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Sensory Adaptation of Antennae and Sex Pheromone-Mediated Flight Behavior in Male Oriental Fruit Moths (Leptidoptera: Tortricidae) After Prolonged Exposure to Single and Tertiary Blends of Synthetic Sex Pheromone

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ABSTRACT Sensory adaptation has been measured in the antennae of male *Grapholita molesta* (Busck) after 15 min of exposure to its main pheromone compound (Z)-8-dodecen-1-yl acetate (Z8-12:OAc) at the aerial concentration of 1 ng/m^3 measured in orchards treated with pheromone for mating disruption. Exposing males to this aerial concentration of Z8-12:OAc for 15 min, however, had only a small effect on their ability to orientate by flight to virgin calling females in a flight tunnel. Experiments were undertaken to determine if exposure to the main pheromone compound in combination with the two biologically active minor compounds of this species, (E)-8-dodecen-1-yl acetate (E8-12:OAc) and (Z)-8-dodecen-1-ol (Z8-12:OH) would induce greater levels of sensory adaptation and have a greater effect on male sexual behavior. The exposure of male antennae to 0.5 g/m^3 air of one of the three pheromone compounds induced sensory adaptation to this compound and to the other two pheromone compounds demonstrating cross adaptation. Average percentage sensory adaptation to a pheromone compound was similar after 15 min of exposure to 1 ng/m^3 air of Z8-12:OAc, or to 1 ng/m^3 air of a 1:1:1 or 93:6:1 blend of Z8-12:OAc, E8-12:OAc, and Z8-12:OH. The exposure of males to 1 ng/m^3 air of Z8-12:OAc or the two ratios of Z8-12:OAc, E8-12:OAc, and Z8-12:OH for 15 min had no effect on their ability to orientate to a virgin calling female. The implications of these results for the operative mechanisms of sex pheromone-mediated mating disruption of this species are discussed.

KEY WORDS *Grapholita molesta*, sex pheromone, sensory adaptation, mating disruption

Mating disruption using synthetic sex pheromone is an effective alternative to the use of insecticide for the control of some moth pests of field crops, orchards, and vineyards (Cardé and Minks 1995). Disruption is accomplished by inundating a cropping environment with synthetic pheromone that is dispensed from atomizers, sealed plastic tubes, open-ended hollow fibers, laminated plastic flakes, or microcapsules (Cardé 2007). A reduction in the responsiveness of antennal sensory neurons to pheromone (sensory adaptation) and/or the central nervous system (habituation), and competition between synthetic and natural sources of pheromone (competitive attraction) have been proposed as modes of action of these pheromone treatments (Bartell 1982; Cardé 1990, 2007; Cardé and Minks 1995; Sanders 1997; Miller et al. 2006a, 2006b). It has been widely assumed that a blend of synthetic pheromone compounds that is qualitatively and quantitatively similar to the natural sex attractant would provide the most efficacious mating disruption of moths, however, there is only limited evidence supporting this assumption (Minks and Cardé 1988).

Sex pheromone-mediated mating disruption has been used to effectively control the oriental fruit moth, *Grapholita molesta* (Busck) (e.g., Trimble et al. 2001, 2004), a worldwide pest of stone and pome fruit (Rothschild and Vickers 1991). Pheromone treatments for the control of *G. molesta* may cause sensory adaptation (Baker et al. 1988; Trimble and Marshall 2007, 2010) and/or habituation (Sanders and Lucuik 1996, Rumbo and Vickers 1997), as well as competitive attraction (Sanders and Lucuik 1996, Valeur and Löfstedt 1996, Maini and Accinelli 2001, Stelinski et al. 2004). A reduction in the mate searching efficiency of male *G. molesta* would either prevent or delay the mating of females (Barclay and Judd 1995); however, the reproductive potential of *G. molesta* declined ≈7% for each day that mating was delayed after emergence (Fraser and Trimble 2001), suggesting that the successful control of this species by mating disruption is

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more likely accomplished by preventing mating than by delaying mating.

The sex pheromone of *G. molesta* contains the main compound (Z)-8-dodecen-1-yl acetate (Z8-12:OAc) (Roelofs et al. 1969) and the minor compounds (E)-8-dodecen-1-yl acetate (E8-12:OAc), (Z)-8-dodecen-1-ol (Z8-12:OH), and dodecanol (12:OH) (Cardé et al. 1979), with the first three of these compounds acting together to elicit male sexual flight behavior (Baker and Cardé 1979). Pheromone blend has been found to affect the efficacy of mating disruption of *G. molesta.* For example, Charlton and Cardé (1981) found that a three compound blend of Z8-12:OAc, E8-12:OAc, and Z8-12:OH (≈100:7:10) was a more effective disruptant of the capture of male moths in pheromone-baited traps than a two compound blend of Z8-12:OAc and E8-12:OAc (100:7). A highly efficacious pheromone dispenser for mating disruption of *G. molesta* contains a three compound blend of Z8-12:OAc, E8-12:OAc, and Z8-12:OH (100:6:1) (e.g., Trimble et al. 2004).

In their study of the relationship between sensory adaptation of *G. molesta* antennae and aerial concentration of pheromone, Trimble and Marshall (2010) estimated a 16 and 28% reduction in sensitivity to pheromone after 15 and 30 min of exposure to the concentration of 1 ng pheromone/m^3 air that has been measured in orchards treated with pheromone for mating disruption. They used only the main pheromone compound, Z8-12:OAc, in their study, and may therefore have underestimated the role of sensory adaptation as an operative mechanism in the mating disruption of this species. The first objective of the current study was to determine if continuous exposure to the minor compounds E8-12:OAc and Z8-12:OH would induce sensory adaptation to these compounds, and if exposure to one of the three compounds would induce adaptation to the other two pheromone compounds (i.e., cross adaptation). The second objective was to compare the degree of adaptation when antennae were exposed only to Z8-12:OAc and to blends of Z8-12:OAc, E8-12:OAc, and Z8-12:OH. The final objective was to compare the sexual flight behavior of males in response to virgin calling females after prolonged exposure only to the main compound or to blends of the main compound and two minor compounds.

