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Effect of lure age and blend on sex pheromone trap catches of the mirid *Sahlbergella singularis* on cacao in Ghana

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ABSTRACT

Mirids, *Sahlbergella singularis* and *Distantiella theobroma* (Heteroptera: Miridae), are the main cacao pests in West Africa. Females of both species produce sex pheromones composed of hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate and hexyl (*R*)-3-hydroxybutyrate, the major and minor components, respectively. Lures composed of 1000:500 μg blends of the two components pre-aged for 2, 4, 8 and 12 weeks in a gauze-walled insectary were compared with fresh lures in a field experiment in Ghana. Lures were replaced monthly. A total of 272 *S. singularis*, all male, was caught. Fresh lures and those pre-aged for 2 and 4 weeks caught similar numbers in a month while lures pre-aged for 8 and 12 weeks caught 34% and 26%, respectively, than fresh lures (83). The attractiveness of five different pheromone blends were compared in a 15-month field trapping experiment. A total of 701 *S. singularis*, all male, was caught. The highest numbers were caught in traps releasing both components with no significant difference among 1000:50, 1000:500 and 1000:1000 μg blends. Traps releasing hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate alone caught over 98% fewer individuals than two-component blends, and those releasing hexyl (*R*)-3-hydroxybutyrate alone caught similarly low numbers to unbaited controls. We recommend that 2:1 blend lures, renewed at least every two months are used for mass-trapping cacao mirids. The results are discussed in relation to previously published mirid pheromone blend optimisation and longevity studies.

1. Introduction

The most damaging pests of cacao (*Theobroma cacao* L.) in West Africa are the mirids *Sahlbergella singularis* Haglund and *Distantiella theobroma* (Distant) (Entwistle, 1972; Collingwood, 1977). Although peak population densities rarely exceed 2500 individuals ha^{-1} (Williams, 1954), annual losses from mirid feeding on cacao have been widely estimated to average 25–30% per annum (Collingwood, 1977; Babin et al., 2004; Anikwe and Makanjuola, 2013) and up to 75% in poorly-managed Ghanaian farms (Stapley and Hammond, 1959; Johnson, 1962). Since 1954, mirids have been controlled by foliar applied insecticides (Johnson, 1962; Owusu-Manu, 2002; Adu-Acheampong et al., 2015). However, an increasing market demand for organically produced cacao (Mahrizal et al., 2012), problems with pesticide-induced secondary pest outbreaks (Entwistle, 1972), farmers' illegal use of pesticides either banned (Mahob et al., 2014) or unapproved (Adu-Acheampong et al., 2015), and loss of diversity and environmental pollution (Mahob et al., 2011), have stimulated research for more ecologically benign methods of control (Babin et al., 2004; Anikwe and Makanjuola, 2013) including sex pheromones (Padi et al., 2002; Ayenor et al., 2007; Mahob et al., 2011; Sarfo et al., 2018a,b).

Female *S. singularis* and *D. theobroma* produce the same two pheromone components in essentially the same ratio (Downham et al., 2002; Padi et al., 2002), however, separation is maintained temporally as adult *S. singularis* reportedly fly at night and *D. theobroma* by day (Leston, 1973). Males of *Bryocoropsis laticollis* Schumacher, a minor mirid pest of cacao (Johnson, 1962), also respond to the same pheromone blend (Sarfo et al., 2018a). The sex pheromone, which is attractive only to males, consists of two components: (I) a diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate and (II) the corresponding monoester, hexyl (*R*)-3-hydroxybutyrate with an estimated naturally-occurring ratio of 2:1 (Downham et al., 2002; Padi et al., 2002).

Sarfo et al. (2018a,b) identified opportunities for managing cacao mirids by mass-trapping using their sex pheromone. To maximise trap catches and minimise expenditure it is important to identify both the length of time lures remain attractive in the field, and the most effective sex-pheromone blend for lures. We investigated the efficacy of lures pre-aged for up to twelve weeks before deployment in order to

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determine the longevity of the lures under field conditions. We also investigated the attractiveness of five different pheromone blends (Mahob et al., 2011) and an untreated control in a 15-month field trapping experiment.

