Monitoring Spodoptera frugiperda in Benin: assessing the influence of trap type, pheromone blends, and habitat on pheromone trapping

Authors: Tepa-Yotto, Ghislain T., Meagher, Robert L., Winsou, Jeannette K., Dahoueto, Borghero T. A., Tamò, Manuele, et al.

Source: Florida Entomologist, 105(1) : 71-78

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.105.0111

Monitoring *Spodoptera frugiperda* in Benin: assessing the influence of trap type, pheromone blends, and habitat on pheromone trapping

Ghislain T. Tepa-Yotto[1,2,], Robert L. Meagher[3], Jeannette K. Winsou[4,5], Borghero T. A. Dahoueto[1,2], Manuele Tamò[1], May-Guri Sæthre[6], and Rodney N. Nagoshi[3]*

Abstract

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has now become a pest of global concern. Originally known to be endemic to the Western Hemisphere, its first detection in Africa was followed by spectacular outbreaks and spread to almost all sub-Saharan countries. The rapid incursion of *S. frugiperda* on maize (*Zea mays* L.; Poaceae) fields in Africa highlighted a crucial need for a comprehensive assessment of integrated pest management strategies in most smallholder farms. However, these strategies cannot successfully function without efficient monitoring and surveillance efforts. These trapping studies were designed to provide an indication as to whether pheromone trap-lure combinations and simple changes in landscape and agricultural practices might mitigate fall armyworm infestations. Our data show that the commercially available Unitrap was the most effective design for fall armyworm captures among the traps tested. The inexpensive home-made 2 L jar trap was capable of consistently collecting fall armyworm during the first season of relatively moderate fall armyworm density. However, the number of fall armyworm captured by home-made trap were several fold lower than by the Unitrap under all conditions, and almost no fall armyworm was captured during the second season by home-made 2 L jar when fall armyworm density was low. Substantial differences were observed among the pheromone blends with respect to numbers of fall armyworm and non-targets captured. The 4-component blend attracted the most fall armyworm under all conditions. The 2-component blend was the most selective, with no non-target species found during the second season experiments.

Key Words: fall armyworm; monitoring; pheromone traps; pheromone lures; cropping system

Resumen

El gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), se ha convertido ahora en una plaga de preocupación mundial. Originalmente conocido por ser endémico del hemisferio occidental, su primera detección en África fue seguida por brotes espectaculares y se extendió a casi todos los países subsaharianos. La rápida incursión de *S. frugiperda* en los campos de maíz (*Zea mays* L.; Poaceae) en África destacó la necesidad crucial de una evaluación integral de las estrategias de manejo integrado de plagas en la mayoría de las fincas pequeñas. Sin embargo, estas estrategias no pueden funcionar con éxito sin esfuerzos eficientes de seguimiento y vigilancia. Estos estudios de trampas se diseñaron para proporcionar una indicación de si las combinaciones de trampas de feromonas y señuelos y los cambios simples en el panorama y las prácticas agrícolas podrían mitigar las infestaciones del gusano cogollero. Nuestros datos muestran que Unitrap, disponible comercialmente, fue el diseño más efectivo para la captura de gusanos cogolleros entre las trampas probadas. La trampa de frasco de 2 L de bajo costo, hecha en casa, fue capaz de recolectar consistentemente el gusano cogollero durante la primera temporada de densidad relativamente moderada del gusano cogollero. Sin embargo, el número de gusanos cogolleros capturados por trampa casera fue varias veces menor que por Unitrap en todas las condiciones, y casi ningún gusano cogollero fue capturado durante la segunda temporada por un frasco casero de 2 L cuando la densidad del gusano cogollero era baja. Se observaron diferencias sustanciales entre las mezclas de feromonas con respecto al número de gusanos cogolleros capturados y no objetivo. La mezcla de 4 componentes atrajo a la mayoría de los gusanos cogolleros en todas las condiciones. La mezcla de 2 componentes fue la más selectiva, y no se encontraron especies no objetivo durante los experimentos de la segunda temporada.

Palabras Clave: gusano cogollero; monitoreo; trampas de feromonas; señuelos de feromonas; sistema de cultivo

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has now become a pest of global concern. Originally known to be endemic to the Western Hemisphere, its first detection in Africa (Goergen et al. 2016) was followed by spectacular outbreaks and

[1]Biorisk Management Facility (BIMAF), International Institute of Tropical Agriculture (IITA-Benin), Cotonou, Benin; E-mail: G.Tepa-Yotto@cgiar.org (G. T. T.-Y.); borgherotar@gmail.com (B. T. A. D.); M.Tamo@cgiar.org (M. T.)
[2]Ecole de Gestion et de Production Végétale et Semencière (EGPVS), Université Nationale d'Agriculture (UNA), Kétou, Bénin
[3]USDA-ARS CMAVE, Insect Behavior and Biocontrol Research Unit, Gainesville, Florida 32608, USA; E-mail: rob.meagher@usda.gov (R. L. M.); rodney.nagoshi@usda.gov (R. N. N.)
[4]Faculty of Biosciences (BIOVIT), Norwegian University of Life Sciences (NMBU), Ås, Norway; E-mail: kossiba.jeannette.winsou@nmbu.no (J. K. W.)
[5]Department for Invertebrate Pests and Weeds in Forestry, Horticulture and Agriculture, Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway
[6]Department for Climate, Energy and Environment, Norwegian Agency for Development and Cooperation (NORAD), Oslo, Norway; E-mail: May-Guri.Saethre@norad.no (M. G. S.)
*Corresponding author; E-mail: G.Tepa-Yotto@cgiar.org

it spread to almost all sub-Saharan countries. The pest was reported 2 yr later in India (Sharanabasappa et al. 2018). To date fall armyworm is present in major maize (*Zea mays* L.; Poaceae) production regions of Africa, Asia (He et al. 2021), Australia (Overton et al. 2021), and Timor-Leste (FAO 2020). The rapid incursion of *S. frugiperda* on maize fields in Africa highlighted a crucial need for a comprehensive assessment of integrated pest management strategies in most smallholder farms. However, these strategies cannot successfully function without efficient monitoring and surveillance efforts (Meagher et al. 2019).

