FACTORS AFFECTING THE EFFICACY OF *BACILLUS THURINGIENSIS* AGAINST *CNEPHASIA JACTATANA* IN KIWIFRUIT

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ABSTRACT

Since the introduction of the Integrated Pest Management programme for kiwifruit known as 'KiwiGreen', late season control of leafrollers has become completely reliant on the use of *Bacillus thuringiensis* (Bt) sprays. The dominant leafroller infesting kiwifruit near the end of the season is the native black-lyre leafroller, *Cnephasia jactatana*. In a laboratory bioassay both the rate of Bt and the larval developmental stage significantly influenced mortality and the extent of feeding of larval *C. jactatana*. Bt caused high mortality amongst 1st instar larvae, but showed little activity against 3rd or 5th instar larvae. Surviving 3rd and 5th instar larvae showed a significant reduction in the amount of feeding on Bt-sprayed leaves compared to those on untreated leaves. Results from a field trial showed that applying Bt to fruit infested with *C. jactatana* larvae decreased levels of infestation, but it was not possible to determine whether the decrease was a result of larval mortality or abandonment of feeding sites.

Keywords: *Bacillus thuringiensis*, kiwifruit, *Cnephasia jactatana*.

INTRODUCTION

The two main pest groups affecting kiwifruit are a complex of leafroller species, and three species of armoured scale insects. Most leafroller damage to fruit occurs in the ten weeks immediately after fruit set (December-February), and the dominant leafroller species responsible is the native brownheaded leafroller, *Ctenopseustis obliquana* (Stevens *et al.* 1995). Nearly all feeding damage caused by *C. obliquana* larvae is superficial, but often the extent of damage prevents the fruit from meeting export quality standards. After February, the rate of new leafroller damage to fruit declines rapidly, but late-season larval infestations occur in some orchard blocks each season. The late-season infestations are dominated by another species of native leafroller, the black-lyre leafroller, *Cnephasia jactatana* (C. McKenna, unpubl. data). *C. jactatana* does not usually cause such extensive scarring as *C. obliquana* as it tends to feed at the base of the fruit, often burrowing into the fruit. The presence of live *C. jactatana* caterpillars within fruit at harvest has serious implications for market access.

For the last four seasons pests of kiwifruit have been managed using the Integrated Pest Management (IPM) system known as 'KiwiGreen' (Steven *et al.* 1994). Since the introduction of KiwiGreen, late season control of leafrollers has become completely reliant on the use of *Bacillus thuringiensis* (Bt) sprays. While Bt sprays have generally proven to be extremely effective, some growers experience difficulties achieving the required level of *C. jactatana* control. Possible explanations for poor control include poor efficacy of Bt against *C. jactatana*, sub-optimal timing or application of sprays, and the ability of larvae to 'avoid' consuming a lethal dose of Bt due to their internal feeding habit. A laboratory bioassay and a small-plot field trial were carried out to assess some of the factors that may influence the effectiveness of Bt against *C. jactatana*.

METHODS

Laboratory bioassays

Circular leaf discs (15 mm diameter) were cut from unsprayed kiwifruit leaves. One of three rates of Bt (Delfin WG, containing 37 000 International Units of *Bacillus thuringiensis* var *kurstaki* (serotype 3a, 3b, strain SA 11)/mg) was applied to both surfaces of the leaf discs using a Potter tower. The rates were half the recommended field rate (25 g product/100 litres applied at a minimum water rate of 2000 litres/ha), field rate (50 g product/100 litres) and twice the field rate (100 g product/100 litres). The tower was operated at 104 kpa (15 psi) and delivered 2 ml of solution followed by a 10 second settling time per test.

After spraying, leaf discs were air dried and individually placed on moist filter paper in a plastic Petri dish (9 cm diameter). A single *C. jactatana* larva was placed on each leaf disc and the Petri dishes sealed with Parafilm. The effects of the 3 rates of Bt and an unsprayed control on 10 x 1st instar (<24 hours old), 10 x 3rd instar and 10 x 5th instar larvae were compared. Each treatment was replicated four times, with the replication based on the sequence of treatment application. Petri dishes with larvae were stored at 18°C (16:8 L:D) and assessed for survival after 1, 3 and 7 days. The extent of feeding was determined after 7 days by measuring the area of the leaf disc eaten using a transparent circular template marked with a 1mm² grid.

The percentage survival of larvae and leaf areas consumed were compared among 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 values shown in tables are untransformed. The analysis was performed using the statistics programme SAS (SAS Institute Inc. 1985).

Field trial

The trial was carried out at Te Puke Research Orchard in an unsprayed block of mature kiwifruit (cv 'Hayward') vines. A two way factorial experiment to compare the effects of canopy density (high versus low) and application of Bt (Dipel 2X (32 000 International Units of *Bacillus thuringiensis* var *kurstaki* (H-3a,3b,HD 1)/mg) @ 50 g/100 litre versus unsprayed) on *C. jactatana* larvae were compared. To achieve a high density of leaf canopy, vines were left unpruned from early December. Vines for treatments requiring a low canopy density received a standard summer prune in the week prior to the commencement of the trial. The trial was repeated using single vine plots grown on T-bar and pergola support structures with four replicates of each treatment allocated in a randomised block design.

