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Comparison of pheromone trap design and lures for *Spodoptera frugiperda* in Togo and genetic characterization of moths caught

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Abstract

Fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), is a pest of grain and vegetable crops endemic to the Western Hemisphere that has recently become widespread in sub-Saharan Africa and has appeared in India. An important tool for monitoring *S. frugiperda* in the USA is pheromone trapping, which would be of value for use with African populations. Field experiments were conducted in Togo (West Africa) to compare capture of male fall armyworm using three commercially available pheromone lures and three trap designs. The objectives were to identify optimum trap × lure combinations with respect to sensitivity, specificity, and cost. Almost 400 moths were captured during the experiment. Differences were found in the number of *S. frugiperda* moths captured in the various trap designs and with the three pheromone lures, and in the number of non-target moths captured with each lure. The merits of each trap × lure combination are discussed with respect to use in Africa. A nearly equal number of *COI*-CS (161) and *COI*-RS (158) moths was captured with no differences found in *COI* marker proportions among traps or lures. However, the diagnostic rice strain marker *Tpi* was rarely found. Overall, the genetic characterization of the pheromone trap collections indicated a consistent distribution of genetic markers from 2016 to 2017, suggesting a population at or near equilibrium.

Introduction

Insect species are constantly shifting among continents and between hemispheres through natural movement and human-aided transport. Species endemic to Africa – such as the small hive beetle, *Aethina tumida* Murray (Neumann et al., 2016), Africanized honey bee, *Apis mellifera scutellata* Lepeletier (Eimanifar et al., 2018), Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (De Meyer, 2005), and longhorn crazy ant, *Paratrechina longicornis* (Latreille) (Deyrup et al., 2000) – have traveled west and became established in North America.

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Recently, an insect species has traveled east from the Western Hemisphere to Africa and Asia. The fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), was discovered in western Africa in early 2016 (Goergen et al., 2016). By late 2017, *S. frugiperda* had invaded most of sub-Saharan Africa where it has the potential to cause maize yield losses between 21–53% in primarily smallholder farms (Abrahams et al., 2017). It was found in the state of Karnataka, India, in 2018 (Ganiger et al., 2018). This species is endemic to the Neotropics, attacking row, vegetable, and turf crops (Luttrell & Mink, 1999; Braman et al., 2000; Nuessly et al., 2007; Souza et al., 2013). In the USA, fall armyworm annually migrates northward each spring from sites in southern Florida (Pair et al., 1986; Mitchell et al., 1991) and southern Texas (Raulston et al., 1986; Pair et al., 1991).

Monitoring, surveillance, and scouting are critical activities to support a successful integrated pest management program that includes resistant varieties, biological control, and cultural control strategies (McGrath et al., 2018). Monitoring actively tracks the presence, population size, and movement of a pest and for fall armyworm, it is best achieved using pheromone-based traps that attract male moths (McGrath et al., 2018). Adult males have been monitored for over 40 years with commercial versions of female-produced sex pheromones as lures, but there are several pheromone blends and styles of traps that are available and the various combinations may provide conflicting results among geographic regions. Field studies in the USA and Central America have shown that the chemicals needed for attraction were two acetates, (*Z*)-9-tetradecenyl acetate (Z9-14:Ac) and (*Z*)-7-dodecenyl acetate (Z7-12:Ac) (Tumlinson et al., 1986; Andrade et al., 2000; Meagher et al., 2013). The addition of two other acetates, (*Z*)-11-hexadecenyl acetate (Z11-16:Ac) and (*Z*)-9-dodecenyl acetate (Z9-12:Ac), did not improve attraction of moths to traps (Tumlinson et al., 1986; Meagher et al., 2013). Studies in Brazil found that the *E* isomer of dodecenyl acetate (E7-12:Ac) significantly increased trap capture (Batista-Pereira et al., 2006). These results indicate that there may be regional differences in attractiveness of pheromone blends.

Differences in pheromone lure composition are also known to influence the number of non-target insects that are collected (Adams et al., 1989; Weber & Ferro, 1991; Meagher, 2001; Spears et al., 2016). This was shown to be an important consideration for monitoring fall armyworm in North America. In Florida, non-target moths rarely comprise more than 10% of the total captures (RL Meagher, unpubl.), but experiments in the northeastern USA showed that commercial lures containing three or four fall armyworm pheromone components attracted large numbers (38–48% of total captures) of the non-target species *Leucania phragmatidicola* Guenée (Fleischer et al., 2005). Traps baited with two-component lures (Z9-14:Ac and Z7-12:Ac) had low *L. phragmatidicola* captures (0.5–1.4%) but also captured fewer *S. frugiperda* (Fleischer et al., 2005). Additional screening or handling of trap samples and possible misidentification of target species decreases the efficiency and value for the monitoring system (Weber & Ferro, 1991; Guerrero et al., 2014; Spears et al., 2016).

