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Visual cues from different trap colours affect catches of *Sahlbergella singularis* (Hemiptera: Miridae) in sex pheromone traps in Cameroon cocoa plantations

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ABSTRACT

Cocoa mirids, *Sahlbergella singularis* and *Distantiella theobroma*, are the most economically important insect pests of cocoa in West and Central Africa, where they can cause up to 40% losses in cocoa yields. *Sahlbergella singularis* is the most common species in Cameroon and was therefore targeted in this study. During a two-year trial in 15 cocoa plantations in Ayos and Konye, in the Centre and Southwest regions of Cameroon respectively, the most effective colour – white, purple or green – of sex pheromone traps was examined for integration into mirid management programmes. Similar temporal patterns in *S. sahlbergella* male trap catches were observed with all three colours, but the green trap caught more *S. singularis* (Ayos: 0.968 ± 0.091 [SEM]; Konye: 0.640 ± 0.076 /trap/month) compared with purple traps (Ayos: 0.674 ± 0.068 ; Konye: 0.441 ± 0.058), and white traps (Ayos: 0.467 ± 0.049 , Konye: 0.253 ± 0.041). Trap reflectance showed wavelength peaks typical for purple in both short (approximately 380–480 nm) and long wavelengths (>650 nm), and ~530 nm for green. Reflectance peaks exceeded 100% of the incident light in the 400–450 nm range from the white, suggesting the presence of optical brighteners. Trap catches support the hypothesis that *S. singularis* ocelli are more responsive to light of shorter wavelengths. Despite the low attractiveness of the sex pheromone to *S. singularis*, a optimisation of the colour of the trap can improve *S. sahlbergella* monitoring, mass trapping, and other control measures which could ultimately lead to reductions of insecticide use and to conservation of beneficial arthropods, particularly cocoa flower pollinators that are critical for improving cacao tree productivity.

1. Introduction

Cocoa (*Theobroma cocoa* L.) is one of the most important cash-crops in West and Central Africa with the Ivory Coast, Ghana, Cameroon and Nigeria being the largest producers in descending order (Wessel and Quist-Wessel, 2015). Damage by insect pests and diseases, such as cocoa mirids, cocoa pod disease, and increasingly cocoa swollen shoot virus disease, continue to be among the most important factors limiting yield (Wessel and Quist-Wessel, 2015). Pesticide treatments, while largely

effective in limiting insect pest and disease damage, are thought to cause considerable negative collateral effects on ecosystem services of beneficial insects, especially cocoa flower pollinators, which are essential for cocoa pollination and pod yield (Toledo-Hernandes et al., 2017).

Cocoa mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.) (Hemiptera: Miridae), are the most important insect pests of cocoa in the cocoa belt of West Africa (Yede et al., 2012; Sarfo, 2013; Mahob et al., 2014; Bagny Beilhe et al., 2018a, Bagny Beilhe et al., 2018b). These mirids cause damage by injecting saliva into feeding sites

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which can lead to desiccation of young shoots and leaves and appearance of cankers on pods and branches (Mahob et al., 2019). Mirid feeding damage can also promote tissue infections by opportunistic pathogenic fungi such as *Fusarium* spp. and *Lasiodiplodia* spp. (Adu-Acheampong et al., 2012, 2014; Voula et al., 2018) that can lead to cocoa dieback (Anikwe and Otuonye, 2015). There is also mounting evidence that yield losses caused by mirid feeding are principally due to abortion of young fruits (Yede et al., 2012). Severe mirid damage can cause up to 40% loss in bean yield and the risk of tree death in the absence of appropriate control (Entwistle, 1972).

