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## CAPTURES AND HOST STRAINS OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) MALES IN TRAPS BAITED WITH DIFFERENT COMMERCIAL PHEROMONE BLENDS

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### ABSTRACT

Traps baited with 4 different commercial sex pheromone lures that contained 2, 3, or 4 components were used to capture male fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] in Alabama, Georgia, Florida, and Texas from 2006-2009. A subset of the moths collected was analyzed for their host strain to determine if there was a difference in attraction to these lures. Chemical analysis of the lures was completed to identify the pheromone components released. Each lure released the number of components expected, but the Trécé lure released relatively higher amounts of the minor component Z7-12:Ac and at a higher percentage of its blend, than the other lures. The 4 lures attracted similar numbers of moths in Alabama, Georgia, and Texas, and there was only a difference among lures in the Florida 2006 trial. More moths were captured in fall 2007 than fall 2008 in Alabama and Georgia. The southern region in Alabama and Georgia averaged more than 13 moths per night, compared to 8.5 in the central region, and 1.9 in the northern region. Lures attracted both host strains of moths, but across years and locations ( $n = 4546$ ), all lures attracted more corn strain than rice strain males (>55% of moths analyzed were corn strain). However, traps baited with Trécé lures captured a 5% lower percentage of corn strain moths than Scenturion-baited lures. Geographic location and time of season appeared to be much more important in determining host strain identity than the specific commercial lure used. Results from these trials suggest that any of the commercial lures tested will attract the numbers of fall armyworm moths necessary for genetic and migration analysis, and that site location (away from trees and in open areas) and periodic trap maintenance (removal of spiders and frogs from clogging the funnel or eating trap catch) are also important in capturing the highest number of moths.

Key Words: *Spodoptera frugiperda*, pheromone trapping, host strains

### RESUMEN

Se utilizó trampas cebadas con 4 diferentes señuelos comerciales de feromonas sexuales que contenían 2, 3 ó 4 componentes para capturar los machos del gusano cogollero [*Spodoptera frugiperda* (J.E. Smith)] en Alabama, Georgia, Florida y Texas, 2006-2009. Se analizó un subconjunto de las polillas recogidas por su cepa hospedera para determinar si había una diferencia en la atracción de estos señuelos. El análisis químico de los señuelos fue hecho para identificar los componentes de la feromona liberada. Cada señuelo liberó el número de componentes esperado, pero el señuelo Trécé liberó relativamente un mayor cantidad de la componente menor Z7-12: Ac y en un mayor porcentaje de su mezcla, que los otros señuelos. Los 4 señuelos atrajeron un número similar de polillas en Alabama, Georgia y Texas, y sólo habían algunas diferencias entre los señuelos en la prueba de la Florida en el 2006. Más polillas fueron capturadas en el otoño de 2007 que el otoño de 2008 en Alabama y Georgia. Un promedio de más de 13 polillas fueron capturadas por noche en la región del sur de Alabama y Georgia, en comparación con 8.5 en la región central, y 1.9 en la región norte. Los señuelos atrajeron polillas de los dos cepas hospederas, pero en años y localidades ( $n = 4546$ ), todos los señuelos atrajeron más machos de la cepa de maíz que los machos de la cepa de arroz

(>55% de las polillas analizadas fueron la cepa de maíz). Sin embargo, las trampas cebadas con los señuelos Trécé capturaron un porcentaje menor de 5% de las polillas de la cepa de maíz que las trampas Scenturion cebadas con el señuelo. La ubicación geográfica y el tiempo de la temporada parecían ser mucho más importante en la determinación de la identidad de la cepa hospedero que el señuelo comercial específico utilizado. Los resultados de estos ensayos sugieren que cualquiera de los señuelos comerciales probados atraerá los números de polillas del gusano cogollero necesarias para el análisis genético y la migración, y que la ubicación del sitio (lejos de los árboles y en áreas abiertas) y el mantenimiento periódico de las trampas (eliminación de arañas y ranas se obstruya el embudo o comen las polillas capturadas en las trampas) también son importantes en la captura del mayor número de polillas.

Palabras Clave: *Spodoptera frugiperda*, trampas de feromonas, cepas hospederas

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) is a migratory, polyphagous moth that attacks a wide variety of crops throughout the Western Hemisphere (Luginbill 1928; Sparks 1979). The species is composed of 2 sympatric, morphologically-identical host strains, which can be distinguished by genetic markers (Levy et al. 2002; Nagoshi & Meagher 2003a; Nagoshi & Meagher 2003b; Nagoshi et al. 2006b). The principal characteristic that distinguishes the strains is their distribution in different habitats as surveyed by larval collection or pheromone trapping. Corn strain populations are preferentially associated with large grasses such as corn (*Zea mays* L.) and sorghum (*Sorghum* spp.), while rice strain populations prefer small grasses such as rice (*Oryza sativa* L.) and forage grasses (*Cynodon* spp.) (Pashley et al. 1985; Pashley 1986; Meagher & Nagoshi 2004; Nagoshi & Meagher 2004). In Florida, corn plants are hosts to both strains whereas forage and turf grasses are infested predominantly by rice strain larvae (Meagher & Gallo-Meagher 2003; Nagoshi et al. 2006a; Nagoshi et al. 2006b).

Adult males have been monitored for over 40 years with sex pheromones. The main component of the pheromone was initially identified as (Z)-9-tetradecenyl acetate (Z9-14:Ac) (Sekul & Sparks 1967), but it was not effective as a lure in the field using the delivery system tested (Mitchell & Doolittle 1976). Field studies concluded that either 2 [Z9-14:Ac and (Z)-7-dodecenyl acetate (Z7-12:Ac)], 3 [Z9-14:Ac, Z7-12:Ac and (Z)-11-hexadecenyl acetate (Z11-16:Ac)], or 4 [Z9-14:Ac, Z7-12:Ac, Z11-16:Ac, and (Z)-9-dodecenyl acetate (Z9-12:Ac)] components were necessary to attract males (Mitchell et al. 1985; Tumlinson et al. 1986). Pheromone differences between strains have been proposed, but the reports don't agree on which component(s) are different (Groot et al. 2008; Lima & McNeil 2009; Unbehend et al. 2013).

