COMPARATIVE TOXICITY OF PESTICIDES TO THE SIXSPOTTED MITE *EOTETRANYCHUS SEXMACULATUS* (RILEY) (ACARI: TETRANYCHIDAE) ON AVOCADOS

P.S. STEVENS, L.E. JAMIESON and J. CAVE

HortResearch, Private Bag 92 169, Auckland
Corresponding author: pstevens@hortresearch.co.nz

ABSTRACT

Sixspotted mite (*Eotetranychus sexmaculatus*) is a serious pest of avocados in New Zealand and their feeding on the leaves can lead to defoliation. There are no pesticides registered for controlling sixspotted mites and there is an urgent need to develop effective control methods. Laboratory bioassays were carried out to evaluate the potential of a range of pesticides against sixspotted mites. A field trial was also carried out comparing mite infestations on trees sprayed with a combination of Avid® + Excel™ Oil® with levels on unsprayed trees. The bioassays indicated that Avid®, Malathion, Basudin 50 WP and Mavrik® Flo all have potential to control mites. The application of two sprays of Avid® + Excel™ Oil® significantly reduced populations of sixspotted mites in avocado trees.
Keywords: sixspotted mite, avocados, chemical control

INTRODUCTION

Sixspotted mite, *Eotetranychus sexmaculatus* (Riley) (Acari: Tetranychidae), has recently been identified as a serious pest of avocados in New Zealand. Although this species has been in New Zealand since at least 1953 and is present in most avocado growing regions throughout the country, populations generally only reach commercially damaging levels in Whangarei. In Whangarei high populations have caused severe defoliation of trees prior to flowering for at least the last few years. Despite the seriousness of the damage caused by this pest, there has been very little research on this mite in New Zealand. There is an urgent need to develop management solutions for sixspotted mites that are compatible with Avogreen®. Monitoring systems, action thresholds and control methods are all required.

All active stages of sixspotted mites feed on vascular plants. During feeding, the mites penetrate the plant with their stylets and suck out the cell contents. Damage initially appears as a purple discolouration on the underside of the leaf veins. On avocados, only low levels of feeding damage are sustained before leaf drop occurs and this may be the most visible sign of sixspotted mite infestations. In California, leaf drop occurs when populations reach an average of 5-10 adult mites per leaf (Bailey and Olson 1990).

There are currently no products registered for control of sixspotted mites on avocados in New Zealand. However, some growers have been applying the

avermectin product Avid®. There have been no trials evaluating the efficacy of Avid® against sixspotted mites in avocado but information from elsewhere suggests that the efficacy of Avid® can be improved by the addition of mineral oil to the mix. Manufacturer information also suggests that Avid® is most likely to be effective during periods of new leaf growth and that maximum effectiveness may not be achieved until seven days after spraying. If the use of Avid® can be optimised, this would be an effective product to assist in reducing the impact of sixspotted mites on avocados. Avid® is compatible with IPM programmes using predatory mites such as *Typhlodromus pyri*, *Phytoseiulus persimilis* and *Galendromus occidentalis*, although predator numbers may be temporarily suppressed.

The use of a product like Avid® needs to be limited to ensure that mites do not develop resistance. Therefore it is essential that there are a range of pesticides available for control of sixspotted mites so that a resistance management strategy can be implemented.

The efficacies of a range of pesticides were compared against sixspotted mites by carrying out laboratory bioassays (Experiment 1). A field trial was also conducted to evaluate the efficacy of a combination of Avid® + Excel™ Oil® against sixspotted mites.

METHODS

Experiment 1: Laboratory bioassays to compare pesticide efficacy against sixspotted mites

The efficacy of seven products were compared (Table 1) for activity against adults and eggs. Sixspotted mites used for bioassays were collected from natural field infestations.

