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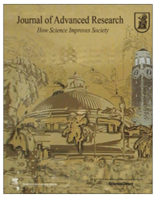

Zoophytophagous predator sex pheromone and visual cues of opposing reflectance spectra lure predator and invasive prey

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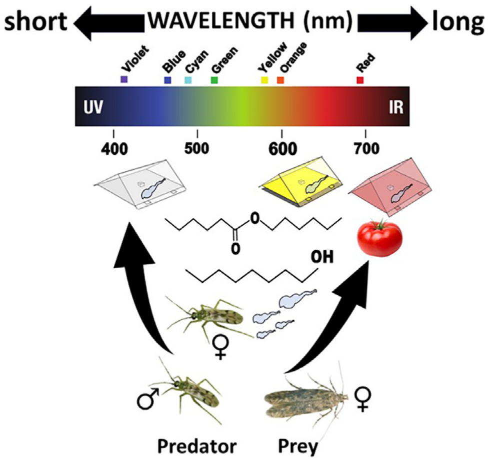
HIGHLIGHTS

- Two sex pheromone components 1-octanol and hexyl hexanoate were identified in the Kenyan population of the mirid predator *Nesidiocoris tenuis*.
- Antennae of both sexes of the predator and invasive tomato prey pest *Phthorimaea absoluta* detect the predator sex pheromone.
- Sex pheromone and specific visual cues combine to attract both predator and prey pest.
- Interplay between sexual communication and visual cues leads to discovery of an effective bio-based management system for females of the invasive pest and male predator.
- Findings from this research offers a promising approach to developing sustainable management tools for other zoophytophagous predators and their invasive prey crop pests.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: In sub-Saharan Africa, the invasive South American leafminer *Phthorimaea absoluta* is the most damaging tomato pest. Females of the pest can reproduce both sexually and through parthenogenesis and lay their eggs on all tomato plant parts. The mirid predator *Nesidiocoris tenuis*, a biological control agent for the pest, is also a tomato pest when prey population is low. To date, however, no study has developed an eco-friendly solution that targets both the predator and its host in a tomato farming system.

Objective: To develop a bio-based management system for both pest and predator based on the combined use of sexual communication in the predator and visual cues.

Methods: We collected volatiles from both sexes of the Kenyan population of the predator *N. tenuis* and identified candidate sex pheromone components by coupled gas chromatography-mass spectrometry (GC–MS). We used electrophysiological assays to identify antennally-active odorants in the volatiles, followed by field trials with different pheromone-baited colored traps to validate the responses of both predator and prey. Thereafter, we compared the reflectance spectra of the colored traps with those of different tomato plant tissues.

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Results: Our results reveal an interplay between different sensory cues which in the predator–prey interaction may favor the predator. Antennae of both sexes of predator and prey detect the predator sex pheromone identified as 1-octanol and hexyl hexanoate. Unexpectedly, our field experiments led to the discovery of a lure for *P. absoluta* females, which were lured distinctly into a pheromone-baited trap whose reflectance spectrum mimicked that of ripe tomato fruit (long wavelength), an egg-laying site for females. Contrastingly, *N. tenuis* males were lured into baited white trap (short wavelength) when the predator is actively searching for prey.

Conclusion: Our results demonstrate the novel use of a predator sex pheromone and different visual cues to assess complex trophic interactions on tomatoes.

Introduction

Predator-prey interaction is an arms race for the survival of both organisms, whose success is determined by the predator's ability to capture and feed on its prey, or the ability of the prey to evade the predator [1–3]. Several cues mediate this interaction, but in insects, olfactory cues play a key role [4]. Insect predators exploit the chemical signatures of their prey such as sex pheromones or host-finding signals to locate their prey [4,5]. Likewise, prey may exploit the olfactory cues of insect predators to evade predation [4,6]. Besides olfactory cues, predator–prey interaction may be influenced by other sensory cues including visual, tactile, and auditory [7,8]. As such, a comprehensive knowledge of the contributing factors mediating predator–prey interaction is critical to developing effective, sustainable, and environmentally sound management options for pests of crops, especially for zoophytophagous predators and prey.

In sub-Saharan Africa (SSA), various food crops are threatened by both below- and above-ground invasive pest species [9,10]. Among these crops is tomato (*Solanum lycopersicum* L), the most universally cultivated vegetable because of its economic and nutritional values [11]. Its annual worldwide production is approximately 189 million metric tonnes, with an estimated economic value of US$ 97.3 billion [11]. Markedly, in SSA, tomato production provides a major source of employment and income for millions of smallholder farmers, despite several production constraints, including damage caused by various insect pests such as the invasive South American tomato leafminer, *Phthorimaea absoluta* Meyrick (formerly known as *Tuta absoluta*) (Lepidoptera: Gelechiidae) [12]. Native to South America, the leafminer was detected in East Africa (Kenya in 2014, Tanzania, and Uganda in 2016), after an earlier invasion of North Africa in 2008 through Spain [13,14]. In Kenya, it has been reported on several Solanaceae host plants, but it is most damaging on tomato, with a mean seasonal production loss due to larval feeding activity estimated at 114,000 tonnes, which translates to about US$ 59.3 million in economic losses annually [14]. Furthermore, it has been reported to have contributed to the near collapse of tomato production in certain countries in West Africa, including Nigeria, Senegal, and Niger [15]. Because of its wide host range and high biotic potential *P. absoluta* control has proven challenging [12,15], hence it is considered a quarantine pest worldwide [16].

Adult females of *P. absoluta* lay up to 260 eggs in their life cycle, producing on average 10–12 generations per year [12]. The eggs are laid on all the aerial parts of the plant, resulting in yield losses of 80–100% caused by larval feeding in unprotected fields [12]. Synthetic chemical control has been the mainstay in managing the tomato leafminer, with limited success because of the cryptic nature of the feeding larvae and the rapid evolution of resistant populations [17,18]. Hence, there is a global demand for more eco-friendly alternatives such as the use of biological control agents and semiochemicals to control this pest [19,20].

The mirid predator, *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae), native to the tropics, has been successfully used in the biological control of *P. absoluta* in greenhouse and open-field tomato production, especially in Europe and North Africa [21,22]. In SSA, it is a naturally occurring biocontrol agent [23,24] used in closed and open field tomato production against several herbivores, especially *P. absoluta*, and whiteflies [24,25]. However, there are risks associated with its use because it feeds on the host plant when the prey population is low or absent resulting in significant crop yield losses [22,26,27]. Although the female-produced sex pheromone of *P. absoluta* has been identified as (3*E*, 8*Z*, 11*Z*)-tetradecatrien-1-yl acetate (TDTA) and (3*E*, 8*Z*)-tetradecadien-l-yl acetate (TDDA) [28,29], and it attracts males, it does not attract the mirid predator [30]. Moreover, the ability of the adult female of *P. absoluta* to reproduce through parthenogenesis makes current pheromone-based management strategies for it inadequate [31]. Our previous laboratory study demonstrated that tomato volatiles, dominated by terpenes attracted both *P. absoluta* and *N. tenuis* [32], but plant kairomones are non-specific, thus limiting the use of this kairomone in managing both species. Given the prolific reproductive potential of *P. absoluta* females and the zoophytophagous behavior of its mirid predator, an effective bio-based management strategy is urgently needed for both predator and prey in a tomato production system.

A recent study identified a female-produced sex pheromone as 1-octanol and octyl hexanoate from a European commercial source of *N. tenuis* strain (Bioline AgroSciences Ltd. Nesiline) with an unknown origin for use in its management [33]. Whether mirid populations in SSA use the same sex pheromone for communication is unknown. It is also unknown whether other sensory cues may contribute to sex attraction in the mirid, and potentially the prey. Based on the patterns of feeding of both predator and prey on tomato plants, especially the invasive leafminer, which feeds and reproduces on all parts of the tomato plant including ripe tomato fruits, we hypothesized that the sex pheromone of the mirid predator may combine with visual cues to significantly reduce both predator and prey populations. Hence, in this study, we first investigated the sex pheromone of the Kenyan population of *N. tenuis*. Secondly, we investigated the use of the sex pheromone in the presence of visual cues to manage both the zoophytophagous mirid predator and the invasive tomato leafminer. Our results demonstrated the potential use of predator sex communication and specific visual cues as a bio-based management strategy for both the zoophytophagous predator and invasive prey in tomato fields.

