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Differential Attraction of *Heliothis subflexa* Males to Synthetic Pheromone Lures in Eastern US and Western Mexico

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Abstract The mate attraction signal of *Heliothis subflexa* (Hs) females consists of a multicomponent sex pheromone blend. In this study, we assessed the intraspecific importance of three groups of compounds found in Hs pheromone glands: three acetate esters (Z7-16:OAc, Z9-16:OAc, and Z11-16:OAc), two 14-carbon aldehydes (14:Ald and Z9-14:Ald), and one 16-carbon alcohol (Z11-16:OH). Because the relative importance of pheromone components may vary in different regions, we conducted experiments in Eastern US (North Carolina) and Western Mexico (Jalisco). Our experiments in Eastern US showed that when the acetates were omitted from a 7-component blend in rubber septa, fewer males were caught in cone traps. Subsequent experiments conducted both in Eastern US and Western Mexico indicated that the addition of Z9-16:OAc alone does not increase attraction of male Hs, while Z11-16:OAc does. The Hs male response to Z7-16:OAc differed between the two regions. In Eastern US, significantly more males were attracted to a minimal three-component blend to which Z7-16:OAc was added, but this was not the case in Western Mexico. The two 14-carbon aldehydes also showed differential attraction between the two regions. 14:Ald and Z9-14:Ald appeared not to play any role in the sexual communication of Hs in Eastern US, but reduced trap catches in Western Mexico. The alcohol Z11-16:OH was tested in two concurrent dose–response studies with Hs males in Western Mexico, one using a minimal blend and one using a complete blend. The minimal three-component blend provided a more discriminating tool for delineating dose–response effects of Z11-16:OH than the seven-component blend. In the minimal blend, the optimal dose of Z11-16:OH was 1%, while in the complete blend similar numbers of males were caught when the alcohol ranged from 1 to 25%.

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Introduction

The evolution of premating signals is generally shaped by both intra- and interspecific selection. In moths, the predominant premating signals consist of multicomponent sex pheromone blends produced by females to which only conspecific males are attracted. To delineate the direction and magnitude of selection exerted on multicomponent pheromone blends, it is important to define the role of each constituent. The behavioral significance of pheromone compounds may vary geographically, and geographic variation in moth pheromone blends has been found in several species (e.g., Klun and Cooperators, 1975; Cardé et al., 1977; Guerin et al., 1984; Gemeno et al., 2000; McElfresh and Millar, 1999, 2001; Gries et al., 2001; El-Sayed et al., 2003). Such variation may be due to drift or to communication interference between closely related species in areas of sympatry. When closely related species with similar pheromone blends cooccur, kairomonal effects of specific compounds in a blend that inhibit attraction of closely related males may be important (Cardé et al., 1977; Löfstedt, 1990, 1993; Löfstedt et al., 1991; Butlin, 1995; Gries et al., 1996; Potting et al., 1999).

Heliothis subflexa (Guenée 1852) (Hs) (Lepidoptera: Noctuidae) is a New World specialist herbivore, and as it only feeds on species in the genus *Physalis* (McElvare 1941; Sheck and Gould, 1995, 1996) its occurrence is limited to habitats where these plants occur. In the US, *Physalis* species (e.g., ground cherry) are weedy plants, growing in disturbed habitats along roadsides and in agricultural fields. However, one species, tomatillo (*Physalis philadelphica* Lam.), is grown commercially in almost every state in Mexico. Thus, Hs appears to be patchily distributed in the US, while it is abundant in Mexico.

The intraspecific importance of each compound in Hs pheromone glands has not been firmly established (Table 1). Some of these compounds may merely be by-products of the pheromone biosynthetic pathway (Teal and Tumlinson, 1987; Jurenka and Roelofs, 1993; Jurenka, 1996, 2004) but most have not been tested systematically. For example, none of the three acetate esters has been tested separately, and research to date has generated contrasting results. Teal et al. (1981) found an increase in trap catch when all three acetates were added to a binary blend of Z11-16:Ald and Z9-16:Ald, whereas Vickers (2002) did not find a change in attraction in a wind tunnel when Z11-16:OAc was added to a three-component blend of Z11-16:Ald, Z9-16:Ald, and Z11-16:OH. We recently found a significant positive relationship between the relative percentage of acetates (sum of Z7-16:OAc, Z9-16:OAc, and Z11-16:OAc) in the pheromone glands of Hs females and the number of males attracted to them in the field (Groot et al., 2006). However, this relationship requires confirmation with synthetic blends, and it remains to be determined whether all three acetates are (equally) important.

