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# Evaluation of pheromone lures, trap designs and placement heights for monitoring the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize fields of Kenya

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#### ABSTRACT

The fall armyworm (FAW), Spodoptera frugiperda (J. E. Smith), is a damaging crop pest that has recently invaded and established across Africa from its native tropical and subtropical regions of the Americas. To develop an improved monitoring system for the FAW, we evaluated five commercial sex pheromone lures (Shenzhen Bioglobal, FALLTRACK, Enlure, P061-Lure and PH-869-1PR), three trap types (delta, bucket, and water-pan) and six placement heights (ground level, 0.5, 1, 1.5, 2 m above the ground and 0.2 m above the crop canopy) in replicated field trials at representative maize growing agroecologies of Kenya. Water-pan traps baited with the lures PH-869-1PR and P061-Lure captured the highest number of moths, whereas delta traps captured the least number of moths regardless of the pheromone lure used. Water-pan traps baited with Enlure and FALLTRACK lures captured more non-target insects than traps baited with the other lures. Traps placed at 1.5 m and 2 m above the ground captured more FAW moths than traps at the other placement heights. Genetic studies revealed no discernible differences between lures in the proportions of FAW strains captured. We recommend PH-869-1PR baited water-pan and bucket traps at a placement height of 1.5 m above ground for monitoring the FAW in Kenya. Moreover, we discussed the merits and drawbacks of different pheromone lure and trap combinations, and placement heights.

# 1. Introduction

Globally, invasive alien species pose a significant threat to agricultural crops which has been predicted to increase with a changing climate and increased international trade (Pratt et al., 2017). Studies have shown that invasive alien species can also displace native organisms, negatively impact biodiversity, and modify ecosystems causing huge economic losses (Kenis et al., 2009; Pratt et al., 2017; Kumar and Singh, 2020; Fortuna et al., 2022). In Africa, invasive alien species include insect pests such as the spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) (Kfir et al., 2002; Tamiru et al., 2011), the tephritid fruit flies, *Bactrocera dorsalis* (Hendel) and *Zeugodacus curcubitae* (Coquillett) (Diptera: Tephritidae) (Lux et al., 2003; Khamis et al.,

2009; Vayssières et al., 2007; Ndlela et al., 2022), the Asian citrus psyllid, *Diaphorina citri* (Oke et al., 2020), and Sirex woodwasp, *Sirex noctilio* (Taylor, 1962; Hurley et al., 2017). The most recent invasive insect species reported in sub-Saharan Africa (SSA) is the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), native to tropical and subtropical regions of the Americas. The FAW was first reported in west Africa in 2016, and it rapidly spread to almost all countries in the continent (Goergen et al., 2016; Rwomushana et al., 2018). The FAW has become a serious and growing threat to food security and livelihoods being a major pest of maize and sorghum, which are staple food and cash crops for more than 300 million people in Africa (Goergen et al., 2016; Day et al., 2017).

FAW moths fly long distances to find a mate and suitable host plants

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for oviposition (Shi-shuai et al., 2021; Sisay et al., 2022). The strong flight capacity, high fecundity, broad host range as well as the region's climate suitability have allowed the pest to quickly spread and fully establish in many African countries (Montezano et al., 2018; Baudron et al., 2019; Paredes-Sánchez et al., 2021). Additionally, older FAW larvae can dominate interspecific competitors and have developed resistance against many classes of insecticides (Yu, 1992; Chapman et al., 2000; Sokame et al., 2022). Moreover, the cryptic feeding behaviour of mature larvae, often concealed in the whorl of host plants, makes FAW control with chemical pesticides challenging. Systemic pesticides, which are absorbed by the plant and spread throughout its tissues, may be more effective in controlling concealed larvae. However, repeated use of chemical pesticides is not only expensive to smallholder farmers but can have increased risks for pollinators, human health, and the environment, including non-target beneficial species and the overall ecosystem (Bruce, 2010). Hence, integrated pest management (IPM) programs incorporating a range of complementary and ecologically friendly tactics are needed for sustainable FAW control.