Materials and Methods

Insects. Pupae were harvested from an insecticide-susceptible laboratory colony (Pree et al. 1998). The larvae were reared on 3-4 cm diameter green apples that had not been sprayed with insecticide (Pree 1985). Male and female pupae were held separately for emergence at 23°C, 60% relative humidity (RH), and at a photoperiod of 16:8 (L:D) h in 33 by 33 by 33 cm Plexiglas cages. The sexes were isolated from each other in separate rooms.

Pheromone. Pheromone compounds were obtained from the Pherobank, Plant Research International, Wageningen, The Netherlands. Z8-12:AOc was 99.0% chemically pure and contained 0.2% (E)-8-dodecen-1-yl acetate (E8-12:OAc). E8-12:OAc and Z8-12:OH were 99% chemically pure.

Pheromone Dose—EAG Response. The effect of Z8-12:OAc, E8-12:OAc, and Z8-12:OH dose on electroantennogram (EAG) response was determined using the Syntech (Hilversum, The Netherlands) EAG system described by Trimble and Marshall (2007). Airflow of 2 liter/min was delivered to the antennal preparation through a 30 cm-long glass air delivery tube with a single 2 mm-diameter hole 10 cm from the outlet. Test stimuli were applied to a 1 by 5 cm piece of Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, Kent, United Kingdom) in 50 μl of 99.9% pure ethanol (Commercial Alcohols Inc., Brampton, Ontario, Canada). The filter paper rectangles were placed in a ≈3 ml-capacity Pasteur pipette after the solvent had evaporated for 30 min in a fume hood. A test stimulus was delivered during 0.5 s in a 5 ml "puff" of air while the tip of Pasteur pipette was inserted through the hole in the air delivery tube. An antenna was first stimulated with the control (i.e., 50 μl ethanol) and then with 10 increasing doses of one compound at 1 min intervals over the range 1.0 by 10^{-11} to 1.0 by 10^{-2} g pheromone. The stimulus source was renewed after 4 h of use. Ten antennae were tested with each pheromone compound.

Comparison of Sensory Adaptation to Z8- and E8-12:OAc and to Z8-12:OH After Prolonged Exposure to One Compound. The EAG system and methods described by Trimble and Marshall (2010) for inducing and measuring sensory adaptation in moth antennae were used to determine if male *G. molesta* antennae exhibit sensory adaptation after continuous exposure to the minor pheromone compounds E8-12:OAc and Z8-12:OH, and if there is cross adaptation between the three pheromone compounds of this species. The pheromone sprayer developed by El-Sayed et al. (1999) and modified by Trimble and Marshall (2007) to permit the atomization of pheromone in ethanol solutions at rates as low as 0.125 μl/min was used to condition air with pheromone. The sprayer components were a microdialysis pump, a 50 μl gas-tight syringe connected to an atomization nozzle with fluorinated ethylene propylene (FEP) tubing, and a function generator that excited a piezo-electric bending motor attached to the nozzle. The tip of the pheromone sprayer nozzle was positioned in the middle of the EAG air delivery tube through a second 2 mm-diameter hole located 20 cm from the outlet end of the tube. Ethanol and the solutions of pheromone in ethanol were atomized at 0.125 μl/min. Antennae were exposed to the following treatments: control 1 (air: standard humidified and activated carbon-filtered airflow @ 2 liter/min), control 2 (ethanol: air + ethanol @ 0.125 μl/min), and Z8-12:OAc, E8-12:OAc, or Z8-12:OH (air + ethanol + pheromone compound @ 8.0 mg/ml ethanol). The 8.0 mg Z8-12:OAc/ml ethanol solution delivered at 0.125 μl/min into the airflow of 2 liter/min produced a resultant aerial concentration of 0.5 ng pheromone/ml air (0.5 g/m^3 air) (Trimble and Marshall 2007). After 15 min of exposure to this

aerial concentration of Z8-12:OAc there was an 80% reduction in EAG response to a 10 μg Z8-12:OAc stimulus (Trimble and Marshall 2007, 2010). An experiment was begun by first measuring an EAG response to the control (i.e., 50 μl ethanol) and then 1 min later to a 10 μg Z8-12:OAc, 100 μg E8-12:OAc, and 100 μg Z8-12:OH stimulus at 1-min intervals. Exposure to the ethanol and ethanol + pheromone compound treatments was begun within 15 s after measuring the last EAG response to pheromone by inserting the spray nozzle into the air delivery tube. After 15 min of exposure to a treatment a second EAG response to the control was measured and 1 min later a second EAG response to the 10 μg Z8-12:OAc, 100 μg E8-12:OAc, and 100 μg Z8-12:OH stimuli was measured. Five antennae were successively tested first with the air treatment, and then with the ethanol, ethanol + Z8-12:OAc, ethanol + E8-12:OAc, and ethanol + Z8-12:OH treatments. A complete treatment delivery system including a 50 μl-capacity syringe, FEP line, sprayer nozzle, and air delivery tube were dedicated to each treatment. The air delivery tube was removed, washed, and rinsed with acetone after each antennae was tested.