Pheromone trapping, using components of sex pheromones to lure adult males into a collection trap, provides an efficient method for detecting fall armyworm that can greatly facilitate monitoring of this pest (Mitchell et al. 1985). However, there is a diversity of pheromone blends and trap styles, and the various combinations can show substantial regional differences in effectiveness with respect to the number of fall armyworm captured and the frequency of non-target Lepidoptera collected (Meagher & Mitchell 2001; Meagher et al. 2013). In perhaps the best documented example, Fleischer et al. (2005) demonstrated that a pheromone blend highly specific for fall armyworm contained components highly attractive to another noctuid species, *Leucania phragmatidicola* Guenée (Lepidoptera: Noctuidae). Trapping with these lures resulted in a high frequency of non-target captures and the possibility of false positives and exaggerated fall armyworm counts. These observations indicate that the effectiveness of any particular pheromone trapping methodology is influenced by the composition of moths in a given region and time, and therefore needs to be empirically assessed rather than assumed.

As a recent intruder into Africa, it would not be surprising if the sexual communication system of fall armyworm overlaps that of native lepidopterans. In fact, it already has been reported that of the 8 *Spodoptera* species known to be in Africa, i.e., *S. apertura* (Walker), *S. cilium* Guenée, *S. exempta* (Walker), *S. exigua* (Hübner), *S. littoralis* (Boisduval), *S. mauritia* Viette, *S. malagasy* (Boisduval), and *S. triturata* (Walker) (all Lepidoptera: Noctuidae) (Pogue 2002), 5 share sex pheromone components with fall armyworm (Hänniger et al. 2020). Such overlaps could compromise the selectivity of commercially available fall armyworm pheromone blends dependent on the lepidopteran species present, which will vary significantly depending on location, habitat, and time.

Meagher et al. (2019) tested different lure and trap combinations in the African nation of Togo and found substantial differences in both number of fall armyworm captured and percentage of moths that were not identified as fall armyworm (percentage non-target captures). However, because native lepidopteran species and agricultural practices can vary substantially across Africa, it is not clear how applicable the results from one region will be to the rest of the continent. To facilitate fall armyworm monitoring in Benin, we tested different trap and lure combinations in the country both for the number of fall armyworm and non-targets captured. An important objective was to assess whether 2 inexpensive home-made trap designs could be effective enough to substitute for commercial traps commonly used by international stakeholders to monitor fall armyworm in Africa (Prasanna et al. 2018; FAO & CABI 2019). While known to be effective, the latter generally are not affordable to small stakeholder farmers in Africa.

These trapping studies were designed to provide an indication as to whether simple changes in landscape and agricultural practices might mitigate fall armyworm infestations. These included testing whether the effectiveness of intercropping strategies could mitigate fall armyworm damage. With respect to the latter, it has been suggested that interspersing the preferred host plant (maize) with a non-host plant (e.g., cowpea, *Vigna unguiculata* (L.) Walp; Fabaceae) might significantly reduce the attractiveness in the field to fall armyworm infestations (Thierfelder et al. 2018; Harrison et al. 2019). Cowpea previously has been shown to be a poor developmental host for fall armyworm (Meagher et al. 2004; Carroll et al. 2008), but is a useful cover crop and food source for humans. These traits could mean that intercropping could reduce local fall armyworm numbers without compromising food yield. A final objective was to assess whether the above treatments, applied at the scale of a single ha, can produce measurable local changes in fall armyworm population density. Because fall armyworm is known to be highly mobile and capable of long-distance flight, it is possible, if not likely, that fall armyworm mitigation will have to be coordinated at the regional level, otherwise individual small farms will be subject to infestations from neighboring farms. The implications of these preliminary findings for future studies are discussed.

Materials and Methods

TRAP TYPES

One inexpensive and recyclable home-made trap initially was tested and compared to the commercially available Unitrap (also known as bucket trap; International Pheromone Systems, Neston, United Kingdom; available from many distributors for about USD $10) that was the most deployed trap in Africa by international stakeholders to monitor fall armyworm during initial outbreaks (Prasanna et al. 2018; FAO & CABI 2019). The tri-colored Unitrap consists of a white bucket container with a green top that provides limited protection from rain and a yellow funnel (total height 21 cm, bucket circumference 50 cm) (Fig. 1A). The pheromone lure was put in a basket within the top. The home-made trap, Jar2, consisted of an inexpensive (about USD 2 cents each) multipurpose transparent 2L plastic bucket with a blue lid (Fig. 1B). Four equidistant 8 × 3 cm opening holes were made on the upper cylindrical surface of the bucket as entrances for attracted moths. The pheromone lure was wrapped in gauze and hung from the bucket lid by a nylon wire. An orange funnel was placed inside the bucket. A 4 L variant of Jar2, designated Jar4, also was tested during the second growing season. All home-made traps were made from items commonly found in local markets and were easily constructed. An odorless alpha-cypermethrin 100 g per L fumigant layer was placed in the bottom of each trap to kill specimens.

PHEROMONE LURES

During the first growing season tests, a 2-component pheromone lure was evaluated. The 2-component lure contained (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac) and (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac) (L976 or fall armyworm-PSU lure) (Scentry Biologicals, Inc., Billings, Montana, USA). Three Pherobank (Wilk bij Duurstede, The Netherlands) custom-made *Spodoptera frugiperda* lures were produced using the chemical analysis from Meagher et al. (2013), and were compared in Benin during the second growing season: 4-component lure type containing *Z*9-14:Ac (78.3%), (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac) (3.6%), *Z*7-12:Ac (11.2%), and (*Z*)-9-dodecenyl acetate (*Z*9-12:Ac) (7.0%); 3-component lure type composed of *Z*9-14:Ac (66.1%), *Z*11-16:Ac (4.7%), and *Z*7-12:Ac (29.3%); and 2-component lure type containing: *Z*9-14:Ac (90.5%) and *Z*7-12:Ac (9.5%).