Materials and methods

Insects

Nesidiocoris tenuis used in this study was reared on potted tomato plants (4–8 weeks old, Moneymaker cultivar) in Plexiglass cages (40 cm × 50 cm × 60 cm) maintained at 25 ± 2 °C, 60 ± 5 % RH, L12:D12 at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The insect colony was established from adults (previously identified using molecular tools) [32] collected from a tomato field in Mwea (1206 m asl, S − 0.601125, E 37.369839), Kenya in March 2022 [32]. The insects were fed with non-viable eggs of *Ephestia kuehniella* Zeuler and *Artemia* sp. (Koppert biological system, Veilingweg, Netherlands). In each cage, 10 g of the non-viable eggs were provided every week, but feeding was supplemented with 80 % honey solution ad libitum. To reduce cannibalism on the young nymphs, adults that emerged from a cage were quickly transferred into a different cage. Only sexually active *N. tenuis* adults (2–7 days old) were used for the experiment, and sexing of the mirid was done as previously described [33]. *Phthorimaea absoluta* used in the study were obtained and reared as described in our previous work [32]. Only *P. absoluta* adults (2–3 days old) were used for the experiment.

Collection of volatiles from predator adults

Air-borne volatiles from both sexes of *N. tenuis* adults (40 each, N = 5), were collected separately in quick-fit glass volatile collection chambers (length = 25 cm, internal diameter = 4 cm) for 24h using a push–pull volatile collection previously described [32]. Super-Q-trapped volatiles were eluted with 150 μL GC grade dichloromethane (Analytical grade, Sigma-Aldrich, St, Louis, MO) under a stream of nitrogen gas into 1.5 mL GC vials. The eluted samples were stored at − 80 °C until required for analysis.

Chemical analysis

Volatiles from *N. tenuis* adults were analyzed using gas chromatography-mass spectrometry (GC–MS) on an HP 7890A series gas chromatograph (Agilent Technologies, Wilmington, USA) coupled to an HP 5975C mass spectrometer (Agilent Technologies, Wilmington, USA). For each sample, an aliquot (1 μL) was injected in the splitless mode (250 °C) and analyzed on Agilent HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm) using an oven temperature programme; 35 °C for 5 min, increased to 280 °C at 10 °C/ min and held for 10.5 min. Helium was the carrier gas at a constant flow rate of 1.0 mL/min. Mass spectra were acquired at 70 eV (mass range of 38–550 Daltons (Da) during a scan time of 0.73 scans/sec). The total ion chromatograms (TICs) of both *N. tenuis* sexes were compared, and the compounds were tentatively identified based on their mass spectral library data (Adams2 [34] and NIST [35]). Compounds were confirmed based on a comparison of their retention times and mass spectral data and co-injection with authentic standards. Quantification of identified compounds was done using external calibration curves (peak area vs. concentration) generated by serial dilutions (1–500 ng/ μL) of the authentic standards 1-octanol and hexyl hexanoate analyzed using the same chromatographic conditions.

Electrophysiology

Antenally-active components were identified using gas chromatography-electroantennographic detection (GC-EAD) as described in our previous study [36]. Briefly, the antennally-active components were analyzed on an HP 7890 Series II gas chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with an HP-5MS capillary column with similar dimensions and oven conditions as described for the GC/MS analysis. Carrier gas was high-purity nitrogen at a flow rate of 1 mL/min. Samples were injected in a splitless mode at 250 °C with a split valve delay of 1 min. Column effluent split was 1:1 with a fused silica outlet splitter (Alltech Associates Inc. Deerfield, IL) for simultaneous detection by an electroantennographic detector (EAD) and flame ionization detector (FID). Silver wires (1.5 mm internal diameter) immersed in glass capillary tubes filled with a ringer solution (1.36 g KH_2PO_4, 0.24g KCl, 0.08 g $CaCl_2$, 1.22 g $MgCl_2$, 4.8 mL KOH, 35.08 g $C_6H_{12}O_6$ (glucose), and 0.35 g NaCl dissolved in 0.5L of distilled water) were used as reference and recording electrodes. The antennae of *N. tenuis* (2–7 days old) and *P. absoluta* (2–3 days old) were connected to the recording electrode (tip of the antennae) and the reference electrode (base of the antennae) which was connected to an AC/DC amplifier in DC mode (Syntech, Kirchzarten, Germany). The FID and EAD signals were detected through an INR-II probe (Syntech, Hilversum, The Netherlands), captured, and processed with an IDAC-2 data acquisition controller. Two microlitres (2 μL) of each volatile sample were analyzed using a GC-EAD 2000 (Syntech, Hilversum, The Netherlands) software on a computer and each was replicated a minimum of five times using fresh antennae in each run. Compounds that elicited consistently at least three EAD responses were considered EAD-active components.

Chemicals

Synthetic standards 1-octanol and the solvents dichloromethane and hexane were purchased from Sigma-Aldrich (Steinheim, Germany). Hexyl hexanoate and octyl hexanoate were generously donated by Prof. Teun Dekker (Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Sweden). All chemicals were at least 95% pure.

Field trials

Experiment 1. We conducted a pilot field study at a farmer's tomato farm in Juja (1442 m asl, S −1.160085, E 37.070442), Kiambu County, Kenya. Here, we tested the attractiveness of the identified pheromones to *N. tenuis* and *P. absoluta* in different traps for four weeks (April – May 2023). This was a mono-crop farm of Nova tomato variety and it measured 215 m × 180 m, which was divided into three blocks. Each block was assigned 12 treatments (Fig. S1a). Treatments included different combinations of trap types; water pan (32 cm o.d x 4 cm, h; black pan), yellow delta trap (25 cm o.d × 20 cm h), and red delta trap (28 cm o.d × 20 cm h) baited with 200 μL each of 0.2 mg, 1 mg, and 2 mg of a hexane solution of a two-component predator sex pheromone blend (1-octanol: hexyl hexanoate 4:1 ratio). All samples were loaded into hexane-extracted rubber septa (1 cm i.d x 2 cm-length) (Sigma-Aldrich, MA, United States) and air-dried in a hood. Control traps were baited with rubber septa loaded with a similar volume of hexane (200 μL) and air-dried. Traps were set 10 m apart within a block and 15 m between blocks (Fig. S1a-b). All the delta traps were placed 15 cm from the base of the tomato plants. Pheromone lures were glued at the center of the sticky surface of each delta trap, but the water pan traps had an odor chamber at the center where the lures were placed. Soap solution (1 L of water + 50 mL of liquid soap) was added to each water pan trap to prevent trapped insects from escaping.

Experiment 2. In this experiment, we compared the attractiveness of our sex pheromone blend (1-octanol: hexyl hexanoate 4:1 ratio) with that of the previously identified pheromone for a strain whose origin was unknown (1-octanol: octyl hexanoate, 1:1). This field trial was carried out for four weeks (May – June 2023) in a farmers' field also in Juja (1452 m asl, S −1.167501, E 37.074844) (190 m × 150 m; mono-crop; Zara tomato variety) (~1 km apart) using a similar experimental layout as described in experiment 1 (ten treatments replicated three times). Based on the results from Experiment 1, here only water pan traps were baited with 2 mg, 4 mg, and 8 mg of the two-component pheromone blend.

Experiment 3. This study was carried out to optimize the different trap colors baited with the identified predator sex pheromone for trapping both predator and prey. The trials were carried out using the same design as in Experiments 1 and 2 at two different farmer-field sites for ten weeks (June – August 2023). These sites were located in Mwea, Kirinyaga County, at a relatively lower altitude than the previous sites: Site 1 (1170 m asl, S −0.628450, E 37.374535), (230 m × 140 m; mono-crop; Ansal tomato variety) and Site 2, (1183 m, S −0.600769, E 37.369595) (140 m × 90 m; Ansal tomato variety; mono-crop). These two sites were ∼3 km apart with 12 treatments replicated five times per site (Fig. S1b). Here, the water pan trap was replaced with a white delta trap (see Results section Field Experiment 3). Traps were baited with 2 mg, 4 mg, and 8 mg of 1-octanol: hexyl hexanoate in a 4:1 ratio.

