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Reduced Mating Success of Female Tortricid Moths Following Intense Pheromone Auto-Exposure Varies with Sophistication of Mating System

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Abstract Mating disruption is a valuable tool for the management of pest lepidopteran species in many agricultural crops. Many studies have addressed the effect of female pheromone on the ability of males to find calling females but, so far, fewer have addressed the effect of pheromone on the mating behavior of females. We hypothesized that mating of female moth species may be adversely affected following sex pheromone auto-exposure, due to abnormal behavioral activity and/or antennal sensitivity. Our results indicate that, for *Grapholita molesta* and *Pandemis pyrusana* females, copulation, but not calling, was reduced following pre-exposure to sex pheromone. In contrast, for *Cydia pomonella* and *Choristoneura rosaceana*, sex pheromone pre-exposure did not affect either calling or copulation propensity. Adaptation of female moth antennae to their own sex pheromone, following sex pheromone auto-exposure, as measured by electroantennograms, occurred in a species for which identical exposure reduced mating success (*G. molesta*) and in a species for which such exposure did not affect mating success (*C. rosaceana*). These results suggest that pre-exposure of female moths of certain species to sex pheromone may further contribute to the success of pheromone-based mating disruption. Therefore, we conclude that, in some species, mating disruption may include a secondary mechanism that affects the mating behavior of female moths, in addition to that of males.

Keywords Mating disruption · Tortricidae · Autodetection · Cross-adaptation · Biorational pest management

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Introduction

Mating disruption is an effective technique for the control of pest insects in a variety of settings. Successful mating disruption techniques have been established for various lepidopteran pests, including *Cydia pomonella* (Pfeiffer et al., 1993), *Grapholita molesta* (Vickers et al., 1985), and various leafroller species (Pfeiffer et al., 1993). Despite extensive research and development, the mechanisms of mating disruption need to be elucidated further. Superficially, mating disruption interferes with a male's ability to locate a female via her pheromone plume; however, the specific mode of action may be due to a number of mechanisms including, habituation to odorant, arrestment of males at high concentration of pheromone, shifting the rhythm of mating behavior, camouflage of the female plume, and competition for males between the mating disruption point source and the calling female (Cardé and Minks, 1995; Miller et al., 2006; Witzgall et al., 2008). It is important to understand the underlying mechanisms of this technology in order to maximize its efficiency and ensure continued success.

Female sex pheromone autodetection, as measured by responses of olfactory receptor neurons, is known to occur in *Panaxia quadripunctaria* (Schneider et al., 1998) and *C. pomonella* (Ansebo et al., 2005). Similarly, Stelinski et al. (2006a) and Gökçe et al. (2007) demonstrated that female *G. molesta* and *Choristoneura rosaceana* are capable of autodetection. Furthermore, intense exposure to sex pheromone had varying effects on female calling behavior in both species (Stelinski et al., 2006a; Gökçe et al., 2007). Although these investigations did not focus on female mating behavior following sex pheromone pre-exposure, it was noticed that the mating behavior of *G. molesta* and *C. rosaceana* females were affected differentially, following otherwise similar pheromone pre-exposure regimes. Specifically, the mating success of *G.*

molesta, but not that of *C. rosaceana*, appeared reduced. Based on these observations, we hypothesized that intense exposure of female moths to sex pheromone may differentially affect subsequent mating behavior, depending on whether or not the mating ritual includes hair pencil exposure during courtship (Baker and Cardé, 1979a) and the need to recognize a male aphrodisiac pheromone for mating success.

Mating has been described for several tortricid pest species. In general, males orient and fly upwind, in a zigzag pattern, toward females releasing pheromone (Marsh et al., 1978). Pulses in pheromone release, resulting in fluctuating concentration packets of pheromone, are necessary for male moths to respond (Baker et al., 1985). Once a male moth reaches a female, several behaviors may ensue in a short period of time. These can include, wing fanning, head or body contact, release of chemical cues (aphrodisiac pheromones) from specialized structures on male moths (hair-pencils), female approach, and copulation (Baker and Cardé, 1979a; Castrovillo and Cardé, 1980). *Grapholita molesta* is a well characterized tortricid pest species with a highly evolved mating ritual. Baker and Cardé (1979b) described the behavioral sequence and associated probabilities for successful mating, including the requirement of a male aphrodisiac to stimulate female acceptance. Although similar male aphrodisiac signals have not been identified in the tortricid, *Pandemis pyrusana* Kearfott, females require a male signal for mate acceptance that suggests the use of a male aphrodisiac (Curkovic et al., 2006). In contrast, both *C. rosaceana* and *C. pomonella* appear to have relatively simple mating behaviors with no evidence of a male aphrodisiac (Castrovillo and Cardé, 1980; Curkovic et al., 2006).

