RESISTANCE TO CHLORPYRIFOS IN THE MEALYBUGS *PSEUDOCOCCUS AFFINIS* AND *P. LONGISPINUS* IN HAWKES BAY AND WAIKATO PIPFRUIT ORCHARDS

J.G.CHARLES¹, J.T.S.WALKER² and V.WHITE²

HortResearch, ¹Private Bag 92 169, Auckland,
and ²Private Bag 1401, Havelock North.

SUMMARY

A residual bioassay was used to measure the responses to chlorpyrifos of two populations of *P. affinis* from Hawkes Bay, and one of *P. longispinus* from Auckland. One population of *P. affinis* exhibited 24 fold level of resistance compared with the other. *P. affinis* from 34 orchards in Hawkes Bay, and *P. longispinus* from five in the Waikato were exposed to diagnostic doses of chlorpyrifos to investigate whether this level of resistance was widespread in pipfruit orchards. It appeared to be restricted to *P. affinis* in old pear orchards in Hawkes Bay. The implications of the results for future mealybug control programmes in New Zealand pipfruit orchards are discussed.

Keywords: mealybug, *Pseudococcus affinis*, *P. longispinus* resistance, chlorpyrifos, diagnostic dose.

INTRODUCTION

Pseudococcus affinis (Maskell)(=*P. obscurus*), is the dominant species of mealybug in Hawkes Bay pipfruit orchards. *P. affinis* in New Zealand has been mistakenly identified as *P. maritimus* or *P. comstocki* in the past, but neither of these species occur in New Zealand. Mealybugs other than *P. affinis* (usually *P. longispinus* or *P. calceolariae*) are also pests in pipfruit orchards outside the Hawkes Bay region (Charles in press).

P. affinis has been a recurring problem in Hawkes Bay pipfruit orchards for many years, and was reportedly resistant to DDT by 1959 (Congdon and Morrison 1959). Three-weekly applications of parathion or dimethoate were recommended for mealybug control through the 1960s, followed by changes to aminocarb (e.g. MacKenzie 1972) or omethoate. Trials in 1980-82 showed that early season applications of chlorpyrifos or prothiofos, in conjunction with a season-long programme of azinphos-methyl, effectively controlled mealybugs in apples (Charles and Walker 1981; Charles 1982). This treatment has been widely adopted by pipfruit growers. Thus organophosphate insecticides have been used for mealybug control for about 30 years. Since 1987, an increasing number of Hawkes Bay orchardists have reported the failure of insecticides to control mealybugs, and resistance to chlorpyrifos began to be suspected. Although apples grown for export receive a similar insecticide programme throughout New Zealand, there have been few reports of mealybug control failure in other regions.

This paper describes some preliminary investigations on the status of mealybug resistance to chlorpyrifos, and, if present, whether resistance was widespread in pipfruit orchards in Hawkes Bay and Auckland/Waikato.

MATERIALS AND METHODS

Laboratory colonies of mealybugs were maintained at ambient temperature (about 20-23°C) on potatoes.

A population of *P. affinis* was arbitrarily designated "susceptible" to insecticides. The mealybugs originated from Hawkes Bay, but had been reared on potatoes, unsprayed with insecticides, for at least 2-3 years. A search for a population with no possible history of exposure to chlorpyrifos was unsuccessful.

Proc. 46th N.Z. Plant Protection Conf. 1993: 120-125

A second population of *P. affinis* ("Hastings pear") was also established, from mealybugs collected in late-November 1990 from a pear orchard in Hastings where mealybug control had become a problem despite frequent applications of chlorpyrifos. Once established in the laboratory, this population was not exposed to further insecticide selection pressure.

A colony of *P. longispinus*, designated "susceptible" to chlorpyrifos, was established from two field populations (collected from citrus in Auckland in December 1990) with no history of exposure to any insecticide.