In experiments 1, 2, and 3, traps were monitored weekly and trapped insects were counted and sexed. In all experiments, pheromone lures were changed weekly and trapped insects were removed and stored in 70 % alcohol for subsequent confirmation of sex in the laboratory. Sexing of the insects was confirmed under a microscope (Leica, MicroPublisher 5.0 RTV).

In Experiment 3, the reflectance spectra of the colored traps, green tomato leaves, and ripe tomato fruits were measured using a handheld spectroradiometer (ASD FieldSpec HandHeld 2 Portable Spectroradiometer, PANalytical Company). The surfaces of the samples were scanned at wavelengths 325–1075 nm with each scan replicated 20 times. The reflectance spectra are expressed as the ratio of energy reflected by the surface of an object to the energy incident on the surface measured as a function of wavelength.

Statistical analyses

Data were analyzed using R software [37] (version 4.3.1) and the R Studio graphical user interface. Count data from the field trials were analyzed using a generalized linear model (GLM) with quasi-Poisson distribution considering trap type and pheromone dose as fixed factors. However, a student *T-test* was used to analyze the binary data obtained from our preliminary greenhouse study where we compared the performance of the water pan and white delta traps. Mean separation (Post hoc test) was done using Tukey's test at a 5 % probability level ($P < 0.05$).

Results

N. tenuis and P. absoluta

Fig. 1 a and b are representative photographs of the zoophytophagous predator *Nesidiocoris tenuis* and invasive leafminer prey *Phthorimaea absoluta.*

Identification of candidate sex pheromone components in N. tenuis volatiles

Coupled gas chromatography-mass spectrometric (GC–MS) analysis of the extract obtained from air-borne volatiles from sexually mature *N. tenuis* adults (2–7 days old) identified two candidate pheromone components, as 1-octanol and hexyl hexanoate, which varied quantitatively between males and females (Fig. 2a-c). Females released ∼1.6- and ∼5.5-fold more 1-octanol (7.0 ± 0.4 ng/h/insect), and hexyl hexanoate (2.0 ± 0.3 ng/h/insect) than males (4.3 ± 0.2 ng/h/insect) and (0.3 ± 0.0 ng/h/insect), respectively, representing a 4:1 ratio of sex pheromone components. These results indicate that the level of pheromone release in *N. tenuis* is sex-dependent.

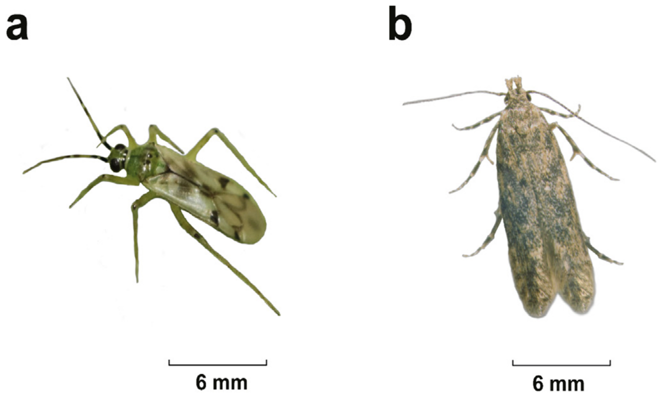

Fig. 1. A picture of an adult (a) predator (*Nesidiocoris tenuis*) male and (b) its prey (*Phthorimaea absoluta*) female.

Antennal responses of N. tenuis and P. absoluta to candidate sex pheromone components

Next, we used coupled GC-electroantennographic detection (EAD) analysis to confirm that the antennae of both sexes of the predator and prey detected 1-octanol and hexyl hexanoate in both the natural volatile extract and synthetic blend (Fig. 3a-d). Antennal detection of the pheromone components in the natural extract varied with the sex of the mirid: females- 1-octanol (0.2 ± 0.0 mV), and hexyl hexanoate (0.1± 0.0 mV); and males- 1-octanol (0.2 ± 0.0 mV), and hexyl hexanoate (0.2 ± 0.0 mV). It also varied quantitatively for the prey: females: 1-octanol (0.1 ± 0.0 mV), and hexyl hexanoate (0.02 ± 0.0 mV); males- 1-octanol (0.1 ± 0.0 mV) and hexyl hexanoate (0.1 ± 0.0 mV) (Fig. 3a-b). A similar pattern was observed in the antennal detection of the predator and prey to the synthetic compounds (200 ng) of 1-octanol and hexyl hexanoate (Fig. 3c-d). Both predator and prey also detected octyl hexanoate, predator (0.2 ± 0.0 mV and 0.1 ± 0.0 mV female: male, respectively) and prey (0.1 ± 0.0 mV and 0.1 ± 0.0mV female: male, respectively) (Supplementary Material Fig. 2a-b).

Field experiment 1: Response of N. tenuis and P. absoluta to sex pheromone combined with visual cues

To test whether the predator sex pheromone attracted males, we carried out a field experiment in a farmer's tomato field using the pheromone blend at three different doses (0.2 mg, 1 mg, and 2 mg) for four weeks (Fig. 4) (Fig. S1a,c). Different trap types including water-pan, red, and yellow delta traps were baited with the pheromone loaded into hexane-cleaned rubber septa. Control traps were baited with similar rubber septa loaded with hexane only and air-dried. *N. tenuis* male trap captures varied significantly with the trap type ($\chi^2 = 32.8$, df = 2, $P < 0.001$) and pheromone dose ($\chi^2 = 99.02$, df = 3, $P < 0.001$) (Fig. 5a). Males were significantly most attracted (96.9 % males, 3.1 % females) to the water-pan trap baited at the highest dose (2 mg) of the pheromone blend ($\chi^2 = 7.7$, df = 6, $P < 0.001$) (Fig. 5a). Pheromone-baited yellow and red delta traps recorded intermediate captures, with their equivalent control traps recording the least numbers of captures (Fig. 5a).

Unexpectedly, the pheromone-baited red delta trap captured significantly more *P. absoluta* females (86.7 % females, 13.3 % males) than other traps ($\chi^2 = 171.5$, df = 2, $P < 0.001$) (Fig. 5b). Likewise, *P. absoluta* trap captures were pheromone dose- ($\chi^2 = 136.7$, df = 3, $P < 0.001$) and sex-dependent (Fig. 5b), indicating that the predator sex pheromone lures both predator and prey in the presence of specific visual cues (Fig. 5b).

