Interfering the mating of *Chilo suppressalis* (Walker): A new role of sex pheromones ((Z)-11-Octadecen-1-ol and (Z)-13-Octadecen-1-ol) from *Cnaphalocrocis medinalis* Guenée

Yu-yong Liang,[1,2] Mei Luo,[1,3] Xiao-gang Fu,[1] Li-xia Zheng,[1] Hong-yi Wei[1,4]

[1] College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, China

[2] Institute of Plant Protection, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China

[3] Department of Entomology, Michigan State University, East Lansing, MI 48864, USA

[4] Corresponding author. E-mail address: hywei@jxau.edu.cn (H-Y Wei)

11 **Abstract**

12 The rice stem borer, *Chilo suppressalis* (Walker), and the rice leaf folder, *Cnaphalocrocis*
13 *medinalis* Guenée are two of the most destructive lepidopteran pests in rice. Since these two
14 lepidopteran insects show occurrence laps in rice paddy fields, farmers prefer to set the
15 pheromone-baited traps of *C. suppressalis* accompany with the pheromone traps of *C.*
16 *medinalis* in the rice fields for convenient observation. However, our field observation
17 demonstrated that no male of the rice stem borer was captured in the traps baited with
18 commercial *C. suppressalis* sex pheromone (CCS) combined with commercial *C. medinalis*
19 sex pheromone (CCM). To confirm the *C. medinalis* sex pheromone component(s) to
20 interfere with the attraction of males of the rice stem borers to their conspecific female moths,
21 the single component of *C. medinalis* sex pheromone combined with CCS in traps were tested
22 in the laboratory and in the rice paddy field. The results revealed the two alcohol components
23 in CM, i.e., (Z)-11-octadecen-1-ol (Z11-18:OH) and (Z)-13-octadecen-1-ol (Z13-18:OH) may
24 cause a significant reduction in the captures by CCS to *C. suppressalis* males. We
25 recommend against using these sex pheromones together in the field and suggested that Z11-
26 18:OH and Z13-18:OH could be potential inhibitors or antagonists of *C. suppressalis* sex
27 pheromone to control the rice stem borer in practice.

28 **Keywords:** *Chilo suppressalis*, *Cnaphalocrocis medinalis*, sex pheromone, antagonist,
29 interfering

Introduction

The rice stem borer, *Chilo suppressalis* (Walker) and the rice leaf folder, *Cnaphalocrocis medinalis* Guenée, are two of the most harmful lepidopteran rice pests throughout China and other Asian countries (Su et al. 2014, Luo et al. 2019). In China, these two pests caused wide and extensive damages in rice and led to great economic loss in recent decades (Sheng et al. 2003, Liu et al. 2008). Currently, chemical control, such as application of insecticides has been utilized as an efficient method to control these two pests in China (Huang et al. 2011, Zheng et al. 2011). However, overuse of insecticides may cause severe insecticide resistance and serious environmental problems (Chen and Klein 2012, Fu et al. 2018, Pu et al. 2019). Therefore, biological control has been raised to be the one of most important strategies to suppress the outbreaks of these rice pests (Lou et al. 2014).

Sex pheromone application is becoming a valuable and efficient strategy of biological control to suppress lepidopteran pests (Chen et al. 2014). For example, pheromone-baited traps were widely used in male flight monitoring, population forecasting, mass trapping, and mating disruption to monitor and control a lot of lepidopteran pest species (Campion and Nesbitt 1983, Witzgall et al. 2010, Chen et al. 2014). Compared to the conventional methods, the pheromone-based control methods represent the advantages via the species-specificity of pheromones. The high biological activity which means that only relatively small amounts are required, and the negligible toxic effects on plants and animals (Campion and Nesbitt 1983). Pheromones generally consist of various component, which may be isomers with respect to the geometry and position of the unsaturation or they may be structurally related compounds

differing in chain length or the nature of the functional group. In most cases, the components are secreted in a very precise ratio (Campion and Nesbitt 1983).

In most lepidopteran insects, sex pheromones are generally secreted by female moths for attracting the males for copulation (Raina 1989). The *C. suppressalis* sex pheromone was first identified by Nesbitt et al. (1975) as a two-component blend of (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-13-octadecenal (Z13-18:Ald). In 1983, an additional compound, (Z)-9-hexadecenal (Z9-16:Ald), was identified (Tatsuki et al. 1983). These three-component blends (Z11-16:Ald, Z13-18:Ald, and Z9-16:Ald) were found at a ratio of 46:6:5, which is used in production of the standard lure in *C. suppressalis* (Tatsuki 1990, Cork 2004). In general, the *C. medinalis* sex pheromone consists of four components, (Z)-11-octadecenal (Z11-18:Ald), (Z)-13-octadecenal (Z13-18:Ald), (Z)-11-octadecen-1-ol (Z11-18:OH) and (Z)-13-octadecen-1-ol (Z13-18:OH) as a mixed ratio of 11:100:24:36 (Kawazu et al. 2000). Since then, the sex pheromones of *C. suppressalis* and *C. medinalis* were widely applied in the rice paddies and has become an ideal way for integrated pest management (IPM) programs (Kawazu et al. 2004, Kawazu et al. 2005, Byers 2007, Litsinger 2009, Cho et al. 2013, Chen et al. 2014).