FIELD EXPERIMENTS

Rainfall in southern Benin has a bimodal regime (Mar–Jul and Sep–Nov). All experiments were conducted at the International Institute of Tropical Agriculture-Benin station, Cotonou, Benin (6.417500°N, 2.331500°E). The early maize variety 'EVDT 99 W STR' was sown dur-

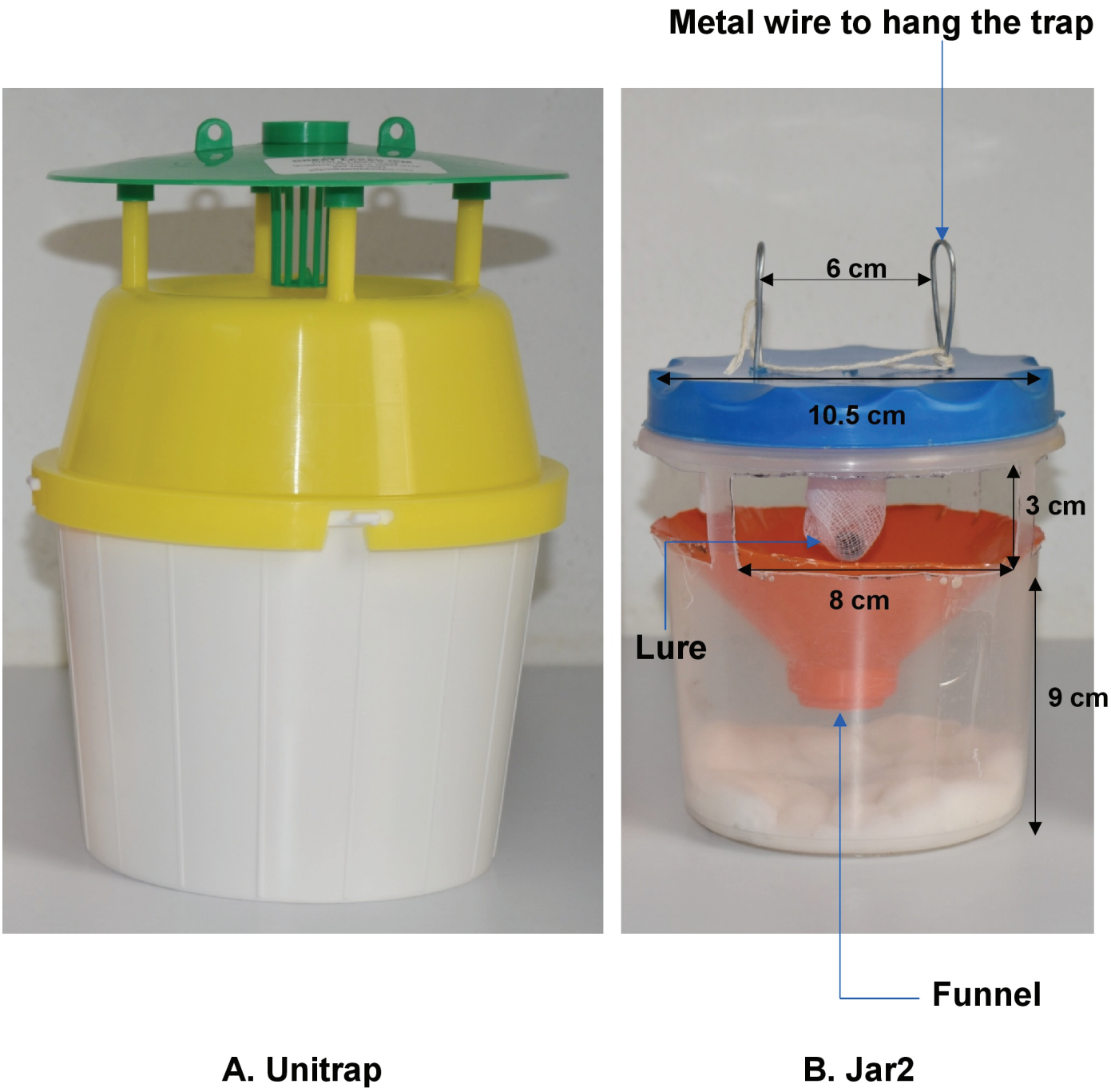

Fig 1. Traps used in study: commercially available Unitrap (A); home-made Jar2 trap constructed from 2 L plastic jar (B). The Jar2 trap was designed by G.T. Tepa-Yotto and J.K. Winsou.

ing preliminary trials at a spacing of 80 cm between rows and 40 cm between plants. The extra-early maize variety '2009 TZEW DT STR' was planted for the second growing season experiments at the same spacing. Nitrogen, phosphorus, and potassium ($N_{15}P_{15}K_{15}$) fertilizer application was done at the dosage of 100 kg per ha^{-1} at 2 wk post maize emergence and just after the first weeding. Urea (46% nitrogen) was applied at the dosage of 50 kg per ha^{-1} 2 wk after the first fertilizer application. In total 3 weedings were done on a 2 to 4 wk interval basis depending on weed density.

In the first growing season experiments, trials were established during the period Jun to Aug 2019 to compare the number of moths captured by Unitraps vs. Jar2 traps. We used untreated maize fields of 0.5 and 1.0 ha, plus two 1.0 ha fields treated with the nucleopolyhedrovirus biopesticides Littovir and Spodovir (Andermatt Biocontrol, Grossdietwil, Switzerland) at the application concentration of 1.1 × 10^{10} occlusion bodies per mL. A total of 4 and 3 traps were tested for Unitraps and Jar2 models, respectively, using the Scentry Biologicals 2-component (L976) lure. Trapped moths initially were collected weekly, but later in the experiment they were collected every 3 d.

In the second growing season experiments, studies were performed from Sep to Dec 2019. Two cropping systems were tested, a maize monoculture and a maize-cowpea intercrop. In the intercrop fields each row of maize was separated by a row of cowpea. Cowpea was sown at the same plant density as maize. Experimental plots were

10 × 100 m, with 4 replicates each for the monoculture and intercrop treatments. Replicate plots within treatments were separated by 20 m, and the monoculture and intercrop fields were separated by a distance of 250 to 500 m. A total of 12 Jar2 and Jar4 traps and 3 control Unitraps in combination with the 3 aforementioned fall armyworm Pherobank-lures were installed per field (i.e., 4-component, 3-component, and 2-component). Traps were set randomly on 1 row in the middle and over the length of the plots, and traps were separated by 15 m. All traps on plots were 12.5 m from the border to avoid edge effects. The moths caught in the traps were collected every 3 d and pheromone lures were changed after 4 wk.

SPECIES IDENTIFICATION OF FIELD-COLLECTED SPECIMENS

Adult males collected in the pheromone traps were examined for fall armyworm identity by morphological criteria as previously described (Huesing et al. 2018). A small subset of specimens was classified as not being fall armyworm (designated as non-targets), and were identified by morphology preliminarily to the genus level and, whenever possible, to the species level.

