A volatile compound, 2-undecanone, increases walking, but not flying, tomato potato psyllid movement toward an odour source

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Abstract *Bactericera cockerelli* (tomato potato psyllid; TPP) is an important pest of solanaceous crops in New Zealand and North America. A volatile compound that alters the behaviour of TPP could be developed into a component of an integrated pest management strategy for solanaceous crops. One compound, 2-undecanone, was found to increase the percentages of female and male TPP (65%, $P < 0.05$) to walk up the odour-laden arm of a Y-tube olfactometer. The presence of 2-undecanone in either a low speed laminar flow wind tunnel or in a potato crop did not substantially affect the response of flying TPP to sticky traps of different colours. The greatest number of TPP adults was recorded on yellow sticky traps, followed by green, with the fewest on clear traps, in both the wind tunnel and field experiments ($P < 0.001$), irrespective of the presence of 2-undecanone.

Keywords *Bactericera cockerelli*, olfactometer, wind tunnel, field, sticky traps, visual cue, olfactory cue.

INTRODUCTION

The tomato potato psyllid, (TPP, *Bactericera cockerelli* (Šulc), Hemiptera: Trioizidae) is a key pest on economically important Solanaceae crops in New Zealand and North America (Teulon et al. 2009; Munyaneza 2012). Currently, effective management of TPP relies on insecticides (Jorgensen et al. 2013; Walker et al. 2013; Butler & Trumble 2012). However, the potential for TPP to develop resistance to insecticides and the economic, health and environmental costs associated with insecticides has prompted the need for alternative strategies.

One such approach is through the development of techniques exploiting behaviour-modifying stimuli, such as semiochemicals.

TPP breed on a range of species belonging to Solanaceae and a few non-Solanaceae species, including Convolvulaceae (Pletsch 1947; Wallis 1955). How TPP locate hosts is still uncertain. TPP, along with other psyllid species, display a strong response to visual stimuli (Mensah & Madden 1992; Brennan & Weinbaum 2001; Taylor et al. 2014). Various psyllid species have also been shown to respond to olfactory cues

emitted by their host plants (Soroker et al. 2004; Kristoffersen et al. 2008; Mann et al. 2012; Yuvaraj et al. 2013). Further, more Asian citrus psyllid, *Diaphorina citri* Kuwayama, were caught on yellow sticky traps located on a jasmine plant within a fallow field, than on traps 1 and 17 m away from the jasmine plant within the fallow field (Hall & Hentz 2011). Either visual and/or olfactory cues from the host plant increased the number of psyllids flying in the area and subsequently caught on the trap on the host plant, and/or the presence of the jasmine plant elicited an odour-induced visual response.

TPP adults have demonstrated a positive olfactory response toward putative pheromones from conspecifics and a negative response towards dimethyl disulfide and plant essential oils in Y-tube olfactometer bioassays (Guédot et al. 2010; Diaz-Montano & Trumble 2012). The purpose of this study was to use a relatively rapid screening technique based on a Y-tube olfactometer to identify a compound active toward walking TPP that could be tested for activity toward flying TPP in (1) a low speed laminar flow wind tunnel and (2) a potato crop in the presence and absence of visual cues.

MATERIALS AND METHODS

Y-tube olfactometer experiment

The glass Y-tube olfactometer and set-up was the same as that used previously for thrips assays (Davidson et al. 2006). The Y-tube has two branching arms at an angle of approximately 45° leading into a single tube (stem), all 60 mm long with an internal diameter of 5 mm. The Y-tube was held at an inclined position of 25° with the branching arms higher than the stem. Glass vials (4 ml) were attached to each arm via Wheaton Micro Kit adapters (room temperature at 23 ± 1°C). Air was drawn through activated charcoal before entering the vials and the Y-tube, using a suction pump (AR Harris Co. Ltd, New Zealand) producing an airflow of 0.4 m/s within the Y-tube. One microlitre of an undiluted compound was applied to filter paper in one 4 ml vial, while blank filter paper was held in the second vial. The compounds tested included β -caryophyllene, R-carvone, S-carvone, 2-undecanone (all from Sigma Aldrich), β -ionone (Riedel-de-Haen),

