The legume pod borer, *Maruca vitrata* (Lepidoptera: Crambidae), is a serious pantropical insect pest of grain legumes such as cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), and common bean (*Phaseolus vulgaris*) (Taylor, 1967; Jackai, 1995; Abate and Ampofo, 1996; Shanower et al., 1999). *M. vitrata* is thought to be native to Indonesia (Dietz, 1914) and is widely distributed across Asia, Africa, North and South America, and Oceania (Sharma, 1998; Adati et al., 1999). The losses in grain yield caused by the legume pod borer are estimated to range from 20% to 60% (Singh and Allen, 1980; Adati et al., 2008). Without control measures, cowpea flower infestation rates have been reported to be up to 80% in West Africa (Afun et al., 1991).

Because of its economic importance, the sex pheromones of the legume pod borer have been the subject of investigations. Adati and Tatsuki (1999) reported that (E,E)-10,12-hexadecadienal (E10,E12–16:Ald) and (E,E)-10,12-hexadecadienol (E10,E12–16:OH) were the major and minor sex pheromone components, respectively, of *M. vitrata*. Downham et al. (2003) extended this work by conducting a field test in Benin and showed that a lure composed of E10,E12–16:Ald, E10,E12–16:OH, and (E)-10-hexadecenal (E10–16:Ald) in a 100:5:5 ratio caught signifi-
cantly more males than any other blend baiting with the major component alone or with a two-component blend, or virgin females in a field test. Experiments for the optimization of traps and lures were continued in Benin (Downham et al., 2004). Lures in polyethylene vials containing 0.1 mg of pheromone attracted more males than other blends of dose or dispenser. The lures remained attractive for at least 4 weeks in the field. A water trap made of a plastic jerry can was the most effective trap design (Downham et al., 2004). Despite this progress in Benin, during the course of further experiments, poor catches were recorded at other locations in West Africa outside Benin (NRI, 2012). Hassan (2007) reinvestigated the sex pheromone blends of M. vitrata, and a new minor component, (E)-10-hexadecen-1-ol (E10–16:OH), was identified in laboratory experiments. The compound improved the effectiveness of the traps in India, but had little effect on catches of M. vitrata males in Benin, Ghana, Burkina Faso, and northern Nigeria. In the subsequent trapping experiments by Hassan (2007), the blends composed of the single major component E10,E12–16:Ald alone were found to be effective in Burkina Faso. The three-component blend reported by Downham et al. (2003) was only effective in Benin. Furthermore, none of the synthetic lures was effective in northern Nigeria and Ghana. These findings suggested sex pheromone polymorphism in M. vitrata across geographic areas, which might limit the potential usefulness of the traps (Adati et al., 2008).

M. vitrata is the primary boring pest of leguminous vegetable crops, particularly cowpea, in China (Ke et al., 1985); it accounts for 85% of all known cowpea borers during the breeding season (Luo et al., 2003). The effectiveness of M. vitrata sex pheromone-based traps in China needs to be assessed. The main cowpea-producing regions in China are located in the south-central part (He, 2002). In this study, two moth populations from Guangdong and Hubei Province, located in the south and central part of China, respectively, were selected for comparison of their sex pheromone blends. Responses of M. vitrata males from the two geographic populations were also investigated using laboratory analyses and trapping tests in the field. From an agricultural standpoint, determination of the sex pheromone components of the two Chinese populations is critical. The life habits of M. vitrata in the two locations are different. The population from southern Huazhou remains viable all year round (Wang et al., 2003a, b). In contrast, it is difficult to detect M. vitrata individuals in northern Wuhan on any alternative host after cowpea harvest at the end of the rainy season, which was also supported by light trap studies. Overwintering pupae could not be found in further surveys (Luo et al., 2003; Lu, 2007). The present study also aimed to determine whether there is variation in sex pheromone components between the two geographically distant populations with different habitats.

