MONITORING AZOCYCLOTIN AND PROPARGITE RESISTANCE IN EUROPEAN RED MITE

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SUMMARY

A comparative study of the toxicity of azocyclotin and propargite to European red mite by leaf disc and plastic petri dish methods was conducted. Although the petri dish method produced lower LC_{50} values than the leaf disc method, reliable results were obtained. The petri dish method was then used to determine the levels of resistance in a number of European red mite populations. The maximum resistance factors detected at the LC_{50} were 13.5 and 33.1 for azocyclotin and propargite respectively.

INTRODUCTION

Two-spotted mite (TSM), (*Tetranychus urticae*) and European red mite (ERM), (*Panonychus ulmi*) reach damaging levels regularly on some horticultural crops. Surveys on the use of pesticides in horticultural crops have shown that the application of miticides remains an important method for controlling spider mites in New Zealand (Chapman and Penman 1987).

Since the withdrawal of cyhexatin from sale in New Zealand (August 1987), propargite and azocyclotin have become the most commonly used miticides for controlling the motile stages of spider mites. In apples, orchardists apply on average 2-4 miticides per season (Chapman and Penman 1987) and in some instances, one chemical has been used by individual growers for 10 or more years (Chapman *et al* 1987). This long period of selection pressure has resulted in the development of miticide resistance in both TSM and ERM (Chapman and Penman 1987).

Several laboratory bioassay methods have been used to assess the levels of resistance in spider mite populations, but each have their particular drawbacks. The slide dip method (Anon. 1974) has been used successfully with TSM, but it is less suitable for ERM because unacceptably high control mortalities can occur (Chapman and Penman 1984). Leaf disc methods have therefore been used more widely for ERM but mite runoff can be a problem especially when the discs are treated with higher concentrations of some miticides, e.g. propargite (Chapman and Penman 1984). Mites can be contained on leaf discs with devices such as the St Jean mite cage (Bostanian 1981) but these are difficult to handle and time-consuming to set up, and mites can avoid the residues by climbing onto the sides.

The main objective of this study was to develop an alternative bioassay method for detecting resistance in ERM. It was adapted from a method originally described by Dennehy *et al* (1987) for testing resistance in *Tetranychus* spp. where adult females were contained on miticide residues in small plastic petri dishes. Two groups of experiments are reported in this paper; first, a comparison of the leaf disc and plastic petri dish methods and second, an estimation of resistance to azocyclotin and propargite in some field populations of ERM using the plastic petri dish method.

METHOD

Comparison of bioassay methods

ERM used for these experiments originated from overwintering eggs collected during the winter of 1986 from the property of A.B. Smith in Hawke's Bay. They were reared on young peach trees (cv. 'Golden Queen') in the laboratory (ca. 22 °C). Bioassay tests completed during 1986/87 using the leaf disc method showed that this population

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was moderately resistant to cyhexatin (Chapman *et al* 1987). The population was allowed to overwinter on these trees in an insectary under natural light and temperature conditions and in the following spring were returned to the laboratory. No selection pressure was applied to the population during this period. Tests commenced when sufficient adult females of the first summer generation were present.

The leaf disc method used was the same as that described by Chapman *et al* (1987), except that adult females were contained on the treated leaf discs by an agar (2% w/v) barrier. This reduced the mite runoff to a level lower than that experienced when leaf discs were placed on damp filter paper. The plastic petri dishes (Falcon 1006 petri dish, Dickinson and Co., Cockeysville, MB 21030 USA) were 50 mm dia. and 9 mm deep and had tight fitting lids.

Aqueous suspensions of propargite (Omite 30% wettable powder) and azocyclotin (Peropal 25% wettable powder) were diluted serially to give a range of concentrations that would produce 5-95% mortality. Petri dishes (internal surfaces of lids and bases) and leaf discs (lower surface) were sprayed with 2ml of each concentration in a Potter tower operated at 70 kPa, allowing a 10 sec. settling period. Distilled water was used for the controls. Once the residues had dried, 10 and about 25 adult female ERM were placed onto the leaf discs and petri dishes respectively. To maintain sufficient humidity in the petri dishes, a small plug of agar 4mm dia. and 3mm long was placed on the inner surface of the lid. Post-treatment conditions for both leaf discs and petri dishes were $23 \pm 1^\circ\text{C}$ and 16:8 hours light:dark.