Comparison of Sensory Adaptation to Z8-12:OAc, E8-12:OAc, and Z8-12:OH After Exposure to Z8-12:OAc Alone and To Two Ratios of Z8-12:OAc, E8-12:OAc, and Z8-12:OH. The EAG system used to compare sensory adaptation to Z8- and E8-12:OAc and to Z8-12:OH after prolonged exposure to one compound in the previous section, were used to compare the amount of sensory adaptation in antennae after exposure to 1 ng/ml air of Z8-12:OAc and two mixtures of Z8-12:OAc + E8-12:OAc + Z8-12:OH. Antennae were exposed to the following treatments: control 1 (air: standard humidified and activated carbon-filtered airflow @ 2 liter/min), control 2 (ethanol: air + ethanol @ 0.125 μl/min), Z8-12:OAc (air + ethanol + pheromone compound @ 1.6 by 10^{-5} mg/ml ethanol, and Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1 or 93:6:1) (air + ethanol + pheromone compounds @ 1.6 by 10^{-5} mg/ml ethanol. Ethanol and the solutions of pheromone and ethanol were atomized at 0.125 μl/min. The 1.6 by 10^{-5} mg pheromone/ml ethanol solutions delivered at 0.125 μl/min into the airflow of 2 liter/min produced a resultant aerial concentration of 1.0 by 10^{-6} ng pheromone/ml air (=1 ng pheromone/m^3 air). The estimated reduction in sensory response was 16% after 15 min of exposure to this aerial concentration of Z8-12:OAc (Trimble and Marshall 2010). An experiment was begun by pairing a randomly selected treatment with a randomly selected pheromone-compound test stimulus (i.e., 10 μg Z8-12:OAc, 100 μg E8-12:OAc, or 100 μg Z8-12:OH). An EAG response was first measured to the control stimulus (i.e., 50 μl ethanol). One minute later a response was measure to the pheromone stimulus and within 15 s exposure to the ethanol, Z8-12:OAc or one of the two Z8-12:OAc + E8-12:OAc + Z8-12:OH treatments commenced. After a 15 min exposure period a second EAG response to the control stimulus was measured and 1 min later a second EAG response to the pheromone stimulus was measured. Five replications of each treatment-pheromone stimulus combination were performed. A complete treatment delivery system including a 50 μl-capacity syringe, FEP line, sprayer nozzle, and air delivery tube were dedicated to each treatment. The air delivery tube was removed, washed, and rinsed with acetone after each antennae was tested.

Comparison of Male Response to Females After Exposure of Males to Z8-12:OAc Alone and Two Ratios of Z8-12:OAc, E8-12:OAc, and Z8-12:OH. The response of a male *G. molesta* to virgin calling *G. molesta* females after 15 min of exposure to one of the same five treatments used to compare sensory adaptation in the previous section was observed in an acrylic plastic flight tunnel (55.5 by 87 by 160 cm, H by W by L) (El-Sayed et al. 2001) using an air velocity of 30 cm/s, temperature of 22–24°C and 50–70% RH. Light intensity was 75 on the floor of the tunnel and 150 lx at the release point of the males. Experiments were conducted during the 3 h before the onset of the scotophase when *G. molesta* males and females exhibit the greatest sexual activity (Baker and Cardé 1979). A method developed by Trimble (2012) was used to pre-expose individual males to one of the five test treatments. One hour before the test period, that is, 3 h before the onset of the scotophase, 1-2 d-old females and 2-3 d-old males were placed individually into a glass tubing "cages" (2 by 2 cm, L by D) that were closed at each end using 0.8 by 0.8 mm-mesh copper screen. Cages holding males were placed on the floor of the flight tunnel for acclimatization. Three female-containing cages were placed on a stand 7.5 cm from the upwind end and 35 cm above the floor of the flight tunnel. One cage was positioned above the centerline of the tunnel (center female) and the other two were positioned 20 cm to the right (right female) and left (left female) of the center female. An experiment was begun when each of the three females were observed calling. The effect of 15 min of exposure to one of the five randomly chosen treatments was tested using the methods of Trimble (2012) and Faraone et al. (2013). The experiment was repeated on 15 d.

Statistical Analysis. Statistical analysis was performed using JMP 7.0 (SAS Institute, Cary, NC). EAG responses and the times to initiate a behavioral response were tested for goodness-of-fit of to the normal distribution using the Shapiro-Wilk W test. The homogeneity of the variances of means was tested using Bartlett's test. Parametric analysis was used to test the significance of treatment effect when data were normally distributed and variances of means were homogeneous. Nonparametric analysis was used if one or both of these criteria was not fulfilled. The significance of Z8-12:OAc, E8-12:OH, and Z8-12:OH dose on EAG response was tested using Friedman's nonparametric analysis of variance (ANOVA) for blocked data (Zar 2010). The Tukey-type nonparametric test for a randomized complete block ANOVA (Zar 2010) was used to identify significantly different mean EAG responses. The effect of the control and the 0.5 ng and 1.0 by 10^{-6} ng pheromone/ml air treatments on mean

EAG response to the control stimulus and net EAG response to the pheromone stimuli (i.e., EAG response to pheromone stimulus–EAG response to control stimulus) was tested using the nonparametric Wilcoxon Sign-Rank test. The percentage reduction in net EAG response to a pheromone stimulus was computed for each antenna exposed to each of the three 1.0 by 10^{-6} ng pheromone/ml air treatments as ([net pretreatment EAG response–net posttreatment EAG response]/net pretreatment EAG response)*100 (Trimble and Marshall 2007). The significance of the effect of treatment on differences in mean percentage reduction in response was tested using the Kruskal–Wallis test. The effect of treatment on the number of males initiating each of the five behavioral phases of upwind flight response was tested using logistic regression analysis as described by Trimble (2012). The Kruskal–Wallis test was used to test the significance of treatment on the mean time required to initiate a behavior.

Results

Pheromone Dose–EAG Response. EAG response was affected by dose of the Z8-12:OAc stimulus ($\chi^2_{10} = 94.0$, $P < 0.001$; Fig. 1). A statistically significant response greater than the response to the control was detected using 1 by 10^{-6} g (i.e., 1 μg) of pheromone. There was no statistically detectable change in response at higher doses of pheromone. The 1 by 10^{-5} g dose (i.e., 10 μg) was selected for use in experiments measuring treatment effect on the responsiveness of antennae. This dose was used by Trimble and Marshall (2007, 2010) in their studies of sensory adaptation in *G. molesta* antennae. EAG response was also affected by dose of the E8-12:OAc ($\chi^2_{10} = 83.4$; $P < 0.001$) and Z8-12:OH ($\chi^2_{10} = 92.8$; $P < 0.001$) stimuli (Fig. 1). A dose of 1 by 10^{-5} g (i.e., 10 μg) of E8-12:OAc and 1 by 10^{-6} g (i.e., 1 μg) of Z8-12:OH were required to elicit a greater response than the response to the control. There was no statistically detectable change in response at higher doses of these compounds. The EAG response to a dose of 1 by 10^{-4} g (100 μg) was ≈4× greater than the response to the control and was therefore was selected for use in experiments measuring treatment effect on the responsiveness of antennae.