2. Material and methods

2.1. Study sites and experimental plots

The study plots were located in organically-managed farmers' cacao at Akwadum, Ghana (06° 05′ N, 0° 21′ W), within 200 ha of mostly contiguous cacao. No insecticides had been applied for at least 5 years at these sites. The cacao trees were irregularly spaced Upper Amazon hybrids shaded by forest trees. The lure longevity experiment was made in a 3 ha plot of 10 year old trees between 3.5 and 6.0 m in height, whereas the pheromone blend experiment was made in a 5 ha plot of *ca.* 30 year old trees which averaged *ca.* 13 m height.

2.2. Lures

The pheromone components were dispensed from polyethylene vials (20 × 8 dia. × 1.5 mm thick; Just Plastics Ltd., Norwich, UK). The pheromone components, diester hexyl (*R*)-3-((*E*)-2-butenoy1)-butyrate, and the corresponding monoester, hexyl (*R*)-3-hydroxybutyrate, were synthesised at the Natural Resources Institute (NRI) as described in Padi et al. (2002) and were >97% pure by gas chromatographic analysis. Lures were prepared by adding a hexane solution (100 μL) containing the appropriate amounts of the pheromone components and an equal quantity of 4-methyl-2,6-di-*tert*-butylphenol as antioxidant, and allowing the solvent to evaporate before closing the lid of the vial. Controls contained antioxidant only.

2.3. Lure longevity experiment

Five treatments were compared in a Randomised Complete Blocks design experiment (RCBD) experiment replicated eight-fold from October 27, 2008 to July 3, 2009. The five treatments were obtained by pre-exposing batches of 50 lures charged with 1.5 mg of a 2:1 ratio of the diester:monoester plus 1.5 mg antioxidant in traps in an outdoor gauze-walled insectary shielded from direct sunlight for zero, two, four, eight and twelve weeks, respectively, and then stored at −18 °C prior to testing.

Traps were constructed from 4.5 L polyurethane bottles (26 H × 16 cm dia.) with two opposed windows (each 7.0 × 20 cm) cut in the sides as illustrated in Sarfo et al. (2018a). The bottles were inverted and filled to just below window level with a dilute solution of detergent in water, the trapping medium, and suspended from cacao trees at a height of 1.8 m, with an inter-trap spacing of 15 m and inter-block spacing of 70 m. Lures were hung on a wire just above the water surface. Traps were emptied and moved to the next location within blocks twice a week to minimise any positional effects to help compensate for the patchy distribution of mirids (Johnson, 1962; Babin et al., 2010). As Perry et al. (1980) pointed out, such a systematic rotation of traps forms a Latin square design. Lures were replaced monthly.

2.4. Pheromone blend experiment

Five pheromone treatments plus a control (Table 1) were compared in a RCBD experiment replicated 8-fold from March 25, 2007–May 5, 2008. The traps, illustrated in Sarfo et al. (2018a), were the rectangular design used by Sarfo et al. (2007, 2018a) and by Mahob et al. (2011) in similar trapping experiments. They were constructed from fluted PVC sheet ('Correx'; Sign Trade Supplies, Maidstone, UK), folded into open-ended boxes 38L x 10 W × 14 H cm lined with a second Correx sheet 38L x 9.6 W × 12 H cm coated with polybutene sticker (Agralan, Ashton Keynes, Wilts. UK) on sides and base, and deployed horizontally. The lure was suspended in the midpoint of the trap. Each experimental block measured 20 × 150 m and was separated from neighbouring blocks by 70 m of cacao tree guards. Traps were suspended on cacao trees 20 m apart in a line about 1.8 m above ground level. That height, although sub-optimal for trapping cacao mirids (Sarfo et al., 2018a), was chosen to maintain direct comparability with pheromone trap studies by Ayenor et al. (2007) and Mahob et al. (2011). The relative positions of treatments within blocks were moved one position within the line every two weeks such that every treatment occupied every position. Lures were replaced at monthly intervals.