Effective management of FAW requires a timely detection of the pest so that appropriate crop protection measures can be taken at early stages of crop infestation. Pheromone-based monitoring, surveillance, and scouting by capturing moths serve as critical activities and key tools for early detection of pests and taking timely and appropriate management decisions (Stokstad, 2017; Hendrichs et al., 2021). Previous studies which investigated pheromones released by the FAW female identified (Z)-9-tetradecen-1-ol acetate (Z9-14:OAc), (Z)-7-dodecen-l-ol acetate (Z7-12:OAc), (Z)-11-hexadecen- 1-ol acetate (Z11-16:OAc), (E)-7-dodecen-1-ol acetate (E7-12:OAc), (Z)-9-dodecen-1-ol acetate (Z9-12:OAc) and (Z)-1 l-tetradecen-1-ol acetate (Z11-14:OAc) (Tumlinson et al., 1986; Andrade et al., 2000; Batista-Pereira et al., 2006). Field trials with synthetic blends of these pheromones showed varying results in different regions of the world (Mitchell et al., 1985; Tumlinson et al., 1986; Meagher and Mitchell, 1998; Meagher et al., 2019). For example, pheromone blends found to work in North America and Europe were not effective in trapping FAW males in Brazil (Batista-Pereira et al., 2006), Costa Rica (Andrade et al., 2000) and Mexico (Malo et al., 2001). These variations in trap catches were attributed mainly to geographic differences in FAW response to pheromone lures though some strain-specific differences cannot be overruled (Unbehend et al., 2014). Furthermore, geographic variability in features such as host plant volatiles, interspecific olfactory cues and environmental factors which influence sexual communication has been ascribed to contribute to the variation in attraction to lures (Delisle and Mcneil, 1987; Royer and McNeil, 1993; Delisle and Royer, 1994; Landolt and Phillips, 1997; Reddy and Guerrero, 2004; Lima and McNeil, 2009; Groot et al., 2010; Unbehend et al., 2013). Similarly, pheromone-based FAW monitoring studies in Africa have revealed varying results (Meagher et al., 2019; Koffi et al., 2021; Tepa-Yotto et al., 2022), necessitating a systematic and well-designed evaluation and optimization of pheromone traps for different agroecologies.

Several studies reported the influence of trap design and placement height on the capture size of male FAW moths (Mitchell et al., 1989; Malo et al., 2001, 2004; Malo et al., 2018). For example, a white plastic jug trap exhibited the highest FAW trapping efficiency compared to a commercial green bucket trap and home-made traps including delta, sleeve and water bottle traps in Mexico (Cruz-Esteban et al., 2022). Meagher et al. (2019) found that bucket traps captured more male FAW moths than delta and locally designed traps in Togo (West Africa). In contrast, Malo et al. (2001) reported more FAW catches in Scentry Heliothis traps than in bucket traps in the coastal region of Chiapas, Mexico. Pertaining to placement height, Nwanze et al. (2021) found that a delta trap baited with the PH-869-1PR lure and placed at a height of 1.5 m captured more FAW moths than the same trap placed at a height of 1 m. Malo et al. (2004) demonstrated that Scentry Heliothis traps baited with Chemtica lures placed at 1.5 m above ground caught significantly more male FAW than those placed at 2 m. Interestingly, equal numbers of male FAW captures were found in bucket traps placed at 1 m, 1.5 m, and 2 m heights (Malo et al., 2004). Hence, there is a need to identify an effective trap design and placement height suitable for African agroecosystems since FAW is a relatively new pest in the continent.

In this study, we hypothesized that lure types, trap designs, and placement height would affect the efficacy of trap catch for FAW monitoring. Additionally, we hypothesize the pheromone lures would equally attract both rice and corn FAW strains. To test these hypotheses, we evaluated trap catches of FAW with five different commercial sex pheromone lures (Shenzhen Bioglobal, FALLTRACK, Enlure, P061-Lure and PH-869-1PR), three different trap designs (delta, bucket, and waterpan) and six different trap placement heights (ground level, 0.5, 1, 1.5, 2 m above the ground and 0.2 m above the crop canopy) in maize fields at representative maize growing agroecologies in Kenya. Furthermore, we characterised the strains of captured moths using molecular techniques.

#### 2. Materials and methods

# 2.1. Study sites

Pheromone lures and trap designs were evaluated during the main cropping season between June 2021 and November 2021 at three main representative maize growing agroecologies in Kenya, namely: Limuru, a highland area 2277 m above sea level (masl) (1.12°S, 36.65°E); Kitale, a mid-altitude area (1730 masl, 1.117°N, 35.12°E) and Kilifi, a lowland area (22 masl, 3.67°S, 39.86°E) (Fig. 1). The trap placement height evaluation experiment was conducted between November 2021 and February 2022 in Embu County (0.82440°S, 37.5116°E and 1113 masl). Laboratory studies were conducted at the International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus, Kasarani, Kenya (1.221°S, 36.896°E; 1616 masl).

# 2.2. Planting materials and trial management

The experiments were conducted in farmers maize fields during the main growing season. Maize planting was carried out on the 9th and 18th June 2021 in Limuru and Kilifi, and on the 14th July 2021 in Kitale. Maize varieties commonly grown by farmers in the study sites, which were H6213, PH4 and DK777 in Limuru, Kilifi and Kitale, were planted respectively. In Embu, hybrid maize variety pioneer (PHB 3253) was planted on the 14th November 2021. The sizes of the maize fields were 5 ha in Limuru and Embu, 4.5 ha in Kilifi, and 4 ha in Kitale. In all sites, maize was planted with 75 cm between rows and 25 cm between plants spacing. The fertilizer, diammonium phosphate (DAP), was applied at planting, while urea was applied 4 weeks after planting at a rate of 100 kg per hectare. No pesticides were applied on the maize fields at the study sites. Other agronomic practices such as weeding were conducted as recommended.