Twenty-five specimens, believed to be fall armyworm, were analyzed further by DNA barcode sequencing to confirm and enhance species identification. The specimen was homogenized in a 5 mL Dounce homogenizer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in 1 mL of phosphate buffered saline (PBS, 20 mM sodium phosphate, 150 mM NaCl, pH 8.0) then pelleted by centrifugation at 6,000 g for 5 min at room temperature. The pellet was resuspended in 800 µL Genomic Lysis buffer (Zymo Research, Orange, California, USA) and incubated at 55 °C for 5 to 30 min. Debris was removed by centrifugation at 10,000 rpm for 5 min. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Orange, California, USA) and processed according to manufacturer's instructions. The DNA preparation was increased to a final volume of 150 µL with distilled water.

Polymerase chain reaction amplification was performed using a 30 µL reaction mix containing 3 µL of 10× manufacturer's reaction buffer, 1 µL 10 mM dNTP, 0.5 µL 20 µM primer mix, 1 µL DNA template (between 0.05–0.5 µg), 0.5 units Taq DNA polymerase (New England Biolabs, Beverly, Massachusetts, USA) with the remaining volume water. The thermocycling program was 94 °C (1 min), followed by 33 cycles of 92 °C (30 s), 52 °C (30 s), 72 °C (30 s), and a final segment of 72 °C for 3 min. The amplified product was gel-purified by the addition of 5 µL of 6× gel loading buffer to each sample, which was then run on a 1.8% agarose horizontal gel containing GelGreen (per manufacturer's instructions, Biotium, Hayward, California, USA) in 0.5× Tris-borate buffer (TBE, 45 mM Tris base, 45 mM boric acid, 1 mM EDTA, pH 8.0). Fragments were visualized on a blue light box. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research, Orange, California, USA) according to manufacturer's instructions. The purified fragments were analyzed by DNA sequence analysis by Genewiz (South Plainfield, New Jersey, USA).

Primers used for the polymerase chain reaction amplification were designed to amplify an approximately 575 bp fragment from a portion of the mitochondrial Cytochrome oxidase subunit I gene (COI) frequently used for DNA barcoding. Two primer pairs were used, 1 specific for fall armyworm, c101F (5'-TTCGAGCTGAATTAGGAACTC-3') and c678R (5'-ATAGGATCACCTCCWCCTGCAG-3'), and the other for *Helicoverpa armigera*, zeaC45F (5'-TTCGAGCAGAATTAGGTAATC-3') and zeaC678R (5'-ATAGGATCACCTCCTCCAGCAG-3'). One and often both pairs of primers were capable of amplifying a COI fragment from 22 of the 25 non-target specimens.

The DNA barcode analysis was based on a 436 bp segment within the amplified COI segment. Blast comparisons were made using the GenBank DNA database via the Geneious Pro 10.1.2 program (Biomatters, Auckland, New Zealand). DNA alignments were performed using MUSCLE (multiple sequence comparison by log-expectation), a public domain multiple alignment software, and phylogenetic trees were graphically displayed in a neighbor-joining tree analysis (Saitou & Nei 1987), both included in the Geneious Pro 10.1.2 program. The relevant DNA sequences were submitted to GenBank. These include accession numbers for non-target sequences: OL539374 for g54g02, g54h01; OL539376 for g54b03; OL539658 for g54b02; OL539375 for g54b01; OL539377 for g54c07, g54c09, g54d02, g54i02; OL539481 for g54c03; OL539482 for g54f01; OL539378 for g54a01, g54c01, g54c02, g54c04, g54c05, g54c06, g54d04, g54e01, g54e02, g54g01.

DATA ANALYSIS

Responses (number of moths trapped) to the combined effect of cropping system and pheromone lure were log-transformed before analysis to meet the assumptions of normality and equal variance. Transformed data then were analyzed using a linear model analysis of variance (ANOVA) type II sum of squares with cropping system (maize monoculture and maize-cowpea intercrops) and pheromone lure (2-component, 3-component, and 4-component) as fixed effect factors. Tukey's post hoc tests at the 5% level were used to test for significant differences among groups, followed by pairwise comparisons (R statistical software; R Core Team 2012).

Results

FIRST SEASON

The Unitrap model was far more effective in fall armyworm pheromone trapping than the home-made trap (Fig. 2A). The mean number of moths captured per trap for Jar2 was 5.1-fold lower than that of Unitrap ($F_{1,68}$ = 25.2; P < 0.0001). The highest numbers of moth catches associated with Unitrap occurred during the first 4 wk of insect collection (Fig. 2B).

SECOND SEASON

Despite the Jar2 model being associated to some level of moth captures during the first season (Fig. 2), the 2 L or 4 L volume styles did not yield substantial results and trapped significantly fewer moths ($F_{2,86}$ = 22.151; P < 0.0001) during the second planting season (Fig. 3). The total number of moths caught did not exceed 2 during the whole season Oct to Dec.

This experiment was designed to compare lures with 2, 3, or 4 pheromone components in a field composed of monocrop and intercrop plots. Fall armyworm and moths of 2 non-target species were caught during the experiments using the Unitraps (Fig. 4). Pheromone lure proved to be a statistically significant main effect (P < 0.0001) but not cropping system (P = 0.95) for *S. frugiperda* catch (Table 1). There was a significant interaction between pheromone lure and cropping system on the trap catch (P = 0.02). The overall number of moths caught (all lures considered) was 1.1 times higher in the maize monoculture compared to the maize-cowpea intercrop system (Fig. 4). In all experiments, the 4-component lure attracted the highest numbers of fall armyworm moths, but 1.2 times fewer moths were collected in the maize-cowpea intercrop plots compared to the maize monoculture plots (Table 1; Fig. 4). Conversely, the 2-component lure was 6.7 times more effective attracting fall armyworm moths in the maize-cowpea intercrops than in the maize monoculture plots (Fig. 4). No fall armyworm moths were caught by the 3-component lure.

