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Comparative Efficacy of Insecticides on *Bactrocera tryoni* and *Zeugodacus cucumis* (Diptera: Tephritidae) in Laboratory and Semifield Trials in Fruiting Vegetables

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Abstract

In-field management of *Bactrocera tryoni* (Froggatt) and *Zeugodacus cucumis* (French) (Diptera: Tephritidae) in fruiting vegetable crops has relied almost exclusively on organophosphate cover sprays. Laboratory and semifield trials were performed to compare a number of alternative insecticides for efficacy against these species. A novel semifield method was used whereby the insecticides were applied to crops as cover sprays under field conditions, and treated plants bearing fruit were transferred to large cages and exposed to fruit flies. Efficacy was assessed in terms of numbers of pupae developing from treated fruit. A laboratory cage method was also used to assess effects on adult mortality and comparative effects of 1- and 3-d-aged residues. The neonicotinoids clothianidin and thiacloprid were very effective against *B. tryoni* and *Z. cucumis*. Clothianidin was the only insecticide other than dimethoate to affect adult mortality. The synthetic pyrethroid alpha-cypermethrin was also very effective, particularly in semifield trials, although higher incidence of aphid and whitefly infestation was observed in this treatment compared to others. Cyantraniliprole was effective against *B. tryoni*, but less effective against *Z. cucumis*. Imidacloprid, bifenthrin, spinetoram, and abamectin were all relatively less effective, although all demonstrated a suppressive effect.

Key words: *Bactrocera tryoni*, *Zeugodacus cucumis*, Tephritidae, insecticide

Tephritid fruit flies are serious pests of horticultural crops in Australia. *Bactrocera tryoni* (Froggatt) attacks a wide range of fruit and vegetable crops in eastern Australia (Drew et al. 1982, Hancock et al. 2000), causing crop loss and threatening market access. There have been increasingly frequent incursions of this pest into the Fruit Fly Exclusion Zone in the southern states of Australia (Dominiak et al. 2015). *Zeugodacus cucumis* (French) causes damage to cucurbit crops and tomatoes in Queensland, northern New South Wales, the Northern Territory, and Torres Strait Islands (Drew et al. 1982, Hancock et al. 2000).

In Australia, in-field management of fruit flies in fruiting vegetable crops has relied almost exclusively on organophosphate cover sprays. However, recent restrictions in the use of dimethoate (Australian Pesticides and Veterinary Medicines Authority [APVMA] 2011, APVMA 2017) and fenthion (APVMA 2015) have greatly limited the available control options. While the use of dimethoate for control of fruit fly in certain fruiting vegetable crops has been retained (e.g. capsicum, melons, zucchini), for others it has been suspended from use, or the withholding period extended rendering it largely ineffective. Alternative approaches for managing

fruit fly in host crops have been explored, such as the use of toxic baits and male annihilation technique (Clarke et al. 2011, Vargas et al. 2015). However, development of these management methods has primarily been focused on tree crops, or exotic tephritid species in vegetable crops. For instance, perimeter baiting, in which a protein bait plus insecticide is applied to a nonhost plant adjacent to the crop, is a commonly used technique for management of melon fly, *Zeugodacus cucurbitae* (Coquillett) in Hawaii (McQuate 2011). It exploits the observation that *Z. cucurbitae* females roost and forage for protein in certain favoured nonhost plants (Nishida and Bess 1957, Prokopy et al. 2003). The efficacy of toxic baits for control of *B. tryoni* and *Z. cucumis* in fruiting vegetable crops is currently unproven. Moreover, vegetable crops are often subject to intensive insecticide regimes for management of other pest species, and it is important to understand the potential impact of these insecticides on fruit flies.

A number of studies have examined the toxicity of pesticides to tephritid fruit flies in the laboratory using a range of methods such as direct application to adult flies (Wang et al. 2013), exposure to residues on artificial substrates (Mosleh et al. 2011), exposure to residues on fruit applied and aged in the laboratory (Maklakov et al.

2001, Yee 2008, Yee and Alston 2012) or the field (Yee et al. 2007, Rahman and Broughton 2016), or application of insecticide sprays to infested fruit (Wise et al. 2009). Small plot field trials have been employed to assess efficacy of insecticides for management of cucurbit-specific fruit flies such as *Z. cucurbitae* in cucurbits (Oke 2008, Khursheed and Raj 2012, Oke and Sinon 2013), and field trials conducted in tree crops for management of *Rhagoletis* spp. (Reissig 2003, Yee and Alston 2006). However, the literature on insecticide efficacy for *B. tryoni* and *Z. cucumis* is scarce. Reynolds et al. (2014) compared insecticides for efficacy against *B. tryoni* in the laboratory and in semifield trials in stonefruit, in which fruit flies were introduced into mesh sleeves enclosing treated fruit on peach trees. Subramaniam (2013) evaluated insecticide cover sprays for management of *B. tryoni* in eggplant; however, efficacy formed part of a systems approach and was not compared with a control. Kay (2004) assessed efficacy of insecticides against *B. tryoni* in a small plot field trial in capsicum, but failed to find significant differences amongst treatments due to high variability of infestation across replicate plots. Atuahene and Hooper (1971) investigated the susceptibility of *B. tryoni* and *Z. cucumis* to DDT, with no recent literature relating to the efficacy of insecticides against the latter species.

Laboratory trials can provide useful information on relative efficacy of insecticides but are limited by their artificial nature (Macfadyen et al. 2014). When insecticides are applied directly or insects are confined in close contact with residues, repellent effects cannot be measured. Laboratory trials often provide no information on the insecticide's performance in the field, such as resistance to weathering, movement within the plant, or the effect of nonuniform application.

Small plot field trials are the conventional method used for many horticultural pests to assess comparative efficacy of insecticides under field conditions. However, there is evidence that because *B. tryoni* invades low growing crops from the field margins, this results in a typically uneven infestation in such crops. Balagawi et al. (2014) recorded higher numbers of male *B. tryoni* in traps placed in vegetation bordering a strawberry crop than traps within the crop, and from the same study Gu (2010) found rates of infestation were correspondingly higher in fruit near the border than within the crop. This makes it difficult to compare treatments within a trial area due to large variability between treatments and replicates, as observed in the small plot field trial performed by Kay (2004) to compare cover sprays for control of *B. tryoni* in capsicum. Kay (2004) speculated that the higher infestation in certain plots was due to proximity to bordering trees and a citrus block. Steiner and Hinman (1952) encountered similar difficulties in small plot tests in tree crops, noting that populations of oriental fruit fly, *Bactrocera dorsalis* (Hendel), in control plots were depressed by nearby treated plots, and that greater movement of fruit flies into the windward side of the trial area resulted in larger infestations in these plots.

