Evaluation of new volatile compounds as lures for western flower thrips and onion thrips in New Zealand and Spain

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Abstract Two glasshouse trials in a capsicum crop near Warkworth, New Zealand, in January 2011 and two field trials in a nectarine orchard near Lleida, Spain, in July 2013 were undertaken to determine if trap capture of *Frankliniella occidentalis* (western flower thrips, WFT) and *Thrips tabaci* (onion thrips, OT) could be increased by alternative volatile compounds beyond the known thrips attractant, methyl isonicotinate (MI). On blue sticky board traps in the crop in New Zealand, none of six alternative compounds tested caught more thrips (mostly WFT, OT and two other species) than the Control traps. In contrast to MI, catches with the alternative lures were mostly significantly lower. For both trials in the orchard in Spain using white water traps, the highest numbers of WFT and OT were caught in traps with MI, significantly higher than the Control (up to 11× for both species) and higher than most of the nine alternative odour compounds tested.

Keywords *Frankliniella occidentalis*, *Thrips tabaci*, lure, methyl isonicotinate, LUREM-TR, nectarine, capsicum.

INTRODUCTION

Methyl isonicotinate, the active ingredient (ai) of the thrips monitoring technology LUREM-TR (Teulon et al. 2011; Koppert 2014; Pherobank 2014), and ethyl isonicotinate have been shown to be effective lures for increasing the behavioural responses of several thrips species (Thysanoptera: Thripidae) in traps or a Y-tube olfactometer in

comparison to other active compounds (Teulon et al. 2007a,b; Davidson et al. 2007, 2008; Wogin et al. 2010; Broughton et al. 2012; Muntyan et al. 2013). However, new potential active ingredients for thrips lures are continually being discovered (e.g. El Sayed et al. 2014), and it is expedient to benchmark the standard ai against new and

alternative ones to improve lure efficacy for thrips monitoring and perhaps pest management through mass trapping (e.g. Sampson & Kirk 2013) and lure and infect (e.g. Niassy et al. 2012).

Key considerations for selecting an optimal ai for a thrips lure include: (1) efficacy against thrips – the ai must increase trap capture of one or more key thrips pest species, (2) non toxic – the ai must be safe for human use, (3) volatility – the ai must be able to vaporise under ambient trapping conditions and (4) cost – the ai must be relatively inexpensive to fit into established pest monitoring programmes.

First and foremost, the ai must attract the targeted thrips species. In this paper four trials are described, two in a capsicum glasshouse in New Zealand and two in a nectarine orchard in Spain, to compare the efficacy of methyl isonicotinate against several alternative active ingredients for use in thrips lures. The trial in New Zealand targeted the response of the cosmopolitan glasshouse inhabiting thrips, *Frankliniella occidentalis* (western flower thrips, WFT) and *Thrips tabaci* (onion thrips OT), to a range of lactone compounds identified from ripening peach fruits and some found to be attractive to the New Zealand flower thrips (*Thrips obscuratus* Crawford) (El-Sayed et al. 2014). The trials in Spain targeted the response of WFT and OT to a wider range of potential active ingredients including selected peach fruit volatiles (γ -decalactone, δ -decalactone, 6-amyl- α -pyrone), known thrips attractants including ethyl nicotinate (Penman et al. 1982; Teulon et al. 1993), cis-jasmone (Teulon et al. 2007a; El-Sayed et al. 2009) and methyl anthranilate (Murai et al. 2000), floral odours (methyl jasmonate) (Teulon et al. 2007a) or vegetable fruit odours (2-isobutyl-3-methoxypyrazine, methyl pyrazinoate). The WFT aggregation pheromone (Hamilton et al. 2005) was not included in these trials as its activity is largely restricted to one thrips species.

