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Cucumber volatile blend, a promising female-biased lure for *Bactrocera cucumis* (French 1907) (Diptera: Tephritidae: Dacinae), a pest fruit fly that does not respond to male attractants

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Abstract *Bactrocera cucumis* (French 1907), the 'cucumber fruit fly', is a horticultural pest in Australia that primarily infests cucurbits and has also been recorded from tomatoes, papaw and several other hosts. It does not respond to known male lures, cue-lure and methyl eugenol, making monitoring and control difficult. A cucumber volatile blend lure was recently developed in Hawaii and found to be an effective female-biased attractant for the melon fly *B. cucurbitae*. This lure was field tested in north Queensland, Australia in McPhail traps in comparison with orange ammonia, Cera Trap® and a control, and was found to more consistently trap *B. cucumis* than the other lures. *B. cucumis* were caught at 41% of the cucumber volatile lure trap clearances, compared with 27% of the orange ammonia, 18% of the Cera Trap and 16% of the control trap clearances. The cucumber volatile lure was more attractive to *B. cucumis* in low population densities and also trapped *B. cucumis* earlier on average than the other lures. Data analysed from the site with highest trap catches (Spring Creek) showed that the cucumber volatile lure caught significantly more *B. cucumis* than the other traps in four of the 11 trap clearance periods, and for the remaining clearances, no other trap type caught significantly more flies than the cucumber volatile lure. The cucumber volatile lure had a strong female-biased attraction but it was not significantly more female-biased than orange ammonia or Cera Trap. Cucumber volatile lure traps were cleaner to service resulting in better quality specimens than the orange ammonia trap or Cera Trap. These findings have potential implications for market access monitoring for determining pest freedom, and for biosecurity monitoring programmes in other countries that wish to detect *B. cucumis* early.

Key words attractant, fruit fly, trapping.

INTRODUCTION

Bactrocera cucumis (French) is a significant pest of cucurbits in Australia that has also been recorded from other commercial hosts including tomatoes, papaw, guava and passion fruit. It is known from the Northern Territory, the Torres Strait Islands, eastern Queensland and north-east New South Wales (Hancock *et al*. 2000). However, as *B. cucumis* does not respond to known male lures, cue-lure and methyl eugenol (Hancock *et al*. 2000), it is difficult to monitor to accurately define its geographic distribution. *B. cucumis* also does not respond to zingerone (Fay 2011, JE Royer unpubl. data 2013), a new male attractant discovered in Malaysia (Tan & Nishida 2007) that was recently found to be an effective lure for another minor pest *B. jarvisi* (Fay 2011). Male lures attract most pest species of tropical fruit fly (Drew 1989) and are central to their monitoring and control.

Export conditions for Australian horticultural produce that are hosts of *B. cucumis* require that it either comes from an area free from *B. cucumis* or has undergone a postharvest treatment to kill viable life stages (EPPO 2005). For example, New Zealand requires that Australian exports of capsicum, tomato, zucchini, cucumber and melons are either free of *B. cucumis* or have been treated with dimethoate (MPI 2012, MAF 2013).

Without an effective lure for *B. cucumis* demonstrating pest, freedom for market access is difficult. Additionally, pre- and postharvest treatment options have recently been limited in Australia by the suspension of dimethoate and fenthion following a review initiated by concerns about their toxicity (APVMA 2013a). These pesticides have been used for the last two decades for disinfestation treatments to control fruit fly so that produce meets market access requirements for domestic and export horticultural trade (Clarke 2012). Consequently, new management methods are needed for this species and identifying a lure that can be used in its monitoring and control is all the more important. The only monitoring options currently available for this species are fruit sampling and trapping with protein bait and orange ammonia traps (Bateman 1982) which result in generally poor quality specimens, while control is limited to cover sprays of broad spectrum insecticides and protein bait sprays (Drew *et al*. 1982, EPPO 2005, APVMA 2013b).