The biology, ecology and management of *S. singularis* and *D. distantiella* have been the subject of numerous studies (Entwistle, 1972; Kumar and Ansari, 1974; De Miré, 1977, 1985; Nwana and Youdewei, 1978; Decazy, 1979; Nwana et al., 1979; Babin et al., 2008, 2010; Mahob et al., 2011; Bagny Beilhe et al., 2018a). Conventional pesticides remain the most widely used option in the control of cocoa mirids (Mahob et al., 2014), which has led to widespread insecticide resistance, secondary pest outbreaks and negative effects on beneficial arthropods, especially cocoa flower pollinators (Sarmah et al., 2004; Tijani, 2006; Geiger et al., 2010; Kibria, 2016; Bagny Beilhe et al., 2018a; Humann-Guillemot et al., 2019). Risks linked to the use of chemical insecticides, including stringent legislation by cocoa-importing countries, have led to numerous efforts to develop alternative mirid control approaches, including host plant resistance (e.g. Sounigo et al., 2003), cultural control through shade management (e.g. Babin et al., 2010), biological control (e.g. Bagny Beilhe et al., 2018b), and semi-chemicals and biopesticides (Padi et al., 2002; Ayenor et al., 2007; Anikwe and Makanjuola, 2013; Mahob et al., 2011; Posada et al., 2010; Sarfo et al., 2018a, b).

Sex pheromones have been identified for a wide range of insect species (e.g., Witzgall et al., 2010; Miller and Gut, 2015; El-Sayed, 2017; Gregg et al., 2018) and used in a variety of ways, e.g., monitoring, pheromone confusion, mass trapping. Female sex pheromones were identified for *S. singularis* and *D. theobroma* by Padi et al. (2000, 2002) and attraction of male mirids to white and clear plastic sticky and water traps baited with the pheromone and their potential for monitoring mirid populations and mass trapping have been demonstrated in Ghana (Ayenor et al., 2007; Sarfo et al., 2018a, b) and in Cameroon (Mahob et al., 2011). None of the previous studies has determined, however, the effect of sex pheromone trap colour on catches of the two mirid species. There is also a need to determine the performance of the sex pheromone traps across a range of abiotic environments and pest pressures.

Trap colour is known to affect attraction of hemipteran and thysanopteran insects to unbaited traps (e.g. Thongjua et al., 2015; Hassan and Mohammed, 2004; Ranamukhaarachchi and Wickramarachchi, 2007; Blackmer et al., 2008; Mazzoni et al., 2011). However, the effect of colour on the attractiveness of traps baited with a chemical attractant has been less frequently investigated (e.g. Sampson et al., 2012; Arnold et al., 2015). In this study, we evaluated three colours of a sticky trap design to optimise mirid sex pheromone trapping in cocoa farms across a range of abiotic environments in Cameroon, with the ultimate aim of improving mirid monitoring and the potential application of pheromone traps in mass-trapping and auto-dissemination of entomopathogenic fungi that are under development for mirid microbial control (Lopes et al., 2014; Mahot et al., 2019).

2. Materials and methods

2.1. Study areas

The study was conducted over a period of 24 months (September 2014 through August 2016) in 15 cocoa plantations in the Centre (Ayos municipality) and Southwest (Konye municipality) regions of Cameroon, two of the areas with the highest cocoa production in Cameroon (Fig. 1) (Jagoret, 2011). In Ayos, the study was conducted in eight plantations in three villages, Ebeck (3), in Meto'o (3) and in Ekok

(2). In Konye, the study was conducted in seven plantations - Kokobuma (2), Baduma (2), Matondo (1) and Diongo (2) (Fig. 1). We used the agro-ecological zone classification developed by the Cameroon Institute of Agronomic Research for Development (IRAD). The Ayos municipality is in the humid forest agro-ecological zone characterised by dense tropical forest and equatorial climate with two rainy seasons (March–July and September–November) separated by two dry seasons (December–February and August). Total rainfall and average temperature and relative humidity are 1440 mm, 25 °C and 78% respectively (IRAD, 2008; Anonymous, 2015, 2016). The Konye municipality is in the lowland humid forest agroecology with equatorial climate characterised by a mono-modal rainfall pattern of abundant precipitation (2461 mm) and average temperature and relative humidity of 27 °C and 80% respectively (IRAD, 2008; Anonymous, 2015, 2106).