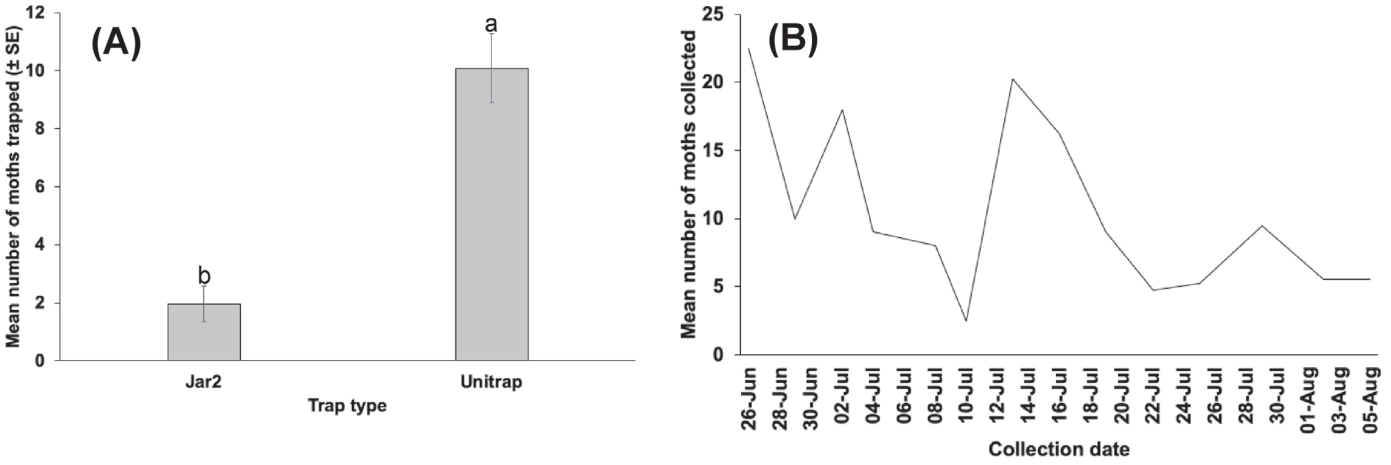

Fig 2. Preliminary field test of pheromone traps using the 2-component fall armyworm pheromone PSU lure during the first maize growing season: comparison between home-made Jar2 trap and Unitrap model (A) (average number per trap type for overall weekly moth collections; error bars represent standard error and different lowercase letters denote statistical difference), and fluctuation in moth trap catch of the Unitrap-2-component lure combination (B) (moth collections were done every 3 d).

NON-TARGET SPECIES

Non-target species were identified initially by adult morphology and could typically be narrowed to a genus and often to a species. DNA barcode sequence data was obtained for 21 specimens and phylogenetic analysis allowed for a molecular taxonomic identification (Fig. 5). There was broad agreement between the morphological and molecular identification. Three specimens identified by morphology as *Chrysodeixis* sp. displayed a barcode sequence most closely similar to that of *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae). Of the 18 samples identified by morphology as *Leucania* sp., 12 were associated with *Leucania curvula* Walker (= *pseudoloreyi* [Rungs]) and 4 with *Leucania loreyi* (Duponchel) (Lepidoptera: Noctuidae). The 2 exceptions were identical sequences that appeared closely related by barcode to *Myrlaea insignella* (Mann) (Lepidoptera: Pyralidae).

Neither pheromone nor cropping system had a significant effect on trap catch for the non-target species when considered individually, but pheromone lure had a significant effect on trap catch of the non-target species combined (Table 1). No non-target species moths were attracted by the 2-component lure. Inversely, the 3-component lure was the most attractive to the non-target species irrespective of the cropping system (Fig. 4). The most common non-target species caught in this study were from *Leucania* (*Mythimna*) spp. (Lepidoptera: Noctuidae) and *Chrysodeixis* sp. (Lepidoptera: Noctuidae). These 2 species were caught in fewer numbers (10.4 times less) compared to fall armyworm in all experiments.

Discussion

The commercially available Unitrap model was the most effective design for fall armyworm captures among the traps tested (Figs. 2A, 3). The highest numbers of moth catches associated with Unitrap oc-

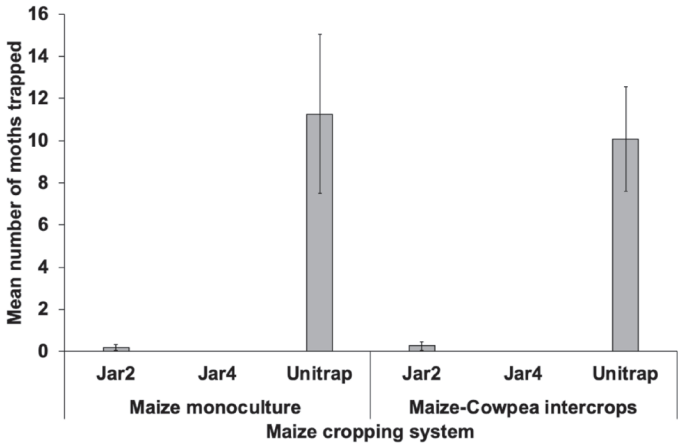

Fig 3. Field screening of home-made trap design (Jar2 and Jar4) in comparison to Unitrap model using pheromone lures (all combined) over 2 maize cropping systems (maize monoculture and maize-cowpea intercrops) during the second planting season. The traps were installed on 30 Sep 2019 during the second maize growing season, and the moth collection period covered Oct to Dec. The data denotes average numbers per trap type for overall 3-d intervals moth collections with standard errors.

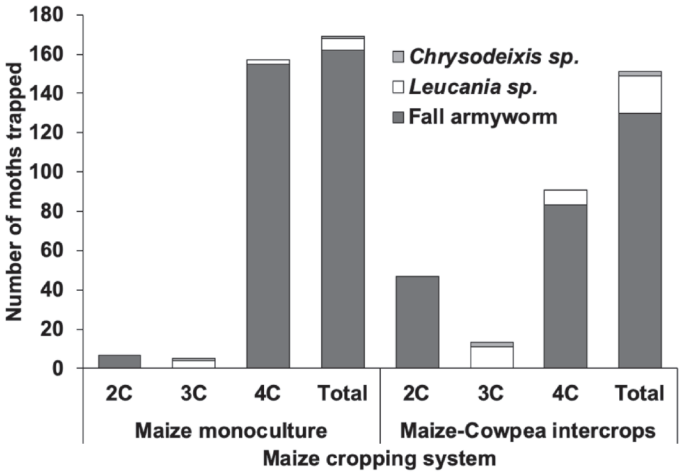

Fig 4. Moth trap catch of 3 pheromone lures over 2 cropping systems (maize monoculture and maize-cowpea intercrops) using Unitraps. The traps were installed on 30 Sep 2019 during the second maize growing season and allowed to collect moths Oct to Dec 2019. The 4-component lure type (4C) contained *Z*9-14:Ac (78.3%), (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac) (3.6%), *Z*7-12:Ac (11.2%), and (*Z*)-9-dodecenyl acetate (*Z*9-12:Ac) (7.0%); whereas the 3-component lure type (3C) was composed of *Z*9-14:Ac (66.1%), *Z*11-16:Ac (4.7%), and *Z*7-12:Ac (29.3%); and the 2-component lure type (2C) of *Z*9-14:Ac (90.5%) and *Z*7-12:Ac (9.5%). The data represents average numbers for overall 3-d intervals moth collections.