# 2.3. Trial 1: evaluation of pheromone lures and trap designs

Five commercially available sex pheromone lures and three trap designs were evaluated for their efficacy and specificity in trapping male FAW moths. Traps without lures were included as negative controls. The lures were Shenzhen Bioglobal (Shenzhen Bioglobal Agricultural Science Co. LTD, China), FALLTRACK (Kenya Biologics Ltd, Nairobi, Kenya), Enlure (Real IPM, Ltd, Nairobi, Kenya), PH-869-1PR (Russell IPM, Deeside, Flintshire, United Kingdom) and P061-Lure (Chemtica International S.A., Heredia, Costa Rica). All pheromone lures were preprepared in rubber septa and contained proprietary blends of synthetic female FAW sex pheromones. Traps included a white delta trap (Area: 20 cm (L)  $\times$  18 cm (W) = 360 cm²), black water-pan trap (trade name: Tutasan) (Diameter = 30 cm; Area:  $\pi$  (15 cm)² = 707 cm²), and white bucket trap with green canopy (Unitrap) (Diameter = 14 cm; Area:  $\pi$  (7 cm)² = 154 cm²). All traps were bought from Koppert Biological Systems

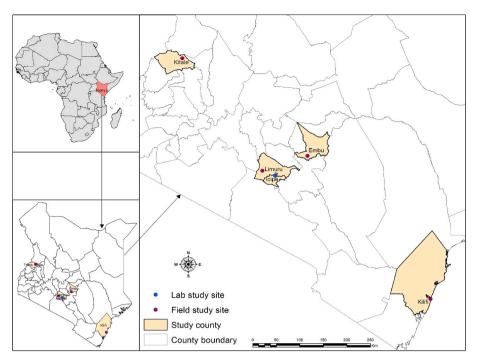


Fig. 1. Map of study sites.

(K) Ltd (Nairobi, Kenya). Lures were placed in the top center of waterpan and bucket traps using standard pheromone baskets. For delta traps, the lures were placed at the center of the sticky papers (18 cm  $\times$  18 cm). Treatments were set up in a randomized block design using 3  $\times$  6 factorial arrangements in maize fields. The treatments were replicated three times in each location. Traps were placed at 30 m intervals into the fields and hung uniformly at 1.5 m above the ground using wooden poles. The traps were set up when the maize plants were 8–9 days old and kept in place until harvest. The sticky pads for the delta traps were changed every fifteen days. Soapy water solution (1 % laundry detergent) was added to water-pan and bucket traps to prevent trapped moths from escaping. The water level in the traps was checked daily and refilled as required. All lures were changed at 30 days intervals.

The number of FAW moths and other non-target insect catches were recorded every 5 days with a total of 20, 22 and 25 sampling dates until silking stage of maize in Kilifi, Kitale, and Limuru, respectively. On each sampling date, FAW moths and non-target insects were recorded and removed from the traps. The non-target insects caught in Limuru alone were identified to order and family levels using previously described morphological keys (Borror and White, 1998; Gibb and Oseto, 2019), due to a very high number of non-target insect catches in other sites.

To determine the most effective trap-lure combination, all trap catches were summed within each lure and trap combination across all sampling dates from the start of the experiment, i.e., when maize plants were 8-9 days old until harvest. To compute the mean number of moths caught per night, trap catches during the first five consecutive sampling dates were summed, divided by the number of days, and expressed as trap catches per day. We chose the first five sampling dates for this variable, as the FAW moth population significantly declined during subsequent sampling dates as the maize plants matured and became less attractive for female FAW moths to lay eggs. To determine the crop stage at which the highest moth catches were recorded, maize growth stages were classified into different levels of vegetative (V) and reproductive (R) stages according to Pringle (2017) and Darby and Lauer (2004). These were V3 (third leaf collar): two weeks after planting, V5 (fifth leaf collar): 3-4 weeks after planting, V7 (seventh leaf collar): 5-6 weeks after planting, V10 (tenth leaf collar): 7-8 weeks after planting, VT (tasseling stage): 9-10 weeks after planting, and R1 (silking stage): over 11 weeks after planting. The FAW catches for each trap x lure combination across these maize growth stages were calculated by dividing the total number of FAW moths caught by the total number of observations at each crop stage and summed within a specific maize growth stage in each location.

# 2.4. Trial 2: evaluation of trap placement height in capturing FAW

The optimal trap placement height was determined at a study site in Embu County using the most effective lure (PH-869-1PR) and trap (water-pan trap) from the previous experiment (2.3). Traps were set up on the ground, 0.2 m above the crop canopy, 0.5, 1, 1.5, and 2 m above the ground, immediately after maize planting, and kept in place for the trial duration (three months). A trap placed at 0.2 m above the crop canopy but without a lure was used as a control. The treatments were set up in a randomized block design with four replications. Traps were checked every five days for a total of 21 observation dates. A stepladder was used to access the 2 m high traps for counting moth captures and refilling the water-soap solution. The moth catches were recorded during the various maize growth stages as described in section 2.3.