Trials were carried out to compare insecticides as cover sprays for efficacy against *B. tryoni* and *Z. cucumis* under semifield conditions. The aims were twofold: first, to obtain information on the comparative efficacy of a series of insecticides, and second, to assess a novel semifield methodology. The insecticides were applied to crops in the ground, and therefore subject to actual use conditions. An artificial infestation method was then used to ensure all fruits from all treatments were subjected to a similar fruit fly pressure.

Materials and Methods

Small Plot Trial Layout

Semifield trials were conducted over two years to evaluate the efficacy of a range of insecticides against *B. tryoni* in capsicum,

Capsicum annuum (commercial variety Warlock), and *Z. cucumis* in zucchini, *Cucurbita pepo* (commercial variety Congo F1). Trials were conducted from January to April 2014 (season one) and January to April 2015 (season two) at Gatton Research Facility (Lockyer Valley, QLD, Australia; 27° 32′ S, 152° 19′ E, elevation 98 m). Crops were planted in January into plastic mulch and irrigated using trickle tape. The trial layout was a randomized complete block design with seven treatments, replicated four times. Treatments were arranged lengthwise, with 2 m no-crop buffers between plots in the lengthwise direction, and 2 m no-crop buffers between replicate blocks. Between-plant spacings were 0.5 m for capsicum and 0.75 m for zucchinis. Each plot was on a 1.5-m-wide bed and plot length varied according to season and crop: season one 9 m (14 capsicum plants) or 9.5 m (11 zucchini plants); season two 10 m (17 capsicum plants) or 10.5 m (12 zucchini plants). Rows of forage sorghum on either side of the trial block provided protection from wind and potential spray drift. Fungicides were applied for disease control: Polyram DF (BASF Australia Ltd, Southbank, VIC), Kocide Blue Xtra (Du Pont (Australia) Pty Ltd, Macquarie Park, NSW), and Dithane Rainshield Neo Tec Fungicide (Dow AgroSciences (Australia) Ltd, Frenchs Forest, NSW). In season one, the insecticides Transform (Dow AgroSciences (Australia) Ltd; active ingredient sulfoxaflor) and Talstar 250 EC (FMC Australasia Pty Ltd, Murarrie, QLD; active ingredient bifenthrin) were applied for control of whitefly and aphids. Application of these insecticides to young plants was made prior to the application of the trial treatments, and was not expected to affect the fruit flies.

Treatments

In each trial, five insecticide treatments were assessed and compared with dimethoate (the industry standard) applied at the rate of 75 ml/ 100 liter and with an untreated control. In season one (2014) the five insecticide treatments were Sumitomo Samurai Systemic Insecticide (Sumitomo Chemical Australia Pty Ltd, Epping, NSW; active ingredient clothianidin) applied at 40 g/100 liter; Confidor 200 SC (Bayer CropScience Pty Ltd, Hawthorn East, VIC; active ingredient imidacloprid) at 25 ml/100 liter; Talstar 250 EC Insecticide/ Miticide (FMC Australasia Pty Ltd, Murarrie, QLD; active ingredient bifenthrin) at 24 ml/100 liter; Fastac Duo Insecticide (BASF Australia Ltd, Baulkham Hills, NSW; active ingredient alpha-cypermethrin) at 55 ml/100 liter; and DuPont Benevia Insecticide (Du Pont (Australia) Pty Ltd, Macquarie Park, NSW; active ingredient cyantraniliprole) at 100 ml/100 liter. In season two (2015) the five insecticide treatments were Sumitomo Samurai Systemic Insecticide (Sumitomo Chemical Australia Pty Ltd; active ingredient clothianidin) applied at 40 g/100 liter and 30 g/100 liter; Calypso 480 SC Insecticide (Bayer CropScience Pty Ltd; active ingredient thiacloprid) at 37.5 ml/100 liter; Vertimec Miticide/Insecticide (Syngenta Australia Pty Ltd, Macquarie Park, NSW; active ingredient abamectin) at 60 ml/100 liter; and Success Neo Insecticide (Dow AgroSciences (Australia) Ltd; active ingredient spinetoram) at 40 ml/ 100 liter. Agral spray adjuvant (Syngenta Australia Pty Ltd) was added to all treatments at the rate of 10 ml/100 liter, with the exception of the Samurai treatments, where Maxx Organosilicone Surfactant (Sumitomo Chemical Australia Pty Ltd) was used at the rate of 50 ml/100 liter.

Insecticides were applied using a gas pressurised sprayer, with a 1.2-m four-nozzle boom. The spray was applied at an operating boom pressure of ~230 kPa and a volume of 700–760 liter/ha (dependent on crop and plot size), achieved through two passes of each plot in order to ensure good coverage. Treatments were applied weekly from first fruit set onwards, until all trials had been

Table 1. Dates of spray application and trials, season one (2014)

Date	Capsicum	Zucchini
18 Feb. 2014		Spray application
25 Feb. 2014	Spray application	Spray application
26 Feb. 2014		Laboratory cage trial one 1 DAT
4 Mar. 2014	Spray application	Spray application
5 April 2014		Field cage trial
		Laboratory cage trial two 1 DAT
11 Mar. 2014	Spray application	
12 Mar. 2014	Laboratory cage trial one 1 DAT	
18 Mar. 2014	Spray application	Spray application
21 Mar. 2014		Laboratory cage trial 3 DAT
25 Mar. 2014	Spray application	
1 April 2014	Spray application	
2 April 2014	Field cage trial	
	Laboratory cage trial two 1 DAT	
4 April 2014	Laboratory cage trial 3 DAT	

DAT—day after treatment.

Table 2. Dates of spray application and trials, season two (2015)

Date	Capsicum	Zucchini
24 Feb. 2015		Spray application
3 Mar. 2015	Spray application	Spray application
4 Mar. 2015		Field cage trial
10 Mar. 2015	Spray application	Spray application
11 Mar. 2015		Laboratory cage trial 1 DAT
13 Mar. 2015		Laboratory cage trial 3 DAT
17 Mar. 2015	Spray application	
24 Mar. 2015	Spray application	
25 Mar. 2015	Laboratory cage trial 1 DAT	
27 Mar. 2015	Laboratory cage trial 3 DAT	
31 Mar. 2015	Spray application	
1 April 2015	Field cage trial	

DAT—day after treatment.

completed, hence the number of treatment applications varied dependent on season and crop (Tables 1 and 2). In season one (2014) four applications were made to the zucchinis and six to the capsicums. In season two (2015) three applications were made to the zucchinis and five to the capsicums. However, due to the rapid rate of development of the zucchini fruit, it was necessary to remove large fruit at regular intervals to ensure continuous production; hence zucchini fruit used in trials had received a maximum of three applications.

