Field evaluation of synthetic sex pheromone traps for the cocoa mirid *Sahlbergella singularis* (Hemiptera: Miridae)

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**Abstract**

**BACKGROUND:** Field trapping experiments were conducted to evaluate the attractiveness of five different pheromone blends as well as the impact of trap design on attractiveness towards cocoa mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.), in Cameroon.

**RESULTS:** A total of 361 adults of *S. singularis* (359 males and two females) were caught. The highest numbers of mirids were found in traps with pheromone blends that combined a monoester and a diester, compared with traps with the diester or the monoester individually and control traps with no pheromone. Rectangular traps caught significantly more mirids compared with delta traps. The mean number of 5.1 mirids trap⁻¹ year⁻¹ caught in rectangular traps was significantly higher compared with the 1.8 mirids trap⁻¹ year⁻¹ for the delta traps.

**CONCLUSION:** The data revealed that rectangular traps containing pheromone blends combining both the monoester and the diester have a good potential to lure and trap adult males of *S. singularis* on cocoa farms. The pheromone blends used were specific for *S. singularis*, and the use of pheromone traps appears to be a promising strategy for incorporation into integrated pest management strategies for the monitoring or even the control of *S. singularis* in cocoa plantations.

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**Keywords:** cocoa mirids; *Sahlbergella singularis*; *Theobroma cacao*; pheromone traps; monitoring; mass trapping

1 **INTRODUCTION**

The cocoa mirids *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.) are the two major pests of cocoa (*Theobroma cacao* L.) in West Africa and can cause production losses anywhere between 30 and 75%.¹–³ Mirids feed on pods and shoots, leading to the desiccation of leaves and the destruction of the plant canopy. Lesions usually develop into cankers, weakening trees and allowing infections by parasitic fungi, particularly *Calonectria rigidiuscula* (Berk et Br.) Sacc.⁴ When control measures are inadequate, the combination of mirid and fungal damage can lead to a rapid decline of cocoa trees.⁵ Currently, insecticide spraying remains the most effective control method in West Africa. However, in Cameroon, farmer spraying practices are often not in line with existing recommendations. Cocoa farmers usually spray insecticides that have been banned, such as endosulfan, and generally do not follow the established spraying calendars.⁶ Moreover, the use of insecticides causes a loss of biodiversity, results in environmental pollution through an accumulation of pesticide residues in soil, water and food chains and decreases biocontrol potential.⁷–⁹

Various approaches to reducing the impact of mirids have been envisioned. For instance, shade management and the elimination of alternative hosts are often recommended for mirid control. Unfortunately, these recommendations are generally not implemented by farmers.¹⁰–¹³ However, a lack of clearly defined methodologies for regulating shade means that farmers often do not know how to do so.¹³ Moreover, control approaches based on the use of resistant cultivars¹⁴,¹⁵ or the use of entomopathogenic fungi such as *Beauveria bassiana* (Bal.) Vuillemin¹⁶ have not yet yielded the desired results and are thus unavailable to farmers.

Insect sex pheromones are used in pest management for both monitoring and control. Monitoring of insect pests using pheromone traps helps pest surveillance and the forecasting of optimal timing for insecticide sprayings.¹⁷,¹⁸ Mass trapping using pheromone traps reduces insect pest populations which can lead to a reduction in damage in field crops and stored products.¹⁹–²²

Only a few studies on the sex pheromones of cocoa mirids in Africa exist. In 1973, King²³ reported the presence of a sex pheromone in virgin adult females of *D. theobroma* attractive to
adult males. In Ghana, sex pheromones were reported for both *S. singularis* and *D. theobroma*.[24,25] Preliminary results obtained in Ghana showed that the synthesised pheromones were capable of luring conspecific adult males of *S. singularis.*[24,25] However, results of long-term trapping experiments are lacking. Moreover, a thorough evaluation of the attractiveness of these pheromone blends and the effect of trap design for mass trapping and monitoring in different countries remains to be done. Therefore, the objective of the present work, using two different trap designs, was to evaluate the attractiveness of five different pheromone blends towards cocoa mirids in Cameroon.