Trap design may also influence moth capture and can significantly affect the cost of a monitoring system (Tingle & Mitchell, 1975; Guerrero et al., 2014). Plastic bucket traps are commonly used for fall armyworm and are available from several sources. Moths are contained in the enclosed bucket and are killed with the use of a fumigant. These traps come in several colors which influences moth capture (Meagher, 2001). Sticky traps come in different shapes (delta, cylindrical), are less expensive, and are easy to transport. But as moths are attached to the sticky surface, the trap's capacity can be exceeded when moth populations are high (Tingle & Mitchell, 1975; Guerrero et al., 2014). Locally produced traps may be constructed of repurposed materials such as plastic containers and soda bottles (Critchley et al., 1997). These traps capture moths in a similar fashion as bucket traps and are usually the least expensive but are variable in effectiveness.

A complication of fall armyworm trapping is that there are two strains that are morphologically identical but differ in their preferred host plant: maize (corn) or rice (Pashley, 1986; Nagoshi, 2012). Pheromone differences have been reported for the strains (Groot et al., 2008; Lima & McNeil, 2009), but the lures tested to date in the Western Hemisphere show no evidence of strain differences in their attractiveness (Meagher et al., 2013; Unbehend et al., 2013). The strains can only be distinguished by genetic markers. However, our studies of fall armyworm from Africa found oddities in the composition and distribution of diagnostic strain markers that bring into question whether the strain preferring rice is present in Africa or is behaving as expected with respect to host preference (Nagoshi et al., 2018). However, these studies used field-collected larvae from strains having maize as their preferred host plants. Collections of free-flying adult males from pheromone traps could provide a more representative description of the African populations.

Accurate monitoring of *S. frugiperda* numbers is a critical element for understanding infestation patterns and forecasting pest movements. Of importance is the specificity of the monitoring method, as measured by the proportion of target to non-targets, because low specificity will lead to overestimations of infestation levels or require additional efforts for identification. We tested two commercially available trap designs in comparison with one locally made device, which might allow for significant cost reductions if shown to be effective, all in combination with three commercial lures. The objectives were to identify the optimal trap and pheromone combinations with respect to the number of *S. frugiperda* males captured, the number and species of non-targets collected, and the cost of monitoring. The specimens obtained were used to confirm whether the unusual distribution of strain markers described from earlier larval surveys can also be observed in pheromone trap collections and to test whether populations in Togo were stable relative to the distribution of genetic markers.

Materials and methods

Trap types

Three categories of traps were tested. A bucket-style trap was represented by a Unitrap (distributed by Great Lakes IPM, Vestaburg, MI, USA). These are commonly used in the USA for *S. frugiperda* monitoring and consist of a white container (bucket) with a green top that provides limited protection from the rain and a yellow funnel (total height 21 cm, bucket circumference 50 cm). The pheromone lure was placed in a basket within the top and a fumigant strip was placed in the bucket to kill specimens. The sticky trap used had a plastic 'Δ-shaped' (delta) configuration with a liner that was inserted into the delta structure (AlphaScents, West Linn, OR, USA). The pheromone lure was placed on the sticky liner and attracted moths became attached to the liner (bottom 28.5 × 19.5 cm; liner 20 × 17.5 cm). The last trap category was one of local (Togo) design. This trap was constructed from a 1.5-l plastic water bottle and was composed of three main parts (Figure 1): (1) the approximately cylindrical transparent or bucket part was 8 cm in diameter and 16 cm high; (2) the conical part of the bottle included the (upside down) bottle neck and had a base diameter of 8 cm and a height of 9 cm, which was stuck into the cylindrical bucket forming the funnel; and (3) a truncated conical yellow plastic plate supported by metal wire at a distance of 3.5 cm from the top of the cylindrical bucket-funnel complex (Figure 1). We used a metal wire to hang the lure inside between the yellow lid and the funnel. The same metal wire passed through the yellow lid to hang the trap. The local trap was based on the bucket trap model but was less costly than commercial products.