2.5. Analysis of data

Data were analysed using GenStat 9 (Payne et al., 2006). All trap data were transformed $\sqrt{(n + 0.5)}$ to stabilize error variances. Total trap-catches were compared by ANOVA, and where ANOVA indicated significant F-ratios ($P < 0.05$), differences between means were tested by Student Newman Keuls (SNK) tests. Few mirids were captured between months 5 and 9 in the 9-month long lure longevity experiment, so GenStat's split-line procedure (R2LINES) was used to model the data for the cumulative catch from each treatment for the full 9-months trap exposure period. There were two internal controls in that experiment, the 2–4 week old lure periods were duplicated in treatments 1 and 2, and 4–6 week old lure periods were duplicated in treatments 2 and 3, so catches in these treatments were partitioned and compared by ANOVA. The lure longevity experiment was designed so that the cumulative catches from each treatment could be combined to produce a single response curve against lure age. Because of the low catch rate after the fourth's months trapping, only the first four months data were modelled. The relationship between catch and lure age from the combined data proved curvilinear so GenStat's FITCURVE directive was used to identify the most parsimonious best-fit explanatory model that minimised residual variance.

3. Results

3.1. Lure longevity experiment

A total of 274 mirids (272 *S. singularis* and two *D. theobroma*), all male, was caught. Cumulative numbers caught increased linearly for the

Table 1
Parameters (SE's in parentheses) for 'broken-stick' regressions (see Fig. 1) for the effects of lures pre-aged from nil (fresh) to twelve weeks on cumulative catch of *Sahlbergella singularis* males during a nine-month experiment. Lures were renewed monthly.

Lure	Break-point (days)			Slope 1[a]	Slope 2	Intercepts	
pre-age	X-axis	CL95%	Y-axis			Y	X_1
fresh	145 (19.3)	107–191	3.05 (0.241)	0.0164 (0.0025)ab	0.0005 (0.00354)	0.66	−40.3
two	124 (11.0)	99–150	3.14 (0.152)	0.0223 (0.0027)a	0.0008 (0.00192)	0.37	−16.6
four	133 (24.1)	69–196	2.43 (0.230)	0.0134 (0.0030)bc	0.0006 (0.00301)	0.64	−47.6
eight	117 (25.6)	42–202	1.82 (0.153)	0.0088 (0.0024)c	0.0006 (0.00174)	0.79	−90.1
twelve	131 (17.2)	96–178	1.71 (0.119)	0.0095 (0.0015)c	0.0006 (0.00149)	0.48	−50.5

[a] Means of slopes 1 followed by the same letter, and all means of slopes 2 are non-significantly different ($P > 0.05$).

first four months of the experiment, but at different rates in each treatment (Fig. 1; Table 1). Consequently the total numbers of mirids trapped also differed significantly between treatments ($F = 5.02$; df = 4,28; $P < 0.01$). There were no significant differences between numbers caught in traps with fresh lures and those with lures pre-aged for up to four weeks (Table 2), but those with lures pre-aged for eight and twelve weeks captured significantly fewer mirids than traps with fresh lures and those pre-aged for two weeks ($P < 0.05$). Traps with lures pre-aged for eight weeks prior to exposure caught 66% fewer mirids each month than did those with fresh lures, whereas those with lures pre-aged for 12 weeks caught 74% fewer (Fig. 2). However, as each lure was tested for four weeks, it follows that fresh lures may be used for up to eight weeks without a significant loss in efficacy. The relationship between lure age and the $\sqrt{(n+ 0.5)}$ numbers of *S. singularis* caught was curvilinear (Fig. 2). An exponential curve provided the best fit line (±Standard Errors for each parameter):

$$y = 2.53\ (\pm 0.095) - 1.81\ (\pm 0.077) \times (0.75\ (\pm 0.036))^{x}$$

Few mirids were caught after the fourth month of trapping (a total of 19 across all treatments), despite lures being replaced at monthly intervals (Fig. 1). The simultaneity of that event in all treatments is confirmed by the break points on the x-axes of all five split-line regressions being non-significantly different (Table 1, see 95% Confidence limits), and by the non-significant differences between second slopes; none of which was significantly different from zero.