Laboratory bioassays

Neonate crawlers were chosen for testing to minimise the physical variability encountered within mealybug instars. Crawlers are a uniform size (about 0.1 mm long), have little protective wax coating, and can also be easily and safely moved with a fine brush while crawling. Subsequent instars are too variable in size, and develop a dense coating of hydrophobic wax. Older mealybugs are also usually feeding, with their proboscis inserted deeply into the phloem tissue, so that it is often difficult to move them without causing injury.

Topical application of pesticide to crawlers was considered impractical, so a residual bioassay was used. Crawlers were released on to an insecticide-treated surface and mortality was assessed after a suitable period. Tests were carried out in 9 cm diameter plastic petri dishes. Chlorpyrifos has a high vapour pressure, so, to minimise any fumigant effect, the dishes were not covered during the tests. Crawlers were restricted to the treated floor of the dishes by painting the inside walls with Fluon (Northeast Chemical Co. Inc., Woonsocket, RI, USA) at least 24 h earlier. Petri-dishes were sprayed evenly with 2 ml of insecticide applied through a Potter spray tower operated at 15 psi, allowing a 10 second settling period. Insecticide dilutions were made with de-ionised water, which was also sprayed on to control dishes.

When dry (<1 hour in the laboratory), about 30 crawlers were transferred by brush to each dish, a process that usually took 5-10 minutes. The dishes were then kept in the laboratory at 25°C ($\pm 2^\circ\text{C}$), and crawler mortality was assessed after 4 h. This assessment period was chosen to minimise mortality from non-treatment effects, particularly desiccation. We found no advantages in extending the assay period to 6 or 8 h, and by 24 h control mortality was unacceptably high (>50%). Crawlers were considered to be affected by the pesticide if they were incapable of crawling, after light prodding with a needle, one body length using coordinated leg movements.

To establish dose/mortality responses of *P. affinis* and *P. longispinus* crawlers were exposed to chlorpyrifos (Lorsban 40EC) residues in January 1991 (the two *P. affinis* populations) and February/March 1991 (*P. longispinus*). At least five concentrations of chlorpyrifos were used, to obtain a range of mortalities from <5% to >90%. At least 100 crawlers (*P. affinis*) and 50 crawlers (*P. longispinus*) were tested at each concentration.

The parents (the F_0 generation) of the "Hastings pear" colony founders (F_1) were the last to receive chlorpyrifos (a cover application in mid-November 1990). The crawlers tested in January 1991 were from the F_1 generation. The experiment was repeated against F_3 crawlers of the "Hastings pear" population in late February 1991. *P. affinis* crawlers from both populations were also exposed to a range of concentrations of prothiofos (Tokuthion 500EC) in January 1991.

Data were analyzed by probit analysis, using the software package POLO-PC (LeOra Software 1988) to generate and compare probit regressions, corrected for mortality in the controls.

The diagnostic dose

The probit analysis identified considerable differences between the susceptibility to chlorpyrifos of the two *P. affinis* populations. We wished to discover whether a difference of similar magnitude was common elsewhere in Hawkes Bay pipfruit orchards. To do this we exposed field collected mealybugs to an appropriate "diagnostic dose" of chlorpyrifos. A diagnostic dose is a less rigorous concept than a "discriminating dose", but more appropriate since we knew nothing of the genetic basis of the differences observed (Halliday and Burnham 1990).

A diagnostic dose for chlorpyrifos was established for each mealybug species, on the basis of the results of the probit analysis in the previous section. This was then used to screen field populations for resistance to chlorpyrifos.

***P. affinis* field populations**

From 8-20 adult female mealybugs or ovisacs were collected on fruit at harvest from 34 apple and pear orchards across the main pipfruit area in Hawkes Bay, during 1991 and 1992. All orchardists had regularly used chlorpyrifos for several seasons prior to 1991. Mealybugs were reared for no longer than the F_1 generation before testing. Adult females were transferred to potatoes to lay eggs. Ovisacs from these females, or ovisacs collected directly from fruit, were placed in small petri dishes for crawler emergence and testing.

Mealybug crawlers (>100 when possible; range = 25-289, mean = 109) were placed on to residues of the chlorpyrifos diagnostic dose in uncovered 9 cm diam. petri-dishes with Fluon-painted sides, at about 30 per dish, and assessed for mortality after 4 h. Water-only treatments were also included.