Despite widespread research documenting how pheromone exposures affect male moth behavior, few studies have addressed how behaviors of females are affected by sex pheromone exposure, and how this might influence the efficacy of mating disruption. In this research, we individually exposed both females and males of four tortricid moth species to their respective female sex attractants, and subsequently assessed ability of females to call and their propensity to copulate.

Methods and Materials

Insects *Cydia pomonella, Grapholita molesta,* and *Choristoneura rosaceana* were obtained from four-, five-, and 8 year-old laboratory colonies, respectively, originally collected as larvae from apple orchards in Southwest Michigan, U.S.A. *Pandemis pyrusana* were from a colony established in 1990 from a commercial apple orchard in Yakima, WA, USA (Curkovic et al., 2006); *Pandemis pyrusana*, collected from an untreated apple orchard in Wenatchee, WA, USA, were added to this colony in 2000 and 2003. All species were reared at 24°C and 60% RH on pinto bean-based diet (Shorey and Hale, 1965), under a 16:8 (L:D) photoperiod. Pupae, sorted by species and sex, emerged in 1 L plastic cages, containing a 5% sucrose solution in a plastic cup with a cotton dental wick protruding from the lid.

Chemicals and Release Devices Moths of each species were pre-exposed to emissions from dispensers containing synthetic components of the specific pheromone. Two types of pheromone dispensers were used to expose moths of each species: 1) red rubber septa (The West Company, Lionville, PA, USA), loaded with partial pheromone blends at dosages known to elicit substantial captures of male moths when deployed in sticky traps in the field; and 2) Isomate polyethylene reservoir dispensers, specifically formulated for mating disruption of each species. The septa were considered a low-release pheromone pre-exposure treatment, while the reservoir dispensers were considered a high release pre-exposure treatment. For *C. pomonella*, septa were loaded with 0.1 mg of (*E*,*E*)-8,10-dodecadien-1-ol (Stelinski et al., 2006a; 99% isomeric and chemical purity; Bedoukian Co., Danbury, CT, USA). The high release dispenser for *C. pomonella* was Isomate-C Plus, containing 205 mg of 53.0% (*E*,*E*)-8,10-dodecadien-1-ol, 29.7% dodecanol, 6.0% tetradecanol, and 11.3% inert ingredients (Shin Etsu, Tokyo, Japan). The release rate of an Isomate-C Plus dispenser is 0.02 mg of pheromone/hr (Stelinski et al., 2009). For *G. molesta*, septa were loaded with 3 μg of (*Z*)-8-dodecenyl acetate, (*E*)-8-dodecenyl acetate, and (*Z*)-8-dodecen-1-ol in a 100: 6 :10 blend (Baker and Cardé, 1979b; pheromone components >95% purity; Shin Etsu). For *G. molesta*, Isomate-M Rosso dispensers, containing 250 mg of 88.5% (*Z*)-8-dodecen-1-yl-acetate, 5.7% (*E*)-8-dodecen-1-yl-acetate, 1.0% (*Z*)-8-dodecen-1-ol, and 4.8% inert ingredients, were used (Shin Etsu). The release rate of an Isomate-M Rosso dispenser is 0.05 mg of pheromone/h (Stelinski et al., 2009). For *C. rosaceana*, rubber septa were loaded with 0.485 mg of (*Z*)- and 0.015 mg (*E*)-11-tetradecenyl acetates (92.2: 3.0 ratio of *Z*: *E*), and 0.026 mg of (*Z*)-11-tetradecenol (Hill and Roelofs, 1979; pheromone components >95% isomeric and chemical purity; Shin Etsu). The high release exposure dispenser for *C. rosaceana* was Isomate OBLR/PLR Plus, containing 274 mg of 93.4% (*Z*)-11-tetradecenyl acetate, 5.1% (*E*)-11-tetradecenyl acetate, and 1.5% (*Z*)-9-tetradecenyl acetate (Shin Etsu). The approximate release rate of this dispenser is 0.04 mg of pheromone/h (Stelinski et al., 2009). For *P. pyrusana*, red septa were loaded with 0.4 mg of a 94: 6 blend of (*Z*)-11-tetradecenyl acetate: (*Z*)-9-tetradecenyl acetate (Roelofs et al., 1977; >95% Purity, Shin Etsu). For this species, the high dosage dispenser was also Isomate OBLR/PLR, as it releases the same major pheromone component for both *P. pyrusana* and *C. rosaceana* and is intended for simultaneous

mating disruption of both species. Pheromone blend solutions used to load rubber septa were prepared in HPLC grade hexane and stored at −18°C between exposure treatments. All Isomate dispensers were aged in a fume hood at 24°C for 3 week prior to pre-exposure experiments, to allow for flash evaporation of pheromone that may have accumulated on the surface during packaging and storage.

