Evaluation of selected entomopathogenic fungi and bio-insecticides against *Bactericera cockerelli* (Hemiptera)

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Abstract Entomopathogenic fungi could provide feasible pest management options for control of *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), an important pest of solanaceous crops in New Zealand. In laboratory trials BotaniGard® ES and BotaniGard® 22WP (*Beauveria bassiana*; GHA strain) resulted in average nymph mortality of 82%, which was significantly greater than the conventional insecticide Oberon® (63%) or the microbial standard eNtocide L™ (63%) ($P < 0.01$). Met52® (*Metarhizium anisopliae*; F52 strain), Mycotrol®O (*B. bassiana*; GHA strain) and *Lecanicillium muscarium* isolates resulted in 91–100% adult and 55–70% nymph mortality. NoFly™ WP (*Isaria fumosorosea*; FE9901 strain), PreFeRal® 20WG (*I. fumosorosea*; Apopka 97 strain) and endemic *I. fumosorosea* isolate were less efficacious than other treatments. In greenhouse trials the application of BotaniGard® ES resulted in the greatest reduction in *B. cockerelli* infestation on both capsicums (88%) and tomatoes (83%). The performance of BotaniGard® ES was not adversely influenced by environmental conditions, unlike Met52® EC.

Keywords entomopathogen, fungi, *Bactericera cockerelli*, psyllid.

INTRODUCTION

Bactericera cockerelli (Sulc) (Hemiptera: Triozidae), commonly known as tomato potato psyllid, is a key pest of solanaceous crops, in particular potatoes (*Solanum tuberosum* L.), tomatoes (*Lycopersicon esculentum*) and capsicums (*Capsicum* L.). Feeding by this sucking insect is recognised as a transmission pathway for the pathogen ‘*Candidatus* *Liberibacter solanacearum*’ (Liefting et al. 2008), the pathogen associated with ‘zebra chip’ disorder in potatoes and ‘psyllid yellows’ in a range of solanaceous crops. Symptoms of these disorders can be severe, and include leaf, fruit and tuber discolouration, reduced plant vigour, quality and yield, and

in some instances plant death (Plant & Food Research 2009).

Of North American origin, *B. cockerelli* has been recorded in the USA, Mexico, southern Canada and New Zealand, where this pest is known to cause major problems for crop growers (Munyanza et al. 2007; Ferguson & Shipp 2002). *Bactericera cockerelli* was first discovered in Auckland, New Zealand, in 2006 (MPI Biosecurity New Zealand 2009), and has subsequently spread throughout crop growing regions of the North and South Islands (Teulon et al. 2009). The spread of *B. cockerelli* can be in part explained by the environmental conditions

being conducive to the development of this pest. In New Zealand *Bactericera cockerelli* exists through several generations per year, developing through three life-stages (egg, nymph and adult) per generation.

Outbreaks of *B. cockerelli* and widespread liberibacter infection continue to challenge New Zealand's primary industries. The economic impact has been substantial, for instance, in 2010-2011 the cost of *B. cockerelli* to the potato industry equated to \$28 million (NZD) (Kale 2011). In addition to the economic impact, the presence of *B. cockerelli* has, and will, continue to put Integrated Pest Management (IPM) programmes within these industries at risk. In New Zealand control of *B. cockerelli* is reliant on frequent pesticide applications. Such a practice is unsustainable in the longer term, with insecticide resistance, outbreaks of secondary pests, safety risks to humans and other mammals, and a decrease in biodiversity all very real concerns for industry. There is a crucial need for the ongoing development of tools that will enable the integration of chemical, cultural and biological control practices.