At least three heliothine species are sympatric with Hs in parts of North America: the tobacco budworm *Heliothis virescens* (Hv) (Fabricius 1777), the corn earworm *Helicoverpa zea* (Hz) (Boddie), and *Heliothis phloxiphaga* (Hp) Grote & Robinson. Hv and Hz are both highly polyphagous species and are important agricultural pests (Laster, 1972; Sparks et al., 1979; Hartstack et al., 1979; Lopez et al., 1994; Parajulee et al., 2004). Hp is an innocuous oligophagous species feeding on asters and *Castilleja indivisa* (Texas

Table 1 Sex pheromone compounds of *H. subflexa* and their inferred role from previously published laboratory or field bioassays

	Gland Content	Volatile Blend	Tested
14:Ald	2, 7		2
Z9-14:Ald	2, 7		2
16:Ald	1, 2, 4, 7	4	1, 2, 3(−)
Z7/9-16:Ald[a]	1, 2, 4, 7	4	1(+), 2, 3, 5(+), 6(+)
Z11-16:Ald	1, 2, 4, 7	4	1(+), 2, 3, 5, 6(+)
16:OAc	2		2
Z7-16:OAc	1, 2, 4, 7	4	1(+), 2, 3
Z9-16:OAc	1, 2, 4, 7	4	1(+), 2, 3
Z11-16:OAc	1, 2, 4, 7	4	1(+), 2, 3, 5(0), 6(0)
Z9-16:OH	4	4	1(−), 3(0)
Z11-16:OH	1, 2, 4, 7	4	1(−), 2, 3, 5(+)

[a] Z7-16:Ald and Z9-16:Ald could not be separated on GC in some studies.

+: increased attraction, - : decreased attraction, 0 : did not affect attraction, no sign: only tested as part of a whole blend, thus a specific function cannot be inferred.

1 Teal et al., 1981 (tested different combinations of blends; found a significant decrease in attraction when 48% Z9-16:OH and 40% Z11-6:OH (relative to Z11-16:Ald) were added to a five-component blend); *2* Klun et al., 1982 (in the field no males were caught in traps with the two-component blend (Z11-16:Ald and Z9-16:Ald; these were tested in different ratios), while the 11-component blend caught males); *3* Heath et al., 1990 (varied the percentage of Z11-16:OH; attraction of Hs males was increased when 0.9–3.5% was released from the septa, but decreased when release rate was higher); *4* Heath et al., 1991; *5* Vickers and Baker, 1997; *6* Vickers, 2002; *7* Groot et al., 2004

paintbrush) (Eger et al., 1982), and it occurs only in the western part of North America (Forbes, 1954; Eger et al., 1982). Females of all four species produce overlapping multicomponent sex pheromone blends (Table 2), so that interspecific cross-attraction may occur at low frequencies (e.g., Chapin et al., 1997; Groot et al., unpublished research). Recently, we found that Hv can exert an impressive selection force on the production of acetate esters in Hs females (Groot et al., 2006).

In this study, we concentrated on three specific questions that emerge from studies on Hs pheromones: (a) What is the intraspecific role of each of the acetate esters found in the pheromone glands of Hs females? (b) Do 14:Ald and Z9-14:Ald, which are present in the Hs pheromone gland, play a role in the attraction of Hs males? (c) Are Hs males differentially attracted to blends with varying amounts of Z11-16:OH and does this response change when tested in a complete blend versus a minimal blend?

In the context of evolution of pheromone blend variants, we hypothesized that the value of pheromone components may differ regionally in response to interspecific interactions with species that have overlapping chemical communication channels. Thus, we reasoned that if the importance of the three acetate esters, 14:Ald, Z9-14:Ald, and Z11-16:OH, in the pheromone communication of Hs depends on the abundance of other heliothine moths and the probability of communication interference, then the following regional differences may be predicted. In areas where Hz and Hv are abundant (North America), Hs males are more likely to be attracted to blends containing the acetates and/or Z11-16:OH because their presence is indicative of, and unique to, the volatile blend of Hs females (Heath et al., 1991). Moreover, both the acetates and the alcohol will be important to antagonize attraction of Hv and Hz males (Hartstack et al., 1980; Vetter and Baker, 1983; Vickers and Baker, 1997; Fadamiro and Baker, 1997; Quero and Baker, 1999). In contrast, Hs males

Table 2 Behaviorally tested and confirmed sex pheromone components of four heliothine species in North America

Compounds found in Hs Glands	*H. subflexa*[1]	*H. virescens*[2]	*Helicoverpa zea*[3]	*H. phloxiphaga*[4]
14:Ald				
Z9-14:Ald		+++		
16:Ald			++	
Z7-16:Ald			++	
Z9-16:Ald	+++		++	++
Z11-16:Ald	++++	++++	++++	++++
16:OAc				
Z7-16:OAc				
Z9-16:OAc				
Z11-16:OAc		−	−	
Z9-16:OH	0			
Z11-16:OH	+++	−	−	+++

[1] Teal et al. (1981); Klun et al. (1982); Heath et al. (1990, 1991); Vickers and Baker (1997); Vickers (2002)

[2] Roelofs et al. (1974); Klun et al. (1979, 1980a); Tumlinson et al. (1975, 1982); Pope et al. (1982); Vetter and Baker (1983)

[3] Klun et al. (1979, 1980b); Vetter and Baker (1984); Pope et al. (1984); Vickers et al. (1991); Fadamiro and Baker (1997); Fadamiro et al. (1999); Quero and Baker (1999); Quero et al. (2001)

[4] Raina et al. (1986)

+: Increased attraction, −: decreased attraction, 0: did not affect attraction, ++++: primary sex pheromone component, +++: secondary sex pheromone component, ++ minor sex pheromone component

should be less attracted to blends containing 14:Ald and Z9-14:Ald in areas with large Hv populations because they risk the possibility of being attracted to Hv females that produce these two compounds in higher amounts (Roelofs et al., 1974; Tumlinson et al., 1975, 1982; Klun et al., 1979, 1980a; Teal et al., 1986).