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Table 1 Summary of cucumber lure trial trapping sites in north Queensland

Site	Map coordinates	Crop and size	Sampling dates	Duration
Dimbulah	−17.10608 145.12229	Organic zucchini crop roughly 250 m × 50 m	11/9/12 to 16/10/12	5 weeks
Dimbulah	−17.10497 145.11826	Organic pumpkin crop roughly 250 m × 30 m	22/10/12 to 26/11/12	6 weeks
Walkamin	−17.11480 145.42829	Cucumber crop roughly 250 m × 50 m	14/9/12 to 15/10/12	5 weeks
Rocky Creek	−17.18077 145.45049	Watermelon crop roughly 170 m × 50 m	16/10/12 to 26/11/12	6 weeks
Spring Creek	−17.19993 145.47569	Pumpkin crop roughly 100 m × 10 m	16/10/12 to 3/1/13	12 weeks

Siderhurst and Jang (2010) identified a cucumber volatile blend that was twice as attractive to the melon fly, *B. cucurbitae*, as Solulys protein bait, with a female-biased attraction. In cage trials conducted in Mareeba, the cucumber volatile lure was found to be more attractive to *B. cucumis* than orange ammonia (GE Lowe & HAC Fay unpubl. data 2011).

In north Queensland, Australia, we field tested the attractiveness of the cucumber volatile lure to *B. cucumis* in wet McPhail traps in comparison to other wet traps: orange ammonia, Cera Trap® (an animal protein trap) and a control.

METHODS

Field testing

Bactrocera cucumis attraction trials were conducted at five sites on the Atherton Tablelands, inland from Cairns in north Queensland, Australia (see Table 1). There were no alternative commercial hosts grown at Dimbulah and Rocky Creek. At Walkamin, zucchinis and capsicums were also grown, and at Spring Creek, zucchinis, cucumbers and tomatoes were grown near the pumpkin crop.

A randomised complete block design was used to compare the efficacy of four lures: cucumber volatile blend lure (McPhail trap with cucumber volatile blend plug suspended inside the top and 300 mL 10% propylene glycol solution. ‘Cucumber volatile plugs’ were sourced from Scentry Biological Inc. in Billings, Montana, USA. McPhail traps are pear-shaped plastic containers with a yellow invaginated base and clear top and were sourced from Entosol, Roselands, New South Wales as AgriSense dome traps); orange ammonia (McPhail trap with 300 mL orange ammonia made from 270 mL orange juice, 30 g ammonium bicarbonate, 1 teaspoon of borax (adapted from Drew *et al*. 1982)); Cera Trap® (a commercially available trap consisting of a clear cylindrical plastic base filled with animal protein liquid lure and a yellow top with 10 mm entry holes, sourced from Barmac Pty Ltd, Darra, Queensland); and a control (McPhail trap with 300 mL 10% propylene glycol solution). Each site contained four blocks. Each block contained one of each of the three lure traps and the control. In each block, the traps were placed at 5 m intervals along the cropping row. Treatment placement within blocks was randomised to prevent bias over relative placement. Blocks were 7–10 m apart.

Traps were cleared weekly and recharged with fresh solution except the Cera Trap solution, which does not spoil and therefore was reused. Spring Creek traps were not cleared at week 11 due to Christmas falling in this period. Cucumber volatile lures were replaced at 6 weeks at Spring Creek. Trials at the other sites ran for 6 weeks or less so there was no replacement of this lure.

Fruit flies were identified to species and sex using a stereomicroscope and the key to tephritid fruit flies prepared by Drew (1989). The number of male and female *B. cucumis* caught was recorded. Week 11 and 12 at Spring Creek were combined due to traps not being cleared at week 11.

Analysis of Spring Creek data

Substantially, more flies were trapped at Spring Creek so this dataset was able to be analysed statistically. Analysis of variance (ANOVA) was used to compare the total number of *B. cucumis* caught per day and the number of female *B. cucumis* caught per day. For each model, a term representing the block was fitted as a random term, with lure type as the fixed term. Each trap clearance was analysed separately. If the F-test was significant ($P < 0.05$), pairwise comparisons between the treatments were made using Fisher’s 95% protected least significant difference (LSD).