Material and Methods

Insect rearing

M. vitrata was reared at 29 °C with a relative humidity of 75–80% and a 14-h light/10-h dark photoperiod. The specimens of M. vitrata used in the study were obtained from two locations in China: (1) Wuhan (WZ): Xinzhou District, Wuhan City, Hubei Province (114°39'11" E, 30°35'51" N), and (2) Huazhou (HZ): Pingding Town, Huazhou City, Guangdong Province (110°23'58" E, 21°57'30" N). Larvae were collected in late June from the flowers and young pods of an infested cowpea (Vigna unguiculata) field. The larvae were mass-reared on intact cowpea flowers in glass containers (20 cm diameter, 15 cm height) up to 3 instars, transferred to separate smaller glass containers (8 cm diameter, 5 cm height), and fed young pods until eclosion. Adults were maintained in a wooden cage (30 cm × 40 cm × 50 cm) with a fine nylon mesh and provided 15% honey solution. The moths were reared in the laboratory for 1–2 generations before testing. Tested adult moths were sexed upon emergence. Moths of the same age and sex were kept together. Cages were kept in two different chambers according to sex until use. Virgin females were used for the preparation of pheromone extracts, while males were used for electrophysiological studies.

Extraction of the female pheromone gland

The sex pheromone glands were removed from virgin 3-day-old calling females at the fifth hour into the scotophase (Lu et al., 2008). The glands were extruded by applying gentle pressure to the tip of the abdomen to evert the ovipositor. The glands were then excised, using iris scissors, into a conical glass vial insert. A single excised
gland was immersed in 10 μL n-hexane (HPLC grade; Sigma-Aldrich Co., St. Louis, MO, USA) containing hexadecyl acetate (16:AC) as internal standard (1 ng/μL) for 30 min at room temperature and then immediately analysed by gas chromatography-mass spectrometry (GC-MS). The remaining extract was transferred to a clean conical glass vial and kept at –20 °C.

**Coupled GC-MS**

Two fused silica capillary columns with different polarities, a medium-polar DB-17 and a polar DB-WAX, were used for the analysis of sex pheromone extracts obtained from females collected from the two study locations.

For the DB-17 column (30 m × 0.25 mm ID × 0.25 μm film thickness; J & W Scientific, Folsom, CA, USA), GC-MS analysis was conducted using a Voyager mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled with a Trace 2000 gas chromatograph (Thermo Fisher Scientific) and Windows NT/Xcalibur software for data analysis. Injections were made in the splitless mode. The initial oven temperature was maintained at 80 °C for 2 min, increased to 200 °C at a rate of 20 °C/min, held for 0 min, increased to 290 °C at a rate of 10 °C/min, and then held for 15 min. Helium was used as the carrier gas (1.5 mL/min). For EI mass spectra, the ionization voltage was 70 eV, and the temperatures of the ion source and interface were both 250 °C. Emission current was 150 μA.

For the DB-WAX column (30 m × 0.25 mm ID × 0.25 μm film thickness; J & W Scientific), GC-MS analysis was conducted using an Agilent Technologies 5973 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) coupled with an Agilent Technologies 6890 N gas chromatograph and Windows NT/mass spectral search program (version 1.7) software for data analysis. Injections were made in the splitless mode. The initial oven temperature was maintained at 50 °C for 1 min, increased to 230 °C at a rate of 5 °C/min, and held for 0 min. Helium was used as the carrier gas (1.0 mL/min). For EI mass spectra, the ionization voltage was 70 eV, and the temperatures of the ion source and interface were 230 °C and 280 °C, respectively. Emission current was 34.6 μA.

**Chemicals**

The sex pheromone compounds, E10,E12–16:Ald, E10,E12–16:OH, E10–16:Ald, and E10–16:OH, were purchased from the Pheromone Bank (Plant Research International, Wageningen, The Netherlands). n-Hexane was redistilled before use. The compounds were found to be 99% pure by GC analysis.

**Electroantennography (EAG) assays**

A micromanipulator assembly (MP-15; Syntech, Hilversum, The Netherlands) was connected to a stimulus controller (CS-55; Syntech). The controller was used for continuous clean airflow or stimulus airpulse. All signal sources were connected to a serial data acquisition interface (IDAC-232; Syntech).