Methods and Materials

Pheromone Lures Pheromone components were obtained from *PHERO*BANK (Wageningen, The Netherlands), Shin-Etsu Chemical (Tokyo, Japan) and Bedoukian Research (Danbury, CT, USA). The following synthetic compounds were tested (% purity by GC indicated): Z11-16:Ald (98.0%; 16:Ald was the largest contaminant at 0.20%), 14:Ald (>98%), Z9-14:Ald (95.5%), 16:Ald (>98%), Z7-16:Ald (98.5%), Z9-16:Ald (96.6%), Z7-16:OAc (97.8%), Z9-16:OAc (99.2%), Z11-16:OAc (95%), and Z11-16:OH (95%).

Compounds were dissolved (*w*/*v*) in dichloromethane (2003) or *n*-hexane (2004 and 2005). Treatment blends were made relative to the primary component, Z11-16:Ald, which was loaded onto rubber septa at 100 μg (2003) or 300 μg (2004 and 2005). The ratios of all other compounds were based on Heath et al. (1991), who determined the release ratios of each of the *H. subflexa* pheromone components from septa similar to those used in this study. BHT was added at 1% (w/*v*) to all solutions, and ratios were confirmed by GC-FID on a DB-WAXETR (extended temperature range) column (30 m×0.25 mm×0.5 μm), which adequately separated Z7-16:Ald and Z9-16:Ald.

Rubber septa (#1780J07, Thomas Scientific, Philadelphia, PA, USA) were first ultrasonicated for 6 hr in hexane, then extracted ×3 with hexane, and air-dried in a fume

hood for at least 48 hr before loading. Each septum was first loaded with 100 µl of one of the treatment solutions, air-dried, then 100 µl of solvent were added to the septum well, air-dried again, and then stored in glass vials at −20°C until used. The septa were hung on a metal paper clip attached to a metal wire with a binder clip at each end, so that they could be positioned in the center of the trap opening without touching any trap surface. All septa were replaced at the same time, every 1–2 wk.

Field Sites Three different field sites were used in Eastern US: the North Carolina State University field station in Clayton, NC (35° 39′ 58″ N, 78° 30′ 36″ W), a commercial soybean field site in Smithfield, NC, USA (35° 30′ 42″ N, 78° 21′ 08″ W, ±20 km south of Clayton), and a commercial tomatillo field site in Oxford, NC (36° 13′ 12″ N, 78° 41′ 16″ W), ~100 km north of Clayton). In Western Mexico, commercial tomatillo fields in the state of Jalisco at Chamela (19° 31′ 49″ N, 105° 03′ 47″ W), Hidalgo (19° 21′ 37″ N, 104° 52′ 28″ W, ~25 km south of Chamela), and Autlán (19° 46′ 03″ N, 104° 20′ 59″ W, ~100 km northeast of Chamela) were used. Table 3 provides a summary of the site(s) used for each experiment.

Species-Specificity To determine species-specificity of the Hs and Hv blends, the following treatments were compared concurrently (Table 4): (1) the complete 7-component Hs blend (HsC treatment), based on Heath et al. (1990, 1991), (2) HsC minus the three acetate esters (HsC minAcs treatment), (3) the complete 7-component Hv blend (HvC treatment), based on Teal et al. (1986) and Heath et al. (1991), (4) HvC plus the three acetate esters (HvC+ Acs treatment), and (5) the control, in which rubber septa were loaded only with solvent and 1% BHT. For all septum loadings, the ratios of all compounds, except Z11-16:OH, were rounded off to the nearest 5% from the ratios reported by Heath et al. (1990). This experiment was conducted twice in both 2003 and 2004 (Table 3). The cotton and soybean

Table 3 Locations and dates of different experiments conducted on the sex pheromones of *H. subflexa* in Eastern US and Western Mexico