Table 1 List of products used in sixspotted mite laboratory bioassays

Product	Active ingredient	Insecticide Class	Rate per 100 L
Avid®	Abamectin	Avermectin	37.5 mls
Basudin 50 WP	Diazinon	Organo-phosphate	100 g
Excel™ Oil®	Mineral oil		1000 mls
Lime Sulphur	Sulphur		7000 mls
Malathion	Malathion	Organo-phosphate	300 mls
Mavrik® Flo	Taufluvalinate	Synthetic pyrethroid	20 mls
Torque®	Fenbutatin oxide	Organo tin	40 g
Water control	-	-	-

For the purposes of the bioassays involving adults, mites were enclosed within a limited area on the lower surface of detached avocado leaves using feeding arenas. Feeding arenas consisted of 20 mm diameter non-toxic clear plastic

tubing (20 mm in length) that had been sealed at one end with metal mesh. Feeding arenas were attached to the leaves covering a leaf vein using Bostick Blu Tack rings. To avoid loss of leaf condition through dehydration, the stems of the avocado leaves were placed in a plastic tube (75 mm length, 10 mm diameter) filled with water and sealed with a cotton-wool plug. The leaves were placed lower surface down on 10 mm thick foam pads in a tray of water held within a plastic bag. After a number of preliminary studies to establish methodology, products were compared using three bioassay methods with ten replicates of each.

Contact toxicity bioassay
Adult mites were placed on filter paper within a Petri dish using a fine camel hair paint brush. Petri dishes were sprayed with a Potter tower operated at 69kpa and delivering 2 ml of solution. Mites (n=20) were then transferred to unsprayed leaves where they were enclosed within feeding arenas attached to the lower leaf surface. The survival and the number of eggs laid were assessed after 24 hours storage at 25^{o}C.

Residual toxicity bioassay
Unsprayed avocado leaves were dipped in insecticide solution for 5 seconds and allowed to dry in ambient conditions. Once leaves were dry, adult mites (n=20) were enclosed within feeding arenas placed on the lower leaf surface using a fine camel hair paint brush. The survival and the number of eggs laid were assessed after 24 hours storage at 25^{o}C.

Ovicidal toxicity bioassay
All motile stages of mites were removed from leaves and the numbers of mite eggs on each leaf was counted prior to dipping leaves in solution for 5 seconds. Leaves were then stored at 18^{o}C before assessment. The numbers of mites and eggs (unhatched versus hatched) were counted after 10 days.

The percentage survival and the numbers of eggs laid were compared among treatments using Analysis of Variance (ANOVA). Least Significant Differences were calculated to separate treatments if the ANOVA resulted in a $P<0.05$. Percentages were angular transformed before analysis but untransformed percentages are shown in tables. The analysis was performed using the statistics programme SAS (Release 6.12).

Experiment 2: Field trial to evaluate the efficacy of Avid® + Excel™ Oil® against sixspotted mites
The trial was conducted in an orchard in Mangatapere, Whangarei. Twenty experimental trees were set aside for the purposes of the trial and no other sprays were applied to these trees over the duration of the trial. The trees were very severely infested with mites and had suffered serious defoliation, although at the commencement of the trial a new foliar flush had just begun.

Samples of 20 leaves per tree were randomly collected into pre-labeled bags on the 8th December 2000, prior to the first spray application. Leaves were transported from the orchard in a chilli-bin cooled with frozen cooler pads. The leaves were microscopically examined and all stages of sixspotted mites, including eggs, were recorded within 36 hours of collection. As a result of the pre-spray assessment, trees were assigned to treatments so that equivalent levels of pre-spray mite numbers occurred. Trees were either sprayed with a combination of 37.5mls Avid® + 0.5% Excel™ Oil® or left unsprayed. There were ten replicates of each treatment. Sprays were applied to run-off (approximately 10 L per tree) using a motorised sprayer and a single nozzle handgun on the 14th and 22nd December 2000. A post-spray sample of leaves was evaluated for mites on the 3rd January 2001 using the methods described above.

The mean number of mites per leaf and the percentage of leaves infested with mites were statistically compared using an independent sample T-test. All analysis was carried out using the statistics programme SAS (Release 6.12). Percentages were angular transformed prior to analysis but untransformed percentages are shown in tables. The percentage of infested leaves was multiplied by the mean number of mites per leaf to give an Infestation Index. The Infestation Index was a useful way of comparing the mite 'load' between treatments.