Pheromone lure types

Three commercially available pheromone blends on rubber septa were tested. They were chosen because they have demonstrated to be effective in pheromone trapping of fall armyworm in the USA and they differ in composition. Chemical analysis of the lure components was not completed; however, the following percentages were determined in a previous study (Meagher et al., 2013). The four-component lure (4C) contained Z9-14:Ac (78.3%), Z11-16:Ac (3.6%), Z7-12:Ac (11.2%), and Z9-12:Ac (7.0%) (L105A or standard FAW lure), whereas the two-component lure (2C) contained Z9-14:Ac (90.5%) and Z7-12:Ac (9.5%) (L976 or PSU lure) (Scentry Biologicals, Billings, MT, USA). A three-component lure (3C), composed of Z9-14:Ac (66.1%), Z11-16:Ac (4.7%), and Z7-12:Ac (29.3%), was provided by Trécé (Adair, OK, USA). Pheromone lures were changed after 30 days.

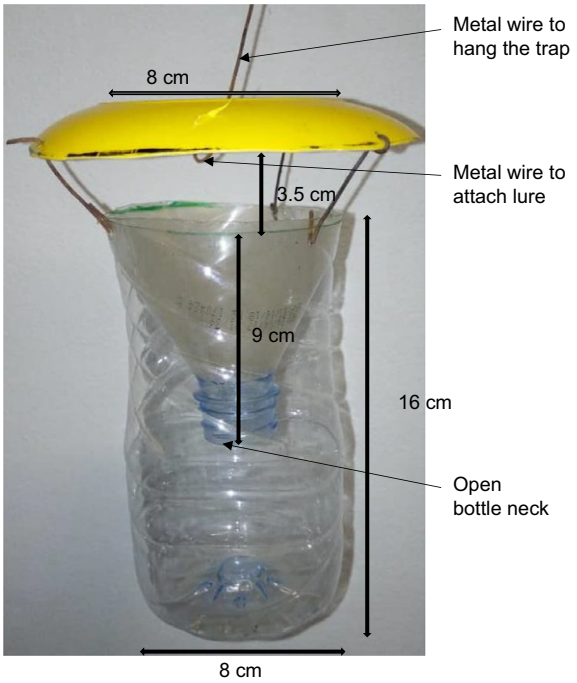

Figure 1 Local Togolese trap constructed from a 1.5-l plastic water bottle, designed by AKM Adjevi, D Koffi, and K Agboka. See the text for a detailed description.

Field study

The trial was conducted during the first maize growing season in 2017 at the Togolese Agricultural Research Institute (Institut Togolais de Recherche Agronomique, ITRA; 06° 10.556N, 001° 12.645E) and at the Station d'Expérimentation Agronomique de Lomé (SEAL) de l'Ecole Supérieure d'Agronomie, Université de Lomé (06° 10.682N, 001° 12.626E), both in southern Togo. Maize ('quality protein maize') was planted on 26 June 2017. Blocks of maize were 8 m wide and 96 m long. Each block contained eight rows of maize separated by a 20-m no-crop zone. The treatments were deployed 2 weeks after planting in a randomized complete block design with four replicates. Trap-lure combinations (n = 9) were placed along a line at the edge of the maize block, with 12 m between traps. Traps were attached to wooden poles at a height of 1.5 m and were checked 21 and 30 July, 8, 17, and 26 August, and 4 September. Fall armyworm and non-target moths were collected, counted and placed into a plastic bag labeled with the date, trap, and lure types, and placed in a freezer. Additionally, Unitraps baited with 4C pheromone lures were placed at SEAL for routine monitoring from 15 May to July 2017. After the experiment was completed, moths were shipped to Gainesville (FL, USA) for genetic analysis.

DNA isolation

Mitochondrial and genomic DNA for use in PCR amplifications were isolated from individual specimens using Zymo-Spin III columns (Zymo Research, Orange, CA, USA) by a modification of protocols described previously (Nagoshi et al., 2018). Individual specimens were obtained either air dried or preserved in alcohol. These were dissected with approximately 1/3 body length used for DNA isolation and the remainder archived in 100% ethanol at −20 °C. The homogenized tissue was pelleted by centrifugation at 6 000 ***g*** for 5 min at room temperature and the pellet was resuspended in 800 µl Genomic Lysis buffer (Zymo Research) and incubated at 55 °C for 5–30 min. Debris was removed by centrifugation at 12 000 g for 5 min. The supernatant was transferred to a Zymo-Spin III column (Zymo Research) and processed according to manufacturer's instructions. The DNA preparation was increased to a final volume of 100 µl with distilled water. Genomic DNA preparations of fall armyworm samples from previous studies were stored at −20 °C.