Pheromone Pre-exposure Protocol Moths destined for pre-exposure were placed in 1-l plastic assay chambers, equipped with two 0.64 cm openings in their lids [See Fig. 1 in Stelinski et al. (2006a)]. Glass inlets and outlets were affixed to the lids, allowing for air to pass through the chambers. Carbon-filtered (filter model 100 Safe Glass Hydrocarbon Trap, Chromatography Research Supplies, Louisville, KY, USA; 50 ml/min) air

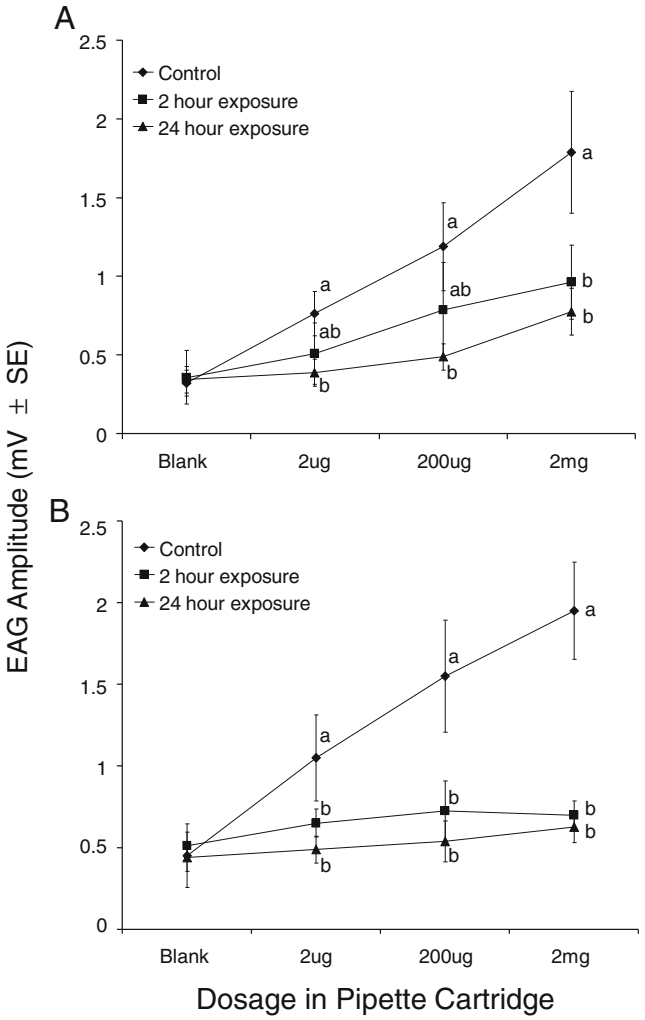

Fig. 1 Electroantennogram (EAG) responses of female *Grapholita molesta* (**a**) and *Choristoneura rosaceana* (**b**) to different dosages of synthetic sex pheromone, following non-exposure (control) or pre-exposure to sex pheromone for 2 or 24 hr. Differences between EAG responses, for a given dosage, are indicated by different letters. *G. molesta* were exposed to Isomate-M Rosso dispensers and *C. rosaceana* were exposed to Isomate OBLR/PLR Plus dispensers

was initially passed through Teflon chambers (described in Stelinski et al., 2003a) containing a given pheromone pre-exposure treatment, described above. From these pheromone-source chambers, air flowed into the exposure chambers, containing moths, via 20 cm of Teflon tubing (Stelinski et al., 2006b). Exposure chambers contained exit valves for air; thus, there was a constant flow of air through the exposure chambers during the pre-exposure period, creating a constant pheromone exposure concentration per unit of air, as described in Stelinski et al. (2003a). Teflon chambers, free of pheromone sources and connected to moth pre-exposure chambers, served as a negative control for each species and dispenser treatment. Each species was tested in separate experiments. Exposure chambers were housed in a wind tunnel, similar to that described in Stelinski et al. (2004), which evacuated pheromone-laden air continuously at 0.5 $m.sec^{-1}$ during pre-exposures. The tunnel was housed in a temperature- and photoperiod-controlled (16:8, L:D) room, maintained at 23°C and 50-70% RH, with light intensity ranging between 3 and 10 lux.

