

Attraction of Fall Armyworm Males (Lepidoptera: Noctuidae) to Host Strain Females

ROBERT L. MEAGHER¹ AND RODNEY N. NAGOSHI

U.S. Department of Agriculture–Agriculture Research Service Center for Medical, Agricultural and Veterinary Entomology, 1700 SW 23rd Drive, Gainesville, FL 32608

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ABSTRACT Attraction of wild male fall armyworm, *Spodoptera frugiperda* (J. E. Smith), was compared in trapping experiments during 2005–2009 in Florida. Traps were baited either with a commercial sex pheromone lure or corn and rice strain females obtained from laboratory colonies. Over 6,900 male moths were collected, and a large subset (>1,500) of these moths was analyzed for their host strain identity. The pheromone lure attracted over four times more males than virgin corn or rice strain females. Almost 60% of males attracted to the pheromone lure were identified as corn strain. However, both corn and rice strain females attracted a higher percentage of rice strain males, providing evidence that the commercial lure used in our study is biased to attract corn strain males and underestimates rice strain population numbers relative to corn strain numbers. Corn and rice strain males were attracted more to corn strain females than rice strain females, although there was variation in response according to location and season. Our results suggest that attraction of males to corresponding-strain females does not appear to be a premating mechanism that results in assortative mating between corn and rice host strains. Clearly other premating or perhaps even postmating mechanisms are important for the maintenance of host strains in *S. frugiperda*.

KEY WORDS *Spodoptera frugiperda*, host strains, premating behavior

Recent studies of closely related herbivore host strains, host races, and insect species has shown that several factors promote sympatric speciation. Adaptation to new or different host plants is one important factor but various premating mechanisms can also provide isolation of populations over time (Drès and Mallet 2002, Malausa et al. 2005, Mallet 2008). For Lepidoptera, premating mechanisms resulting in assortative mating between types have generally included some disruption in the female-produced pheromone–male response system such as a change in pheromone blend composition, temporal partitioning of female pheromone release (calling), or change in host site of female calling (Emelianov et al. 2003, Thomas et al. 2003, Dopman et al. 2004).

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) provides a potentially useful system to study certain aspects of sympatric speciation. This migratory, polyphagous noctuid moth attacks a wide variety of crops throughout the Nearctic and Neotropical Western Hemisphere (Luginbill 1928, Sparks 1979). The species is composed of two morphologically identical

strains that are defined by their host plant preferences (reviewed in Nagoshi and Meagher 2004a). One strain was identified from populations feeding on corn and sorghum (corn strain) and the other was identified from populations feeding on rice (*Oryza sativa* L.) and forage grasses (*Cynodon* spp.) (rice strain) (Pashley et al. 1985, Pashley 1986). The two strains can be distinguished by genetic markers (Levy et al. 2002, Nagoshi and Meagher 2003, Nagoshi et al. 2006). Pheromone trapping studies in agricultural habitats routinely attract males of both strains, though the proportion will vary depending on the dominant plant type, indicating that the strains overlap substantially in their distribution and are mutually attracted to a common pheromone source (Meagher and Nagoshi 2004, Nagoshi and Meagher 2004b).

One way to test whether premating mechanisms exist for *S. frugiperda* is to place virgin females of both strains separately in the field and identify the host strain of responding males. An experiment was completed in one season (June through September) in Louisiana and suggested that males are attracted, although modestly, to like-strain females (Pashley et al. 1992). However, studies in Florida show dramatic differences in corn and rice strain numbers throughout a year as measured by captures in pheromone-baited traps (Meagher and Nagoshi 2004, Nagoshi and Meagher 2004b). Our article expands upon the limited

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¹ Corresponding author, e-mail: rob.meagher@ars.usda.gov.

field research to date by comparing wild male attraction to colony females and to a commercial pheromone lure across different seasons and in different locations in Florida.