Table 1. Number of moths caught (Mean ± SE) with Unitraps and 3 pheromone lure combinations under maize monoculture and maize-cowpea intercrops field conditions.

Source	*Spodoptera frugiperda*	*Leucania* sp.	*Chrysodeixis* sp.	Combined non-target moths
Cropping system				
Maize monoculture	3.60 ± 1.41 a	0.13 ± 0.07 a	0.02 ± 0.02 a	0.11 ± 0.07 a
Maize-cowpea intercrop	2.84 ± 0.83 a	0.53 ± 0.23 a	0.04 ± 0.04 a	0.57 ± 0.23 a
	$F_{1,84}$ = 0.003; P = 0.954	$F_{1,86}$ = 2.94; P = 0.090	$F_{1,86}$ = 0.099; P = 0.753	$F_{1,86}$ = 3.13; P = 0.080
Pheromone lure				
2-component	1.80 ± 0.74 b	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 b
3-component	0.00 ± 0.00 c	0.50 ± 0.30 a	0.10 ± 0.07 a	0.60 ± 0.31 a
4-component	7.86 ± 2.11 a	0.50 ± 0.19 a	0.00 ± 0.00 a	0.50 ± 0.19 a
	$F_{2,84}$ = 25.96; P < 0.0001	$F_{2,86}$ = 3.22; P = 0.054	$F_{2,86}$ = 1.94; P = 0.149	$F_{2,86}$ = 3.54; P = 0.033
Cropping system × pheromone lure	$F_{2,84}$ = 3.669; P = 0.029	–	–	–

Means within a column and within a cropping system or lure type followed by the same letter are not significantly different (Tukey's tests at the 5% level). ANOVA was conducted on log-transformed data. Non-significant interactions were excluded from full models and they were not considered in final reduced models.

curred during the first 4 wk of insect collection matching the early-late whorl stages of the maize crop (Fig. 2B). The inexpensive home-made Jar2 trap was capable of consistently collecting fall armyworm during the first season of relatively moderate fall armyworm density, with a capture profile similar to that displayed by the Unitrap. However, the number of fall armyworm captured by Jar2 were several fold lower than by Unitrap under all conditions, and almost no fall armyworm was captured during season 2 when fall armyworm density was low. These observations suggest that Jar2 could be used to monitor for moderate to high fall armyworm density, particularly since its low cost would allow many more traps to be deployed than using the Unitrap. We believe further optimization of the Jar2 design should be pursued in view of area-wide practical applications.

Substantial differences were observed between the pheromone blends. The 4-component blend attracted the most fall armyworm under all conditions by a substantial margin. The 2-component blend was the most selective, with respect to the number of fall armyworm captured and the percentage of all moths captured that were not fall armyworm during the second season. An unexpected finding was the ineffectiveness of the 3-component blend, which attracted no fall armyworm but did attract *Leucania* and *Chrysodeixis* species at levels approaching that of the 4-component blend. It is widely agreed that the 2 acetates, *Z*9-14:Ac and *Z*7-12:Ac, are the most crucial for fall armyworm male attraction (Tumlinson et al. 1986; Andrade et al. 2000; Meagher et al. 2013, 2019; Hänniger et al. 2020). Our data clearly show that the addition of the acetate *Z*9-12:Ac improved attraction in the 4-component pheromone blend, whereas *Z*11-16:Ac did not in the 3-component lure (Fig. 4). This component may have been too high of a concentration in these lures but is still surprising to have not attracted any fall armyworm. This component is known to be attractive to *Leucania* spp. moths (Fleischer et al. 2005). Recent investigations revealed that fall armyworm males from African populations displayed highest pheromone sensitivity towards *Z*7–12:Ac in electroantennogram experiments, whereas American males exhibited the highest sensitivity towards the major component *Z*9–14:Ac (Hänniger et al. 2020). The same authors suggested that increasing the production of and response to the critical minor component *Z*7–12:Ac may reduce communication interference with other African *Spodoptera* species that share the same major pheromone component. Our work is in agreement with the conclusions that *Z*9–14:Ac, *Z*7–12:Ac, and *Z*9–12:Ac are the most promising candidates that should be used to formulate fall armyworm lures in Africa, which is not the case in America. Indeed, it was found that only these 3 compounds are present within the female gland and induced higher electroantennogram responses (Hänniger et al. 2020).

Tests comparing traps (Unitraps vs. a Togolese homemade trap) and lures (commercial 2-component, 3-component, and 4-component) were conducted in neighboring Togo (Meagher et al. 2019). Our results that the Unitrap was superior compares favorably with the previous study; however, the 3-component lure attracted more moths in the study in Togo than with ours. The study in Togo also showed that 15 to 36% of moths captured were not fall armyworm, with as much as 12% being *L. loreyi*. Trap-lure combinations can differ significantly in the number and species collected depending on the region. This is likely due to subpopulations being genetically variable and differences in the types and numbers of related non-target species. Previous work in West Africa showed that the agricultural habitat (Agro-Ecological Zone) where experiments were conducted had a significant effect on fall armyworm populations (Koffi et al. 2020).