Today many species of entomopathogenic fungi are formulated as effective bio-insecticides, many of which are based on the fungi *Lecanillicum lecanii*, *Beauveria bassiana* and *Metarhizium* spp. Bio-insecticides could provide feasible options for inclusion in IPM programmes as these tend to have targeted and multiple modes of action, are host specific and inherently less toxic than conventional insecticides, and have the potential to disseminate and cycle through the pest population. The objective of this study was to evaluate a range of fungal entomopathogens, including fungal isolates and commercially available bio-insecticides, against *B. cockerelli* under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Bio-insecticides and isolates

From August 2011 to January 2012 six bio-insecticides and four fungal isolates were received for screening against *B. cockerelli* in New Zealand (Table 1). The viability of all bio-insecticides and isolates was confirmed upon receipt.

In November 2011 *B. cockerelli* adults were inoculated with four fungal isolates as a means of re-activating the isolates through the target host; such a method is utilised when isolates have been in culture collection or sub-cultured multiple times. The inoculation method was adapted from a technique for inoculating aphids and armoured scale insects with entomopathogenic fungi (Butt & Goettel 2000; Mauchline et al. 2011). Each isolate was prepared by adding 3–4 ml of distilled water and surfactant Tween80 (0.05%) to a sporulating culture plate. Anaesthetised (using carbon dioxide) adult *B. cockerelli* were transferred, using a fine-tipped paint brush, onto filter paper (47 mm diameter) contained within plastic, unvented Petri dishes (90 mm diameter). Each filter paper was transferred with sterile forceps onto a millipore filtration device attached to a Venturi vacuum pump. A 1 ml aliquot of the suspension was pipetted from the culture plate onto filter paper containing ten adult *B. cockerelli*. All insects were immersed for approximately 10 s after which the vacuum was activated drawing away the suspension. Each filter paper containing the inoculated insects was transferred into a Petri dish and maintained at 25°C. After 14 days fungal re-isolations were made from the insect cadavers, with subsequent culturing performed using the passaged isolates. Isolates were sub-cultured onto 50% strength potato dextrose agar (PDA, Oxoid) fortified with chloramphenicol (0.05 g/litre, BDH), and were maintained at 23–25°C, 16:8 h light:dark.

Plant propagation and insect supply

Plants were grown from seed in 2-litre pots within the greenhouse facilities at The New Zealand Institute for Plant & Food Research Limited (PFR), Te Puke. Plant varieties included capsicum 'Californian Wonder' (Watkins) and tomato 'Tiny Tim' (Yates). All plants were fed with Osmocote® controlled release plant food and maintained without fungicides or insecticides. When required, supplementary capsicum (variety 'Marconi Red') and tomato (Variety 'Russian Red') seedlings were supplied by Oakdale Organics (Pukekohe).

Bactericera cockerelli were reared on capsicum variety 'Giant Bell' at the Mt Albert Research Centre

(PFR), and transported to the Te Puke laboratory in vials (adults) or on capsicum leaves (nymphs). On receipt, insects were transferred onto caged capsicum or tomato plants within the laboratory or greenhouse. This process enabled good control of infestation levels and the age distribution of *B. cockerelli* within a particular trial.

Laboratory screening

In February 2012 10 *B. cockerelli* adults were aspirated into a single vial. Thirteen replicate vials were provided for each treatment (Table 1). Carbon dioxide was administered to each vial

for up to 10 s in order to anesthetize the insects. Isolates were suspended as described above, with concentration standardised to approximately 10^7 conidia/ml. Bio-insecticides were reconstituted to the rates outlined in Table 1. Entocide L™ (active ingredient *Lecanicillium muscarium*) was selected as a microbial standard due to the established use of this bio-insecticide for sucking insects in New Zealand commercial greenhouse crops. Oberon® (active ingredient spiromesifen) was selected as a conventional insecticide standard due to widespread use for control of *B. cockerelli* in New Zealand.

Table 1 Treatments evaluated against *Bactericera cockerelli*.