3.2. Pheromone blend experiment

A total of 703 male mirids (701 *S. singularis* and two *D. theobroma*) was caught from March 25, 2007–May 5, 2008. The results are summarised in Table 3. Although the 1000:500 μg blend had the highest mean catch it was not significantly higher than catches with any other binary blend or the diester alone. Catches with all binary blends were significantly higher than those in traps baited with the monoester alone and unbaited control ($P < 0.05$).

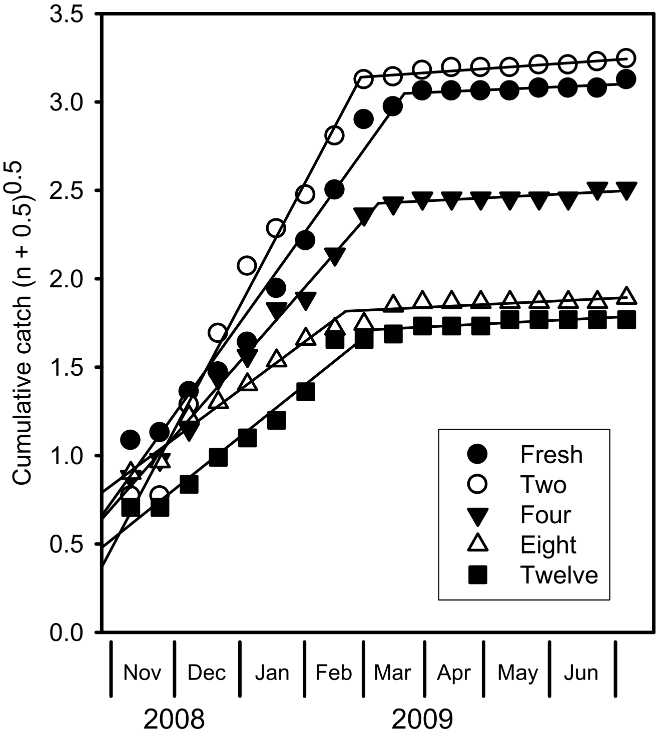

Fig. 1. Mean cumulative catches of *Sahlbergella singularis* in pheromone traps with lures either fresh (●) or pre-aged for two (○), four (▼), eight (Δ) and twelve (■) weeks. Lures were replaced monthly.

Table 2
Effect of pre-aged lures on captures of male *Sahlbergella singularis*, 27 October 2008–3 July 2009. Means followed by the same letter are non-significantly different $P > 0.05$ by SNK tests.

Lure pre-aged (weeks)	Mean $\sqrt{(x + 0.5)}$ ±SE catch *Sahlbergella singularis*
0	3.16 ± 0.504a
2	3.21 ± 0.703a
4	2.51 ± 0.744 ab
8	1.91 ± 0.471bc
12	1.77 ± 0.085c