Test	Eastern US	Western Mexico (all *Physalis*)
Species-specificity	Clayton, NC: 28 Jul–18 Sep, 2003 (cotton) 30 Jul–31 Aug, 2004 (cotton) 16 Sep–6 Oct, 2004 (soybean) Smithfield, NC: 30 Sep–21 Oct, 2003 (soybean)	
Role of each acetate	Clayton, NC: 27 Jul–29 Aug, 2005 (cotton, *Physalis*)	Autlan, Jalisco 18 Oct–25 Oct, 2005 Hidalgo, Jalisco 17 Nov–23 Nov, 2005 21 Nov–24 Nov, 2005
Role of 14:Ald and Z9-14:Ald	Clayton, NC 1 Jul–22 Jul, 2005 (*Physalis*) Oxford, NC 12 Sep–28 Sep, 2005 (*Physalis*)	Hidalgo, Jalisco 29 Oct–3 Nov, 2005
Dose–response of Z11-16:OH		Hidalgo, Jalisco 3 Nov–8 Nov, 2005 Chamela, Jalisco 3 Nov–8 Nov, 2005

Table 4 Attraction of *H. subflexa* males in Eastern us to different synthetic blends to determine species-specificity

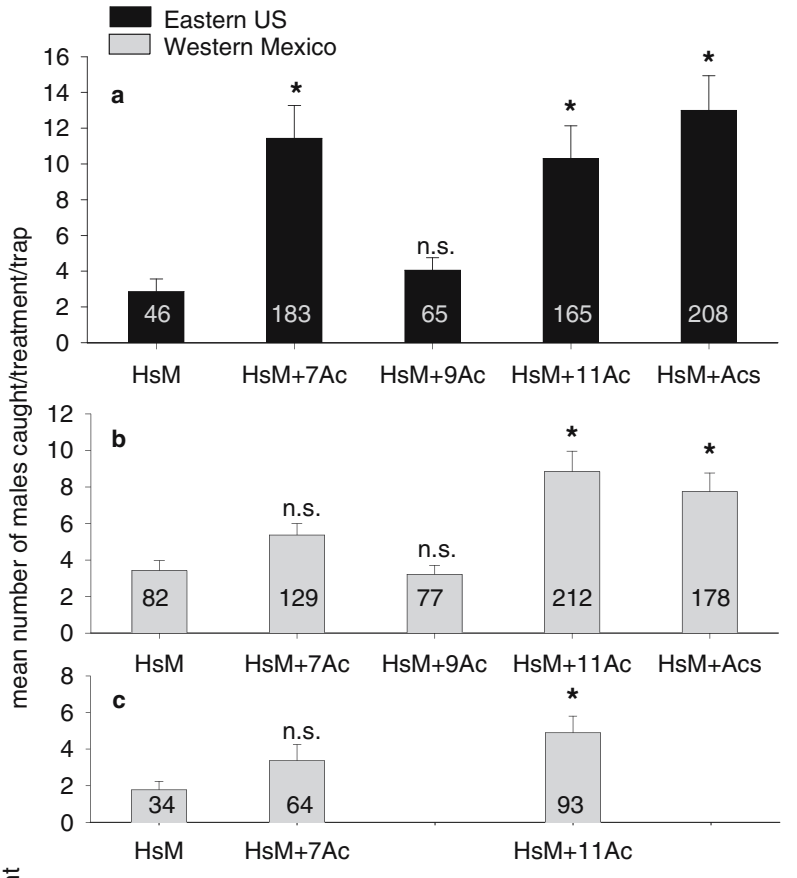
Treatment Name	Treatment Composition										Hs Males Caught Per Trap (mean± SEM)		Total No. of Males Caught
	14: Ald	Z9-14: Ald	16: Ald	Z7-16: Ald	Z9-16: Ald	Z11-16: Ald	Z7-16: OAc	Z9-16: OAc	Z11-16: OAc	Z11-16: OH			
HsC			5		50	100	5	10	20	10	0.49±0.088	a	45
HsC minAcs			5		50	100				10	0.29±0.083	b	26
HvC	5	5	10	2	2	100				1	0	–	0
HvC+Acs	5	5	10	2	2	100	5	10	20	1	0.022±0.022	c	2
Control											0.022±0.015	–	2

The experiment was conducted twice in 2003 and twice in 2004 (Table 3). Statistical analysis showed a significant date effect but not a significant year effect; therefore, we combined all data for further analysis. The treatment names are explained in the text, while their composition is shown in the table (the numbers refer to the percentages of each compound relative to Z11-16:Ald). Different letters indicate significant differences in mean number of males caught (ANOVA, Tukey's adjustment for multiple comparisons, $P < 0.05$). HvC treatment was not included in the analysis because no Hs males were caught in traps baited with this blend. The control treatment was omitted from the analysis because the specific question was whether the attraction of the treatments differed from each other.

fields in Clayton were about 100 m apart, and about 150 m from a field in which *Physalis* species had been planted to attract and generate an Hs population. Hartstack cloth traps (Hartstack et al., 1979) were arranged 15–20 m apart in a completely randomized Latin square design consisting of three replicates (rows), for a total of 15 traps in each field. These traps consist of a conical base leading to a removable top in which males are trapped. The five treatments were rotated every 3–4 d, the tops were replaced and males caught in the traps during 3–4 nights were sorted by species under a microscope.