An unexpected result was that attraction of fall armyworm to the 2-component pheromone blend appeared to dramatically increase as a result of intercropping (Fig. 4). Less than 10 moths were captured in the

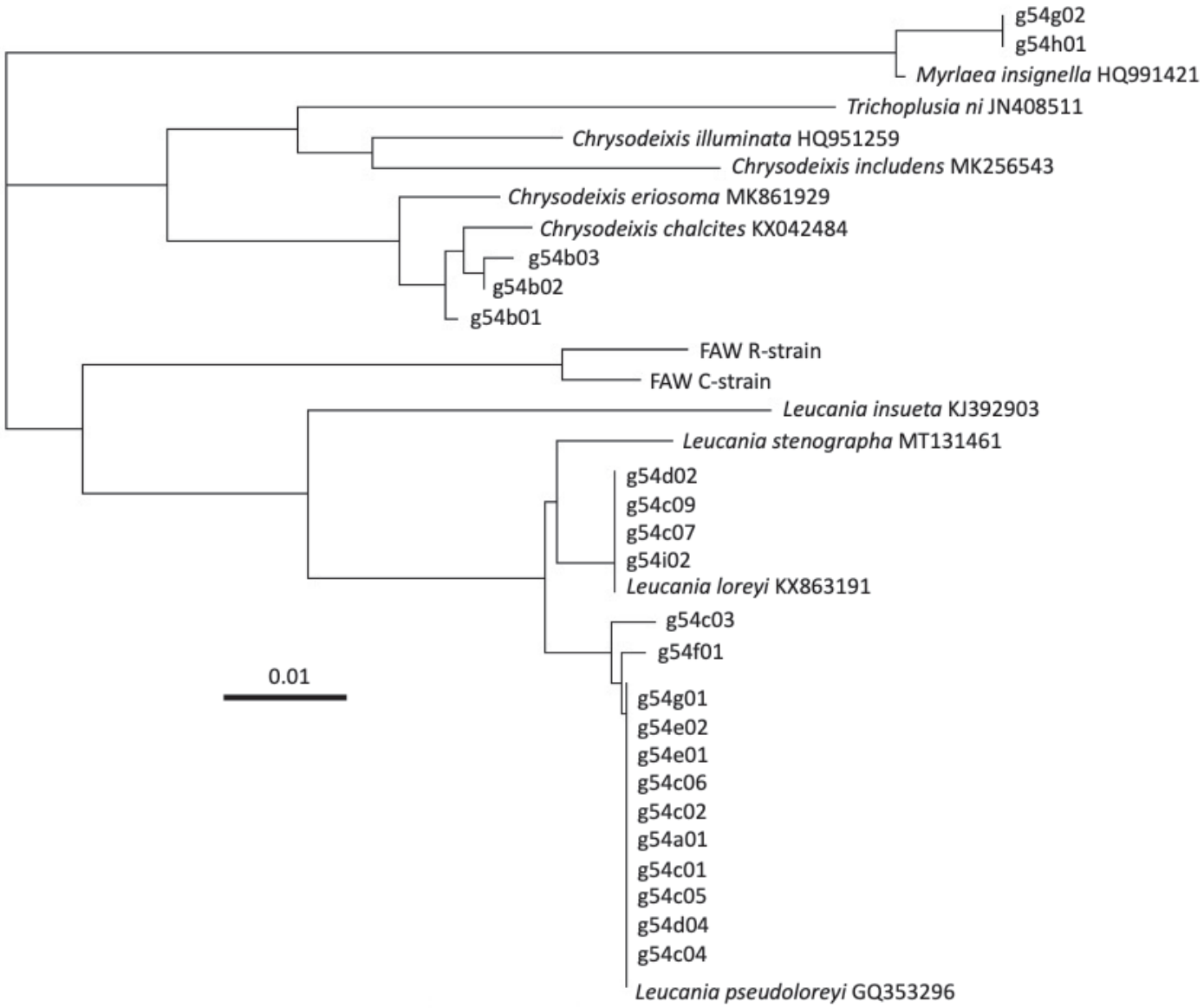

Fig 5. Phylogenetic tree based on a portion of the COI barcoding segment showing the relationships of selected non-target moth specimens (g54xxx) isolated from fall armyworm pheromone traps relative to selected GenBank sequences. GenBank sequences are indicated by species name followed by accession number. Fall armyworm R-strain and fall armyworm C-strain are consensus sequences for the 2 fall armyworm host strains.

maize monoculture treatment by 2-component baited traps compared to nearly 50 in the maize-cowpea intercrop treatment. In contrast, the 4-component pheromone profile show the opposite trend, declining from over 150 moths captured in the monoculture treatment to about 80 moths in the intercrop fields. The reason for this is unclear, but it could indicate an as yet unknown influence of habitat on the response of fall armyworm to pheromones, which has been shown previously (Unbehend et al. 2013, 2014). Overall numbers of moths captured in monocrop vs. intercrop fields was not significantly different, although there were slightly fewer moths found in the intercrop field. It is not known whether there was a low level of infestation with fewer larvae or less maize damage in the intercrop field compared to the monoculture field. This research should be expanded to compare different landscape techniques to manage fall armyworm populations (Midega et al. 2018).

In summary, this study confirms that trap design has substantial impact on fall armyworm trapping efficiency with the Unitrap outperforming the home-made Jar2 and Jar4 designs. However, the inexpensive Jar2 design could be useful under conditions of higher fall armyworm densities. Future research should attempt to optimize the Jar2 trap to yield better results under lower fall armyworm population densities. We also identified the best pheromone blends to use in Benin depending on whether one wants to optimize fall armyworm captures or decrease non-targets.

Acknowledgments

The authors are obliged to Georg Goergen for helpful arrangements to procure experimental pheromone lures at discounted prices at Pherobank. This work was partly funded by the Royal Norwegian Embassy in Mali for 'climate smart agricultural technologies for improved rural livelihoods and food security' in Mali (Grant MLI-17-0008) and Niger (Grant NER-17-0005). We acknowledge the funding from the International Development Association of the World Bank to the Accelerating Impact of CGIAR Climate Research for Africa (AICCRA, P173398) project.

References Cited

Andrade R, Rodriguez C, Oehlschlager AC. 2000. Optimization of a pheromone lure for *Spodoptera frugiperda* (Smith) in Central America. Journal of the Brazilian Chemical Society 11: 609–613.

Carroll MJ, Schmelz EA, Teal PEA. 2008. The attraction of *Spodoptera frugiperda* neonates to cowpea seedlings is mediated by volatiles induced by conspecific herbivory and the elicitor inceptin. Journal of Chemical Ecology 34: 291–300.

FAO – Food and Agriculture Organization of the United Nations. 2020. Fall armyworm control in action newsletter. Issue #1. http://www.fao.org/3/cb1938en/cb1938en.pdf (last accessed 10 Dec 2021).