limonene (Merck) and methyl salicylate (Ajax). Air was drawn through the Y-tube for at least 15 min before introducing the first TPP adult (age or mated status unknown). TPP adults (male and female; *Candidatus* Liberibacter solanacearum status undetermined) were collected from a laboratory colony (maintained on tomato plants (*Solanum lycopersicum* 'MoneyMaker') at Plant & Food Research, Lincoln, in a controlled temperature room at 25 ± 1°C, 14:10 h light:dark) and starved for at least 4 h (access to water only). Whether the psyllid walked up the odour-laden arm, clean-air arm or did not walk up either arm, was recorded, with the psyllid being observed in the Y-tube for a maximum of 3 min. After each psyllid had walked up either arm of the Y-tube or 3 min had passed, the psyllid was collected into a separate 1.5 ml Eppendorf tube containing 96% ethanol. The sex of each psyllid was then determined under 20× magnification using a stereomicroscope. The Y-tube, Wheaton apparatus and connector tubing was swapped to alternate sides to avoid position effects after every five psyllids tested. One replicate comprised 20 individual TPP adults to choose the odour-laden or clean-air arm, or neither, with one replicate done in a day. There were three replicates for each compound/blank. Between replicates, the Y-tube, Wheaton apparatus and connector tubing were cleaned in 99% hexane and allowed to air dry.

Numbers of TPP choosing the treatment arm out of the total psyllids choosing an arm were analysed with a binomial generalized linear model (McCullagh & Nelder 1989). Comparisons between the compounds and sexes were made by including contrasts within the analysis of deviance that were assessed with χ^2 tests. Confidence limits (95%) for the percentages were obtained on the transformed (logit) scale and back-transformed. Since 50% has a value of zero on the logit scale, a comparison with 50% (no preference between arms) was made for each treatment using t-tests of the percentages on the logit scale. Any compounds where more than 50% of the TPP adults chose the odour-laden arm ($P < 0.05$) were used in subsequent experiments.

Wind tunnel experiment

The low speed laminar flow wind tunnel was the

same as that used for thrips assays (Davidson et al. 2006). The Perspex® observation chamber measured 0.58 m wide by 0.55 m high by 1.7 m long and was illuminated by five 36W/840 cool lights alternating with five 36W/965 true light fluorescent tubes, producing an average of 10,000 lux (Beha, Digital Lux Hi Tester 93-1065L, Germany). The frame and floor of the wind tunnel were painted charcoal grey to facilitate locating insects and to reduce potential visual stimuli. During bioassays, room air filtered through activated charcoal was passed through the chamber in a laminar flow. Airflow for the experiments was on average 0.25 m/s and the room temperature was $23 \pm 1^\circ\text{C}$.

A single yellow (Bugscan, Biobest), green (IVOG, Germany) or clear sticky trap, 10×25 cm, was suspended with the bottom of the trap 7 cm above the floor, in the centre of the wind tunnel, 40 cm from the upwind end of the chamber. Clear sticky traps were made using acetate (OfficeMax A4 OHP transparency sheets, 100 μm) cut into rectangles (10×25 cm). Glue from extra yellow sticky traps was transferred to the clear rectangles by repeatedly pressing the clear rectangle on to additional yellow sticky traps. Glass vials (4 ml) with white filter paper (5×6 cm, Whatman No. 1) rolled into a cylinder to form a wick, projecting 1 cm above the opening, either had 0.5 ml of 2-undecanone (99% purity, Aldrich, Germany) added or no compound (control). Wire (0.7 mm) was wrapped around the top of the vial to form a hook, with the top of the vial hanging approximately 10 cm from the top of the sticky trap with the 25 cm edge of the trap hanging vertically. The six treatment combinations were each replicated five times, with a complete set of the six treatments run in each of 5 days. Within a day, the first three runs were all done with odour (or all without), and the second, the reverse. This gave a split-plot design, with odour as the main plot treatment and trap as the split plot treatment. Row-column designs were used for both the odour main plots and the colour split-plots to balance the odours and colours approximately across the six sessions over the 5 days (CycDesign Software 2009).

Adult TPP (no detectable *Candidatus* *Liberibacter solanacearum*) were collected from

a colony at Plant & Food Research Lincoln, reared on *Candidatus* *Liberibacter solanacearum*-negative capsicum (*Capsicum annuum* 'Giant Bell') plants in a controlled temperature room ($25 \pm 1^\circ\text{C}$, 40% humidity, 16:8 h light:dark). Thirteen to 16 adults were held in Petri dish lids (7 cm diameter) covered with stretched Parafilm®. A few drops of water were placed on the Parafilm®, with a smaller Petri dish (3 cm) used to contain the water. Adults were held for at least 4 h before starting the experiments. Adults were placed on a raised Perspex® platform (13 cm above the wind tunnel floor, 70 cm downwind of sticky trap) and the Parafilm® was removed. The numbers of TPP on the sticky traps, wind tunnel (floor, ceiling, walls) or still on the release platform (and whether dead or alive), were recorded after 20 min. All TPP that could be found were removed from the wind tunnel before wiping the wind tunnel down with 1:1 acetone:water (BDH, 99.5%), and clean air was flushed through for 5-10 min before starting the next treatment.