### 2 MATERIALS AND METHODS

#### 2.1 Study site and plot description

The study was conducted in two experimental plots (plot 1: 30 x 36 m; plot 2: 36 x 36 m) located at the Institute of Agricultural Research for Development (IRAD), Nkoemvone research station. Nkoemvone is located in the southern evergreen forest area of Cameroon (2° 40’ N and 11° 20’ E), at about 630 m above sea level. The climate is equatorial, with two rainy seasons and two dry seasons. Annual rainfall varies between 1500 and 1700 mm, and mean temperature is relatively constant, around 25°C.[26,27] The soil is ferrallitic, nutrient poor and acidic.

Experimental plots were homogeneous for shade and flora, with three species of shade trees: *Cassia spectabilis* DC. (Fabaceae), *Inga edulis* (Vellozo) Martius (Fabaceae) and *Maesopsis sp.* (Rhamnaceae). Cocoa trees were spaced 3 x 3 m, and plots were surrounded by other cocoa plantations. Experimental plots were chosen on the basis of the presence of mirid damage on cocoa such as feeding lesions on pods and shoots and cankers on branches, and the absence of insecticide treatments for the last 3 years.

#### 2.2 Pheromones, traps and experimental design

In experiment 1, five different blends of the two components hexyl ([R]-3-((E)-2-butenoyloxy)-butyrate and hexyl ([R]-3-hydroxybutyrate) of the *S. singularis* female sex pheromone, namely A, B, C, D and E (Table 1), were tested over two consecutive years, 2006 and 2007. Dispensers were polyethylene vials (20 x 8 x 1.5 mm thick; Just Plastics, UK) and traps were delta traps (28 x 20 x 11.5 cm). In experiment 2, two different trap models were compared, the delta trap and a rectangular trap (45.1 x 10 x 15 cm) (Fig. 1). Both traps were white in colour and made out of recycled polyethylene and cardboard. The bottom part of the interior of the traps was covered with water-resistant glue (‘Trappit’ insect glue; Agrisense-BCS Ltd, UK), while the pheromone lure was suspended from the centre of the roof of the trap with respect to length and cross-section. The pheromone blends and traps used in this study were provided by Dr M Downham of the Natural Resources Institute (University of Greenwich, UK).

In both experiments, a completely randomised design was used. Traps were spaced at 6 m intervals approximately 2 m above ground level and oriented at random. The pheromone blends were changed every 2 months because of their anticipated temporal degradation.[28] In experiment 1, established in plot 1, three delta traps were used for each pheromone blend (Table 1). Three traps were used as control and did not contain any pheromone blend. Thus, a total of 18 traps were used. For experiment 2 in plot 2, a total of 24 traps, 12 delta and 12 rectangular, were used. For each pheromone blend (Table 1), two traps of the two designs were used. The four remaining traps (two of each model) served as controls.

<table>
<thead>
<tr>
<th>Table 1. Code and chemical composition of tested pheromone blends</th>
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<tr>
<td><strong>Pheromone blend code</strong></td>
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<tr>
<td>A</td>
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\[ a \text{ Hexyl ([R]-3-((E)-2-butenoyloxy)-butyrate.} \]

\[ b \text{ Hexyl ([R]-3-hydroxybutyrate.} \]

Recent work by Babin et al.[13] has shown that mirid populations are highly aggregated in cocoa plantations. Therefore, to control any other spatial effects, trap position was randomly permuted once every month. Traps were checked weekly, and mirids were removed from the trap, preserved in haemolysis tubes containing alcohol (70°) and identified and sexed in the laboratory.