After 24 h the mites were scored as alive, moribund or dead. The criterion for moribund mites was the inability to walk one body length after being lightly prodded. For this study, the numbers of moribund and dead mites were pooled and median lethal concentration values were calculated using the computer programme POLO (Russell *et al* 1977).

Resistance to azocyclotin and propargite

ERM were collected from 10 orchards from Hawke's Bay, Nelson and Canterbury during the summer. As a basis for comparison, a susceptible population was collected from an orchard at Lincoln College which had not been sprayed with any pesticides for at least 7 years. Infested leaves that were collected from Hawke's Bay apple orchards were air-freighted to Lincoln College and stored at 4°C until testing. Most tests were completed within several days of the receipt of samples. The plastic petri dish method was used to assess the levels of resistance in these populations. The methods used were identical to those described above.

RESULTS

Comparison of bioassay methods

The median lethal concentration values for azocyclotin and propargite tested against the A.B. Smith population using the leaf disc and petri dish methods are listed in Table 1.

TABLE 1: The responses of European red mite (Smith strain) to propargite and azocyclotin using the leaf disc and petri dish methods.

Miticide	Method	LC ₅₀ g ai/ litre	95% CI	Slope $\pm$ SE		No. of mites Tested
propargite	leaf disc	0.214	0.115-0.386	1.32	0.13	1149
	petri dish	0.014	0.010-0.017	2.25	0.32	397
azocyclotin	leaf disc	0.134	0.096-0.176	2.68	0.23	871
	petri dish	0.021	0.014-0.032	3.78	0.57	357

Comparison of LC₅₀ values showed that residues of propargite and azocyclotin on the petri dishes were respectively 15.3 and 6.4 times more toxic to ERM adult females, compared to those on the leaf discs. The slopes of the concentration-mortality curves also differed and for both chemicals the petri dish method produced a higher slope

value. A test of the hypothesis that the slopes of the concentration-mortality curves were equal was rejected (Russell *et al* 1977).

Resistance to azocyclotin and propargite

The responses of three ERM populations to azocyclotin or propargite tested by the petri dish method are listed in Table 2. These populations represent the extremes of the range of response to these chemicals and all other populations tested fell between them (Chapman and Bowie, unpublished).

TABLE 2: The toxicity of propargite and azocyclotin to susceptible and resistant populations of European red mite using the petri dish method.

Miticide	Population	LC ₅₀ g ai/litre	95% CI	Slope ± SE		No. of mites Tested
propargite	Lincoln	0.012	0.008-0.016	4.17	0.43	445
	WES	0.397	0.154-0.827	1.25	0.13	562
azocyclotin	Lincoln	0.012	0.006-0.026	1.91	0.14	1318
	Kerr	0.162	0.133-0.210	3.08	0.71	414

By comparing LC₅₀ values, the greatest difference between the susceptible population (Lincoln) and the resistant populations was 33.1 and 13.5 times for propargite and azocyclotin respectively. The slopes of the concentration-mortality curves also differed between populations and miticides. For propargite, the resistant population (WES) produced a much lower slope value than the susceptible population but the reverse was true for azocyclotin. Tests of the hypothesis that the slopes of the concentration-mortality curves were equal was rejected in both instances (Russell *et al* 1987).

DISCUSSION

An ability to characterise the type and level of resistance in a pest population is commonly regarded as a prerequisite for successful resistance management. Ideally bioassay methods should be simple, produce consistent results and reflect the responses of pests to field applications of pesticides. The results of this study are considered from these viewpoints.

The modified petri dish method developed by Dennehy *et al* (1987) for testing *Tetranychus* spp. resistance has been found to be suitable for ERM. The main modifications were the use of the Potter tower to apply the miticide residues and the inclusion of an agar plug to act as an humidity source. It was decided to use the Potter tower to apply the miticide to ensure that an even deposit was applied to all internal surfaces and an equivalent deposit was applied to the leaf discs. By comparison, Dennehy *et al* (1987) pipetted 1 ml of miticide solution in ethanol into the dish, swirled for 5 sec. and then poured off the excess. Application of miticide by the Potter tower also allows the amount of deposit per unit area to be determined, thus enabling other researchers to prepare identical residue deposits for comparative resistance studies.

