

Geographic variation in sexual communication in the cotton bollworm, *Helicoverpa armigera*

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Abstract

BACKGROUND: Geographic variation in male response to sex pheromone lures has been studied in the field in a number of moth species. However, only a few studies have investigated geographic variation in female calling and sex pheromone under field conditions. For an effective field implementation of sex pheromone lures, it is essential to know the local sex pheromone blend and local timing of sexual communication. We investigated the level and extent of geographic variation in the sexual communication of the important agricultural pest *Helicoverpa armigera* (Lepidoptera, Noctuidae) in three continents.

RESULTS: We found there is no genetic variation in the calling behavior of *H. armigera*. In the female sex pheromone, we found more between-population variation than within-population variation. In male response experiments, we found geographic variation as well. Strikingly, when adding the antagonistic compound Z11-16:OAc to the pheromone blend of *H. armigera*, significantly fewer males were caught in Australia and China, but not in Spain. This variation is likely not only due to local environmental conditions, such as photoperiod and temperature, but also to the presence of other closely related species with which communication interference may occur.

Conclusion: Finding geographic variation in both the female sexual signal and the male response in this pest calls for region-specific pheromone lures. Our study shows that the analysis of geographic variation in moth female sex pheromones as well as male responses is important for effectively monitoring pest species that occur around the globe.

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Keywords: sexual behavior; sex pheromone; communication interference; cotton bollworm

1 INTRODUCTION

To reduce the use of chemical insecticides against pests in agriculture, several environmentally friendly approaches have been developed in recent years.^{1–3} One effective method is the behavioral manipulation of pest species.⁴ For example, manipulation of sexual communication of insects has been widely used in integrated pest management, from monitoring to controlling pest populations through mate disruption.^{5–7} However, the effectiveness of behavioral manipulation methods can be greatly increased by taking into account behavioral and ecological variation of the pests, especially for pests with wide geographical distributions.⁸

Geographic variation in sexual communication of insects is a common phenomenon.^{9–13} For an effective field implementation, it is essential to know the local sex pheromone blend and local timings of sexual communication. Variation in timing of sexual behaviors in moths, such as female calling and pheromone release, has been extensively studied under laboratory conditions, showing that these sexual behaviors are affected by abiotic factors, such as photoperiod,^{14–16} temperature,¹⁷ relative humidity^{18,19} and wind speed.²⁰ Sexual behaviors in moths may also be affected by biotic and physiological factors, such as age,^{21–23} host plants,^{24,25} larval diet,²⁶ pupal period²⁷ and insecticides.^{28,29} In addition, variation in moth sexual communication may be caused through communication interference between closely

related species in areas of sympatry.^{13,30,31} Although moth sex pheromone signals are species-specific, closely related species often share common sex pheromone components and some components in the pheromone blend may play roles as antagonists to avoid heterospecific attraction.^{10,13,32}

Geographic variation in male response to pheromone lures has been studied in the field in a number of species.^{10,33–35} However, only a few studies have investigated geographic variation in

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female calling and sex pheromone release under natural conditions in the field, i.e. under local prevailing temperatures, photoperiodic conditions and the presence of other related species.^{36–38} Consequently, our understanding of how abiotic and biotic factors under natural conditions affect sexual communication in moths is still scarce, particularly under rapid environmental changes.³⁹

The cotton bollworm, *Helicoverpa armigera* (Hübner), is an important multivoltine pest, occurring throughout Africa, Europe, Asia and Oceania,⁴⁰ and has a long history of worldwide insecticide resistance to several chemicals.⁴¹ Recently, *H. armigera* has been found as an invasive species and causes significant economic losses in South and Central America^{42–44} and threatens to spread further.⁴⁵ The larvae are highly polyphagous and feed on a variety of host plants, including economically important crops, such as cotton, corn, soybean and tomato.⁴⁶ In addition, *H. armigera* has the ability to undergo facultative diapause and seasonal migration with long-distance dispersal.⁴⁷ Among *H. armigera* populations, geographic variation in host plant preference have been found,^{48,49} but as far as we know geographic variation in sexual communication has not been documented yet.

Application of sex pheromone has become increasingly important for integrated pest management of *H. armigera* throughout the world.^{6,50} In this study, we investigated the level and extent of geographic variation in the sexual communication of the moth *H. armigera*, combining field experiments under natural conditions and laboratory experiments. To determine geographic variation in the timing of female calling and sex pheromone production, and male attraction to different synthetic pheromone blends we investigated *H. armigera* populations in Spain, China and Australia. To determine the consistency of circadian rhythms of female sexual activities, we also assessed variation in the timing of female calling in the laboratory.

2 MATERIALS AND METHODS

2.1 Field locations

Field experiments were conducted between 2016 and 2017 during *H. armigera* flight seasons in three continents: Guadajira, Badajoz, Spain, which is a major processing tomato growing area; Dali County, Shaanxi, China, which is a cotton growing area; and Gatton, Brisbane, Australia, which is a mixed horticultural cropping area (Fig. 1(a) and Table 1).

