LARVAL FEEDING BEHAVIOUR AND TOXICITY OF *BACILLUS THURINGIENSIS* AGAINST *CTENOPSEUSTIS* *OBLIQUANA* ON AVOCADOS

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ABSTRACT

Ctenopseustis obliquana is a serious pest of avocado in New Zealand. Laboratory experiments to gain a greater understanding of the interaction between larval feeding behaviour and environmental degradation of *Bacillus thuringiensis* (Bt) residues were carried out. Larval *C. obliquana* were found to feed equally during light and dark periods and the application of Bt sprays in the evening did not consistently result in higher mortality than sprays applied in the morning. First instar larvae were more susceptible to Bt sprays than third instar larvae. However, when first instar larvae were provided with leaf discs that had been sprayed with Bt seven days previously, mortality was equivalent to that of larvae feeding on unsprayed leaves. Mortality of first and third instar larvae provided with Bt-treated leaf discs collected from within the shade of the avocado tree canopy (zero and one day after spraying) was significantly greater than that of larvae provided with Bt-treated leaf discs collected from the outer perimeter of the canopy. Results indicated that even very short exposure to sunlight (i.e. 30-60 minutes) was sufficient to cause a significant reduction in the toxicity of Bt-sprayed leaves to *C. obliquana* larvae.

Keywords: *Bacillus thuringiensis*, residues, feeding behaviour, avocado.

INTRODUCTION

A complex of leafroller species are important pests of avocado in New Zealand. Larvae feed on the surface of fruit causing scarring that makes fruit unacceptable for export. Between 80-95% of all larvae found on fruit have been identified as the brownheaded leafroller *Ctenopseustis* spp. (either *C. obliquana* (Walker) or *C. herana* (Felder and Rogenhofer) (Stevens et al. 1995). Larvae of *C. obliquana* and *C. herana* cannot be morphologically distinguished (Foster et al. 1991) but pheromone trap catches in avocado orchards suggest that the majority of larvae are *C. obliquana* (P.S. Stevens, unpubl. data). Control of leafrollers in avocados has been reliant on applications of broad-spectrum insecticides, but there is an increased interest in moving towards the use of selective products and those that are acceptable for organic production.

Products based on *Bacillus thuringiensis* (Bt) have been extensively used to control leafrollers in New Zealand kiwifruit (Steven et al. 1994). However Bt products have not been as successfully developed for use in other crops due to limited efficacy. Bt residues are rapidly degraded by ultraviolet light (Bailey et al. 1996) and the impact of ultraviolet degradation may be influenced by plant species, canopy management system and the location of the target insect within the canopy. If UV degradation is an important factor limiting the effectiveness of Bt in a particular crop, it may be possible to manipulate the situation, for example by using UV protectants or by spraying in the evening. Measures such as these may increase the probability that larvae ingest a lethal dose of Bt before residues decay to non-lethal levels. The influence of UV degradation on the efficacy of Bt may also be influenced by any diurnal feeding patterns displayed by the insect. Chapman (1998) states that most

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insects eat discrete meals separated by relatively long periods of non-feeding. Some lepidopteran larvae have been found to have diurnal feeding patterns with most feeding occurring at night, while others appear to have feeding patterns that are independent of light conditions (Benz 1991). This paper describes experiments evaluating the daily feeding pattern of larval *C. obliquana*, as well as the interaction between timing (morning versus evening) of spray application. The persistence of Bt, and the effect of exposure to UV degradation on the efficacy of Bt against larval *C. obliquana* on avocados, was also investigated.

METHODS

All insects used in experiments were from a laboratory colony of *C. obliquana* maintained at HortResearch, Mt Albert. All experiments were carried out at 18°C.

Experiment 1: Determining the daily pattern of larval feeding

Circular leaf discs (15 mm diameter) were cut from unsprayed 'Hass' avocado leaves. Leaf discs were individually placed in a plastic Petri dish (9 cm diameter). A single *C. obliquana* larva was placed within each Petri dish and held at 18°C (12:12 h light:dark) and 12 replicates of first, third and fifth instar larvae were set up. Each Petri dish was checked every 30 min and the behaviour (feeding, resting or walking) of the larvae was recorded over a 24 h period.

Experiment 2: The persistence of Bt sprayed on avocado leaves against larval *C. obliquana*

Individual leaves on a 'Hass' avocado tree were sprayed at 7am on the 23 September 2000 with 50 g/100 litres Delfin® WG (containing 37 000 International Units of *Bacillus thuringiensis* var. *kurstaki* [serotype 3a, 3b, strain SA 11]/mg) using a hand-held sprayer. All leaves were located on the outside of the tree and were not subject to any shading. Leaves were allowed to dry (approximately 30-60 min) before sub-samples of treated and untreated leaves were picked. Leaf discs (15 mm) were cut from leaves and placed on moist filter paper within a plastic Petri dish (9 cm). A single *C. obliquana* larva was placed on each leaf disc. The survival of 10 x first instar larvae (<24 h old), and 10 x third instar larvae was measured after 9 days. Each treatment was replicated four times. Further leaves were sub-sampled from the avocado tree one and seven days after spraying and used for bioassays using the methods described above.