Each vine was artificially infested with 1st instar *C. jactatana* larvae by using net bags to cage single egg batches onto each of 50 fruit clusters per vine. Egg batches were 24 hours or less from hatch. The net bags were removed from the fruit clusters after three weeks and fruit infested with *C. jactatana* were tagged. Very low numbers of caged fruit resulted in *C. jactatana* infestations so all fruit on each vine were checked, and fruit with natural infestations were also tagged.

The Bt spray was applied by handgun from a tractor mounted small-plot sprayer 24 hours after infested fruit were tagged. T-bar trained vines were sprayed on 9.2.98 and pergola trained vines on 10.3.98. Tagged fruit were harvested two weeks later and checked under a microscope for the presence of *C. jactatana*. The numbers of fruit infested with live caterpillars after spraying were compared among treatments by ANOVA using initial infestation levels as a covariance. The analysis was performed using the statistics programme MINITAB (MINITAB Inc., State College, PA, 1991).

RESULTS

Laboratory bioassay

The survival of *C. jactatana* feeding on leaves treated with Bt was affected by the larval developmental stage (Table 1). There was a significantly higher mortality of 1st instar larvae on Bt-treated leaf discs than on unsprayed control leaf discs after 3 days, and after 7 days almost 100% of 1st instar larvae on Bt-treated leaf discs were dead.

Only the highest rate of Bt resulted in significant levels of 3rd instar larval mortality. No mortality of 5th instar larvae fed on Bt-treated leaf discs was observed after 1 and 3 days. Some mortality was observed after 7 days, but this was not significantly different from the control.

TABLE 1: Percentage survival of larval *C. jactatana* feeding on kiwifruit leaf discs sprayed with three rates of Bt (mean $\pm$ SE)

Treatment (g /100 litres)	1 day after treatment	3 days after treatment	7 days after treatment
1st instar			
Bt @ 25 g	87.5 $\pm$ 2.5	15.0 $\pm$ 8.7 ab ¹	0 a
Bt @ 50 g	77.5 $\pm$ 10.3	25.0 $\pm$ 2.9 b	2.5 $\pm$ 2.5 a
Bt @ 100 g	80.0 $\pm$ 4.1	0 a	0 a
Unsprayed	92.5 $\pm$ 7.5	87.5 $\pm$ 6.3 c	85.0 $\pm$ 11.9 b
P>F	ns	<0.001	<0.001
3rd instar			
Bt @ 25 g	100	100 a	90.0 $\pm$ 7.1 a
Bt @ 50 g	100	95.0 $\pm$ 5.0 a	90.0 $\pm$ 7.1 a
Bt @ 100 g	100	77.5 $\pm$ 6.3 b	42.5 $\pm$ 4.8 b
Unsprayed	100	100 a	100 a
P>F	ns	<0.005	<0.001
5th instar			
Bt @ 25 g	100	100	97.5 $\pm$ 2.5
Bt @ 50 g	100	100	92.5 $\pm$ 9.6
Bt @ 100 g	100	100	92.5 $\pm$ 2.5
Unsprayed	100	100	100
P>F	ns	ns	ns

¹For each instar, figures within a column followed by the same letter are not significantly different (P<0.05)

The area of leaf discs consumed by surviving caterpillars was significantly affected by spraying leaves with Bt (Table 2). There were no surviving 1st instar larvae from any of the Bt treatments after 7 days so it was not possible to record the effects of Bt on feeding of 1st instar larvae. The surviving 1st instar larvae feeding on unsprayed leaf discs consumed a mean of 3.5 $\pm$ 0.4 SE mm² over 7 days. However, 3rd instar larvae on leaf discs sprayed with 50 g or 100 g Bt/100 litres consumed significantly less than larvae on unsprayed leaf discs. The 3rd instar larvae feeding on the lowest rate of Bt did not have reduced leaf consumption compared to those feeding on unsprayed leaf discs. The average consumption of 5th instar larvae feeding on leaf discs sprayed with 25 g and 100 g Bt/100 litres was significantly less than that of larvae feeding on unsprayed leaves. The 5th instar larvae feeding on leaf discs sprayed with 50 g Bt also showed a reduced consumption of leaf discs compared to those on unsprayed leaf discs, but this was not significant.

Field trial

The application of Bt significantly reduced the numbers of live *C. jactatana* larvae remaining in infested fruit (T-bar P<0.05, Pergola P<0.005) (Table 3). Canopy density had no significant effect on the numbers of live larvae remaining on fruit after spraying, and there was no significant interaction between Bt-spraying and canopy density.