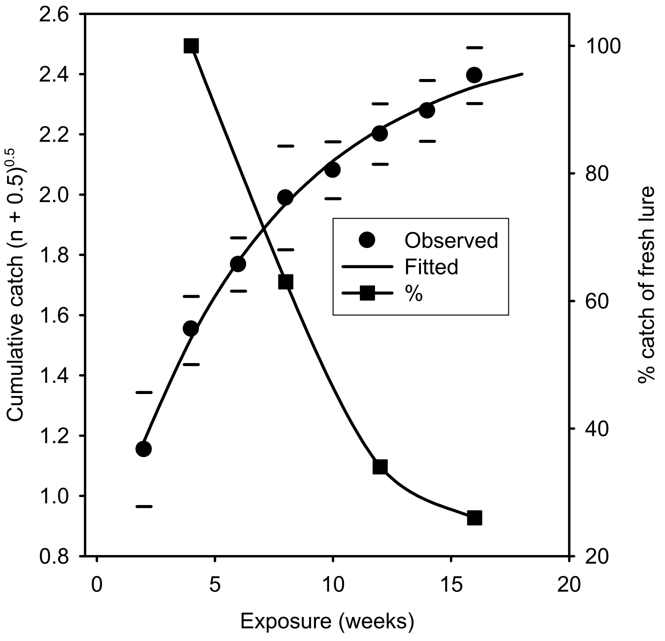

Fig. 2. Effect of lure age on pheromone trap catches of *Sahlbergella singularis* and the % catch compared with a fresh lure after four, eight and twelve weeks pre-exposure. Horizontal lines above and below observed mean cumulative catches are ±1SD. The equation for the best-fit exponential curve (fitted line) is $y = 2.53–1.81 \times (0.75)^{x}$.

4. Discussion

Lures baited with cacao mirid sex pheromones maintain their initial efficacy for eight weeks exposure in the field, but trap one third fewer than a fresh lure when exposed for a further month (Fig. 2, Table 2). Ayenor et al. (2007) replaced lures after three months which our results suggest is likely to have induced regular periodic oscillations in their data as lures lost efficacy. We recommend that lures should be changed

Table 3
Effect of pheromone blend on captures of male *Sahlbergella singularis* A: March 25, 2007–May 5, 2008 B: A plus data from Mahob et al. (2011) Experiment 1 in 2006 and 2007.

Blend diester: monoester (μg)	Mean[a] $\sqrt{(x + 0.5)}$ ± SE catch *Sahlbergella singularis*	
	A	B
1000:0	3.53 ± 0.504a	3.42 ± 0.404b
1000:50	4.07 ± 0.703a	4.21 ± 0.563 ab
1000:500	5.05 ± 0.744a	5.08 ± 0.587a
1000:1000	4.91 ± 0.471a	4.97 ± 0.584a
0:1000	0.88 ± 0.119b	1.37 ± 0.339c
Unbaited control	0.84 ± 0.085b	0.95 ± 0.104c

[a] Means in the same column followed by the same letter are non-significantly different $P > 0.05$ by SNK tests.

every two months if the aim is either to maximise catches or to quantify population fluctuations, as in Ayenor et al. (2007), although they may be used for at least another month if the aim is simply to monitor the presence of mirids. Indeed, Sarfo and Ackonor (1997) reported that initial 1000:500 μg blend lures exposed continuously for 6 months still trapped some cacao mirids.

Age associated decline in the attractiveness of pheromone lures has been widely observed in the Lepidoptera (Leonhardt et al., 1990; Lopez, 1998; Showler et al., 2005), and among Miridae (Millar et al., 1997; Millar and Rice, 1998; Innocenzi et al., 2004; Yasuda and Higuchi, 2012). Under constant temperature (27 °C) and wind speed (8 kph) in a laboratory wind tunnel, Padi et al. (2002) showed that 65% of the cacao mirid pheromone diester had evaporated in 30 days from rubber septa compared with 50% from the polyethylene vials used here and in other field experiments (Ayenor et al., 2007; Mahob et al., 2011; Mahot et al., 2020; Sarfo et al., 2018a,b).

Traps in all treatments virtually ceased catching mirids from March 2009 after four months running of the experiment. The months of shutdown (March–June) coincided with annual periods of low flight activity by *S. singularis* in Ghana (Ayenor et al., 2007; Sarfo, 2013).

