USE OF A DISCRIMINATING CONCENTRATION FOR MONITORING PROPARGITE RESISTANCE IN TWOSPOTTED SPIDER MITE

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SUMMARY

Several ways of selecting a discriminating concentration (DC) were examined for monitoring propargite resistance in twospotted spider mite (TSM) using the petri dish method. The selected DC of propargite (0.105% ai) identified the presence of resistance in different TSM strains as accurately as did multiple concentration (MC) tests. Considering time involvement and the number of test individuals required for conducting a MC bioassay, the DC technique would be a cost effective approach for resistance monitoring. Despite the long history of propargite use in New Zealand, resistance in TSM was found at only low levels from some locations.

Keywords: discriminating concentration, propargite, resistance monitoring, twospotted spider mite

INTRODUCTION

At least 447 species of insects and mites have developed resistance to one or more classes of pesticides (Georghiou 1986). Both overuse and misuse of pesticides may lead to the development of resistance. However, poor field control by a pesticide does not necessarily mean that a pest population has developed resistance. Field control failure may occur due to poor spray timing, inadequate coverage, inappropriate selection of pesticide and the presence of resistant individuals. As several factors may be responsible for poor field control, correct identification of the cause of control failure is essential for effective pest management.

Pesticide resistance in a pest population can be detected by one or more of the following methods: (a) multiple concentration (MC) tests; (b) discriminating or diagnostic concentration (DC) tests and (c) biochemical and immunological tests. MC and DC tests are most commonly used as biochemical and immunological tests have not yet been developed for most species.

Despite the wide scale use of the DC test in monitoring pesticide resistance in insects and mites, the criterion for selecting a DC has not yet been standardized. Often a DC is selected arbitrarily (e.g., LC_{99} x 2 or 3, LC_{100}, LC_{100} x 2 etc.) (e.g. Collins and Wilson 1986) from the concentration-response regression line of a susceptible strain. Dennehy *et al* (1983) has indicated the dangers of using an arbitrarily selected DC several times higher than the LC_{99}. Roush and Miller (1986) also noted that the use of a high concentration as a DC could impose higher estimation errors than a lower concentration. McCutchen *et al* (1989) suggested a DC should be any selected concentration between the LC_{80}-LC_{90} while Halliday and Burnham (1990) recommended that concentrations producing 94.0-99.2% mortality of the susceptible strain would have greater chance of detecting resistant phenotypes.

The selective miticide propargite has been used in New Zealand for many years to control spider mites on a range of horticultural crops. Resistance to this miticide has been reported only sporadically (Chapman and Penman 1984; Bowie *et al* 1988) but it is probable that low levels of resistance exist in many populations. To determine the level and distribution of resistance to propargite in spider mite population a rapid and reliable resistance monitoring technique is required. The main objectives of this research were to evaluate procedures for selecting a propargite concentration that would

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discriminate between resistant and susceptible mites and to determine the reliability of tests using the selected DC by comparing the results with those from MC tests.

METHODS

Both the DC and MC test techniques using the petri dish residue-Potter tower method (PDR-PT) (Kabir *et al* 1990) were used for testing the responses of various TSM strains to propargite (Omite 30% wettable powder). Mites were held in treated dishes for 24 h in a controlled temperature cabinet at 23 ± 1 °C and 70 ± 10% R.H. before mortality was assessed. The mortality criterion was inability to walk at least one body length when lightly prodded.

Twenty three TSM strains were collected from different locations throughout New Zealand (Table 2) in conjunction with the Plant Protection Research Unit, Lincoln University. Apart from the locally collected strains, all were colonised and reared on bean plants (*Phaseolus vulgaris*) to obtain the required number of adult females for testing. Locally collected strains were tested directly after collection with the DC whenever possible and remaining mites were then colonised for 3-5 generations before further testing. A laboratory susceptible strain (LS) (Kabir *et al* 1990) was used as a reference strain.

Selection of a discriminating concentration

The pooled responses of 2400 susceptible mites to five concentrations of propargite and a water-treated control were analysed by the log-probit method to estimate the LC_{98}, LC_{99} and LC_{100} values. Five hundred adult females were then exposed to each of the LC_{98} and LC_{99} in 25 replications to determine mortalities at these concentrations. A water-treated control using 80 mites in four replications determined natural mortality. Five concentrations between $>LC_{99}$-LC_{99} x 3.3, with 80 mites each, were also tested to determine a concentration that would result in 100% mortality of the susceptible strain.

