to concentrations of synthetic pyrethroid, organophosphate, and carbamate insecticides that killed 95% of the susceptible population. These survival rates were not associated with control problems and demonstrated the difficulty of relating laboratory assays to field efficacy in New Zealand. In the present study, the significance of resistance in New Zealand populations of diamondback moth was determined by comparing test populations with a standard North American population.

METHODS

Insects

In April 1993, two New Zealand diamondback moth populations were established from collections made from cabbage at the Pukekohe Research Station (Pukekohe 1) in South Auckland and from a commercial cabbage crop near Napier in Hawke's Bay, New Zealand. Approximately 100 larvae were collected from each site and reared as separate populations on cabbage seedlings at the Mt Albert Research Centre in Auckland. In July 1993, c. 60 pupae from each culture were shipped to the New York State Agricultural Experiment Station in Geneva, NY, United Sates and reared on rape seedlings (Shelton et al. 1991). The susceptibility of these populations to insecticides was compared with a standard susceptible diamondback moth population (Geneva 88) collected from cabbage near Geneva NY (Shelton et al. 1993b). A further New Zealand population was established in February 1994 from c. 100 diamondback moth larvae collected from a commercial brassica field at Pukekohe. Failure to control diamondback moth larvae had been reported in this crop following the application of a sequence of synthetic pyrethroid, organophosphate, and carbamate insecticides. The susceptibility of this population (Pukekohe 2) was compared with the Pukekohe 1 population, which served as the standard population in New Zealand.

Insecticide use

Information on the insecticides used by brassica growers in the Pukekohe area was gained by interviewing individual growers and asking them to identify the materials they had used in the last 12 months from a list of trade names. Twenty-six growers were interviewed in 1988 and 1989 and this information was informally updated in discussions with growers.

Bioassays

Measurements of the susceptibility of diamondback moth larvae were based on leaf-dip bioassays developed by Shelton et al. (1993a,b). For comparison of populations, the assays for each insecticide were carried out simultaneously. In Geneva, leaves from the outer layers of cabbage heads were cut into 25 mm diameter disks that were dipped into test dilutions for 5 s; held to drain for 5 s, dried at room temperature for 1–2 h and then placed in 40 ml plastic cups with vented lids. Five larvae were placed in each cup. Second instar larvae each weighing between 0.2 and 0.4 mg were used for tests with *Bacillus thuringiensis*, and 3rd instar larvae each between 0.7 and 0.9 mg were used for synthetic insecticides. Larval mortality was assessed after 48 h at 28°C for tests with synthetic insecticides and 96 h for *B. thuringiensis*. Larvae that did not move after being prodded with a brush were considered to be dead. Synthetic insecticides were obtained as the following commercial materials: permethrin (Ambush 2E), methomyl (Lannate 1.8L), and methamidophos (Monitor 4E). Two *B. thuringiensis* formulations were used: Javelin WG, Sandoz (32 000 International Units per mg), a commercial formulation of *B. thuringiensis* subsp. *kurstaki*, and XenTari, Abbot Laboratories (15 000 International Units per mg), a commercial formulation of *B. thuringiensis* subsp. *aizawai*. Bond sticker spreader (Loveland Industries, Loveland, CO, United States) was added at the rate of 0.25 ml/100 ml dilution for each concentration.

In Auckland, assays were performed as in Geneva except leaf dip assays used 40 mm diameter leaf disks and larvae were held at 26°C prior to assessment. The same source of permethrin was used at both test locations and lambdacyhalothrin, as Karate (50 g/litre) was also tested in New Zealand. Methomyl was obtained as Lannate L (200 g/litre) and methamidophos as Tamaron (600 g/litre). *B. thuringiensis kurstaki* was obtained as Dipel 2X, Abbot Laboratories (32 000 International units/mg).

