EVALUATION OF ACARICIDES FOR CONTROL OF *MELITTIPHIS ALVEARIUS* MITE IN EXPORT BEE PACKAGES

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SUMMARY

In a set of bioassays, three rates of each of three acaricides were evaluated for the control of *Melittiphis alvearius*, a mite with potential to threaten New Zealand's bee export industry. Although bromopropylate had no harmful effect on bees, mortality of mites was poor. Cyhexatin was highly toxic to bees and cannot be recommended for use. Fluvalinate at 0.1 g/m² caused a 99% mortality of the mite within 30 hours and showed no toxic effects on bees held in the containers for 9 days. Bee tolerance of low relative humidity (40%) was improved by some acaricide treatments.

INTRODUCTION

The opportunity for New Zealand beekeepers to export honey bees (*Apis mellifera*) to Canada may be jeopardised by possible restrictions on New Zealand exports imposed because of *Melittiphis alvearius* (Berlese), a mite widespread in this country. The mite has contaminated previous consignments of bee packages and this led to *M. alvearius* being found in beehives in Nova Scotia (Delfinado-Baker 1988). Gibbins and van Toor (in prep.) have shown the mite not to be parasitic on bees but merely commensal in the beehive and feeding on pollen. Nevertheless Canada and other countries with potential and existing markets for live bees may request that all mites contaminating bee packages be dead on arrival.

Bees are exported in vertical cardboard tubes (1.3 m x 95 mm internal diameter) (Berry 1987) where they are kept for up to 8 days before release as booster colonies into beehives in Canada. Commercially available acaricides which are relatively non-toxic to bees were chosen for evaluation in containers approximating those used for export. Bromopropylate has been found to be effective in controlling *Varroa jacobsoni* mites in beehives (Aleksenko *et al* 1985) and was moderately effective in reducing populations of *Acarapis externus* when it was ingested by bees (Clinch and Faulke 1977). Fluvalinate is a pyrethroid insecticide considered 'bee-safe' (Rouas 1987) and has been shown to be effective on *Tetranychus urticae* (Penman *et al* 1986) a mite pest on fruit trees. Cyhexatin has been used in New Zealand's horticultural crops for control of European red mite and two-spotted spider mite (Penman and Ferro 1977).

This report describes laboratory bioassays evaluating the effect of these materials at three rates on *M. alvearius* and honey bees caged in containers under conditions simulating those likely to occur in bee packages exported to Canada. The objective was to find an acaricide which gave 100% mortality of mites with no adverse effect on bees.

METHODS

During October 1987 *M. alvearius* nymphs and adults were collected from six hives at Outram, Eastern Otago. They were found between hive mat sacking under the roof and the top bars of frames immediately above the brood chamber. The mites were transferred directly to the glass slides or containers used in the tests.

Toxicity of liquid suspensions

Direct toxicity of bromopropylate (Neoron 50% EC), fluvalinate (Mavrik Aquaflo 24%) and cyhexatin (Plictran 600FL) was evaluated at 0.01 g, 0.1 g and 1.0 g/litre by the slide dip method. An average of 3.3 adults and 3.4 nymphs were tested in each of 10 replicates. Mites were placed ventral side up on double-sided sticky tape on a glass slide. Slides were dipped in the acaricide suspensions for 5 seconds. A control was

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prepared by dipping mites in deionised water. The mites were held in vented petri dishes at 28°C and $85 \pm 3\%$ relative humidity. Mortality was assessed by microscopic examination after 6, 22, and 30 hours with death being determined by the absence of leg movement following prodding with a camel hair brush.

Toxicity by surface contact with dried acaricides

Contact activity of bromopropylate, fluvalinate and cyhexatin was evaluated at 0.01, 0.1 and 1.0 g/m² of surface area by applying 0.56 ml of 0.01, 0.1 and 1.0% acaricide solutions onto 85 mm diameter cardboard discs. Deionised water was used as a control. The discs were made from the same ridged box-board used in cardboard tubes for exporting bees. The moist discs were oven dried for 30 minutes at 35°C. The discs were then attached by double-sided sticky tape to the inside of the lid of an 86 mm diameter clear-plastic petri dish. The petri dish bottom which had a 10 mm diameter vent hole for accommodating a removable plug covered with gauze of 6 x 150 micron size apertures, was placed on top of the cardboard and the joins sealed with a water-based solution of 3% methyl cellulose, 1.0% agar and 0.2% sodium chloride. A wire clip clamping the lid and base together combined with the sealant properties of the solidified gel prevented the mites from escaping.