#### 2.5. Molecular identification of captured FAW strains

DNA analysis was carried out to identify FAW strains (corn, rice). Genomic DNA was isolated from 50 fresh moths caught from Limuru and Kilifi using traps baited with Shenzhen Bioglobal, P061-Lure, PH-869-1PR, FALLTRACK and Enlure lures (10 moths from each lure) with the ISOLATE II Genomic DNA Kit (Bioline, London, UK) according to the manufacturer's instructions. The mitochondrial COI gene was targeted using LepF1 5'ATTCAAC-CAATCATAAAGATAT-TGG 3' and LepR1 5' TAAACTTCTGGATGTCCA- AAAAATCA 3' (Hajibabaei et al., 2006) markers in addition to the traditional barcode region markers LCO 1490 5' GGTCAACAAATCATAAAGATATTGG 3' and HCO2198 5' TAAACTTCAGGGTGACC-AAAAAATCA 3' (Folmer et al., 1994). The PCRs were conducted in a total reaction volume of 20  $\mu$ L containing 5  $\times$  My Taq Reaction Buffer (5 mM dNTPs, 15 mM MgCl2, stabilizers and enhancers), 0.5 pmol  $\mu$ l $^{-1}$  of each primer, 0.5 mM MgCl2, 0.0625 U  $\mu$ l $^{-1}$  My Taq DNA polymerase (Bioline, London, UK) and 15 ng  $\mu$ l $^{-1}$  of DNA

template. The reactions were set up in the Nexus Mastercycler gradient (Eppendorf, Germany) with the following cycling conditions: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s annealing (52 °C for LepF1/R1 and 54.1 °C for LCO/HCO), extension for 1 min at 72  $^{\circ}$ C, then a final elongation step of 10 min at 72  $^{\circ}$ C. The PCR products were resolved through a 1.2% agarose gel, then visualized and documented using KETA GL imaging system trans-illuminator (Wealtec Corp, Meadowvale Way Sparks, Nevada, USA). These were further purified using Isolate II PCR and Gel Kit (Bioline) following the manufacturer's instructions then shipped to Macrogen Europe BV (Meibergreef, Amsterdam, the Netherlands), for bi-directional sequencing. The successful sequences were assembled and edited using Geneious Version 8 (http://www.geneious.com) (Kearse et al., 2012). For conclusive identification of the rice and corn strain, similarity searches were conducted by querying the consensus sequences via BLASTn at the GenBank database hosted by National Centre of Biotechnology Information (NCBI). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version X (Kumar et al., 2018) using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The reliability of the tree was assessed using 1000 bootstrap replications.

#### 2.6. Data analysis

All data were checked for normality and homogeneity using the Shapiro–Wilk and Bartlett tests, respectively. In Trial 1, due to over-dispersion of the count data, a generalized linear model (GLM) with a negative binomial distribution was employed to compare the number of FAW moths and non-target insects captured by each trap  $\times$  lure combination. GLM with Poisson distribution was used to analyze the number

of FAW moths caught per day over the first five sampling dates. FAW moth catches across all traps  $\times$  lure combination within a specific maize growth stage were summed and converted into percentages of total moth catch over the entire crop duration in each location. An analysis of variance (ANOVA) was used to determine the effect of crop stage on moth catches and percentage of non-target insect catches. Similarly, in Trial 2, the number of FAW moths caught at different trap placement heights was compared using a GLM with a negative binomial distribution. Differences between lures in attracting corn and rice strains of FAW were determined using chi-square tests. All treatment means were compared using post-hoc Tukey's HSD tests (P < 0.05). All statistical analyses were done using R statistical software (v. 4.0.4) (R Core Team, 2021).

#### 3. Results

# 3.1. Evaluation of pheromone lures and trap designs

Overall, 10183 FAW male moths were caught across all study sites. Of these, the highest number of moths (38.1%) was captured by the PH-869-1PR lure, followed by P061-Lure (31.1%), Shenzhen Bioglobal lure (19.2%), FALLTRACK (6.8%) and Enlure (3.3%), with the lowest catch recorded in the control trap (1.3%). Number of FAW moths captured varied significantly among lures and traps in the three study locations (Fig. 2a, b, c). PH-869-1PR lure-baited traps captured significantly higher number of moths than P061-Lure and Shenzhen Bioglobal in Limuru (Fig. 2a) and Kilifi (Fig. 2b), but not in Kitale (Fig. 2c). PH-869-1PR and P061-Lure baited bucket traps caught significantly more moths than delta traps baited with the same lures in Limuru and Kilifi (Fig. 2a and b; Table 1). FALLTRACK and Enlure lures caught the fewest number