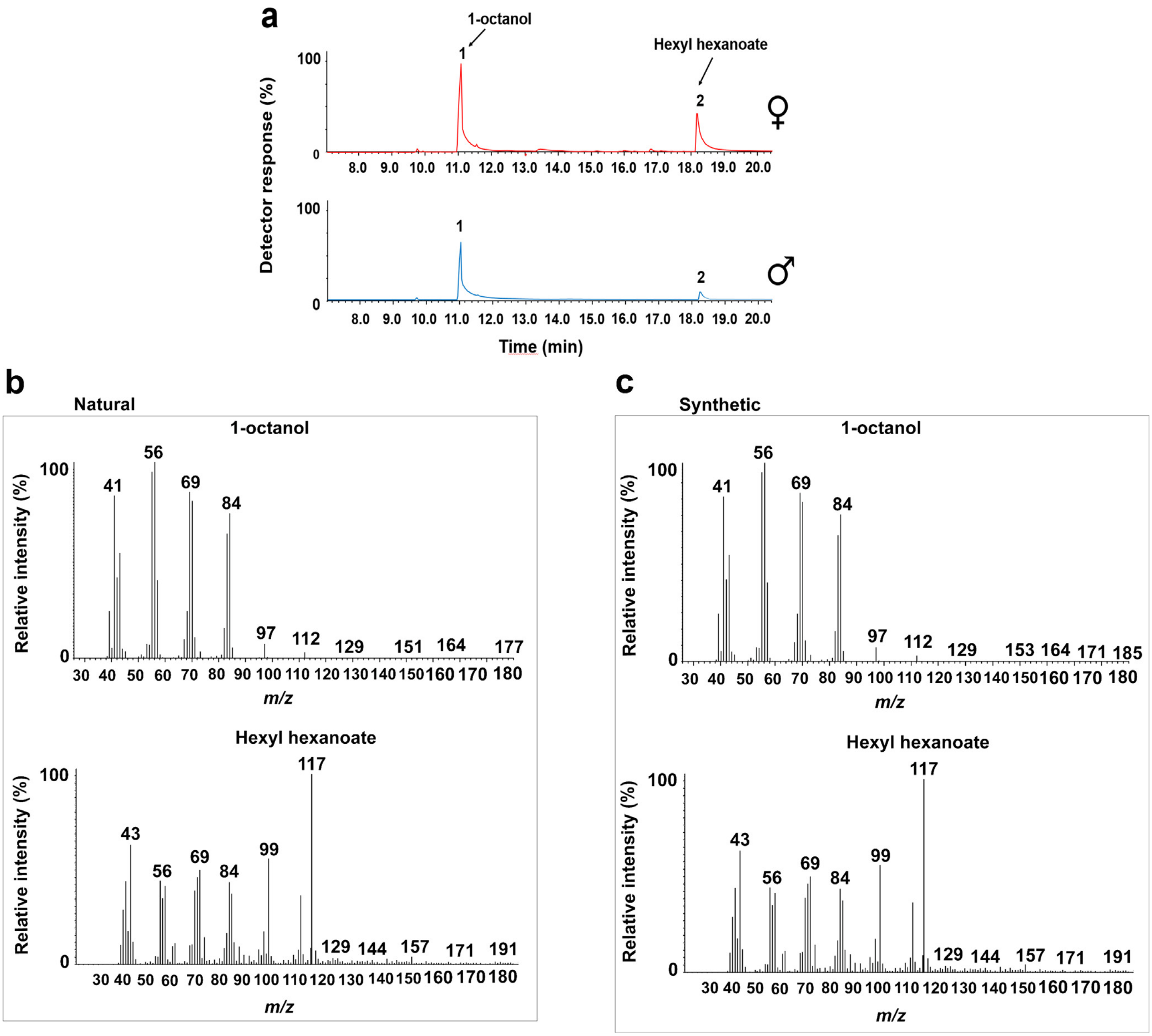

Fig. 2. Representative total ion chromatograms (TICs) of air-borne volatiles from (a) *Nesidiocoris tenuis* female (♀) and male (♂), and mass spectra of *N. tenuis* sex pheromone components 1-octanol and hexyl hexanoate identified in (b) natural volatile extract and (c) synthetic standards.

Field experiment 2. Response of N. tenuis to different sex pheromone compositions

Since a previous study had identified the sex pheromone of *N. tenuis* from a commercial source with unknown origin as 1-octanol and octyl hexanoate (1:1) [33] (Fig. S2a-b), we compared responses of the Kenyan population of the mirid to this sex pheromone blend and our identified blend (1-octanol and hexyl hexanoate 4:1) in a tomato field (Fig. 4, Fig. S1a,d) for four weeks. Field experiment 2 had the same design as field experiment 1, using water-pan traps baited with relatively higher doses of 2 mg, 4 mg, and 8 mg of the two pheromone blends. We found that trap captures of *N. tenuis* males varied significantly with the pheromone blend (χ^2 = 190.7, df = 6, P < 0.001) (Fig. 5c). Male captures followed a pheromone dose-dependent response irrespective of the pheromone blend. However, significantly more males were captured in traps baited with the highest dose (8 mg) of our identified pheromone blend (χ^2 = 190.7, df = 6, P < 0.001) (Fig. 5c) than the pheromone blend identified for the unidentified commercial population of *N. tenuis.* Nonetheless, the performance of both pheromone blends in attracting *N. tenuis* males was significantly higher than the control (Fig. 5c).

Interestingly, we found that trap captures of the tomato leafminer, *P. absoluta* were generally low irrespective of the pheromone blend and dose and were not significantly different from the control (χ^2 = 6.2, df = 6, P > 0.05 (Fig. 5d). These results confirm the important role visual cues play in the attraction of both *N. tenuis* and *P. absoluta.*

Field experiment 3: Optimisation of trap color baited with predator sex pheromone

Next, we optimized the different trap colors baited with the predator sex pheromone blend in field trials at two different farmer-field sites (Sites 1 and 2) (Fig. 4, Fig. S1b,e-f) for 10 weeks. The sites were located at a relatively lower altitude (1170 m – 1183 m asl) than the previous sites (1442–1452 m asl). This field experiment also had the same design as field experiment 1, but

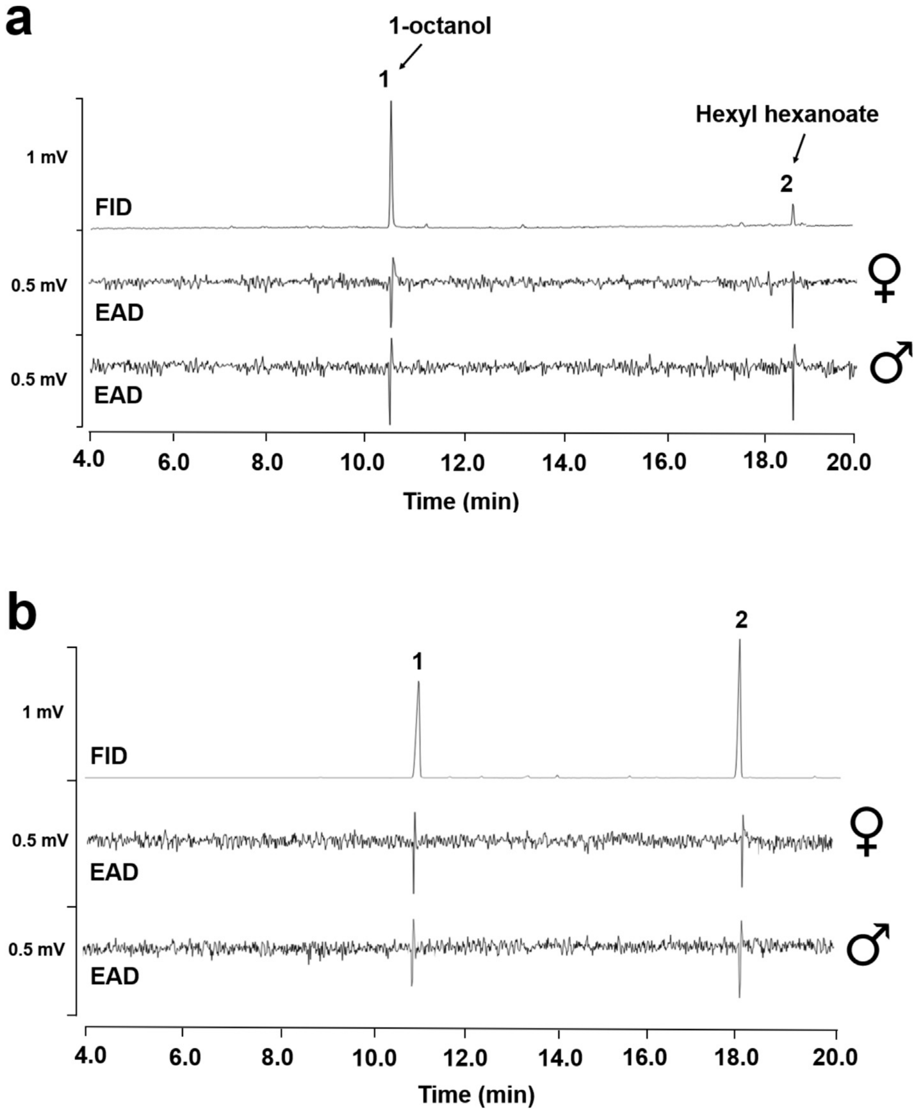

Fig. 3. Coupled GC-EAD analysis showing antennal detection of the identified *Nesidiocoris tenuis* sex pheromone components by conspecific adults to (a) natural volatile extract and (b) synthetic components, and *Phthorimaea absoluta* antennal responses to (c) natural volatile extract and (d) synthetic components. (♀) denotes female and (♂) male. In each analysis, 200 ng of the synthetics were injected.