2.2 Effects of larval diet (host plants) on sexual communication

To compare within-population variation to between-population (i.e. geographic) variation, at each field site we compared female calling and sex pheromone amount and composition from females that were reared as larvae on three different host plants per site in semi-outdoor conditions. At each field site, we collected 50–90 gravid *H. armigera* females with an entomological net. These field-collected females were placed singly in plastic beakers (473 mL; Solo, Lake Forest, IL, USA) provided with 10% sugar water and covered with a gauze cloth, on which the females oviposited eggs. After the eggs hatched, the larvae were placed individually into transparent plastic cups (37 mL; Solo) and supplied with fruits of local host plants, i.e. tomato, corn and pepper in Spain, and tomato, corn and cotton in China and Australia. The host plants were replaced every day until pupation. Pupae were checked daily for emerging adults, and these were sexed and placed separately into transparent cups (37 mL) containing wool

cotton soaked with 10% sugar water. Newly emerged virgin females were used in the calling observations and pheromone extractions at each field site.

2.3 Female calling behavior

To evaluate variation in the time of female calling behavior at each field site, the newly emerged virgin females were placed singly in clear transparent plastic beakers (473 mL) covered with a gauze cloth. We kept track on the host plants on which the females had been reared. The night of emergence was defined as age 0. The observations started with 1-day-old females and were repeated on successive nights. The age at which females initiated calling, as well as the duration of calling behavior, were observed every 30 min with a red light throughout the night, i.e. from sunset to sunrise. Female calling behavior was noted when the ovipositor with pheromone gland was clearly extruded from the abdomen. During the observational nights, fluctuations in temperature and relative humidity were recorded by a hygrothermograph (TFA Dostmann, Wertheim, Germany) at 1 h intervals.

To assess whether variation in female calling behavior was due to geographic differences, calling behavior was also determined in the laboratory. For these experiments, eggs and larvae of *H. armigera* in the fields were collected from the same three field sites in Spain, China and Australia in 2018, and shipped to the laboratory at the University of Amsterdam, where they were reared individually on artificial pinto bean diet in climate chambers [60% relative humidity (RH); 25 °C; 14 h light:10 h dark with lights off at 11 am]. Upon hatching, newly emerged adults were sexed and placed separately into transparent cups (37 mL) containing cotton wool soaked with 10% sugar water. In this study, 3- to 6-day old virgin females from each population were observed in the climate chamber in which the larvae were reared. These observations were conducted every 30 min with a red light throughout scotophase (10 h from 11 am to 9 pm) and repeated for two consecutive nights.

2.4 Female pheromone analysis

To compare within-population variation to between-population variation in the sex pheromone quantity and composition, female pheromone glands were extracted individually from the different groups of females that were reared on the different diets, after the females were used for calling observations in each region. Glands were extracted at 2-hour intervals throughout the night after all observation of calling. As the night in Spain lasted only 9 h, glands were extracted at four timepoints (1, 3, 5 and 7 h into the night). In China and Australia, the night lasted 10 and 11 h, respectively, so that in these regions glands were extracted at five timepoints (1, 3, 5, 7 and 9 h into the night). At each time point, glands were dissected with fine scissors and forceps, and deposited individually in conical vials. The conical vials, containing 200 ng pentadecane within 50 µL hexane as internal standard, had been prepared in advance at the laboratory in GC vials with Alu Crimp caps (11 mm) and spring inserts, which minimizes evaporation and allowed the extracts to be transported. After 30–40 min, the glands were removed from the solution with forceps, and the extracts were sealed and kept at –20 °C until shipping.

All pheromone samples were analyzed at the University of Amsterdam, in a HP7890 Gas Chromatograph (GC) with a 7683 automatic injector, as detailed in Groot *et al.*⁹ and summarized here. The hexane extracts were reduced to 2 µL under a gentle stream of N₂, after which each sample with 1 µL octane was injected into the GC. The sex pheromone peaks were identified