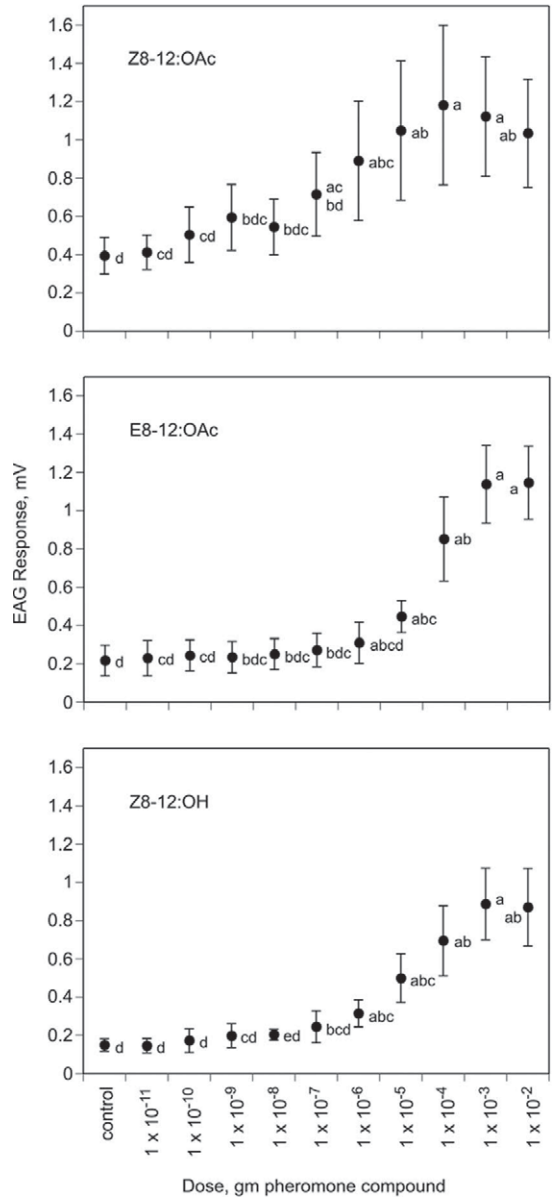

Fig. 1. Mean ± SD EAG response (mV) of male *G. molesta* antennae after stimulation with increasing doses of Z8-12:OAc, E8-12:OAc, and Z8-12:OH (g) at 1 min intervals. Means followed by the same letter are not significantly different (Friedman's nonparametric ANOVA for blocked data followed by Tukey-type nonparametric test for a blocked ANOVA.)

Comparison of Sensory Adaptation to Z8-12:OAc, E8-12:OAc, and Z8-12:OH After Prolonged Exposure to One Compound. Reduction in mean EAG response to the control stimulus was 49.7, 33.5 and 63.2% after 15 min exposure to Z8-12:OAc, E8-12:OAc, and Z8-12:OH, respectively (Table 1). After 15 min of exposure to air, mean net EAG response was reduced by 37% when Z8-12:OAc was used as a stimulus and by 30% when E8-12:OAc was used as a stimulus (Table 2). There was a marginally insignificant (i.e., $P = 0.0625$) reduction of 28% in mean net EAG response to Z8-12:OH after 15 min of exposure to air. After 15 min of exposure to ethanol, there was a marginally insignificant (i.e., $P = 0.0625$) reduction in mean net EAG response of 22 and 9% when Z8-12:OAc and E8-12:OAc were used as stimuli. Reduction in mean net EAG response to Z8-12:OAc, E8-12:OAc, and Z8-12:OH was ≥98% after 15 of exposure to Z8-12:OAc, E8-12:OAc, and Z8-12:OH. The Wilcoxon Sign-Rank test was not used to test the significance of these very large reductions in EAG response.

Comparison of Sensory Adaptation to Z8-12:OAc, E8-12:OAc, and Z8-12:OH After Exposure to Z8-12:OAc Alone and to Two Ratios of Z8-12:OAc, E8-12:

Table 1. Mean ± SD electroantennogram response (EAG) (mV) of male *G. molesta* antennae to a control stimulus before and after 15 min of continuous exposure to five treatments when using three pheromone stimuli

Treatment	Mean ± SD EAG response		Wilcoxon Sign-Rank test	
	Preexposure	Postexposure	*Z*	*P*
Control 1 (air)	0.126 ± 0.020	0.117 ± 0.013	−4.5	0.2
Control 2 (air + ethanol)	0.200 ± 0.025	0.179 ± 0.024	−5.5	0.09
Z8-12:OAc	0.167 ± 0.042	0.084 ± 0.017	−7.5	0.03
E8-12:OAc	0.158 ± 0.020	0.105 ± 0.015	−7.5	0.03
Z8-12:OH	0.261 ± 0.057	0.096 ± 0.002	−7.5	0.03

Five antennae were exposed to each treatment. Antennae were exposed to 6.25 μl ethanol/ml air in the control 2 and pheromone treatments, and to 0.5 ng pheromone/ml air in the Z8-12:OAc, E8-12:OAc, and Z8-12:OH treatments. Treatment effect was measured using stimuli of 10 μg Z8-12:OAc, 100 μg E8-12:OAc, and 100 μg Z8-12:OH delivered at 1-min intervals in the order listed before and after the 15 min treatment exposure period.