Chilo suppressalis occurs four generations a year in most parts of Jiangxi province, China. Moreover, the emergence period of *C. suppressalis* and *C. medinalis* overlapped extensively. In this case, the two sex pheromones may be used at the same time by the famers to control *C. suppressalis* and *C. medinalis* together. However, previous studies have reported that mixing sex pheromones of two pest species resulted in interference of capture (Haynes et al. 2002, Gemeno et al. 2006). In addition, weather mixing the sex pheromones of *C.*

suppressalis and *C. medinalis* is risky in interference of capture is unknown now. Therefore, in this study, we aimed to determine whether mixing the sex pheromones of *C. suppressalis* and *C. medinalis* has negative effects in the IPM. We also want to determine the mechanism if mixing the sex pheromones has negative effects in pest control. This study is very important for guiding farmers in intensive application of various sex pheromones in practice.

Materials and Methods

Insects

A laboratory colonized population of *C. suppressalis* was collected from Shang-gao county, Jiangxi, China (E115°04′53.34″, N28°19′10.87″). Naturally overwintering larvae were collected from the paddy field in late March 2012, then were successively reared in cylindrical glass jars (10.0 cm in height and 12.0 cm in diameter) with several rice stems. Cotton stoppers for jars to prevent insects from escaping. The rice stems were changed every two days. Pupae were collected and transferred to 24-well plates for emergence individually. New emerged female and male adults were maintained separately in transparent plastic bags with a 10% sucrose solution. The insects were maintained in a growth room under a 14:10 (L: D) photo regime at 25 ± 1C° and 85 ± 5% RH.

Chemicals

The commercial sex pheromones of *C. suppressalis* (CCS) and *C. medinalis* (CCM) were purchased from Pherobio Technology Co., Ltd (Beijing, China). The synthetic chemicals Z11-16:Ald, Z13-18:Ald, Z9-16:Ald, Z11-18:Ald, Z11-18:OH and Z13-18:OH with more than 99% purity obtained from Sigma® Chemical Co. (St. Louis, MO, U.S.A.).

Experimental devices

94 The trap 1 (Fig. 1a) is custom-made, and it consists of a basin (25.0 cm in diameter and
95 10.0 cm in deep) and a triangle bracket. Two drainage holes were formed away from the dish
96 bottom 6.0-7.0 cm. The basin was fixed on the bracket, and the height of the basin was
97 always adjusted to 10.0-20.0 cm higher than the rice. 2.0 g washing powder were added to the
98 water in the basin. Subsequently, the basin was filled with water until the drainage holes. The
99 lures were always fixed 0.5-1.0 cm above the water.

100 The trap 2 (Fig. 1b) was purchased from Pherobio Technology Co., Ltd (Beijing, China),
101 which consists of a ship-type trap and a trestle. The ship-type trap consists of a sticky board, a
102 bezel and a jack device for lures.

103 The olfactometer (Fig. 1c) is custom-made, and it consists of a cuboid reaction section of
104 60.0 cm long by 15.0 cm inner diameters. The cuboid was averagely divided into three parts,
105 an insect-releasing region of the middle part and two testing regions at the two ends,
106 respectively. The insect-releasing region and testing regions was baffled with filter papers. An
107 exit opening was located on the back of the device. The olfactometer was kept on the table,
108 humidified and purified air at 1.0 L/min on both ends was injected into the device. The lures
109 were made of strips of filter paper (1.0 cm × 5.0 cm), which loaded with either 100 μl of the
110 test stimulus, or redistilled hexane (control).

111 **Experimental design**

112 To determine whether mixing the sex pheromones of *C. suppressalis* and *C. medinalis*
113 interfere the capture of *C. suppressalis* and *C. medinalis*, we tested the attraction of the traps
114 baited with CCS and CCM to these two pests in the field (Experiment 1) (Table 1). The traps

115 (trap 1) with two sex pheromones and CCS (control) were tested with 3 traps for 3 replicates
116 from July to September 2012. Trap captures were counted every day.