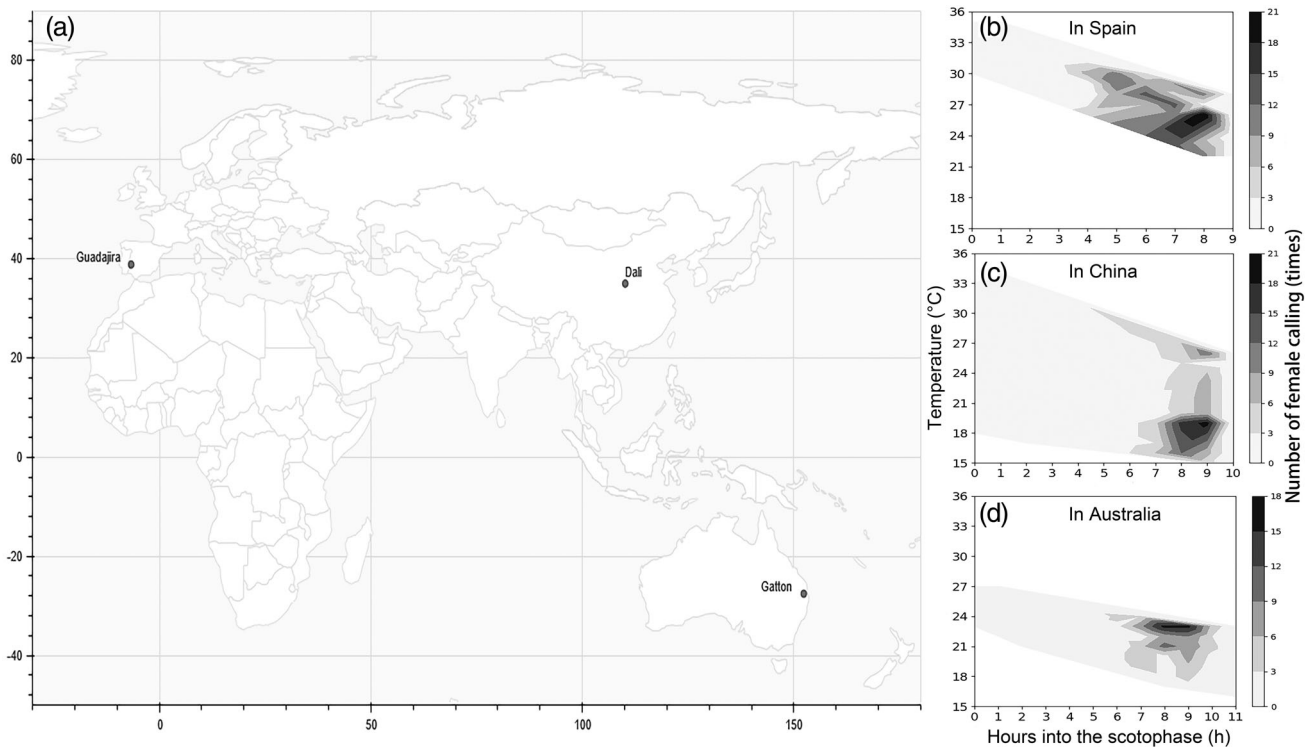


Figure 1. Field experiments conducted in different geographic regions. (a) Field locations. (b)–(d) Correlations between timing of female calling and temperature in the three different regions. Shaded areas showed the measured fluctuations of temperature during the observation nights.

Table 1. Geographic locations of *H. armigera* populations studied in the field

| Country | Location | Coordinates | Main crop | Date | Photoperiod |
|-----------|----------------------|-----------------------------------|-----------|---------------------|-------------|
| Spain | Guadajira, Badajoz | 38° 51' 08.8" N, 6° 40' 48.9" W | Tomato | June–August 2016 | 15 L:9D |
| China | Dali county, Shaanxi | 34° 45' 01.9" N, 110° 09' 56.1" E | Cotton | July–September 2017 | 14 L:10D |
| Australia | Gatton, Brisbane | 27° 32' 11.6" S, 152° 20' 16.3" E | Lucerne | January–March 2017 | 13 L:11D |

and integrated based on their retention times, which were compared to a synthetic pheromone blend of *H. armigera*, which was injected before and after each round of 30 injections. The amount of each pheromone component was calculated relative to the 200 ng of internal standard.

2.5 Male response experiments

To test geographic variation in male attraction to pheromone lures in different regions, two field trapping experiments, each with two to four replicates were conducted at each of the three sites in a complete randomized block design (Table 2). In experiment 1, the attraction of five synthetic pheromone blends was compared, which were prepared at Pherobank BV (Wijk bij Duurstede, the Netherlands). Each pheromone lure consisted of a red rubber septum that was loaded with 100 µL of hexane containing 5 mg of the major component Z11-16:Ald (100%), while the other compounds were loaded in amounts relative to Z11-16:Ald (see Table 2). In experiment 2, the effect of the antagonist pheromone compound Z11-16:OAc on *H. armigera* male attraction was tested. We focused on this antagonistic compound because it is part of the sex pheromone blend of the closely related species *H. assulta* that occurs sympatrically with *H. armigera* in China.⁵¹

For experiment 2, we prepared two synthetic pheromone lures in the laboratory. Each pheromone lure consisted of a red rubber septum that was loaded with 100 µL of hexane containing 300 µg of Z11-16:Ald (100%) and other compounds relative to 300 µg of Z11-16:Ald, so that one treatment consisted of 100% Z11-16:Ald and 5% Z9-16:Ald (which are the two critical sex pheromone components of *H. armigera* and to which we refer as the H.a blend), and another treatment consisted of 100% Z11-16:Ald and 5% Z9-16:Ald + 10% Z11-16:OAc (H.a + Z11-16:OAc blend) (Table 2).

All lures were hung in bucket traps (Pherobank BV) attached to a wooden pole and positioned at a height of approximately 1.5 m above the ground, and distributed at least 30 m apart in the field. The males caught in the traps were collected and counted every day. All the lure experiments were conducted in the field in each region (Table 1). For experiment 1, at all field sites two rubber septum lures per treatment at the same time were used and the treatments were rotated daily over five nights (ten replicates per treatment) to minimize possible position and odorant effects. For experiment 2, in Spain, four rubber septum lures per treatment at the same time were used and the treatments were rotated daily over two nights (eight replicates per treatment). In Australia, two rubber septum lures per treatment at the same time