OAc, and Z8-12:OH. There was a 40.9% reduction in EAG response to the control stimulus in the Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1) treatment–Z8–12:OAc stimulus combination (Table 3). After 15 min of exposure to ethanol there was a marginally insignificant (i.e., $P = 0.0625$) reduction of 20% in mean EAG response to the control stimulus when Z8-12:OAc was used as the pheromone stimulus compound. There was a marginally insignificant (i.e., $P = 0.0625$) reduction of 25% in mean EAG response to the control stimulus after 15 min of exposure to a 1:1:1 blend of Z8-12:OAc, E8-12:OAc, and Z8-12:OH when E8-12:OH was used as the stimulus compound. After 15 min of exposure to a 93:6:1 blend of Z8-12:OAc, E8-12:OAc, and Z8-12:OH, there was a marginally insignificant (i.e., $P = 0.0625$) reduction of 46% in mean EAG response to the control stimulus when Z8-12:OAc was used as the stimulus compound. Exposure of antennae to air or ethanol for 15 min did not significantly affect mean net EAG response to the three pheromone stimulus compounds (Table 4). After 15 min of exposure to Z8-12:OAc, there was a marginally insignificant reduction (i.e., $P = 0.0625$) of 33 and 64% in mean net EAG response to E8-12:OAc and Z8-12:OH. Mean net EAG response to Z8-12:OAc was reduced by 70% after 15 min of exposure to a 1:1:1 blend of Z8-12:OAc, E8-12:OAc, and Z8-12:OH. After 15 min of exposure to a 93:6:1 blend of Z8-12:OAc, E8-12:OAc, and Z8-12:OAc, mean net EAG response to Z8-12:OAc and E8-12:OAc was reduced by 79 and 32%, respectively. There was a marginally insignificant reduction (i.e., $P = 0.0625$) of 49% in mean net EAG response to Z8-12:OH. Composition of the pheromone treatment did not significantly effect the percent adaptation to Z8-12:OAc, E8-12:OAc, or Z8-12:OH (Table 5).

Comparison of Male Response to Females After Exposure of Males Z8-12:OAc Alone and Two Ratios of Z8-12:OAc, E8-12:OAc, and Z8-12:OH. Fifteen minutes of exposure to the air, ethanol, Z8-12:OAc and two Z8-12:OAc + E8-12:OAc + Z8-12:OH treatments had no effect on the number of male *G. molesta* initiating the activation, take-off, lock-on, close-in, and touchdown phases of upwind flight behavior in response to a virgin, calling *G. molesta* female (Table 6). These treatments also had no effect on the number of males initiating these behaviors in response to the first of the three females that were used test male responsiveness (Table 7). The time required to initiate each of the six upwind flight behaviors was not affected by treatment (Table 8).

Discussion

The prolonged exposure to a complete, three-compound blend of synthetic sex pheromone does not

Table 2. Mean ± SD net electroantennogram response (EAG) (mV) of male *G. molesta* antennae to three pheromone stimuli before and after 15 min of continuous exposure to five treatments

Treatment	Stimulus compound	Mean ± SD net EAG response		Wilcoxon Sign-Rank test	
		Preexposure	Postexposure	*Z*	*P*
Control 1 (air)	Z8-12:OAc	0.879 ± 0.210	0.553 ± 0.180	−7.5	0.03
	E8-12:OAc	0.601 ± 0.117	0.420 ± 0.084	−7.5	0.03
	Z8-12:OH	0.390 ± 0.084	0.279 ± 0.057	−6.5	0.06
Control 2 (air + ethanol)	Z8-12:OAc	1.176 ± 0.244	0.920 ± 0.125	−6.5	0.06
	E8-12:OAc	0.524 ± 0.097	0.476 ± 0.108	−6.5	0.06
	Z8-12:OH	0.418 ± 0.071	0.430 ± 0.079	1.5	0.6
Z8-12:OAc	Z8-12:OAc	0.677 ± 0.204	0.008 ± 0.008	—	—
	E8-12:OAc	0.538 ± 0.128	0.004 ± 0.007	—	—
	Z8-12:OH	0.434 ± 0.109	0.005 ± 0.007	—	—
E8-12:OAc	Z8-12:OAc	0.535 ± 0.169	0.011 ± 0.018	—	—
	E8-12:OAc	0.378 ± 0.058	0.000 ± 0.000	—	—
	Z8-12:OH	0.244 ± 0.052	0.002 ± 0.005	—	—
Z8-12:OH	Z8-12:OAc	0.980 ± 0.175	0.011 ± 0.016	—	—
	E8-12:OAc	0.728 ± 0.153	0.000 ± 0.000	—	—
	Z8-12:OH	0.644 ± 0.155	0.000 ± 0.000	—	—

Five antennae were exposed to each treatment. Antennae were exposed to 6.25 μl ethanol/ml air in the control 2 and pheromone treatments, and to 0.5 ng pheromone/ml air in the Z8-12:OAc, E8-12:OAc, and Z8-12:OH treatments. Treatment effect was measured using stimuli of 10 μg Z8-12:OAc, 100 μg E8-12:OAc, and 100 μg Z8-12:OH delivered at 1-min intervals in the order listed before and after the 15 min treatment exposure period.

Table 3. Mean ± SD electroantennogram response (EAG) (mV) of male *G. molesta* antennae to a control stimulus before and after 15 min of continuous exposure to five treatments when using one of three pheromone stimulus compounds

Treatment	Stimulus compound	Mean ± SD EAG response		Wilcoxon Sign-Rank test	
		Preexposure	Postexposure	*Z*	*P*
Control 1 (air)	Z8-12:OAc	0.164 ± 0.060	0.149 ± 0.075	−5.5	0.09
	E8-12:OAc	0.163 ± 0.072	0.165 ± 0.051	0.0	0.5
	Z8-12:OH	0.119 ± 0.052	0.109 ± 0.048	−5.5	0.09
Control 2 (air + ethanol)	Z8-12:OAc	0.125 ± 0.030	0.100 ± 0.017	−6.5	0.06
	E8-12:OAc	0.125 ± 0.074	0.155 ± 0.060	3.5	0.8
	Z8-12:OH	0.114 ± 0.021	0.103 ± 0.016	−4.5	0.2
Z8-12:OAc	Z8-12:OAc	0.146 ± 0.066	0.128 ± 0.047	−2.5	0.3
	E8-12:OAc	0.103 ± 0.035	0.101 ± 0.049	−2.5	0.3
	Z8-12:OH	0.123 ± 0.043	0.096 ± 0.018	−5.5	0.1
Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1)	Z8-12:OAc	0.137 ± 0.055	0.081 ± 0.021	−7.5	0.03
	E8-12:OAc	0.150 ± 0.036	0.112 ± 0.032	−6.5	0.06
	Z8-12:OH	0.120 ± 0.023	0.093 ± 0.030	−5.5	0.09
Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1)	Z8-12:OAc	0.172 ± 0.033	0.093 ± 0.023	−6.5	0.06
	E8-12:OAc	0.149 ± 0.041	0.113 ± 0.030	−5.5	0.09
	Z8-12:OH	0.136 ± 0.056	0.102 ± 0.028	−5.5	0.09