Treatment name	Manufacturer/ Supplier	Active ingredient (ai)	Rate (ai/litre unless otherwise stated)
BotaniGard® ES	BioWorks Inc., USA	<i>Beauveria bassiana</i> (GHA strain)	2.5 ml
BotaniGard® 22WP	BioWorks Inc., USA	<i>Beauveria bassiana</i> (GHA strain)	1.2 g
Mycotrol® O	BioWorks Inc., USA	<i>Beauveria bassiana</i> (GHA strain)	2.5 ml
NoFly™ WP	Natural Industries Inc., USA	<i>Isaria fumosorosea</i> (FE 9901 strain)	2.4 g
PreFeRal® 20WG	BioBest, Belgium	<i>Isaria fumosorosea</i> (Apopka 97 strain)	2.1 g
Met52® EC	Novozymes Biologicals Australia Pty Ltd	<i>Metarhizium anisopliae</i> (F52 strain)	2.5 ml
Isolate F421 ¹	AgResearch, NZ	<i>Lecanicillium muscarium</i> ²	10^7 conidia/ml
Isolate F425 ¹	AgResearch, NZ	<i>Lecanicillium muscarium</i> ²	10^7 conidia/ml
Isolate F426 ¹	AgResearch, NZ	<i>Lecanicillium muscarium</i> ²	10^7 conidia/ml
Isolate F129 ¹	AgResearch, NZ	<i>Isaria fumosorosea</i> ³	10^7 conidia/ml
eNtocide L™ (microbial standard)	Biotelliga Limited, NZ	<i>Lecanicillium muscarium</i>	10^9 conidia/ml
Oberon® (conventional standard)	Bayer CropScience	Spiromesifen	0.6 ml
Water (control)			

¹Number refers to the isolate number within the AgResearch culture collection.

²*Lecanicillium muscarium* were isolated from *Scolypopa australis*.

³*Isaria fumosorosea* were isolated from *Galleria mellonella*.

All treatments were applied using the methodology described above. Petri dishes were sealed with Parafilm® and held at 25°C ($\pm 1.1^\circ\text{C}$) for up to 122 h. The Parafilm® was removed after 24 h.

Assessments were completed at 0, 24, 28, 72, 96 and 122 h after treatment. Each insect was scored as live or dead, where dead insects were defined as showing no response to touch and having a desiccated appearance. After the final 122 h assessment dead insects were randomly selected from each treatment, and plated onto 50% strength PDA. Any resulting fungal growth was used to diagnose the cause of death.

In February 2012 an experiment to test efficacy of bio-insecticides against *B. cockerelli* nymphs was set up using 56 capsicum leaves ('Californian Wonder') prepared by placing each leaf stalk into a 2 ml Eppendorf tube containing water and plugging with cotton wool. Half of the leaves ($n=28$) were each infested with 50 1st to 2nd instar nymphs and half with 50 3rd to 5th instar nymphs. For each of the two instar groups, two replicate leaves were assigned to each of 12 treatments (Table 1) and a water control. Approximately 1 ml of suspension was applied to each leaf until just before run-off using a plastic trigger sprayer (500 ml). Leaves were maintained in vented plastic containers in the laboratory at 25°C ($\pm 1.0^\circ\text{C}$). Assessments were completed at 0, 3, 5 and 7 days after treatment, with nymphs scored as live (responsive to touch, turgid) or dead.

Caged greenhouse trials

Caged greenhouse trials were performed using capsicum ('Marconi Red') and tomato ('Tiny Tim') plants. Fifty nymphs ($20\times 1^{\text{st}}$ to 2^{nd} instar, $30\times 3^{\text{rd}}$ to 5^{th} instar) were transferred onto each plant ($n=5$ plants per cage) and 300 were adults released into each cage (0.5 mm gauge; 47 cm width $\times$ 47 cm depth $\times$ 74 cm height) to provide plants infested with mixed-age *B. cockerelli*. The sides of each cage were covered with polythene to restrict spray movement to within the cage. Three cages containing capsicum plants (from a total of 12 cages) were assigned to each of four treatments (Table 2). The first spray was applied to the capsicum plants on 29 March and the second on 2 April 2012. In addition, two