Table 2. Compositions of pheromone lures used in the fields

| Experiment | Lures | Z11-16:Ald | Z9-16:Ald | Z7-16:Ald | Z9-14:Ald | Z11-16:OAc |
|------------|------------------|------------|-----------|-----------|-----------|------------|
| 1 | Blend1 | 100 | | | | |
| | Blend2 | 100 | 2.5 | 0.6 | | |
| | Blend3 | 100 | 1.4 | | 0.3 | |
| | Blend4 | 100 | 4 | | | |
| | Blend5 | 100 | 6 | | | |
| 2 | H.a | 100 | 5 | | | |
| | H.a + Z11-16:OAc | 100 | 5 | | | 10 |

In experiment 1, compound concentrations were as follows: 100% = 5 mg, 2.5% = 0.125 mg, 1.4% = 0.07 mg, 4% = 0.2 mg, 6% = 0.3 mg, 0.6% = 0.03 mg, 0.3% = 0.015 mg.
In experiment 2, compound concentrations were as follows: 100% = 300 µg, 5% = 15 µg, 10% = 30 µg.

were used and rotated daily over five nights (ten replicates per treatment). In China, three rubber septum lures per treatment at the same time were used and rotated daily over three nights (nine replicates per treatment).

2.6 Data analysis

All statistical analyses were performed in R software, version 3.4.1 (R Core Team, 2018). The effect of larval diets (host plants) on the calling behavior of females within each geographic region was tested using a generalized linear model (GLM) with a binomial distribution, where the percentage of calling was the response variable, time and larval diet were the independent variables. Difference in the age of female initial calling among the three populations was compared using a Kruskal–Wallis test, followed by Dunn's test for multiple comparisons. Under laboratory conditions, the calling behaviors of females among the three populations were compared using a GLM with a binomial distribution, where the percentage of calling was the response variable, and time and population were the independent variables. To compare the pheromone signal between females, we conducted the following analyses. Within each region, the effect of larval diets (host plants) on the total amount of pheromone was tested using a GLM with a negative binomial distribution. To assess geographic variation in the relative amounts of the compounds in the pheromone blend among the three populations, female pheromone data from the main calling time (i.e. the last two time points in each region) were $\log(x + 1)$ -transformed to stabilize the variance and then compared using a GLM with a negative binomial distribution, after which a MANOVA analysis was performed. Each compound among the three populations was compared with ANOVA, followed by Tukey–Kramer HSD test at the 5% probability level for multiple comparisons. To compare male responses between the treatments at each field site, the number of males caught in each field site were analyzed using a generalized linear model with a Poisson distribution, followed by a Tukey–Kramer HSD test at the 5% probability level for multiple comparisons in experiment 1. The difference of males caught in experiment 2 was compared by nonparametric Wilcoxon rank-sum test.

3 RESULTS

3.1 Variation in the timing of female calling

Within each population, approximately 75% of females observed exhibited calling behavior (total number of females observed: in Spain, $n = 85$; in China, $n = 67$; in Australia, $n = 53$). Larval diets

(host plants) did not affect adult female calling behavior in Spain ($P = 0.699$), China ($P = 0.921$) or Australia ($P = 0.837$). Among the three populations, the age of female initial calling varied: in Spain, females started calling at significantly younger age ($n = 38$, 1.8 ± 0.1 days) compared to females in China ($n = 28$, 2.7 ± 0.2 days) ($P = 0.005$) and Australia ($n = 30$, 3.5 ± 0.3 days) ($P < 0.001$). Within all three populations, the calling patterns were significantly different between the first calling night and later calling nights (Spain: $\chi^2 = 8.41$, $df = 1$, $P = 0.004$; China: $\chi^2 = 5.96$, $df = 1$, $P = 0.015$; Australia: $\chi^2 = 6.71$, $df = 1$, $P = 0.01$) (Fig. 2(a)).

In all three populations, female calling was very low in the first half of the night and then increased sharply in the second half of the night (Fig. 2(a)). A similar pattern was shown in all three regions, even though there were differences in night lengths and temperatures in the three geographic regions, i.e. *H. armigera* females showed similar temporal patterns of calling behavior, namely in the last part of the night (Figs. 1(b) and 2(a)). When we recorded the female calling behavior in all three sites, temperatures generally decreased throughout the nights. Changes in temperature during the nights were smaller in Spain (22–35 °C) and in Australia (16–27 °C), with female calling during the optimum temperature at 24–26 °C in Spain and at 20–23 °C in Australia. However, in China, even when the temperature during the nights fluctuated a bit more, namely between 15–34 °C, the females still exhibited calling behavior at lower temperature (i.e. lower temperature coincided with rainfall during the nights) (Fig. 1(b)). Under laboratory conditions, the percentage of females calling were similar among the three populations ($P = 0.884$) (Fig. 2(b)).

3.2 Variation in female pheromone amount and composition

Within each population, larval diets did not affect the pattern of female sex pheromone titers, as this increased in a similar pattern throughout the night (Fig. 3). Larval diets also did not affect pheromone amounts in Australia ($\chi^2 = 0.44$, $df = 1$, $P = 0.51$) (Fig. 4(e)). However, in Spain and China, females that were reared on corn contained significantly more pheromone than females reared on tomato (Spain: $P = 0.015$; China: $P = 0.042$) (Figs. 4(c),(d)). The pheromone composition (i.e. relative amounts) was unaffected by larval diets in all three regions: (Spain: $df = 2$, $P = 0.091$; China: $df = 2$, $P = 0.37$; Australia: $df = 1$, $P = 0.051$) (Figs 4(c)–(e)).