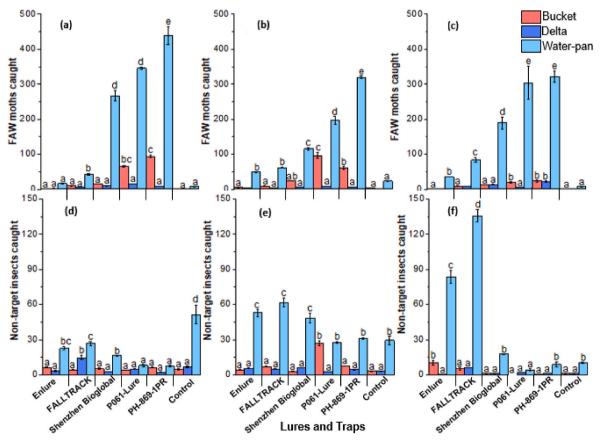


Fig. 2. Total numbers of FAW male moths and non-target insects caught by three traps baited with different lures. Mean ( $\pm$ SE) trap catches of male FAW moths in Limuru (a), Kilifi (b), Kitale (c) and non-target insects in Limuru (d), Kilifi (e), Kitale (f) are shown. Bars with different letters indicate significant differences in lure \* trap designs interaction at P  $\leq$  0.05 using Tukey's HSD test [N = 25 (Limuru), N = 20 (Kilifi), and N = 22 (Kitale)].

Table 1
Statistical analysis comparing effects of lures, traps and their interactions in FAW and non-target insects catches in the three study locations using GLM with a negative binomial distribution.

Locations	Source of variations	Degrees of freedom	FAW			Non-target insects		
			Deviance	Residual deviance	Pr (>Chi)	Deviance	Residual deviance	Pr(>Chi)
Limuru	NULL			46.4			43.5	
	Lures	5	11.3	35.1	0.04	13.3	30.2	0.02
	Traps	2	15.8	19.3	0.00013	20.3	9.9	< 0.0001
	Lures × Traps	10	1.6	17.7	0.04	0.8	9.1	0.02
Kilifi	NULL			62.8			106.8	
	Lures	5	12.4	50.4	0.04	14.4	92.4	0.03
	Traps	2	29.1	21.3	< 0.0001	71.6	20.8	< 0.0001
	Lures $\times$ Traps	10	1.4	19.9	0.03	2.5	18.3	0.03
Kitale	NULL			58.7			126.9	
	Lures	5	12.1	46.6	0.04	43.3	83.5	< 0.0001
	Traps	2	27.1	19.5	< 0.0001	63.3	20.2	< 0.0001
	Lures $\times$ Traps	10	0.5	19.0	0.03	2.9	17.3	0.01

Pr (>Chi) is P-value for the chi-square statistic.

of FAW moths, regardless of trap design used in all locations (Fig. 2a, b & c). The interaction effects of lure  $\times$  trap combination showed significant differences in FAW catches (P < 0.05) across the three locations (Table 1).

There were also significant differences between the lures and trap designs in capturing non-target insects at the three locations (Fig. 2d, e, f). For example, the water-pan traps baited with FALLTRACK, Enlure, and Shenzhen Bioglobal captured significantly higher numbers of non-target insects than bucket and delta traps (P < 0.001). On the other hand, PH-869-1PR or P061-Lures baited traps captured significantly fewer non-target insects in the respective study location, the lowest across all locations being delta trap design (Fig. 2d, e, f). The interaction effects of lure  $\times$  trap combination showed significant differences in non-target insect catches (df = 10; P < 0.05) across the three locations (Table 1).

Total number of FAW captured in water-pan traps were significantly more than bucket and Delta traps ( $\chi^2=5280.8, P<0.0001$ ). Moreover, the bucket trap captured significantly more moths ( $\chi^2=5280.8, P<0.001$ ) than the delta trap. The Delta trap captured the least number of

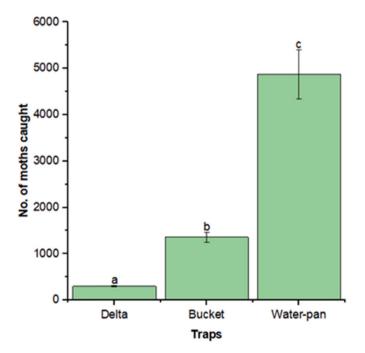


Fig. 3. Total male FAW moths caught by three pheromone-baited traps (Waterpan, Bucket, Delta) across the study locations. Bars with different letters are significantly different at  $P \leq 0.05$  using the chi-square test.

FAW moths (Fig. 3).

The number of FAW moths captured per day varied with the type of pheromone lure used (Fig. 4). Significantly more moths were caught per day in traps baited with PH-869-1PR than with the other lures (df = 5,  $\chi^2$  = 186.4, P < 0.01). Traps baited with P061-Lure and Shenzhen Bioglobal lure caught significantly more moths per night than FALLTRACK and Enlure (Fig. 4).