A laboratory colony of the most resistant population was maintained for 5 months (2-3 generations). Crawlers were exposed to the diagnostic dose of chlorpyrifos in January (F_2 generation since last possible exposure to chlorpyrifos), March (F_3) and May (F_{4-5}) 1991. Finally, crawlers from the "Hastings pear" population (F_{8-10} , estimated under laboratory conditions) were exposed to the diagnostic dose of chlorpyrifos in January 1992, after the mealybugs had spent 13 months in the laboratory, growing on potatoes with no exposure to any pesticide.

***P. longispinus* field populations**

Five orchardists from Te Kauwhata (Waikato) reported difficulty in controlling mealybugs in their pipfruit crops. Mealybugs from these orchards were all identified as *P. longispinus*, and crawlers (mean $n = 47$, range 29-76) from these populations were exposed for 4 h to the diagnostic dose of chlorpyrifos for this species, as described above for *P. affinis*.

RESULTS AND DISCUSSION

Dose/mortality responses

Reliable estimates of mealybug responses to increasing concentrations of chlorpyrifos, but not prothiofos, were obtained. The technique was clearly unacceptable for prothiofos, as mealybugs showed no observable response to increasing concentrations above 100 ppm in the susceptible population or 400 ppm in the "Hastings pear" population. It was therefore not possible to statistically compare the responses of the "susceptible" and "Hastings pear" populations to prothiofos. However, the latter population was clearly more tolerant of prothiofos. Only 3-20% of crawlers from the "susceptible" population survived doses of 100-1000 ppm, whereas 80% of crawlers from the "Hastings pear" population survived doses of 400-40,000 ppm.

The "Hastings pear" population was significantly more tolerant to chlorpyrifos than the "susceptible" population, with an RF (resistance factor: $-(LC_{50 RF2} + LC_{50 SF1})$) of 24.3 in January 1991 (Table 1). This difference was sufficiently large to indicate a real and severe problem. Comparison with a susceptible population with known freedom from any chlorpyrifos sprays in its past may increase this difference.

The RF for the "Hastings pear" population dropped significantly in February 1991 to 9.8. Unfortunately, we did not repeat the bioassay for the "susceptible" population at this time, so cannot be sure that the change in response was not due solely to being reared on potatoes.

The diagnostic dose

The calculated LC_{99} for the susceptible population was 2090 ppm chlorpyrifos (Table 1). We chose a diagnostic dose for *P. affinis* of 5000 ppm. By interpolation, we would expect this dose to kill c 60% of crawlers from the resistant "Hastings pear" population (F_2 generation) or c 87% of the F_1 generation (Table 1). We considered that similar levels of survival against this dose from field collected populations would be a good indicator of a similar level of resistance. We were aware of some of the pitfalls

of using a diagnostic dose. We were especially aware that we lacked any knowledge of the genetics of the resistance, so that particularly at higher mortalities (ie closer to the LC_{99} of the susceptible population), the less certain we were that we had identified a resistant population. However, because the diagnostic dose exceeded the 95% CL for the calculated LC_{99} response of the “susceptible” population (Table 1), we considered that all but the highest mortalities were indicative of at least some comparative level of tolerance to chlorpyrifos.

TABLE 1: Responses of susceptible (S) *P. affinis* and *P. longispinus*, and “Hastings pear” (R) *P. affinis*, crawlers to chlorpyrifos residues (ppm ai) in petri-dishes

	<i>P. affinis</i>		<i>P. long.</i>	
	S (n ¹ =552)	R(F ₂) (n ¹ =148)	R(F ₃) (n ¹ =177)	S (n ¹ =312)
slope ² ± SE	2.0 ± 0.14	1.58 ± 0.24	1.98 ± 0.36	2.4 ± 0.3
LC ₅₀	143	3370	1379	11
(95% CL)	(107-183)	(2366-4748)	(534-2434)	(7.8-14.6)
LC ₆₀		4874		
		(3498-7230)		
LC ₈₇			5124	
			(2878-15835)	
LC ₉₉	2090			105
(95% CL)	(1344-3909)			(63-265)
Diagnostic Dose	5000			200
RF ³	-	24.3	9.8	

¹ n = total number of crawlers tested to obtain each regression equation

² For *P. affinis*, slopes and intercepts of lines are not equal (P=0), but slopes are parallel and therefore comparable (P=0.335) (LeOra Software 1988).