During 16 November to 27 November 1988, an average of 14 mites (adults or nymphs) for each of 10 replicates of each treatment were transferred from the hive through the petri dish vent hole and placed onto the acaricide-treated cardboard. A fresh brush was used for each treatment to prevent contamination from the other acaricides. Approximately 2 g of pollen-impregnated propylis, a substance which the mites were observed to feed on (Gibbins and van Toor, in prep.), were placed in each petri dish as a food source. Within 2 hours of collecting the mites, the containers were incubated at 28°C and $85 \pm 3\%$ relative humidity. Mortality was assessed after 6, 22 and 30 hours with death being the absence of movement after 10 seconds observation. Immobile mites displaying twitching limbs were considered moribund and, if in subsequent assessments had not recovered, were judged dead in the first instance. Inspections of mites were made through the clear plastic with no dismantling of the containers required.

Toxicity of acaricides to bees

Cardboard, cut to a trapezium 72 mm x 67 mm sides by 67 mm tall and treated with three rates of the three acaricides as above was inserted as a cylinder into 78 mm x 24 mm tapered clear polycarbonate tubes. At the bottom of the tube, a food source of 4 ml of candy gel comprising 60% sucrose and 0.4% agar was available to the bees. The cardboard was positioned flush with the tube entrance allowing for a gap of 3 mm between the top of the sugar candy and lower end of the cardboard. This space prevented the candy from soaking into the cardboard. The cap had a 22 mm diameter gauze vent containing 0.25 mm x 1.25 mm apertures. The vials represented conditions in the cardboard tubes used for export.

On 9 December 1987 bees collected from the brood chamber of a hive were anaesthetised in a collecting chamber with carbon dioxide and five bees transferred to each tube. Each of the 10 treatments were replicated 10 times to give 100 vials per set. The vials were randomly positioned in racks and held at 10° below horizontal.

The humidities likely to be experienced by the bees in transit from New Zealand to Canada can range from 10% RH ambient in the hold of a Boeing 747 to 90% RH ambient at Auckland airport terminal (N. Taylor, Mangere Jet Base, pers. comm.). To evaluate the effect of humidity on acaricidal toxicity to bees, each set of vials were held at 40%, 65% and 85% relative humidities and a uniform temperature of 28°C. Mortality was assessed daily for 9 days. Dead bees were removed at each inspection.

Consumption of sucrose by the bees during the course of the experiment was estimated by subtracting the remaining sucrose dry matter at day 9 from the 2.4 grams administered per tube initially, and dividing the amount consumed by the aggregate of surviving bees per day to give the amount of sugar consumed/day/bee.

Statistical analysis

Bee and mite survival in acaricide treatments was adjusted in relation to that in the controls as if the latter was 100%. Analysis was by probit of log dose.

RESULTS AND DISCUSSION

Acaricidal activity on mites

Mortality of mites dipped in acaricides occurred with all chemicals within 6 hours leading to at least 90% mortality at the 1.0 g/litre rate after 22 hours. Mite mortality in untreated slides was 18%. Results showed that all three chemicals in the form of a liquid suspension were toxic to the mites.

Results were similar for mites in contact with acaricides applied to the cardboard surface (Table 1). Fluvalinate and cyhexatin gave high mortalities within 6 hours and 99% mortality for 0.1 and 1.0 g/m² rates after 30 hours. Fluvalinate demonstrated immediate knockdown at all rates, leaving mites moribund within an hour. The maximum mortality of 87% from bromopropylate was not considered high enough for the purpose of exterminating mites in bee packages.

There was no significant difference ($P > 0.05$) between the mortality of nymphs and adults at the three rates in either the dip or contact methods of evaluation.

TABLE 1: Proportion of total mites surviving after the mites were placed on cardboard treated with acaricides.

Treatment	Rate g ai/m ²	Number hours of contact		
		6	22	30
untreated		.98	.87	.79
fluvalinate	0.01	.24	.13	.08
	0.1	.03	.02	.01
	1.0	.06	.02	.01
bromopropylate	0.01	.89	.63	.52
	0.1	.88	.53	.35
	1.0	.32	.10	.06
cyhexatin	0.01	.90	.68	.49
	0.1	.21	.02	.01
	1.0	.14	.02	.00

Formula for comparisons of treatments within columns.

$$LSD_{(.05)} = 1.96 \sqrt{\frac{p_1(1-p_1) + p_2(1-p_2)}{140}}$$

where P = proportion of mites surviving

(All treatment data are significantly different ($P < 0.05$) from untreated control data).