Non-target insects varied with the trap design and lure, with the highest proportion recorded from the family Muscidae while the least from the families Apidae, Sphecidae and Chysomelidae (Table 2). Traps baited with FALLTRACK lures captured predominantly insects belonging to Muscidae and Apidae followed by Enlure lures (P < 0.05). The most abundant non-target insects came from the order Diptera while the lowest from Coleoptera (Table 2).

Across the study sites, trap catches decreased by more than 50% with increasing maize maturity (Fig. 5). The highest FAW catches were recorded at V3 and V5 maize growth stages, while the lowest at tasseling (VT) and silking (R1) stages (df = 5; P < 0.001) (Fig. 5).

# 3.2. Evaluation of trap placement height

Trap height significantly affected captures of FAW male moths (Fig. 6). Traps placed at 1.5 and 2 m above ground caught the greatest number of moths at V3 ( $\chi^2=49.9,\ P<0.0001$ ), V5 ( $\chi^2=43.7,\ P<0.0001$ ), V7 ( $\chi^2=17.06,\ P<0.01$ ) and V10 ( $\chi^2=18.2,\ P<0.01$ ) maize growth stages. There were no significant differences in the number of FAW moths caught between different heights when traps were deployed at VT ( $\chi^2=10.4\ P>0.05$ ) and RI ( $\chi^2=2.05,\ P>0.05$ ) growth stages. At

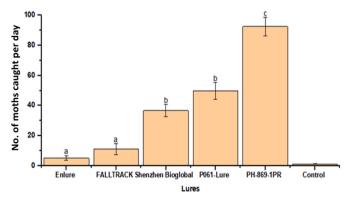


Fig. 4. Mean FAW male moths captured per day by five lures (Shenzhen Bioglobal, FALLTRACK, Enlure, P061-Lure and PH-869-1PR) and control across study locations. Mean ( $\pm$ SE) number of moths caught per day has been shown. Bars with different letters are significantly different at P  $\leq$  0.05 using Tukey's range test (N = 5).

Table 2 Non-target insects captured during lure  $\times$  trap design evaluation study in Limuru (Jun–Nov 2021).

Lures	Percentage of non-target insect caught						
	Diptera	Hymenop	tera	Coleoptera	Total		
	Muscidae	Apidae	Sphecidae	Chysomelidae			
P061-Lure	$5.6 \pm 0.6$	0.6 ±	$1.5\pm0.7$	$1.1\pm0.8~\mathrm{Bb}$	8.8		
	Ca	0.2 Cb	Bb				
PH-869-1PR	$6.4\pm1$	0.4 $\pm$	$0.6\pm0.3$	$0.8\pm0.5~Bb$	8.2		
	Ca	0.2 Cb	Cb				
Shenzhen	$9.2\pm3$	$1.3~\pm$	$1.3\pm0.4$	$0.8\pm0.5~Bb$	12.6		
Bioglobal	Ba	0.7 Bb	Bb				
Enlure	12.0 $\pm$	2.4 $\pm$	$1.5\pm0.6$	$2.3\pm0.3~\text{Ab}$	18.2		
	5.9 Ba	0.6 Bb	Bb				
FALLTRACK	$26.9 \pm$	8.3 $\pm$	$3.2\pm0.8$	$2.6\pm0.2~Ac$	41		
	6.2 Aa	2.6 Ab	Ac				
Control	$8.5\pm2.9$	$1.3~\pm$	$1.3\pm0.5$	$0.2\pm0.1~\text{Cc}$	11.3		
	Ba	0.75 Bb	Bb				
Overall percentage of non-target	68.6	14.3	9.4	7.8			
insect							

Means followed by different letters within a column (upper case letters) and within a row (lower case letters) are significantly different at  $P \leq 0.05$  using chisquare test.

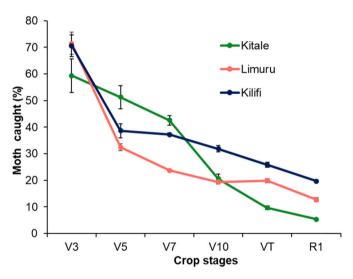


Fig. 5. Percentage of male FAW moths caught at V3, V5, V7, V10, VT and R1 stages of the maize plants in Kitale, Limuru and Kilifi. FAW moth catches (mean  $\pm$  SE) were compared by ANOVA. Non-overlapping error bars within each location indicate statistically significant difference in moth catches at  $P \leq 0.05$  using Tukey's HSD test.

the V3 stage, traps placed 0.5 and 1 m above the ground caught more moths ( $\chi^2 = 46.9$ , P < 0.05) than traps set at the ground and 0.2 m above the crop canopy. The least number of moth captures were recorded in traps placed on the ground and 0.2 m above the crop canopy (Fig. 6).