Initial analyses using binomial-beta hierarchical generalized linear models (Lee et al. 2006) were carried out to see whether there were differences relating to days or orders within days (random effects). However, such effects were largely negligible, so were ignored in the final analyses. As a second initial analysis, the data for all four locations (TPP on trap, wind tunnel or on the platform (dead or alive)) were analysed simultaneously, using a log-linear model (McCullagh & Nelder 1989) for multinomial response data. This assessed whether the pattern of percentages at each location varied between the treatments, and indicated that the pattern did vary between treatments, primarily between trap colours. Therefore, the data for the four locations were analysed separately. For each of the four locations, the percentage of TPP was analysed with a binomial generalised linear model with a logit link (McCullagh & Nelder 1989). These analyses are sub-analyses of the log-linear analysis described above. The effects of colour, odour and the interaction between them were assessed with F-tests, within an analysis of deviance, as part of the analysis. The percentage at each location for each treatment and associated 95% confidence limits were obtained on the transformed (logit)

scale and back-transformed. Confidence limits were based on the Pearson deviance obtained from the log-linear analysis. The analyses were carried out with GenStat (GenStat Committee 2013a).

Field experiment

Clear (acetate, 200 µm), green and yellow sticky traps (described above) with five replicates of the six treatments (three colours, with or without 2-undecanone) were laid out in an incomplete Latin Square (CycDesign Software 2009). Traps were at least 15 m apart, with the top of the trap at the crop canopy level, attached vertically to 1.2 m poles using fold-back clips and twist ties. Glass vials (4 ml) with or without 3 ml of 2-undecanone were hung from the sticky traps (top of glass vial approximately 10 cm from top of trap) using 0.7 mm wire hooks, as in the wind tunnel experiment. Traps were left in the field for 3 days before being removed to the laboratory, where male and female TPP were identified and counted using a stereomicroscope (up to 100× magnification).

Total TPP, female and male were analysed, with a separate analysis for each. Data were analysed with a Poisson-gamma hierarchical generalized linear model with logarithmic links (HGLM, Lee et al. 2006). This included colour, odour and the interaction between these as fixed effects with a Poisson distribution, and random potential effects (replicates, columns of traps as in the design) with a gamma distribution. The importance of random effects was assessed with a χ^2 test of the change in deviance on dropping a term, as implemented in GenStat's HGRTEST

procedure (GenStat Committee 2013a). Replicates but not columns were found to be important, so replicates alone were included in the final analysis. The fixed effects were also assessed with a χ^2 test of the change in deviance, as implemented in GenStat's HGFTEST procedure. Mean trap counts and associated confidence limits were obtained on the transformed (log) scale and back transformed for presentation. The analyses were carried out with GenStat (GenStat Committee 2013b).

RESULTS

In the Y-tube, a range of compounds was tested. However, only the results for the blank (filter paper in each arm) and 2-undecanone are presented in this paper. The percentage of TPP to walk up the odour-laden arm was significantly higher in the presence of 2-undecanone ($P=0.011$, Table 1). There were no significant differences between the sexes in the percentage of psyllids choosing the treatment arm, either overall ($P=0.2$) or in terms of modifying the effect of compounds ($P=0.8$ for the sex by compound interaction).

The percentage of TPP released into the wind tunnel was not strongly affected by the presence of 2-undecanone at any of the four locations: trap, wind tunnel or platform (alive or dead) ($0.08 < P < 0.93$ for the main effects and the interaction with colour for the four locations, Figure 1). The percentage of released TPP on the trap or elsewhere in the wind tunnel (floor, ceiling or walls, i.e. the percentage that flew but did not land on the trap) varied strongly between colours ($P < 0.001$), with the percentage on yellow traps (42%) > green (26%) > clear

Table 1 The percentage of female and male *Bactericera cockerelli* (tomato potato psyllid; TPP) $\pm$ 95% confidence limits choosing the odour-laden arm of a Y-tube containing only filter paper or 1 µl of 2-undecanone.