Among the populations, the pheromone compositions were significantly different ($df = 2$, $P < 0.001$) (Figs. 4(a),(b)). Specifically, females from China contained significantly more of the major sex

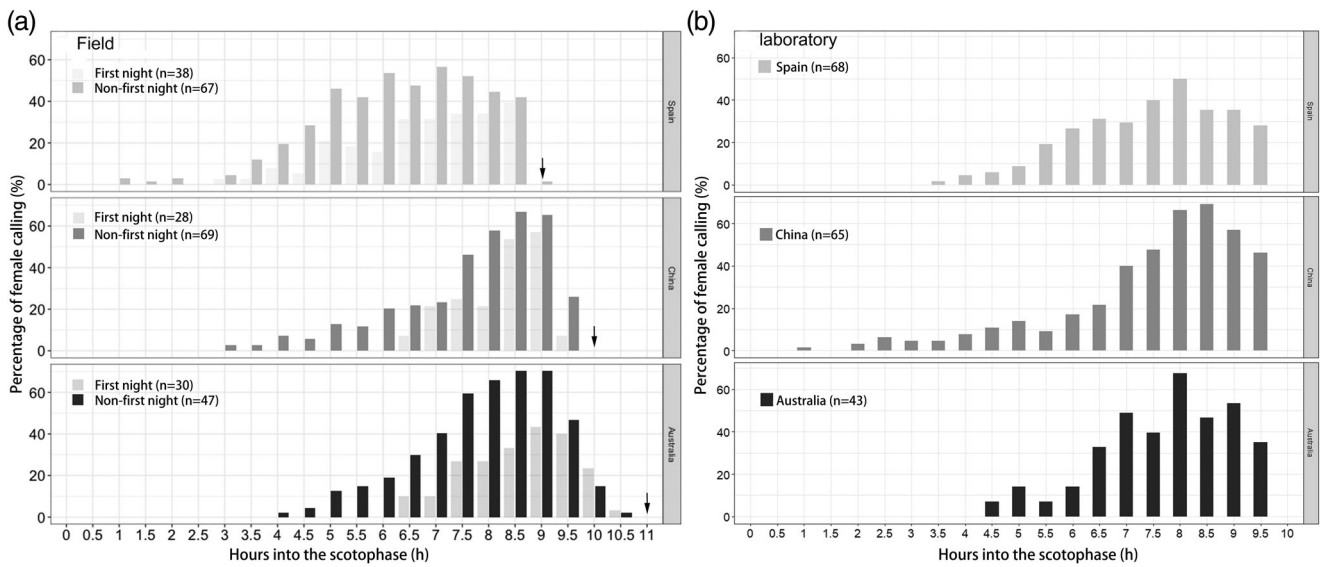


Figure 2. Female calling patterns (a) at the three field locations and (b) in the laboratory. The black arrows in (a) indicate the end of scotophase in Spain, China and Australia. *n*, number of females exhibiting calling behavior.