Materials and Methods

Field Sites. Moths were collected at two sites in northern Florida and two sites in southern Florida (Table 1). One site in northern Florida (Alachua Co.) was the University of Florida Dairy Research Unit, Hague. The research dairy and surrounding farm comprise 344 ha and has continuous production of field corn for silage from March through October. During 2005, traps were placed in September and October; in 2007, traps were placed in October (Table 1). The second site was a 400-ha commercial peanut field near Williston in Levy Co. During 2009, traps were placed at various dates between July and August.

The Miami-Dade Co. site near Homestead in southern Florida was an area of fresh market sweet corn produced from October to March. After harvest (April to June), fields are planted with sorghum-sudangrass [*Sorghum bicolor* x *S. bicolor* variety *sudanense* (Piper) Stapf.] (SSG) cover crop that is an excellent host for fall armyworm (Meagher et al. 2004). In the fall season of 2005, traps were placed from November to January 2006. Sampling during summer 2006 in the SSG fields showed large larval populations of fall armyworm, therefore, traps were placed in June. An unusual seasonal planting of sweet corn was sampled in June 2007, while a normal seasonal planting was sampled in November 2007. The second southern Florida site was in western Palm Beach Co. at the University of Florida Everglades Research and Education Center (EREC) in Belle Glade. This center contains experimental plots of sweet corn, sugarcane, rice, vegetables, and turfgrass. Traps in 2007 were placed next to sweet corn plots in May and November.

Host Strain Females. Females for these tests were from several sources and were confirmed to carry the mitochondrial marker of either corn or rice strain (Meagher and Gallo-Meagher 2003, Nagoshi and Meagher 2003). All colonies (except CS-Lab) originated from laboratory pair matings of field-collected larvae that, unless otherwise stated, completed development on and were subsequently reared on pinto bean diet (Guy et al. 1985, Stuhl et al. 2008).

The Hague-September 2005 and Hague-October 2005 trials used two corn strain (CS) and two rice strain (RS) colonies (Table 1). CS-Lab larvae were from a laboratory colony reared on artificial diet that originated from individuals received from U.S. Department of Agriculture-Agriculture Research Service, Tifton, GA. This colony had been in the laboratory for several years and had introductions of field material before we received individuals. CS-JS (generation at first testing was F_3) females were from a merged colony collected from sweet corn in Miami-Dade Co. from two different sites in October and November 2004. Larvae from the two colonies were merged in February 2005 to form the CS-JS colony.

Table 1. Field locations with host plants, sampling dates, and colony for female corn and rice strain fall armyworm used to attract males to standard Unitraps, Florida, 2005–2009

Location	Coordinates	Sampling dates	Colonies used	Host plants
Northern Florida UF-DRU, Hague, Alachua Co. (Hague-Sept. 2005)	29° 47.123' N, 82° 25.050' W	13, 26, 28 Sept. 2005	CS-Lab, CS-JS; RS-Ona03, RS-MS	Field corn
UF-DRU, Hague (Hague-Oct. 2005)	29° 47.123' N, 82° 25.050' W	5, 12, 20 Oct. 2005; 9–11 Oct. 2007	CS-Lab, CS-JS; RS-Ona03, RS-MS	Field corn
Williston (Levy Co.) (Williston, 2009)	29° 20.469' N, 82° 34.152' W	9 July–20 Aug. 2009	CS-DRU; RS-Ona05	Mostly peanuts but large areas of pasture grass
Southern Florida Homestead, Miami-Dade Co. (Homestead 2005)	25° 27.994' N, 80° 24.233' W	16, 29 Nov., 6 Dec. 2005; 16 Jan. 2006	CS-JS; RS-Ona03	Sweet corn
Homestead and UF-EREC, Belle Glade, Palm Beach Co. (south Florida spring)	25° 27.994' N, 80° 24.233' W and 26° 40.067' N, 80° 37.911' W	Homestead: 27–28 June 2006, 19–20 June 2007; Belle Glade 29–30 May 2007	2006: CS-JS; RS-MS 2007: CS-Hague; RS-MS	Homestead: sorghum-sudangrass and sweet corn; Belle Glade mixed
Homestead and UF-EREC, Belle Glade (south Florida fall)	25° 27.994' N, 80° 24.233' W and 26° 40.067' N, 80° 37.911' W 26° 40.067' N, 80° 37.911' W	Homestead: 19–20 Nov. 2007; Belle Glade 12–13 Nov. 2007	CS-Hague; RS-MS; RS-Ona05	Homestead: sweet corn; Belle Glade mixed