The results from the blends experiment confirm some findings of Mahob et al. (2011) from their 2006 data. They found no significant differences between any pheromone treatment in 2007 despite trapping similar numbers of mirids in both years (93 vs 100, respectively). We confirm their finding of no significant differences in the response of *S. singularis* males between any of the two-component blend treatments. However, owing to our greater replication (8 vs 3) and higher catch (703 vs 93), we found that catches with the diester alone treatment, the major component, were not significantly less than any two-component blend, whereas catches with the monoester alone and unbaited controls were not significantly different confirming the findings of Mahob et al. (2011). Combining the 2006 and 2007 data of Mahob et al. (2011) with those from the present study as two additional blocks in a meta-analysis of 894 individuals (Table 3B) provides greater clarity into the response of *S. singularis* to sex-pheromone blends ($F = 16.03$; df = 5,45; $P < 0.001$). Overall, catches of male *S. singularis* were highest in traps baited with the 1000:500 μg blend, which mimics its natural sex pheromone (Padi et al., 2002), although, as before, not significantly greater than with either of the other two-component blends. However, catches with the 1000:500 μg and 1000:1000 μg blends were significantly greater than with the diester (I) alone ($P < 0.05$), while as before catches with the monoester (II) and untreated were significantly less (Table 3B).

Similar synergism between a major and minor pheromone component has been reported previously in mirid species that utilise two-component pheromone blends (Lowor et al., 2009; Zhang et al., 2015), and, as here, these mirids responded similarly to the range of two-component blends presented, with a greater response to the major component than the minor. The same behavioural flexibility to blends is also shown in mirids utilising two or more major components and one or more minor ones (Kakizaki and Sugie, 2001; Zhang and Aldrich, 2003, 2008; Byers et al., 2013; Yasuda et al., 2008, 2013; Yang et al., 2014, 2015; Zhang et al., 2016). Under constant temperature (27 °C) and wind speed (8 kph) in a laboratory wind tunnel, Padi et al. (2002) found that the monoester (II) of the cacao mirid pheromone volatilised faster than the diester (I) from a polyethylene vial such that after 9 d the diester: monoester ratio in the volatile blend released from a vial initially loaded with the 1000:500 μg ratio of components was approximately 3.5:1. On this basis, the 1000:50 μg initial loading of the two components would release nearer a 200:1 blend under the same conditions after 9 days.

Only four *Distantiella theobroma* were trapped in this study. The low numbers here and in our previous studies at Akwadum (Sarfo et al., 2018a) and Acherensua (Sarfo et al., 2018b), probably reflects a decline in its abundance in Ghana first suggested by Owusu-Manu (2002) and since confirmed from an analysis of 34 years monitoring data of mirids from the Eastern Region of Ghana by Adu-Acheampong et al. (2017).

Synthesising the present results with those from our previous experiments (Sarfo et al., 2018a,b) suggests that the current best strategy for mass-trapping cacao mirids would require 150 pheromone traps ha^{-1} (Sarfo et al., 2018b) deployed from November - end March (Sarfo, 2013) at around canopy height (Sarfo et al., 2018a) using a 2:1 diester: monoester blend released from a polyethylene lure renewed every two months. Further work is needed to establish whether such a high trap density is needed universally, or whether it might be confined to outbreak areas, *i.e.* only in so-called ‘capsid pockets’ (Entwistle, 1972). Although the precise blend of the two pheromone components is not critical, a 2:1 blend would ensure that both components were still being released at lure renewal time. Trap design is also non-critical although water traps made from discarded polyethylene terephthalate single-use bottles provide a cheap and effective option as they are readily available and would allow the external surface to be treated with a killing agent thereby, increasing trap efficiency by ca. four-fold (Sarfo et al., 2018a). Colouring traps green also more than doubles the catch (Mahot et al., 2020).

Currently, the increasing numbers of West African cacao farmers growing crops organically (Mahrizal et al., 2012) have few mirid control options other than the adoption of specific good agronomic practices (Baah et al., 2009) such as pruning and shade-tree management (Bisseleua et al., 2013). Mass-trapping with sex pheromones may provide some remedy for them and could help reduce the current reliance on synthetic pesticides for conventionally farmed cacao, and help prevent the development of resistance to the narrow range of approved insecticides (Ninsin and Adu-Acheampong, 2017).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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