2.2. Experimental design

The study was carried out in 15, 20–40 year-old traditional cocoa plantations with relatively high occurrence of *S. singularis* and absence of insecticide treatments for at least two years before the initiation of experiments. One experimental plot (100 × 50 m) was delimited within each of the selected cocoa plantations. Each plot contained at least 600 cocoa trees with spacing that ranged between 2.5 × 2.5 m and 3 × 3 m. Six pheromone traps of each of the three colours (see below for description) were placed in each experimental plot. Traps were randomly assigned to trees within each plot with approximate spacing of 12.5 m between traps within a row and 15 m between rows. Traps were rerandomised in each experimental plot at two-month intervals because of the aggregated distribution of *S. singularis* (Babin et al., 2010). According to our trap deployment design, a trap is a sampling unit and a plantation is the experimental unit (or replication).

2.3. Trap design

The standard rectangular sticky trap (40 × 10 × 15 cm) was used due to its proven effectiveness in pheromone trapping of *S. singularis* (Mahob et al., 2011; Sarfo, 2013). The traps were fabricated locally with a 2-mm thick Plexiglas sheet in three colours, green and purple that resembles the colours of cocoa leaves and pods, and white to match traps used in studies by Mahob et al. (2011) and Sarfo et al. (2018a). The internal trap surface was lined on three sides (base and two vertical sides) with thin polyethylene sheet coated with a film of clear Tanglefoot® (Seabright Laboratories, Emeryville, CA, USA). Pheromone lures were prepared at the Natural Resources Institute and consisted of polyethylene vials (20 × 8 × 1.5 mm; Just Plastics, UK). The vials were impregnated with 1 mg of hexyl (R)-3-[(E)-2-butenyloxy]-butyrate + 0.5 mg hexyl (R)-3-hydroxy butyrate and an equal amount of butylated hydroxyl toluene as antioxidant (Padi et al., 2000, 2002; Mahob et al., 2011; Sarfo et al., 2018a). One lure was suspended with a wire from the inside centre roof of the trap. Traps were suspended in the canopy of cocoa trees at approximately 2 m above ground to facilitate trap servicing. Damaged or missing traps were replaced as needed. The experiments did not include unbaited control traps because the attractiveness of the pheromone lure to *S. singularis* has been demonstrated previously (Mahob et al., 2011; Sarfo, 2013).

Traps were monitored at 14-day intervals. Captured insects were removed and placed in tubes with 70% alcohol for later identification using the dichotomous keys of Entwistle (1972) and Lavabre (1977). The sex of trapped mirids was determined by the presence or absence of an ovipositor in females and males, respectively. The polyethylene sheet lining the inside of the traps and the pheromone lures were replaced every 2 months as loss of attractiveness usually occurs after eight weeks of pheromone placement in the field (Sarfo, 2013).