Regional monitoring programs have been developed to map the annual northward migration of pest Lepidoptera (e.g., [www.pestwatch.psu.edu](http://www.pestwatch.psu.edu)). These programs use cooperators in

many locations that gather moth numbers from sex pheromone-baited traps. Several companies produce lures to attract fall armyworm, however, these lures also attract large numbers of nontarget Lepidoptera which complicates species identification and counting of individuals (Adams et al. 1989; Weber & Ferro 1991). Experiments in Pennsylvania showed that commercial lures containing 3 or 4 pheromone components attracted large numbers of the nontarget species *Leucania phragmatidicola* Guenée (Noctuidae) (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). Another concern for our monitoring program is the ability to capture high numbers of corn strain rather than rice strain moths, as currently only corn strain moths have the genetic markers necessary to identify geographic haplotypes (Nagoshi et al. 2007; Nagoshi et al. 2008).

The first objective of this study was to chemically analyze the commercial lures to identify the pheromone components released and their relative rates of release. The second objective included field studies in Alabama, Georgia, Florida, and Texas to compare capture numbers of fall armyworm males in traps among lures, specifically to determine if the two-component lure captured fewer moths. In Alabama and Georgia, this second objective was expanded to determine if season (fall 2007 or fall 2008) or region (north, central, or south) were important variables in moth capture. Finally, the third objective was to determine if there is a difference in attractiveness of these lures to corn strain or rice strain males.

## MATERIALS AND METHODS

### Pheromone Confirmation

Volatile chemicals were collected from fall armyworm pheromone lures using collector traps with super-Q as the adsorbent (Analyti-

cal Research Systems, Inc., Gainesville, Florida). Volatile-collection traps were cleaned by soxhlet extraction using methylene chloride for 24 h and dried in a fume hood prior to use. Fall armyworm rubber septa pheromone lures were purchased from 3 companies: Scentry Biologicals, Inc. (Billings, Montana) provided the "standard" fall armyworm lure, which contains all 4 components (L105A) and the "PSU" lure (L976) that has Z9-14:Ac and Z7-12:Ac, while Trécé, Inc. provided the "FAW" lure (Adair, Oklahoma) and Suterra LLC (Bend, Oregon) provided the Scenturion® fall armyworm lure both of which contained Z9-14:Ac, Z11-16:Ac, and Z7-12:Ac. Five newly opened lures of each type were introduced separately into a cylindrical glass chamber (2.5 cm diam., 38.1 cm length). Purified air was introduced into the chamber (75 mL per min) and headspace volatiles collected for 5 h. Volatile chemicals were eluted from the super-Q adsorbent using 100 µL of high purity methylene chloride (99.5% pure; ACROS, Morris Plains, New Jersey). An aliquot of C<sub>14</sub> standard (5 µg) was added to each sample for quantitative analysis. The samples were then concentrated to 50% by solvent evaporation before injection in a gas-chromatograph (ThermoQuest Trace GC-FID 2000, Austin, Texas) with a fused silica DB-5 column (J&W Scientific, Agilent Technologies, Santa Clara, California), 10 m long, 0.18 mm I.D, with film thickness 0.18 µm. The GC temperature was programmed from 50 °C for 1 min, 50 to 220 °C at 35 °C min<sup>-1</sup>, and held at 220 °C for 2 min. Chemical emissions from the lures were identified by comparing their Retention Indexes (RI) to the RI of the 4 commercial synthetic pheromones: Z-11-hexadecenyl acetate, Z-9-dodecenyl acetate, Z-7-dedecen-1-yl acetate, Z-9-tetradecen-1-yl acetate (Bedoukian Research Inc., Danbury Connecticut). Three replicates per type of lures were analyzed.

#### Trapping and Locations

Sampling was conducted in Alabama, Georgia, northern Florida, and southern Texas from 2006-2009 (Table 1, Fig. 1). Standard tricolor (green top, yellow funnel, white bucket) or all green Universal moth traps (Great Lakes IPM, Vestaburg, Michigan) were placed on 1.5-m poles. Each trap contained insecticide strips containing 10% 2,2-dichlorovinyl dimethyl phosphate (Hercon® Environmental, Emigsville, Pennsylvania). Either all standard tricolor or all green traps were used during a sampling date. In Alabama and Georgia, each site contained 1 trap for each pheromone lure. Traps were placed next to corn, cotton, or mixed crop plantings in an effort to obtain corn strain moths. During fall 2007, there were 5 sampling

dates with 16, 17, 17, 17, and 11 sites, respectively ( $n = 312$ ); during fall 2008, there were 4 sampling dates with 11, 7, 12, and 10 sites, respectively ( $n = 160$ ). In Florida, the Williston site contained 5 replications of the 4 lures sampled at 7 dates in 2006 ( $n = 140$ ) or 5 dates in 2009 ( $n = 100$ ). Traps in Williston were placed next to large peanut fields with abundant pasture grass and weedy patches between fields. In Texas, the Weslaco site contained 4 replications of the 4 lures sampled for 5 dates ( $n = 80$ ), and traps were placed near corn fields and other row crops.