PCR amplification and DNA sequencing

PCR amplification of the mitochondrial *Cytochrome Oxidase subunit I* gene (*COI*) was performed in a 30-µl reaction mix containing 3 µl of the 10× manufacturer's reaction buffer, 0.5 µl 10 mM dNTP, 0.5 µl 20 µM primer mix, 1–2 µl DNA template (0.05–0.5 µg), 0.5 U Taq DNA polymerase (New England Biolabs, Beverly, MA, USA). The thermocycling program was 94 °C (1 min), followed by 33 cycles of 92 °C (30 s), 56 °C (45 s), 72 °C (45 s), and a final segment of 72 °C for 3 min. Typically 96 PCR amplifications were performed at the same time using either 0.2-ml tube strips or 96-well microtiter plates. Two segments of the *COI* gene were amplified as needed and one segment of the *Triosephosphate Isomerase I* (*Tpi*) gene. All primers were obtained from Integrated DNA Technologies (Coralville, IA, USA) and included *JM76* (5′-GAGCTGAATTAGGGACTCC-3′), *JM77* (5′-ATCACC TCCACCTGCAGGATC-3′), *COI*-891F (5′-TACACGAG-CATATTTTACATC-3′), *COI*-1472R (5′-GCTGGTGGTA AATTTTGATATC-3′), *Tpi-412F* (5′-CCGGACTGAAGG TTATCGCTTG-3′), and *Tpi*-1195R (5′-AGTCACTGAC CCACCATACTG-3′). The PCR-amplified fragments were isolated from 1.8% horizontal agarose gels using the *Z*ymoclean Gel DNA Recovery Kit (*Z*ymo Research) and was directly DNA sequenced by Genewiz (South Plainfield, NJ, USA). DNA alignments and consensus building were performed using MUSCLE (multiple sequence comparison by log-expectation), a public domain multiple alignment software incorporated into the Geneious Pro v.10.1.2 program [Biomatters, New Zealand; http://www.geneious.com (Kearse et al., 2012)].

Analyzing *COI* strain markers

The primer pair *COI*-891F and *COI*-1472R generates a 603-bp fragment that contains two polymorphic sites that we previously used to identify strains (Nagoshi et al., 2008). In Western Hemisphere fall armyworm populations, sites $CO1_{1164}$ and $CO1_{1287}$ identify one rice-strain ($CO1_{1164}$ = T, $CO1_{1287}$ = A) and four maize-strain haplotypes ($A_{1164}A_{1287}$, $A_{1164}G_{1287}$, $G_{1164}A_{1287}$, and $G_{1164}G_{1287}$). Haplotypes $A_{1164}A_{1287}$ and $G_{1164}A_{1287}$ are generally infrequent, whereas $A_{1164}G_{1287}$ and $G_{1164}G_{1287}$ frequencies vary by region, with h2 predominant in Texas and h4 mostly in Florida. Specimens that differed from the *S. frugiperda* consensus were examined using the *JM76*/*JM77* 569-bp PCR amplification product that includes the region previously used for barcode comparisons of *Spodoptera* species (Nagoshi et al., 2011). These sequences were then compared to the GenBank database by Basic Local Alignment Search Tool analysis for species identification.

Analyzing *Tpi* strain markers

This method was originally based on the presence of 10 single nucleotide polymorphisms in the product amplified by *Tpi*-412F/*Tpi*-1195R (Nagoshi, 2010). Two additional polymorphic sites, g*Tpi*192Y and g*Tpi*198Y, are located on the same exon 9 and 15 bp downstream of $e4_{183}$. Both show a much lower strain-bias than $e4_{183}$ (Nagoshi et al., 2017a) and together produce two *Tpi*-C subgroups in the Africa collections, *Tpi*Ca1 ($C_{192}C_{198}$) and *Tpi*Ca2 ($T_{192}T_{198}$), whereas the *Tpi*-R marker is always associated with $T_{192}T_{198}$ (*Tpi*Ra1). No other variants at these loci have so far been found in the Africa specimens. The PCR amplified fragment produced by the primer pair *Tpi*412F/1140R contains all relevant *Tpi* markers and can be simultaneously read from a single sequencing run using *Tpi*412F as primer.

The *Tpi* gene is located on the *Z* sex chromosome that is present in one copy in females and in two copies in males. As males can be heterozygous for *Tpi*, there is the potential for the simultaneous display of alternatives at each of the polymorphic sites. This would be indicated by an overlapping C and T DNA sequence chromatograph (Nagoshi, 2010). Heterozygosity at the strain-diagnostic $e4_{183}$ is denoted as *Tpi*H, whereas heterozygosity at sites $e4_{192}$ and e_{4198} is indicated by a Y (i.e., *Tpi*C-YY, *Tpi*R-YY, *Tpi*H-YY).