117 To determine the optimal concentration of CS (three components of *C. suppressalis* sex
118 pheromone (Z11-16:Ald, Z13-18:Ald, Z9-16:Ald=46:6:5)) in behavioral experiments, we
119 tested responses of *C. suppressalis* males in the wind tunnel to its sex pheromones at different
120 concentrations (Experiment 2) (Table 1). The wind tunnel was a cylindrical (2.0 m in length
121 and 0.5 m in diameter) aluminum frame with glass walls. The experimental operation of the
122 wind tunnel is the same with it in our another experiment (Luo et al. 2020). The pheromone
123 source with different concentrations (0.1, 1.0, 10.0 μg/μl) of CS were placed in a 4.0 cm^2 mesh
124 cage and were placed 20.0 cm from the upper air outlet of wind tunnel. One-day-old male adults
125 were placed in a steel screen platform which was placed above the floor of the wind tunnel.
126 Thirty males were tested for each treatment and each male was tested only once. After release,
127 male behavior was observed for 2.0 min. Behaviors recorded included exciting, taking flight
128 from the release cage, oriented flight, contacting the pheromone source.

129 To determine the response of male *C. suppressalis* to sex pheromone components of *C.*
130 *medinalis*, we tested the effects of different sex pheromone components of *C. medinalis* to male
131 *C. suppressalis* with a two-way choice bioassay by using a custom-made olfactometer
132 (Experiment 3) (Table 1). For each bioassay, male *C. suppressalis* individually was introduced
133 into the insect-releasing region of the device. The males were released after the barriers (two
134 filter papers) were pulled out. The male *C. suppressalis* initiated flight and migrated to one of
135 the testing ends (set over half of the choice chambers) and the choice was recorded after it

136 remained for at least 60.0 s in that testing end. If the male did not make a choice within 5.0 min
137 after being released into the olfactometer, it was considered as a non-responder. We rotated the
138 olfactometer 180° to randomize any positional effects after five males have been tested. We
139 cleared the olfactometer with 95% ethyl alcohol after ten males have been tested. The test filter
140 papers were used as the lures in this study, which containing different sex pheromone
141 components. The lures were CCM; CCM+CCS; Z11-18:Ald; Z13-18:OH; Z11-18:OH; four-
142 component blend of CCM, at a ratio of 100:36:11:24, of the Z13-18:Ald, Z13-18:OH, Z11-
143 18:Ald and Z11-18:OH (CM); the components of CCS and CCM, at a ratio of
144 140:117:15:36:11:24, of the Z11-16:Ald, Z13-18:Ald, Z9-16:Ald, Z13-18:OH, Z11-18:Ald,
145 Z11-18:OH (CS+CM). Thirty males were tested in each treatment.

146 To determine the effects of the sex pheromone components of *C. medinalis* on *C.*
147 *suppressalis* in the paddy field, we tested the attraction of male *C. suppressalis* to the traps
148 baited with a combination of CCS and the components of CCM by the trap 2 (Experiment 4)
149 (Table 1). Combined Z11-18:Ald, Z13-18:OH and Z11-18:OH with CCS respectively were the
150 treatments and CCS alone was the control treatment. Each treatment was tested with 3 traps for
151 3 replicates from June to August 2014. Trap captures were also counted every day.

152 **Data analysis**

153 We compared the difference of capture of *C. suppressalis* males between treatments and
154 control treatment with a *t*-test by SPSS 24.0 (SPSS Inc., Chicago, IL). Differences in
155 olfactometer experiments were analyzed by a *Chi*-square test. The percentage data were

arcsine-square-root transformed and statistically evaluated by one-way ANOVA followed by Duncan's new multiple range test. Significance level was set at 0.05.

Results

Mixing the sex pheromones of *C. suppressalis* and *C. medinalis* interfered the capture of *C. suppressalis*

During the experimental period (July to September 2012), we found that no *C. suppressalis* male and ten *C. medinalis* males were caught in the traps baited with combination of CCS and CCM. However, the total captures by CCS were 254 and the average trap caught by CCS during the trial was 2.41 males per trap per day, which were extremely significant higher than those in treatment of combining CCS and CCM ($t_{(76)} = 6.045$, $P < 0.001$) (Fig. 2). The peak point of male *C. suppressalis* captured by its sex pheromone (CCS) appeared on August 29th, 2012. While, there was zero *C. suppressalis* caught by the combination of CCS and CCM during the whole period. This result suggested that mixing the sex pheromones of *C. suppressalis* and *C. medinalis* interfered the capture of *C. suppressalis* in the field.