#### Strain Analysis

The host strain of collected moths was determined using the following methods (Nagoshi et al. 2006b). Individual specimens were homogenized in 4 mL of phosphate buffered saline (PBS, 20 mM sodium phosphate, 150 mM NaCl, pH 8.0) in a 15 mL test tube using a tissue homogenizer (PRO Scientific Inc., Oxford, Connecticut). Cells and tissue were pelleted by centrifugation at 6000 g for 5 min at room temperature. The pellet was resuspended in 800 µL cell lysis buffer (0.2 M sucrose, 0.1 M Tris-HCl at pH 8.0, 0.05 M EDTA, and 0.5% sodium dodecyl sulfate), transferred to a 1.5 or 2.0 mL microcentrifuge tube and incubated at 55 °C for 5 min. Proteins were precipitated by the addition of 100 µL of 8M potassium acetate. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Irvine, California) and processed according to manufacturer's instructions. The DNA preparation was increased to a final volume of 40 µL with distilled water. Each PCR reaction required 1 µL of the DNA preparation.

PCR amplification of the mitochondrial *COI* gene was performed in a 30 µL reaction mix containing 3 µL 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 unit Taq DNA polymerase (New England Biolabs, Beverly, Massachusetts). 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. Primers were synthesized by Integrated DNA Technologies (Coralville, Iowa). Amplification of the *COI* region used the primer pair *COI-893F* (5'-CAC-GAGCATATTTTACATCWGCA-3') and *COI-1303R* (5'-CAGGATAGTCAGAATATCGACG-3') to produce a 410 bp fragment.

For fragment isolations 6 µL of 6 × gel loading buffer was added to each amplification reaction and the entire sample run on a 1.8% aga-

TABLE 1. LOCATION, CROP HABITAT, AND DATES OF SAMPLING OF THE PHEROMONE LURE EXPERIMENT ON ATTRACTING FALL ARMYWORM MALE MOTHS, 2006-2009.

| Site (State, County) | Region  | Latitude/Longitude           | Predominant Crop Plants | Dates Sampled  |
|----------------------|---------|------------------------------|-------------------------|--|
| <b>ALABAMA</b>       |         |                              |                         |  |
| Autauga              | central | N 32° 26.622', W 86° 24.937' | mixed                   | 2008: 4/23, 5/07, 5/28, 8/20, 10/23                            |
| Baldwin              | south   | N 30° 27.706', W 87° 36.422' | mixed                   | 2008: 4/22, 5/08, 5/28, 9/16, 10/20                            |
| Cleburne             | north   | N 33° 36.367', W 85° 35.657' | corn                    | 2008: 5/29   |
| Coffee               | south   | N 31° 24.455', W 86° 10.004' | corn                    | 2008: 4/22, 5/08, 5/28, 8/19, 9/16, 10/20                      |
| Henry                | south   | N 31° 22.634', W 85° 18.648' | mixed                   | 2008: 4/22, 5/08, 5/28, 8/19, 9/16, 10/20                      |
| Macon                | central | N 32° 25.429', W 85° 56.285' | corn                    | 2008: 4/23, 5/08, 5/29   |
| Russell              | central | N 32° 24.654', W 85° 06.975' | corn                    | 2008: 5/29   |
| Shelby               | central | N 33° 22.795', W 86° 25.023' | corn                    | 2008: 5/28, 10/23  |
| Talladega            | north   | N 33° 33.356', W 86° 01.340' | corn                    |  |
| <b>FLORIDA</b>       |         |                              |                         |  |
| Levy                 |         | N 29° 20.493', W 82° 34.381' | peanuts                 | 2006: 8/11, 8/15, 8/21, 8/25, 8/29, 9/15, 9/25                 |
| <b>GEORGIA</b>       |         |                              |                         |  |
| Decatur              | south   | N 30° 57.649', W 84° 43.091' | corn                    | 2007: 8/21, 9/11, 9/25, 10/15, 11/06                           |
| Haralson             | north   | N 33° 47.930', W 85° 16.698' | corn                    | 2008: 5/28, 8/20   |
| Lowndes              | south   | N 30° 48.566', W 83° 21.568' | cotton                  | 2008: 4/22, 5/06, 5/27, 8/19, 9/16                             |
| Muscogee             | central | N 32° 23.273', W 84° 58.459' | mixed                   | 2008: 4/23, 5/06, 5/27   |
| Newton               | north   | N 33° 40.483', W 83° 52.219' | mixed                   | 2008: 5/28, 8/21, 9/10, 9/18, 10/23                            |
| Peach                | central | N 32° 33.524', W 83° 48.376' | cotton                  | 2008: 4/23, 5/06, 5/27, 8/21, 9/10, 9/18, 10/23                |
| Taylor               | central | N 32° 31.550', W 84° 14.938' | mixed                   | 2007: 9/11, 9/25, 10/15, 11/07                                 |
| Thomas               | south   | N 30° 48.937', W 83° 52.010  | cotton                  | 2008: 4/22, 5/06, 5/27, 8/19                                   |
| <b>TEXAS</b>         |         |                              |                         |  |
| Hidalgo              |         | N 26° 09.670', W 97° 57.511  | mixed                   | 2008: 5/29, 6/05, 6/12, 6/19, 6/26, 10/16, 10/23, 10/30, 11/06 |



Fig. 1. Map of trap locations in Alabama, Florida, and Georgia. The full name of each County can be found in Table 1.

rose horizontal gel containing GelRed (Biotium, Hayward, California) in 0.5X Tris-borate buffer (TBE, 45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). Fragments were visualized on a long-wave UV light box and cut out from the gel. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research, Orange, California) according to manufacturer's instructions. The isolated fragments were analyzed by DNA sequencing performed by Northwoods DNA, Inc. (Bemidji, Minnesota) or the University of Florida ICBR Center (Gainesville, Florida). All other DNA sequences were obtained from NCBI GenBank. DNA comparisons, alignments, and restriction site mapping were performed using the DS Gene program (Accelrys, San Diego, California).

#### Statistical Analysis

All analyses were conducted using SAS (SAS 9.2, SAS Institute 2008). Release rates of 3 pheromone components (Z7-12:Ac, Z9-14:Ac, and Z11-16:Ac) were compared among pheromone lures using analysis of variance of non-transformed data (PROC MIXED, LSMEANS).