Five antennae were exposed to each treatment–stimulus compound combination. Antennae were exposed to 6.25 μl ethanol/ml air in the control 2 and pheromone treatments, and to 1.0 by 10^{-6} ng pheromone/ml air in the Z8-12:OAc and Z8-12:OAc + E8-12:OAc + Z8-12:OH treatments.

induce greater levels of sensory adaptation or reductions in the mate-seeking ability of male *G. molesta* than prolonged exposure to only the main pheromone compound. There is evidence of cross adaptation because the exposure of antennae to one compound induces sensory adaptation to this compound as well as to the other two pheromone compounds. It is unlikely that sensory adaptation is an operative mechanism of disruption in this species because the prolonged exposure of males to the main pheromone compound alone, or to blends of each of the three pheromone compounds at the aerial concentration measured in orchards treated with pheromone for mating disruption, has no effect on their ability to locate virgin calling *G. molesta* females in a flight tunnel.

The change in EAG response with each 10-fold increase the amount of Z8-12:OAc used in the stimulus delivery was similar to that previously recorded by Trimble and Marshall (2007), but in the current study the EAG system had greater "sensitivity." In the current study for example, a detectible response to pheromone was obtained using a dose of 0.1 ng, whereas in the earlier study a dose of 100 ng was required to elicit a detectable EAG response. In addition, the maximum average EAG response was −1.160 mV in the current study, but only −0.655 in the previous study. One possible factor contributing to this difference could have been the smaller volume (5 vs. 36 ml) of air used to deliver the pheromone stimulus in the current study. All of the other EAG parameters, that is, the pulse duration of 0.5 s and 3 ml-capacity Pasteur pi-

Table 4. Mean ± SD net electroantennogram response (EAG) (mV) of male *G. molesta* antennae to one of three pheromone stimuli before and after 15 min of continuous exposure to five treatments

Treatment	Stimulus compound	Mean ± SD EAG response		Wilcoxon Sign-Rank test	
		Preexposure	Postexposure	*Z*	*P*
Control 1 (air)	Z8-12:OAc	0.732 ± 0.312	0.712 ± 0.368	1.5	0.6
	E8-12:OAc	0.424 ± 0.244	0.368 ± 0.136	−2.5	0.3
	Z8-12:OH	0.179 ± 0.058	0.209 ± 0.048	4.5	0.8
Control 2 (air + ethanol)	Z8-12:OAc	0.430 ± 0.177	0.468 ± 0.330	0.5	0.5
	E8-12:OAc	0.453 ± 0.175	0.521 ± 0.241	4.5	0.8
	Z8-12:OH	0.199 ± 0.078	0.300 ± 0.070	7.5	1.0
Z8-12:OAc	Z8-12:OAc	0.664 ± 0.339	0.574 ± 0.047	−3.5	0.2
	E8-12:OAc	0.240 ± 0.100	0.162 ± 0.018	−6.5	0.06
	Z8-12:OH	0.257 ± 0.093	0.092 ± 0.080	−6.5	0.06
Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1)	Z8-12:OAc	0.800 ± 0.243	0.242 ± 0.278	−7.5	0.03
	E8-12:OAc	0.324 ± 0.151	0.235 ± 0.309	−4.5	0.2
	Z8-12:OH	0.233 ± 0.152	0.228 ± 0.130	−1.5	0.4
Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1)	Z8-12:OAc	0.496 ± 0.371	0.105 ± 0.124	−7.5	0.03
	E8-12:OAc	0.386 ± 0.227	0.263 ± 0.271	−7.5	0.03
	Z8-12:OH	0.197 ± 0.056	0.101 ± 0.097	−6.5	0.06

Five antennae were exposed to each treatment–stimulus compound combination. Antennae were exposed to 6.25 μl ethanol/ml air in the control 2 and pheromone treatments, and to 1.0 by 10^{-6} ng pheromone/ml air in the Z8-12:OAc and Z8-12:OAc + E8-12:OAc + Z8-12:OH treatments.

Table 5. Mean ± SD percent adaptation of male *G. molesta* antennae to one of three pheromone stimuli after 15 min of continuous exposure to three pheromone treatments

Simulus compound	Treatment	Percent adaptation	Kruskal–Wallis test	
			χ^2	*P*
Z8-12:OAc	Z8-12:OAc	32.3 ± 39.4	4.0	0.1
	Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1)	66.2 ± 34.2		
	Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1)	76.2 ± 27.5		
E8-12:OAc	Z8-12:OAc	30.1 ± 19.4	0.6	0.8
	Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1)	56.3 ± 51.6		
	Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1)	43.0 ± 43.3		
Z8-12:OH	Z8-12:OAc	60.7 ± 35.9	4.4	0.1
	Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1)	14.1 ± 13.1		
	Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1)	56.4 ± 39.4		

Five antennae were exposed to each treatment-stimulus compound combination. Antennae were exposed to 1.0 by 10^{-6} ng pheromone/ml air in the Z8-12:OAc and Z8-12:OAc + E8-12:OAc + Z8-12:OH treatments. Doses of pheromone stimuli used in the EAG were 10 μg Z8-12:OAc and 100 μg E8-12:OAc and Z8-12:OH.

pette, where the same as those used by Trimble and Marshall (2007). The EAG response of *G. molesta* antennae to the minor pheromone compounds E8-12:OAc and Z8-12:OH was demonstrated for the first time. The relative response of antennae to these compounds was only 30% of the response to the main pheromone compound Z8-12:OAc at a stimulus dose of 10 μg.