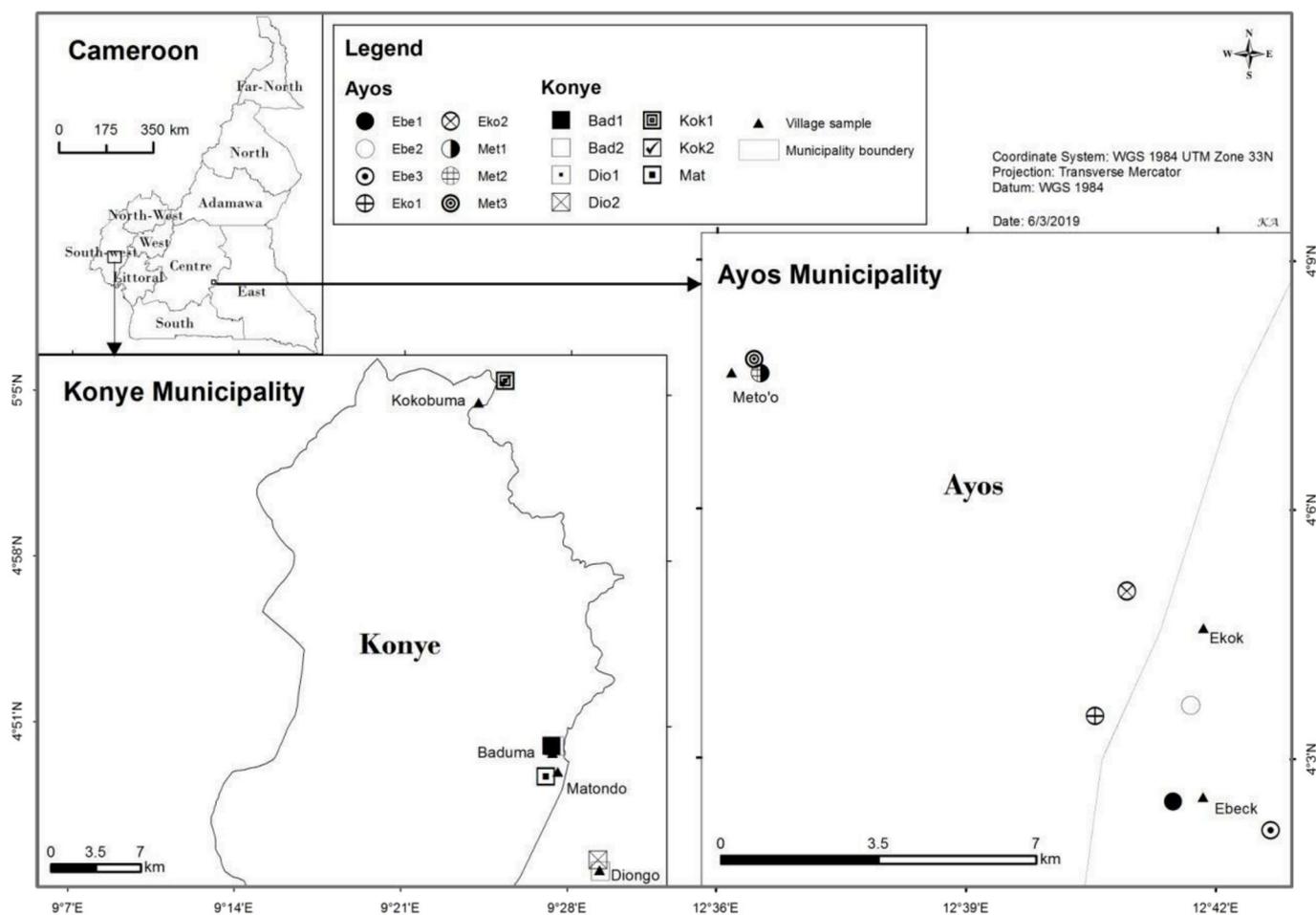


Fig. 1. Location of the 15 plantations used in the experiments during the two years of the experiments in Ayos and Konye Municipalities, respectively in Cameroon's Centre and Southwest regions. Each circle or square represents a plantation. Six traps of the three colours (green, purple and white) were deployed at each site.

2.4. Spectral reflectance of trap materials

Spectral reflectance curves for the trap materials were measured according to Chittka and Kevan (2005) using an Avantes AvaSpec-2048 spectrophotometer and an AvaLight-DH-S-BAL Deuterium-Halogen light source (Avantes, Leatherhead, Surrey, UK), calibrated relative to a BaSO₄ white standard (Avantes WS-2). Measurements were taken with a fine probe (FCR7UV200-2-1.5 x 100) at 45° to the stimulus surface. The resulting reflectance values are the mean of three measurements on each of the front and reverse side of each green and purple samples, corresponding to the outside and inside of the trap. Only the front side of white traps was taken because the reverse side of the white trap is the same as the front side.

2.5. Data analysis

Sahlbergella singularis catches for each trap colour within each plantation (six traps of each colour in each plantation) were averaged on a monthly basis after summing two consecutive, 14-day counts. Monthly catch counts were then compared separately for each location (Ayos or Konye) by univariate repeated measures analysis of variance on trap counts, with trap color and month as fixed factors and plantation (i.e. replication) as random factor.