Moth number data were analyzed using Box-Cox (PROC TRANSREG) and PROC UNIVARI-

ATE (Osborne 2010) to find the optimal normalizing transformation. Box-Cox selects the optimal transformation based on the best lambda fitted to the regression and the univariate procedure provides further evidence of skewness, kurtosis, and a bell-shaped curve. For the Alabama and Georgia results, a reciprocal 4th root transformation was suggested. This was followed by analysis of variance (PROC GLM, LSMEANS) to separate means among regions, pheromone lure treatments, and the region by lure interaction. The Williston, Florida 2006 data used a reciprocal square root transformation and the Williston 2009 data used a 4th root transformation. The Weslaco, Texas data were analyzed using a reciprocal square root transformation. Analysis of variance (PROC MIXED, LSMEANS) was then used to separate means among pheromone lure treatments for the Florida and Texas results.

To test if the number of corn strain moths collected was independent of pheromone lure, a Pearson Chi-Square test was used (PROC FREQ, 2 (host strain)  $\times$  4 (commercial lures) table, Pearson exact result). A significant *P* value rejects the null hypothesis that host strain and pheromone lure are independent. The frequency of corn strain

to rice strain moths for each pheromone lure was analyzed (PROC FREQ, exact chi-square test) to determine if the ratio was different from 50%, i.e. did the sample contain more corn strain (>50%), more rice strain (<50%), or an equal frequency of the 2 strains?

RESULTS

Pheromone Components

The PSU and Scenturion lures released relatively large amounts (>50 ng/h) of Z9-14:Ac that composed > 90% of their blends (Fig. 2). The Scentry and Trécé lures released smaller amounts, which were also a lower percentage of their blends. All lures released Z7-12:Ac, with Trécé containing > 16 ng/h which was nearly 30% of its blend. Other lures release < 5 ng/h which comprised < 10% of their blends. The Scentry, Scenturion, and Trécé lures released small amounts (< 3 ng/h) of Z11-16:Ac; only the Scentry lure released Z9-12:Ac.

Moth Numbers

In Alabama and Georgia, fall armyworm moths were collected at all sites where traps were placed, although several county sites had very low numbers. Data were not recorded for several traps due to broken trap tops, traps with the funnels closed due to spider webs, traps with frogs, or where moths were unable to be counted due to wet conditions. There were 5 x more moths per night collected during fall 2007 than fall 2008 (mean ± SE; 9.9 ± 0.8 vs. 1.8 ± 0.3; *F* = 107.9, *df* = 1, 458, *P* < 0.0001, *n* = 460). However, there was no difference in the number of moths captured among lures in the fall 2007 or fall 2008 seasons (Table 2).

County sites in Alabama and Georgia were analyzed according to 3 regions (Table 1), and all had significantly different numbers of moths per night. For fall 2007, the south region (Baldwin, Coffee, and Henry Counties in Alabama; Decatur, Lowndes, and Thomas Counties in Georgia) averaged more moths per night than the central region (Autauga, Macon, and Russell Counties in Alabama; Muscogee, Peach, and Taylor Counties in Georgia), which captured more moths than the north region (Cleburne, Shelby, and Talladega Counties in Alabama; Haralson and Newton Counties in Georgia) (Table 2). For fall 2008, the south averaged more moths per night than both the central and the north (Table 2). The region by lure interaction was not significant in either season (*P* > 0.92).

Populations in Williston, Florida in 2006 were relatively low with seasonally less than 5 moths per night collected. However, a difference among lures was found as traps with the PSU lure caught more moths and traps baited with the Scenturion and the Scentry lure caught fewer moths (Table 2). This was the only test where a difference in trap capture occurred among lures and this result could be an artifact of low trap numbers. Moth numbers captured in 2009 were over 3 times higher than in 2006, with no difference among the various lures.

Fall armyworm populations in southern Texas were relatively high in spring 2008 and fall 2009 (Table 2). Moth numbers decreased during fall 2008 and remained so low during spring 2009 that statistical analyses were not conducted. There was no difference in spring 2008 or fall 2009 among lures in male trap captures.

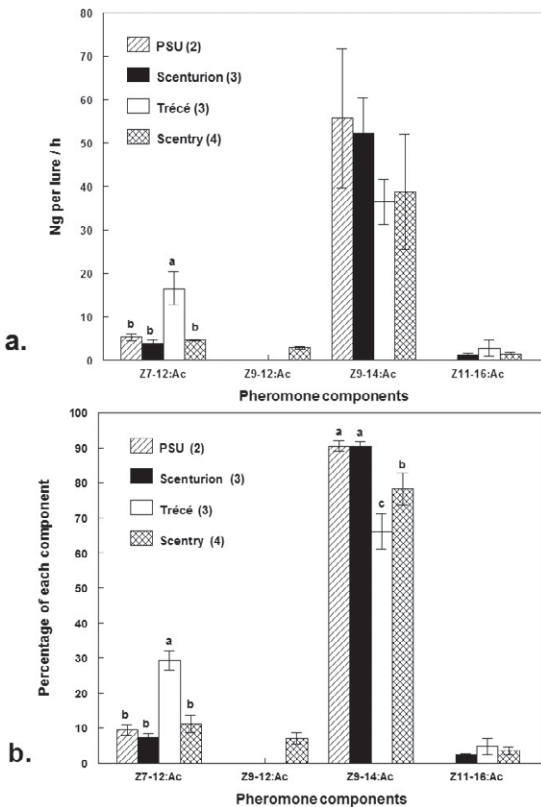


Fig. 2. Quantity (µg) and percentage of each component released per pheromone lure based on a 5 h collection period (mean ± SE). The same letters above Z9-14:Ac or Z7-12:Ac indicates no significant differences (*P* < 0.05).

Host Strains

Host strain analysis was conducted on over 3,500 moths collected in Alabama and Georgia in 4 sample periods from Aug through Nov 2007. In the north region, moths collected during Aug and Sep were predominantly corn strain whereas moths in Oct were a mix of both strains (Fig.