The antennae from the heads of male *M. vitrata* were excised using microscissors and mounted on the antenna holder with two metal electrodes by using conductive gel (Spectra 360; Parker Lab, Fairfield, NJ, USA). The electrode holder was then inserted into the EAG probe. A relative stable base line should be visible before testing. All tested antennae were from 2- to 3-day-old unmated males. Each treatment was performed three times using a different male each time. In all, six antennae were tested.

Different doses of synthetic sex pheromones were used as stimulants. The sequence of tested compounds was random. Stimulation duration was 0.1 s, and stimuli were given at 1-min intervals to allow the olfactory sensilla to recover. Absolute net EAG responses to the test components were obtained by subtracting the mean absolute EAG responses of the control stimulations (with n-hexane) immediately preceding (control) and following (control+1) the test components from the absolute EAG responses of the test components (EAGx):

\[
\text{absolute net EAG}_x (\text{mV}) = \text{EAG}_x - [(\text{control}_x + \text{control}_{x+1})/2].
\]

**Field evaluation**

Field trials were conducted using a variety of synthetic blends in cowpea fields in the two locations, *i.e.* Xinzhou District and Pingding Town. In each location, the trials were carried out by randomized complete-block designs, with six blocks comparing the attractiveness of a blend in various
ratios. The six blocks were separated by a minimum distance of 300 m, and were usually situated in separate fields. Sticky delta traps (30 cm length × 20 cm width × 16 cm height) were used (Geruibiyuan Technology Company, Beijing, China). In each block, traps consisting of all treatments were positioned randomly in a grid formation at 20-m intervals. Traps were suspended by wires from sticks approximately 1.2 m high.

One-, two-, and three-component blends of synthetic \(E_{10},E_{12}–16\text{:Ald, }E_{10},E_{12}–16\text{:OH, and }E_{10}–16\text{:Ald, as well as gland extracts were used as lures. The component ratios in the two- and three-component blends were developed on the basis of the natural composition of the gland extracts. The lures consisted of red rubber septa impregnated with \(n\)-hexane solutions of the synthetic pheromone components and female extract (2FE). Unbaited controls (\(n\)-hexane) were also used to compare the relative attractiveness of the different blends. Catches were removed from the traps throughout the day, and the number of males trapped was recorded at each time interval. The traps were cleaned and rerandomized after each recording.

**Statistical analysis**

Male EAG response to the three synthetic sex pheromone components at different doses and male catches with different compositions of the three components at two locations were analysed by one-way analysis of variance (ANOVA). The means were separated by Tukey's multiple-range tests. Significant differences in male EAG response to each synthetic sex pheromone of the same concentration between the two populations, the composition of three sex pheromone gland extracts between the two populations, and field catches using the synthetic lures at the two locations were analysed by the Mann-Whitney U-tests. All data were analysed using SPSS version 16.0.

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**Fig. 1.** Total ion chromatograms of GC-MS analysis of sex pheromone gland extracts (1, \(E_{10}–16\text{:Ald; 2, }E_{10},E_{12}–16\text{:Ald\}) of *M. vitrata* from (A) Wuhan and (B) Huazhou. DB-17 column.
Results

**GC-MS analysis of the sex pheromone gland extracts of the two populations**

Compounds in female extracts were identified by comparing the retention times and mass spectral matches on DB-17 and DB-WAX GC columns with those of synthetic standards (Figs. 1 and 2).

Peak 1 was identified as E10–16:Ald (Figs. 1 and 2), which had the molecular ion [M⁺] at m/z 238 and a significant [M⁺–18] ion at m/z 220, suggesting that the compound was a monounsaturated hexadecenal isomer. The mass spectrum of peak 1 (Fig. 3A) exhibited the following peaks [m/z (relative abundance)]: 41, 55 (100, base), 69, 81, 98, 220 [M⁺–18], and 238 [M⁺]; it was identical to that of E10–16:Ald (Fig. 3B). In addition, the retention times of peak 1 matched those of E10–16:Ald on both columns (9.93 min and 27.87 min for DB-17 and DB-WAX, respectively). Thus, the compound was identified as E10–16:Ald. Gland extracts of the Wuhan population contained approximately eight times more E10–16:Ald than those of the population from Huazhou.