Z11-18:OH and Z13-18:OH are two major components of CCM that inhibited the attraction of male *C. suppressalis* in olfactory behavior

To determine the optimal concentration of CS components in behavioral experiments, we tested the response of *C. suppressalis* males to CCS and three concentrations of CS (Z11-16Ald: Z13-18Ald: Z9-16Ald = 140:17:15) in the wind tunnel experiment (Table 2). The result showed that the CCS gave the highest percentage of *C. suppressalis* males exciting,

177 taking flight, orienting flight and contacting source among the five treatments. Among the
178 three concentrations of the three components (Z11-16Ald: Z13-18Ald: Z9-16Ald =
179 140:17:15) of CS, *C. suppressalis* males showed high levels of exciting, taking flight,
180 orienting flight and contacting source at the concentration of 1.0 μg/μl. In addition, there was
181 no difference observed in the four behaviors in the concentrations of 0.1, 10.0 μg/μl and
182 control. Therefore, 1.0 μg/μl was used as the optimal concentration of CS in the further
183 behavioral experiments.

184 To determine the response of *C. suppressalis* male to sex pheromone components of *C.*
185 *medinalis*, we tested the attractiveness of the seven lures to *C. suppressalis* males by a
186 custom-made olfactometer (Fig. 3). We found that the lures of Z11-18:Ald (χ^2 = 1.067, P =
187 0.302), Z13-18:OH (χ^2 = 1.067, P = 0.302) and CCS+CCM (χ^2 = 1.067, P = 0.302) have no
188 significant effects on responses of *C. suppressalis* males. However, the males were
189 predominantly attracted to the control than to Z11-18:OH (χ^2 = 32.267, P < 0.001), CM (χ^2 =
190 17.067, P < 0.001), CS+CM (χ^2 = 38.400, P < 0.001) and CCM (χ^2 = 13.067, P < 0.001)
191 treatments. Which suggested that Z11-18:OH is the major component of *C. medinalis* to
192 inhibit responses of *C. suppressalis* males.

193 **Adding Z11-18:OH and Z13-18:OH to the CCS significantly decreased the captures of**
194 ***C. suppressalis* males in the field experiment**

195 We further determined the inhibition effects of sex pheromone components of *C.*
196 *medinalis* on response of *C. suppressalis* male in the field. The results showed that adding
197 Z11-18:OH ($t_{(26)}$ = 2.334, P < 0.05) or Z13-18:OH ($t_{(26)}$ = 2.252, P < 0.05) to the CCS

198 obviously inhibited the capture of *C. suppressalis* male in the field (Fig. 4). However,
199 compared with control treatment (CCS), adding Z11-18:OH ($t_{(26)} = 2.042$, $P = 0.051$) has no
200 significant difference on response of *C. suppressalis* male. Which suggested that adding Z11-
201 18:OH or Z13-18:OH to the CCS could significantly inhibit the attraction of CCS to *C.*
202 *suppressalis* male in the field.
203 We further investigated the response of combination Z11-18:OH, Z13-18:OH and CCS to
204 the *C. suppressalis* male. We also found that adding both Z11-18:OH and Z13-18:OH ($t_{(24)} =$
205 4.677, $P < 0.001$) to the CCS could significantly inhibit the attraction of CCS to *C.*
206 *suppressalis* male in the field (Fig. 5). The means of total captures by CCS without and with
207 both Z11-18:OH and Z13-18:OH were 18.66 and 2.33, respectively.

208 ## Discussion

209 In this study, we found that mixing the sex pheromones of *C. suppressalis* and *C.*
210 *medinalis* interfered the capture of *C. suppressalis* male in the field experiment. Our finding
211 agreed with the result of Gemeno et al. (2006), which has shown that mixing sex pheromones
212 of two insect species resulted in significantly lower captures of one of these species.
213 Similarly, attraction of *Tetanolita mynesalis* (Lepidoptera: Noctuidae) to its own pheromone
214 was inhibited when mixing with the sex pheromone of *Lacinipolia renigera* (Lepidoptera:
215 Noctuidae) (Haynes et al. 2002), and the sex pheromone of *Adoxophyes orana* (Lepidoptera:
216 Corynidae) inhibited the attraction of *Cydia pomonella* (Lepidoptera: Tortricidae) (Potting et
217 al. 1999). Such phenomena of inhibition or antagonism in the sex pheromone systems of
218 several insect species probably contribute to the reproductive isolation of closely related

219 species (same genus) (Roelofs and Cardé 1974, Borden 1997, Cardé and Haynes 2004). In
220 addition, pheromone inhibition or antagonism may occur between species that are not closely
221 related (different genera). For instance, Lopez et al. (1990) found that multispecies sex
222 pheromone traps caused a reduction of baiting in more than one species that the baiting in
223 individual species traps. Our results demonstrated that CCM inhibited the attraction of *C.*
224 *suppressalis* and we did not recommend the use of these two sex pheromones together in the
225 field.