Role of Each of the Acetate Esters To specifically determine whether Z7-16:OAc, Z9-16:OAc, or Z11-16:OAc are important in the attraction of conspecific males, an experiment consisting of five treatments was conducted in 2005 (see Fig. 1): a treatment consisting of the primary component Z11-16:Ald and the two secondary sex pheromone components Z9-16:Ald and Z11-16:OH, considered the Hs minimal blend (HsM) (based on Vickers, 2002); three treatments in which each of the three acetate esters was added to HsM (HsM+7Ac, HsM+9Ac, HsM+11Ac), and one treatment in which all three acetates were added (HsM +Acs). This experiment was conducted both in Eastern US and Western Mexico to investigate possible geographic differences in response to the different treatments. In Eastern US, the experiment was conducted in a field planted with *P. angulata*, *P. pubescens*, *P. cordata*, and *P. heterophylla* at Clayton (Table 3). Two replicates per treatment were used and the treatments were rotated every 2–3 d. In Autlán, Western Mexico, the experiment

Fig. 1 The effect of adding three acetate esters, separately and in combination, to the minimal blend on the attractiveness of lures to male *Heliothis subflexa* in Eastern United States and Mexico. A significant increase in number of Hs males caught is indicated by asterisks in a, b, and c. Asterisks in d indicate a significant difference between Eastern US and Western Mexico in the elevated attraction of Hs males compared to the minimal blend (i.e., the addition of Z7-16:OAc and the three acetate esters resulted in a significantly higher increase in attraction in Eastern US than in Western Mexico). ns represents "not significant", the error bars show +SEM, and numbers within bars indicate total number of Hs males caught. Each blend is represented at the bottom of the figure by percentages of each compound relative to Z11-16:Ald



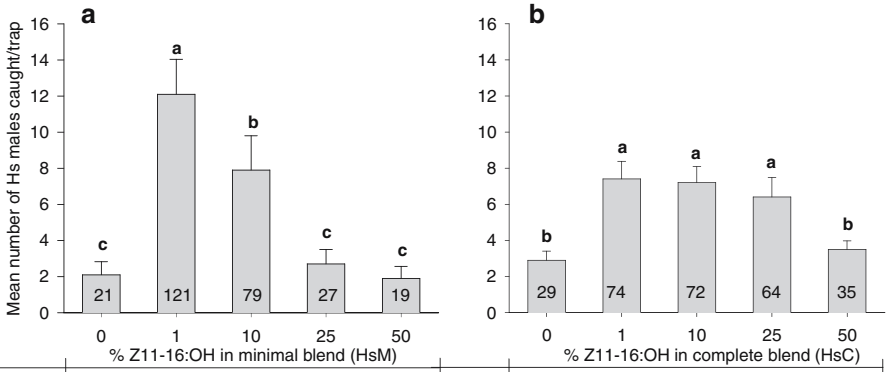
	HsM	HsM+7Ac	HsM+9Ac	HsM+11Ac	HsM+Acs
14:Ald					
Z9-14:Ald					
16:Ald					
Z7-16:Ald					
Z9-16:Ald	50	50	50	50	50
Z11-16:Ald	100	100	100	100	100
Z7-16:OAc		5			5
Z9-16:OAc			10		10
Z11-16:OAc				20	20
Z11-16:OH	10	10	10	10	10

consisted of three replicates per treatment, rotating the treatments every day. In addition to the complete experiment, an additional experiment was conducted in Hidalgo, Western Mexico, where HsM+7Ac and HsM+11Ac were compared with HsM. This experiment was replicated twice (Table 3), once with two replicates per treatment and once with three replicates per treatment, rotated every day.

Role of 14:Ald and Z9-14:Ald To determine whether 14:Ald and Z9-14:Ald are important in the attraction of Hs males, an experiment was conducted in 2005 comparing HsC and HsC with 14:Ald and Z9-14:Ald added (HsC+14Alds). This experiment was conducted at Clayton, using five replicates per treatment that were rotated every 3–4 d, eight times in total, and in Oxford, where both treatments were rotated every 2 d. This experiment was also conducted in Hidalgo, Western Mexico, where both treatments were replicated four times and rotated every 1–2 d, four times in total.

Dose–Response Test of Z11-16:OH To determine the effect of Z11-16:OH at different doses, two dose–response experiments were conducted concurrently. In Chamela, Z11-16:OH was varied from 0 to 50% in the minimal blend (HsM), while in Hidalgo the same doses were tested when added to the complete blend, HsC (see Fig. 2). In both experiments, two replicates per treatment were used and all treatments were rotated daily over 5 d.