For both populations (Figs. 1 and 2), the mass spectrum of peak 2 (Fig. 4A) yielded a distinct molecular ion at m/z 236 [M⁺], but no ions at [M⁺–18] or [M⁺–28], suggesting that it was a diene C₁₆ aldehyde. The comparatively strong molecular ion peak and retention times that were significantly higher than those of E10–16:Ald suggested that the double bonds were conjugated. Strong fragment ions at m/z 67 (100, base), 81, 95, and 109 were also found on DB-17 and DB-WAX columns, and the intensities of the fragment ions, which are typical ions found in a conjugated diene (Fig. 4B), were quite high (Fig. 4A). The compound was a conjugated 10,12-hexadecadienal, with a molecular weight of 236 according to an EI mass spectrum analysis of lepidopteran sex pheromones.

![Fig. 2. Total ion chromatograms of GC-MS analysis of sex pheromone gland extracts (1, E10–16:Ald; 2, E10,E12–16:Ald; 3, E10,E12–16:OH) of M. vitrata from (A) Wuhan and (B) Huazhou. DB-WAX column.](image-url)
pheromones conducted using a conjugated diene system. The pheromone components were further confirmed to have an $E_{10},E_{12}$ geometry by comparing their retention times and mass spectra on DB-17 and DB-WAX columns by using the standards of the $E_{10},E_{12}$ isomer, which matched peak 2 on both columns (10.70 min and 31.13 min for DB-17 and DB-WAX, respectively). This information allowed the identification of the insect-produced compound as $E_{10},E_{12}$-16:Ald.

Similarly, peak 3 was identified as $E_{10},E_{12}$-16:OH (Fig. 2). The component yielded a molecular ion at $m/z$ 238 [M$^+$], which is consistent with a C$_{16}$ di-unsaturated alcohol. Its relatively large molecular ion peak and retention time, which was significantly higher than that of 16:OH, suggested that it was a conjugated dienol. Strong fragment ions at $m/z$ 67 (100, base), 81, 96, and 109, which are typical ions of 10,12-hexadecadienol, were also diagnostic on the DB-17 and DB-WAX columns (Figs. 5A, B). Similar to the aldehyde, the alcohol was determined to be an $E_{10},E_{12}$ geometric isomer by comparing its

Fig. 3. Mass spectra of (A) peak 1 and (B) $E_{10}$-16:Ald.
pheromone gland extracts in individual calling females are shown in Table I. The titer of the major component of the pheromone, E10,E12–16:Ald, ranged from 2.7 to 8.5 and 3.5 to 8.4 ng/female in the extracts from the Wuhan and Huazhou populations, respectively.

Dose-response of males to synthetic pheromones

The dose-response relationship to the chemicals in the two populations suggested that male antennae responded to all three synthetic components (Figs. 6A, B). In particular, E10,E12–16:Ald elicited the largest male EAG response, followed by E10,E12–16:OH and E10–16:Ald. For E10,E12–16:Ald, an initial EAG response could be elicited at a stimulus dose of 0.001 μg in both populations. The magnitude of the response increased considerably between doses of 0.01 and 100 μg. The responses to E10,E12–16:OH and E10–16:Ald were low at a dose of 0.001 μg for both populations; increases were observed at doses of 0.1 and 100 μg in the Wuhan and Huazhou populations, respectively (Figs. 6A, B). Between 0.1 and 100 μg, the responses to all three compounds increased strongly – especially, the response to E10,E12–16:Ald increased significantly. Similar increases were observed for the other two compounds.

Field-trapping experiment with synthetic sex pheromone lures

Field-trapping experiments were conducted to assess the responses of males from the two populations to different blends containing single, binary, and ternary synthetic sex pheromone lures in various ratios on the basis of the natural composition in the extracts (Table II).

Field tests conducted with single (S) synthetic sex pheromone lures indicated that E10,E12–16:OH alone did not attract males (treatment S3), while both E10–16:Ald and E10,E12–16:Ald on their own were able to attract a few males (treatments S1 and S2).