Treated plants were exposed to fruit flies using two methods. First, fruit flies were exposed to intact fruit on plants removed from the field and placed into large field cages. Second, fruit flies were exposed to fruit in small laboratory cages, in order to assess adult mortality and efficacy of aged residues.

Fruit Flies

All fruit flies were obtained from colonies maintained by the Market Access research group at the Department of Agriculture and Fisheries (DAF) (Brisbane, QLD, Australia). *Bactrocera tryoni* colonies were established from collections of host fruit (*Endiandra* sp, *Barringtonia calyptrata*, and *Terminalia catappa*) in the Cairns region in January 2012 and reared according to the method of Heather and Corcoran (1985). Adults used in season one (2014) were 10–16 d post emergence and 16–18 d post emergence in season two (2015). *Zeugodacus cucumis* colonies were established from collections of zucchini in the Ayr and Cairns regions in September and October 2010. They were reared according to the method of Heather and Corcoran (1985) using the pumpkin diet described by Swaine et al. (1978). *Zeugodacus cucumis* were 13–15 d post emergence. Prior to use in the tests all fruit flies were provided with sugar, water, and protein (autolysed yeast) and allowed to mate, hence were ready to oviposit.

Field Cage Infestation

Trials were performed in four metal frame, netted cages (3 m by 3 m base, 2.5 m high), each cage representing one replicate. One day following treatment application, treated plants bearing fruit were selected at random from each replicate of each treatment, dug up, placed in large pots, watered and transferred to the field cages. Fruit flies were therefore exposed to residues on the foliage as well as on the fruit. Three zucchini plants or four capsicum plants were used per treatment replicate, with plants from each treatment grouped together, meaning that all seven treatments were present in each replicate cage. Approximately 600 mixed sex adult fruit flies, determined by pupal weight, were released into each cage. In the first season trial, all fruit flies were left for ∼4 h to oviposit. In the second season trial, based on results from the first season, the exposure time was decreased to 3 h for *Z. cucumis*, and due to adverse weather conditions (cool with light rain), *B. tryoni* were left to oviposit overnight (∼ 20–21 h). Fruit were then harvested and transported to the laboratory for subsequent assessment of infestation. The size and number of fruit per treatment replicate varied between replicates and treatments, due to variable fruit production of the plants. Therefore, fruit in each treatment replicate were weighed (not counted), and the weight range for each of the field cage trials presented in the results.

In the laboratory, the harvested fruit from each treatment replicate were placed on shallow plastic containers covered with net, within ventilated containers. A layer of vermiculite on the base of the container was provided as a substrate for pupation. The fruit were held in a controlled environment room (26 °C, 70% relative humidity) for ∼2 wk to allow any eggs laid to develop to the pupal stage. The vermiculite was then sieved and the number of pupae counted. Additional fruit were harvested from untreated control plants in the trial block to assess the level of infestation in the field, prior to artificial infestation in the field cages. However, no pupae developed from any of these fruit and therefore these results were not included in the analyses.

Laboratory Cage Infestation

Fruit were removed from plants in the trial plots either 1 or 3 d after treatment application (1 and 3 DAT). Two 1 DAT and one 3 DAT laboratory cage trials were conducted for the first season; one 1 DAT and one 3 DAT trial were conducted for the second season. Bifenthrin (Talstar 250 EC Insecticide/Miticide) was omitted from the 3 DAT first season cucumber fly trial due to poor efficacy at 1 DAT. Three fruit from each treatment replicate were exposed to fruit flies in small laboratory cages (wire frame, netted cages, 21 cm wide, 21 cm high, 33 cm deep). Each cage contained 10 male and
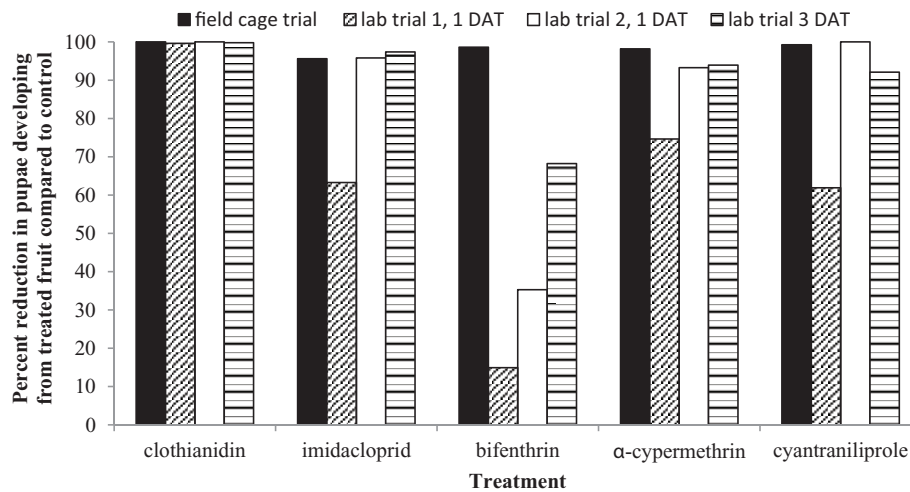
Fig. 1. Effect of treatments on reduction in numbers of *B. tryoni* pupae developing from treated capsicum compared with control fruit in season one trials.

10 female fruit flies, provided with sugar and water. Exposure times varied dependent on the fruit type, fruit fly species, and time of day that fruit were harvested. In season one, zucchinis in the 1 DAT trials were picked and placed into cages in the afternoon, and hence infested overnight (13 and 18 h infestation periods). This was reduced to 3 h for the 3 DAT trial. Capsicums were infested for 3 h (1 DAT trial one), overnight (~ 15 h, 1 DAT trial two) or 2.5 h (3 DAT trial). In season two, exposure was reduced due to overinfestation in season one, and all laboratory cage trial fruit was infested for between 2 and 3 h. Following exposure to the fruit flies, fruit were held in ventilated containers under controlled conditions as described previously, and the number of pupae counted. Mortality of adult fruit flies was assessed 1 d (~ 24 h) after placement of fruit into the cages.