TABLE 2. NUMBER OF MOTHS CAPTURED PER NIGHT AMONG TRAPS WITH DIFFERENT PHEROMONE LURES AND IN DIFFERENT REGIONS IN ALABAMA AND GEORGIA IN FALL 2007 AND FALL 2008; IN WILLISTON, FLORIDA IN 2006 AND 2009, AND IN WESLACO, TEXAS IN 2008 AND 2009. THE SAME LETTER IN EACH GROUPING INDICATES NO SIGNIFICANT DIFFERENCE. NUMBERS AFTER THE PHEROMONE LURE NAME INDICATES THE NUMBER OF COMPONENTS.

| Alabama and Georgia Fall 2007             |                            | Alabama and Georgia Fall 2008             |                            |
|---|----------------------------|---|----------------------------|
| Pheromone Lure                            | Mean $\pm$ SE ( <i>n</i> ) | Pheromone Lure                            | Mean $\pm$ SE ( <i>n</i> ) |
| Trécé (3)                                 | 11.0 $\pm$ 1.6 a (76)      | Trécé (3)                                 | 2.1 $\pm$ 0.8 a (39)       |
| Scenturion (3)                            | 10.2 $\pm$ 1.6 a (75)      | Scentry (4)                               | 1.9 $\pm$ 0.6 a (40)       |
| PSU (2)                                   | 9.6 $\pm$ 1.6 a (76)       | PSU (2)                                   | 1.6 $\pm$ 0.5 a (40)       |
| Scentry (4)                               | 8.9 $\pm$ 1.3 a (74)       | Scenturion (3)                            | 1.6 $\pm$ 0.5 a (40)       |
| $F = 0.97$ ; $df = 3, 289$ ; $P = 0.4060$ |                            | $F = 0.46$ ; $df = 3, 147$ ; $P = 0.7138$ |                            |
| Region                                    | Mean $\pm$ SE ( <i>n</i> ) | Region                                    | Mean $\pm$ SE ( <i>n</i> ) |
| south                                     | 16.5 $\pm$ 1.3 a (116)     | south                                     | 4.0 $\pm$ 0.8 a (52)       |
| central                                   | 8.8 $\pm$ 1.2 b (110)      | central                                   | 1.1 $\pm$ 0.3 b (32)       |
| north                                     | 1.4 $\pm$ 0.2 c (75)       | north                                     | 0.6 $\pm$ 0.1 b (75)       |
| $F = 69.3$ ; $df = 2, 289$ ; $P < 0.0001$ |                            | $F = 23.4$ ; $df = 2, 147$ ; $P < 0.0001$ |                            |
| Williston, Florida 2006                   |                            | Williston, Florida 2009                   |                            |
| Pheromone Lure                            | Mean $\pm$ SE ( <i>n</i> ) | Pheromone Lure                            | Mean $\pm$ SE ( <i>n</i> ) |
| PSU (2)                                   | 4.3 $\pm$ 0.6 a (35)       | Trécé (3)                                 | 15.8 $\pm$ 2.9 a (25)      |
| Trécé (3)                                 | 3.8 $\pm$ 1.0 ab (35)      | Scentry (4)                               | 12.5 $\pm$ 2.3 a (25)      |
| Scentry (4)                               | 2.6 $\pm$ 0.4 b (35)       | Scenturion (3)                            | 11.3 $\pm$ 1.8 a (24)      |
| Scenturion (3)                            | 1.2 $\pm$ 0.2 c (35)       | PSU (2)                                   | 10.4 $\pm$ 1.8 a (25)      |
| $F = 8.0$ ; $df = 3, 18$ ; $P = 0.0013$   |                            | $F = 1.5$ ; $df = 3, 12$ ; $P = 0.1358$   |                            |
| Weslaco, Texas 2008                       |                            | Weslaco, Texas 2009                       |                            |
| Pheromone Lure                            | Mean $\pm$ SE ( <i>n</i> ) | Pheromone Lure                            | Mean $\pm$ SE ( <i>n</i> ) |
| Trécé (3)                                 | 17.9 $\pm$ 4.5 a (19)      | Trécé (3)                                 | 16.9 $\pm$ 4.2 a (16)      |
| Scenturion (3)                            | 14.5 $\pm$ 3.7 a (19)      | PSU (2)                                   | 13.7 $\pm$ 2.9 a (16)      |
| PSU (2)                                   | 11.5 $\pm$ 2.8 a (19)      | Scenturion (3)                            | 9.5 $\pm$ 2.3 a (16)       |
| Scentry (4)                               | 10.3 $\pm$ 2.7 a (19)      | Scentry (4)                               | 9.4 $\pm$ 2.1 a (16)       |
| $F = 0.6$ ; $df = 3, 65$ ; $P = 0.6276$   |                            | $F = 1.3$ ; $df = 3, 54$ ; $P = 0.2823$   |                            |

3). For all 3 sampling times, there was no association between host strain and pheromone lure ( $P = 0.5069, 0.4985, \text{ and } 0.5606$  for Aug, Sep, and Oct, respectively). Moths collected in central region sites provided a different host strain picture. In Aug, statistically more of the moths captured were of the corn strain except for moths captured in Trécé-baited traps (Fig. 4). In Sep and Oct there was an even percentage of corn and rice strain moths captured. By Nov, there was an abundance of corn strain moths in all traps. As in the north, there was no association between host strain and pheromone lure ( $P = 0.4609, 0.2516, 0.8073, \text{ and } 0.3086$  for Aug, Sep, Oct, and Nov, respectively). High percentages of corn strain moths were collected from the Scentry- and Scenturion-baited traps in the south in Aug, but traps with the PSU and Trécé lures captured similar numbers of both strains, and this was enough of a difference to

reject the null hypothesis that host strain and pheromone lure were independent ( $P = 0.0011$ ) (Fig. 5). Moths captured in Sep were a mix of the corn and rice strains. Three of the 4 lures attracted more rice strain moths in Oct, but by Nov, mostly similar percentages of each host strain were captured.