TABLE 2: Area of kiwifruit leaf disc consumed by surviving 3rd and 5th instar *C. jactatana* larvae seven days after spraying with three rates of Bt (mean $\pm$ SE).

Treatment	Sample size	Leaf area consumed (mm ²)
3rd instar		
Bt @ 25 g	36	12.6 $\pm$ 1.3 a ¹
Bt @ 50 g	35	6.9 $\pm$ 1.5 b
Bt @ 100 g	17	2.1 $\pm$ 0.5 b
Unsprayed	40	15.8 $\pm$ 1.8 a
P>F		<0.001
5th instar		
Bt @ 25 g	37	21.5 $\pm$ 4.4 ab
Bt @ 50 g	35	28.5 $\pm$ 5.2 bc
Bt @ 100 g	35	10.5 $\pm$ 2.1 a
Unsprayed	40	40.5 $\pm$ 5.6 c
P>F		<0.001

¹For each instar, figures within a column followed by the same letter are not significantly different (P<0.05)

TABLE 3: Numbers of fruit infested with live *C. jactatana*, pre- and post-spray (50 g/100 litres Bt), on high and low canopy densities and two types of vine support structures (mean $\pm$ SE).

Treatment	Number fruit infested with live <i>C. jactatana</i>		% fruit with live larvae after spraying
	Pre-spray	Post-spray	
T-bar			
Bt-sprayed	14.0 ± 2.0	5.9 ± 0.9	43.6 ± 4.9
Unsprayed	20.6 ± 3.5	16.5 ± 3.5	73.6 ± 7.5
Low canopy density	18.9 ± 4.0	12.0 ± 3.8	54.8 ± 7.9
High canopy density	15.8 ± 1.7	10.4 ± 2.5	62.4 ± 8.8
Pergola			
Bt-sprayed	13.1 ± 1.1	2.3 ± 0.8	15.9 ± 5.7
Unsprayed	12.5 ± 2.5	6.1 ± 2.1	39.6 ± 7.8
Low canopy density	12.5 ± 1.6	3.5 ± 1.4	24.2 ± 7.5
High canopy density	13.1 ± 2.2	4.9 ± 2.0	31.2 ± 8.6

DISCUSSION

The results of the laboratory bioassay show that Bt causes high mortality of 1st instar *C. jactatana* larvae, but is less effective against 3rd and 5th instar larvae. Doubling the field rate of Bt increased the mortality of later larval instars, but more than 40% of 3rd instar and 90% of 5th instar larvae were still alive after 7 days. Those larvae, which survived 7 days exposure to Bt-treated leaves, showed a reduced leaf consumption of up to 85%. Other studies have also shown one of the effects of Bt is a reduction in feeding (Bailey *et al.* 1996; Jyoti *et al.* 1996). Another behaviour observed in association with Bt is increased walking and abandonment of feeding sites (Harris *et al.* 1997). The field trial described here showed a significant reduction in fruit infested with live larvae after spraying with Bt, although it was not possible to

determine whether this reduction was due to larval mortality or active abandonment of feeding sites. However, up to 48% of infested kiwifruit were still infested with live *C. jactatana* after spraying with Bt suggesting that larvae could readily survive commercial applications of Bt.

Insects may increase their chance of survival after exposure to Bt by temporarily reducing their feeding rate until the Bt residues have declined, or by actively abandoning the feeding site. In a field situation there would possibly be increased photo-degradation of Bt residues compared to those occurring in a laboratory bioassay, although kiwifruit are protected from direct sunlight. Although the residual biological activity of Bt on kiwifruit in the field is not known, it is possible that larvae can survive for a sufficient period to avoid ingesting a lethal dose. An additional feature of *C. jactatana* biology, which may enhance survival when kiwifruit is sprayed with Bt, is their habit of feeding within the fruit. The use of leaf discs does not allow larvae to easily avoid feeding on Bt. However, in a more natural situation, larvae may be able to almost completely avoid ingesting a lethal dose of Bt. Stapel *et al.* (1998) offered *Spodoptera exigua* a choice between Bt incorporated within artificial diet and artificial diet sprayed with Bt. Larvae accumulated on the Bt-sprayed diet and it was suggested that the larvae may have eaten through the thin Bt layer and thereby only been exposed to a small quantity of Bt. The larvae were then able to recover by feeding on the Bt-free diet located under the surface. A similar situation may occur with kiwifruit, as *C. jactatana* larvae initially establish feeding sites on, or within fruit.

These results suggest that there is potential to improve the control of *C. jactatana*. There may be benefits in adding feeding stimulants (e.g. Pheast) to Bt sprays to increase effectiveness. For example, feeding raspberry leaves treated with Bt + Pheast to larval *Choristoneura rosaceana*, resulted in a 93% greater mortality than that observed in larvae feeding on Bt alone (Li and Fitzpatrick 1997). Additional control techniques, such as mating disruption, may allow more reliable control of *C. jactatana* and reduce the industry reliance on Bt.

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