Tpi haplotype frequencies were calculated on a per chromosome basis. Larval data from Africa were used from a previous study (Nagoshi et al., 2018). The sex of the larvae was not determined so these could be hemizygous or homozygous for the *Tpi* haplotype. We assumed a 1:1 sex ratio and so multiplied the number of apparent homozygous larvae (i.e., those that showed an unambiguous

*Tpi*Ca1, *Tpi*Ca2, and *Tpi*Ra1 haplotype) by 1.5 to estimate chromosome number. Pheromone trap collections are all males, so these homozygotes were multiplied by 2. Because only three *Tpi* haplotypes have so far been found in Africa, the heterozygous types were limited to *Tpi*C-YY, *TpiR*-YY, and *Tpi*H-YY, and the contributing haplotypes could be unambiguously identified. Based on these considerations, *Tpi* haplotype numbers were calculated as follows for larvae, *Tpi*Ca1 = 1.5[*Tpi*Ca1]+[*Tpi*C-YY]+[*Tpi*H-CC]; *Tpi*Ca2 = 1.5[*Tpi*Ca2]+[*Tpi*C-YY]+[*Tpi*H-YY]; *Tpi*Ra1 = 1.5[*Tpi*Ra1]+[*Tpi*R-YY]+[*Tpi*H-YY]. For the pheromone trap collections, the 1.5 was replaced by 2.

Statistical analysis

All analyses were conducted in SAS (SAS Institute, 2012). All data were first analyzed using Box-Cox (Proc TRANSREG) and Proc UNIVARIATE to find the optimal normalizing transformation (Osborne, 2010); as many traps contained no moths, all data were transformed with y + 0.1. Trap and lure differences were compared with a randomized complete block design in Proc MIXED, with trap, lure, and the trap*lure interaction as the fixed variables and block as the random variable. In all analyses, LSMEANS with an adjusted Tukey test was used to separate variable means.

Trap sensitivity was measured in two ways, regardless of which pheromone lure was used. First, the number of times (sampling date × blocks across all lures) that a trap contained zero moths. The other calculation was the number of times that a trap was the only trap of the three with zero moths. Trap sensitivity was analyzed using Proc MIXED, with trap as the fixed variable and sampling date as the random variable. Pheromone lure sensitivity was tested in the same way, regardless of which trap was used. Frequency analysis (Proc FREQ, exact χ^2 test) was used to compare *COI* and *Tpi* markers among traps and lures.

Results

Trap types and lures differ in their specificity

A total of 216 traps collected 304 *S. frugiperda* moths over the 45-night survey period for an average of 1.41 moths per trap. The trap*lure interaction was not significant ($F_{4,24}$ = 1.31, P = 0.30), but the effects of both trap type and lure were significant (Table 1). Bucket traps captured more fall armyworm than the local and sticky traps combined, and the 3C lures attracted more fall armyworm moths than the 4C or 2C lures (Figure 2). Bucket traps with 3C lures caught the most fall armyworm moths, and significantly more than the sticky traps with 2C and 4C lures, and then local traps with 2C and 4C lures ($F_{8,24}$ = 7.08, P<0.001).

Trap selectivity

Although the capability of a trap to capture large numbers of moths is important, another factor to consider in selecting a trap is its capability to capture moths when populations are low. That a trap contained zero moths occurred between 30 and 50% of the samples, and there was no difference among traps (Table 2). However, in 18% of the samples, a sticky trap was the only one with zero moths, compared to 2.8 and 4.2% for bucket and local traps, respectively. These results indicated that under conditions of low populations, sticky traps performed poorly in capturing moths (Table 2). There was no difference in the sensitivity of pheromone lures in attraction of fall armyworm moths, although there was a trend for traps with 3C lures to contain moths when the other traps were empty (Table 2).

Cost analysis

Cost analysis is provided as a rough guideline because it is based on small samples of moths. Sticky traps appear to be cheaper than the bucket traps, but if populations are high, then the inserts for the sticky traps need to be changed frequently. The cost analysis for the 7 weeks of trapping with the bucket traps is (USD) $13.15 per trap (trap cost = $9.95 + two fumigant strips @ $3.20); the cost for the sticky traps is $15.60 (trap cost = $5.10 + weekly inserts @ 7 × $1.50). The local traps are easily made from materials at hand and are variable in cost but are the least expensive (estimated at $3.50 for materials). Therefore, the traps used in this experiment could potentially be the most efficient, catching slightly over 21 moths per dollar (75/$3.50). The bucket trap was next, at about 12 moths per dollar (156/$13.11), and the sticky traps did the poorest at under five moths per dollar (73/$15.60). Over 4× more moths could be captured in local traps per dollar than in sticky traps in a monitoring program.