RS-Ona03 (F_{12}) females were from individuals collected from various forage grasses at the Range Cattle Research and Extension Center, Ona, Hardee Co., FL in May 2003. This colony was originally reared on both grasses [Bermuda grass, *Cynodon dactylon* (L.) Pers. and stargrass, *C. nlemfuensis* Vanderyst variety *nlemfuensis* 'Florona'] and on pinto bean diet. The grass and pinto bean diet cultures were merged in May 2004 and became the RS-Ona03 colony. RS-MS (F_8) larvae were from individuals collected from Bermuda grass in Washington Co., MS, in August 2004. The colony was developed from parents carrying both the mitochondrial and genomic marker for rice strain (Nagoshi and Meagher 2003). The Williston 2009 trials used CS-DRU (F_{9-11}), which was derived from individuals collected from field corn at Hague in May 2008. RS-Ona05 (F_{56-58}) was an extension of the RS-Ona03 colony but with additional material collected from pasture grasses in 2004 and 2005. The Homestead fall and winter 2005–2006 trials used CS-JS (F_{4-6}) and RS-Ona03 (F_{14-16}). The Homestead summer 2006 trial used CS-JS (F_{11}) and RS-MS (F_{15}). The Homestead and Belle Glade summer 2007 trials used CS-Hague (F_{20-21}) and RS-MS (F_{25-26}). Finally, the Homestead and Belle Glade fall 2007 trials used CS-Hague (F_{25-26}) and RS-MS (F_{32}) and RS-Ona05 (F_{28}).

Trapping. Male fall armyworm moths were collected using standard plastic Universal moth traps (Unitraps – green top, yellow funnel, white buckets; Great Lakes integrated pest management (IPM), Vestaburg, MI). All traps contained insecticide strips (Hercon Vaportape II containing 10% 2,2-dichlorovinyl dimethyl phosphate, Hercon Environmental Co., Emigsville, PA) to kill moths. Traps were placed on 1.5-m metal poles next to the corn fields and each treatment was at least 30 m apart.

Sex pheromone-baited traps were composed of lures with three components ((*Z*)-9-tetradecen-1-ol acetate, (*Z*)-11-hexadecen-1-ol acetate, and (*Z*)-7-dodecen-1-ol acetate; Scenturion, Suterra, LLC, Bend, OR). Two to 4-d-old virgin corn or rice strain females were placed within a small screen insert that had a piece of cotton with 10% honey/sugar solution in the bottom. This insert holding two females was then placed inside the green top of the Unitraps in the late afternoon or early evening. During a sampling night, two pheromone-baited traps and from three to five corn and rice strain female-baited traps each were placed in the field ($n = 8-12$ traps). Males were collected the next morning and new females were used for the next night. Trapped moths were separated from other species, counted, and a subset randomly selected for host strain analysis.

Moth DNA Preparation. 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, CT). Cells and tissue were pelleted by centrifugation at $6,000 \times g$ for 5 min. at room temperature. The pellet was resuspended in 800 μ l 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 μ l of 8M potassium acetate. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Orange, CA) and processed according to manufacturer's instructions. The DNA preparation was increased to a final volume of 40 μ l with distilled water. Each polymerase chain reaction (PCR) reaction required 1 μ l of the DNA preparation ($\approx 0.02 \mu$ g).

Moth PCR Analysis and Cloning. PCR amplification of the mitochondrial *COI* gene was performed in a 30 μ l reaction mix containing 3 μ l 10 \times manufacturer's reaction buffer, 1 μ l 10 mM dNTP, 0.5 μ l 20 μ M primer mix, 1 μ l DNA template (between 0.05–0.5 μ g), and 0.5 U *Taq*DNA polymerase (New England Biolabs, Beverly, MA). 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, IA). Amplification of the *COI* region used the primer pair *COI*-893 *F* (5'-CACCAGCATATTTTACATCWGCA-3') and *COI*-1303R (5'-CAGGATAGTCAGAATATCGACC-3') to produce a 410 bp fragment.