226 Sex pheromones in many insect species are composed of multiple components. In such
227 cases, air permeation with an individual component can often disrupt the sexual
228 communication between male and female. Our olfactory experiments and field tests showed
229 that adding Z11-18:OH and Z13-18:OH to the traps baited with CCS caused the antagonism
230 in attraction of *C. suppressalis* male. The results are similar to the studies of the yellow stem
231 borer *Scirpophaga incertulas* (Lepidoptera: Pyralidae), which showed that Z-11-hexadecenol
232 from *S. incertulas* sex pheromone inhibited the captures of *C. suppressalis* males (Cork and
233 Basu 1996). Some alcohols have been shown could be synergists or inhibitors of many insect
234 pheromones. Yu et al. (2014) reported that 1-undecanol acted as sex pheromone synergist to
235 enhance the attraction of male *Grapholita molesta* (Lepidoptera: Tortricidae) pheromone
236 traps. Several previous studies have demonstrated that Z-11-hexadecenol inhibited the
237 attraction of male *Mamestra brassicae* (Lepidoptera: Noctuidae) (Struble et al. 1980), as well
238 as the attraction of *Cydia pomonella* (Lepidoptera: Tortricidae) was inhibited by Z-9-

239 tetradecenol (Chisholm et al. 1983). Our results indicated that the alcohols Z11-18:OH and
240 Z13-18:OH may act as the inhibitors for *C. suppressalis* sex pheromone.

241 In summary, we found that combined using of CCS and CCM in the trip caused no
242 capture of *C. suppressalis* male in the field experiment. Our results also demonstrated that
243 Z11-18:OH and Z13-18:OH could improve the efficiency of mating disruption by inhibiting
244 the attraction of male *C. suppressalis* to CCS. Therefore, we do not suggest that using sex
245 pheromones of these two species together in the field. Our finding is very important to guide
246 farmers in agricultural activates. In addition, we also suggest that Z11-18:OH and Z13-18:OH
247 could be potential repellents or antagonists of *C. suppressalis* sex pheromone, which may
248 contribute to biological control in sex pheromone-mediate mating disruption in future.

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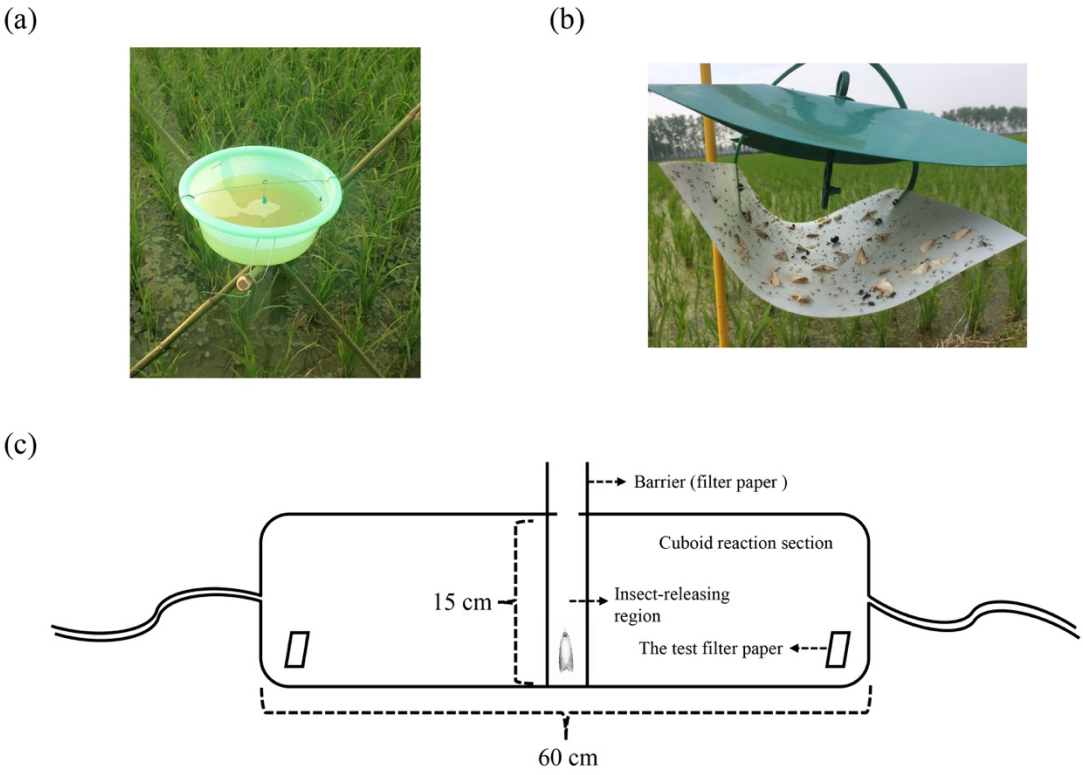

Fig. 1 The schematic diagrams of three experimental devices in this study. (a) trap 1, (b) trap 2 and (c) olfactometer.