Preliminary assays without replication were used to select an appropriate concentration range for the assays. Five to six concentrations were used for each insecticide, but the range of concentrations

for each population sometimes differed when populations showed differing susceptibility. The concentration ranges for each insecticide were: permethrin (0.003–1.0 mg (a.i.)/ml), lambdacyhalothrin (0.0001–0.1 mg (a.i.)/ml), methomyl (0.1–10 mg (a.i.)/ml), methamidophos (0.003–1.0 mg (a.i.)/ml), Javelin (0.03–10 mg/litre), XenTari (0.1–31.6 mg/litre), Dipel 2X (0.1–30 mg/litre).

The results were analysed by probit analysis fitted to log (concentration) and the response of different populations to the same insecticide compared using POLO (Robertson & Preisler 1992). Where there were statistically significant ($P < 0.05$) differences between the responses of the test and standard populations, the ratio of the LC_{50} of these populations was calculated and recorded as the resistance ratio (RR). Where the dose-response lines were not parallel, the data was plotted and resistance ratios at other percentage mortalities were examined.

RESULTS AND DISCUSSION

Insecticide use

The survey of insecticide use by vegetable brassica growers in the Pukekohe area in 1988 and 1989 indicated that a wide variety of insecticides was applied for control of Lepidoptera. The synthetic pyrethroid permethrin was the most commonly used insecticide; 24% of growers identified this material. Together with three other synthetic pyrethroids (cyfluthrin, deltamethrin, and fenvalerate), this insecticide class accounted for 51% of the insecticides named. Organophosphates were the other major class identified (43%), mainly parathion methyl, mevinphos, and methamidophos with <2% use of another 10 insecticides in this class. Five percent of growers used the carbamate, methomyl. No growers used *Bacillus thuringiensis*. By 1993, with the addition of the new materials, lamdacyhalothrin and cypermethrin, the synthetic pyrethroids remained the predominant insecticide class. The frequency of methamidophos applications increased as this insecticide was applied to control aphids as well as Lepidoptera. Amongst growers surveyed, the use of *B. thuringiensis* was still not identified by 1993 and application to one crop was reported by 1995.

Field control

Diamondback moth showed no signs of field resistance in various field trials conducted in the Pukekohe area from 1989 to 1991 with the commonly used insecticides permethrin, lambdacyhalothrin, or methamidophos (Beck & Cameron 1990; Beck et al. 1992). In 1991, Bell & Fenemore (1990) established discriminating concentrations of several synthetic insecticides based on the LC_{95} for a susceptible population. They detected <50% mortality of their Pukekohe 1 population to discriminating concentrations of the synthetic pyrethroids esfenvalerate and permethrin, the organophosphate diazinon, and the carbamate carbaryl. These levels of resistance appeared to have no effect on field control and their significance in the development of control failures is unclear. In 1993, applications of lambdacyhalothrin and the *B. thuringiensis* product Thuricide in field trials gave effective control of diamondback moth in cabbages just prior to the collection of the standard Pukekohe 1 population (P. J. Cameron unpubl. data). In 1994, there were several anecdotal reports of control failures in the Pukekohe area, including that at the Pukekohe 2 site.

Bioassays

Bioassays comparing the New Zealand populations with the standard susceptible Geneva 88 population provided a means for assessing the susceptibility of New Zealand populations compared with the range of populations surveyed by Shelton et al. (1993a,b). The repeatability of the assay technique in different locations was demonstrated by assaying the Pukekohe 1 population, using the same insecticides, at both the New York Experiment Station laboratory in the United States and Crop & Food Research's laboratory in Auckland. Despite minor differences in the methodology, the LC_{50}s for the Pukekohe 1 population for permethrin and methamidophos were not significantly different between the laboratories. This calibration of the Pukekohe 1 population against the Geneva 88 population has allowed the use of this New Zealand population as a new baseline for comparison of resistance.

Synthetic insecticides

Tests with permethrin in New York (Table 1) showed that both New Zealand populations were less susceptible ($P < 0.05$) than the Geneva 88 population. In our assays, the LC_{50} of the Geneva 88 population was lower than recorded earlier by Shelton et al. (1993b) and equivalent to field populations such as Purdue, which Shelton et al.