the water-pan trap was replaced with a white delta trap. In a greenhouse study, we found that trap captures of *N. tenuis* males in pheromone-baited white delta (8.8 ± 0.39) and water pan (7.9 ± 0.37) traps were not significantly different (*t*-test, t (18) = 1.66, P > 0.05). This trap replacement solved a logistical problem of frequent refilling of the water-pan trap with water when temperatures were high. Interestingly, trap captures of male *N. tenuis* and female *P. absoluta* followed a similar pattern as previously recorded in field experiment 1. There was a significant treatment effect: for *N. tenuis* at Site 1, trap type (χ^2 = 108.4, df = 2, *P* < 0.001) and pheromone dose (χ^2 = 224.4, df = 3, *P* < 0.001) (Fig. 6a); and for Site 2, trap type (χ^2 = 35.9, df = 2, P < 0.001) and pheromone dose (χ^2 = 96.5, df = 3, P < 0.001) (Fig. 6c). *N. tenuis* males were significantly most attracted to pheromone-baited white delta traps in both experimental sites, with trap captures in yellow and red delta traps not significantly different from each other. The least number of captures was recorded in the control traps (Fig. 6a,c).

Remarkably, *P. absoluta*'s response to pheromone-baited traps mirrored the captures previously obtained in field experiment 1. Site 1- trap type (χ^2 = 447, df = 2, *P* < 0.001), pheromone dose (χ^2 = 199, df = 3, *P* < 0.001) (Fig. 6b); and Site 2, trap type (χ^2 = 667.7, df = 2, P < 0.001), and pheromone dose (χ^2 = 182.8, df = 3, P < 0.001) (Fig. 6d). The pheromone-baited red delta trap at the highest dose (8 mg) was significantly most attractive, the yellow and white traps recorded intermediate captures, whereas control traps recorded the least captures (Fig. 6b,d). Trap captures of *N. tenuis* females (Fig. S3a) with the different baited colored traps at both Sites 1 and 2 were ~ 1 % out of the total number of trap captures. On the other hand, trap captures of *P. absoluta* with the pheromone-baited red delta trap were predominantly females (Fig. S3b) ~79.6–90.6 %, with males ~9.4–20.4 % at both experimental sites. These results demonstrate that visual cues enhance the performance of the predator sex pheromone (white for the predator and red for the prey) in attracting both *N. tenuis* and *P. absoluta*.

Reflectance spectra of traps and tomato plant tissues

Does the spectral reflectance of the commercial red trap mirror that of a ripe tomato? To address this question, we used a handheld reflectance spectroradiometer to compare the spectral reflectance of the different delta traps with ripe tomato fruit. We found an

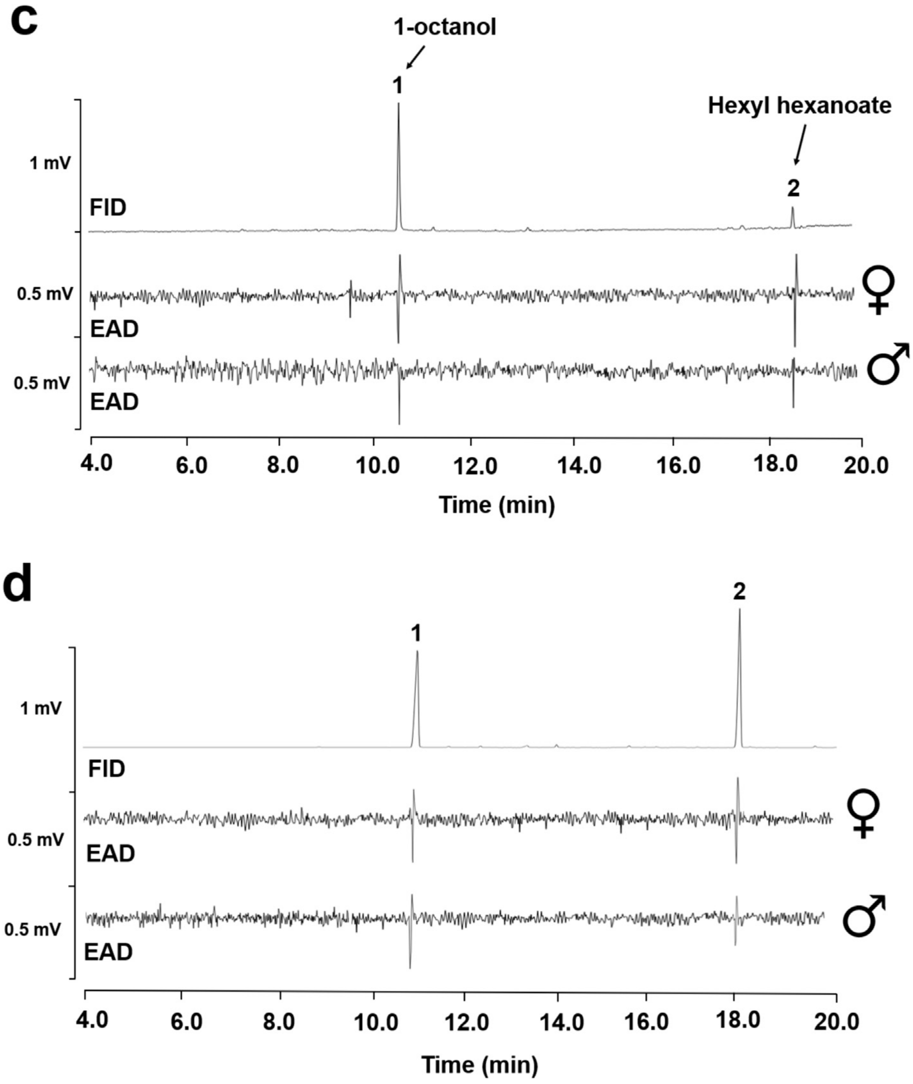

Fig. 3 (*continued*)

overlap between the reflectance spectra of the red delta trap (on average 74 ± 2.8 % reflectance at 854 nm dominant wavelength) which captured predominantly females of *P. absoluta* and ripe tomato fruits (on average 52 ± 4.9 % reflectance at 805 nm dominant wavelength) (Fig. 7). On the other hand, the white traps which captured predominantly males of *N. tenuis* had 92 ± 28.7 % reflectance at 432 nm dominant wavelength, with the yellow trap (92 ± 1.7 % reflectance at 650 nm dominant wavelength), and green tomato leaves (58 ± 1.8 % reflectance at 936 nm dominant wavelength) (Fig. 7). These results suggest that *P. absoluta* females respond to visual cues that mimic ripe tomato fruits at long wavelength, whereas *N. tenuis* males respond to visual cues at short wavelength.

