THE PRECISION AND UTILITY OF BIOASSAY METHODS FOR TESTING MITICIDES AGAINST SPIDER MITES

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SUMMARY

Six commonly used bioassay methods were evaluated for precision and utility using propargite (WP) and fenbutatin oxide (WP and SC) with twospotted spider mite (TSM) and European red mite (ERM). The petri dish residue-Potter tower method estimated the responses of TSM and ERM to propargite with high precision. It did not result in similar precision with both formulations of fenbutatin oxide. Significant mite run off occurred with leaf disc methods. The toxicity of the candidate miticides was method- and species- dependent, and variation of LC values between methods within species was statistically significant.

INTRODUCTION

Bioassay of spider mites is a challenging problem. Several bioassay methods have been developed to overcome problems associated with their small size, the difficulties of administering pesticide treatments and confining subjects to treated substrates. There is no universally accepted method as each has particular advantages and drawbacks (Bowie *et al* 1988). Slide dip and leaf disc methods are widely used with a range of pesticides. However, the slide dip method has been highly criticized for producing high control mortality, inappropriate response estimates and underestimating field resistance problems (e.g. Chapman and Penman 1984; Dennehy *et al* 1983). The major drawback of the leaf disc method is mite run off (Chapman and Penman 1984; Thwaite and Hatley 1987). Hoy and Conely (1989) scored run off mites as dead to simplify the analysis.

A petri dish bioassay method has recently been introduced (Dennehy *et al* 1987) to overcome some of the problems associated with leaf disc and slide dip methods. It appears to be a relatively temperature-insensitive method, but few chemicals have been tested by this method to date.

The reported shortcomings, and criticism for the slide dip and leaf disc methods, and a concern that sole reliance on a particular method could be unwise have led us to assume that: (a) the response of mites to miticides could be method dependent; (b) if the test method is inappropriate, then the toxicity of a chemical, as well as the level of resistance of a field population could be underestimated; (c) the precision of estimates which can be achieved using any method may be chemical- and species- dependent.

Bioassay methods need to be simple, to provide reproducible results, and ideally to simulate field treatment conditions as closely as possible. No previous attempt has been made to document the reliability and utility of a wide range of bioassay methods for determining the response of spider mites to miticides. In this paper we report on the precision and utility of six commonly used test methods (with necessary modifications) for propargite (WP) and fenbutatin oxide (WP and SC) against twospotted spider mite (*Tetranychus urticae*) and European red mite (*Panonychus ulmi*).

METHODS

TSM used for these experiments were reared on French dwarf bean (*Phaseolus vulgaris*) in the laboratory. Mites had not been exposed to any pesticides for more than 5 years.

ERM used in this study were collected from the peach orchard at Lincoln University which had not been sprayed with propargite and fenbutatin oxide for at least 2 years.

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In all cases (species vs miticides vs test methods) preliminary experiments were done to select a series of concentrations that would produce 5-95% mortality for the final assessment. The number of concentrations including control (6-9), replications (4), test individuals (80-120) in each replication and post-treatment conditions ($23 \pm ^\circ\text{C}$ and $70 \pm 10\%$ RH) were similar in all experiments. Fresh aqueous suspension of miticides were used. Propargite (Omite 30% wettable powder (WP)) and fenbutatin oxide (Torque 50% (WP) and 55% suspension concentrate (SC)) were used in this study.

Slide dip (SD)

Twenty adult females were affixed by their dorsal surfaces to double-sided adhesive tape attached to glass microscope slides. Slides were dipped for 5 s in the required concentrations, allowed to dry at room temperature and then transferred to a controlled temperature (CT) cabinet for 24 h. The mortality criterion was an inability to move a leg when lightly prodded.

Leaf disc direct-Potter tower (LDD-PT)

Five leaf discs (bean for TSM and peach for ERM), each 18 mm diam. were placed upside down in plastic petri dishes containing moistened cotton wool or a semi-solid agar pad. Five to 10 adult females were released on to each disc and sprayed with 2 ml of solution of the required concentration under a Potter tower, operated at 55 kPa with 10 s settling period. Treated dishes were placed on covered plastic trays and transferred to a CT cabinet for 48 h. The mortality criterion was an inability to walk at least one body length when lightly prodded. Run off mites were not included in the analysis.

Leaf disc residue-Potter tower (LDR-PT)

The same procedure was used as for the LDD-PT method except that the adult female mites were released onto the leaf discs after spraying.

Leaf disc residue-dipping (LDR-D)

The same procedure was used as for the LDR-PT method except that the leaf discs were dipped for 5 s in the required concentration, and adult females were placed on the discs once the deposit had dried.

Petri dish residue-Potter tower (PDR-PT)

The method used was the same as that described by Bowie *et al* (1988) except that the Potter tower was operated at 55 kPa. The mortality criterion was the same as the LDD-PT method.

Petri dish residue-rinsing (PDR-R)

The same procedure was used as for the PDR-PT method except that 2 ml of solution of the required concentration was placed in each dish, the lid closed tightly, and the dish gently swirled for 5 s in both an upright and inverted position. Excess solution was poured off and then the dish was allowed to dry at room temperature.

Statistical analysis

Data from all replicates were pooled and subjected to log-probit analysis using the computer program POLO (Robertson *et al* 1980) to determine median lethal concentration (LC) values. Significant differences in LC values were determined by non-overlap of 95% confidence intervals (CI). CIs were used to rank the precision of test methods; the smaller the CI, the greater the precision (Finney 1971).