RESULTS AND DISCUSSION

Experiment 1: Laboratory bioassays to compare pesticide efficacy against sixspotted mites

Contact toxicity bioassay

Mortality of mites directly sprayed with all treatments, apart from Basudin 50 WP, was significantly higher than for mites sprayed with water (Table 2). Mortality of mites sprayed with either Avid® or Malathion was significantly higher than for all other treatments. The number of eggs laid by mites sprayed with Avid®, Excel™ Oil®, Lime Sulphur, Malathion and Mavrik® Flo was significantly less than for mites sprayed with water, Basudin 50 WP or Torque®.

Table 2 Direct toxicity of pesticides against sixspotted mites. Mortality and oviposition (mean ± SEM) 24 hours after treatment.

Treatment	% mortality[1]	Number eggs laid (24 hours)
Avid®	99.4 ± 0.6 a[2]	0.3 ± 0.2 c
Basudin 50 WP	11.5 ± 2.2 de	5.1 ± 1.6 ab
Excel™ Oil®	22.8 ± 3.8 d	1.1 ± 0.6 bc
Lime Sulphur	43.6 ± 5.3 c	1.5 ± 0.4 bc
Malathion	96.1 ± 2.3 a	0 c
Mavrik® Flo	84.4 ± 5.1 b	0 c
Torque®	17.3 ± 3.5 d	8.7 ± 2.6 a
Water Control	8.3 ± 1.1 e	7.9 ± 0.9 a
	P=0.0001	**P=0.0001**

[1]Percentage mortality corrected for number mites recovered.
[2]Values within a column followed by the same letter are not significantly different (P<0.05).

Residual toxicity bioassay

The mortality of mites placed on insecticide-dipped leaves was significantly higher than that for mites placed on leaves dipped in water, with the exception of those mites exposed to Torque® residues (Table 3). Mortality of mites placed on leaves dipped in either Avid®, Basudin 50 WP, or Malathion was significantly higher than levels recorded for other products. Oviposition was significantly lower for mites placed on insecticide-dipped leaves when compared with oviposition for mites placed on leaves that had been dipped in water.

Table 3 Residual toxicity of pesticides against sixspotted mites. Mortality and oviposition (mean ± SEM) after 24 hours exposure.

Treatment	% mortality[1]	Number eggs laid (24 hours)
Avid®	95.8 ± 2.6 a[2]	0.2 ± 0.1 cd
Basudin 50 WP	88.3 ± 3.8 ab	0 d
Excel™ Oil®	35.7 ± 4.5 d	0 d
Lime Sulphur	43.8 ± 8.6 d	2.8 ± 0.6 b
Malathion	85.5 ± 3.3 b	0 d
Mavrik® Flo	67.9 ± 5.1 c	0 d
Torque®	18.5 ± 5.2 e	2.5 ± 0.8 bc
Water control	11.8 ± 1.2 e	4.8 ± 0.6 a
	P=0.0001	**P=0.0001**

[1]Percentage mortality corrected for number mites recovered.
[2]Values within a column followed by the same letter are not significantly different (P<0.05).

Ovicidal toxicity bioassay

Prior to treatment, individual leaves were infested with an average of 10-14 unhatched eggs (Table 4). More than 50% of the eggs had hatched in 10 days on the leaves dipped in water. Leaves that were dipped in Excel™ Oil® or Mavrik® Flo had the highest percentage of unhatched eggs.

Table 4 Ovicidal activity of pesticides against sixspotted mites

Treatment	Mean number eggs/leaf prior to treatment	% eggs unhatched 10 days after treatment
Avid®	10.8 ± 1.5 a[1]	73.5 ± 5.8 ab
Basudin 50 WP	11.6 ± 3.2 a	52.3 ± 5.8 bc
Excel™ Oil®	11.9 ± 1.6 a	80.7 ± 5.2 a
Lime Sulphur	10.5 ± 2.2 a	62.8 ± 7.8 ab
Malathion	12.5 ± 3.5 a	66.2 ± 9.6 ab
Mavrik® Flo	14.1 ± 4.7 a	79.9 ± 7.3 a
Torque®	14.4 ± 2.9 a	49.2 ± 7.1 bc
Water control	12.6 ± 2.7 a	42.4 ± 6.0 c
	P=0.9752	**P=0.0018**

[1]Values within a column followed by the same letter are not significantly different (P<0.05).