METHODS

New Zealand glasshouse trials

Two trials were conducted in one-half of a 12,560 m² capsicum (*Capsicum annuum*) glasshouse near Warkworth (36° 24' 29" S, 174° 36' 58" E)

from 17-21 January (Trial 1) and 24-28 January (Trial 2) 2011 to test the response of thrips to methyl isonicotinate and six alternative volatile compounds. Blue HORIVER sticky board traps, 10 × 25 cm (Koppert Biologicals, The Netherlands) were hung 10-20 cm above the crop canopy, 10 m apart and at least 20 m from the glasshouse wall in a 3 × 5 incomplete Latin square. In the centre of each sticky trap a 10 mm diameter hole was pre-drilled and a cotton dental roll (size 2, South Island Medical Wholesalers, New Zealand, 3.5 cm long) placed in the hole to which 1 ml (in most cases – see Table 1) of the odour compound was added. All the sticky traps were hung in the greenhouse before treatments were applied. In each trial, three different alternative odour compounds, a positive control (MI) and a neutral control (water) were included. The trials ran from Monday to Friday. All traps were replaced after 2 days, giving two sets of traps per trial (Mon–Wed, Wed–Fri). The numbers of thrips caught on each trap were counted directly on the traps using a stereo microscope (>100× magnification). All thrips from the 20 traps (five from Mon–Wed, five from Wed–Fri for each Trial) from the middle row (i.e. one-third of the traps) were removed, slide mounted and identified to species/sex under a compound microscope (>400× magnification) using Mound & Walker (1982) and Moritz et al. (2004). Only the results of total number of thrips were analysed.

Spain orchard trial

Two field trials were conducted in a nectarine (*Prunus persica* var. Fantasia) orchard near Lleida, Spain (41° 34' 55" N, 0° 25' 59" E) between 1-3 July (Trial 1) and 3-5 July (Trial 2) 2013 to test the response of WFT and OT to ten volatile compounds. Air temperatures were 15.6°C (mean daily minimum) and 33.1°C (mean daily maximum). The fruit on the trees was immature. The water traps used were 2 litre plastic containers (178 × 178 × 80 mm) (Viscount Plastics, New Zealand) laid out in incomplete Latin square designs. Traps were spaced at 20 m intervals in a 5 × 7 grid (Trial 1) and a 5 × 6 grid (Trial 2). The water traps were placed on top of inverted brown/terracotta coloured

plant pots (16 cm high) on the ground, just below the trees. Each trap contained approximately 1.7 litres of water and a few drops of detergent. A 4 ml glass vial (Arthur Holmes Ltd, New Zealand) was suspended over the middle of each water trap with a piece of wire (0.5 mm diameter). Each vial contained a roll of filter paper (50 × 60 mm, Whatman International, England) that projected ca 1 cm above the top of the vial to create a wick. Five and four volatile compounds were included in Trial 1 and Trial 2, respectively, as well as a positive (MI) and a neutral control (water) (Table 1).

Trials were run over 48 h. Traps were placed in the field and the appropriate treatment vial added. All insects in the trap were extracted within 24 h by sieving the water through a fine mesh. All thrips were removed, counted and stored in 96% ethanol. A subsample was mounted on microscope slides for identification using the following criteria: (a) for ≤50 per trap, all thrips were mounted, (b) for 51 to 500 per trap, 50 thrips were mounted. The number of thrips for each species and their sex was determined and the number per trap estimated. Thrips were identified according to Moritz et al. (2004).

Compounds tested in all four trials, their purity (%) and the amount (ml) used is listed in Table 1.

Statistical analyses

Data for the four trials were analysed separately using a Poisson-gamma hierarchical generalised linear model approach (McCullagh & Nelder 1989; Lee et al. 2006). This used a Poisson distribution for the fixed effects (treatments) and a gamma distribution for random effects, both with logarithmic links. The analyses of data for the species included the log(number in mounted sub-sample/total on trap) as an ‘offset’ (McCullagh & Nelder 1989), to adjust results to estimated numbers of each species/sex per trap. The importance of possible random effects (such as the two sets of traps for the glasshouse trials (i.e. Mon–Wed and Wed–Fri.) and spatial position for the Spanish trials) were explored by fitting the model with various combinations of the effects, and comparing the models with X^2 tests of the change in deviance, as implemented in GenStat’s HGRTEST procedure (GenStat Committee 2013a). For the glasshouse trials, there were important differences between traps

Table 1 Treatments used in the capsicum glasshouse (New Zealand) and nectarine orchard (Spain). All compounds were applied as 1 ml of liquid, except cis-jasmone that was applied at 0.2 ml per dispenser due to a limited amount available and methyl pyrazinoate that was dissolved in absolute ethanol to produce 1 ml of solution at 0.21 g/ml.