The prolonged exposure of *G. molesta* antennae to a high-aerial concentration of Z8-12:OAc (0.5 ng pheromone/ml air, i.e. 0.5 g pheromone/m^3 air) induced sensory adaptation to this compound as well as to the minor pheromone compounds E8-12:OAc and Z8-12:OH. This "cross adaptation" also occurred when antennae were exposed to the same aerial concentration of the minor compounds, demonstrating that the exposure to one compound induces adaptation to the other two compounds. One explanation for this phenomenon may be that at least some of the olfactory receptor neurons (ORs) on the antennae of male *G. molesta* respond to all three of this species' pheromone compounds. Single cell recordings from antennal ORs of the codling moth, *Cydia pomonella* L. demonstrated three different types of receptor neurons. The most abundant type was most sensitive to the main pheromone compound (E,E)-8,10-dodecadienol (E8,E10-12:OH). This OR also responded to the geometric isomers E,Z-, Z,E-, and Z,Z- of E8,E10-12:OH, and to (E,E)-8,10-dodecadienyl acetate, an attraction inhibitor of *C. pomonella* (Bäckman et al. 2000). Another possible explanation for the cross adaptation in male *G. molesta* antennae may be interaction of pheromone compound-specific ORs originating in adjacent sensilla. Approximately 40% of the sensilla on the antennae of male corn earworms, *Helicoverpa zea* (Boddie) with a large-spiking OR responding to this species' main pheromone compound (Z)-11-hexadecenal also exhibit small spiking action potentials when the antenna is stimulated with this compound. Lee and Baker (2008) provided experimental evidence that some sensilla of this species are not completely isolated from neighboring sensilla and the small spikes in some recordings originate from large-spiking ORs in neighboring sensilla.

There was no reduction in EAG response to the control stimulus (i.e., 50 μl ethanol) when antennae were exposed to air or ethanol for 15 min, but response to the control declined by 34–63% when antennae were exposed to the high-aerial concentration of Z8-12:OAc, E8-12:OAc, or Z8-12:OH. One possible explanation for these results is that exposure to the high-aerial concentration of pheromone reduced the vitality of an antenna. Another possible explanation is that the stimulation of an antenna with each pheromone compound at 1-min intervals before exposure to pheromone caused a greater reduction in vitality than

Table 6. Number of male *G. molesta* initiating successive phases of upwind flight to a virgin calling *G. molesta* female in a flight tunnel after 15 min of exposure to one of five treatments and results of logistic regression analyses

Treatment	Number				
	Activation	Take-off	Lock-on	Close-in	Touchdown
Control 1 (air)	15	15	15[a]	15[a]	15[a]
Control 2 (air + ethanol)	15	15	15[a]	15[a]	15[a]
Z8-12:OAc	15	15	15[a]	15[a]	15[a]
Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1)	15	15	14	14	14
Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1)	15	15	13	13	13
χ^2	—	—	0.4	0.4	0.4
df	—	—	1	1	1
P	—	—	0.5	0.5	0.5

[a] Excluded from analysis. Fifteen males were tested with each treatment. Males were exposed to 6.25 μl ethanol/ml air in the control 2 and pheromone treatments, and to 1.0 by 10^{-6} ng pheromone/ml air in the Z8-12:OAc, Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1), and Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1) treatments.

Table 7. Number of male *G. molesta* initiating successive phases of upwind flight to a virgin calling *G. molesta* female in a flight tunnel on the first of three attempts after 15 min of exposure to one of five treatments and results of logistic regression analyses

Treatment	Number				
	Activation	Take-off	Lock-on	Close-in	Touchdown
Control 1 (air)	15	15	12	12	12
Control 2 (air + ethanol)	15	14	11	11	11
Z8-12:OAc	15	15	13	13	13
Z8-12:OAc + E812:OAc + Z8-12:OAc (1:1:1)	15	15	11	11	11
Z8-12:OAc + E812:OAc + Z8-12:OAc (93:6:1)	15	15	7	7	7
χ^2	—	—	6.6	6.6	6.6
df	—	—	4	4	4
P	—	—	0.2	0.2	0.2

Fifteen males were tested with each treatment. Males were exposed to 6.25 μl ethanol/ml air in the control 2 and pheromone treatments, and to 1.0 by 10^{-6} ng pheromone/ml air in the Z8-12:OAc, Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1), and Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1) treatments.

when an antenna was first stimulated with the pheromone compounds and exposed to only air or to ethanol. In contrast to the lack of a reduction in EAG response to the control stimulus after exposure to air or to ethanol, net EAG response to Z8-12:OAc and to E8-12:OAc declined by 37 and 30%, respectively, after exposure to air. These reductions may have been caused by stimulation with the three pheromone compounds at 1-min intervals before exposure to air, as postulated above as a potential cause for the reduction in response to the control stimulus. The lack of a significant reduction in net response when antennae were exposed to ethanol suggests that exposure to alcohol reduced the sensory fatigue that may have been caused by stimulation of antennae with Z8-12:OAc, E8-12:OAc, and Z8-12:OH at 1-min intervals. The percentage reduction of 99% in EAG response after exposure to the high-aerial concentration of the main pheromone compound was 20 percentage points greater than observed in previous studies by Trimble and Marshall (2007, 2010). The use of the net EAG response in the current study versus the use of a response that was not adjusted for response to the control stimulus in the previous studies may be one reason for this discrepancy. Other possible reasons for the difference may be the greater sensitivity of EAG system in the current study and the stimulation of antennae with Z8-12:OAc, E8-12:OAc, and Z8-12:OH at 1-min intervals before exposure to the high-aerial concentration of pheromone.