The proportion of female *B. cucumis* caught at each sampling time out of the total *B. cucumis* caught at that time was also analysed. To account for the varying numbers of total *B. cucumis* caught, a generalised linear mixed model (GLMM) with a binomial distribution was used. A probit link function was used with a random term representing block and the fixed term representing the lure type. An across times GLMM analysis was also conducted on the proportion of female *B. cucumis* caught using the approach above, with the random model now including a term representing sampling time.

All statistical analyses were performed using GenStat for Windows 14th Edition (VSN International 2011).

RESULTS

All sites

Few *B. cucumis* were trapped at Dimbulah, Rocky Creek and Walkamin. These trials ran between September and November 2012 and likely reflect lower populations of *B. cucumis* during spring. Lower numbers of *B. cucumis* were also trapped at Spring Creek during this period but dramatically increased in December. Results for all sites are summarised in Table 2. Due to the low numbers at Dimbulah, Rocky Creek and Walkamin, data were not analysed further.

Table 2 Summary of *B. cucumis* trap catches at five sites on the Atherton Tablelands from September 2012 to January 2013

	Cucumber volatile lure			Orange ammonia			Cera Trap			Control		
	Traps with *B. cucumis*†	Weeks til first *B. cucumis*	Total *B. cucumis*	Traps with *B. cucumis*†	Weeks til first *B. cucumis*	Total *B. cucumis*	Traps with *B. cucumis*†	Weeks til first *B. cucumis*	Total *B. cucumis*	Traps with *B. cucumis*†	Weeks til first *B. cucumis*	Total *B. cucumis*
Cucumber Walkamin 14/9–15/10/12	8/20	2	17	3/20	2	3	0/20	nil	0	0/20	nil	0
Zucchini Dimbulah 11/9 –16/10/12	1/20	2	3	1/20	2	1	0/20	nil	0	1/20	5	1
Watermelon Rocky Ck 16/10–26/11/12	2/24	1	3	3/24	5	3	0/20	nil	0	0/20	nil	0
Pumpkin Dimbulah 22/10–26/11/12	6/24	2	9	3/24	6	6	0/20	nil	0	0/20	nil	0
Pumpkins Spring Ck 16/10–3/1/13	37/44	1	1033	26/44	4	925	24/44	5	129	20/44	1	152
Percentage traps with B. cucumis‡	41%			27%			18%			16%		
Average weeks until detection		1.6			3.8			NC§			NC§	
Total B. cucumis			1065			938			129			153

†Trap clearances with *B. cucumis*/total trap clearances.
‡Total trap clearances with *B. cucumis* all sites/total trap clearances all sites × 100.
§NC = not calculated, as there were several weeks where no flies were caught.

Table 3 Number of traps out of four that caught *B. cucumis* at Spring Creek over 11 sampling periods from 22 October 2012 to 3 January 2013

Week	Cucumber volatile lure	Orange ammonia	Cera Trap	Control
1	2	0	0	1
2	3	0	0	0
3	4	0	0	0
4	4	1	0	1
5	2	1	1	2
6	2	4	3	1
7	4	4	4	1
8	4	4	4	2
9	4	4	4	4
10	4	4	4	4
11/12	4	4	4	4
Total	37	26	24	20

Bactrocera cucumis were caught in 41% of the cucumber volatile lure trap clearances, 27% of the orange ammonia clearances, 18% of the Cera Trap clearances and 16% of the control clearances. The average trapping time until the first *B. cucumis* detection was 1.6 weeks for the cucumber volatile lure and 3.8 weeks for orange ammonia. *B. cucumis* was detected in the Cera Traps at only one of the five sites, and it took 5 weeks for the first detection. The control detected *B. cucumis* at two sites, and it took on average 3 weeks for the first of these detections (see Table 2).

Spring Creek: total *Bactrocera cucumis* per day

Table 3 shows the number of traps at Spring Creek that caught *B. cucumis* over the 11 sampling periods for each trap type. The cucumber volatile lure trap was the only trap type to consistently catch *B. cucumis* across the 12 weeks. Orange ammonia traps did not catch any *B. cucumis* until week 4, while the Cera Traps did not catch any *B. cucumis* until week 5.