Testing for resistance

For each strain, 100-250 mites were exposed to the selected DC in four-ten replications and 20-40 mites in a water-treated control in one-two replications. Following tests with the DC, mites of each strain were tested by a MC test. For the MC test, five serially diluted concentrations were chosen considering one of the following criteria: (a) if the DC yielded >90% mortality of a test strain, concentrations between 0.005-0.08% ai of propargite were used; (b) if the mortality was 70-90%, the range tested was 0.01-0.16% ai; (c) if the mortality was 50-70%, the range tested was 0.02-0.32% ai; (d) if the mortality was <50%, the range tested was 0.04-0.64% ai. In almost all cases 80 mites were used for each concentration in four replications.

The level of resistance of each strain was calculated using a resistance percentage (RP) for the DC test and a resistance ratio (RR) for the MC test. The resistance percentage was calculated by the formula adopted by McCutchen *et al* (1989): RP = 100-(MT/MS x 100); where MT = % mortality of the test strain and MS = % mortality of the susceptible strain. In both cases, the observed mortality at the DC was corrected for control mortality using Abbott's (1925) formula. The resistance ratio was calculated by the LC_{50} or LC_{90} of the test strain divided by the LC_{50} or LC_{90} of the susceptible strain.

RESULTS

Selection of a discriminating concentration

The estimated LC_{98}, LC_{99} and LC_{100} values of propargite for the susceptible strain were 0.086, 0.105 and 1.52% ai respectively but the observed mortalities at the LC_{98} and LC_{99} were lower than predicted (Table 1). The observed mortalities at concentrations between $>LC_{99}$-LC_{99} x 3.3 were almost identical to those at the LC_{98} and LC_{99} and none of the concentrations yielded 100% mortality. Based on these results, the LC_{99} of propargite (0.105% ai) was selected as a DC. Any test strain giving mortality below this level would then be suspected of being resistant.

Testing for resistance

The responses of different TSM strains to the DC of propargite are summarized in Table 2. Thirteen of the 24 strains tested were as susceptible as the LS strain. The

resistance percentage in the remaining strains ranged from 1.5-79.1. Tests with strains J1 and J3 were conducted twice, directly after collections and after 2-3 months of rearing. Direct tests resulted in 1.7-2.0 x higher resistance percentage than tests after rearing.

TABLE 1: The predicted and observed corrected percentage mortalities of the susceptible TSM strain at different concentrations of propargite.

Concentration (% ai)	Predicted mortality (%)	Observed mortality (SD) (%)
0.086	98	94.87 (5.74)
0.105	99	95.69 (3.53)
0.150	>99	93.59 (4.91)
0.200	>99	96.15 (2.56)
0.250	>99	96.15 (2.56)
0.300	>99	98.72 (2.57)
0.350	>99	97.44 (2.96)

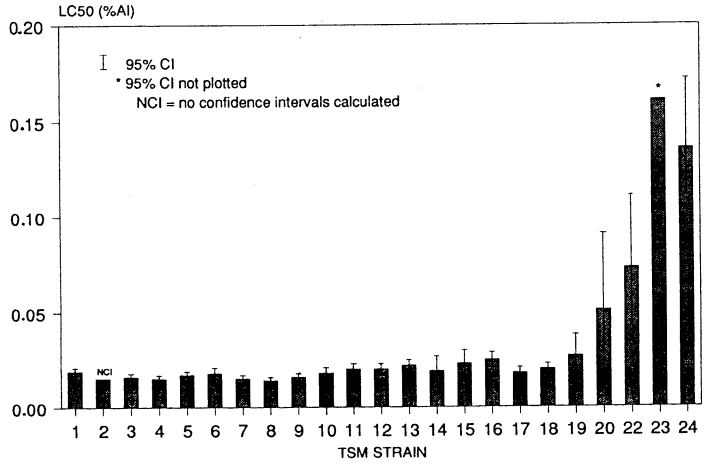
TABLE 2: The responses of different TSM strains to the discriminating concentration (0.105% ai) of propargite.