#### 2.3 Statistical analysis

The number of mirids caught per week for each individual trap was recorded. The number of mirids per trap per year was transformed by log(x + 1), and a comparison between pheromone blends and/or trap design was done with the appropriate generalised linear model (GLM) procedure using SAS (v.9.1).[29] Means were subsequently separated using Duncan’s multiple range test (P < 0.05).

### 3 RESULTS

#### 3.1 General

In total, 354 adult *Sahlbergella singularis* males were caught in traps containing pheromone blends. For experiment 1, 93 males were caught in 2006 (6.2 mirids trap−1 year−1) and 100 males in 2007 (6.7 mirids trap−1 year−1). For experiment 2, 97 males were caught in 2006 (4.9 mirids trap−1 year−1) and 64 in 2007 (3.2 mirids trap−1 year−1). A total of seven adult mirids (three males in 2006; two males and two females in 2007) were caught in the control traps. Besides *S. singularis*, no other mirid species common on cocoa, such as *D. theobroma* or *Helopeltis* spp., were caught, even in the control traps.

#### 3.2 Experiment 1, comparison of pheromone blends

Significant differences between the different pheromone blends were found with respect to the total number of mirids caught in 2006 and 2007 (for 2006: F = 12.82, df = 5, P < 0.001; for 2007: F = 7.56, df = 5, P < 0.01). In 2006, pheromone blends B, C and D attracted significantly more mirids than blends A and E and the control. Blends A and E attracted significantly more mirids than the control (Fig. 2). In 2007, the results were similar, except that blends A and E did not differ significantly from the other blends and the control (Fig. 2).

#### 3.3 Experiment 2, comparison of trap model

The second experiment revealed that catches were significantly different between trap models. In 2006 and 2007, significantly more mirids were caught in rectangular traps than in delta traps,
irrespective of the pheromone blend (2006: $F = 59.08$, df $= 1$, $P < 0.0001$; 2007: $F = 57.79$, df $= 1$, $P < 0.0001$) (Fig. 3). Similarly to experiment 1, significant differences between pheromone blends were observed in 2006 and 2007 (2006: $F = 29.33$, df $= 5$, $P < 0.0001$; 2007: $F = 30.16$, df $= 5$, $P < 0.0001$). In 2006, pheromone blends A and E attracted significantly more mirids than the control. Pheromone blend B was significantly better than both blends A and E and equal to blend C. Traps containing blend C were equal to B and D, whereas blend D was again the best-performing blend, attracting more mirids than all other pheromone blends. In 2007, the control traps and traps with pheromone blend A attracted significantly fewer mirids than all other blends. Blends D and E were significantly better than blend A and the control, while blends B and C attracted significantly more mirids than any other blend or the control. No interaction was found between the kind of trap and the pheromone blend (2006: $F = 2.05$, df $= 5$, $P = 0.14$; 2007: $F = 2.93$, df $= 1$, $P = 0.06$) (Table 2).

3.4 Annual variation of catches

Fluctuations in the number of mirids caught per trap per month followed similar patterns for the different pheromone blends and over the course of 2006 and 2007 (Fig. 4). Three phases are recognisable in the trapping datasets. The first phase, approximately from January to March, is characterised by apparent absence of mirids. Only one mirid was caught in February 2006 in a trap with pheromone blend C (Fig. 4). The second phase, from April to June, is generally characterised by a gradual increase in the number of trapped mirids. The third and final phase, from July to January, is described by a gradual decrease in trapped mirids and ends with the ‘disappearance’ of mirids in the traps.
4 DISCUSSION

The present results clearly demonstrated the specific attractiveness of the tested pheromone blends towards conspecific males of \textit{S. singularis}. The absence of \textit{D. theobroma}, a species very close to \textit{S. singularis}, with similar morphology and biology, was particularly notable. Although the pheromone has proven to be fairly specific,\textsuperscript{24} the fact that no individuals of \textit{D. theobroma} were caught could also indicate that this species is rarely found on cocoa in this region, which would confirm the findings of Babin \textit{et al.}\textsuperscript{13}

The numbers of \textit{S. singularis} captured in the traps baited with pheromone blends B, C and D, made up of both diester and monoester, were higher than those obtained in traps with pheromone blends A and E which only contained the diester or the monoester respectively (Fig. 2). It seems that there is a positive interaction between the diester and monoester that causes them to act more effectively when combined than when used individually. In Ghana, Sarfo and collaborators (private communication, 2009) also noted that a diester/monoester mixture attracted more mirids than the individual components.