The results from the ANOVA on the total number of *B. cucumis* detected per day for each sampling period suggests that significant differences between the treatments were found in weeks 2, 3, 4, 8 and 11/12 ($P < 0.05$) (Table 4). At weeks 2, 3, 4 and 8, the cucumber volatile lure caught significantly more flies per day than the other three traps. At week 11/12, the orange ammonia trap caught more flies per day than the control and Cera Trap, but not significantly more than the cucumber volatile lure trap.

Figure 1 shows the mean number of *B. cucumis* detected per day across the 12 week sampling period for each trap. The *x*-axis represents the number of days since the traps were first put in the field and the bars represent the 95% LSD at sampling times where there were significant treatment differences ($P < 0.05$).

Spring Creek: female *Bactrocera cucumis* per day

The results from significant ANOVA ($P < 0.05$) on the number of female *B. cucumis* caught per day are shown in Table 5.

Table 4 Mean total *B. cucumis* per day at four trap types at Spring Creek from 22 October 2012 to 3 January 2013, showing weeks with significant differences between trap types

	Week 2	Week 3	Week 4	Week 8	Week 11/12
Trap	29/10/12	3/11/12	12/11/12	10/12/12	3/01/13
Cucumber volatile lure	0.429a	0.600a	0.361a	6.536a	7.154ab
Orange ammonia	0.000b	0.000b	0.028b	0.464b	14.115a
Cera trap	0.000b	0.000b	0.000b	0.286b	0.692b
Control	0.000b	0.000b	0.028b	0.321b	1.288b
95% LSD	0.3364	0.3918	0.2100	4.7487	9.8437

Means in the same column with a letter in common are not significantly different ($P > 0.05$).

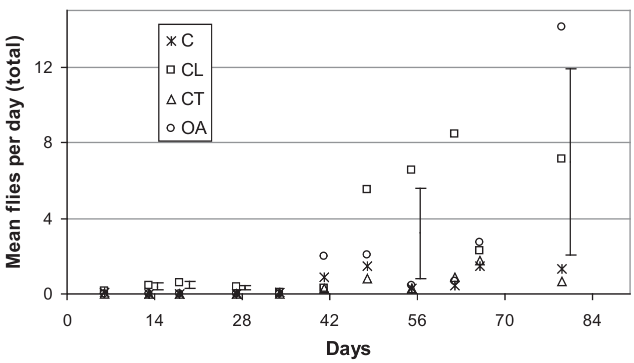

Fig. 1. Mean *B. cucumis* per trap per day at four trap types at Spring Creek from 22 October 2012 to 3 January 2013. Bars represent the 95% least significant difference (LSD). C = control, CL = cucumber volatile lure, CT = Cera Trap, OA = orange ammonia.

There were significant differences between lure type in weeks 2, 4, 7, 8 and 11/12. At weeks 2, 4 and 8, the cucumber volatile lure caught significantly more female *B. cucumis* per day than the other three traps. At weeks 7 and 12, there was no significant difference between the number of female *B. cucumis* caught per day in the cucumber volatile lure and orange ammonia traps. Significantly, more female *B. cucumis* were caught per day with the cucumber volatile lure compared with the control and Cera Trap in week 7.

Figure 2 shows the mean number of *B. cucumis* females detected in each trap at each sampling time and the bars represent the 95% LSD. The 95% LSD is only shown for sampling times where there were significant treatment differences ($P < 0.05$).

Spring Creek: proportion of female *Bactrocera cucumis* trapped

The results from the GLMM applied to the proportion of female *B. cucumis* caught in samples 6–11 are presented in Figure 3. The GLMM was not able to converge for the first five sampling periods, most likely due to the low number of traps that caught flies and hence no results are presented. It shows the back-transformed mean proportions of female *B. cucumis* caught per day obtained from the GLMM. The noticeable feature is the steady increase in the proportions for the orange ammonia trap, which show signs of plateauing after week 9 (63 days).

Pairwise comparisons of the overall proportion of females caught per day shows that there was no significant difference between cucumber volatile lure (0.87) and orange ammonia (0.84), but cucumber volatile lure did trap a significantly higher proportion of females than the control (0.75).