No.	Strain and location	N[1]	Mean mortality (SD) (%)	RP[2]
1	LS, Laboratory	500	95.69 (3.53)	-
2	KF1, Kerikerki	200	100.0 (0.00)	-
3	KF3, Kerikeri	200	100.0 (0.00)	-
4	CRSO, Gisborne	160	100.0 (0.00)	-
5	PBLTN, Christchurch	200	100.0 (0.00)	-
6	FRNHO, Gisborne	200	99.50 (1.58)	-
7	KF2, Kerikeri	200	99.46 (1.71)	-
8	KF4, Kerikeri	200	99.44 (1.76)	-
9	THIP, Gisborne	160	99.34 (1.86)	-
10	UQUT, Nelson	200	98.97 (2.16)	-
11	NLTS, Kerikeri	200	98.38 (2.16)	-
12	HDY, Nelson	200	98.33 (2.69)	-
13	SMNR, Christchurch	200	98.03 (2.72)	-
14	OFRAO, Opotiki	250	97.45 (2.98)	-
15	ESTN, Nelson	160	94.23 (5.08)	1.53
16	HNRS, Havelock North	160	93.75 (8.34)	2.03
17	LIN, Christchurch	200	91.35 (5.22)	4.54
18	GRRD, Christchurch	100	90.00 (2.48)	5.95
19	HTCN Christchurch	200	88.42 (7.36)	7.60
20	MSN, Ohope	200	86.50 (4.74)	9.60
21	CRNTN, Christchurch	100	85.26 (6.86)	10.90
22	J1, Christchurch	200	71.53 (9.35)	25.21
23	WBSTR, Puhoi	160	66.89 (12.41)	30.10
24	J3, Christchurch	200	53.85 (12.08)	43.72
25	J1, Christchurch[3]	100	46.32 (19.48)	51.59
26	J3, Christchurch[3]	100	24.21 (13.21)	74.70
27	J2, Christchurch[3]	100	20.00 (5.00)	79.10

[1]Number of mites tested; [2]Resistance percentage; [3]Tested directly after collection

Figure 1 shows the LC_{50} values of propargite for 22 TSM strains calculated from the results of the MC test. Eleven strains yielded lower LC_{50} and LC_{90} values than the LS strain. These strains displayed no resistance by the DC test. LC values of the strains ESTN, HNRS, GRRD and HTCN also were not significantly different from the LS and exhibited resistance ratios of 1.03-1.34. These strains had 1.5-7.6% resistant mites by the DC tests.

Fig. 1: The responses of different TSM strains to propargite using the multiple concentration test of the petri dish method.



Four strains (MSN, J1, WBSTR and J3) gave significantly higher LC values (by non overlap of 95% CI) than the LS with resistance ratios of 2.7-62.2. By the DC tests, these strains had 9.6-43.7% resistant mites. The calculated resistance ratio at the LC_{50} was always lower than at the LC_{90}. The highest resistance ratio was found for the WBSTR strain where the resistance ratio was 8.4 at the LC_{50} and 62.2 at the LC_{90}. Lower slope values were generally found with resistant strains.

DISCUSSION

In a rountine resistance monitoring programme, often an investigator wishes to use a simple cost effective technique. The discriminating or diagnostic concentration test is such a technique. Roush and Miller (1986) have compared the DC and MC test techniques and concluded that the DC was more efficient for resistance monitoring as it accurately distinguished between resistant and susceptible individuals.

Selection of an appropriate concentration as a DC is an important factor when using a single concentration approach in resistance monitoring. DC selection should represent a compromise between using too low a value which would give false detection of resistance versus too high a value which may allow low levels of resistance to go undetected. The slightly lower mortalities obtained at the LC_{98} and LC_{99} than were predicted from log-probit analysis demonstrate that testing for the observed mortality in the susceptible strain at any selected DC is crucial. The present results (Table 1) show that none of the arbitrarily selected concentrations yielded 100% mortality. Selecting any one of these concentrations as a DC, assuming that any survivors in the test are resistant, might lead to overestimation of resistance. Subramanyan *et al* (1989) noted that monitoring resistance with an arbitrarily selected DC is risky because naturally tolerant individuals that survive a DC are classified as resistant. Because of such a complicating factor Halliday and Burnham (1990) have suggested that a DC should be used with caution. However, selection of the LC_{99} as a DC in the present study is in agreement with several workers (e.g. Gunning and Easton 1989).