³ RF = resistance factor, $LC_{50R} \div LC_{50S}$

Our diagnostic dose for *P. longispinus* was more arbitrarily chosen, as we had data from only one population. We chose the dose to be close to twice the LC_{99} response of the susceptible population, and planned to investigate any survivors further.

***P. affinis* field populations**

Some crawlers from each of 19 of the 34 populations examined survived the diagnostic dose (Table 2). None of the populations tested showed a higher tolerance to the insecticide than the “Hastings pear” population. Mealybugs exhibiting the highest chlorpyrifos tolerance (from grower 1, with 63% mortality) (Table 2) were from a property immediately adjacent to that of the “Hastings pear” population, and should perhaps be considered part of the same population.

The tolerance to chlorpyrifos in property 1 decreased markedly over 2-3 generations in the laboratory (Table 2), following a pattern similar to that observed in the “Hastings pear” population. Whether these observations were a result of a rapid shift in genotype in the absence of chlorpyrifos selection, or a result of rearing on a new host plant (potatoes vs pear tree) has not been investigated. Such host-plant mediated expression of resistance has recently been demonstrated for leafrollers (Robertson *et al.* 1990). However, mealybugs collected from pears at two properties in 1991 and again in 1992 were screened. Resistance was detected in both properties in both years, but was lower in 1992 than 1991 (Table 2). Unfortunately it was not possible to correlate the two measurements with the number of applications of chlorpyrifos made in each season, but it is probable that fewer applications were made in 1992, lending some support to a shift in genotype.

TABLE 2: Responses of “hard-to-kill” *P. affinis* from 34 Hawkes Bay pipfruit orchards to a diagnostic dose of 5000 ppm ai chlorpyrifos, in petri-dish bioassays.

Orchard number	Mealybugs tested per orchard	% kill
1 F ₁	70	63
F ₃	95	86
F ₄₋₅	109	100
2	100	77
3	90	87
4	135	90
5 (1991)	211	91
(1992)	328	99
6 (1991)	126	94
(1992)	130	97
7	101	94
8	289	94
9	71	96
10	99	97
11-16	99-163	98
17-19	91-99	99
20-34	24-140	100

Highest survival was found among mealybugs from pear orchards (especially properties 1-5). Mealybugs had been a problem in each of these properties in the 1970's, a period during which excessive use was made of parathion and other organophosphate insecticides in an attempt to control mealybugs (Murray Ross, pers. comm.)

Other survivors came from both pears and “old” apple trees at properties 6-19. No crawlers from 15 populations survived exposure to the diagnostic dose (Table 2). Because we have no genetical or toxicological evidence to demonstrate that our diagnostic dose does, in fact, discriminate between genotypes, we are reluctant to say that survivors from any of these populations are “resistant” to chlorpyrifos. In addition, the numbers of mealybugs sent from all properties was minimal for carrying out testing, and it is quite probable that the full complement of mealybug genotypes present in any orchard was not screened. Nevertheless, we are confident that the diagnostic dose did demonstrate that mealybugs from these properties exhibited a range of responses to chlorpyrifos.

The bioassay even demonstrated differences (albeit subjectively) between populations exhibiting 100% mortality. Crawlers that died early in the test dehydrated rapidly at 25°C, and were very shrivelled after 4 h. Those dying closer to examination (presumably as a result of being more tolerant to the residues) were turgid and often were able to move legs and antennae.

In January 1992 91% of crawlers (n=181) of the “Hastings pear” orchard died when exposed to the diagnostic dose, thus continuing the trend in this population towards increasing susceptibility to chlorpyrifos.