Compound	Sex	%	95% confidence limits	Prob. > 50%
Blank	Female	52.9	36.5, 68.8	0.366
	Male	51.9	33.6, 69.6	0.424
	Both	52.5	40.0, 64.6	0.351
2-undecanone	Female	64.1	48.2, 77.4	0.041
	Male	66.7	44.7, 83.2	0.067
	Both	65.0	52.2, 75.9	0.011

traps (2%). The opposite pattern was found for the percentages of TPP found elsewhere in the wind tunnel (clear (60%) > green (45%) > yellow (19%), Figure 1). The percentage of TPP remaining on the platform, dead or alive, did not vary strongly with colour ($P=0.790$ and $P=0.185$ for the overall difference between colours for live and dead TPP on the platform respectively).

As with the wind tunnel experiment, adding 2-undecanone to sticky traps in the field had little effect on capture of total TPP ($P=0.848$ for the colour by odour interaction and $P=0.247$ for the main effect, Figure 2). However, trap catches varied strongly with trap colour ($P<0.001$ for the colour main effect): total TPP caught with yellow (34/trap) > green (26/trap) > clear (11/trap, Figure 2). This pattern was similar for female and male TPP (Figure 2).

DISCUSSION

The presence of 2-undecanone increased the percentage of TPP walking up an odour-laden arm of a Y-tube olfactometer. However, the addition of this compound to sticky traps of different colours did not substantially increase the percentage of TPP landing on the sticky traps in the present study. It may be that 2-undecanone provides a short-range chemical cue, hence its influence on walking, but not on

flying TPP. Alternatively, 2-undecanone may require the presence of other chemicals to elicit a response in flying TPP. In a Y-tube olfactometer, 2-undecanone combined with lactic acid had a stronger effect than the individual compounds on the mosquito *Aedes (Stegomyia) aegypti* L. (Haas et al. 2006). The compound, 2-undecanone is a constituent of a range of plant species including Solanaceae (e.g. Carter et al. 1989; Antonius et al. 2005; Ali et al. 2013). It has been shown to have predominantly toxic and/or repellent properties against a range of insects (e.g. Lin et al. 1987; Antonius et al. 2005; Ali et al. 2013). A previous study reported that 2-undecanone, among a range of other compounds tested individually, was attractive to the olive bark beetle, *Phloeotribus scarabaeoides* Bernard (Szauman-Szumski et al. 1998). However, attraction was only demonstrated in a short-range (50×3 cm) olfactometer (Szauman-Szumski et al. 1998).

The use of clear traps may provide a means of differentiating the response of TPP toward visual and olfactory cues. The response of TPP to spectrally characterised sticky traps, including the yellow and green traps used in the present study, has been previously documented (Taylor et al. 2014). Yellow traps with a spectral reflectance maximum of ca 580 nm caught significantly more TPP than green traps with a peak

Figure 1 The percentage of *Bactericera cockerelli* (tomato potato psyllid; TPP) adults caught on sticky traps (yellow (Y), green (G) or clear (C)) or on the chamber (floor, walls or ceiling) or on the release platform (alive or dead), without or with an odour (0.5 ml of 2-undecanone) in the wind tunnel. The lowest, highest and a mid range 95% confidence limit are shown.

Figure 2 The percentage of total, female and male *Bactericera cockerelli* (tomato potato psyllid; TPP) adults caught on sticky traps (yellow (Y), green (G) or clear (C)) without or with an odour (3 ml of 2-undecanone) in a potato crop. Note the different scales on the Y axes. Error bars are 95% confidence limits.

reflectance of around 520 nm (Taylor et al. 2014). It is not known how TPP use visual and olfactory cues to locate potential hosts. One possibility is that flying TPP use host plant derived volatile compounds. However, semiochemicals that elicit a response from flying TPP over and above their response to visual cues have not been identified yet. Alternatively, TPP in flight use visual cues as a principal means of locating their hosts, and olfactory cues provide shorter range signals.

ACKNOWLEDGEMENTS

We are grateful to Kye Chung Park and Jessica Dohmen-Vereijssen for reviewing this manuscript. We would also like to thank Brad Whitfield for allowing access to his potato crop. We also extend our thanks to Corina Till for assisting with the Y-tube olfactometer experiments. This work was funded by The New Zealand Institute for Plant & Food Research Limited.

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