Statistical Analyses The number of males caught per treatment in each experiment were square-root transformed, when needed, to normalize the variance. To determine the role of each of the acetate esters, a mixed model ANOVA was carried out using proc GLM in SAS



14:Ald										
Z9-14:Ald										
16:Ald						5	5	5	5	5
Z7-16:Ald										
Z9-16:Ald	50	50	50	50	50	50	50	50	50	50
Z11-16:Ald	100	100	100	100	100	100	100	100	100	100
Z7-16:OAc						5	5	5	5	5
Z9-16:OAc						10	10	10	10	10
Z11-16:OAc						20	20	20	20	20
Z11-16:OH	0	1	10	25	50	0	1	10	25	50

Fig. 2 Dose–response experiment with Z11-16:OH in Hidalgo and Chamela, Western Mexico. a: Z11-16:OH added to the minimal three-component blend, b: Z11-16:OH added to the complete seven-component blend. Different letters within each graph indicate significant differences in mean number of males caught and the error bars show +SEM. Numbers within bars indicate total number of males caught. Each blend is represented by percentages of each compound relative to Z11-16:Ald

9.1 with "treatment" and "day" as fixed effects, and "replicate" and "replicate by treatment" as random effects. To determine whether the addition of each of the acetate esters to HsM increased attraction, the number of Hs males captured in each treatment was compared to the number attracted to HsM alone, using Dunnett's procedure with "replicate by treatment" as the error term.

Geographic differences in attraction between Eastern US and Western Mexico to the acetate treatments were compared after normalizing the trap catches relative to those in HsM baited traps for each region. Subsequently, the increase in attraction to each blend in Eastern US was compared to the increase in attraction to each blend in Western Mexico, using proc MIXED in SAS 9.1, where "treatment", "region", and "day" were considered fixed factors, and "replicate", "trap position" and "treatment by trap position" per site were considered random effects.

Differences in attraction of (a) the species-specific blends, (b) the HsC and HsC+14Alds, and (c) the alcohol treatments, were compared using ANOVA (proc MIXED in SAS 9.1). Within each experiment, the numbers of males attracted to the different treatments were compared, where "treatment" and "day" were considered fixed factors, and "replicate" and "trap position" were regarded as random. The treatment means were then separated using Tukey's adjustment for multiple comparisons.

Results

Species-Specificity The HsC blend attracted significantly more Hs males than the HsC minAcs blend, while the Hv blend, with or without the acetates, did not differ from the control (Table 4).

Role of Each of the Acetate Esters Significantly more Hs males were caught in traps with HsM+11Ac and HsM+Acs than in traps with HsM alone in both regions, while the addition Z9-16:OAc did not increase attraction (Fig. 1a, b). In the case of HsM+7Ac, trap catches only increased in Eastern US (Fig. 1a). The lack of attractiveness of Z7-16:OAc for Mexican Hs males was confirmed in independent trials (Fig. 1b, c).

After normalizing the relative trap catches to that of the minimal blend, there were significant effects of region, treatment, day and their interactions (see Table 5), and there were contrasts between Eastern US and Western Mexico when a) HsM+7Ac, and b) HsM+Acs were compared (Fig. 1d). In both cases, more Hs males were caught in Eastern US than in Western Mexico.

Table 5 Type 3 tests of fixed effects when *H. subflexa* trap catches of the five acetate treatments were compared between Eastern US and Western Mexico

Effect	Degrees of Freedom	*F* value	Pr>*F*
Region	1	8.27	0.027
Treatment	4	33.26	<0.001
Region×treatment	4	5.69	<0.001
Day	7	5.92	<0.001
Region×day	7	10.13	<0.001
Treatment×day	28	1.68	0.031
Region×treatment×day	28	1.50	0.072

Role of 14:Ald and Z9-14:Ald In Eastern US, the addition of 14:Ald and Z9-14:Ald did not increase the attractiveness of the HsC blend (Table 6). However, in Western Mexico, more Hs males were caught in traps baited with the HsC (N=166) than those with HsC+14Alds (n=110, P=0.014).

Dose–Response of Z11-16:OH Trap catches of Hs males showed a nonmonotonic function in relation to increasing Z11-16:OH doses in both HsC and HsM blends, represented by parabolic, or inverted U-shaped curves (Fig. 2). Omission of the alcohol, as well as high doses of it, suppressed trap catch, whereas intermediate doses increased trap catch. However, the specific effective doses depended upon whether the alcohol was in the minimal or complete blend. Similar numbers of Hs males were caught with 1 to 25% Z11-16:OH in HsC, whereas significantly more males were only caught in traps baited with HsM and 1% Z11-16:OH (Fig. 2).

During these experiments, we also deployed pheromone traps in both Eastern US and Western Mexico to estimate the relative abundance of *H virescens*. A comparison of traps baited HvC and HsC in Eastern US showed that there were similar, or more, Hv males as Hs males caught in all 3 yr (Fig. 3). In contrast, in Western Mexico only three Hv males were captured in fields of commercially grown *Physalis*, compared with 785 Hs males.

Discussion

A thorough evaluation of the contribution to mating success of each pheromone compound found in the glands of *H. subflexa* females is critical to our goal of understanding the evolutionary diversification of heliothine moth pheromone signals (Groot et al., 2004, 2006; Sheck et al., 2006) In this study, we assessed the importance of several compounds whose roles in Hs sexual communication were equivocal.