The average number of \textit{S. singularis} trapped in July (1 – 2.5 per trap) seems quite high, especially when considering the fact that only males are trapped. When taking the trap density to the hectare level, based on 167 traps ha\textsuperscript{−1}, and taking into account that the adult sex ratio is approximately 1:1\textsuperscript{10} and the adult larvae ratio is approximately 1 : 5,\textsuperscript{13} this would mean that, at the peak of July, between 2000 and 5000 mirids ha\textsuperscript{−1} are present. This is in line with previous estimations of Williams\textsuperscript{31} and Gibbs \textit{et al.}\textsuperscript{32} who estimated maximum densities of between 2500 and 7000 mirids ha\textsuperscript{−1} respectively. These densities are equivalent to approximately 2.5 – 7 mirids per tree at the peak of the pullulation period, while the economic threshold level for cocoa mirids in Cameroon has been determined at just 0.7 mirids per tree.\textsuperscript{33} The relatively large number of \textit{S. singularis} males, especially in traps with pheromone blends B, C or D, might indicate that the pheromones lure mirids from large distances away from the experimental blocks. Unfortunately, to date it has not been possible to test this hypothesis. There is a need for future research into how large-scale pheromone trapping could be integrated into IPM programmes, for example with regard to the density of pheromone traps needed per hectare. However, the present results suggest that mass pheromone trapping for \textit{S. singularis} has to be considered.

Rectangular traps captured almost 3 times more mirids than the delta traps. The fact that trap design can influence capture results has also been confirmed through observations made in Ghana, where Padi \textit{et al.}\textsuperscript{24} found that the standard delta trap caught slightly fewer males than a larger ‘modified sticky trap’. Foster and Muggleston\textsuperscript{34} studied the influence of trap design on the catch of light-brown apple moth, \textit{Epiphyas postvittana} (Walker) (Lepidoptera: Tortricidae), and found that the field catch of male \textit{E. postvittana} was linearly related to the length of the delta traps used.

The fluctuations in mirid captures were in accordance with mirid population dynamics as described by Lavabre\textsuperscript{35} and Bruneau de Miré.\textsuperscript{36} The pullulation peak that occurs from June to September was thought to be linked to the abundance of food in the form of pods on cocoa trees.\textsuperscript{37} In Cameroon, spraying calendars for cocoa mirids are based on the knowledge of population fluctuations: two insecticide applications are recommended, the first in May/June, at the beginning of the pullulation period, and the second in November/December, after cocoa harvesting.\textsuperscript{38} Rectangular traps containing pheromone blend D could be used for early detection of mirid populations. As soon as populations surpass the economic threshold of 0.7 mirids tree\textsuperscript{−1}, spraying should start. Yet, based on the results presented here, traps might be sufficient to control mirid populations, although more research is needed to answer this question unequivocally. As part of this research, the possible use of sex pheromone traps for mating disruption should be considered.\textsuperscript{23,39,40}

5 CONCLUSIONS

Irrespective of the trap design, although rectangular traps are more efficient, three pheromone blends B, C and particularly D
(1000 µg diester 1000 µg⁻¹ monoeaster) are effective in luring adult males of S. singularis. However, the impact of the use of pheromone traps on cocoa production remains to be analysed. Nonetheless, these results indicate that there is a real potential for the use of pheromone traps to monitor or even control S. singularis populations on cocoa farms in Cameroon, especially when incorporated in broad-scale IPM strategies.

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