cages containing tomato plants were assigned to each of five treatments (a total of 10 cages) (Table 3). The first spray was applied to the tomato plants on 24 April and the second on 30 April 2012. Sprays were applied using portable pressure sprayers (Solo® 402, 2 litres). Approximately 20–30 ml was applied to each plant, including the upper and lower leaf surfaces. One hour after application the polythene covers were removed from the sides of the cages. Temperature and relative humidity (RH) were monitored. From late March to early April, the temperature was above 25°C for 41% of the first 3 days following application (average temperature of 19°C). Relative humidity averaged 72%, and was above 90% RH for 42% of the time. From late April to early May the temperature was greater than 25°C for 3% of the first 3 days after application (average temperature 15.8°C). Relative humidity averaged 86%, and was greater than 90% RH for 66% of the time.

Mortality assessments of early nymphs (1st to 2nd instars), late nymphs (3rd to 5th instars) and adults were completed 2 weeks after the first application. Each plant was cut into sections and examined under a dissecting microscope (10 $\times$ magnification). All insects were scored as live or dead.

Data analysis

Data were summarised using Microsoft® Excel 2007 and GenStat, 13th edition. Greenhouse mortality data were corrected using the standard correction applied to proportions (Abbott 1925). Corrected data were presented as percent reduction in *B. cockerelli* numbers. Logistic models were used to fit the mortality data for comparison of treatment effects to the control at the pooled data level and at different life stages. The GENMOD procedure in SAS 9.2 was used. The least squares means of treatments were compared using the Wald chi-square test and the differences were interpreted as odds ratios of mortality between treatments. The significance levels were defined at $P=0.05$.

RESULTS

Laboratory screening

With the exception of PreFeRal® 20WG, all treatments resulted in significantly greater

Figure 1 Average cumulative mortality (%) of *Bactericera cockerelli* adults from 0 to 122 h after application of six bio-insecticides, four fungal isolates, a microbial standard (eNtocide LTM), a conventional standard (Oberon[®]) or a water control under laboratory conditions.

mortality of adult *B. cockerelli* than the water control 72 h after application ($P < 0.001$) (Figure 1). Greater adult mortality was observed as a result of the application of bio-insecticide BotaniGard[®] ES than the conventional standard insecticide, Oberon and microbial standard, eNtocide LTM ($P < 0.07$). Adult mortality ranged from 91% to 100% 72 h following the application of Met52[®], Mycotrol[®]O and *Lecanicillium muscarium* isolates (Figure 1). *Isaria fumosorosea*-based bio-insecticide, PreFeRal[®] 20WG, was less successful at killing adult *B. cockerelli*, resulting in adult mortality of 44% 72 h after application.

Seven days after application, the mortality of nymphs treated with bio-insecticides or isolates was significantly greater than nymphs treated with water ($P < 0.001$) (Figure 2). Among the entomopathogen treatments, BotaniGard[®] ES and BotaniGard[®] 22WP gave the best result with 82% nymph mortality, which was greater than the conventional insecticide Oberon[®] (63%) or the microbial standard eNtocide LTM

(63%) ($P < 0.01$). Met52[®], Mycotrol[®]O and *L. muscarium* isolates resulted in nymph mortality ranging from 55% to 70%, but PreFeRal[®] 20WG, No FlyTM WP and isolate F129 resulted in lower mortality ranging from 45% to 55%. Differences in mortality between 1st to 2nd instars and 3rd to 5th instars were apparent for all treatments, with younger nymphs succumbing more quickly than the older nymphs ($P < 0.001$). No instar-related differences were detected in the water control.

Caged greenhouse trials

The mortality of mixed-age *B. cockerelli* on capsicums treated with BotaniGard[®] ES, Met52[®] EC or Oberon[®] was significantly greater than those treated with water ($P < 0.0001$). Data pooled across all life-stages showed greater insect mortality after application of BotaniGard[®] ES (overall reduction of 88%) than Met52[®] EC (66%) or Oberon[®] (49%) ($P < 0.0001$) (Table 2). A 75% reduction in adults after application of

Figure 2 Average cumulative mortality (%) of *Bactericera cockerelli* nymphs (1–2 instar and 3–5 instar) from 0–7 days after application of six bio-insecticides, four fungal isolates, a microbial standard (eNtocide L™), a conventional insecticide standard (Oberon®) or a water control under laboratory conditions.