The results of these bioassays indicate that Avid®, Malathion and Basudin 50 WP have the most immediate 'knock-down' effect on survival of mites. Mavrik® Flo also showed good potential for control through mortality of all life stages and reduction in oviposition. However, while the bioassay techniques described here were able to identify products that result in immediate kill, consideration must be given to more realistic effects for products that take a longer period to result in death, or that have good persistent activity against mites. For example, Torque® is likely to perform at a higher level in the field than is indicated in these bioassays. A limitation to these bioassays was that unacceptably high levels of control mortality occurred when the time between treatment and assessment was extended, as mites died of 'old age'. In an orchard situation, the overlapping life-stages and population structure would allow products with less immediate effects to cause mite population decreases.

Experiment 2: Field trial to evaluate the efficacy of Avid® + Excel™ Oil® against sixspotted mites

Prior to the spray applications there were no differences between trees in the mean number of mites per leaf, the percentage of infested leaves or in the calculated value of the Infestation Index (Table 5). However, in January, trees that had been sprayed with two applications of Avid® + Excel™ Oil® had significantly fewer mites, lower percentages of leaves infested with mites, and lower Infestation Index values compared with unsprayed trees.

Table 5 Numbers of sixspotted mites, percentage of leaves infested, and Infestation Index values (Number of mites/leaf x % infested leaves) on avocado leaves (Mean ± SEM) 6 days before and 12 days after 2 sprays of Avid® + Excel™ Oil®.

	Assessment time	Unsprayed Control	Avid® + Excel™ Oil®
Number mites/leaf	Pre-spray	12.5 ± 3.4 a[1]	13.0 ± 5.0 a
	Post-sprays	5.8 ± 1.0 a	0.2 ± 0.1 b
% infested leaves	Pre-spray	70.0 ± 8.8 a	63.5 ± 9.4 a
	Post-sprays	83.5 ± 5.4 a	5.5 ± 1.9 b
Infestation index	Pre-spray	1091.6 a	1167.5 a
	Post-sprays	631.9 a	1.9 b

[1]Numbers within a row followed by the same letter are not significantly different (P>0.05).

The results of this trial indicate that a combination of Avid® and Excel™ Oil® has the potential to reduce infestations by sixspotted mites on avocados. However, the effectiveness of applying Avid® alone, at different rates, or at other times of the year is unknown. Results from the bioassay in Experiment 1 suggest that both the Avid® and the Excel™ Oil® are contributing to control. The rate of Avid® used in this trial was equivalent to that recommended for control of two-spotted mites in apples and pears however, a range of rates is recommended for different crops. The high costs of Avid® mean that it would be advantageous to reduce the rate of Avid® if possible.

A key issue relating to the use of Avid® in avocados is to ensure that sixspotted mites do not develop resistance. If control of mites is reliant on chemical methods, at least in the short term, an additional product with a completely different mode of action will be required.

In the longer term it would be desirable to develop non-chemical means of controlling sixspotted mites in avocados. A number of predators have been observed in avocado orchards in association with sixspotted mites, including *Stethorus* ladybirds, and predatory mites. The very promising results from California, where releases of predatory mites are providing good control of *Oligonychus perseae*, suggest useful future research directions (Hoddle *et al.* 2000, Kerguelen and Hoddle 1999). However, the New Zealand avocado orchard environment is much more toxic to natural enemies than would be typical of orchards in California, due to the need to spray for other pests. More information about the compatibility of various pesticides with biological control agents would be extremely useful.

CONCLUSIONS

Laboratory bioassays indicate that Avid®, Malathion and Basudin 50 WP have the most immediate 'knock-down' effect on mites. Mavrik® Flo also showed good potential for control through mortality of all life stages and reduction in oviposition. The largest reduction in percentage egg hatch resulted from dipping eggs either in Excel™ Oil® or Mavrik® Flo. The results of the spray trial indicate that a combination of Avid® and Excel™ Oil® has the potential to reduce infestations by sixspotted mites on avocados. However, the effectiveness of applying Avid® alone, at different rates, or at other times of the year is unknown. Results from the bioassay in Experiment 1 suggest that both the Avid® and the Excel™ Oil® are contributing to control.

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