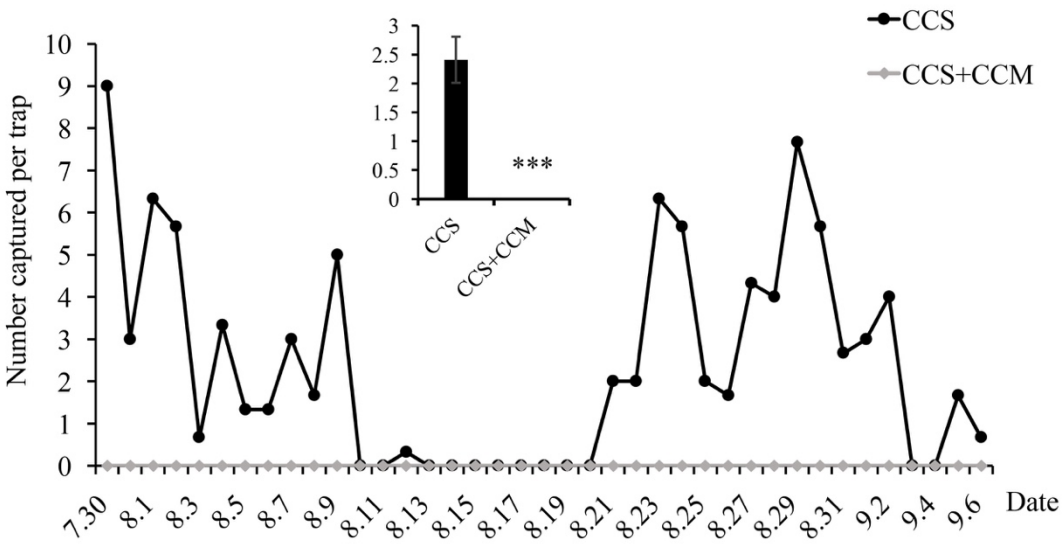

Fig. 2 The number of *C. suppressalis* males captured by commercial *C. suppressalis* sex pheromone alone (CCS) (black) and combined with commercial *C. medinalis* sex pheromone (CCS+CCM) (gray) in Jiangxi, China, from July to September 2012. We compared the difference of capture of *C. suppressalis* males between treatments and control treatment with a *t*-test by SPSS 12.0. ***, $P < 0.001$.

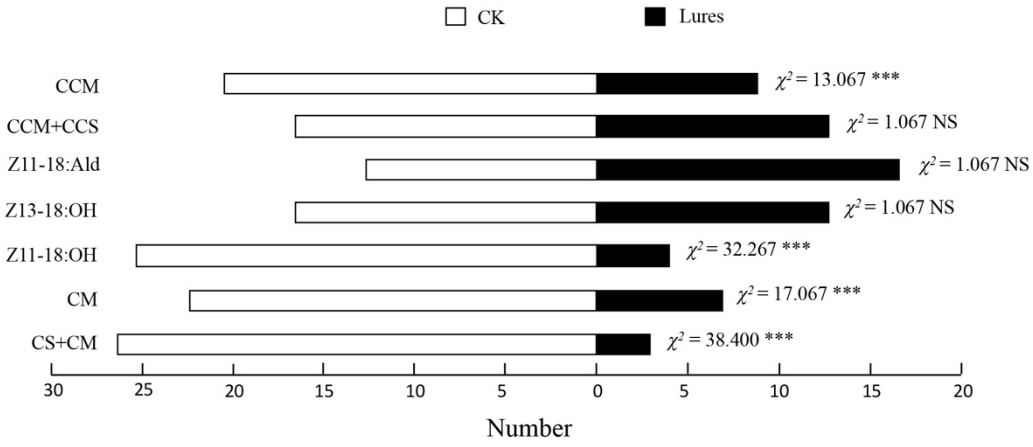

Fig. 3 Response of male *C. suppressalis* to seven lures over controls in an olfactometer. CCM means commercial *C. medinalis* sex pheromone; CCM+CCS means combination of commercial *C. medinalis* and *C. suppressalis* sex pheromones; CM means the four components of *C. medinalis* sex pheromone (Z13-18:Ald, Z13-18:OH, Z11-18:Ald, Z11-18:OH=100:36:11:24); CS+CM means combination of the components of *C. suppressalis* and *C. medinalis* sex pheromones (Z11-16:Ald, Z13-18:Ald, Z9-16:Ald, Z13-18:OH, Z11-18:Ald, Z11-18:OH=46:106:5:36:11:24). The concentrations of the lures are 1 μg/μl except the two commercial sex pheromones. The control (CK) is redistilled hexane. Significance levels of χ^2 (*Chi*-square test) indicated by *** ($P < 0.001$) or NS (no significant difference). $N = 30$.