Compound	CAS number	Glasshouse trials	Nectarine trials	Purity
Water (control)		1 & 2	1 & 2	100%
methyl isonicotinate (MI)	2459-09-8	1 & 2	1 & 2	>98%
γ-decalactone	706-14-9	1	1	98%
γ-heptalactone	105-21-5	1		≥98%
6-amyl-α-pyrone	27593-23-3	1	2	>96%
γ-octalactone	104-50-7	2		≥97%
δ-decalactone	705-86-2	2	2	≥98%
γ-nonolactone	104-61-0	2		≥98%
cis-jasmone	488-10-8		1	>85%
methyl jasmonate	39924-52-2		1	95%
methyl anthranilate	134-20-3		1	>98%
2-isobutyl-3-methoxypyrazine	24683-00-9		2	99%
methyl pyrazinoate	6164-79-0		2	21%
ethyl nicotinate	614-18-6		2	99%

on Mon–Wed and Wed–Fri, whilst for both Spanish field trials no spatial random effects were required. For all sets, the dispersion was estimated, and in addition for the Spanish trials, it was found necessary to adjust for aggregation of thrips by including a saturated random effect for traps. Once the important random effects had been identified, the model was re-fitted including these effects, and the overall treatment effect assessed similarly to the random effects, using GenStat's HGFTEST procedure. Comparisons of each treatment with the Control or MI were assessed by including a contrast that was assessed similarly to the overall treatment effect.

In the results, mean numbers per trap and associated 95% CIs were obtained on the link (logarithmic) scale, and back-transformed to counts. For the species/sexes, these means were obtained by setting the offset to $\log(1)$, thus giving estimated mean counts per trap. All analyses were carried out with GenStat (GenStat Committee 2013b).

RESULTS

New Zealand glasshouse trials

For both trials, total thrips trap catch varied significantly between the treatments ($P<0.001$ and $P=0.001$ for Trials 1 and 2, respectively). For Trial 1, all compounds tested caught more than the Control, significantly so ($P<0.05$) for MI,

γ -heptalactone and 6-amyl- α -pyrone (Table 2). All of the alternative compounds caught significantly less than MI. In Trial 2, all compounds caught more than the Control, with MI and γ -octalactone significantly more. However, all of the alternative compounds caught fewer thrips than MI, with δ -decalactone significantly less ($P<0.001$). Of the 327 thrips mounted and identified, 46% were WFT, 11% were OT, 23% were other *Thrips* spp. (mostly NZFT), and 20% were *Frankliniella intonsa*.

Spain nectarine orchard trials

In all, 436 thrips were caught in Trial 1 and 1043 in Trial 2, which equated to 12.5 and 34.8 thrips per trap respectively. Around 82% of the total thrips counted in water traps in Trial 1 and 78% in Trial 2 based on the sub-sampling criteria (see above) were mounted on microscope slides and identified to species and sex. WFT females were the most common (54% in Trial 1, 43% Trial 2) of those identified. WFT males made up around 19% and 32%, and OT 21% and 17% in Trial 1 and 2, respectively. *Thrips major* females and males made up less than 6% of those identified in either trial. Numbers of total thrips (sexes combined), WFT (both sexes) and OT (females only) only were analysed formally.

Total thrips numbers varied significantly between treatments in both trials ($P=0.010$ and $P=0.001$ for Trial 1 and Trial 2, respectively for the

Table 2 Mean number of total thrips per trap (95% CI) and ratios of mean counts to the Control or methyl isonicotinate (MI) for the two New Zealand (capsicum greenhouse) trials. **Bold** ratios are larger ($P<0.05$) and *italic* ratios are smaller ($P<0.05$) than Control or MI.