When the ratio of E10–16:Ald and E10,E12–16:Ald in binary lures corresponded to that found in the female sex pheromone...
gland, many males were attracted. Wuhan (W) males were most attracted to lures containing 80 μg E10–16:Ald and 100 μg E10,E12–16:Ald (treatment W2), whereas Huazhou (H) males were most attracted to lures containing 10 μg E10–16:Ald and 100 μg E10,E12–16:Ald (treatment H2) (Table II).

In both populations, three-component lures were more effective than binary lures. In the comparative field studies, distinct differences were noted in the responses of Wuhan and Huazhou males, respectively, to various doses of the three pheromone components. The addition of a minor amount of E10,E12–16:OH to two-component lures increased the number of catches. Both Wuhan and Huazhou males responded optimally to blends containing lower doses of E10,E12–16:OH.

Wuhan males were most attracted to lures (treat-

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Table I. Composition of the female sex pheromone of *M. vitrata* in gland extracts from calling females of Wuhan (WH) and Huazhou (HZ) populations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount in female extracts (% relative to E10,E12–16:Ald)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WH</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>E10–16:Ald</td>
<td>79.5*</td>
</tr>
<tr>
<td>E10,E12–16:Ald</td>
<td>100</td>
</tr>
<tr>
<td>E10,E12–16:OH</td>
<td>12.1*</td>
</tr>
</tbody>
</table>

* Percent of female extracts with detectable amounts (n = 30, Wuhan population; n = 32, Huazhou population). The asterisks indicate significant differences in the composition of three sex pheromone gland extracts between both populations (Mann-Whitney U-tests, *P* < 0.05). SD, standard deviation.
ment W1) containing 80 μg $E_{10-16}$:Ald, 100 μg $E_{10,E12-16}$:Ald, and 10 μg $E_{10,E12-16}$:OH, whereas the values were 10 μg, 100 μg, and 10 μg, respectively, for Huazhou males (treatment H1) (Table II). Fewer males were captured, when the blend that was most attractive to one geographic strain, or crude gland extract, were offered to the respective other strain.

In the field test, the number of captured males reached a maximum at a dose of 300 μg/lure in both populations (Table III); a further increase in the dose reduced the number of catches.

Discussion

Female sex pheromone polymorphism has previously been demonstrated for African and Indian populations of *M. vitrata* of different geographic origins (Adati and Tatsuki, 1999; Downham *et al*., 2003; Hassan, 2007). Similarly, in our study, geographic variation in the sex pheromone composition of *M. vitrata* was found between populations sampled at two locations ranging from 30°35’51” N to 21°57’30” N. Our laboratory analyses revealed that the sex pheromone of the Huazhou population was composed of $E_{10-16}$:Ald, $E_{10,E12-16}$:Ald, and $E_{10,E12-16}$:OH in the ratio of 10.3:100:0.7, with a relatively low proportion of $E_{10-16}$:Ald, while a high proportion of $E_{10-16}$:Ald was detected in the sex pheromone of the Wuhan population (79.5:100:12.1). In the field-trapping experiments, fewer males were captured when the blend of one strain was offered to the other strain.

A discrepancy was noted between some of the previously reported pheromone components of a population in Benin and those identified in this study in the two Chinese populations. First, while we clearly detected $E_{10-16}$:Ald by GC-MS in the two Chinese populations and found it to be biologically active, Downham *et al*. (2003) did not detect this compound by GC-MS, but reported it as a minor component in wind-tunnel and field bioassay experiments. Konno *et al*. (1982) identified $E_{10-16}$:Ald as a sex pheromone component of the yellow peach moth, *Dichocrocis punctiferalis* (Lepidoptera: Pyralidae); we obtained identical mass spectral data. In our field trials, another pyralid moth, *Pleuroptia chloropahanta* (Butler) (Lepidoptera: Pyralidae), was frequently captured when the extracts from *M. vitrata* were used in the two geographic populations (unpublished data). Honda and Kimura (2004) confirmed that $E_{10-16}$:Ald was an effective attractant for monitoring *Pleuroptia chloropahanta* and is a sex pheromone component of that species. $E_{10-16}$:Ald has been reported as a pheromone component in additional moth species, including *Dichocrocis punctiferalis* Guenee (Konno *et al*., 1982) and *M. vitrata* (Downham *et al*., 2003), all of which belong to the subfamily Pyraustinae. Second, $E_{10,E12-16}$:OH was occasionally not detected in the full-scan mass spectrum using a DB-17 column in our study. However, laboratory EAG and field-trapping experiments provided evidence that this alcohol was a synergistic component of the pheromone of the population captured at both locations, although it was not the sole attractant. A possible reason why it was not detected by GC-MS is a difference in the column efficacy between DB-WAX and DB-17. Electro-
physiological testing indicated that the Huazhou population was more sensitive to $E_{10-16}:Ald$ and $E_{10}, E_{12-16}:OH$ than the Wuhan population. Accordingly, the amounts of the two components in the Huazhou female extracts were lower than those in the Wuhan population. Finally, $E_{10-16}:OH$ could not be identified in the M. vitrata sex pheromone gland extracts of the two Chinese populations by chromatography on the two columns of differing polarity, even though this compound had been identified as a sex pheromone component in the laboratory experiments by Hassan (2007).