For fragment isolations 6 μ l of 6 \times gel loading buffer was added to each amplification reaction and the entire sample run on a 1.8% agarose horizontal gel containing GelRed (Biotium, Hayward, CA) in 0.5X Tris-borate buffer (TBE, 45 mM Tris base, 45 mM boric acid, and 1 mM EDTA pH 8.0). Fragments were visualized on a long-wave ultraviolet light box and cut out from the gel. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research, Orange, CA) according to manufacturer's instructions. The isolated fragments were analyzed by DNA sequencing performed by Northwoods DNA, Inc. (Bemidji, MN) or the University of Florida Interdisciplinary Center for Biotechnology Research (Gainesville, FL). 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, CA).

Statistics. Low numbers of moths were collected at some sites, therefore data were combined across locations to include: Hague-September 2005, Hague-October 2005, Williston 2009, Homestead 2005, south Florida spring (Homestead SSG 2006, Homestead sweet corn 2007, and Belle Glade sweet corn 2007), and south Florida fall (Homestead fall 2007 and Belle Glade fall 2007) (Table 1). Differences in moths per night among traps were determined using a mixed analysis of variance (ANOVA) test (PROC MIXED, SAS 9.2, SAS Institute 2008) with Log_{+1} transformed data and means were separated using the Least Square Means Test. Differences between host strain captures were determined using the Exact χ^2 Test (PROC FREQ, SAS 9.2, SAS Institute 2008).

Table 2. Number per trap per night (mean \pm SEM), and host strain of male fall armyworm moths attracted to a commercial pheromone lure, corn strain (CS) females, or rice strain (RS) females in several locations in Florida

Location	Lure	Total collected	No. per trap/night	Total analyzed	No. corn strain males (%)	No. rice strain males (%)	<i>P</i> value ^a
Hague-Sept. 2005	CS-females	365	16.2 \pm 3.4b ^b	129	41 (31.8)	88 (68.2)	<0.0001
	RS-females	88	2.6 \pm 1.3c	65	3 (4.6)	62 (95.4)	<0.0001
	Pheromone	2,009	59.9 \pm 11.0a	125	55 (44.0)	70 (56.0)	0.2103
Hague-Oct. 2005	CS-females	100	5.3 \pm 2.0b	81	36 (44.4)	45 (55.6)	0.3742
	RS-females	55	2.0 \pm 0.7b	46	19 (41.3)	27 (58.7)	0.3020
	Pheromone	406	24.8 \pm 5.9a	128	88 (68.75)	40 (31.25)	<0.0001
Homestead 2005	CS-females	178	14.8 \pm 3.9a	150	30 (20.0)	120 (80.0)	<0.0001
	RS-females	49	4.1 \pm 1.5b	56	2 (3.6)	54 (96.4)	<0.0001
	Pheromone	136	12.4 \pm 2.7a	118	54 (45.7)	64 (54.2)	0.4075
South Florida-spring	CS-females	128	6.4 \pm 2.3b	48	26 (54.2)	22 (45.8)	0.6655
	RS-females	97	4.9 \pm 1.7b	85	35 (41.2)	50 (58.8)	0.1284
	Pheromone	568	71.0 \pm 12.4a	112	87 (77.7)	25 (22.3)	<0.0001
Combined	CS-females	771	10.1 \pm 1.5b	408	133 (32.6)	275 (67.4)	<0.0001
	RS-females	289	3.4 \pm 0.7c	252	59 (23.4)	193 (76.6)	<0.0001
	Pheromone	3,119	40.0 \pm 5.3a	483	284 (58.8)	199 (41.2)	<0.0001
South Florida-fall	CS-females	125	6.9 \pm 3.6a	37	7 (18.9)	30 (81.1)	<0.0001
	RS-females	111	9.3 \pm 3.7a	33	7 (21.2)	26 (78.8)	0.0013
	Pheromone	15	3.8 \pm 0.5a	—	—	—	—
Williston 2009	CS-females	2,160	179.8 \pm 49.1a	225	149 (66.2)	76 (33.8)	<0.0001
	RS-females	—	—	—	—	—	—
	Pheromone	336	38.8 \pm 16.1b	118	92 (78.0)	26 (22.0)	<0.0001

^a Probability that the percentage of corn or rice strain is significantly different from 50% (Exact χ^2 Test).