Statistical Analyses

Numbers of pupae developing from the fruit were analyzed using a generalized linear mixed model (GLMM) assuming a Poisson distribution and a log link function. Due to variability in number and size of fruit sampled from each replicate of each treatment, fruit weight was initially used as a covariate in the field cage analyses. However, with the exception of season one (2014) trial with *B. tryoni* in capsicum, the effect of fruit weight was found to be not significant and therefore removed. Where a significant effect of treatment was found, pairwise comparisons between the transformed means were made using the 95% least significant difference (LSD). In some instances, no pupae developed in a treatment, or pupae developed in only one replicate of a treatment, resulting in an overinflated standard error. Means with an overinflated standard error were not included in the pairwise comparisons; however, when no pupae developed we can intuitively say that there was a significant effect of treatment. Control corrected means (Abbott 1925) were also calculated and presented to facilitate comparison of treatment efficacy.

The proportion of dead fruit flies at one day after exposure to the treated fruit was analyzed using a GLMM assuming a binomial distribution and logit link function. Where a significant treatment effect was found, pairwise comparisons between the transformed means were made using the 95% LSD. Mortality was expressed as the mean number of dead fruit flies per cage for presentation in results.

Statistical analyses were performed in GenStat for Windows 16th Edition (VSN International 2013).

Results

Season One (2014) *B. tryoni* in Capsicum

Results of a field cage trial found a significant effect of treatment on development of pupae from treated fruit ($P = 0.002$; Table 3). All insecticide treatments resulted in significantly fewer pupae than the control. Weight of fruit harvested from each replicate of each treatment varied between 908 g and 1984 g. The first 1 DAT laboratory cage trial and the 3 DAT laboratory cage trial both found a significant effect of treatment ($P < 0.001$), with significantly fewer pupae in all treatments compared to the control at 3 DAT and significantly fewer pupae in all treatments except bifenthrin compared to the control at 1 DAT. There was no significant effect of treatment in the second laboratory cage trial ($P > 0.05$), due in part to large variability in the control treatment (pupal counts ranged from 0 to 108). The effect of treatment on development of pupae from treated fruit was also expressed as control corrected means (Fig. 1). Clothianidin consistently resulted in circa 100% reduction in development of pupae in all four trials; imidacloprid, alpha-cypermethrin, and cyantraniliprole resulted in greater than 90% reduction in development of pupae in three of the four trials; and bifenthrin was effective only in the field cage trial.

Mortality of the adult fruit flies in the laboratory cage trials was assessed at ~ 24 h (Table 4). There was a significant effect of treatment on fruit flies exposed to 1-d residues (1 DAT), in both trials ($P \leq 0.011$), with significantly higher mortality in the dimethoate treatment compared to the control, and in the clothianidin treatment compared to the control in the first trial only. Three-day-old residues had no significant effect on mortality of the adult fruit flies ($P > 0.05$).

Season One (2014) *Z. cucumis* in Zucchini

Results of a field cage trial found a significant effect of treatment on the number of pupae developing from the fruit ($P = 0.011$; Table 5). Clothianidin, alpha-cypermethrin, cyantraniliprole, and dimethoate resulted in significantly fewer pupae compared to the untreated control. Weight of fruit harvested from each replicate of each treatment varied between 892 g and 1200 g. Two laboratory cage trials conducted at 1 DAT and a laboratory cage trial at 3 DAT all found a significant effect of treatment ($P \leq 0.004$). Clothianidin and dimethoate consistently resulted in the fewest pupae, and bifenthrin

Table 3. Mean number of pupae developing from capsicum exposed to *B. tryoni* in season one trials; back-transformed means (BTM) and predicted means on the log scale $\pm$ 1 standard error (PM)

Treatment		Field cage trial, 1 DAT		Lab cage trial 1, 1 DAT		Lab cage trial 2, 1 DAT		Lab cage trial, 3 DAT	
		BTM	PM	BTM	PM	BTM	PM	BTM	PM
Clothianidin		0.0	$-16.82 \pm \delta$	0.5a	-0.69 ± 1.90	0.0	$-16.48 \pm \delta$	0.3	$-1.39 \pm \delta$
Imidacloprid		3.1a	1.12 ± 1.13	46.7b	3.84 ± 0.20	0.7	-0.32 ± 1.66	3.3a	1.18 ± 1.28
Bifenthrin		1.0a	-0.02 ± 1.62	108.2c	4.68 ± 0.14	11.1	2.41 ± 0.80	39.3a	3.67 ± 0.37
α-Cypermethrin		1.3a	0.24 ± 1.65	32.2b	3.47 ± 0.24	1.2	0.15 ± 1.38	7.5a	2.02 ± 0.85
Cyantraniliprole		0.5a	-0.63 ± 2.22	48.5b	3.88 ± 0.20	0.0	$-16.48 \pm \delta$	9.8a	2.28 ± 0.74
Dimethoate		1.0a	-0.04 ± 1.63	24.0ab	3.18 ± 0.28	1.3	0.26 ± 1.32	7.3a	1.98 ± 0.86
Untreated control		70.2b	4.25 ± 0.48	127.2c	4.85 ± 0.13	17.2	2.85 ± 0.77	123.5b	4.82 ± 0.21
GLMM	F	5.41		12.59		2.10		6.31	
	df	6, 17.7		6, 20		6, 18.2		6, 21	
	P	0.002		<0.001		0.104		<0.001	

Means with a letter in common are not significantly different (LSD test; $P > 0.05$).
δ indicates an overinflated standard error.

Table 4. Mean mortality per cage of 20 adult *B. tryoni* exposed to insecticide residues on capsicum in season one trials; back transformed means (BTM) and predicted means on the logit scale $\pm$ 1 standard error (PM)

Treatment		Lab cage trial 1, 1 DAT		Lab cage trial 2, 1 DAT		Lab cage trial, 3 DAT	
		BTM	PM	BTM	PM	BTM	PM
Clothianidin		5.5d	-0.97 ± 0.24	3.5bc	-1.55 ± 0.38	1.5	-2.51 ± 0.48
Imidacloprid		0.5a	-3.66 ± 0.68	1.5ab	-2.51 ± 0.54	1.5	-2.51 ± 0.48
Bifenthrin		2.5bc	-1.95 ± 0.32	0.5a	-3.66 ± 0.91	0.5	-3.66 ± 0.80
α-Cypermethrin		1.3ab	-2.71 ± 0.44	0.5a	-3.66 ± 0.91	1.5	-2.51 ± 0.48
Cyantraniliprole		1.8ab	-2.35 ± 0.38	1.5ab	-2.51 ± 0.54	1.5	-2.51 ± 0.48
Dimethoate		4.8cd	-1.17 ± 0.25	5.5c	-0.97 ± 0.32	3.3	-1.64 ± 0.34
Untreated control		1.8ab	-2.35 ± 0.38	0.8ab	-3.25 ± 0.75	2.0	-2.20 ± 0.42
GLMM	F	5.62		3.76		1.25	
	df	6, 21		6, 21		6, 21	
	P	0.001		0.011		0.320	

Means with a letter in common are not significantly different (LSD test; $P > 0.05$).

was ineffective. Only clothianidin resulted in over 90% reduction in development of pupae in all four trials (Fig. 2).