In addition to the selection of an appropriate DC, sound interpretation of resistance results is vitally important. According to McCutchen *et al* (1989), the formula used in this study would eliminate the survival of susceptible mites and death of resistant mites as a complicating factor and give a more accurate figure of resistance in the test population. Therefore, resistance is not overestimated. With results of MC tests, resistance is usually expressed by a resistance ratio. With this approach a test strain could be considered resistant if it produced a resistance ratio of >1 when the LC

values were not significantly different from the LC values of the susceptible strain. However, Tabashnik *et al* (1987) categorized a test strain as resistant only when it yielded significantly higher LC values than the susceptible strain. Collins and Wilson (1986) classified the tested strains as being resistant when they gave a resistance ratio of >1 but did not indicate whether LC values should be significantly different. We suggest that a test strain should only be considered as resistant when it produces significantly higher LC values and a resistance ratio of >1.

Ideally, the DC and MC tests should give a similar indication of propargite resistance in different strains. Five strains (i.e. ESTN, HNRS, LIN, GRRD and HTCN) showed 1.5-7.6% resistance by the DC tests while MC tests yielded resistance ratios of 1.03-1.62 for the same strains. These strains cannot be considered as resistant by the MC tests as their LC values were not significantly different from the LS strain. Propargite resistance in four strains (i.e. MSN, J1, WBSTR and J3) was confirmed by both the DC and MC tests (Table 2 and Fig. 1). The calculated resistance ratios for these strains are comparable with those reported by Chapman and Penman (1984).

In conclusion, the results of this study indicate that a DC test using the petri dish method could be used to detect propargite resistance in TSM populations. Further work is required to determine what level of mortality in a DC test would indicate that control failure was likely following a field application of propargite.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol. 18:* 265-267.

Bowie, M.H., Chapman, R.B. and Walker, T.S., 1988. Monitoring azocyclotin and propargite resistance in European red mite. *Proc. 41st N.Z. Weed and Pest Control Conf.:* 189-192

Chapman, R.B. and Penman, D.R., 1984. Resistance to propargite by European red mite and twospotted mite. *N.Z. J. Agric. Res. 27:*103-105.

Collins, P.J. and Wilson, D., 1986. Insecticide resistance in the major coleopteran pests of stored grain in Southern Queensland. *Queensland J. Agricultural and Animal Sci. 43(2):* 107-114

Dennehy, T.J., Granett, J. and Leigh, T.F., 1983. Relevance of slide-dip and residual bioassay comparison for detection of resistance in spider mites. *J. Econ. Entomol. 76:* 1225-1230.

Georghiou, G.P., 1986. The magnitude of the resistance problem. *In* Pesticide Resistance: Strategies and Tactics for Management. National Academy Press, Washington, D.C., 1986. pp. 14-43.

Gunning, R.V. and Easton, C.S., 1989. Pyrethriod resistance in *Heliothis armigera* (Hubner) collected from unsprayed maize crops in New South Wales 1983-1987. *J. Aust. Ent. Soc. 28:* 57-61.

Halliday, W.R. and Burnham. K.P., 1990. Choosing the optimal diagnostic dose for monitoring insecticide resistance. *J. Econ. Entomol. 83:* 1151-1159.

Kabir, K.H., Chapman, R.B. and Penman, D.R., 1990. The precision and utility of bioassay methods for testing miticides against spider mites. *Proc. 43rd N.Z. Weed and Pest Control Conf.:* 272-281.

McCutchen, B.F., Plapp, Jr., F.W., Nemec, S.J. and Campanhola, C., 1989. Development of diagnostic monitoring techniques for larval pyrethroid resistance in *Heliothis* spp. (Lepidoptera:Noctuidae) in cotton. *J. Econ. Entomol. 82:* 1502-1507.

Roush, R.T. and Miller, G.L., 1986. Considerations for design of insecticide resistance monitoring programmes. *J. Econ. Entomol 79:* 293-298.

Subramanyam, Bh., Harein, P.K. and Cutkomp, L.K., 1989. Organophosphate resistance in adults of red flour beetle (Coleoptera:Tenebrionidae) and sawtoothed grain beetle (Coleoptera:Cucujidae) infesting barley stored on farms in Minnesota. *J. Econ. Entomol. 82:* 989-995.

Tabashnik, B.E., Cushing, N.L. and Johnson, M.W., 1987. Diamondback moth (Lepidoptera:Plutellidae) resistance to insecticides in Hawaii: Intra-island variation and cross resistance. *J. Econ. Entomol. 80:* 1091-1099.