^b Means within a location followed by the same letter are not significantly different at $\alpha = 0.05$ (Least Square Means Test); "combined" does not include numbers from south Florida fall or Williston 2009.

Results

Moths per Trap. Over 6,900 male moths were captured in trials conducted in Florida during a 5-yr period. In the four trials where comparisons were made among the three lure types, the 3-component pheromone lure on average captured four times more males than laboratory-reared corn strain females, which attracted almost three times as many moths as rice strain females (Table 2). Variation in trap capture was high, as female-baited traps attracted from 0 to 505 males per night.

Host Strain of Captured Males. The host strain of over 1,100 male fall armyworm moths was determined in four locations that compared pheromone-baited-, corn strain female baited-, and rice strain female-baited traps. Pheromone-baited traps at Hague attracted an equal percentage of corn and rice strain males in the September samples ($P = 0.2103$), but by October, significantly more corn strain males were collected ($P < 0.0001$) (Table 2). Corn strain females attracted a lower percentage of corn strain males in September, but attracted an equal percentage of both strains in the October samples. Rice strain females attracted an even lower percentage of corn strain males in September, but this percentage increased to over 40% corn strain males in the October collections that were similar to the percentage of rice strain males collected.

An equal percentage of both strains were found in samples from pheromone-baited traps at the Homestead site from November 2005 to January 2006 ($P = 0.4075$; Table 2). However, only 20 and 3.6% of males collected in female corn strain- and rice strain-baited traps were corn strain males, respectively. In 2006 and 2007, samples from Homestead (SSG and sweet corn) and Belle Glade (sweet corn and other crops) were combined for the May–June (spring) collections.

Traps baited with pheromone attracted a higher percentage of corn strain males, but equal numbers of both strains when traps were baited with females. When the results were combined across the four trials, almost 59% of the 483 males attracted to the pheromone lure were identified as corn strain. Both corn and rice strain females attracted a higher percentage of rice strain males than corn strain males (Table 2).

Two sites had pair-wise comparisons between corn and rice strain female baited-traps (south Florida fall, $n = 70$) and between corn strain female- and pheromone-baited traps (Williston 2009, $n = 343$). The south Florida fall locations showed that females only attracted $\approx 20\%$ corn strain males (Table 2). At the Williston 2009 site, males attracted to the pheromone traps in late summer were predominantly corn strain (Table 2). Corn strain females also attracted more corn strain than rice strain males. Even though rice strain females were placed in traps, they attracted <40 males throughout the sampling effort. Unfortunately strain identification of these males was not possible because the samples were lost.

Selection of Same-Strain Females. Another way to look at the results is to determine the destination of corn strain and rice strain males to female-baited traps. The data were combined for all sites except Williston 2009. For corn strain males, 140 of 206 or 68% were caught in traps baited with corn strain females (Fig. 1a). Over 80% of the corn strain males were caught in these traps in the Hague-September 2005, Hague-October 2005, and Homestead 2005 trials, while corn strain males in south Florida spring and south Florida fall failed to respond significantly to corn strain females. Rice strain males also favored corn strain females (58.2%, $n = 524$), but at a lower percentage. In fact, rice strain males responded