Discussion

Our study demonstrates a novel and promising management option for the zoophytophagous predator *N. tenuis* and its invasive prey *P. absoluta* based on a two-component female-produced sex pheromone identified as 1-octanol and hexyl hexanoate (4:1 ratio) from the Kenyan population of the mirid predator *N. tenuis*. An analogous two-component sex pheromone blend had previously been reported for the strain of the mirid sourced from a commercial supplier (Bioline AgroSciences Ltd. Nesiline) with an unknown origin as 1-octanol and octyl hexanoate in a 1:1 ratio [33]. These results indicate that *N. tenuis* exhibits geographic variation in sexual communication. Whether the difference in pheromone composition may be a consequence of intensive rearing of the biocontrol agent *N. tenuis* over a long period in the laboratory is unknown. Biosynthetically, it appears that different populations may use different fatty acid and alcohol templates to enzymatically synthesize specific esters required for their sex communication. Understanding the geographic and population genetic basis for these differences will shed light on sex pheromone biosynthesis in this mirid species. Interestingly, consistent with the results of the present study, previous studies had identified diverse sex pheromones for various mirids as comprised of saturated and unsaturated straight-chain esters, aldehydes, and alcohols [38,39]. For example, the female-produced sex pheromones of two mirid pests of rice *Trigonotylus caelestialium* and *Stenotus rubrovittatus* comprise a mixture of hexyl hexanoate, (*E*)-2-hexenyl hexanoate for the former pest, and octyl butyrate, hexyl butyrate, (*E*)-2-hexenyl butyrate, and (*E*)-4-oxo-2-hexenal for the latter pest [40]. Likewise, hexyl butyrate, (*E*)-2-hexenyl butyrate, and (*E*)-4-oxo-2-hexenal are shared female-produced sex pheromone components of five

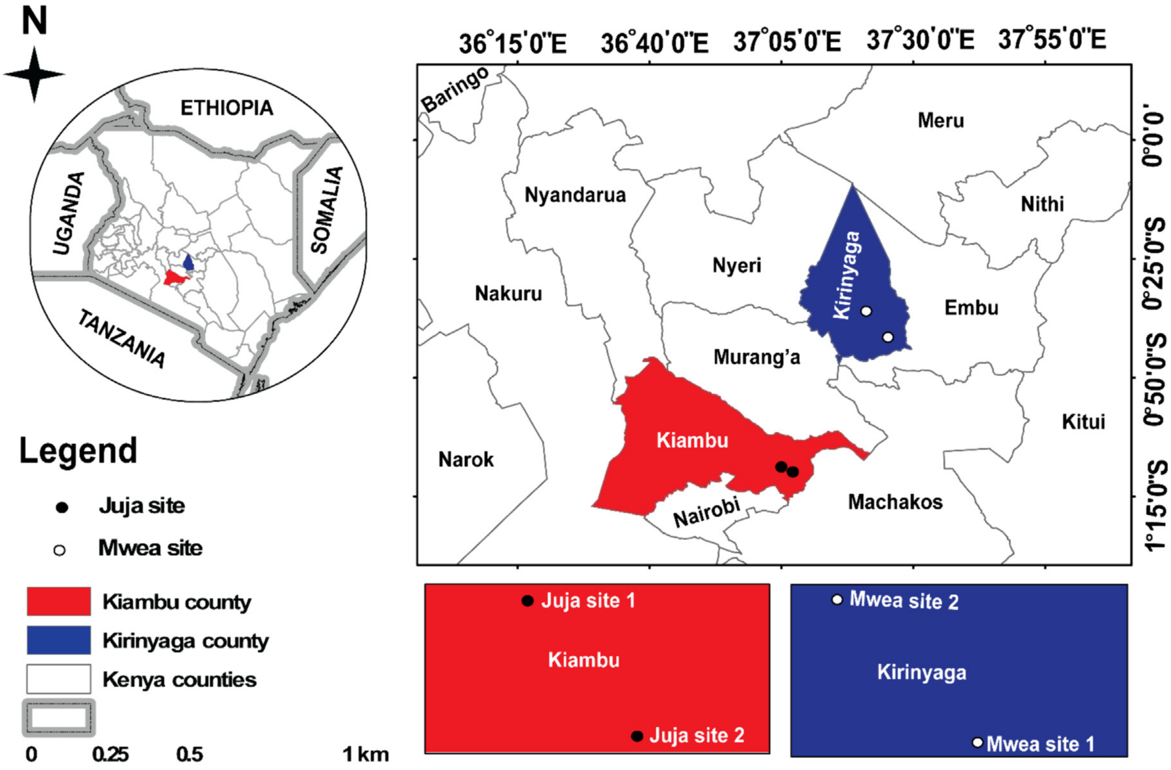

Fig. 4. A map showing the experimental sites in Kiambu and Kirinyaga counties, Kenya.

other related mirid species including *S. rubrovittatus*, *Apolygus spinolae*, *Apolygus lucorum*, *Taylorilygus apicalis*, and *Orthops campestris* [39]. Studies on the geographic variation in sex pheromone production in mirid species have been previously demonstrated in *Lygus pratensis* [41,42] and *Apolygus lucorum* [43]. Likewise, it is widely documented in lepidopteran species to which the tomato leafminer belongs. For example, the sex pheromone of the Indian and Philippine populations of the rice leaf folder C*naphalocrocis medinalis* consists of (*Z*)-11-hexadecenyl acetate and (*Z*)-13-octadecenyl acetate whereas the Japanese population of the mirid consists of (*Z*)-11-octadecenal, (*Z*)-13-octadecenal, (*Z*)-11-octadecen-1-ol, and (*Z*)-13-octadecen-1-ol [44,45]. Similarly, the sex pheromone of the West African population of the legume pod borer, *Maruca vitrata*, is comprised of (*E*, *E*)-10,12-hexadecadienal, (*E*, *E*)-10,12-hexadecadienol, and (*E*)-10- hexadecenal differs from the Asian population of the moth comprising of the first two pheromone components with the absence of the latter component (*E*)-10- hexadecenal [46]. Similar geographic differences in sex pheromone production have been reported in different populations of the fall armyworm *Spodoptera frugiperda* [47,48].

In the current study, interestingly, antennae of both sexes of *N. tenuis* and *P. absoluta* detected the natural and synthetic female-produced sex pheromones of *N. tenuis*. We also show that they detect the analogous pheromone components previously identified for the population obtained from a commercial source (Fig. S2a-b), indicating the presence of chemoreceptors in the antennae of both insect species that are sensitive and broadly tuned to detect alcohols and esters. Given that the native predator *N. tenuis* shares no evolutionary history with the invasive leafminer, it is intriguing that the latter detects the pheromone blend of the native predator. The possible explanation could be that the female-produced sex pheromone of *P. absoluta* is also comprised of esters, (3*E*, 8*Z*, 11*Z*)-tetradecatrien-1-yl acetate (TDTA) and (3*E*, 8*Z*)-tetradecadien-l-yl acetate (TDDA) [28,29]. Additionally, previous electrophysiological assays have shown that *P. absoluta* females detect several constitutive and herbivore-induced volatiles including the alcohol (*Z*)-3-hexen-1-ol [49,50]. Thus, it is possible that odorant receptors of *P. absoluta* may be broadly tuned to detect esters and alcohols of different chain lengths including the sex pheromone components of the mirid predator identified in the current study. Although research is yet to be carried out to identify the specific receptors involved in responding to these compounds in both insects, our field experiments revealed remarkable findings from an ecological perspective.

Firstly, our field trapping results showed that *N. tenuis* males were attracted to the female-produced sex pheromone in the presence of background host plant volatiles, indicating specificity of the identified sex pheromone. Furthermore, the obvious dose-dependent responses of *N. tenuis* males to our identified sex pheromone compared to that identified for the other population of unknown origin suggested both phenotypic plasticity and specificity in male responses. Whether these responses to the different sex pheromone blends may be due to the presence of different populations of the mirid at the experimental site is unknown. Further studies on the molecular identification of trapped *N. tenuis* are required. The specificity of sex pheromone responses in different mirid species has been previously reported [39,51]. For example, whereas the female-produced sex pheromones including (*E*)-4-oxo-2-hexenal and (*E*)-2-hexenyl butyrate are most essential for attracting *Lygus lineolaris* and *Lygus elisus* males, (*E*)-4-oxo-2-hexenal and hexyl butyrate are essential for attracting *Lygus hesperus.* However, all the sex pheromone components are produced in the three mirid species [51]. Likewise, in another field study, a three-component blend consisting of (*E*)-4-oxo-2-hexenal, (*E*)-2-hexenyl butyrate, and hexyl butyrate produced as sex pheromone components by five mirid species including *A. lucorum*, *A. spinolae*, *O. campestris*, *S. rubrovittatus,* and *T. apicalis* attracted only *A. lucorum*, *O. campestris*, and *T. apicalis* males. On the other hand, a fourth component (*E*)-2-octenyl butyrate produced by *A. lucorum* and *T.*

apicalis females attracted only conspecific males of the mirids [39]. Future studies on sex pheromones of other species in the genus *Nesidiocoris* would be useful in understanding sex communication in this group, and their potential use in monitoring populations of mirid predator species that are also agricultural pests.