Treatment	Trial 1			Trial 2		
	Mean	Ratio to		Mean	Ratio to	
		Control	MI		Control	MI
Control	7.2 (3.7,13.9)	-	<i>0.3</i>	17.0 (9.8,29.5)	-	<i>0.6</i>
methyl isonicotinate	26.0 (14.6,46.4)	3.6	-	30.8 (18.5,51.3)	1.8	-
γ -decalactone	9.0 (4.7,17.1)	1.3	<i>0.3</i>	-	-	-
γ -heptalactone	12.5 (6.8,23.1)	1.7	<i>0.5</i>	-	-	-
6-amyl- α -pyrone	15.8 (8.7,28.9)	2.2	<i>0.6</i>	-	-	-
γ -octalactone	-	-	-	25.2 (14.9,42.4)	1.5	0.8
δ -decalactone	-	-	-	12.8 (7.2,23.0)	0.8	<i>0.4</i>
γ -nonalactone	-	-	-	23.0 (13.6,39.0)	1.4	0.7

overall test). For both trials, the highest numbers of total thrips were caught in the traps with MI, significantly higher than those caught on the Control ($P < 0.05$, Table 3). Significantly more total thrips were caught in traps with γ -decalactone and δ -decalactone in Trial 1, and with methyl pyrazinoate and ethyl nicotinate in Trial 2 than in the Control traps. However, in each case, traps with these treatments caught around half the numbers of total thrips in traps with MI.

For WFT, there were significant differences between treatments in numbers of females in both trials ($P = 0.006$, and $P = 0.038$, Table 4) and in numbers of males in Trial 2 ($P = 0.007$, Table 5). However, numbers of males in Trial 1 did not vary so strongly ($P = 0.056$ respectively), although numbers were highest in traps with MI and δ -decalactone (10 $\times$ and 12 $\times$ more than Control traps respectively). For WFT females, traps with MI caught the most thrips (11.5 $\times$ ($P = 0.009$) and 3.4 $\times$ ($P = 0.007$) more than Control traps in Trial 1 and 2, respectively). In Trial 1, numbers of both sexes were higher for treatments γ -decalactone and δ -decalactone than for the Control ($P = 0.047$, 0.046 for females,

$P = 0.072$, 0.028 for males, respectively). However, numbers for treatments other than δ -decalactone were lower than those for MI (not significantly in all cases). In Trial 2, only numbers caught with MI were significantly higher than those for the Control ($P = 0.007$ females, $P = 0.009$ males).

Trap capture of OT females was significantly different between the treatments in both trials ($P = 0.020$ and $P < 0.001$, respectively, Table 6). In Trial 1, only MI caught significantly more than the Control ($P = 0.010$), whilst in Trial 2, MI and ethyl nicotinate caught significantly more than the Control ($P < 0.001$). However, numbers caught with ethyl nicotinate were only just above half of those caught with MI.

DISCUSSION

This work substantiates the relative greater utility of MI, compared to a range of alternative volatile compounds, for increasing trap capture of the key cosmopolitan pest thrips species, WFT and OT, and reinforces previous work comparing MI with a larger range of volatile compounds (e.g. Teulon et al. 2007a; Davidson et al. 2007, 2008). Additionally, MI has activity against a

Table 3. Mean number of total thrips per trap (95% CI) and ratios of mean counts to the Control or methyl isonicotinate (MI) in Spain (nectarine orchard) trials. **Bold** ratios are larger ($P < 0.05$) and *italic* ratios are smaller ($P < 0.05$) than Control or MI.

