DIFFERENTIAL TOXICOLOGICAL RESPONSES OF GREEDY SCALE STAGES TO DIAZINON

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SUMMARY

A laboratory dipping technique was used to investigate detailed toxicological responses of different greedy scale (*Hemiberlesia rapax*) stages to diazinon. The LC_{50} and LC_{90} values increased with age or size of each stage. At current recommended field rates of diazinon all immature scale would be expected to be killed but approximately 17% of the mature egg-laying stages may survive.

INTRODUCTION

Greedy scale is an important quarantine pest of kiwifruit. Detection of even relatively low infestations at harvest can markedly increase packing costs, whilst whole crops may be rejected at moderate to high infestations.

The value of a laboratory technique for screening toxicants against greedy scale has been recognised for some time. Ferguson and Wallace (1979) developed a dipping technique using natural scale infestations on fruit but had to contend with high natural mortalities. Berry (1983) developed a dipping technique where white caps (first instar) of known age were reared on potatoes and the infested potatoes dipped. This technique showed promise for carrying out detailed toxicological testing of insecticides. Greedy scale has, however, a number of different stages which may vary in their response to insecticides. The white cap moults into the yellow cap (second instar) and black cap (third instar) with a corresponding increase in size of each stage. With each moult, the dorsal half of the cast skin is incorporated into the protective covering, further thickening it. The immature black cap then undergoes a massive increase in size as the eggs develop, forming the conspicuous dome-shape of the mature scale. Mother scale have eggs or hatched crawlers under the protective silken cover.

This study investigated the feasibility of using the potato-dipping technique for detailed toxicity comparisons of diazinon to the different scale stages. Diazinon was used as it has been shown to be effective in field studies and is commonly recommended for scale control (Sale 1972; Sale and Steven 1984).

MATERIALS AND METHODS

The greedy scale colony was established on potatoes in 1984 from scale obtained from infested kiwifruit vines in the Maunu area, near Whangarei. The greedy scale colony was held at $22 \pm 1^\circ\text{C}$ and $74 \pm 10\%$ relative humidity. New potatoes were inoculated by placing them amongst a colony infesting potatoes and allowing crawlers to transfer. A uniformly aged white cap infestation was obtained by inoculating new potatoes with crawlers for 2-3 days and then isolating the new potatoes from the main colony until toxicity testing 10-11 days later. Some crawlers settled immediately whilst others took 2-3 days, so the age of the white cap population was approximately 8-11 days. Potatoes containing a mixed aged scale population, with yellow cap, black cap, mature and mother scales in sufficient numbers for toxicity testing, were selected from lightly infested potatoes from the main colony.

Infested potatoes were dipped for 5 seconds in a range of diazinon (Basudin 80 EC) concentrations in addition to a control which was dipped in water. These potatoes were held at $20 \pm 2^\circ\text{C}$ for 14-21 days before mortality assessment. Preliminary investigations had shown that the criteria for distinguishing live from dead scales were best applied about 14 days after treatment. This allowed plenty of time for affected scale to be killed by the toxicant with a subsequent desiccation and shrinking of the scale body which

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changes colour from yellow to brown. A high humidity was maintained after dosing by enclosing moistened cotton wool with the potatoes in a plastic container.

Preliminary trials were carried out to establish a range of concentrations to be used for the main toxicity tests. Ten day old white cap dose responses were determined from three test runs carried out on separate occasions. Each test run involved six-eight concentrations of insecticide as well as the water control and there were two-three repeats (potatoes) per dose. Mortality was assessed from 50-100 scales per potato. Mixed scale stage dose responses were determined from two-three test runs. Each test run involved eight-15 insecticide concentrations but for any scale stage only six-seven doses were evaluated. There were two or four repeats per run and mortality was assessed for 50 scale stages per repeat.

Log concentration probit regression analysis was used to calculate the LC_{50} and LC_{90} , the lethal concentrations required to kill 50% and 90% respectively, and 95% confidence intervals. This analysis took into account Abbott's formula (Busvine 1971) for correcting for control mortality which did not exceed 16%.

The size of the greedy scale was estimated for 10 individuals per stage. The shape of the body of the scale was assumed to be a flattened circular disc. The diameter was taken as the average of the widest and longest measurements which were often identical. The height of the disc was taken as the average of the minimum and maximum heights which showed most variation for mature scale.

RESULTS

The quantal response data for yellow cap, mature and mother stages gave an inflection of the log concentration percentage mortality lines indicating heterogeneity of the test population. As a consequence the yellow cap and mature scale (but not the mother scale) critical concentration levels could only be estimated using log concentration probit analysis. The simplified straight line plots of log concentration percentage mortality are shown in Figure 1. The critical concentration levels and slopes of the probit regression lines for each of the stages tested are shown in Table 1. There was a clearly defined increase in concentration to obtain an equivalent LC_{50} and LC_{90} with increasing scale age. For all stages, the log concentration probit regression showed evidence of flattening out near the LC_{90} level and this was reflected in the wide 95% confidence limits.