Secondly, in the field trapping experiments, there were species- and sex- discriminatory responses to the pheromone in the presence of different visual cues, indicating the presence of light-sensitive detectors in both species. For *N. tenuis*, it appeared that males who were in search of the opposite sex for reproduction were attracted to our pheromone-baited water-pan or white delta traps, but were weakly attracted to brightly colored pheromone-baited traps. This suggested that in *N. tenuis* light-sensitive detectors may be tuned to light of short wavelength when the predator is usually active in search of prey. This is in line with a previous study which demonstrated that indeed Hemipterans respond more to shorter wavelengths (395 nm) in comparison to insects from different orders including Hymenoptera, Coleoptera, and Neuroptera [52]. In contrast, females of the predator were weakly attracted to all the pheromone-baited traps irrespective of the color of the trap. It appears that females perceiving their own pheromone avoided it in order to find another suitable oviposition site to lay eggs to increase the fitness of their progeny. On the other hand, we obtained unexpected results for *P. absoluta* responses in the field study. We discovered that females were strongly lured into pheromone-baited red delta traps, whereas males were weakly attracted to all the different colored traps. These differential responses to the combined chemical and visual stimuli might be

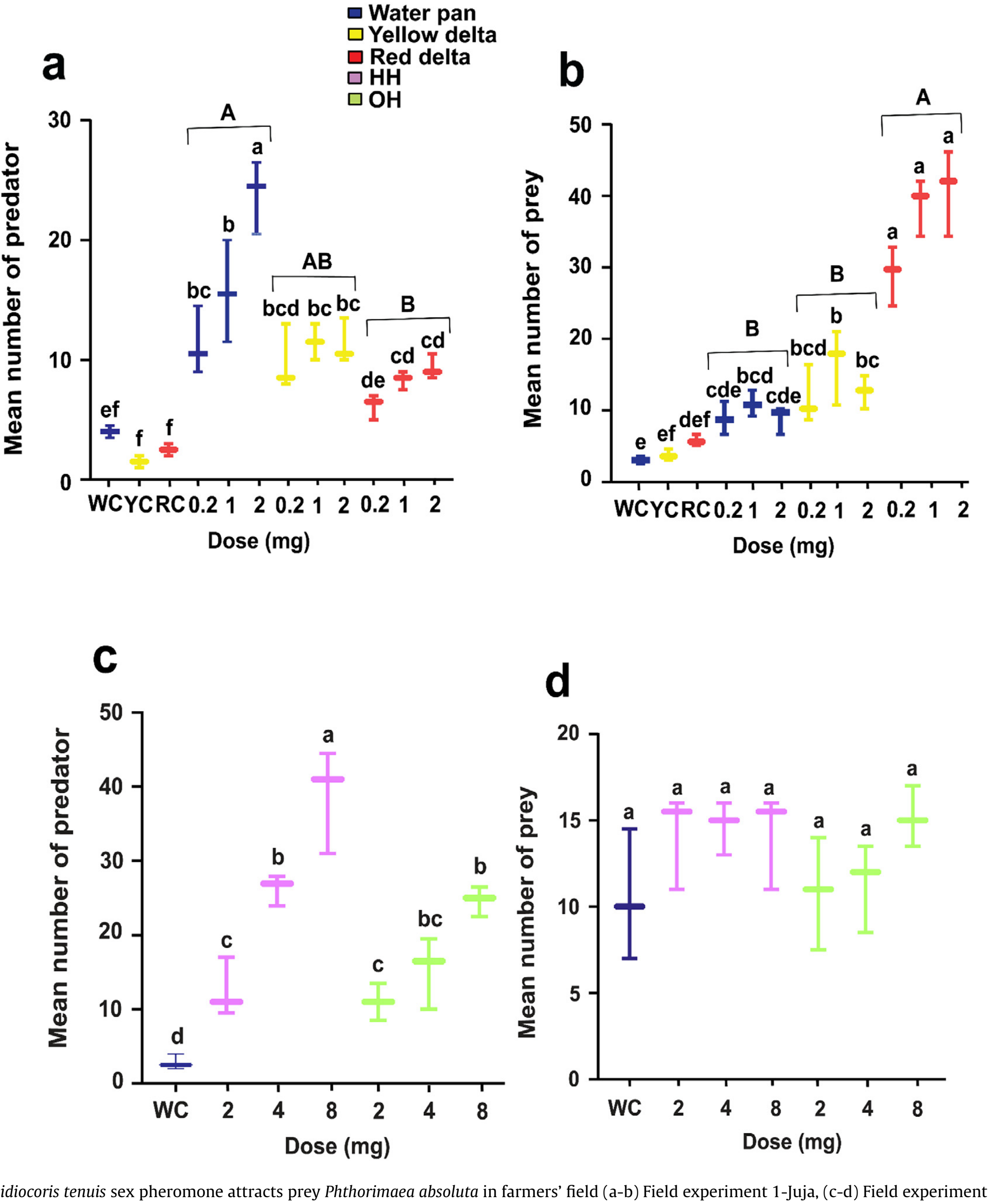

Fig. 5. Predator *Nesidiocoris tenuis* sex pheromone attracts prey *Phthorimaea absoluta* in farmers' field (a-b) Field experiment 1-Juja, (c-d) Field experiment 2-Juja where only water pan traps were baited with a blend of 1-octanol and hexyl hexanoate or octyl hexanoate in the experiment. WC = water pan trap control, RC = Red delta trap control, YC = Yellow delta trap control, HH = Hexyl hexanoate, OH = Octyl hexanoate, and 0.2, 1, 2, 4, 8 mg are different doses of the blends tested. Means with different letters differ significantly (Tukey's post hoc test, $P < 0.05$).