Inclusion of the agar plug markedly improved the survival of ERM, reducing the control group mortality, on average, from 16.6% (without agar plug) to 6.5%. Overall, the petri dish method was quicker to set up, mite mortality in untreated control groups was low and there was no loss of mites through the irritant action of higher miticide concentrations. The technique would also be readily transportable and allow mites to be transferred to dishes directly from the field.

The relationship between the laboratory bioassays and field performance of a miticide was not specifically investigated in this study. However, it might be expected that the leaf disc method would more closely approximate field conditions. It is possible that contact with the miticide on the smooth petri dish surfaces would be greater than on leaf surfaces and that the vapour effect would also be enhanced in the petri dish, especially with propargite (400 Pa at 20°C) which has a relatively higher vapour pressure than azocyclotin (5 mPa at 25°C). These factors may be the reasons why the

LC₅₀ values for both miticides were lower on the petri dishes than on the leaf discs.

Resistance by ERM to miticides has not been well documented in New Zealand and this may be due to the difficulty in conducting bioassays with this species. The orchards from which the most resistant populations were collected (WES and Kerr, Table 2) had experienced control problems (J.T.S. Walker, unpublished) with the miticides used. Although the tests conducted in this study confirmed high levels of resistance, the problem, still, is to determine the level of resistance at which significant field control failure occurs. One approach to this problem is to define a discriminating concentration and a level of mortality below which resistance allows enough mites to survive to cause control failure. Dennehey *et al* (1987) suggested an action threshold of 80% mortality of *Tetranychus* spp. at the discriminating concentration of dicofol (0.056 g/litre) and that below this percentage mortality the miticide should not be used. This cannot be done at present for ERM as we have insufficient knowledge of the effects of field applications against a range of populations. However, on the basis of the results presented in Table 2 discriminating concentrations for propargite and azocyclotin respectively would be 0.03 g/litre and 0.06 g/litre. This would produce > 90% mortality in susceptible populations and < 10% mortality in resistant populations using the petri dish method.

CONCLUSION

This study has demonstrated that a petri dish bioassay method can give reliable results for testing the responses of ERM to azocyclotin and propargite. Resistance to both these miticides was detected in some populations and the data presented will provide a basis for comparison in future studies. A concentration of 0.06 g/litre for azocyclotin and 0.03 g/litre for propargite is suggested for discriminating between susceptible and resistant populations. However, verification with field control failures will be necessary.

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REFERENCES

- Anonymous, 1974. Recommended methods for detection and measurement of resistance of agricultural pests to pesticides. 10. Tentative methods for spider mites and their eggs, *Tetranychus* spp., *Panonychus ulmi* (Koch). *F.A.O. Plant Protection Bulletin* 22: 103-107.
- Bostanian, N.J., 1981. The St. Jean mite cage. *Canadian Entomologist* 113: 359-360.
- Chapman, R.B. and Penman, D.R., 1984. Resistance to propargite by European red mite and two-spotted mite. *N.Z. J. Agric. Res.* 27: 103-105.
- Chapman, R.B. and Penman, D.R., 1987. Miticide use and spider mite resistance in New Zealand. *Proc. of the Symposium on Mite Control in Horticultural Crops*. Orange, N.S.W. Australia, July 29-30 1987, 54-59.
- Chapman, R.D., Penman, D.R. and Walker, J.T.S., 1987. European red mite resistance to organotin miticides in Hawke's Bay apple orchards. *Proc. 40th N.Z. Weed and Pest Control Conf.*: 94-98.
- Dennehy, T.J., Grafton-Cardwell, E.E., Granett, J. and Barbour, K., 1987. Fractioner-assessable bioassay for detection of dicofol resistance in spider mites (Acari : Tetranychidae). *J. Econ. Entomol.* 80: 998-1003.
- Russell, R.M., Robertson, J.L. and Savin, N.E., 1977. POLO: A new computer program for probit analysis. *Bull. Entomol. Soc. of America* 23: 209-213.