Non-target moths

Other moth species were collected in traps and some of these could be confused with *S. frugiperda*. One species that was identified using DNA sequencing was *Leucania* (*Mythimna*) *loreyi* (Duponchel). Moths were collected in similar numbers across traps but 7× more were attracted to the 3C pheromone lure than to the 2C and 4C lures (Table 1). The bucket-3C combination caught more *L. loreyi* than bucket-2C, bucket-4C, and sticky-4C combinations ($F_{8,24}$ = 3.9, P = 0.0045; Figure 2). Of the 24 *L. loreyi* moths identified, 13 were collected on one sample date (26 August). One other species, *Spodoptera triturata* (Walker), was collected once and is similar in appearance to *S. frugiperda*. Other non-target moths were present but would likely not be misidentified as fall armyworm. These include

Table 1 Mean (± SE; n = 12) number of moths per trap, captured in bucket, local, or sticky traps baited with sex pheromone lures comprising two (2C), three (3C), or four (4C) components, in a maize field in Togo, 2017

Trap		*Spodoptera frugiperda*	*Leucania loreyi*	Other non-target moths	Combined non-target moths
Bucket		13.0 ± 1.5a	0.92 ± 0.5a	1.41 ± 0.38ab	2.33 ± 0.64a
Local		6.25 ± 1.3b	0.75 ± 0.33a	0.83 ± 0.27b	1.58 ± 0.38a
Sticky		6.08 ± 0.9b	0.33 ± 0.14a	3.08 ± 0.69a	3.42 ± 0.65a
		$F_{2,24}$ = 19.0, P<0.0001	$F_{2,24}$ = 0.43, P = 0.65	$F_{2,24}$ = 7.0, P = 0.004	$F_{2,24}$ = 3.0, P = 0.068
Lure	2C	7.0 ± 1.5b	0.25 ± 0.13b	2.58 ± 0.71a	2.83 ± 0.69a
	3C	11.1 ± 1.7a	1.67 ± 0.48a	1.17 ± 0.32a	2.83 ± 0.55a
	4C	7.25 ± 1.2b	0.08 ± 0.08b	1.58 ± 0.48a	1.67 ± 0.51a
		$F_{2,24}$ = 6.7, P = 0.0048	$F_{2,24}$ = 11.4, P = 0.0003	$F_{2,24}$ = 2.3, P = 0.12	$F_{2,24}$ = 1.4, P = 0.27

Means within a column and within a trap type (bucket/local/sticky) or lure type (2C/3C/4C) followed by the same letter are not significantly different (Adjusted Tukey test: P>0.05).

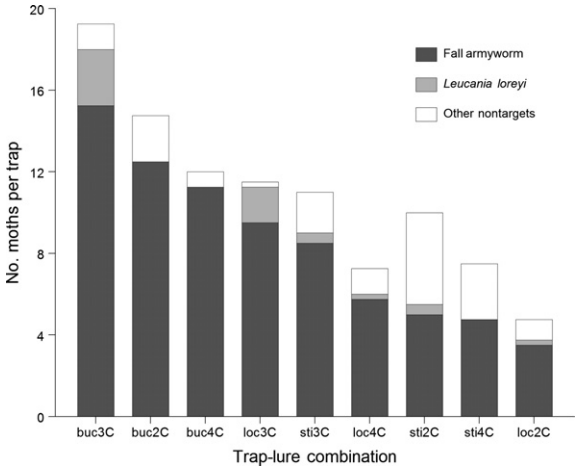

Figure 2 Number of fall armyworm, *Leucania loreyi*, and other non-target moths per trap across nine trap-lure combinations, comprising bucket (buc), local (loc), and sticky (sti) traps, and pheromone lures of two (2C), three (3C), or four (4C) components.

Table 2 Mean (± SE) sensitivity of bucket, local, or sticky traps and pheromone lures comprising two (2C), three (3C), or four (4C) components, to capture fall armyworm moths in a maize field in Togo, 2017

Trap		Frequency (%) of events that a trap or lure contained zero moths	Frequency (%) of events that a trap or lure does not contain a moth when the others do
Bucket		31.9 ± 11.3a	2.8 ± 1.8b
Sticky		51.4 ± 12.8a	18.1 ± 5.9a
Local		45.8 ± 8.0a	4.2 ± 1.9b
		$F_{2,10}$ = 1.9, P = 0.21	$F_{2,10}$ = 5.2, P = 0.028
Lure	2C	47.2 ± 10.2a	8.3 ± 3.0a
	4C	47.2 ± 12.5a	5.6 ± 1.8a
	3C	34.7 ± 9.2a	6.9 ± 4.0a
		$F_{2,10}$ = 1.1, P = 0.39	$F_{2,10}$ = 0.2, P = 0.82