Bee survival

Bees exported to Canada need to be established in their new hives within 6 days after departure from New Zealand apiaries, so discussion of the survival of bees in the bioassay will emphasise the 6 day assessment. Survival was high in untreated vials until the 4th day. Thereafter, survival deteriorated with the greatest decreases occurring at 40% RH (Table 2) where presumably all the available moisture contained in the sugar candy had been extracted and the bees suffered from dehydration.

At all humidities cyhexatin was extremely toxic to bees causing up to 98% mortality at the high rate after 2 days. Bee mortality at 0.1 g/m² was apparently not due to direct toxicity but it caused the bees to smear sugar candy over themselves leading to death from asphyxiation.

Bromopropylate caused no reduction in bee survival in comparison with the untreated control. However, at relative humidities of 40% the chemical improved survival at the 1.0 g/m² rates. The same effect was observed for fluvalinate at all rates (Table 2). This suggests that these chemicals may offer some physiological advantage in combating moisture stress e.g. by lowering the bees metabolic activity.

TABLE 2: Toxicity of acaricides to bees in packages held at 40%, 65% or 85% relative humidities and a constant temperature of 28°C. Data are the proportion of bees surviving per treatment.

Treatment	Relative humidity No. days of contact g ai/m ²	40%			65%			85%		
		3	6	9	3	6	9	3	6	9
untreated		1.00	.12	.00	0.96	.86	.50	0.98	.74	.52
fluvalinate	0.01	1.00	.42*	.00	1.00	.92	.52	1.00	.68	.44
	0.1	1.00	.32*	.00	0.96	.86	.60	1.00	.74	.54
	1.0	1.00	.40*	.02	0.94	.70*	.48	0.94	.54*	.40
bromopropylate	0.01	0.96	.06	.00	1.00	.70*	.40	0.98	.72	.46
	0.1	1.00	.04	.00	1.00	.88	.50	1.00	.86	.50
	1.0	1.00	.46*	.18*	0.98	.96	.60	0.98	.80	.46
cyhexatin	0.01	1.00	.38*	.00	1.00	.86	.28*	0.98	.78	.34
	0.1	0.22*	.08	.00	0.22*	.00*	.00*	0.26*	.02*	.00*
	1.0	0.00*	.00*	.00	0.00*	.00*	.00*	0.02*	.00*	.00*

Formula for comparisons of treatments within columns

$$LSD_{(0.05)} = 1.96 \sqrt{\frac{P_1(1 - P_1) + P_2(1 - P_2)}{50}}$$

where P = proportion of bees surviving

* Means which are significantly different ($P < 0.05$) from untreated controls.

Fluvalinate was 'bee safe' at 0.01 and 0.1 g/m² but at 1.0 g/m² caused up to 27% mortality of bees after 5 days of contact. The symptoms of toxicity which occurred in two replicates at 65% humidity and six replicates at 85% humidity, as indicated by a slight smearing of sugar candy over the vial interior, were a milder form of that noted for cyhexatin. These results were confirmed in a repeat of the experiment at 85% relative humidity using 4 bees/vial, the bees being collected from the brood chamber of another hive.

Sugar consumption averaged over all treatments was 28.5 mg/bee/day (SEM = 0.6) and this was not significantly ($P > 0.05$) altered by the different chemical rates or humidities. This suggests the treatments did not affect feeding.

Fluvalinate at 0.1 g/m² appears suitable to control the mites in packages. At current prices of \$167 + G.S.T./litre, the cost of the chemical at 0.1 g/m² is 2.7 cents per tube which is economically feasible.

In export bee packages to Canada the influence of humidity should not be a problem, despite the relative humidity in the baggage hold of a Boeing 747 reaching as low as 10% with maximum flight durations of 12 hrs (N. Taylor, Mangere Jet Base, pers. comm.). The bees can modify atmospheric conditions themselves inside the tubes by forming clusters for warmth or dispersing for cooling and utilise the water contained in the 1 kg of sugar gel within the package to maintain suitable humidity levels. What these levels are requires further study.

CONCLUSION

While bromopropylate demonstrated no ill effects on bees at all rates provided RH was above 65%, its efficacy on mites at the highest rate tested was relatively poor (87%). Higher rates may give the near 100% control required. Fluvalinate at 0.1 g/m² produced acceptable control of mites after 30 hours (99%) and showed no toxic effect on bees after 9 days. Fluvalinate is recommended for further evaluation in commercial bee packages with particular emphasis on determining at what level the acaricide becomes toxic to bees. Because of its severe toxicity to bees, cyhexatin cannot be recommended for use in bee packages.

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