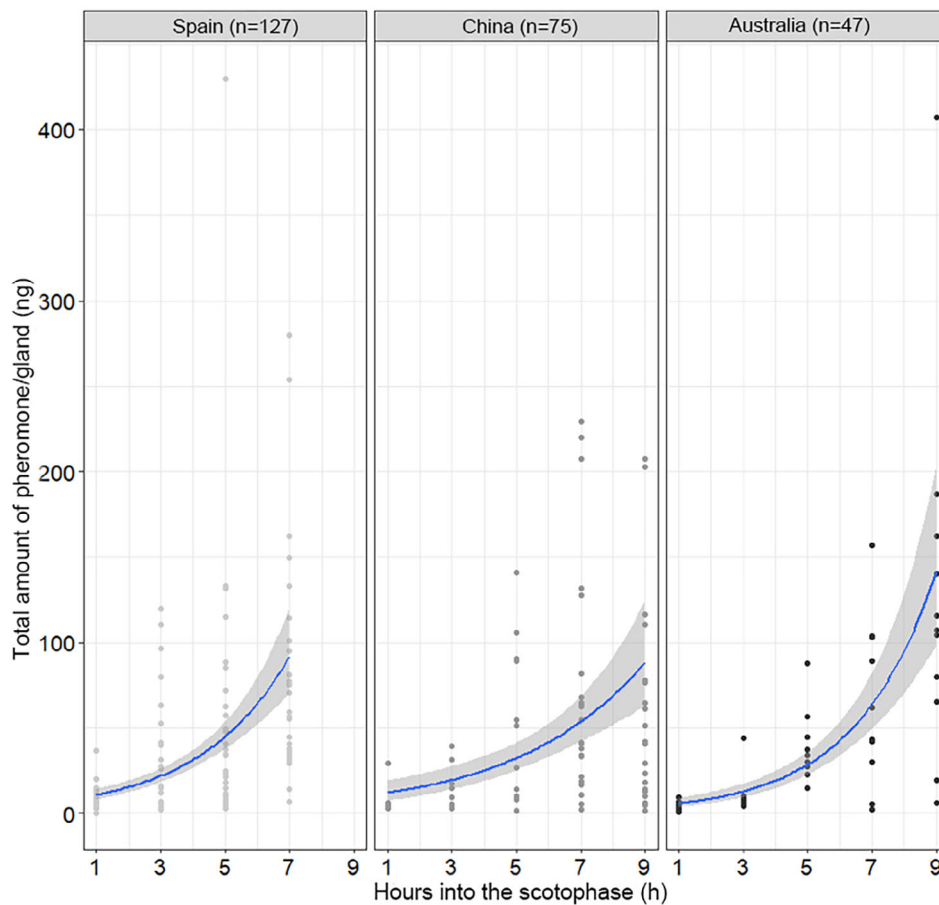


Figure 3. Total pheromone amounts in females (ng) when glands were extracted at different time points into the scotophase in the three field locations. The gray areas represent 95% confidence intervals. *n*, total number of females extracted. [Color figure can be viewed at wileyonlinelibrary.com]

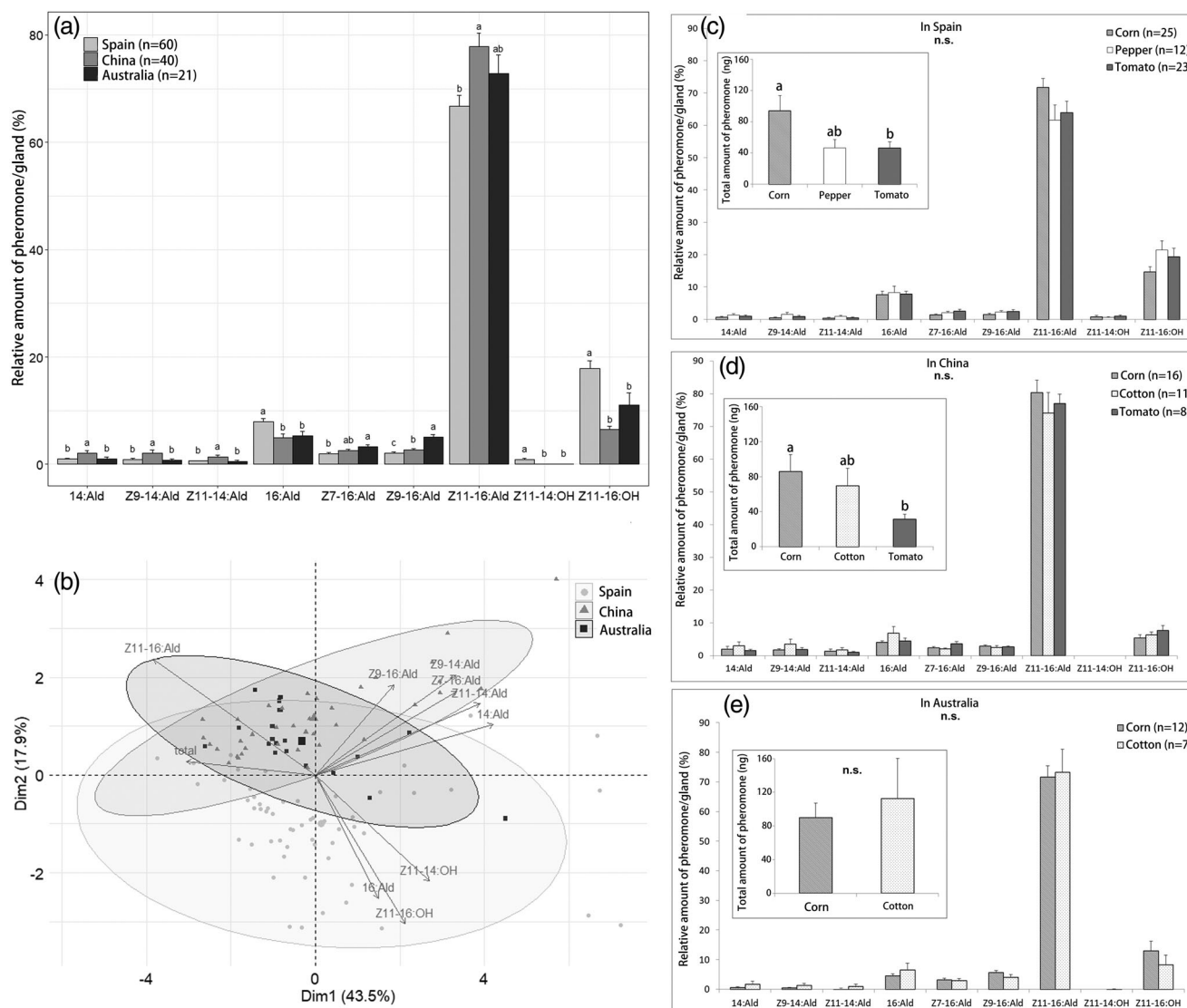


Figure 4. Phomone composition of *H. armigera* females in Spain, China and Australia. (a) Relative amount (%) of female phomone compounds. (b) Principal component analysis (PCA) of female phomone blends. PCA plot shows the first two principal components and the arrows represent each phomone component. (c)–(e) Quantity (in ng, inserts) and quality (in %, main panels) of phomone blends from females reared on different larval diets (host plants) in (c) Spain, (d) China and (e) Australia. Significant differences are indicated by different letters ($P < 0.05$). n.s., not significant; n, total number of females extracted.

phomone component Z11-16:Ald, and the minor compounds 14:Ald, Z9-14:Ald and Z11-14:Ald, than females from Spain. In contrast, females from Spain contained significantly more 16:Ald and Z11-16:OH, while females from Australia contained significantly more Z7-16:Ald and the critical secondary sex phomone component Z9-16:Ald. Interestingly, Z11-14:OH was present in Spain, but not in China and Australia.