# 3.3. Molecular identification of captured FAW strains

All commercial lures captured both corn and rice strains of FAW moths (Fig. S1). Of the FAW moths captured in traps baited with P061-Lure, PH-869-1PR and FALLTRACK lures, 60% comprised rice strains while 40% were corn strains. Conversely, 40% rice strain and 60% corn strain were captured in traps baited with Enlure lure. Shenzhen Bioglobal caught equal proportions of the rice and corn strains (Fig. 7). However, the differences in proportion of corn and rice FAW strain captured by various lures were not statistically significant (df = 6,  $\chi$ 2 =

10.27, P > 0.05) (Fig. 7).

#### 4. Discussion

The fall armyworm (FAW) is a new invasive pest of maize crop in Africa, including Kenya. Commercially produced FAW sex pheromones have been successful used to monitor the FAW population in the Americas, where the pest originated. However, the efficacy of commercially available pheromone lures towards the African populations of FAW is largely unknown. In this study, we evaluated the efficacy of five pheromone lures, three trap designs, and six trap placement heights on the capture of FAW male moths at representative maize growing agroecologies in Kenya. Our results showed that Russell IPM lure "PH-869-1PR" and Chemtica International lure "P061-Lure" captured a greater number of FAW male moths in all study sites. The maximum number of moths caught per day was recorded in PH-869-1PR baited traps, followed by P061-Lure and Shenzhen Bioglobal lure. Enlure caught the lowest number of FAW moths per day. Our results agree with previous studies conducted in Niger, Argentina, and Mexico where highest FAW trap captures were recorded with the Russell IPM and Chemtica lures (Bratovich et al., 2019; Nwanze et al., 2021; Malo et al., 2001). Several synthetic pheromone blends are currently available, but their performance in attracting FAW males varies across regions (Batista-Pereira et al., 2006; Meagher et al., 2019). The variations in the ratio and composition of pheromone molecules released by the different commercial lures may have contributed to the differences in their attractiveness to male FAW moths (Mitchell et al., 1985; Spears et al., 2016; Meagher et al., 2019). Moreover, several studies have demonstrated significant intraspecific geographic variation in moth pheromone composition (Andrade et al., 2000; El-Sayed et al., 2003; Batista-Pereira et al., 2006). This could be due to reproductive isolation of the populations arising from geographical isolation, which may in turn elicit correspondingly adaptions in male FAW response (Malo et al., 2004).

Our results showed that, although there is genetic variation in FAW populations, both corn and rice FAW strains were attracted to the different commercial pheromone lures tested. Moreover, there were no significant differences in the proportion of rice and corn strain moths attracted to the pheromone blends under field conditions. The relatively higher capture of rice strains than corn strains, though not statistically significant, could be attributed to the high population of rice strains found in Africa, as described by Nagoshi et al. (2018) and Gichuhi et al. (2020). Our findings from the field are supported by wind-tunnel assays results of Unbehend et al. (2013) which demonstrated an absence of differential attraction of FAW males towards females from the two strains despite strain-specific differences in the female pheromone composition (Groot et al., 2008; Unbehend et al., 2013). Nevertheless, in corn fields, Unbehend et al. (2013) observed that the corn strain-specific pheromone blend attracted more males of both strains than the rice strain-specific pheromone blend, whereas, in grass fields there was no significant difference between the strain-specific blends. Earlier, Meagher and Nagoshi (2004) reported substantial overlap in strain distribution and attraction of both strains during pheromone trapping although proportions varied based on dominant host plant species. These studies highlight the influence of environmental factors such as background plant volatiles on FAW pheromone blend attractiveness. Hence, further studies with larger FAW sample size collected from diverse agroecologies and cropping systems could better decipher the key factors modulating variations in strain-specific pheromone attractiveness.

Trap design has also been shown to influence moth capture (Mitchell et al., 1985; Guerrero et al., 2014). In our study, pheromone-baited water-pan traps captured a higher number of FAW moths compared to bucket and delta traps. Specifically, the water-pan traps baited with PH-869-1PR lure recorded the highest FAW catches, followed by the same trap type baited with P061-Lure. The open top design of the

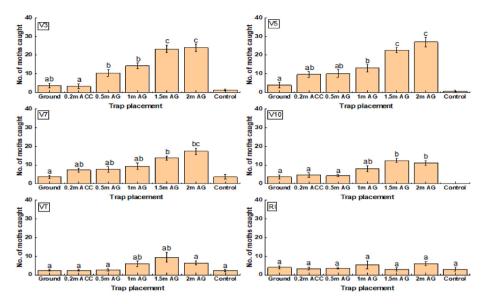
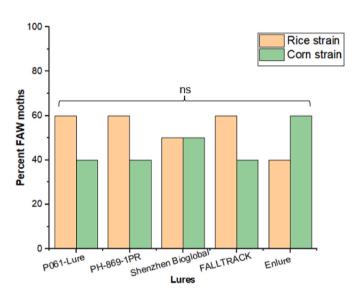


Fig. 6. Number of FAW male moths captured by pheromone baited traps placed at different heights and growth stages of maize plant. The maize growth stages were V3 (third leaf collar), V5 (fifth leaf collar), V7 (seventh leaf collar), V10 (tenth leaf collar), VT (tasseling stage) and R1 (silking stage). ACC = above crop canopy, AG = above ground. Data are means ( $\pm$ SE) number of FAW male moths captured. Bars with different letters within each growth stage are significantly different using Tukey's HSD test (N = 8; P < 0.05).