The prolonged exposure of *G. molesta* antennae to a low aerial concentration of pheromone (i.e., 1.0 by 10^{-6} ng/ml air) induced a detectable reduction in net EAG response to Z8-12:OAc when antennae were exposed to a 1:1:1 or a 93:6:1 blend of Z8-12:OAc:E8-12:OAc:Z8-12:OH, but not when using only Z8-12:OAc. The quadratic polynomial regression model of Trimble and Marshall (2010) predicted a reduction in EAG response of 16% after 15 min of exposure to 1.0 by 10^{-6} ng Z8-12:OAc/ml air, and Faraone et al. (2013) measured a 47% reduction in net EAG response of male *G. molesta* antennae after 15 min of exposure to this concentration of pheromone. In the current study, the decline of 14% in net EAG response was similar to that predicted by Trimble and Marshall (2010), but the reduction was not statistically significant ($P = 0.2$). Adaptation to the minor compounds was detectable only for E8-12:OAc (32%) when using the 93:6:1 blend, although after exposure to Z8-12:OAc alone there were marginally insignificant reductions ($P = 0.06$) in net EAG response to E8-12:OAc (33%) and Z8-12:OH (64%), and after exposure to the 93:6:1 blend of compounds there was a marginally insignificant reduction ($P = 0.06$) in EAG response to Z8-12:OH (49%). Average percentage sensory adaptation to a pheromone compound based on both significant

Table 8. Mean (±SD) time (seconds) required for male *G. molesta* to initiate successive phases of upwind flight to a virgin calling *G. molesta* female in a flight tunnel after 15 min of exposure to one of five treatments and results of the Kruskal–Wallis test

Treatment	Activation	Take-off	Lock-on	Close-in	Touchdown
Control 1 (air)	4.13 ± 2.94 (15)	57.51 ± 59.94 (15)	3.88 ± 4.75 (15)	3.24 ± 1.61 (15)	1.53 ± 0.96 (15)
Control 2 (air + ethanol)	5.34 ± 4.14 (15)	53.79 ± 49.50 (15)	4.34 ± 2.03 (15)	2.95 ± 0.97 (15)	2.20 ± 1.54 (15)
Z8-12:OAc	5.54 ± 2.40 (15)	81.41 ± 116.22 (15)	3.90 ± 2.59 (14)	3.17 ± 1.60 (15)	1.73 ± 0.99 (15)
Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1)	4.80 ± 3.53 (15)	74.91 ± 117.15 (15)	5.61 ± 5.85 (14)	3.32 ± 1.54 (14)	1.95 ± 1.33 (14)
Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1)	6.74 ± 6.09 (15)	118.74 ± 137.32 (15)	3.54 ± 1.97 (13)	2.45 ± 1.21 (13)	1.80 ± 1.01 (13)
χ^2	2.9	3.6	4.4	3.6	3.3
df	4	4	4	4	4
P	0.6	0.5	0.4	0.5	0.5

Fifteen males were tested with each treatment. Number of males initiating a behavior in parentheses. Males were exposed to 6.25 μl ethanol/ml air in the control 2 andpheromone treatments, and to 1.0 by 10^{-6} ng pheromone/ml air in the Z8-12:OAc, Z8-12:OAc + E8-12:OAc + Z8-12:OH (1:1:1), and Z8-12:OAc + E8-12:OAc + Z8-12:OH (93:6:1) treatments.

and nonsignificant reductions in net EAG response was not affected by the composition of pheromone to which antennae were exposed, suggesting that there would be no advantage to using a complete blend for mating disruption if sensory adaptation is an operative mechanism of disruption in this species.

There was no reduction in response to the control stimulus (i.e., 50 μl ethanol) when antennae were exposed to the low aerial concentration of Z8-12:OAc, whereas there was a reduction in EAG response to the control of 50% when antennae were exposed the high-aerial concentration of this compound. These contrasting results may be because of the very large difference between the low and high-aerial pheromone concentrations (i.e., ≈31,000×), or to the stimulation of antennae with only one pheromone compound when using the low aerial contraction of pheromone.

It is likely that a longer period of exposure of antennae to the low aerial concentration of Z8-12:OAc would have induced greater adaptation to this compound. The model of Trimble and Marshall (2010) predicted a 1.8-fold increase in adaptation (i.e., 16 vs. 28%) when the exposure period was doubled from 15 to 30 min. Therefore, it is also possible that a longer exposure period would result in significant reductions in response to the minor compounds when using Z8-12:OAc alone and in combination with E8-12:OAc and Z8-12:OH.

It is unlikely that sensory adaptation is an operative mechanism of pheromone-mediated mating disruption of *G. molesta.* The prolonged exposure of males to a blend of synthetic pheromone compounds used in a highly efficacious and commercially available pheromone dispenser at the aerial concentration of 1 ng/m^3 air measured in orchards treated with pheromone had no effect on the mate seeking behavior of males. The exposure of males to a blend containing equal amounts of each of this species' three pheromone compounds, or to the main pheromone compound alone, also had no effect on mate seeking behavior. The results corroborate those of Trimble (2012) and Faraone et al. (2013) who found that the exposure of male *G. molesta* to 1 ng Z8-12:OAc/m^3 air for 15 min had no effect on their ability to orientate to virgin calling females in a flight tunnel. In another flight tunnel experiment, Linn and Roelofs (1981) found that 5 min of pre-exposure to E8-12:OAc did not affect the response of male *G. molesta* to optimally attractive blends of Z8-12:OAc, E8-12:OAc, and Z8-12:OH emitted from a red rubber septum.

In the current study male *G. molesta* were exposed to atmospheric pheromone for 15 min, but under natural conditions they could be exposed to pheromone for many hours before females become sexually active and begin emitting pheromone. In a flight tunnel study, Trimble (2012) observed a 10% reduction in the number of males successfully locating a virgin calling female after 30 min of exposure to 1 ng Z8-12:OAc/m^3 air. It is possible that a longer period of exposure could have a greater impact on male behavior and/or reveal effects of pheromone composition on male behavior.

The ideal conditions of the flight tunnel may have compensated for any impairment of mate seeking behavior induced by preexposure to atmospheric pheromone. *G. molesta* males were positioned 130 cm downwind from a female in a nonturbulent air stream within the zone where the female's pheromone was most likely to occur. In an orchard, males could be much further from a female, and turbulent airflow would likely cause a noncontinuous plume with a lower concentration of pheromone. Under these conditions the degree of sensory adaptation observed after exposure to atmospheric pheromone may reveal effects on the ability of males to locate females.

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