FAO and CABI – Food and Agriculture Organization of the United Nations, and Centre for Agriculture and Bioscience International. 2019. Community-based fall armyworm (*Spodoptera frugiperda*) monitoring, early warning and management – Training of Trainers Manual, First edition. https://www.fao.org/3/CA2924EN/ca2924en.pdf (last accessed 10 Dec 2021).

Fleischer SJ, Harding CL, Blom PE, White J, Grehan J. 2005. *Spodoptera frugiperda* pheromone lures to avoid nontarget captures of *Leucania phragmatidicola*. Journal of Economic Entomology 98: 66–71.

Goergen G, Kumar PL, Sankung SB, Togola A, Tamò M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS ONE 11: e0165632. doi: 10.1371/journal.pone.0165632

Hänniger S, Goergen G, Akinbuluma MD, Kunert M, Heckel DG, Unbehend M. 2020. Sexual communication of *Spodoptera frugiperda* from West Africa: adaptation of an invasive species and implications for pest management. Scientific Reports 10: 2892. doi: 10.1038/s41598-020-59708-7

Harrison RD, Thierfelder C, Baudron F, Chinwada P, Midega C, Schaffner U, van den Berg J. 2019. Agro-ecological options for fall armyworm (*Spodoptera frugiperda* JE Smith) management: providing low-cost, smallholder friendly solutions to an invasive pest. Journal of Environmental Management 243: 318–330.

He L, Zhao S, Ali A, Ge S, Wu K. 2021. Ambient humidity affects development, survival, and reproduction of the invasive fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), in China. Journal of Economic Entomology 114: 1145–1158.

Huesing JE, Prasanna BM, McGrath D, Chinwada P, Jepson P, Capinera JL. 2018. Integrated pest management of fall armyworm in Africa: an introduction, pp. 1–9 *In* Prasanna BM, Huesing JE, Eddy R, Peschke VM [eds.], Fall Armyworm in Africa: A Guide for Integrated Pest Management, First editon. CIMMYT, El Batán, Mexico State, Mexico.

Koffi D, Agboka K, Adenda DK, Osae M, Tounou AK, Agbeko KT, Adjevi MKA, Fening KO, Meagher Jr RL. 2020. Maize infestation of fall armyworm (Lepidoptera: Noctuidae) within agro-ecological zones of Togo and Ghana in West Africa 3 years after its invasion. Environmental Entomology 49: 645–650.

Meagher Jr RL, Mitchell ER. 2001. Collection of fall armyworm (Lepidoptera: Noctuidae) using selected pheromone lures and trap designs. Journal of Entomological Science 36: 135–142.

Meagher Jr RL, Nagoshi RN, Stuhl C, Mitchell ER. 2004. Larval development of fall armyworm (Lepidoptera: Noctuidae) on different cover crop plants. Florida Entomologist 87: 454–460.

Meagher RL, Agboka K, Tounou AK, Koffi D, Agbevohia KA, Amouze TR, Adjevi KM, Nagoshi RN. 2019. Comparison of pheromone trap design and lures for *Spodoptera frugiperda* in Togo and genetic characterization of moths caught. Entomologia Experimentalis et Applicata 167: 507–516.

Meagher RL, Nagoshi RN, Armstrong JS, Niogret J, Epsky N, Flanders KL. 2013. Captures and host strains of fall armyworm (Lepidoptera: Noctuidae) males in traps baited with different commercial pheromone blends. Florida Entomologist 96: 729–740.

Midega CAO, Pittchar JO, Pickett JA, Hailu GW, Khan ZR. 2018. A climate-adapted push-pull system effectively controls fall armyworm, *Spodoptera frugiperda* (J E Smith), in maize in East Africa. Crop Protection 105: 10–15.

Mitchell ER, Tumlinson JH, McNeil JN. 1985. Field evaluations of commercial pheromone formulations and traps using a more effective sex pheromone blend for the fall armyworm (Lepidoptera: Noctuidae). Journal of Economic Entomology 78: 1364–1369.

Overton K, Maino JL, Day R, Umina PA, Bett B, Carnovale D, Ekesi S, Meagher R, Reynolds O. 2021. Global crop impacts, yield losses and action thresholds for fall armyworm (*Spodoptera frugiperda*): a review. Crop Protection 145: 105641. doi: 10.1016/j.cropro.2021.105641

Pogue M. 2002. A world revision of the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae). Memoirs of the American Entomological Society 43: 1–202.

Prasanna BM, Huesing JE, Eddy R, Peschke VM [eds.]. 2018. Fall Armyworm in Africa: A Guide for Integrated Pest Management, First Edition. CIMMYT, El Batán, Mexico State, Mexico.

R Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/ (last accessed 10 Dec 2021).

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Sharanabasappa SE, Kalleshwaraswamy CM, Asokan R, Swamy HMM, Maruthi MS, Pavithra HB, Hegde K, Navi S, Prabhu ST, Goergen G. 2018. First report of the fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. Pest Management in Horticultural Ecosystem 24: 23–29.

Thierfelder C, Niassy S, Midega C, Subramanian S, van den Berg J, Prasanna BM, Baudron F, Harrison R. 2018. Biological control and biorational pesticides for fall armyworm management, pp. 89–96 *In* Prasanna BM, Huesing JE, Eddy R, Peschke VM [eds.], Fall Armyworm in Africa: A Guide for Integrated Pest Management. CIMMYT, El Batán, Mexico State, Mexico.

Tumlinson JH, Mitchell ER, Teal PEA, Heath RR, Mengelkoch LJ. 1986. Sex pheromone of fall armyworm, *Spodoptera frugiperda* (J. E. Smith): identification of components critical to attraction in the field. Journal of Chemical Ecology 12: 1909–1926.

Unbehend M, Hänniger S, Meagher RL, Heckel DG, Groot AT. 2013. Pheromonal divergence between two strains of *Spodoptera frugiperda*. Journal of Chemical Ecology 39: 364–376.

Unbehend M, Hänniger S, Vásquez GM, Juárez ML, Reisig D, McNeil JN, Meagher RL, Jenkins DA, Heckel DG, Groot AT. 2014. Geographic variation in sexual attraction of *Spodoptera frugiperda* corn- and rice-strain males to pheromone lures. PLoS ONE 9: e89255. doi.org/10.1371/journal.pone.0089255