**Fig. 7.** Percentage of corn and rice strain of *S. frugiperda* moths collected by traps baited with different lures. Chi-Square analysis showed no statistically significant differences between lures in catching corn and rice *S. frugiperda* strains (N = 50).

water-pan trap may have also contributed to its effectiveness. On the other hand, water-pan traps needed frequent soapy water refilling due to high evaporation rates, especially during hot weather, caused by direct exposure of trap water solution to sun and wind as well as the trap having a larger surface area. Previous studies suggested adding unused motor oil on the trap's water to enhance surface water retention and reduce evaporation in water-pan traps (Aksoy and Kovanci, 2016). We found that bucket traps captured more FAW moths than delta traps as previously reported by Meagher et al. (2019). The restricted entrance of the bucket trap may account for the relatively lower moth catches compared to water-pan traps. The poor performance of delta traps could be due to narrow trapping surface area, which loses its effectiveness as they become saturated with high moth populations in a shorter time (Aksoy and Kovanci, 2016).

Another important parameter to enhance trap catch is determining

the optimal placement height of traps (Malo et al., 2004). Our result revealed that there was a direct correlation between the number of FAW male moth captures and trap placement height. Traps placed at 1.5 m and 2 m above ground level captured a greater number of male moths than traps at lower placement heights. Interestingly, no statistically significant difference was detected on the numbers of male moths captured at these two placement heights, i.e., 1.5 and 2 m. However, it was difficult to monitor and refill water pan traps placed 2 m above the ground without a stepladder. Hence, we recommend 1.5 m trap placement height for convenient monitoring and management of trapped insects including checking and refilling of the trapping water as necessary. Our results agree with previous studies which showed that traps baited with PH-869-1PR (Nwanze et al., 2021) and Chemtica lures (Malo et al., 2004) placed at 1.5 m height captured more FAW than traps at other placement heights. Furthermore, our study showed decreased FAW trap catches with increasing maize maturity. This suggests that for effective management of the FAW, pheromone-baited traps should be set up during the early maize growth stage, as previously found by Malo et al. (2001). The reduced trap catches at the later stage of the plant could be attributed to low oviposition preference of FAW moths to older and matured plants. Evidence of a higher oviposition preference of FAW moths for younger maize plants has previously been documented (Harrison, 1984).

Our results also revealed that pheromone-baited traps captured several non-target insects from different orders such as Diptera, Coleoptera, and Hymenoptera. Notably, water-pan traps baited with FALL-TRACK and Enlure lures, including control, captured more non-target flying insects. There could be different possible reasons for this finding besides the open-end design of water-pan traps. The quest for moisture by some non-target insect species may contribute to their accidental capture, while others may be attracted to certain chemical components of the pheromone lures. Furthermore, insects that feed on decaying or decomposing matter, such as house flies, stable flies and beetles, may be attracted to odors of trapped dead moths in the traps. Interestingly, we found that PH-869-1PR and P061-Lure captured the lowest number of non-target insects, whereas FALLTRACK and Enlure lures captured the highest numbers of non-target insects. Previous studies reported diverse factors including the type of pheromone lures, trap design, background host plant volatiles and surrounding environments to influence the captures of non-target insects (Adams et al., 1989; Mitchell et al., 1989;

Gross and Carpenter, 1991; Meagher and Mitchell, 1999; Malo et al., 2001; Spears et al., 2016). Indiscriminate trapping of large number of non-target species can influence the effectiveness of monitoring by reducing the available space for target species and may negatively impact beneficial insect populations such as predators and pollinators (Spears et al., 2016). Hence, selecting a pheromone lure and a trap design with minimal non-target trap catch is necessary. Moreover, further research to better understand the contributing factors of non-target captures will help to enhance the performance and efficacy of pheromone-baited traps.

In conclusion, our study provides useful insight into the choice of lure, trap design and placement height for robust monitoring of FAW populations in Kenya and East Africa at large. Although PH-869-1PR lure gave the highest trap catches, we recommend using either PH-869-1PR or P061-lure as both lures resulted in reasonably high FAW catches and minimum trapping of non-target insects. The water-pan traps baited with PH-869-1PR lure gave the highest trap catch followed by bucket traps. Bucket traps may have the advantage of slowing the evaporation of the soapy water solution, which is used to prevent trapped moths from escaping, for a longer period due to the protection provided by its canopy. We recommend placing pheromone traps at a height of 1.5 m for optimal capture of the FAW in maize fields. We also recommend studying the pheromone composition of FAW strains from wider geographic locations and examining effects of trap color in the attraction of FAW and non-target insect species to further improve monitoring of the pest.

# 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.

# Data availability

Data will be made available on request.

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# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2023.106523.

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