3.3 Variation in male response

In experiment 1, *H. armigera* males were caught in all tested traps, although traps with lures containing only the major component Z11-16:Ald (blend 1) caught significantly fewer males in all fields compared to blends 2–5. In Spain and China, males were equally attracted to traps with blend 2–5, to which Z9-16:Ald, Z7-16:Ald or Z9-14:Ald was added. However, in Australia about twice as many males were caught in blend 5 containing 6% Z9-16:Ald than in blends 2–4 (Fig. 5(a)).

In experiment 2, the addition of 10% Z11-16:OAc significantly reduced the number of males captured in Australia ($P < 0.0001$) and China ($P < 0.0001$), but not in Spain, where equal numbers of males were caught in traps with or without this acetate ester ($P = 0.599$) (Fig. 5(b)).

4 DISCUSSION

Although *H. armigera* is widely distributed across a large latitudinal gradient and thus experiences different photoperiodic conditions, we found that overall female calling behavior was similar among the three geographic regions. However, we did find that *H. armigera* females in Spain initiated calling at a significantly younger age than in Australia and China. This is likely due to the fact that in Spain nights were shorter and the temperature at night was higher compared to China and Australia (Fig. 1(b)). Previous studies also found that photoperiod and temperature affect

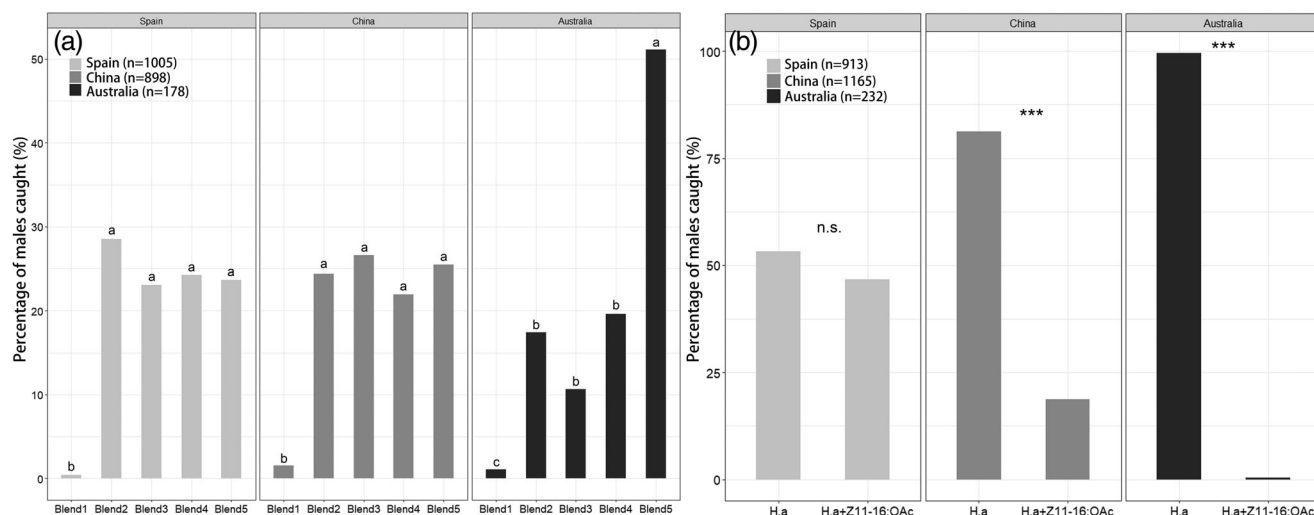


Figure 5. Field trapping experiments in the three field locations, showing the percentage of *H. armigera* males caught. (a) Attraction of *H. armigera* males to five blends, as specified in Table 2. (b) Attraction of *H. armigera* (H.a) males to H.a pheromone blends with or without 10% Z11-16:OAc, as specified in Table 2. Significant differences are indicated by different letters ($P < 0.05$) or asterisks ($***P < 0.001$). n.s., not significant; n, total number of males caught.

the age that females initiate calling.^{14,52} This variation hints at physiological differences in sexual maturation, which could be an adaptation to changes in different photoperiodic and temperature conditions, especially for migratory species.^{22,52,53}

Previous studies have suggested that there could be genetic variation in the calling behavior of *H. armigera*.^{38,54} However, as we did not find differences in the timing of calling among the *H. armigera* populations in Spain, China and Australia under laboratory conditions, genetic variation in calling behavior is unlikely, at least for these populations.