Experiment 3: The effect of time of spray application on the efficacy of Bt against *C. obliquana*

Individual leaves on a 'Hass' avocado tree were sprayed at either 7am or 7pm on the 23 September 2000 with 50 g/100 litres Delfin® WG (containing 37 000 International Units of *Bacillus thuringiensis* var. *kurstaki* [serotype 3a, 3b, strain SA 11]/mg). All leaves were located on the outside of the tree. Leaves were allowed to dry (approximately 30-60 min) before sub-samples of treated and untreated leaves were picked. A further sample of leaves was picked 12 h after spraying. Immediately after picking, larvae were set up in individual Petri dishes with leaf discs as described for experiment 2 above.

Experiment 4: The effect of exposure to sunlight on the efficacy of Bt against *C. obliquana*

Individual leaves on a 'Hass' avocado tree were sprayed at 7am on the 23 September 2000 with 50 g/100 litres Delfin® WG (containing 37 000 International Units of *Bacillus thuringiensis* var. *kurstaki* [serotype 3a, 3b, strain SA 11]/mg). Half the leaves were located on the outside of the tree and were therefore potentially subject to UV degradation ('sun leaves'), while half were located within the shade of the canopy ('shade leaves'). Leaves were allowed to dry (approximately 30-60 min) before sub-samples of treated and untreated leaves were picked. Further samples of sprayed and unsprayed leaves were picked 24 h after spraying. Immediately after picking, larvae were set up in individual Petri dishes with leaf discs as described for experiment 2 above.

Statistical analysis

Differences in larval feeding were analysed using t-tests. The percentage survival of larvae was compared between treatments using analysis of variance (ANOVA). Least

significant differences (LSDs) 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).

RESULTS

Experiment 1: Determining the daily pattern of larval feeding

Larvae were observed to feed at similar levels during the light and dark periods (Fig. 1). There were no significant differences in the mean percentage of larvae feeding during the day versus the night for any instar. Means were 11.1% and 16.6% for first instar larvae feeding during the light and dark phases respectively ($P > 0.05$) and 16.7% and 16.3% for third instar larvae feeding during the light and dark phase respectively ($P > 0.05$). A mean of 16.7% of fifth instar larvae fed during the light phase versus a mean of 20.1% feeding during the dark phase ($P > 0.05$).

FIGURE 1: Percentage of *C. obliquana* larvae observed actively feeding over 24 hours under a 12:12 h light:dark photoperiod.

Experiment 2: The persistence of Bt sprayed on avocado leaves against larval *C. obliquana*

The mortality of first instar *C. obliquana* provided with leaves collected on the same day and one day after being sprayed with Bt was significantly higher than for larvae provided with unsprayed leaves ($P < 0.001$) (Table 1). However, the mortality of first instar larvae provided with leaves that had been sprayed with Bt seven days previously was not significantly higher than for larvae provided with unsprayed leaves. The mortality of third instar *C. obliquana* provided with Bt-sprayed leaves was not significantly higher than for larvae provided unsprayed leaves, even when the larvae were provided with leaves immediately after spraying ($P > 0.05$).

TABLE 1: The percentage mortality of first and third instar *C. obliquana* larvae feeding on avocado leaves collected 0, 1 and 7 days after spraying with Bt (mean $\pm$ SEM).

Treatment	Residue age (days after spraying)	% mortality after 9 days feeding	
		first instar	third instar
Bt	0	37.5 $\pm$ 8.5 a ¹	10.0 $\pm$ 5.8 a
	1	45.0 $\pm$ 6.5 a	7.5 $\pm$ 4.8 a
	7	15.0 $\pm$ 8.7 b	0 a
Unsprayed	0	0 c	0 a
	1	5.0 $\pm$ 5.0 b	2.5 $\pm$ 2.5 a
	7	2.8 $\pm$ 2.8 bc	2.5 $\pm$ 2.5 a

¹Figures within a column followed by the same letter are not significantly different ($P < 0.05$).

Experiment 3: The effect of time of spray application on the efficacy of Bt against *C. obliquana*

Mortality of first instar larvae provided with leaf discs that had been sprayed with Bt was significantly higher than for larvae provided with unsprayed leaves ($P < 0.001$) (Table 2). Mortality of first instar larvae provided with leaves immediately after spraying with Bt in the morning was significantly lower than for larvae provided with freshly sprayed leaves in the evening. However, the mortality of larvae provided with leaves that had been sprayed in the morning and exposed to 12 hours of sunlight was significantly greater than for larvae provided with leaves that had been sprayed in the evening and exposed to 12 hours of darkness.

As with experiment 2, the mortality of third instar larvae provided with leaves that had been sprayed with Bt was not significantly different from that of larvae provided with unsprayed leaves ($P > 0.05$).