A second analysis of variance was used to compare the effect of colour (sum of average monthly *S. sahlbergella* trap counts over 12 consecutive months of sampling) and location (Ayos and Konye) as fixed factors, with year as time factor and plantation (replicate) as random factor. Where needed, Tukey's HSD was used for post-hoc comparison of

means at $\alpha = 0.05$. Monthly trap counts were log-transformed before each analysis to correct for unequal variances inherent in count data. All statistical analyses were performed with JMP Pro 14.3. (SAS Institute, 2019).

3. Results

The number of *S. singularis* caught in traps depended on trap colour (Ayos: $F_{2,14} = 94.8$, $P < 0.001$, Konye: $F_{2,12} = 44.2$, $P < 0.001$) and month of sampling (Ayos: $F_{23,483} = 24.8$, $P < 0.001$; Konye: $F_{23,405} = 9.29$, $P < 0.001$) in each of the two locations (Fig. 2). Green traps caught the highest number of *S. singularis* (Ayos: 0.968 ± 0.091 ; Konye: 0.640 ± 0.076 /trap/month), followed by purple (Ayos: 0.674 ± 0.068 ; Konye: 0.441 ± 0.058), and white traps (Ayos: 0.467 ± 0.049 , Konye: 0.253 ± 0.041). Mean catches in the three differently coloured traps were significantly different at each location (Tukey HSD, $P < 0.05$).

Differences in trap colours at each location were not consistent across months as evident in sampling month and trap colour interactions before critical F value sphericity adjustment (Ayos: $F_{46,483} = 1.61$, $P = 0.009$; Konye: $F_{46,405} = 1.46$, $P = 0.030$). The highest trap catches occurred during the rainy season from May–October with another peak after harvest in February–March (Fig. 2). In Ayos, the variance components of the random factors - plantations, plantations x trap colour and plantation x trap colour x month explained 3.39, 0 (negative), and 7.93% of the variance with Wald P values of 0.075, 0.034, and < 0.001 respectively. In Konye the random factors plantations, plantations x trap colour were not significant while plantation x trap colour x month

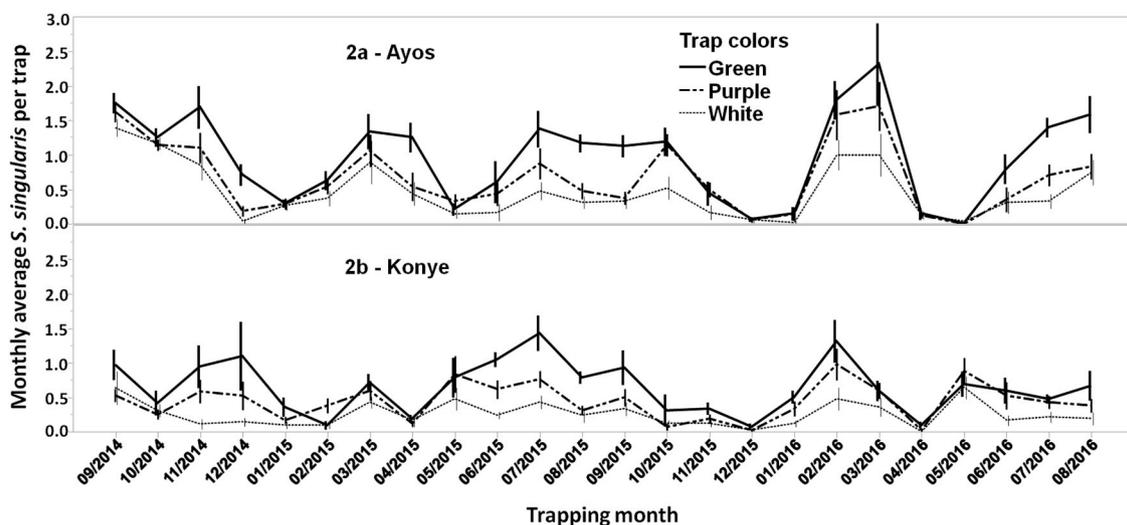


Fig. 2. Monthly counts (mean ± SEM) of the cocoa mirid, *Sahlbergella singularis*, in three colours of sex pheromone trap in Ayos (upper) and Konye Municipalities (lower).

explaining 6.48% of the variance and a Wald $P < 0.001$.