Means within a column and within a trap type (bucket/sticky/local) or lure type (2C/3C/4C) followed by the same letter are not significantly different (Adjusted Tukey test: P>0.05).

brightly colored erebids [*Spilosoma* sp., *Polypogon fractalis* (Guenée), and *Metarctia* sp.], and unidentified smaller 'micros' in the superfamily Gelechioidea. Higher numbers of these non-target moths were in the sticky traps compared to the bucket and local traps (Table 1). Higher numbers of non-target moths were found in traps baited with the 2C lure, but the difference among lures was not significant ($F_{8,199}$ = 1.64, P = 0.12).

Host strain marker comparison

A nearly equal number of *COI*-CS (161) and *COI*-RS (158) moths was found in the trap-lure study with no significant differences found in *COI* marker proportions among traps (*COI*-RS: local = 0.5, sticky = 0.4068, bucket = 0.3664; P>0.3) or lures (*COI*-RS: 3C = 0.43, 4C = 0.4054, 2C = 0.3816). However, fewer moths from bucket traps were *COI*-RS than *COI*-CS (χ^2 = 9.4, P = 0.0028). In comparison, the diagnostic rice strain *Tpi* marker, *Tpi*R, was rarely found in all collections (frequency <0.06), consistent with earlier findings based on a survey of larval collections (Nagoshi et al., 2017b). This earlier study characterized Togo fall armyworm populations for *COI*-CS and *Tpi* haplotype distributions using larval collections from field crops (Nagoshi et al., 2017b). No significant differences were observed between those results and that obtained from the pheromone trap collections (Table 3). These data also indicate consistency in the haplotype frequency from 2016 to 2017 in Togo.

A subset of *COI* haplotypes based on polymorphisms at sites 1 164 and 1 287 shows reproducible regional differences in frequency in the Western Hemisphere, with the $G_{1164}G_{1287}$ variant predominating in the Caribbean, Florida, and the eastern coast of the USA, whereas $A_{1164}G_{1287}$ is the majority elsewhere. Previous surveys of Togo and parts of Africa by larval collections only identified the $G_{1164}G_{1287}$ subtype. For the first time, the $A_{1164}G_{1287}$ variant was found in Togo where it represented 4 of 163 (2.5%) of the pheromone trap specimens tested (Figure 3).

Discussion

Pheromone traps are an effective method for pest monitoring as they do not require direct collection from plant hosts and the pheromone lure should in principle be specific to the targeted pest. In practice, however, trap-lure combinations can differ significantly in both sensitivity and specificity, depending on region. This is likely due to genetic variation between geographically separated subpopulations and differences in the types and numbers of related non-target species. It is therefore important to field test trap-lure combinations in previously unmonitored areas to assess their sensitivity and specificity.

The part of non-targeted pests was variable, ranging from 15 to 36% of total captures with the highest frequency found with sticky traps. However, only *L. loreyi* and *S. triturata* were similar enough to fall armyworm to present an identification challenge. *Spodoptera triturata* is found throughout continental Africa south of the Sahara and attacks grass crops (Fletcher, 1956; Vermeulen & Catling, 1980). Only a single specimen was found in our collections and because its pheromone composition is different from that of fall armyworm (Blair & Tannock, 1978), it is doubtful that this species will be a consistent non-target in fall armyworm pheromone trapping surveys.

Table 3 Comparison of fall armyworm *COI* (calculated on a per individual basis) and *Tpi* (calculated on a per chromosome basis) frequencies between larval and pheromone trap collections using χ^2 analysis. Larval data are from Nagoshi et al. (2017b)

		COI strain marker		*Tpi* strain marker		
	n_{ind}	*COI*-CS	n_{chrom}	*Tpi*-Ca1	*Tpi*-Ca2	*Tpi*-Ra1
Larvae	74	0.6945	131	0.4351	0.4084	0.1565
Traps	250	0.6520	500	0.5320	0.4040	0.0640
χ^2		0.1341		0.9709	0.0024	3.88
$P<\chi^2$		0.32		0.32	0.96	0.049