In all three geographic sites, the total amount of pheromone increased throughout the night, which reflects the calling behavior patterns. Such a synchronization between female calling and pheromone release has been previously shown in many other moth species from several families, i.e. *Helicoverpa assulta* (Noctuidae), *Phthorimaea operculella* (Gelechiidae), *Agrotis ipsilon* (Noctuidae) and *Choristoneura rosaceana* (Tortricidae).^{16,55–57}

Within each geographic region, we found that larval diet did not affect the composition of the pheromone blends, but it did affect the total amount of pheromone. Larval nutrition can directly influence pupal weight or adult body size, which has been found to be positively correlated with pheromone titer.^{24,58} Since females that were reared on corn produced more pheromone than females reared on tomato both in Spain and China (Figs. 4(c),(d)), possibly females that were reared on corn acquired more nutrition than larvae fed on tomato. Alternatively, geographic variation in host plant suitability may induce larval stress, which may indirectly affect adult pheromone amounts.⁵⁹ Although *H. armigera* can utilize a large range of host plants, host preference and suitability varies in different geographic regions. For example, *H. armigera* in Australia prefers tobacco and corn,⁶⁰ while tomato is the main host plant in Spain.⁴¹ Interestingly, tomato seems an unsuitable host for *H. armigera* in China.⁶¹

Among the three geographic regions, we found some differences in the relative amounts of the pheromone compounds, especially between the populations from Spain and China. Specifically, we found a significantly lower amount of the major pheromone component Z11-16:Ald, but higher amount of the minor

compounds 16:Ald, Z9-16:Ald and Z11-16:OH in Spain than in China. In addition, we detected a very low amount of Z11-14:OH in females from Spain, but not in China and Australia. Konyukhov et al.⁶² first reported the presence of Z11-14:OH in *H. armigera* female pheromone blends. However, further study will be needed to confirm the biological function and relevance of Z11-14:OH in *H. armigera* female pheromone glands.

One possible explanation for the geographic variation in the relative amounts of some pheromone compounds could be potentially interference between closely related species.^{13,63} In China *H. armigera* co-occurs with *H. assulta*, and in Australia with *H. punctigera*. Selection may be exerted to reduce cross-attraction and hybridization in these areas of sympatry, so that females vary the relative amounts of pheromone compounds.^{10,64} For example, tuning the major compounds Z11-16:Ald or Z9-16:Ald or adding other components could increase the attraction of intraspecific males in different regions.^{65,66}

We also found geographic variation in male response to different pheromone lures. In general, the two pheromone components for *H. armigera* are Z11-16:Ald and Z9-16:Ald, and the combination of these two components is recommended as the standard blend for attracting the species.^{67,68} Zhang et al.⁶⁹ found that the addition of Z9-14:Ald into the standard blend attracted more males, while the addition of Z7-16:Ald did not increase attraction. However, the results of our field tests showed that neither Z9-14:Ald nor Z7-16:Ald increased the attraction of males in any of the three sites. Interestingly, the addition of 6% of Z9-16:Ald in blend 5 did cause a significant increase in the number of males trapped in Australia, but not in Spain or China (Fig. 5(a)). This coincides with our finding that females in Australia produced higher relative amounts Z9-16:Ald in their pheromone blends compared to females in China and Spain, suggesting some selection for Z9-16:Ald which may maximize attraction of conspecific males and possibly avoid interspecific matings in Australia.

Interestingly, we found significant geographic differences in male response when adding a potential sex pheromone antagonist, Z11-16:OAc. The fact that Z11-16:OAc dramatically inhibited attraction of *H. armigera* males in Australia and China suggests

that Z11-16:OAc is an antagonist to avoid heterospecific attraction between closely related species.^{13,51,63,70} Specifically, *H. punctigera* co-occurs in Australia,⁷¹ which also use Z11-16:Ald as their major sex pheromone component, and has Z11-16:OAc in their sex pheromone as well.^{72,73} Similarly, *H. assulta* is sympatric with *H. armigera* in China.⁷⁴ Even though the main sex pheromone component of *H. assulta* is Z9-16:Ald instead of Z11-16:Ald, the female blend also contains Z9-16:OAc and Z11-16:OAc.^{74,75} In Spain, such communication interference does not seem to be present, as *H. armigera* males were not deterred by Z11-16:OAc in this region.

Our findings of geographic differences in *H. armigera* male response are important for the development of region-specific lures.^{13,34} For example, using 6% of Z9-16:Ald instead of 1.4–4% of Z9-16:Ald in synthetic pheromone lures is probably more effective in monitoring and controlling *H. armigera* populations in Australia. Furthermore, Z11-16:OAc has a potential application as an antagonist in pest management strategies, such as mating disruption against other closed related species in sympatric regions.

5 CONCLUSIONS

We found geographic variation in the sexual signals and responses of *H. armigera*, which is likely due to local environmental conditions, such photoperiod and temperature, but also due to the presence of other closely related species with which communication interference could occur. Most importantly, we found that the male response window varies in the three continents and is wider in Spain than in China and Australia. The fact that we found not only geographic variation in both the female signal and the male response indicates that sexual communication is not fixed in this species, which may have important consequences for the development of this pest in its newly invaded area of South America.

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