DISCUSSION

The cucumber volatile lure traps attracted *B. cucumis* more consistently, catching them in 41% of all the trap clearances compared with 27% of the orange ammonia, 18% of the Cera Trap and 16% of the control trap clearances. At Spring Creek, it was the only lure type to trap *B. cucumis* consistently across the 12 weeks (see Table 3). The cucumber volatile lure also caught *B. cucumis* earlier on average (1.6 weeks) than the orange ammonia (3.8 weeks), Cera Trap or control (which often did not trap any flies, see Table 2).

The cucumber volatile lure was more attractive at low population densities than the other traps (see Tables 2 and 3). Low numbers of *B. cucumis* were trapped at all sites from September to November. The data from this period indicate that in areas of low pest prevalence, the cucumber volatile lure is able to detect *B. cucumis* earlier, in more traps and in higher numbers than the other lures.

Little is known of the biology and seasonal phenology of *B. cucumis*, likely due to its lack of response to known male lures, making it difficult to monitor. It appears from this study that on the Atherton Tablelands, populations are low in spring from September to November and rapidly increase in December, the start of summer. Trapping throughout the year and at different sites throughout the pest's distribution would help to clarify seasonal population trends for this species.

At Spring Creek, where the highest numbers of *B. cucumis* were collected, the cucumber volatile lure trapped a higher mean number of *B. cucumis* per day than the other traps for all trap clearances except weeks 6, 10 and 11/12. The lures were recharged after week 6 so the decline in attractiveness of the cucumber volatile lure may be an indication of its reduced longevity in the tropics, with attractiveness declining after 4–5 weeks. Cucumber volatile lures are replaced every 6 weeks in Hawaii (M Siderhurst unpubl. data 2012) though Hawaii has a more subtropical climate so lures would be expected to have better field longevity. The cucumber volatile lure caught significantly more *B. cucumis* than the other traps in weeks 2, 3,

Table 5 Mean total female *B. cucumis* per day at four trap types at Spring Creek from 22 October 2012 to 3 January 2013, showing weeks with significant differences between trap types

	Week 2	Week 4	Week 7	Week 8	Week 11/12
Trap	29/10/12	12/11/12	3/12/12	10/12/12	3/01/13
Cucumber volatile lure	0.286a	0.278a	3.357a	5.821a	6.173ab
Orange ammonia	0.000b	0.000b	1.321ab	0.357b	12.827a
Cera Trap	0.000b	0.000b	0.571b	0.286b	0.558b
Control	0.000b	0.028b	0.607b	0.179b	1.135b
95% LSD	0.2086	0.1629	2.0566	4.1331	8.8533

Means in the same column with a letter in common are not significantly different ($P > 0.05$).

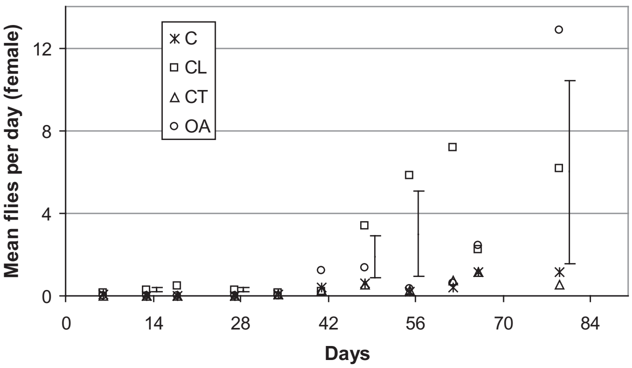

Fig. 2. Mean female *B. cucumis* per trap per day at four trap types at Spring Creek from 22 October 2012 to 3 January 2013. C = control, CL = cucumber volatile lure, CT = Cera Trap, OA = orange ammonia.

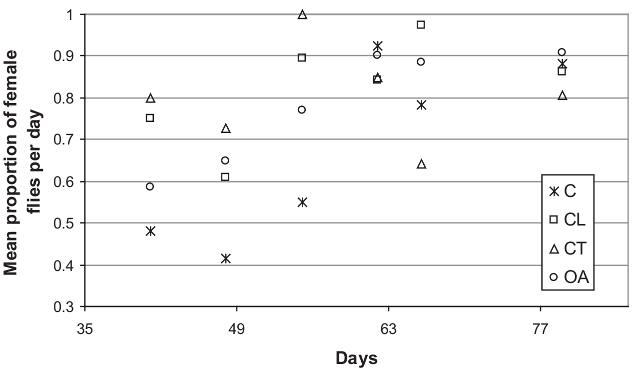

Fig. 3. Proportion of female *B. cucumis* caught at different trap types at Spring Creek from 22 October 2012 to 3 January 2013. C = control, CL = cucumber volatile lure, CT = Cera Trap, OA = orange ammonia.