In the second analysis, where monthly counts were pooled for each year (12 consecutive months) of sampling, similar differences in male *S. singularis* counts were observed with trap colours independent of year for both locations (Tables 1 and 2). Counts of *S. singularis* in traps were higher overall in Ayos than in Konye and followed similar patterns in both years (Tables 1 and 2). Trap counts were higher in year 1 than in year 2 in both locations. The random factors plantation and plantation by trap color contributed insignificantly to total variation of the random factors in both locations (Wald $P = 0.075-0.882$).

Spectral reflectance of trap materials from the front (external) and reverse (internal) side of each sample were similar, but overall reflectance was lower from the reverse surface, which corresponds to the inside surface the trap (Fig. 3). Front and reverse surfaces of white traps had similar reflectance values. The purple trap material had reflectance peaks in both short (approximately 380–480 nm) and long wavelengths (>650 nm). The reflectance curve for the green trap material was typical for a “plastic” green with a peak at approximately 530 nm, similar to that from green leaves. The white trap presumably contained fluorescent optical brightener(s) as the reflectance peaks exceeded 100% of the incident light in the 400–450 nm range (Fig. 3) (Goulson et al., 2000; Arnold et al., 2015).

4. Discussion

The principal objectives of our study were to determine the effect of trap colour on attraction of male *S. singularis* to pheromone traps, and the level of consistency in the attractiveness across a large number of cocoa plantations in different environments (i.e. areas with different climates), all using a single trap design recommended by Mahob et al. (2011). Our results clearly support the hypothesis that appropriately

Table 1

Number of male *Sahlbergella singularis* (mean ± SE) in three colours of sex pheromone traps over two years (24 consecutive months) of trapping in Ayos and Konye municipalities of Cameroon.

Trap colour	Ayos		Konye	
	Year 1	Year 2	Year 1	Year 2
Green	12.3 ± 0.90a	11.1 ± 1.41a	8.50 ± 0.89a	6.57 ± 1.21a
Purple	8.63 ± 0.89b	7.54 ± 1.05b	5.48 ± 0.57b	4.93 ± 0.88b
White	6.50 ± 0.59c	4.67 ± 0.78c	3.21 ± 0.50c	2.76 ± 0.54c

*Values followed by the same letter in a column are not significantly different (Tukey HSD, $P > 0.05$).

Table 2

Analysis of variance of the effect of trap colour on counts of male *Sahlbergella singularis* in sex pheromone traps over a period of two years (24 consecutive months) in Ayos and Konye municipalities in Cameroon.

Sources of variation	Ndf ^a	Ddf ^a	F ratio	Prob > F
Ayos				
Trap colour (Green, Purple, White)	2	14	94.8	<0.001
Year (1 and 2)	1	21	7.12	0.014
Trap colour ^a Year	2	21	0.204	0.817
Konye				
Trap colour (Green, Purple, White)	2	12	46.3	<0.001
Year (1 and 2)	1	18	5.92	0.026
Trap colour ^a Year	2	18	1.41	0.269

^a Ndf and Ddf are respectively numerator and denominator degrees of freedom.

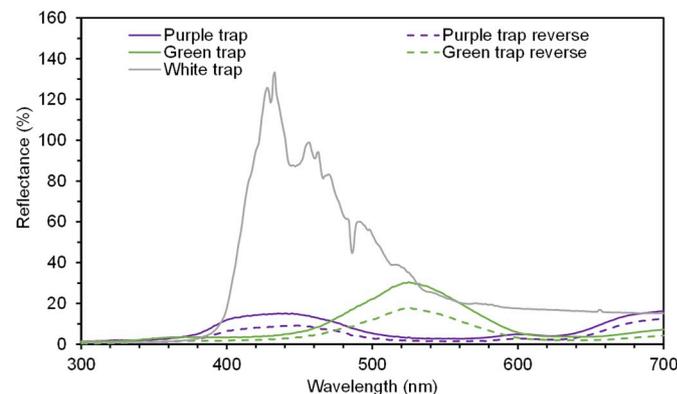


Fig. 3. Relative reflectance from two sides of purple, green and one side of white materials used in the construction of traps in the experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