BotaniGard® ES was recorded compared with an 18% reduction after the application of Oberon® ($P=0.04$). Met52® EC resulted in greater nymph mortality than Oberon® ($P<0.0001$). No differences were apparent between BotaniGard® ES and Met52® EC when applied to 3rd to 5th instar nymphs ($P=0.29$).

The mortality of mixed-age *B. cockerelli* on tomatoes treated with BotaniGard® ES, Met52® EC, eNtocide L™ or Oberon® was significantly greater than those treated with water ($P<0.0001$). Data pooled across all life-stages showed greater mortality after application of BotaniGard® ES (overall reduction of 83%) than Met52® EC (33%), eNtocide L™ (63%) or Oberon® (40%) ($P<0.0001$) (Table 3). Greater than 65% mortality of 1st to 2nd instar nymphs was recorded for all treatments. BotaniGard® ES and eNtocide L™ were the most effective against 3rd to 5th instar nymphs. Oberon® resulted in reduced adult mortality compared with Met52® EC ($P=0.0054$).

DISCUSSION

Entomopathogenic fungi have long been associated with Hemipteran species including psyllids, scale insects, aphids and whitefly, and there are reports of fungal epizootics naturally regulating some psyllid species (Jaques & Patterson 1962; Villacarlos & Robin 1989; Lacey et al. 2009). Evaluations of fungi against *B. cockerelli* are limited to trials performed on potatoes in Central America. These evaluations include inundative introductions of *M. anisopliae*, *B. bassiana* and *I. fumosorosea*. These introductions significantly reduced the number of psyllid eggs and nymphs compared with the controls, and decreased plant damage and increased tuber yield (Lacey et al. 2009, 2011).

The laboratory and greenhouse trials reported herein demonstrated efficacy of certain entomopathogens against *B. cockerelli*. Under laboratory conditions significant treatment effects were observed within 3 days and 5

Table 2 Mortality (%) ($\pm$ SEM) and percent reduction in numbers of mixed-age *Bactericera cockerelli* 7 days following application of two bio-insecticides, one conventional insecticide standard (Oberon®) or a water control on capsicums grown under greenhouse conditions.

Treatment	Life-stage	Mortality	% reduction
BotaniGard® ES (2.5 ml/litre)	1–2 instar	99.5 $\pm$ 0.5	97.0
	3–5 instar	97.0 $\pm$ 1.3	92.5
	Adult	76.4 $\pm$ 3.3	74.6
Met52® EC (2.5 ml/litre)	1–2 instar	84.1 $\pm$ 2.1	81.6
	3–5 instar	98.5 $\pm$ 0.8	94.0
	Adult	23.9 $\pm$ 3.4	22.1
Oberon® (0.6 ml/litre)	1–2 instar	68.3 $\pm$ 3.9	65.7
	3–5 instar	68.3 $\pm$ 3.3	63.8
	Adult	20.2 $\pm$ 4.0	18.4
Water	1–2 instar	2.1 $\pm$ 0.9	
	3–5 instar	4.8 $\pm$ 1.3	
	Adult	1.8 $\pm$ 0.5	

Table 3 Mortality (%) ($\pm$ SEM) and percent reduction in numbers of mixed-age *Bactericera cockerelli* 7 days following application of two bio-insecticides, one microbial standard (eNtocide L™), one conventional insecticide standard (Oberon®) or a water control on tomatoes grown under greenhouse conditions.