4 and 8, and in the remaining weeks, none of the other traps caught significantly more of this species than the cucumber volatile lure. Although the orange ammonia caught considerably more *B. cucumis* in weeks 6, 10 and 11/12, this was not significant. These data indicate that this lure may be more effective in monitoring abundance but further trials are needed to confirm this.

The female trap catches at Spring Creek reflect the findings for both sexes discussed earlier. The cucumber volatile lure had a higher mean female trap catch at Spring Creek in all weeks except weeks 6, 10 and 11/12, at which time orange ammonia had a higher mean female catch. The cucumber volatile lure caught significantly more females at Spring Creek in weeks 2, 4 and 8 than the other three traps, but the other traps did not catch significantly more females than the cucumber volatile lure for the remaining weeks. There was no significant difference in the proportion of females caught in the cucumber volatile lure compared with orange ammonia and the Cera Trap. While the cucumber volatile lure did not have a significantly more female-biased attraction than the other lures (except the control), it still had a female-biased attraction (see Fig. 2), catching between 60% and 95% females. The female-biased attraction confirms findings of Siderhurst and Jang (2010) with this lure and *B. cucurbitae*.

The peak in *B. cucumis* numbers at Spring Creek for orange ammonia traps occurred in weeks 6 and 11/12. This may be due to the declining attractiveness of the cucumber volatile lure.

Of interest were the total numbers of *B. cucumis* at the control trap, which were higher than that in the Cera Trap. This seems to illustrate the importance of trap colour and shape in attracting this species. Fruit flies are attracted to hosts through a combination of olfactory and visual stimuli. They are attracted from a distance to fruit by olfactory cues then orientate short range to the fruit using visual stimuli such as colour, shape and size (Economopoulos 1989). It is surmised that *B. cucumis* was attracted to the crop by the odour of the hosts and to the McPhail control traps by the yellow colour and round shape which mimicked the colour and shape of pumpkin, a *B. cucumis* host. *B. cucumis* may also have been less attracted to the Cera Trap protein lure if they had been foraging for protein from other sources such as bird faeces and bacteria on fruit (Drew & Lloyd 1987; Prokopy *et al*. 1993). Protein fed flies are known to be less attracted to protein lures (Vargas & Prokopy 2006). The shape of the Cera Trap may have also contributed to its lower trap catches. While it does have a yellow top giving a colour cue to flies, it is cylindrical rather than round perhaps giving less visual cues than the McPhail trap that it is fruit like. In summary, if *B. cucumis* were protein fed, they may have been responding more strongly to the colour and shape cues of the McPhail control than the protein cue of the Cera Trap.

The results from this study indicate that the cucumber volatile lure would be a useful monitoring tool for early detection and routine monitoring of *B. cucumis*. The lure detected

B. cucumis earlier, and more consistently throughout the trapping periods and at lower population densities than the other wet traps. Cucumber volatile lure traps are cleaner to service, and as flies are trapped in clear 10% propylene glycol solution, specimens are of a better quality than those from orange ammonia or Cera Trap, making accurate identification easier. The cucumber volatile lure had a female-biased attraction, which would have the added advantage in a trapping programme in controlling the sex that causes the damage to fruit through egg laying and subsequent larval development.

Further studies are needed to determine whether cucumber volatile lure will consistently catch significantly more *B. cucumis* than orange ammonia (particularly if the lures are replaced more regularly, e.g. at 4–5 weeks), the effective radius of traps baited with cucumber lure and the proportion of the population captured by traps baited with this lure. This would clarify its potential application in monitoring populations to determine area freedom and areas of low pest prevalence and its potential use as a mass trapping tool to reduce pest pressure.

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