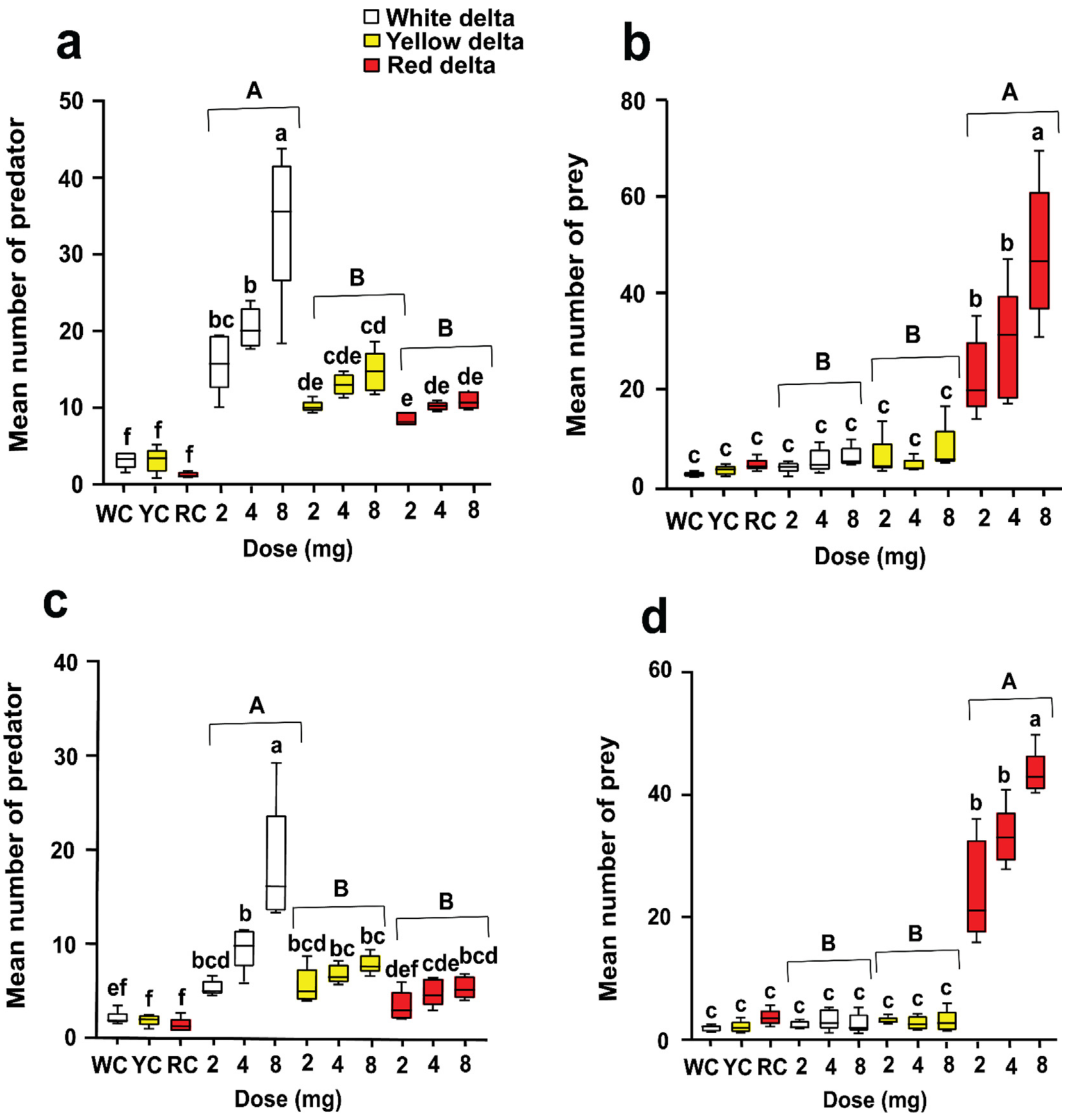

Fig. 6. Predator *Nesidiocoris tenuis* sex pheromone attracts prey *Phthorimaea absoluta* in farmers' field (a-b) Field experiment 3-Mwea showing Site 1, and (c-d) Site 2. WC = white delta trap control, RC = Red delta trap control, YC = Yellow delta trap control, and 2, 4, and 8 mg are different doses of the blends tested. Means with different letters differ significantly (Tukey's post hoc test, $P < 0.05$).

explained by the different sexes searching for different resources; mates in the case of the males, but food or near food in the case of females, which ripe tomato fruits and the greener plant parts may provide in terms of nutrition for their offspring. In fact, because *P. absoluta* females can oviposit on all the aerial parts of a tomato plant including fruits at different ripening stages [31,53], it is reasonable for them to respond to a pheromone-baited trap that may simulate the reflectance spectra of ripe tomato fruits, as found in the current study. It is possible that the trapping system may have favored the predator in the predator–prey interaction. In fact, both the predator and prey respond to tomato plant volatiles [30,32]. Hence, pheromone-releasing *N. tenuis* females present on tomato plants bearing ripe fruits may have the advantage of attracting *P. absoluta* females to lay their eggs on various parts of the plant including ripe tomato fruits. Because the predator responds to the reflectance spectrum of short wavelength (white traps), eggs laid by *P. absoluta* females may readily be detected by the predator to feed on, especially during the day. Furthermore, we note that the reflectance spectrum of green leaves was slightly higher than that of ripe tomato fruits, but the reflectance spectra of both the former and latter tissues were completely different from the white delta trap avoided by *P. absoluta* females. These results emphasize the need to study other visual cues as well as geographic and genetic differences in populations that may influence the responses of the insects in the predator–prey interaction.

Thirdly, the ability of a predator to influence prey behavior, physiology, and morphological traits mediated by predator cues is well documented [54,55]. For instance, the exposure of the prey Colorado potato beetle, *Leptinotarsa decemlineata* to pheromones of the associated spined soldier stink bug predator, *Podisus maculiventris* reduced the prey's feeding on potato plants [55]- a behavioral response that may reduce the prey's vulnerability to predation. Likewise, our previous work has shown that while both the prey *P. absoluta* and predator *N. tenuis* are attracted to intact tomato volatiles [32], induction of herbivore-induced plant volatiles (HIPV) by the predator elicits an avoidance behavior in the prey [56]. Additionally, we have shown in a previous study that intraguild interactions between the greenhouse whitefly *Trialeurodes vaporariorum* and *P. absoluta* causes the latter herbivore to lay more eggs on the basal stratum of tomato plants, which is away from its preferred oviposition site, the apical stratum [36]. The shift

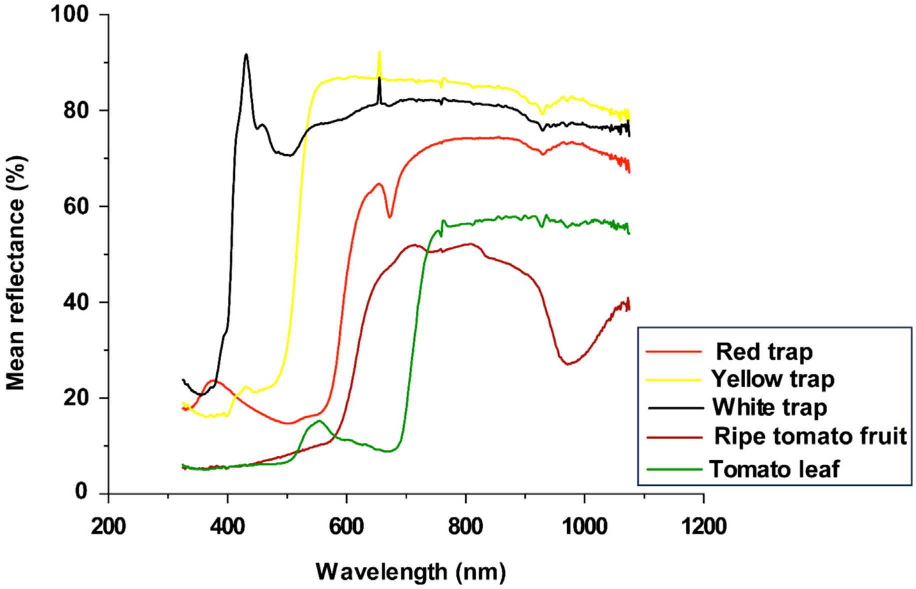

Fig. 7. Mean percent reflectance of white, yellow, and red delta traps, ripe tomato fruit, and green tomato leaves which measures the percentage of energy/radiation reflected by the objects out of the total energy/radiation incident upon them.

in oviposition behavior in *P. absoluta* led to decreased parasitism by the parasitoid *Bracon nigricans* on herbivore larvae [36]. Thus, given the complexity of the interactions between the different herbivores and natural enemies on tomato plants, and balancing between competition and utilization of the same resource for survival, it requires that the different insects on the resource adopt different strategies to increase their fitness. Natural enemies are known to benefit from preying on weaker herbivores on well-defended plants and vice-versa [57]. As such, in the current study, it appears that to increase its fitness, the predator may have adopted the strategy of using its sex pheromone combined with a specific visual cue to manipulate the behavior of a stronger herbivore on the tomato hostplant, known to draw in multiple herbivores and natural enemies [57]. Further studies with ripe tomato fruits are required to validate prey recruitment by the predator with its sex pheromone.

Conclusion

Firstly, in this study the Kenyan population of the zoophytophagous predator *N. tenuis* was found to produce a sex pheromone which varied from that reported for a strain sourced from a commercial supplier in Europe, clearly showing geographic variation in sexual communication in the predator. Secondly, we conclude that in the predator–prey interaction, the interaction may favor the predator since it can lure the prey with its sex pheromone in the presence of specific visual cues. In field trials, the invasive prey responded to the predator sex pheromone when combined with a visual cue that mimicked the reflectance spectrum of one of the preferred oviposition sites- ripe tomato fruits.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Credit author statement

FK, BT, and BA conceptualized and designed the study. BA conducted the experiments, BA FK, and AY analyzed the data, and BA wrote the first draft of the manuscript. BA, AY, BT, and FK revised the manuscript and approved the final draft. FK and BT sourced funding for the study. All authors approved the submitted version.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2024.05.005.

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