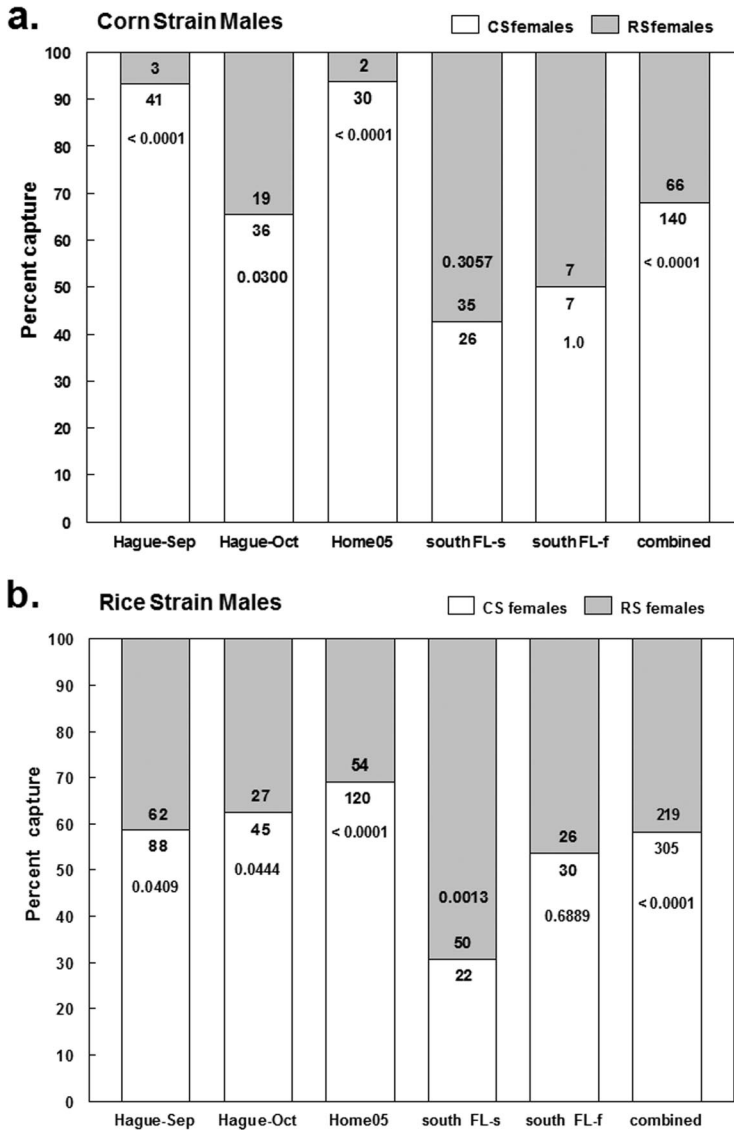


Fig. 1. Selection of corn (a) or rice (b) strain males to virgin corn strain (CS) or rice strain (RS) female-baited traps in several Florida locations (northern Florida: Hague-September 2005 and Hague-October 2005; southern Florida: Homestead 2005, south Florida spring, and south Florida fall). Numbers in each bar indicated number of males tested for strain identification. CS or RS significance is indicated by the probability value using an Exact χ^2 Test.

more to rice strain females only in the south Florida spring trial and were unbiased in their attraction in the south Florida fall trial (Fig. 1b).

Discussion

Our results showed that commercial pheromone-baited traps provide a much more efficient attractant than female-baited traps, as over four times more moths per night were collected. Females, at times, attracted large numbers of males, as shown by corn strain females in Williston 2009. However, the opposite result can occur as rice strain females in the same trial attracted males for only three of the 12 testing nights. The

condition of the females was critical to attracting males, as handling and weather conditions at night affected the female’s health and ability to release pheromone. By using different females each night and including multiple sites and seasons, the infrequent inability of females to attract males was minimized.

Studies comparing the host strains of pheromone-trapped fall armyworm adults with either nonpheromone adult sampling (such as black-light trapping) or larval sampling have not been completed. However, in the four trials where pheromone- and female-baited traps were compared, there were two outcomes. The first was that pheromone traps collected an even number of both strains while females attracted more rice

strain males (Hague-September 2005, Homestead 2005). The second outcome was that pheromone traps collected more corn strain males while the females attracted an even number of both strains (Hague-October 2005, South Florida spring). Both outcomes result in pheromone traps having a higher relative percentage of corn strain moths than female-baited traps. The pheromone blend identified by Tumlinson et al. (1986) and commercialized was undoubtedly isolated from corn strain females collected as larvae in corn and sorghum in Florida, since the research of those involved focused on chemical ecology in those habitats (Tingle and Mitchell 1975; Mitchell and Doolittle 1976; Mitchell et al. 1984, 1985). Therefore, it appears that at least the commercial lure used in our study is biased to attract corn strain males and underestimates rice strain population numbers relative to corn strain numbers.