coloured traps can improve catches of *S. singularis*. Green (30.5% and 18.3% reflectance on front and reverse side respectively) was more attractive than purple (16.2% and 12.5% reflectance) followed by the standard white trap colour (reflectance: 133.4%). Green coloured traps resulted in nearly two-fold increase in catches of male *S. singularis* over those in the standard white trap. Of particular significance, the

difference among trap colours was independent of regions (despite agro-ecological differences), abundance of *S. singularis* or time-period, thus supporting the conclusion that using green traps is a reliable method of increasing catches of *S. singularis* in sex pheromone trap across a broad spectrum of the species densities and geographic locations.

When testing and optimising coloured traps, it is essential to work with quantified stimuli, i.e. colours that have been measured and reported in a way that is free from the bias of human visual perception. Extensive research has shown that colours that appear similar to the human eye can appear different to insects (Chittka et al., 1994). Complementary control approaches that include visual recognition and facilitate identification of host-plant cues might therefore improve insect monitoring tools (Mazzoni et al., 2011). Vision in Hemiptera is less well-characterised than in the Hymenoptera, Diptera or Lepidoptera. Vision studies in Hemiptera have been limited to aphids (Kirchner et al., 2005; Döring and Chittka, 2007), backswimmers *Notonecta* spp. (Bruckmoser, 1968; Bennett and Ruck, 1970), the stink bug *Nezara viridula* (L.) (Nobuyuki et al., 2014) and the psyllids *Ctenarytaina eucalypti* (Maskell), *C. bipartite* sp. n., *Anoconeossa bundoorensis* sp. n., and *Glycaspis brimblecombei* Moore (Farnier et al., 2014). All of these groups, as for most insects, have three (trichromatic) sensitivity peaks: in the UV (< 400 nm), blue (400–500 nm) and green (500–570 nm) (Fennell et al., 2019). Probably *S. sahlbergella* also has three photoreceptors like the majority of Hemiptera, and uses the green:blue opponency or green: (blue + UV) opponency to locate its host plant. So green or yellow traps are likely to be preferred during landing. Additional longer wavelength (>570 nm) sensitivity in tetrachromate Lepidoptera, Hymenoptera and Coleoptera may exist in *S. singularis* which would promote attraction by purple with high reflectance peak at ~700 nm (Fennell et al., 2019). Stimuli appearing blue, green and yellow to the human eye are likely to be highly prominent to Hemiptera species, but long-wavelength reflecting stimuli (i.e. red colours) are less readily detected. Ashfaq et al. (2005) reported that short wavelengths attract more insects than long wavelengths and insect ocelli are more responsive to light of shorter wavelength.

Reflectance measurement of the purple trap material showed two peaks; at the short and longer wavelength bands, but also an intermediate peak like that from green leaves. In comparison, reflectance from foliage tends to peak around 550 nm (Chittka et al., 1994). The white reflectance peaks exceeded 100%, most probably due to the presence of a fluorescent optical brightener (Goulson et al., 2000; Arnold et al., 2015). It is possible that mirids may be repelled by this fluorescence, leading to lower catches in white traps, although this was shown not to be the case for the maize weevil, *Sitophilus zeamais* (Motsch.) (Arnold et al., 2015). Moericke (1950, 1952) also demonstrated that aphids were not attracted to white colour and the landing response to other tested colours was highest against achromatic stimuli.