There was a significant effect of treatment on adult fruit fly mortality in all three laboratory cage trials ($P < 0.001$; Table 6). Mortality was highest in the dimethoate and clothianidin treatments at 1 DAT, and in the dimethoate treatment at 3 DAT.

Although not quantified, it was observed that occurrence of aphids (*Myzus persicae* and *Aphis gossypii*) and sweetpotato whitefly (*Bemisia tabaci*) was much higher in plots treated with alpha-cypermethrin, bifenthrin, and dimethoate compared with other treatments.

Season Two (2015) *B. tryoni* in Capsicum

Results of a field cage trial found no significant effect of treatment on the development of pupae from treated fruit ($P > 0.05$; Table 7). Weight of fruit harvested from each replicate of each treatment varied between 421 g and 2836 g. Laboratory cage trials at 1 DAT and 3 DAT both found a significant effect of treatment ($P \leq 0.018$), with no pupae developing in the clothianidin treatments, and significantly fewer pupae in the thiacloprid and dimethoate treatments compared to the control. In addition, pupal counts were significantly lower than the control for abamectin in the 1 DAT trial and spinetoram in the 3 DAT trial. Only the higher rate of clothianidin (40 g/100 liter)

and thiacloprid resulted in greater than 90% reduction in development of pupae in all three trials (Fig. 3).

There was a significant effect of treatment on adult fruit fly mortality in both the 1 DAT and 3 DAT laboratory cage trials ($P \leq 0.007$; Table 8). Significantly higher mortality compared to other treatments was observed for dimethoate and both rates of clothianidin, in both trials.

Season Two (2015) *Z. cucumis* in Zucchini

Results of a field cage trial found no significant effect of treatment on the number of pupae developing from the zucchinis ($P > 0.05$; Table 9). Weight of fruit harvested from each replicate of each treatment varied between 1172 g and 2696 g. Laboratory cage trials at 1 DAT found a significant effect of treatment on number of pupae developing from the fruit ($P < 0.001$), with significantly fewer pupae in all treatments compared to the untreated control. Clothianidin and dimethoate resulted in the fewest pupae, and abamectin was the least effective treatment. There was no significant effect of treatment for 3 DAT residues ($P > 0.05$). Only clothianidin resulted in close to 100% reduction in development of pupae in all three trials (Fig. 4).

There was a significant effect of treatment on adult fruit fly mortality in both the 1 DAT and 3 DAT laboratory cage trials ($P \leq 0.008$; Table 10). The highest mortality was observed in the clothianidin and dimethoate treatments.
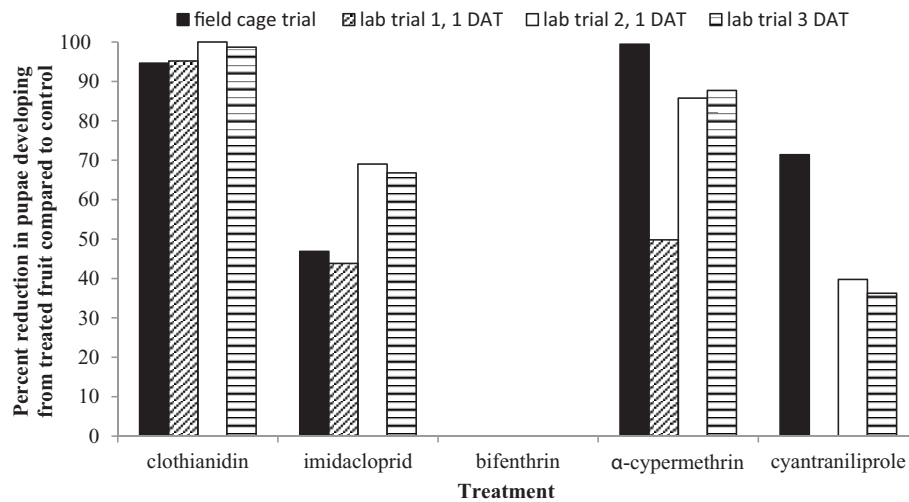
Fig. 2. Effect of treatments on reduction in numbers of *Z. cucumis* pupae developing from treated zucchini compared with control fruit in season one trials.

Table 5. Mean number of pupae developing from zucchini exposed to *Z. cucumis* in season one trials; back-transformed means (BTM) and predicted means on the log scale $\pm$ 1 standard error (PM)

Treatment	Field cage trial, 1 DAT		Lab cage trial 1, 1 DAT		Lab cage trial 2, 1 DAT		Lab cage trial, 3 DAT	
	BTM	PM	BTM	PM	BTM	PM	BTM	PM
Clothianidin	23.4a	3.16 ± 1.21	20.5a	3.02 ± 0.92	0.0	−8.69 ± δ	11.0a	2.40 ± 0.97
Imidacloprid	231.5ab	5.45 ± 0.49	238.6b	5.48 ± 0.29	177.0c	5.18 ± 0.19	281.2c	5.64 ± 0.19
Bifenthrin	601.7c	6.40 ± 0.39	432.0b	6.07 ± 0.23	590.8e	6.38 ± 0.10		[a]
α-Cypermethrin	2.5	0.91 ± δ	213.1b	5.36 ± 0.31	81.5b	4.40 ± 0.28	104.0b	4.64 ± 0.32
Cyantraniliprole	124.7a	4.83 ± 0.60	432.0b	6.07 ± 0.23	344.5d	5.84 ± 0.14	539.6d	6.29 ± 0.14
Dimethoate	18.7a	2.93 ± 1.35	50.4a	3.92 ± 0.59	17.8a	2.88 ± 0.60	17.2a	2.85 ± 0.77
Untreated control	435.8bc	6.08 ± 0.41	424.6b	6.05 ± 0.23	571.8e	6.35 ± 0.11	846.6e	6.74 ± 0.11
GLMM *F*	3.92		4.83		17.22		18.28	
df	6, 17.9		6, 17.8		6, 21		5, 15	
P	0.011		0.004		< 0.001		< 0.001	

Means with a letter in common are not significantly different (LSD test; *P* > 0.05).

δ indicates an overinflated standard error.

[a] Bifenthrin was omitted from the 3 DAT trial due to poor results in the 1 DAT trials.