Host strain analysis was conducted on over 500 moths captured in Williston, Florida in 2006. In both the Aug and Sep samples, pheromone lure influenced the collection of the host strains (Fig. 6a,  $P = 0.0205$  and  $0.0057$ , respectively). In Aug the Scenturion lure attracted significantly more corn strain moths, while the other lures attracted equal numbers of both strains (Fig. 6a). For the samples collected in Sep, all lures attracted more rice strain moths. However, only 1 corn strain moth (2.4%) was identified from the PSU-baited trap. For Weslaco, Texas, host strain analysis was determined



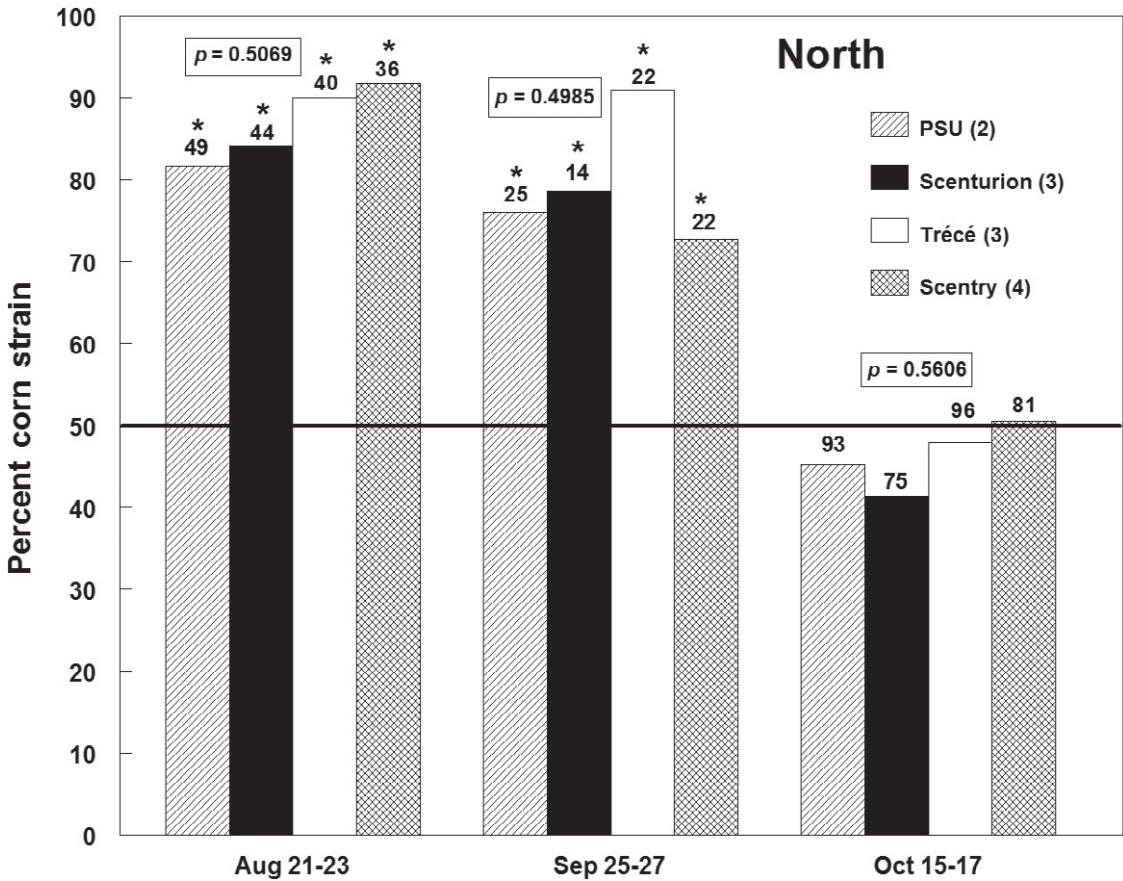


Fig. 3. Percent of corn strain of male fall armyworm moths collected in pheromone-baited traps located in the north region of Alabama and Georgia during 3 sample periods in 2007. For each sampling period, the  $P$  value from a Pearson Chi-Square analysis tested the null hypothesis that host strain and pheromone lure were independent. For each pheromone lure individually, exact chi-square analysis determined whether the sample contained more or less than 50% corn strain moths (\*,  $P < 0.05$ ). Tested numbers of moths for strain analysis are above each bar.

with samples from 29 May to 26 Jun 2008 ( $n = 286$ ). Greater than 85% of the moths analyzed were of the corn strain (Fig. 6b).

#### DISCUSSION

The release of Z7-12:Ac in this study was different than in a previous study (Meagher & Mitchell 2001). Scentry, Scenturion, and Trécé lures released relatively equal percentages of their blends in the 2001 study (mean release = 5.8%). Our analysis of this more recent study showed higher amounts released (mean release = 9.3% excluding Trécé), especially the release from Trécé lures (mean release = 14.3% including Trécé). The earlier study suggested that the Scentry-baited traps would not capture as many moths as Scenturion- or Trécé-baited traps, but the Scentry lures in our recent test attracted moths as well as the other lures. There was a concern that the PSU lure would not be as attractive

as the other lures (Fleischer et al. 2005), but our results agree with Tumlinson et al. (1986) that a blend of Z9-14:Ac and Z7-12:Ac attracts similar numbers of moths as the other commercial lures tested. In fact, Tumlinson et al. (1986) suggested that the addition of the other acetates did not significantly increase trap capture, a result that we also found in this recent study. This is important, because it appears that the two-component lure attracts fewer nontarget noctuid species in the eastern and central USA (Fleischer et al. 2005; Meagher unpublished).