(1993b) considered to exhibit baseline susceptibility to permethrin. By comparison with this baseline LC_{50} of 0.005 mg (a.i.)/ml, the resistance ratio for the Pukekohe 1 population was 10 and for Hawke's Bay was 3.8. The Pukekohe and Geneva populations also differed in their response to methamidophos (Table 1). Comparison of the LC_{50}s indicated that the Pukekohe population was less susceptible than the Geneva 88 population to methamidophos, with a resistance ratio of 8.7. These resistance ratios suggest that there has been selection for resistance to both insecticides in the New Zealand populations. Comparison of these results with those of Shelton et al. (1993b) showed that populations with resistance ratios similar to Pukekohe 1 (e.g., the Funks MS) were still susceptible to field rates of these insecticides. This observation was confirmed by the complete mortality of our test larvae at discriminating concentrations suggested by Shelton et al. (1993b) to survey for field resistance to permethrin (discriminating concentration of 1.0 mg (a.i.)/ml) and methamidophos (0.32 mg (a.i.)/ml).

In New Zealand, the Pukekohe 1 population was used as a standard to estimate the significance of reported resistance in a commercial brassica crop (Pukekohe 2). A significantly higher LC_{50} ($P < 0.05$) was detected in the Pukekohe 2 population for the synthetic pyrethroid, lambdacyhalothrin (Table 1). By comparison with the Pukekohe 1 population, the resistance ratio for lambdacyhalothrin in the Pukekohe 2 population was 4.9. As the slopes of these lines were significantly different, it was necessary to calculate the resistance ratio at other percentage mortalities—at the LC_{90} it was 13.0. By comparison with the more susceptible Geneva 88 population the resistance ratio could be estimated by mutiplying the two ratios: 4.9×10. This 49-fold resistance ratio was equivalent to some of the most resistant populations assayed by Shelton et al. (1993b), which included populations that originated from locations where attempts to control field populations had failed.

By contrast, significant but minor changes occurred in the susceptibility to methomyl of the Pukekohe 2 compared with Pukekohe 1 populations (resistance ratio 2.1, Table 1). No further resistance appeared to have developed to methamidophos. The pattern of reduced development of resistance to methamidophos is consistent with the results of Shelton et al. (1993b).

Bacillus thuringiensis

Neither of the New Zealand populations tested in New York showed significantly different LC_{50}s ($P < 0.05$) compared with the Geneva 88 population (Table 2). This susceptibility of the Pukekohe 1 and Hawke's Bay populations is consistent with the

Table 1 LC_{50}s of *Plutella xylostella* from Geneva (New York State, United States) and New Zealand sites exposed to permethrin, methamidophos, and methomyl. (NC = not calculable.)

Insecticide	Population	n[a]	LC_{50} (95% CI) mg (a.i.)/ml	Slope ± SE	Het[b]	RR[c]
Tests in New York						
Permethrin	Geneva 88	100	0.0055 (0.003–0.008)	1.87 ± 0.40	0.30	–
	Pukekohe 1	149	0.055 (0.037–0.080)	1.68 ± 0.22	0.42	10.0
	Hawke's Bay	123	0.021 (0.015–0.029)	2.50 ± 0.38	0.30	3.8
Methamidophos	Geneva 88	100	0.015 (NC)	4.00 ± 0.71	6.08	–
	Pukekohe 1	150	0.13 (0.065–0.28)	2.12 ± 0.31	1.71	8.7
Tests in New Zealand						
Permethrin	Pukekohe 1	100	0.087 (0.053–0.194)	2.48 ± 0.45	1.54	–
Lambdacyhalothrin	Pukekohe 1	150	0.0013 (0.001–0.002)	3.11 ± 0.50	0.84	–
	Pukekohe 2	150	0.0062 (0.004–0.009)	1.53 ± 0.21	0.07	4.9
Methomyl	Pukekohe 1	147	0.20 (0.13–0.28)	1.90 ± 0.33	0.59	–
	Pukekohe 2	148	0.42 (0.33–0.54)	3.60 ± 0.57	0.64	2.1
Methamidophos	Pukekohe 1	100	0.070 (0.052–0.096)	3.38 ± 0.64	0.25	–
	Pukekohe 2	112	0.075 (0.033–0.201)	2.45 ± 0.43	1.41	NS

[a]No. of insects tested.
[b]Heterogeneity = chi^2/d.f.
[c]RR = significant ($P<0.05$) resistance ratio comparing LC_{50} of test population to LC_{50} of standard population; NS = lines not significantly different.