For each species, experiments were conducted either by exposing females only and pairing them with unexposed males for subsequent behavioral assays, or *vice versa*. When one sex was exposed, the other sex was obtained from the rearing method described above without previous exposure to synthetic pheromone. Durations of either two or 24 h exposure were conducted for each pheromone pre-exposure treatment and for each species. For the 2 h pre-exposure treatment, moths in chambers were exposed to various treatments (no pheromone control, low dosage dispenser, high dosage dispenser) for 2 h prior to the period of maximal calling behavior of females and sexual responsiveness by males. For *C. pomonella*, *C. rosaceana*, and *P. pyrusana*, exposures were initiated 2–2.5 h prior to the end of the 16 h photophase. For *G. molesta*, exposure was initiated 5 h prior to the end of the 16 h photophase. For 24 h exposures, durations were adjusted, such that moths were similarly pre-exposed starting 24 h prior to onset of maximal diel sexual responsiveness, as described above. In assays in which females were pre-exposed, and males left unexposed, 50 replicates of females and males were bioassayed. In cases in which males were exposed, and females were not, 20 replicates of pairs were bioassayed. The male exposure treatment served as a type of positive control, to confirm that pheromone exposures affected moth behavior, as it is well established that such exposures decrease pheromone-mediated behavior of male moths (Witzgall et al., 2008).

Behavioral Assays Following Pheromone Exposure Directly following pre-exposure, pairs of moths (one sex pheromone exposed and the other not) were placed into plastic cylindrical cages, similar to those described in Curkovic et al. (2006). In brief, cages were transparent, 30 cm length x

10 cm diam. tubes of acetate sheet, sealed at both ends with fine wire mesh, which prevented moth escape but allowed airflow. These cages were placed into the wind tunnel under conditions described above, with airspeed of 0.3 m.sec^{-1}. Cages were suspended approximately 30 cm above the tunnel floor, using ring stands in an orientation that allowed direct airflow through the cylinders. Mating pairs were established initially by placing females at the upwind end of the tubes and males at the down-wind end, and the ends sealed with wire-mesh caps. Mating pairs of moths within cylinders were observed over a period of 60 min, during which incidences of female calling or female–male copulation were recorded. Five to eight tubes were observed simultaneously during a given session. Calling behavior of female moths was noted by the characteristic posture of females, of wings elevated slightly and displaced laterally, with the abdomen concurrently raised, as described (Baker and Cardé, 1979a; Curkovic et al., 2006). Copulation was recorded when a male clasped a female's abdomen. In experiments with pheromone-exposed females and un-exposed males, males behaved normally by walking toward calling females, wing fanning, and attempting copulation.

Antennal Response of Female Moths Following Pheromone Pre-exposure We sought to determine whether auto-exposure of female moths to sex pheromone resulted in antennal adaptation in the subsequent response to sex pheromone. Two (of the four) representative species were tested. Female moths of the two species were pre-exposed to the high rate of pheromone, for either two or 24 h, as described above. Females in blank untreated chambers, for the same times as the exposure treatments, served as controls. Each species was exposed to the respective mating disruption dispenser, described above. Soon after exposure (1–2 min), antennal responses were tested by electroantennogram (EAG). The EAG system (Syntech, Kirchzarten, Germany) and test protocols have been described elsewhere (Stelinski et al., 2003a, b). EAG cartridges were made by pipetting various dosages (2 μg–2 mg) of pheromone in hexane (20 μl total solution) onto 1.4×0.5 cm strips of Whatman No. 1 filter paper. The blends used were the same as in the low dosage pre-exposure lures, described above. After 5 min. in a fume hood for solvent evaporation, pheromone-treated filter paper strips were inserted into disposable glass Pasteur pipettes. EAGs were measured as the maximum amplitude of depolarization to 1-ml puffs of air through EAG-cartridges directed over live-insect preparations.

Female moths of each species were 2–4 d-old when tested. A live insect was restrained on a 3.5 cm diam. Petri dish, filled with wax, by placing a strip of clay (8×3 mm) over the thorax and abdomen. The terminal two segments of the antenna used for recording EAGs were excised, and the recording electrode attached at the severed end. The reference electrode was gently inserted into the head capsule near the base of the antenna used for recording. EAGs were measured for 8 moths per pheromone pre-exposure treatment or control. Solvent-only stimulations (using filter paper impregnated with 20 μl of hexane) were administered as a control for subsequent pheromone stimulations. Each replicate moth was stimulated with the blank solvent, followed by each successive pheromone dosage within the range of dosages administered (Fig. 1a,b). Successive stimulations were applied 12–20 sec apart, to minimize potential onset of additional adaptation due to the stimulus puff.