Differences in the geographic strains used in the various studies are possible reasons for the discrepancy between the reported sex pheromone components of legume pod borers. Moths used by Adati and Tatsuki (1999) were from Ghana, whereas those used by Downham et al. (2003) were a mixed laboratory population from India, Nigeria, Benin, and Taiwan. In China, M. vitrata is found from northern to southern regions, but infestations in the southern and central regions are more severe. Therefore, we used populations from central and southern China, respectively. Geographic variation in the sex pheromone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Composition of lures [μg]</th>
<th>Total number of males caught per trap at the two locations Mean (SD)^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>WH</td>
<td>EH 10–16:Ald</td>
<td></td>
</tr>
<tr>
<td>HZ</td>
<td>EH 10,E12–16:Ald</td>
<td></td>
</tr>
<tr>
<td>WH</td>
<td>EH 10,E12–16:OH</td>
<td></td>
</tr>
<tr>
<td>HZ</td>
<td>EH 10</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>100</td>
<td>2.0 (1.4)f</td>
</tr>
<tr>
<td>S2</td>
<td>100</td>
<td>4.8 (1.7)def</td>
</tr>
<tr>
<td>S3</td>
<td>100</td>
<td>0.0 (0.0)f</td>
</tr>
<tr>
<td>W1</td>
<td>100</td>
<td>19.5* (3.6)a</td>
</tr>
<tr>
<td>W2</td>
<td>100</td>
<td>11.2* (2.1)bc</td>
</tr>
<tr>
<td>W3</td>
<td>100</td>
<td>6.5 (2.2)de</td>
</tr>
<tr>
<td>W4</td>
<td>100</td>
<td>3.7* (1.9)ef</td>
</tr>
<tr>
<td>H1</td>
<td>100</td>
<td>8.2 (1.3)cd</td>
</tr>
<tr>
<td>H2</td>
<td>100</td>
<td>7.7 (2.7)cde</td>
</tr>
<tr>
<td>H3</td>
<td>100</td>
<td>0.0 (0.0)f</td>
</tr>
<tr>
<td>H</td>
<td>Huazhou extract (2FE)</td>
<td>14.3* (2.6)b</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>7.8 (1.7)cde</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0 (0.0)f</td>
</tr>
</tbody>
</table>

^a The comparative field studies were conducted at two locations: (1) WH: Xinzhou District, Wuhan City, Hubei Province, China, from July 15 to August 12, 2010; and (2) HZ: Pingding Town, Huazhou City, Guangdong Province, China, from August 18 to September 15, 2010.
^b S1–S3, single component; W1–W4, subsets of Wuhan blends; H1–H3, subsets of Huazhou blends.
^c The ratios of female sex pheromone gland extracts were simplified based on Table I.
^d Values are presented as means and SD for total catches throughout the experiment per trap. Numbers in the same column with the same letters are not significantly different according to Tukey’s multiple range test at a level of 5%. The asterisks indicate significant differences in field catches using identical synthetic lures at two locations (Mann-Whitney U-tests, $P < 0.05$).