Table 2 LC_{50}s of *Plutella xylostella* from Geneva (New York State, United States) and New Zealand sites exposed to *Bacillus thuringiensis* preparations.

Insecticide	Population	n[b]	LC_{50} (95% CI) mg (a.i.)/ml	Slope ± SE	Het[c]	RR[d]
Tests in New York						
Javelin	Geneva 88	146	0.40 (0.16–0.63)	2.18 ± 0.57	0.26	–
	Pukekohe 1	100	0.11 (0.058–0.19)	1.34 ± 0.25	0.40	NS
	Hawke's Bay	148	0.52 (0.30–0.93)	1.68 ± 0.23	1.03	NS
XenTari	Geneva 88	148	4.35 (2.24–9.70)	0.77 ± 0.13	0.19	–
	Pukekohe 1	150	4.21 (1.58–7.05)[a]	2.67 ± 0.61	1.65	NS
Tests in New Zealand						
Dipel 2X	Pukekohe 1	120	5.87 (3.08–16.55)	1.18 ± 0.30	0.25	–
	Pukekohe 2	150	7.82 (3.08–27.07)	0.62 ± 0.62	0.28	NS

[a]90% CI (95% CI not calculable).
[b]No. of insects tested.
[c]Heterogeneity = chi^2/d.f.
[d]RR = significant ($P<0.05$) resistance ratio comparing LC_{50} of test population to LC_{50} of standard population; NS = lines not significantly different.

infrequent use of *B. thuringiensis* in vegetable crops in New Zealand before 1993. The more recently collected Pukekohe 2 population was as susceptible as the standard Pukekohe 1 population, showing that the limited use of *B. thuringiensis* has had no effect on resistance in New Zealand.

Failure to control diamondback moth following the correct application of synthetic pyrethroids in the Pukekohe area may be the result of resistance to insecticides that have been used frequently over the last several years. Prior to 1994, the level of resistance appeared to be insufficient to cause control failures at the population densities encountered. Although our investigations of some reported control failures in New Zealand implicate poor application procedures, the level of resistance in the Pukekohe 2 population was equivalent to that found in North American populations where control had failed. It is unclear if resistance to methamidophos or methomyl in New Zealand is sufficient to cause control failures.

This study illustrates the difficulty of interpreting the significance of laboratory bioassays of resistance before the levels of resistance are sufficient to cause control failures in the field. In Victoria, Australia, the identification of definite control failures allowed the discrimination of LC_{50}s and resistance ratios that were associated with susceptible or resistant field populations (Endersby & Ridland 1994). This method of interpretation is of use only after field resistance has developed, thereby delaying the implementation of resistance management strategies. Comparisons with an overseas standard such as the North American Geneva 88 population provide an alternative approach. Following our detection of moderate resistance in 1993, a resistance management strategy was developed for the Insecticide Resistance Task Group of the New Zealand Committee on Pesticide Resistance (Cameron 1996). The identification of high levels of resistance in 1994 suggested the rapid development of resistance to synthetic pyrethroids, and, in response the vegetable industry has supported further research. Our results, showing the susceptibility of New Zealand diamondback moth to *B. thuringiensis,* indicate that resistance management strategies would benefit from the inclusion of *B. thuringiensis* preparations as one class in planned alternation of insecticide classes. The use of such alternation strategies, as described by Deuter (1989), will have a greater probability of successfully managing insecticide resistance if they are implemented before the efficacy of any one insecticide class is reduced.

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