Table 6. Mean mortality per cage of 20 adult *Z. cucumis* exposed to insecticide residues on zucchini in season one trials; back transformed means (BTM) and predicted means on the logit scale $\pm$ 1 standard error (PM)

Treatment	Lab cage trial 1, 1 DAT		Lab cage trial 2, 1 DAT		Lab cage trial, 3 DAT	
	BTM	PM	BTM	PM	BTM	PM
Clothianidin	9.0b	−0.20 ± 0.48	5.7b	−0.91 ± 0.27	1.7a	−2.35 ± 0.44
Imidacloprid	1.0a	−2.93 ± 0.65	0.3a	−4.38 ± 0.96	0.3a	−4.38 ± 1.08
Bifenthrin	0.2a	−4.62 ± 1.15	0.5a	−3.67 ± 0.69		[a]
α-Cypermethrin	0.6a	−3.48 ± 0.76	1.7a	−2.35 ± 0.40	0.3a	−4.38 ± 1.08
Cyantraniliprole	0.6a	−3.48 ± 0.76	0.0	−16.75 ± δ	0.0	−16.75 ± δ
Dimethoate	3.4b	−1.60 ± 0.52	7.5b	−0.51 ± 0.25	11.0b	0.20 ± 0.27
Untreated control	0.0	−17.71 ± δ	0.3a	−4.38 ± 0.96	0.0	−16.75 ± δ
GLMM *F*	9.95		9.36		10.64	
df	6, 18.2		6, 17.9		6, 15	
P	< 0.001		< 0.001		< 0.001	

Means with a letter in common are not significantly different (LSD test; *P* > 0.05).

δ indicates an overinflated standard error.

[a] Bifenthrin was omitted from the 3 DAT trial due to poor results in the 1 DAT trials.

Table 7. Mean number of pupae developing from capsicum exposed to *B. tryoni* in season two trials; back-transformed means (BTM) and predicted means on the log scale ± 1 standard error (PM)

Treatment		Field cage trial, 1 DAT		Lab cage trial, 1 DAT		Lab cage trial, 3 DAT	
		BTM	PM	BTM	PM	BTM	PM
Clothianidin 40 g/100 liter		0.0	−13.68 ± δ	0.0	−11.69 ± δ	0.0	−18.71 ± δ
Clothianidin 30 g/100 liter		15.3	2.73 ± 0.74	0.0	−11.69 ± δ	0.0	−18.71 ± δ
Thiacloprid		1.4	0.36 ± 2.29	13.2a	2.58 ± 0.64	3.4a	1.22 ± 1.10
Abamectin		35.0	3.56 ± 0.52	47.7ab	3.87 ± 0.34	41.8bc	3.73 ± 0.37
Spinetoram		42.2	3.74 ± 0.49	103.7bc	4.64 ± 0.24	11.1ab	2.41 ± 0.63
Dimethoate		6.5	1.87 ± 1.10	20.4a	3.02 ± 0.51	2.2a	0.78 ± 1.37
Untreated control		35.5	3.57 ± 0.52	182.5c	5.21 ± 0.19	75.2c	4.32 ± 0.31
GLMM	*F*	0.94		6.32		3.53	
	df	6, 18.2		6, 18.1		6, 17.7	
	P	0.492		0.001		0.018	

Means with a letter in common are not significantly different (LSD test; *P* > 0.05).

δ indicates an overinflated standard error.
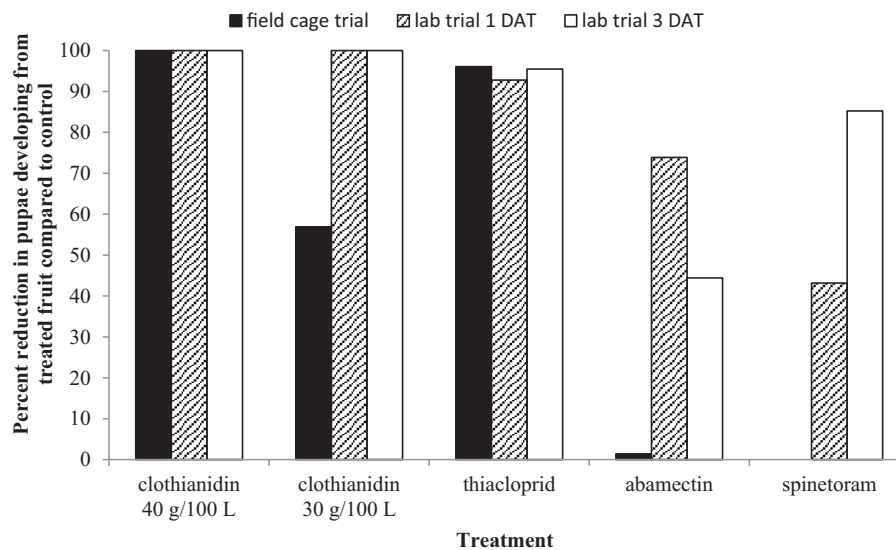
Fig. 3. Effect of treatments on reduction in numbers of *B. tryoni* pupae developing from treated capsicum compared with control fruit in season two trials.

Weather Data

Rainfall for the 2014 trial period was minimal except for a period following the penultimate spray application made to capsicum (25th March). Rainfall during the 2015 trial period was minimal in the 48-h periods following spray applications with the exception of those applied to capsicum on 17th and 24th March.

Discussion

All the insecticides demonstrated some level of efficacy compared to the untreated control. Efficacy was generally lower for *Z. cucumis* than *B. tryoni*. This may in part have been because the zucchinis developed more quickly than capsicums, were picked more frequently and hence received fewer sprays. However, it is likely that the comparative vigour of the two fruit fly species also had an effect; the number of *Z. cucumis* pupae developing in all treatments, including the control, was generally much higher than for *B. tryoni*. Unpublished data collected for the two fruit fly colonies used in the trials found that egg hatch and total survival to adult in *Z. cucumis* (94–98% and 78–87%, respectively) was higher than *B. tryoni* (76–87% and 65–79%).