We deployed traps with medium-wavelength reflectance (green to humans) and traps with both short and long-wavelength reflectance peaks (purple to humans). The green traps would likely stimulate the long wavelength receptor of a trichromatic insect, whilst the purple traps would likely stimulate the short and medium wavelength receptors. Holopainen et al. (2001) reported that unbaited blue sticky traps were far more attractive to *Lygus rugulipennis* (Poppius) than yellow. The former had a reflectance peak at 450 nm like that of the green trap used in our study, while the latter showed peak reflectance >600 nm. Previous work on *Lygus* spp. showed poor responses to “red” and “orange” unbaited traps (Blackmer and Cañas, 2005), although both trap colours had reflectance around 500 nm in addition to the long wavelength reflectance. Conversely, green, yellow, purple, blue and clear traps caught larger numbers of insects (Blackmer and Cañas, 2005). These are traps that all have relatively good reflectance in medium wavelengths (around 500 nm) in addition to short- and long-wavelength reflectance, which correspond to the green trap, but not the purple trap used in our experiments. While the white trap had

reflectance around 500 nm, the optical brighteners reduce major reflectance to less than 450 nm. Consequently, based on results of Blackmer and Cañas, 2005, we would predict that green traps work most effectively, and indeed this was the case in our experiments.

Insect response to colour also appears to be species-specific (Blackmer et al., 2008). This specificity is supported by the evidence of numerous studies which have shown diverse preferences of colours (Ranamukhaarachchi and Wickramarachchi, 2007; Mazzoni et al., 2011; Thongjua et al., 2015). Green was preferred for the capture of the hemipteran bug, *Neomegalotomus parvus* (Westwood) (Ventura and Panizzi, 2004) and *C. eucalypti* (Farnier et al., 2014). During autumn 2004 and summer 2005, the preference order of *Lygus* spp. was green followed by purple then white (Blackmer et al., 2008). These results highlight the green colour attractiveness to hemipteran bugs and correspond well with our results on the attractiveness of green to *S. singularis*.

Combining colour and odour cues can have additive or even synergistic effects in mediating insect responses to a stimulus source (Giurfa et al., 1995; Raguso and Willis, 2005; Arnold et al., 2015). Traps containing both types of cues can have greater catches, compared with traps with only a visual or odour-based attractant. Conversely, presenting competing cues, e.g. odour from a different source to a visual stimulus, can provide information about the relative importance of visual versus odour cues in different species (Otalora-Luna et al., 2013; Pan et al., 2015; Arnold et al., 2016).

In our study, *S. singularis* catches in white sex pheromone traps were similar to those reported by Mahob et al. (2011) and Sarfo (2013). This relatively low catch was a reflection of the low abundance of *S. singularis* in Cameroon (Babin et al., 2008, 2010). Recent research by Sarfo et al. (2018a) showed that *S. singularis* sex pheromone trap catches can be improved substantially with trap placement in the middle of the cocoa canopy. Furthermore, Babin et al. (2010) showed that the highest densities of *S. singularis* occur in “pockets” where light incidence through the canopy is highest. Cocoa trees receiving light display microclimatic conditions suitable for development of mirid nymphs.

The higher trap catches reported by Sarfo et al. (2018a) from the middle of the cocoa tree canopy would substantially improve the potential for the sex pheromone and trap to be used in mass trapping (Gregg et al., 2018; Sarfo et al., 2018b), mating disruption (Lowor et al., 2009) or auto-dissemination of entomopathogenic fungi (Lopes et al., 2014; Tewaril et al., 2014). In the latter, male *S. singularis* are attracted to the trap where they are inoculated with spores of entomopathogens for wider dissemination to the target insect population in and around the cocoa plantations as part of the integrated control of mirid pests. Such improvement in the control of *S. singularis* with green traps could potentially lead to substantial reductions in the use of insecticides that have been implicated in the reduction in cocoa flower pollinators which have been shown to be essential for improving cocoa pod yield (Toledo-Hernandez et al., 2017).

Trap catches can also be used in time series analysis to model the effect of climate (i.e., temperature, rainfall and relative humidity) and plant phenology on the population dynamics of *S. singularis* and to compare these with a recent model from Djoukwe Tapi et al. (2018) to provide insights on the seasonality of this pest.

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