Treatment	Trial 1			Trial 2		
	Mean	Ratio to		Mean	Ratio to	
		Control	MI		Control	MI
Control	3.2 (1.0,10.4)	-	<i>0.1</i>	19.8 (11.9,33.0)	-	0.3
methyl isonicotinate	35.2 (12.5,99.2)	11.0	-	71.4 (44.7,114.1)	3.6	-
cis-jasmone	2.8 (0.8,9.3)	0.9	<i>0.1</i>	-	-	-
methyl jasmonate	3.4 (1.1,11.0)	1.1	<i>0.1</i>	-	-	-
γ -decalactone	16.2 (5.7,46.5)	5.1	<i>0.5</i>	-	-	-
methyl anthranilate	7.4 (2.5,22.1)	2.3	<i>0.2</i>	-	-	-
δ -decalactone	19.0 (6.7,54.3)	5.9	<i>0.5</i>	-	-	-
2-isobutyl-3-methoxypyrazine	-	-	-	18.6 (11.1,31.1)	0.9	0.3
6-amyl-alpha-pyrone	-	-	-	15.6 (9.2,26.4)	0.8	0.2
methyl pyrazinoate	-	-	-	41.8 (25.9,67.6)	2.1	0.6
ethyl nicotinate	-	-	-	41.4 (25.6,66.9)	2.1	0.6

Table 4 Mean number of western flower thrips females per trap (95% CI) and ratios of mean counts to Control or methyl isonicotinate (MI) in Spain (nectarine orchard) trials. **Bold** ratios are larger ($P < 0.05$) and *italic* ratios are smaller ($P < 0.05$) than Control or MI.

Treatment	Trial 1			Trial 2		
	Mean	Ratio to		Mean	Ratio to	
		Control	MI		Control	MI
Control	1.8 (0.5,7.1)	-	<i>0.1</i>	8.4 (4.4,16.0)	-	0.3
methyl isonicotinate	20.6 (6.5,65.5)	11.5	-	28.6 (16.1,50.9)	3.4	-
cis-jasmone	1.0 (0.2,4.6)	0.6	<i>0.0</i>	-	-	-
methyl jasmonate	1.0 (0.2,4.6)	0.6	<i>0.0</i>	-	-	-
γ -decalactone	11.2 (3.5,36.2)	6.2	0.5	-	-	-
methyl anthranilate	2.8 (0.8,10.1)	1.6	<i>0.1</i>	-	-	-
δ -decalactone	11.3 (3.5,36.3)	6.3	0.5	-	-	-
2-isobutyl-3-methoxypyrazine	-	-	-	9.8 (5.2,18.3)	1.2	0.3
6-amyl-alpha-pyrone	-	-	-	8.4 (4.4,16.1)	1.0	0.3
methyl pyrazinoate	-	-	-	12.3 (6.2,24.2)	1.5	0.4
ethyl nicotinate	-	-	-	17.5 (9.7,31.5)	2.1	0.6

Table 5 Mean number of western flower thrips males per trap (95% CI) and ratios of mean counts to Control or methyl isonicotinate (MI) in Spain (nectarine orchard) trials. **Bold** ratios are larger ($P < 0.05$) and *italic* ratios are smaller ($P < 0.05$) than Control or MI.

Treatment	Trial 1			Trial 2		
	Mean	Ratio to		Mean	Ratio to	
		Control	MI		Control	MI
Control	0.4 (0.1,2.7)	-	<i>0.1</i>	6.6 (3.5,12.6)	-	<i>0.3</i>
methyl isonicotinate	4.0 (1.2,13.6)	10.1	-	21.6 (12.3,37.8)	3.3	-
cis-jasmone	0.2 (0.0,2.4)	0.5	<i>0.0</i>	-	-	-
methyl jasmonate	1.0 (0.2,4.4)	2.5	<i>0.2</i>	-	-	-
γ -decalactone	3.2 (0.9,10.9)	8.0	0.8	-	-	-
methyl anthranilate	2.2 (0.6,7.9)	5.5	0.5	-	-	-
δ -decalactone	5.0 (1.6,16.4)	12.6	1.3	-	-	-
2-isobutyl-3-methoxypyrazine	-	-	-	4.4 (2.2,8.9)	0.7	<i>0.2</i>
6-amyl-alpha-pyrone	-	-	-	4.9 (2.5,9.9)	0.7	<i>0.2</i>
methyl pyrazinoate	-	-	-	12.3 (6.4,23.4)	1.9	0.6
ethyl nicotinate	-	-	-	9.8 (5.3,17.9)	1.5	0.5

further eight thrips species (Teulon et al. 2011). This is also the first published report of MI increasing trap capture of Spanish WFT and OT populations.