The importance of pheromone compounds may differ geographically when their behavioral significance partly depends on the presence and/or abundance of closely related species that are attracted to similar pheromone blends. Of the common heliothines in

Table 6 Effects of 14:Ald and Z9-14:Ald on *H. subflexa* trap catches in Eastern US and Western Mexico

Treatment Name	Treatment Composition										Hs Males Caught Per Trap (mean±SEM)	
	14:Ald	Z9-14:Ald	16:Ald	Z7-16:Ald	Z9-16:Ald	Z11-16:Ald	Z7-16:OAc	Z9-16:OAc	Z11-16:OAc	Z11-16:OH	Eastern US	Western Mexico
HsC			5		50	100	5	10	20	10	3.63±0.46 a (N=232)	10.38±1.47 a (N=166)
HsC+14Alds	2	2	5		50	100	5	10	20	10	3.48±0.45 a (N=230)	6.88±0.78 b (N=110)

Different letters within region indicate significant differences in mean number of males caught (P<0.05). Each blend is represented by percentages of each compound (w/v) relative to Z11-16:Ald. HsC is the complete seven-component blend, and HsC+14Alds represents the addition of 14:Ald and Z9-14:Ald to the complete blend.

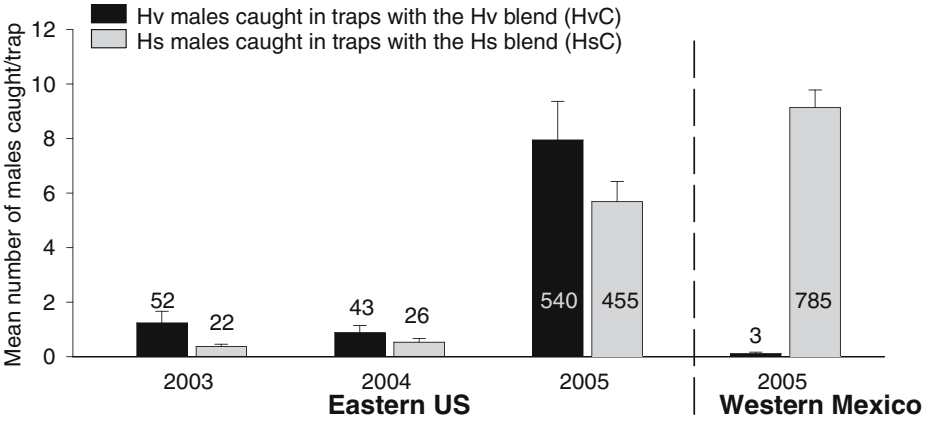


Fig. 3 Mean number of Hv and Hs males caught in traps with their respective pheromone lure during the trapping experiments conducted in Eastern US (years 2003–2005) and Western Mexico (2005). Numbers above/in bars indicate total number of males caught and the error bars show +SEM

Eastern US, Hv is the most closely related species to Hs (Mitter et al., 1993). Females of both species elicit interspecific precopulatory behavior from males in close proximity (Klun et al., 1982), and they can be hybridized in the lab, producing sterile male, and less fit fertile female, offspring (Laster, 1972; Sheck and Gould, 1995). However, males of these two species are rarely attracted to heterospecific females in the field (Groot et al., 2006). Trap catches in pheromone traps baited with the complete Hv blend confirm our supposition that Hv is much less abundant in Western Mexico than in Eastern US. Therefore, the chance of communication interference—that is, the likelihood of Hs females attracting heterospecific Hv males—is much greater in Eastern US than in Western Mexico.

Role of the Acetate Esters The species-specificity experiments conducted in Eastern US showed that one or more of the three acetate esters contribute to greater attraction of Hs males (Table 4). This experiment supports our finding that backcross Hs females who produce a very low amount of the acetate esters (<5% relative to all compounds present in the gland) were less attractive to Hs males than those with higher amounts (Groot et al., 2006). Notably, the context in which the acetates are presented is important as adding the three acetates to an HvC blend failed to attract more Hs males.

Of the three acetate esters emitted by calling Hs females, Z9-16:OAc does not appear to play a role in the long distance attraction of conspecific males in either Eastern US or Western Mexico. Hence, the presence of this compound in the Hs pheromone gland may just be a by-product of the biosynthetic pathway that produces the critical secondary pheromone component Z9-16:Ald, or may serve an antagonistic function to suppress attraction of other species, as has been found for Z11-16:OAc.

The addition of Z7-16:OAc to the HsM blend increased captures of Hs males in Eastern United States, whereas in Western Mexico HsM and HsM+7Ac gave similar results (Fig. 1). This geographic difference was even more evident when normalized trap catches were contrasted between the two regions (Fig. 1d).