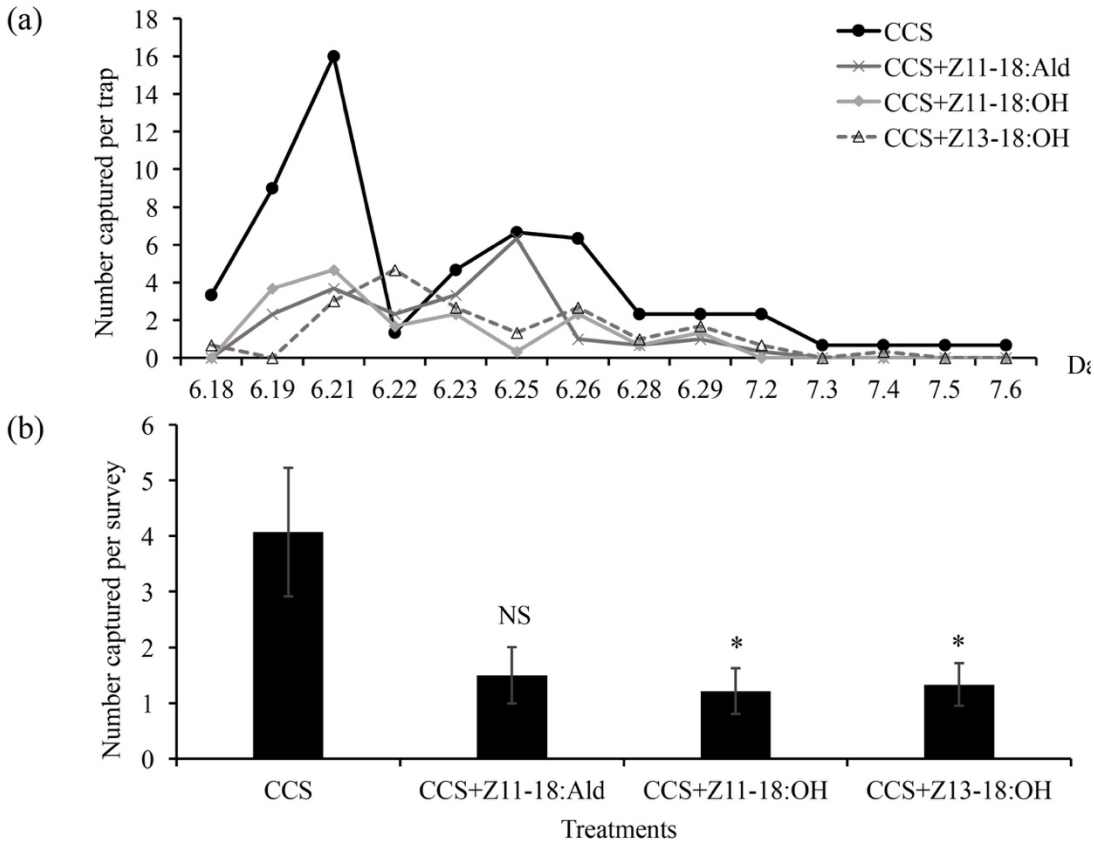

Fig. 4 The dynamic linear graph (a) and summary graph (b) of number of *C. suppressalis* males captured by commercial *C. suppressalis* sex pheromone alone (CCS) and combined with the components of *C. medinalis* sex pheromone (Z11-18:Ald, Z11-18:OH, Z13-18:OH) in Jiangxi, China, from June to July 2014. The difference of capture of *C. suppressalis* males between treatments and control treatment were analyzed with a *t*-test. *, $P < 0.05$; NS, no significant difference.