Treatment	Life-stage	Mortality	% reduction
BotaniGard® ES (2.5 ml/litre)	1–2 instar	100 $\pm$ 0.0	86.1
	3–5 instar	89.5 $\pm$ 3.3	89.1
	Adult	75.8 $\pm$ 4.4	72.5
Met52® EC (2.5 ml/litre)	1–2 instar	64.5 $\pm$ 8.6	50.6
	3–5 instar	35.9 $\pm$ 5.1	35.5
	Adult	15.8 $\pm$ 2.8	12.5
eNtocide L™ (2 ml/litre)	1–2 instar	79.1 $\pm$ 4.0	65.1
	3–5 instar	77.5 $\pm$ 4.7	77.0
	Adult	49.5 $\pm$ 5.2	46.2
Oberon® (0.6 ml/litre)	1–2 instar	86.0 $\pm$ 4.9	72.1
	3–5 instar	45.8 $\pm$ 4.8	45.3
	Adult	7.1 $\pm$ 1.6	3.9
Water	1–2 instar	13.8 $\pm$ 4.5	
	3–5 instar	0.5 $\pm$ 0.5	
	Adult	3.3 $\pm$ 0.9	

days after application to adults and nymphs, respectively. These results are encouraging, as bio-insecticides tend to be slower in their action compared to conventional chemical controls.

BotaniGard® ES and Met52® EC showed efficacy against *B. cockerelli* and outperformed the conventional insecticide, Oberon. Both bio-

insecticides were effective against adults and nymphs, resulting in significant population reduction and control. The efficacy on adult *B. cockerelli* is an important aspect of regulating population density, as effective control of adults would reduce the number of eggs laid in subsequent generations. Furthermore the

presence of infected adults would enable the dissemination of fungi within localised insect populations. Despite the lower comparative efficacy of *I. fumosorosea*-based bio-insecticides and isolates, other isolates of this species have provided good insecticidal activity against *B. cockerelli* (Lacey et al. 2009, 2011).

It is well documented that temperature and relative humidity have profound effects on germination, infection and survival of entomopathogens (Vidal & Fargues 2007). Entomopathogenic fungi can tolerate a wide range of environmental conditions, but the conditions required for infection and growth are usually more restrictive. The optimal temperature for *B. bassiana* is 23–25°C, although growth can occur between 5 and 35°C (Goettel & Inglis 1997). The optimal temperature for infection of *M. anisopliae* (strain F52) is 25–30°C. No infection or sporulation occurs below 10°C or above 35°C, and extended periods of relative humidity above 90% are required for germination and infection to occur (ECHCD 2008). The environmental conditions experienced throughout late March to early April (first greenhouse trial) were conducive for germination and infection, but conditions were sub-optimal during late April–early May (second greenhouse trial). Sub-optimal conditions could slow the germination and infection of most entomopathogens.

BotaniGard® ES demonstrated more consistent efficacy in response to the change in environmental factors. This could be partially explained by the unique colloidal delivery system of BotaniGard® ES where micelles are formed. This delivery system buffers the active ingredient against environmental factors such as temperature and ultraviolet radiation. Therefore sub-optimal environmental conditions have less effect on the performance of BotaniGard® ES.

In general commercial capsicum and tomato greenhouses are kept above 15°C but below 32°C. Controlled-environment greenhouses for growing capsicums can range in temperature from 18 to 28°C, depending on the development stage of the plant (Nederhoff 2008). Both *B. bassiana* and *M. anisopliae* are suited to such temperatures, but the germination and

growth of these fungi will be dependent on the amount of time conditions are optimal, that is 23–25°C and 25–30°C, respectively. As such, timing spray applications to correspond to periods in the growing season when higher greenhouse temperatures are maintained is likely to maximise the effectiveness of the active ingredient. In greenhouse environments where environmental conditions are not controlled, the use of these entomopathogens would be best suited to late spring, early and late summer, and early autumn. Further work is recommended to test candidate entomopathogens under a broader range of environmental conditions, and on targeted crops throughout a growing season.

Entomopathogens could be utilised as part of an integrated system that includes multiple tools to manage *B. cockerelli*. It is recommended that the compatibility of entomopathogens with current crop management practises is assessed. In addition, the effects of candidate entomopathogens on non-target organisms, such as predators and parasitoids of *B. cockerelli*, should be examined so that the scope of inclusion into IPM programmes can be determined.

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