The HsM+Acs lure was more attractive than HsM in both sites (Fig. 1a, b) but the addition of all three acetate esters resulted in a greater increase in Eastern US than in Western Mexico (Fig. 1d). This finding confirmed our prediction, based on our earlier

observations (Groot et al., 2006), that in Eastern US, where Hv and Hz are abundant, the acetates would not only function to enhance intraspecific communication but also to prevent the attraction of heterospecific males. Where the probability of communication interference with Hv and Hz is low, as in Western Mexico, selection pressure to produce acetates may be attenuated and Hs males may be more finely tuned to the other pheromone components (Z11-16:Ald, Z9-16:Ald, and Z11-16:OH). It will be interesting to investigate the interspecific interaction between Hs and Hp because their pheromone blends are more similar to each other than to Hv or Hz (Table 2), and one or more of these acetate esters might serve to reduce interspecific attraction of Hp males. However, Hp is an innocuous nonpest species and its population densities may be relatively low in areas where tomatillo is a major agricultural crop. In Western Mexico, no Hp males were caught in traps regardless of the Hs lure used in this study, suggesting that the species was not present at the time of our experiments.

Role of 14:Ald and Z9-14:Ald Both 14:Ald and Z9-14:Ald are found in the pheromone glands of Hv and Hs, and Z9-14:Ald is the critical secondary sex pheromone component of Hv (Table 2) (Roelofs et al., 1974; Tumlinson et al., 1975, 1982; Klun et al., 1979, 1980a, 1982; Teal et al., 1986; Groot et al., 2005). It is not known, however, whether they are released by calling Hs females, and their contribution to conspecific attraction had not been investigated. Vickers (2002) showed that 0.05–50% Z9-14:Ald (relative to Z11-16:Ald) failed to attract Hs males in a wind tunnel, but in his experimental design Z9-14:Ald replaced the critical secondary pheromone component Z9-16:Ald. Since Hv is abundant in Eastern US and scarce in Western Mexico, we expected blends containing 14:Ald and Z9-14:Ald to be less attractive to Hs males in the former site, if these compounds are used by Hs males to distinguish Hs from Hv blends. However, while their addition did not affect catches in the Eastern United States, they significantly reduced the attractiveness of lures in Western Mexico (Table 6). This suggests that these do not to function as pheromone components for the intraspecific attraction in either region, but it would be instructive to conduct dose-response studies with each of these compounds in HsM to rule out the possibility that they only function optimally at very precise release ratios.

Role of Z11-16:OH In Western Mexico, the response of Hs males to different doses of Z11-16:OH to the lure depended on whether it was added to the HsC or HsM blend (Fig. 2). In the minimal blend, 1% Z11-16:OH caught more Hs males than any other treatment, while in the complete blend a range of doses between 1 and 25% Z11-16:OH caught similar numbers of Hs males. These results suggest that males respond more precisely to component ratios when tested in a minimal blend in the field, whereas in a more complex blend, males respond to broader component ratios as there might be some redundancy of components (Linn and Roelofs, 1989). We, therefore, suggest that the importance of heliothine pheromone compounds may be better discerned when compounds are added singly to a minimal blend in dose–response studies, rather than when compounds are omitted from a complete blend. This approach will be used in future studies to elucidate the optimal percentage of Z11-16:OH needed to attract Hs males in Eastern US, as well as the role of other compounds, such as 16:Ald and Z7-16:Ald, that are present in Hs pheromone glands.

Our results confirm the importance of Z11-16:OH to Hs males, but differ from previously published data. Vickers (2002) found no difference in male responses when Z11-16:OH varied from 1 to 50% in HsM on filter papers in wind tunnel assays, but it is

possible that more replications would be needed to delineate subtle dose-response effects in no-choice wind-tunnel tests. In the field, Heath et al. (1990) reported significantly higher attraction of HsC to rubber septa when Z11-16:OH was loaded between 3 and 6%. The broader range we observed may be related to close-range male orientation. We used Hartstack traps that captured males before they reach the pheromone source, whereas Heath et al. (1990) used sticky and bucket traps, which require that males land close to the lure to be captured. The requirement for close-range orientation to sticky traps may produce sharper dose–response curves, even to the complete blend. In addition, the climatic conditions in Western Mexico at the time that we conducted our experiments may have been different from those when Heath et al. (1990) ran their experiments in Florida, which could differentially affect the release rates of compounds.

Geographic variation in the response of pest moths to sex pheromone blends has important practical implications. Pheromone traps are widely used to monitor pest moth populations with the catch data being used to determine if and when insecticide applications are needed. Geographic differences in male response have led to the development of region-specific pheromone lures for several other moth species (e.g., Klun and Cooperators, 1975; Arn et al., 1983; Hansson et al., 1990; Cork et al., 1992; Tóth et al., 1992; McElfresh and Millar, 1999, 2001; Gemeno et al., 2000; Gries et al., 2001; El-Sayed et al., 2003). Our finding of geographic variation in Hs male response indicates that the development of region-specific lures is an important consideration for heliothine moths as well. Monitoring the presence of Hs with pheromone traps can be a first step towards improved management of Hs populations, especially in tomatillo fields where it is an important pest. Understanding the evolution of pheromone communication in this context is critical because changes in population densities may result in shifts in both the pheromone blend and behavioral responses of males.

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