Attraction of corn and rice strain males to the different commercial lures varied slightly among locations and time of season. But when host strain data were taken into account across locations ( $n = 4,546$  moths analyzed), all lures attracted significantly more corn strain males than rice strain males (PSU 54.9%, Trécé 56.6%, Scentry 59.5%, and Scenturion 60.0%). These differences among lures was significant

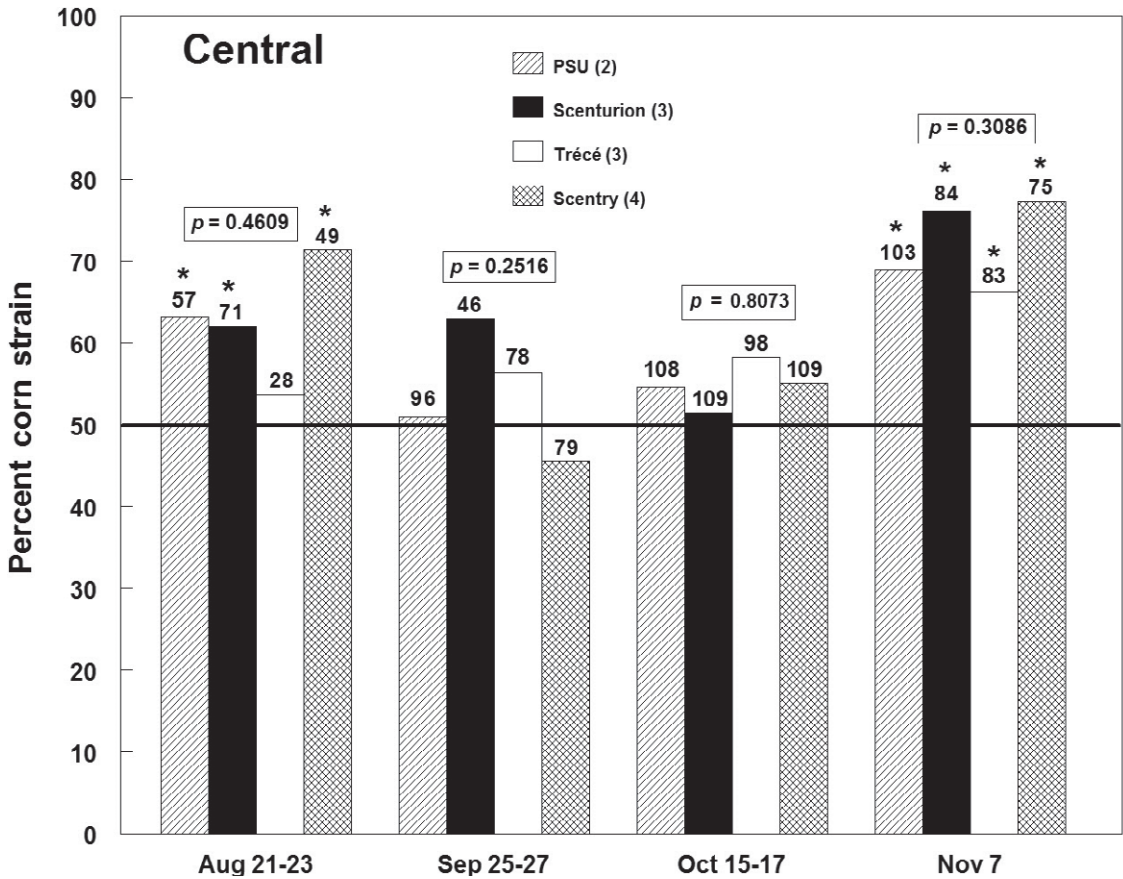


Fig. 4. Percent of corn strain of male fall armyworm moths collected in pheromone-baited traps located in the central region of Alabama and Georgia during 4 sample periods in 2007. For each sampling period, the  $P$  value from a Pearson Chi-Square analysis tested the null hypothesis that host strain and pheromone lure were independent. For each pheromone lure individually, exact chi-square analysis determined whether the sample contained more or less than 50% corn strain moths (\*,  $P < 0.05$ ). Tested numbers of moths for strain analysis are above each bar.

( $P = 0.0399$ ), suggesting that lures differentially attract the strains. Research completed after our field tests seemed to suggest that the Trécé lures might attract fewer corn strain males, since those lures contained the highest amounts of Z7-12:Ac, and Unbehend et al. (2013) showed that corn strain males are attracted to low amounts of this component. However, with other factors such as geographic location, time of season, and crop habitat seemingly more important, this slight difference of 5% between the PSU and Scenturion lures in the collection of one strain over the other should not influence future haplotype research.

There are several studies that provide examples of factors other than pheromone lure being important in the capture of host strains. Results of trials in the overwintering areas of southern Florida suggested both strains were present in spring samples (Mar-May) but rice

strain moths were more common in fall samples (Oct-Dec), even though traps were located in corn habitats (Meagher & Nagoshi 2004; Nagoshi & Meagher 2004). Our results from locations north of the overwintering areas showed corn and rice strain populations also varied seasonally. Late Aug samples had higher percentages of corn strain males, but by Oct, the ratios were either equal or biased to rice strain. Corn strain moths generally rebounded in Nov, but only in the southern regions. Host strain populations vary within a season probably because of the availability of preferred hosts, but local or regional movement of moths can't be ignored as a possible cause for fluctuating host strain ratios.