RESULTS

Test methods were ranked by the 95% CI and listed in Table 1 along with their respective LC₅₀ values. Potter tower methods resulted in shorter CIs than either dipping or rinsing methods with some exceptions. With propargite, the PDR-PT method yielded highest precision for ERM, and it held third position for TSM. On the contrary, for fenbutatin oxide the PDR-PT method produced 2.3-10.9 and 6.4 times greater CIs than LDD-PT (WP vs TSM and ERM; SC vs ERM) and LDR-D (SC vs TSM) methods respectively. The SD method did not produce a CI (G value >0.4 , Finney 1971) with propargite (WP) and fenbutatin oxide (WP) for either TSM or ERM but performed better than the petri dish methods with fenbutatin oxide (SC). Significant variation in LC values was found between methods within species and ERM was relatively more susceptible than TSM. Fenbutatin oxide (SC) and propargite (WP) were more toxic than fenbutatin oxide (WP) in almost all cases.

TABLE 1: Relative rank of test methods (based on 95% confidence intervals) and respective LC₅₀ values for different miticides against spider mites. See text for explanation of the abbreviations for methods.

Rank	Miticide		
	propargite (WP)	fenbutatin oxide (WP)	fenbutatin oxide (SC)
Twospotted spider mite			
1	LDD-PT 0.009a;0.003*	LDD-PT 0.025a**;0.007	LDR-D 0.017a;0.013
2	LDR-PT 0.014b;0.004	LDR-PT 0.049b;0.014	LDD-PT 0.011a;0.014
3	PDR-PT 0.013ab;0.005	LDR-D 0.027a;0.016	LDR-PT 0.024b;0.017
4	LDR-D 0.011ab;0.012	PDR-PT 0.134c;0.076	SD 0.107c;0.078
5	PDR-R 0.045c;0.050	PDR-R 0.179c;0.124	PDR-PT 0.114c;0.083
6	SD 0.140;NCI	SD 0.235;NCI	PDR-R 0.100c;0.115
European red mite			
1	PDR-PT 0.009a;0.006	LDD-PT 0.020a;0.007	LDD-PT 0.010a;0.004
2	LDD-PT 0.014ab;0.007	LDR-PT 0.034b;0.013	LDR-PT 0.020b;0.009
3	LDR-D 0.012a;0.007	PDR-PT 0.046b;0.016	LDR-D 0.024b;0.010
4	PDR-R 0.010a;0.008	LDR-D 0.036b;0.024	PDR-PT 0.033b;0.017
5	LDR-PT 0.021b;0.009	PDR-R 0.119c;0.061	SD 0.076c;0.058
6	SD 0.110;NCI	SD 0.092 NCI	PDR-R 0.077bc;0.090

* LC values in % AI; length of CI

** values with same letter(s) indicate no significant difference based on overlap of the 95% CI.

NCI = no confidence intervals calculated.

DISCUSSION

Rational selection of test methods is an important prerequisite for miticide screening programmes and resistance studies with spider mites. Selection of the test method should be made by considering the precision of LC estimates, cost, reliability and simplicity. Our results have clearly indicated that the responses of TSM and ERM to propargite (WP) and fenbutatin oxide (WP and SC) were method-dependent and the precision of LC₅₀ estimates varied between methods.

The smallest CI was correlated with lowest LC value in most cases, which led us to conclude that the bioassay method in which the miticides reach the target most efficiently produces the lowest LC value. With propargite the highest precision was obtained by the PDR-PT method for ERM and although it gave third highest level of precision for TSM the variation indicated by the CI was still narrow. The PDR-PT method did not produce similar precision for fenbutatin oxide (WP and SC) for either TSM or ERM. Although LDD-PT and LDR-PT methods produced acceptable levels of precision with propargite, the mite run off was unacceptably high (20-50%). This is an important limiting factor of using these methods when limited numbers of test individuals are available. The distinct advantages of the petri dish method over leaf discs are the lower time requirement (38 min to test 100 mites compared to 46 min with LDD-PT) to conduct the bioassay, production of reproducible results within 24 h and no run off. These conclusions are similar to those of Pree and Cole (1989) who used dicofol in their studies. Doubt has been cast by Bowie *et al* (1988) about the potential of the petri dish method to enhance the vapour effect of propargite (WP). Our studies have demonstrated that propargite does not induce vapour activated mortality when dishes were held at 23 and 30 °C for 24 h after treatment (Kabir unpubl.).

Leaf disc methods gave greater precision than the PDR-PT method in estimating LC₅₀ values of both formulations of fenbutatin oxide with TSM and ERM. Although mite run off was high (20-40%) with the leaf disc methods, in this case they would be more appropriate than the petri dish method because of the high variability in precision of estimates achieved with the latter. Scoring run off mites as dead (Hoy and Conely 1989) might be one way to simplify the analysis of results using the leaf disc methods.

The overall performance of leaf disc methods, if run off mites are excluded from the analysis, was higher than for the other methods. Huge run-off from the treated discs however, could impair the reliability of estimates and because of this the precision of leaf disc methods is difficult to estimate.

CONCLUSION

The results of this study suggest that the petri dish bioassay method would be the most appropriate method for testing the response of TSM and ERM to propargite. Although lower precision was achieved with the petri dish method compared to leaf disc methods using fenbutatin oxide, it may still be the preferred method as it overcomes the mite run-off problem. Investigation of factors that may influence the response of spider mites to miticides in petri dishes is being carried out.

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