There were large increases in diameter and thickness as the scale matured from white cap to mother scale (Table 2). There was also a massive increase in volume of the immature black cap as eggs developed to form the mature scale. The toxicity of diazinon to the older stages up to the mature stage decreased in proportion to an increase in thickness or diameter, but not to volume. The low toxicity of diazinon to the mother scale could not be related to the size of this stage.

Fig. 1: Plots of the logarithm concentration of diazinon against percentage mortality for each greedy scale stage.

TABLE 1: Total numbers of the different greedy scale stages tested (n) using diazinon, critical concentration levels (95% confidence intervals) and slopes of the logarithm concentration probit regression curves.

Stage	n	LC ₅₀ (95% CI) (g ai/100 litres)	LC ₉₀ (95% CI) (g ai/100 litres)	Slope ± SE
white cap	9143	0.3 (0.2-0.4)	1.7 (1.4-2.4)	1.73 ± 0.04
yellow cap	2807	0.6 (0.4-0.8)	3.2 (2.3-5.0)	1.76 ± 0.07
black cap	2178	0.7 (0.5-0.9)	2.8 (2.1-4.1)	2.15 ± 0.08
mature	2370	1.1 (0.8-1.5)	6.1 (4.3-9.8)	1.71 ± 0.06
mother	3032	5.3 (3.5-7.5)	115.9 (64.6-267.5)	0.95 ± 0.03

TABLE 2: Diameter and thickness of greedy scale stages and a comparison of toxicity and thickness ratios relative to the white cap stage.

Stage	Diameter (mm)	Thickness (mm)	Thickness ratio	LC ₅₀ ratio	LC ₉₀ ratio
white cap	0.22	0.08	1.0	1.0	1.0
yellow cap	0.31	0.11	1.4	2.0	1.9
black cap	0.67	0.19	2.4	2.3	1.6
mature	0.97	0.26	3.3	3.7	3.6
mother	0.92	0.32	4.0	17.3	68.2

The recommended field concentration of diazinon (48 g ai/100 litres) for control of greedy scale on kiwifruit can be compared with the quantal response data (Fig. 1). Greater than 99% mortality of the immature and non-egg laying mature stages may be expected using the field concentration, compared with 83% mortality of mother scales.

DISCUSSION

Earlier attempts at investigating the susceptibility of scale stages to diazinon have been somewhat inconclusive. Ferguson and Wallace (1979) found no difference in the mortality of immature and mature scale with diazinon at field concentration. Berry (1983) investigated the toxicity of diazinon to white caps of different ages but did not compare susceptibility of the different stages. Blank *et al* (1985), in their field screening trials, compared the susceptibility of white cap, yellow cap and black cap stages to a field rate of diazinon and considered there was little difference in mortality between these stages, but mature stages were not present when tests were carried out.

The estimate for the LC₅₀ for 10-day-old white caps (Table 1) was similar to the estimate of 0.25 g ai/100 litres obtained by Berry (1983). However, her estimates for the LC₉₀ of 14 g ai/100 litres and confidence intervals were markedly higher than those obtained in this study.

These results clearly show that scale stage influences susceptibility to diazinon. Toxicity tests should thus include both immature and mother (egg-laying matures) scales in order to establish the range of concentrations required to kill all stages. The toxicity testing procedures used in these trials show considerable potential for evaluation of the efficacy of different insecticides and formulations against greedy scale as they can be carried out far more precisely and expeditiously than current field testing techniques.

It is of interest to speculate on the heterogeneity amongst some stages of the test population. All stages, with the exception of the 10-day old white cap, comprised mixed age populations and this may have contributed to the heterogeneity. It is possible that different cohorts were present within each stage which differed in their response to the toxicant due to differences in size, thickness of the protective silken cover, feeding activity, or some other physical or physiological feature. Another possibility is that the population tested comprised of a mixture of diazinon susceptible and resistant scale. There was evidence of a small proportion of hard-to-kill individuals with all stages tested. Further work is required to establish the cause of this heterogeneity.

In these tests 17% of mother scale were not killed at current recommended field rates. In the field higher rates of diazinon may improve control of established populations containing mature scale, as for example during winter spray applications. However, given the relatively flat slopes of the logarithm dose mortality curves at the LC_{50} level, a doubling of the field rate may still not control all individuals. A better strategy would be to target spray applications at the most sensitive immature scale stages.

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