Another interesting result of the study was that there was a seasonal difference in the host strain of the males that were attracted to the pheromone lure and to the females. This was most obvious in the trials from south Florida. Traps baited with pheromone in the summer attracted higher numbers of corn strain males (south Florida spring), but this difference was not significant by late fall (Homestead 2005), perhaps indicating an increase in rice strain numbers. Previous collections with pheromone-baited traps in southern Florida showed an asymmetric distribution, with fall-winter samples producing larger rice strain populations (generally over 80%) and late winter-spring collections resulting in a larger percentage of corn strain moths (as much as 50%) (Meagher and Nagoshi 2004, Nagoshi and Meagher 2004b). Females of both strains in the spring were unbiased in the males they attracted (south Florida spring), but by fall attracted significantly more rice strain males (Homestead 2005 and south Florida fall), again indicating an increase in the number of rice strain moths. This pattern was not clear in traps placed in northern Florida.

A closer look at corn and rice strain males separately in the southern Florida trials also shows a seasonal difference (Fig. 1). Corn strain males in the spring were found in equal numbers between female-baited traps (south Florida spring), but corn strain males in the fall responded more to corn strain females (Homestead 2005). Rice strain males appeared to change their response to females, as males in the spring were more attracted to rice strain females (south Florida spring) while males in the fall were either found in higher numbers in corn strain female traps (Homestead 2005) or were equally distributed between strain female traps (south Florida fall).

The only other study that documented attraction of males to corn or rice strain females was completed in Louisiana (Pashley et al. 1992). Results from their study were reported in two ways. First, the host strain of all males attracted to either strain female was reported. In this case, females of both strains attracted large numbers of rice strain males, in fact, 85.3 and 94.2% of males attracted to either corn or rice strain females, respectively, were rice strain males. The females that we used also attracted more rice strain

males, but our percentages were not as high and our results had seasonal variation (Table 2).

Pashley et al. (1992) also reported to which female-baited trap corn or rice strain males were collected. This analysis is not influenced by the large number of rice strain males attracted to the traps and only determines where a particular strain male was found. The results showed a slight but significant bias for males to be attracted to same-strain females (64.9% of corn strain and 60.1% of rice strain males were captured in same-strain female baited traps). Our results showed that three of the five trials tested had high percentages of corn strain males attracted to corn strain females. However, rice strain males were also captured at a higher percentage in corn strain female-baited traps in these same trials (Fig. 1b). Overall, a higher percentage of males of both strains were captured in corn strain female-baited traps.

Pheromone differences between strains have been proposed, but the reports do not agree on which component(s) are different. Females used in the two experiments were either from Florida (Groot et al. 2008) or Louisiana (Lima and McNeil 2009), which could be an important detail because our research suggests that populations migrating to Louisiana most likely arrive from Texas (Nagoshi et al. 2007, Nagoshi et al. 2008). It is not clear whether the Pashley et al. (1992) field study used Louisiana or Florida females, and although our corn strain females were from Florida, our rice strain females were from Florida and Mississippi (Mississippi receives Texas migrants; Nagoshi et al. 2008). Therefore, there still is a question as to the importance of natal origin in describing pheromone differences between strains.

Attraction of males to corresponding-strain females does not appear to be a premating mechanism that results in assortative mating between corn and rice strains. However, our study only looked at attraction of males and not to potential mating. In laboratory studies Pashley et al. (1992) found that females chose like-strain males $\approx 85\%$ of the time, and Schöfl et al. (2009, 2011) found males to be less restricted than females in regard to the timing of scotophase activity and mate preferences. Clearly other pre- or postmating mechanisms are important for the maintenance of host strains in *S. frugiperda*.

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