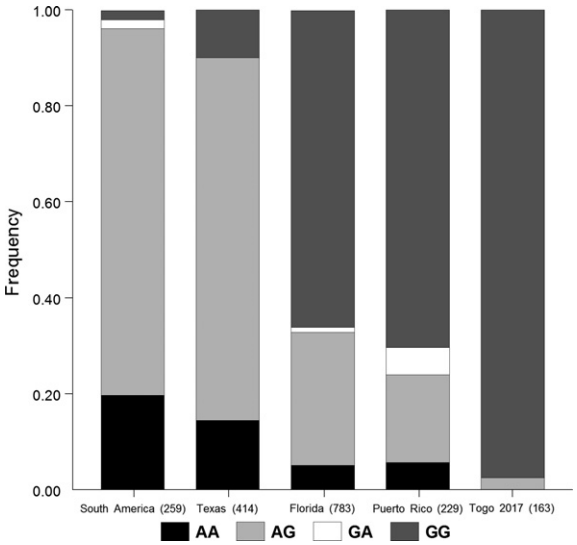

Figure 3 Distribution of fall armyworm *COI*-CS haplotypes based on polymorphisms at sites 1164 and 1287. Four haplotype variants (AA, AG, GA, and GG) have been observed that differ in their frequencies by region. Numbers in brackets are the total *COI*-CS found in the collections. Collection data was used from South America (SA) (Nagoshi et al., 2012, 2015), Texas, USA (TX) (Nagoshi et al., 2011), Florida, USA (FL) (Nagoshi et al., 2011), Puerto Rico (PR) (Nagoshi et al., 2015), Togo 2016 (larvae) (Nagoshi et al., 2017b), and Togo 2017 (traps) from this paper.

In contrast, *L. loreyi* made up as much as 12% of trap collections and its pheromone composition is a mixture of three components shared by fall armyworm (Sato et al., 1980; Takahashi et al., 1980). Specifically, the *L. loreyi* pheromone composition (Z9-14:Ac, Z7-12:Ac, and Z11-16:Ac) is comparable to the 3C lure, consistent with traps with this lure having the highest *L. loreyi* capture frequency. Captures of *L. loreyi* were reduced to less than 5% with the 4C (contains an additional acetate, Z9-12:Ac) and 2C lures (lacks Z11-16:Ac). These observations are remarkably similar to the relationship between fall armyworm and *L. phragmatidicola* in North America, where in certain regions the 3C and 4C lures attracted both species but the 2C lure was only effective with fall armyworm (Fleischer et al., 2005). *Leucania loreyi* is distributed throughout Africa, Asia, and the Middle East, attacking grain and sugar crops (Joomun et al., 2012; Qin et al., 2017). This broad distribution and similarity in host range indicates that it may be a potential non-target of fall armyworm pheromone trapping throughout the Eastern Hemisphere, with the degree of contamination varying with the size of the *L. loreyi* local population.

Our results provide some general guidance for the choice of trap type and lure in western Africa. Highest fall armyworm captures were obtained with the commercial bucket trap regardless of lure and with 3C in all trap types. Not surprisingly, the bucket trap-3C combination showed the highest sensitivity and would be the preferable choice if only a few traps are to be deployed. Whereas 3C had the highest attraction for *L. loreyi*, this may be a manageable problem in Togo where the contamination was only 12%, but would likely be problematic in habitats with higher endogenous *L. loreyi* density. If these moths are counted as fall armyworm in a continent-wide trapping study, the results could influence numbers for population dynamics and migration patterns research. At a local level, misidentification of moths in traps could affect management decisions by causing insecticide applications when not needed. If morphologically similar non-targets are a concern, the combination of bucket trap with the 2C lure provides a reasonable compromise.

When taking cost into consideration, the local traps had clear advantages. The lower sensitivity of these traps can be compensated by their affordability, which allows more traps to be deployed. This is an important consideration as indicated by the variability of the trap collection results. Although the bucket trap had statistically significant overall higher fall armyworm collection rate, and was highest in four of the six collection periods, collection rates in only two of these four periods were significantly higher. This indicates the influence of the microenvironment on trap efficiency, which can be overcome by using multiple trap placements.

Overall, the pheromone trap collections gave similar results with respect to genetic marker frequencies as the larval collections, consistent with an unbiased attraction for the two host strains. The *Tpi*-R haplotype remained rare, suggesting that its low frequency is not the result of sampling bias (larval collections are generally limited to strains preferring maize as host plant) but reflects actual scarcity in the field. The similarity of the fall armyworm populations in Togo from 2016 to 2017 is unexpected, given the rapid spread of fall armyworm across the continent from its first detection in 2015–2016. The relative stability of the genetic marker frequencies in Togo is more suggestive of an established population in equilibrium, so it will be interesting to see if this stability is maintained in subsequent seasons.

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