Clothianidin (Sumitomo Samurai Systemic Insecticide) was the most effective of the eight insecticides assessed, with both 1- and 3-d-aged residues consistently demonstrating efficacy comparable to dimethoate in terms of numbers of pupae developing from treated fruit. Clothianidin was the only insecticide other than dimethoate to significantly affect mortality of adult fruit flies. Two other neonicotinoid insecticides, thiacloprid (Calypso 480 SC Insecticide) and imidacloprid (Confidor 200 SC), also demonstrated efficacy comparable to dimethoate against *B. tryoni* in terms of reduction in pupal development. However, they were generally much less effective against *Z. cucumis*, and had no effect on adult mortality in either species. Reynolds et al. (2014) found clothianidin to be moderately effective in semifield trials with *B. tryoni* in stonefruit. Neonicotinoids have also proven to be effective against a number of other tephritid species. Rahman and Broughton (2016) found clothianidin and thiacloprid significantly reduced infestation of stonefruit by Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in laboratory trials of 24-h field aged residues. Efficacy was reduced when residues were aged for 7 d. Yee and Alston (2006) found thiacloprid and imidacloprid significantly suppressed infestation by western cherry fruit fly *Rhagoletis indifferens* Curran in field trials

in cherry orchards. Reissig (2003) found imidacloprid to be more effective than thiacloprid in laboratory trials with apple maggot, *Rhagoletis pomonella* (Walsh), but thiacloprid was more effective in orchard trials. The author suggested efficacy was primarily a result of reduced oviposition; neither insecticide resulted in high adult mortality. Hu and Prokopy (1998) demonstrated that whilst imidacloprid was effective against *R. pomonella* via contact (residues applied to glass) and ingestion, it was ineffective when applied to foliage. Similarly, Hu et al. (1998) found imidacloprid to be ineffective for control of *R. pomonella* in apple orchards, suggesting that this was due to rapid absorption by the foliage and degradation by sunlight.

Alpha-cypermethrin (Nufarm Fastac Duo Insecticide) was effective against *B. tryoni* in capsicums in laboratory and field cage trials, with efficacy of 1-d and 3-d residues comparable to dimethoate. Alpha-cypermethrin was also effective against *Z. cucumis* in zucchini when tested in the field cage trial, but was less effective in

Table 8. Mean mortality per cage of 20 adult *B. tryoni* exposed to insecticide residues on capsicum in season two trials; back transformed means (BTM) and predicted means on the logit scale ± 1 standard error (PM)

Treatment	Lab cage trial, 1 DAT		Lab cage trial, 3 DAT	
	BTM	PM	BTM	PM
Clothianidin 40 g/100 liter	6.3b	-0.79 ± 0.25	3.2b	-1.65 ± 0.29
Clothianidin 30 g/100 liter	8.0b	-0.41 ± 0.23	2.5b	-1.95 ± 0.31
Thiacloprid	0.3a	-4.37 ± 1.03	0.3a	-4.38 ± 0.86
Abamectin	0.0	$-14.75 \pm \delta$	0.0	$-16.75 \pm \delta$
Spinetoram	0.3a	-4.37 ± 1.03	0.3a	-4.38 ± 0.86
Dimethoate	9.0b	-0.20 ± 0.23	2.7b	-1.84 ± 0.30
Untreated control	0.0	$-14.75 \pm \delta$	0.3a	-4.38 ± 0.86
GLMM F		5.62		4.32
df		6, 21		6, 18.2
P		0.002		0.007

Means with a letter in common are not significantly different (LSD test; $P > 0.05$).

δ indicates an overinflated standard error.

laboratory trials. Reynolds et al. (2014) found that efficacy of alpha-cypermethrin against *B. tryoni* was comparable to fenthion in semifield tests. Various isomers of cypermethrin have demonstrated efficacy against tephritids, for example, cypermethrin reduced infestation and increased yield in a trial to manage *Bactrocera* spp in cucumber (Sharma et al. 2016); residues of zeta-cypermethrin on cherries effectively reduced oviposition by *R. indifferens* (Yee and Alston 2012); fresh cypermethrin residues on cucumber prevented oviposition by *Dacus ciliatus* Loew, the lesser pumpkin fly (Maklakov et al. 2001). Efficacy of the second synthetic pyrethroid assessed, bifenthrin (Talstar 250 EC Insecticide/Miticide) was also comparable with dimethoate in a field cage trial against *B. tryoni*. However, efficacy in laboratory trials was generally low and this insecticide was ineffective against *Z. cucumis*. Maklakov et al. (2001) found that fresh bifenthrin residues prevented oviposition by *D. ciliatus* in cucumbers, suggesting that cypermethrin and bifenthrin had a repellent effect on fruit flies. Repellency of pyrethroids has been documented in a variety of insects, including mosquitoes, houseflies, honey bees, Lepidoptera, and mites (Virgona et al. 1983, Rieth and Levin 1988, Hirano 1989, Siegert et al. 2009). A repellent effect could explain the better efficacy achieved for alpha-cypermethrin and bifenthrin in field cage trials, where fruit flies were presented with a choice of treated and untreated fruit, as opposed to laboratory cage trials, where fruit flies were confined in close proximity with treated fruit.

Cyantraniliprole (DuPont Benevia Insecticide) demonstrated efficacy in field cage trials against both fruit fly species, and laboratory cage trials against *B. tryoni*. It was less effective against *Z. cucumis*. There is relatively little published data on the effect of this new insecticide on tephritid flies. Reynolds et al. (2014) found cyantraniliprole to have little impact on mortality of adult *B. tryoni* in laboratory tests. Cyantraniliprole significantly reduced adult emergence of *B. dorsalis*, *Z. cucurbitae*, and *C. capitata*, when applied as a soil drench (Stark et al. 2013), and resulted in high adult mortality of *B. dorsalis* when ingested (Zhang et al. 2015).

Spinetoram (Success Neo Insecticide) was not effective in the field cage trials and had mixed efficacy in the laboratory cage trials. Reynolds et al. (2014) found spinetoram to be moderately effective on mortality of *B. tryoni* in laboratory tests. Likewise, Yee and Alston (2012) found that this chemical resulted in 81% mortality of *R. indifferens* females exposed to residues on cherries at

Table 9. Mean number of pupae developing from zucchini exposed to *Z. cucumis* in season two trials; back-transformed means (BTM) and predicted means on the log scale ± 1 standard error (PM)

Treatment	Field cage trial, 1 DAT		Lab cage trial, 1 DAT		Lab cage trial, 3 DAT	
	BTM	PM	BTM	PM	BTM	PM
Clothianidin 40 g/100 liter	2.8	1.02 ± 4.25	1.7a	0.56 ± 1.75	0.0	$-13.69 \pm \delta$
Clothianidin 30 g/100 liter	0.0	$-15.52 \pm \delta$	12.7a	2.54 ± 0.65	1.5	0.39 ± 3.97
Thiacloprid	376.4	5.93 ± 0.47	90.3b	4.50 ± 0.25	189.1	5.24 ± 0.38
Abamectin	375.3	5.93 ± 0.47	478.3c	6.17 ± 0.12	385.9	5.96 ± 0.28
Spinetoram	361.0	5.89 ± 0.47	151.2b	5.02 ± 0.20	253.8	5.54 ± 0.34
Dimethoate	44.8	3.80 ± 1.09	9.0a	2.20 ± 0.77	236.1	5.46 ± 0.35
Untreated control	423.7	6.05 ± 0.45	797.4d	6.68 ± 0.10	243.7	5.50 ± 0.34
GLMM F		0.91		32.06		0.87
df		6, 17.9		6, 18		6, 18.1
P		0.507		< 0.001		0.538

Means with a letter in common are not significantly different (LSD test; $P > 0.05$).