Results from these trials suggest that any of the commercial lures tested will attract the numbers of moths necessary for genetic and migration analysis, provided the site has the preferred host

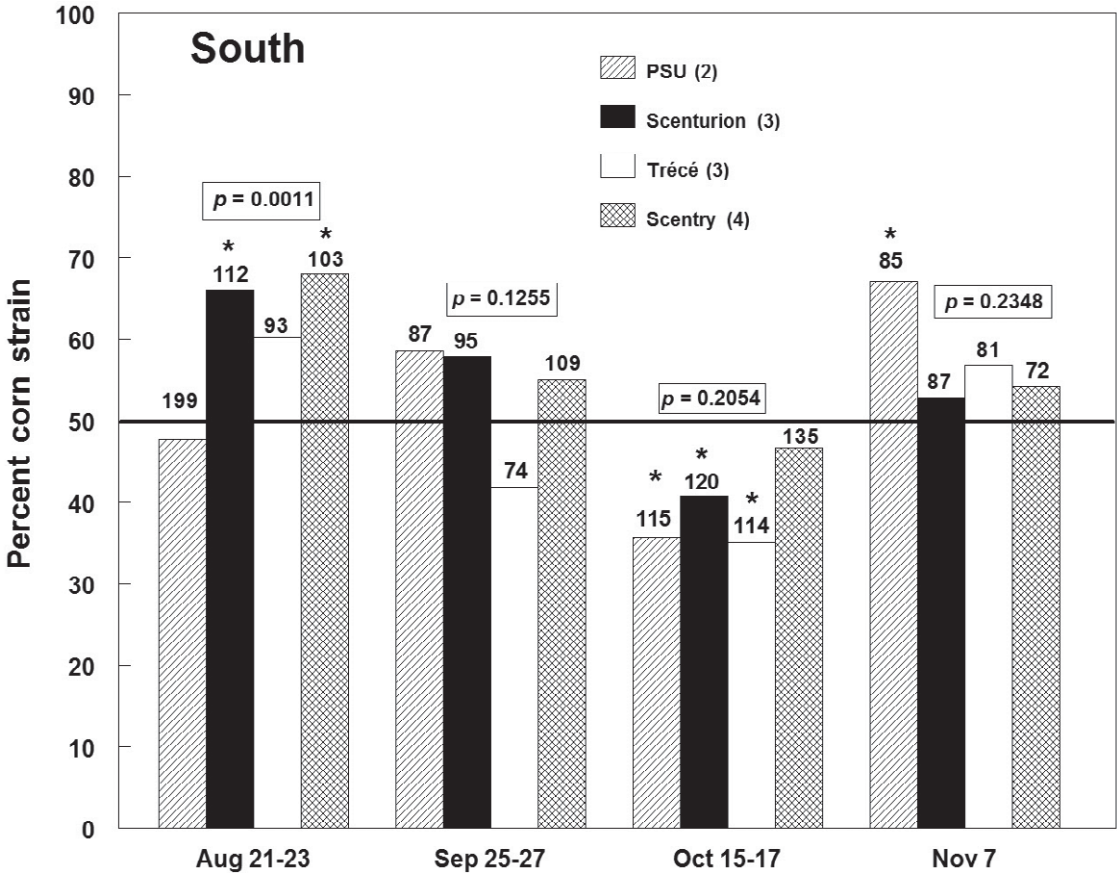


Fig. 5. Percent corn strain of male fall armyworm moths collected in pheromone-baited traps located in the south region of Alabama and Georgia during 4 sample periods in 2007. For each sampling period, the  $P$  value from a Pearson Chi-Square analysis tested the null hypothesis that host strain and pheromone lure were independent. For each pheromone lure individually, exact chi-square analysis determined whether the sample contained more or less than 50% corn strain moths (\*,  $P < 0.05$ ). Tested numbers of moths for strain analysis are above each bar.

plants available to attract corn strain males. Site location (away from trees and in open areas) and periodic trap maintenance (removal of spiders and frogs clogging funnels or eating trap catches) are also important in collecting the highest number of moths. Additional studies are needed to determine why the captured host strain composition changes within a season, i.e., is the change caused by a decrease in the numbers of one strain or caused by a large increase of the other as the result of population movement. Finally, research should be expanded to determine if trap capture represents the predominant host strain of the population in the field at the time of sampling by using other adult sampling techniques. A recent report that compared capture of males using Scenturion-baited traps versus female-baited traps concluded that the lure is biased to attract

corn strain males and underestimates rice strain numbers relative to corn strain numbers (Meagher & Nagoshi 2013).

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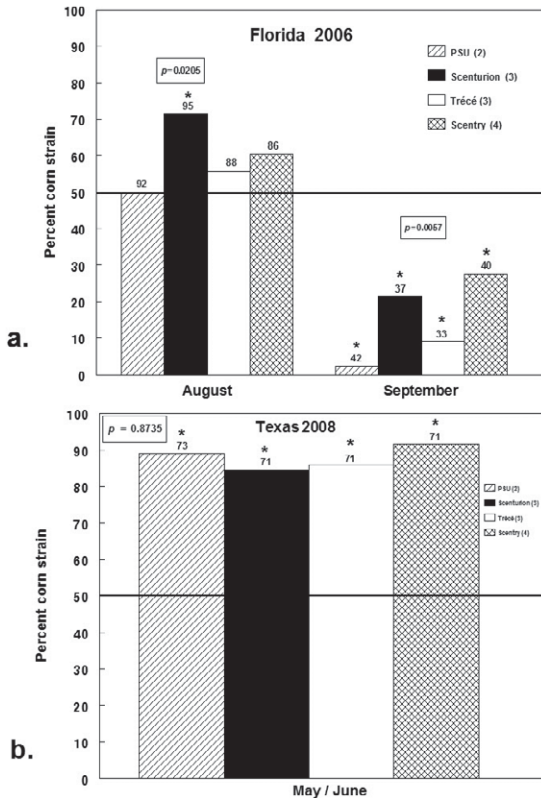


Fig. 6. Percent corn strain of male fall armyworm moths collected in pheromone-baited traps in Florida or Texas in 2006 or 2008, respectively. The  $P$  value from a Pearson Chi-Square analysis tested the null hypothesis that host strain and pheromone lure were independent. For each pheromone lure, exact chi-square analysis determined whether the sample was more or less than 50% (\*,  $P < 0.05$ ). Tested numbers of moths for strain analysis are above each bar.

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