The present study also widens the reported activity of another group of recently discovered thrips active volatile compounds, the lactones from ripening peach fruit (El-Sayed et al. 2014). While El-Sayed et al. (2014) found 6-amyl- α -pyrone (6-pentyl-2H-pyran-2-one) to be the most effective compound for increasing trap capture of the New Zealand flower thrips (*Thrips obscuratus*) in comparison to a range of other lactone compounds, the present results indicate that other lactone compounds may be better for other thrips species. γ -Decalactone and δ -decalactone both increased WFT capture more than 6-amyl- α -pyrone, although not as much as MI (with one exception). Conversely, none of the lactone compounds tested in Spain increased trap capture of OT.

The ratios for the number of total thrips caught in traps with MI and Control traps in the New Zealand trials (Table 2) were lower than for similar ratios in the Spain trials (Table 3). Another study in Spain (M.M. Davidson, Plant

& Food Research, unpublished data) has shown that the MI/Control ratio is similar (although total numbers differ) between sticky board and water traps in the same trial so trap type does not appear to influence this ratio. The different ratios may reflect specific characteristics of the different crops, their environments and the thrips complex present. In particular, the enclosed nature of the glasshouse may have allowed for contamination of the Control traps where odour from a MI baited trap influenced capture of thrips in the Control trap. This may have resulted in increased thrips capture in the Control traps and a lower MI/Control ratio. This may also be true for outdoor traps as far apart as 10 m (Teulon et al 2007b, 2014). Such an effect might be expected to be worse in greenhouses where the enclosed structure and relative lack of air movement would inhibit the dissipation of odours into the wider environment.

Based on these and other published results, MI remains the ai of choice for trapping WFT and OT, especially when they occur together, and possibly other thrips pest species, particularly in temperate conditions, due to its chemical attributes (e.g. volatility and stability) and its

Table 6 Mean number of onion thrips females per trap (95% CI) and ratios of mean counts to Control or methyl isonicotinate (MI) in Spain (nectarine orchard) trials. **Bold** ratios are larger ($P < 0.05$) and *italic* smaller ($P < 0.05$) than Control or MI.

Treatment	Trial 1			Trial 2		
	Mean	Ratio to		Mean	Ratio to	
		Control	MI		Control	MI
Control	0.8 (0.2,3.6)	-	<i>0.1</i>	1.6 (0.7,3.5)	-	<i>0.1</i>
methyl isonicotinate	9.3 (3.5,25.1)	11.7	-	16.7 (12.0,23.1)	10.4	-
cis-jasmone	1.0 (0.2,4.1)	1.2	<i>0.1</i>	-	-	-
methyl jasmonate	0.8 (0.2,3.6)	1.0	<i>0.1</i>	-	-	-
γ -decalactone	1.3 (0.3,5.0)	1.6	<i>0.1</i>	-	-	-
methyl anthranilate	2.0 (0.6,6.5)	2.5	<i>0.2</i>	-	-	-
δ -decalactone	1.5 (0.4,5.3)	1.8	<i>0.2</i>	-	-	-
2-isobutyl-3-methoxypyrazine	-	-	-	2.4 (1.2,4.6)	1.5	<i>0.1</i>
6-amyl-alpha-pyrone	-	-	-	0.4 (0.1,2.0)	0.3	<i>0.0</i>
methyl pyrazinoate	-	-	-	3.3 (1.7,6.2)	2.0	<i>0.2</i>
ethyl nicotinate	-	-	-	9.1 (6.2,13.3)	5.7	<i>0.5</i>

relatively low toxicity profile to mammals (Acros Organics 2014; D.A.J. Teulon, Plant & Food Research, unpublished data).

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