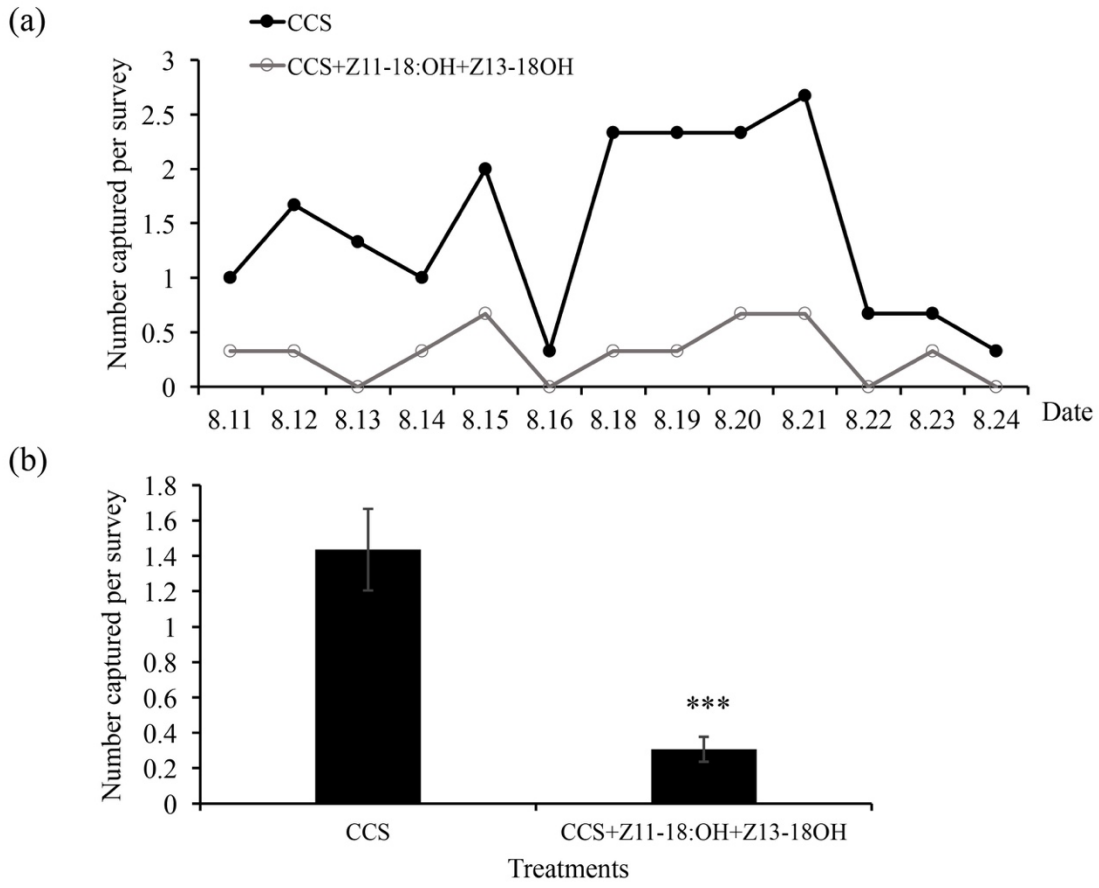

Fig. 5 The dynamic linear graph (a) and summary graph (b) of number of *C. suppressalis* males captured by commercial *C. suppressalis* sex pheromone alone (CCS) and CCS plus individual components (Z11-18:OH and Z13-18:OH) in Jiangxi, China, from 11th to 24th August, 2014. The difference of capture of *C. suppressalis* males between treatments and control treatment were analyzed with a *t*-test. ***, $P < 0.001$.

Table 1 The experimental design

Experiment	Experimental purpose	Experimental device	Time
Experiment 1	To determine whether mixing the sex pheromones of *C. suppressalis* and *C. medinalis* interfere the capture of *C. suppressalis* and *C. medinalis*	Trap 1	July to September 2012
Experiment 2	To determine the optimal concentration of CS components in behavioral experiments	Wind tunnel	July to August 2013
Experiment 3	To determine the response of *C. suppressalis* male to sex pheromone components of *C. medinalis*	Olfactometer	July to August 2013
Experiment 4	To determine the effects of the sex pheromone components of *C. medinalis* on *C. suppressalis* in the field	Trap 2	June to August 2014

Table 2 The different behavioral responses of *C. suppressalis* males in the wind tunnel to the sex pheromones at different concentrations

Treatments	Response (%)			
	Exciting	Taking flight	Orienting flight	Contacting source
CCS	53.3 ± 5.8a	36.7 ± 11.5a	23.3 ± 5.8a	13.3 ± 5.8a
CS (0.1 μg/μl)	16.7 ± 5.8c	10.0 ± 0.0b	6.7 ± 5.8b	6.7 ± 5.8b
CS (1 μg/μl)	36.7 ± 5.8b	20.0 ± 0.0b	10.0 ± 10.0b	6.7 ± 5.8b
CS (10 μg/μl)	20.0 ± 0.0c	10.0 ± 10.0b	0.0 ± 0.0b	0.0 ± 0.0b
CK	20.0 ± 10.0c	13.3 ± 5.8b	0.0 ± 0.0b	0.0 ± 0.0b

Data in the table are means ± *SE*, and those in the same column followed by different letters are significantly different ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). CCS means commercial *C. suppressalis* sex pheromone. CS means the three components of *C. suppressalis* sex pheromone (Z11-16:Ald, Z13-18:Ald, Z9-16:Ald=46:6:5). The control (CK) is redistilled hexane. $N = 30$.