Experiment 4: The effect of exposure to sunlight on the efficacy of Bt against *C. obliquana*

The mortality of both first and third instar larvae provided with Bt-sprayed leaf discs collected from within the tree canopy (shade leaves) was significantly higher than for larvae provided with Bt-sprayed leaf discs collected from the outside of the canopy (sun leaves) ($P < 0.001$ and $P < 0.01$ for first and third instar respectively) (Table 3). This effect was observed even with leaves that were collected only 30–60 min after spraying (residue age zero days).

DISCUSSION

The results of these experiments indicate that applications of Bt are not likely to result in significant mortality of third instar *C. obliquana*, although a proportion of first instar larvae are likely to be affected. Similar results were obtained for *Cnephasia*

TABLE 2: The percentage mortality (mean $\pm$ SEM) of larval *C. obliquana* provided with avocado leaves sprayed with Bt at either 7am or 7pm.

Treatment and time of spray application	Residue age (hours after spraying)	% mortality after 9 days feeding	
		first instar	third instar
Bt-AM	0	37.5 $\pm$ 8.5 bc ¹	10.0 $\pm$ 5.8 a
Bt-PM	0	62.5 $\pm$ 4.8 a	15.0 $\pm$ 6.5 a
Bt-AM	12	50.0 $\pm$ 0 ab	15.0 $\pm$ 9.8 a
Bt-PM	12	27.5 $\pm$ 6.3 c	20.0 $\pm$ 10.8 a
Unsprayed-AM	0	0 d	0 a
Unsprayed-PM	0	0 d	0 a
Unsprayed-AM	12	0 d	0 a
Unsprayed-PM	12	5.0 $\pm$ 5.0 d	2.5 $\pm$ 2.5 a

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

jactatana on kiwifruit (Stevens & McKenna 1999). However, even first instar larvae are not likely to be affected by Bt residues within seven days of a spray application. Larval *C. obliquana* appear to feed equally during the light and dark periods. While the results of experiment 3 indicate that spraying in the evening is not likely to provide improved efficacy of Bt, this result was unexpected as UV degradation during the day would be expected to reduce the efficacy of Bt residues against larvae. In addition, results of experiment 4 showed that the location of leaves within the canopy (and the degree of shading) significantly affected the mortality of larvae provided leaves sprayed with Bt. It is difficult to explain this apparent contradiction but possibly the occurrence of a light dew overnight affected the drying and stability of the Bt spray deposits on the leaves sprayed in the evening. An analysis of the actual residues remaining on all types of leaves would be needed to clarify the cause of changes in larval mortality.

The most important factor influencing the effectiveness of Bt in these experiments was the location of the leaf within the canopy. The degree of shading is known to have a large effect on the efficacy of Bt, presumably through protection from UV degradation of residues. The results of the experiments described in this paper indicate that even very short exposure to

TABLE 3: The percentage mortality (mean $\pm$ SEM) of larval *C. obliquana* provided with 'sun' leaves versus 'shade' avocado leaves sprayed with Bt.

Treatment	Residue age (days after spraying)	% mortality after 9 days feeding	
		first instar	third instar
Bt-Sun	0	37.5 $\pm$ 4.8 b ¹	10.0 $\pm$ 5.8 b
Bt-Shade	0	87.2 $\pm$ 4.7 a	30.0 $\pm$ 11.5 a
Bt-Sun	1	45.0 $\pm$ 6.5 b	7.5 $\pm$ 4.8 b
Bt-Shade	1	94.7 $\pm$ 3.1 a	35.0 $\pm$ 15.0 a
Unsprayed-Sun	0	0 d	0 b
Unsprayed-Shade	0	10.3 $\pm$ 4.1 c	0 b
Unsprayed-Sun	1	5.0 $\pm$ 5.0 cd	2.5 $\pm$ 2.5 b
Unsprayed-Shade	1	5.0 $\pm$ 5.0 cd	0 b

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

sunlight (i.e. 30-60 minutes drying time) is sufficient to cause a significant reduction in the toxicity of Bt-sprayed leaves to larval *C. obliquana*. Bailey et al. (1996) showed that the activity of Bt on grape leaves exposed to the sun decreased by more than 50% within 24 hours. By contrast, shaded leaves retained over 60% activity after 32 hours. However, they concluded that reduced feeding rates of larvae on Bt-treated foliage was a more important factor limiting the efficacy of Bt than rapid inactivation due to UV degradation. Reduced feeding activity as a result of jaw paralysis is known to be an early symptom of Bt toxicity. However, reduced feeding in the presence of Bt residues may also be a result of 'avoidance' behaviour. For example, neonate lightbrown apple moth (*Epiphyas postvittana*) exhibit Bt avoidance behaviour when provided diet that contains Bt (Harris et al. 1997). When *C. jactatana* are offered a choice between leaf discs sprayed with Bt and unsprayed leaf discs, larvae were found to preferentially feed on the untreated food (P. Stevens, unpubl. data).

In conclusion, applications of Bt in avocados will need to focus on periods when small larvae are present. The limited persistence of the product will mean that repeated applications will be needed. Spraying in the evening is not likely to provide benefits over spraying in the morning. As many larvae are located in leaf shoots on the outside of the tree, decreased toxicity due to rapid UV degradation will continue to be an issue.

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