<table>
<thead>
<tr>
<th>Dose [μg]</th>
<th>Total number of males caught per trap at the two locations Mean (SD)^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>WH</td>
<td></td>
</tr>
<tr>
<td>HZ</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.3 (0.8)c</td>
</tr>
<tr>
<td>100</td>
<td>10.3 (2.4)b</td>
</tr>
<tr>
<td>300</td>
<td>12.3 (2.3)a</td>
</tr>
<tr>
<td>500</td>
<td>10.0 (2.4)b</td>
</tr>
<tr>
<td>1000</td>
<td>1.0 (0.9)c</td>
</tr>
<tr>
<td>2000</td>
<td>1.2 (0.8)c</td>
</tr>
<tr>
<td>Control</td>
<td>0c</td>
</tr>
</tbody>
</table>

^a The comparative field studies were conducted at two locations: (1) WH: Xinzhou District, Wuhan City, Hubei Province, China, from July 6 to August 5, 2011; and (2) HZ: Pingding Town, Huazhou City, Guangdong Province, China, from August 8 to September 4, 2011.
^b The ratios of chemicals in multicomponent lures were 80:100:10 and 10:100:10 in Wuhan and Huazhou, respectively, corresponding to $E_{10-16}:Ald$, $E_{10}, E_{12-16}:Ald$, and $E_{10}, E_{12-16}:OH$.
^c Values are presented as means and SD for total catches throughout the experiment per trap. Numbers in the same column with the same letters are not significantly different according to Tukey’s multiple range test at a level of 5%.

Differences in the geographic strains used in the various studies are possible reasons for the discrepancy between the reported sex pheromone components of legume pod borers. Moths used by Adati and Tatsuki (1999) were from Ghana, whereas those used by Downham et al. (2003) were a mixed laboratory population from India, Nigeria, Benin, and Taiwan. In China, M. vitrata is found from northern to southern regions, but infestations in the southern and central regions are more severe. Therefore, we used populations from central and southern China, respectively. Geographic variation in the sex pheromone components of M. vitrata males changes from one geographic region to another.
composition has been reported for several other moth species (Guerin et al., 1984; Baltensweiler and Priesner, 1988; Whittle et al., 1991; Huang et al., 1998, 2002; McElfresh and Millar, 1999, 2008; Krokos et al., 2002). Differential male responses to pheromone blends from different geographic populations have also been well documented (Carde and Baker, 1984; Ando, 2009). The presence of sex pheromone polymorphisms in other species indicates that polymorphic pheromones in geographic strains may not be unusual. Different life habits between two populations may contribute to sex pheromone polymorphisms. *M. vitrata* produces 9 generations every year and has no need to overwinter in southern Huazhou (Wang et al., 2003b). However, the pest could not finish its life cycle in the more northern region of Wuhan (Lu, 2007). *M. vitrata* individuals were hardly detected on any alternative host in northern Wuhan, when cowpea had been harvested at the end of the rainy season; this finding is also supported by light trap studies. As overwintering pupae could not be found, the Wuhan population found in the following year may actually consist of migratory individuals. *M. vitrata* has in fact been reported to be a migratory insect (Taylor, 1978; Ke et al., 1985; Luo et al., 2003; Adati et al., 2008; Margam et al., 2010). Therefore, further studies are required to examine this hypothesis. However, even if evidence was found for *M. vitrata* migration to Wuhan, the insects could hardly be from Huazhou because of the dramatic difference in pheromone blends and male response specificities between the two populations. A migratory effect on a moth pheromone system was reported by Gemen et al. (2000) for the black cutworm moth, *Agrotis ipsilon*, and by Huang et al. (1998) for the Asian corn borer, *Ostrinia furnacalis*.

However, the factors that promote directional changes in the pheromone composition are yet unknown. In order to determine the genetic control underlying the sex pheromone polymorphism of *M. vitrata*, cultures of pure geographic strains should be established and crossing experiments between such strains performed; such studies allowed to determine that the sex pheromone blend of *Ostrinia nubilalis* is controlled by an autosomal locus with two codominant alleles. Additional sampling of *M. vitrata* populations from more regions of China should be done in the future to determine the possible existence of sex pheromone variation in further locations.

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