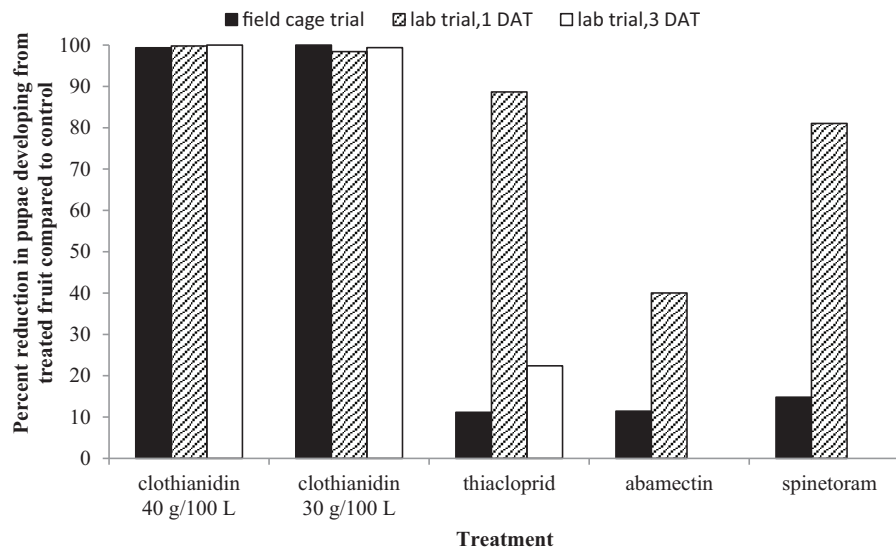δ indicates an overinflated standard error.

Fig. 4. Effect of treatments on reduction in numbers of *Z. cucumis* pupae developing from treated zucchini compared with control fruit in season two trials.

Table 10. Mean mortality per cage of 20 adult *Z. cucumis* exposed to insecticide residues on zucchini in season two trials; back transformed means (BTM) and predicted means on the logit scale ± 1 standard error (PM)

Treatment	Lab cage trial, 1 DAT		Lab cage trial, 3 DAT	
	BTM	PM	BTM	PM
Clothianidin 40 g/100 liter	7.5b	-0.52 ± 0.25	5.5bc	-0.97 ± 0.38
Clothianidin 30 g/100 liter	4.5a	-1.25 ± 0.28	2.5ab	-1.95 ± 0.50
Thiacloprid	0.0	$-18.75 \pm \delta$	0.3a	-4.37 ± 1.45
Abamectin	0.0	$-18.75 \pm \delta$	0.3a	-4.37 ± 1.45
Spinetoram	0.0	$-18.75 \pm \delta$	0.3a	-4.37 ± 1.45
Dimethoate	11.3c	-0.25 ± 0.25	10.0c	0.00 ± 0.34
Untreated control	0.0	$-18.75 \pm \delta$	0.0	$-15.75 \pm \delta$
GLMM *F*		4.24		9.36
df		6, 18		6, 18.1
P		0.008		0.004

Means with a letter in common are not significantly different (LSD test; $P > 0.05$).

δ indicates an overinflated standard error.

24 h, and reduced oviposition compared to controls. Both Reynolds et al. (2014) and Yee and Alston (2012) applied treatments and aged residues under laboratory conditions. Yee et al. (2007) found that aging of spinetoram residues under field conditions resulted in reduced mortality of adult *R. pomonella*; however, residues remained effective up to 14 d in terms of preventing oviposition.

Abamectin (Vertimec Miticide/Insecticide) had no effect in field cage trials. One-day residues had a small but significant effect on *B. tryoni* and *Z. cucumis* in laboratory trials. Kay (2004) found abamectin residues on capsicum resulted in up to 70% mortality of *B. tryoni* up to 4 h after dipping, but 24-h residues were less effective. Reynolds et al. (2014) found abamectin residues on stonefruit resulted in high mortality of adult *B. tryoni* and reduced oviposition. Abamectin is rapidly degraded by exposure to light and air (MacConnell et al. 1989).

In summary, the neonicotinoid clothianidin demonstrated efficacy comparable to dimethoate against both *B. tryoni* and *Z. cucumis*. Thiacloprid and imidacloprid were also generally effective against *B. tryoni*. However, there are concerns about effects of neonicotinoids on bees and other pollinator species (Blacquière et al. 2012, Godfray et al. 2014). The synthetic pyrethroid alpha-cypermethrin was also very effective. However, observations of increased aphid and whitefly activity in alpha-cypermethrin and bifenthrin plots compared with other treatments suggest a disruptive effect on natural enemies. Cyantraniliprole was effective against *B. tryoni* and is claimed by the manufacturer to be soft on beneficials. Spinetoram, abamectin, and bifenthrin were generally less effective than the other treatments. However, these insecticides are registered in fruiting vegetable crops for control of other pests, and it is likely that their use would have a suppressive effect on fruit flies. It should also be noted that treated fruit were exposed to much higher fruit fly pressure than could be expected in the field, and it is possible that greater efficacy of treatments would be observed in actual use conditions. Large variability between replicates was a problem and may have obscured some treatment effects; incomplete coverage by insecticides may have accounted for some of this variability.

These data represent the first successful trial of the efficacy of insecticides, applied to a vegetable crop as cover sprays, against *B. tryoni* and *Z. cucumis*. Although Kay (2004) applied insecticides using a similar small plot layout, background fruit fly pressure alone was not sufficient to result in a significant difference amongst treatments. Reynolds et al. (2014) also used a semifield method to compare insecticides for efficacy against *B. tryoni*; however, although insecticides were applied to fruit in the field, fruit flies were then caged in close proximity to the treated fruit. The field cage method described here allowed for comparison of a number of insecticides under semirealistic conditions. Insecticides were applied to plants and the residues aged under field conditions; fruit flies were exposed to entire plants bearing fruit; and fruit flies were able to choose where to land and oviposit. This allowed for evaluation of effects other than mortality, for example, repellent effects resulting in reduced oviposition. This is significant, as few of the insecticides affected adult mortality in the laboratory cage trials, but the majority

demonstrated at least a suppressive effect on infestation of fruit in field cage trials.

Acknowledgments

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