***P. longispinus* field populations**

Only *P. longispinus* were found in the five Waikato orchards. All of the mealybug crawlers exposed to the diagnostic dose were killed, providing no evidence for resistance in these properties.

CONCLUSIONS

These experiments provided convincing evidence of the existence of *P. affinis* resistance to chlorpyrifos in at least one Hawkes Bay pipfruit orchard. Use of a

diagnostic dose provided evidence that mealybugs were also tolerant to chlorpyrifos in other apple and pear orchards. The diagnostic dose indicated that the highest levels of tolerance were associated with old pear orchards, and excessive use of organophosphate insecticides over a long period. There is a very low tolerance for mealybugs in export apples, so even slight resistance is likely to lead to economically significant levels of fruit infestation.

Experiments are now required to determine the genetic and toxicological characteristics of the resistant genotype. These are needed to help determine whether the apparently rapid loss of tolerance to chlorpyrifos in the absence of selection pressure is "real" (ie. due to changing genotype) or merely an artefact of the laboratory rearing procedure. If there is a rapid loss of tolerance to chlorpyrifos in the absence of selection pressure, then it may be of considerable value (together with the use of non-OP insecticides) in designing future insecticide resistance management programmes for mealybugs.

Widespread resistance should be of concern to the pipfruit industry, since chlorpyrifos remains, with prothiofos, perhaps the last effective OP insecticide available for mealybug control. These tests provided no evidence of resistance in *P. longispinus* in the Waikato, and, for pipfruit growers outside Hawkes Bay, chlorpyrifos appears to remain an effective insecticide for mealybug control.

Nevertheless, in the immediate future, successful control of chlorpyrifos resistant mealybugs in a few properties will rely on a non-OP insecticide regime. Fortunately, field control can be achieved using an insect growth regulator, buprofezin (Walker *et al* 1993).

Finally, this bioassay technique is suitable for resistance work, where the aim is to compare the genotypes of different strains through their phenotypic responses to a pesticide. It is not appropriate for determining concentrations of pesticides that will control orchard populations of mealybugs.

ACKNOWLEDGEMENTS

We thank Mark Maunder for assisting with the laboratory bioassays, and Bruce Chapman for helpful and constructive comments on an earlier draft of the manuscript. The work was partly funded by the New Zealand Apple and Pear Marketing Board.

REFERENCES

- Charles, J.G., 1982. Commercial control of mealybugs in apple orchards. *Orchardist of N.Z.* 55: 298-299.
- Charles, J.G. A survey of mealybugs and their natural enemies in horticultural crops in North Island, New Zealand, with implications for biological control. *Biocontrol Science and Technology*: in press.
- Charles, J.G. and Walker, J.T.S., 1981. Mealybug control in apples. *Orchardist of N.Z.* 54: 252-253.
- Congdon, N.B. and Morrison, L.G., 1959. Mealybug resurgence in Hawke's Bay orchards. *N.Z. J. Agr.* 99: 481-487.
- Halliday, W.R. and Burnham, K.P., 1990. Choosing the optimal diagnostic dose for monitoring insecticide resistance. *J. Econ. Entomol.* 83: 1151-1159.
- LeOra Software. 1988. POLO-PC: A User's Guide to Probit or Logit Analysis. LeOra Software, Berkeley, California, 22pp.
- MacKenzie, D.J., 1972. Control of codling moth, leafroller and mealybug in apples. *Orchardist of N.Z.* 45: 155.
- Robertson, J.L., Armstrong, K.F., Suckling, D.M. and Preisler, H.K., 1990. Effects of host plants on the toxicity of azinphosmethyl to susceptible and resistant light brown apple moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* 83: 2124-2129.
- Walker, J.T.S., White, V. and Charles, J.G., 1993. Field control of chlorpyrifos resistant mealybugs (*Pseudococcus affinis*) in a Hawkes Bay orchard. *Proc. 46th N.Z. Plant Prot. Conf.*: this volume.