Data Analysis To investigate the effects of pheromone dosage and exposure interval on the probability of calling and copulation by four moth species, logistic regression was used and significance tested using the log-likelihood ratio χ^2 (The LOGISTIC Procedure, SAS 9.1). Calling or copulation data were analyzed separately as dependent variables, with dosage, exposure duration, and the interaction between dosage and exposure duration, considered the independent variables (SAS INSTITUTE, 2005). EAG data were subjected to analysis of variance (ANOVA), and differences in pairs of means over time, and between treatments, were separated using Tukey's multiple comparison tests (SAS INSTITUTE, 2005). Since EAG responses of female moths exposed to clean air for two or 24 h were not different, these were combined as a single control for the pheromone exposure treatments for each species.

Results

Effect of Pheromone Pre-exposure on Male Moths To confirm that pheromone exposure dosages were sufficient to affect male moth behavior, we conducted calling and copulation assays with pre-exposed males (Table 1). In all cases, calling behavior of females was unaffected. Copulation, for each species, was greatly reduced following 2 h exposure to the lowest dosage of pheromone (Table 1). Copulation frequency was zero or near zero for each species after 24 h pheromone pre- exposure (Table 1).

Effect of Pheromone Pre-exposure on Female Moths *Choristoneura rosaceana* and *Cydia pomonella* females appeared unaffected in calling or copulation behavior (or interaction between the two), at either dose or duration of pheromone pre-exposure (Tables 2 and 3). The average proportion of calling among all treatments for *C. rosaceana* and *C. pomonella* females was 84 and 82%, respectively, while the average proportion of copulation among all treatments was 80 and 80.6%, respectively.

Table 1 Proportions of female calling and copulation observed in four species of tortricid moths in which males were pre-exposed to female sex pheromone and then mated with naive females. Males were pre-exposed for two or 24 hr at either low or high doses of female pheromone

[a]Denotes an effect (relative to the control of untreated males; $P<0.05$, log-likelihood ratio χ^2) of pre-exposure on calling or copulation

		Proportion of females exhibiting					
	Hours of pre-exposure	Calling			Copulation		
		Control	Low Dose	High Dose	Control	Low Dose	High Dose
Grapholita molesta	2	0.70	0.65	0.80	0.65	0.20[a]	0.00[a]
	24	0.70	0.80	0.75	0.60	0.00[a]	0.00[a]
Pandemis pyrusana	2	0.60	0.70	0.55	0.50	0.35[a]	0.00[a]
	24	0.65	0.75	0.85	0.60	0.00[a]	0.00[a]
Choristoneura rosaceana	2	0.85	0.80	0.75	0.75	0.35[a]	0.00[a]
	24	0.90	0.85	0.85	0.80	0.00[a]	0.00[a]
Cydia pomonella	2	0.75	0.85	0.80	0.70	0.55[a]	0.10[a]
	24	0.90	0.80	0.75	0.80	0.00[a]	0.00[a]

Similarly, dose, exposure duration, and the interaction between these factors, had no effect on calling for *G. molesta* and *P. pyrusana* females (Tables 2 and 3). However, the proportion of females that copulated was affected for both species. The proportion of copulations for *G. molesta* was affected by dosage, exposure duration, and the interaction of these two variables (Table 3). For this species, copulation success was reduced by about 40% in the high dose treatment, following 2 h pre-exposure, and by 53 and 63% for the low and high dosages, respectively, after 24 h pre-exposure (Table 2). For *P. pyrusana*, the proportion of females that copulated was affected by exposure duration, and the interaction of exposure duration and dosage, but not by dosage (Tables 2 and 3). For *P. pyrusana*, there was no reduction in copulation following 2 h pre-exposure to either dosage, but there was a reduction following 24 h pre-exposure to either dosage (Table 2).

Antennal Response of Female Moths Following Pheromone Pre-exposure For *G. molesta* females, pheromone pre-exposure for 24 h reduced ($P<0.05$) antennal responses to all pheromone dosages tested (range: 2 μg–2 mg), compared with the control (Fig. 1a). For the 2-h pre-exposure, EAG responses were reduced ($P<0.05$) only to the two mg cartridge dosage (Fig. 1a). For *C. rosaceana* females, antennal responses to the full dosages tested were reduced ($P<0.05$), compared with the control, following either two or 24 h pre-exposure (Fig. 1b).

Discussion

Humans rely on artificial methods of pest control to maintain food production. With the threat of evolution of pesticide resistance (Bates et al., 2005), and the increasing food requirements of the human population (Pimentel et al., 1994), ecologically responsible pest management methods are imperative. Pheromone-based mating disruption, which exploits natural behaviors to control insect pests, has been a valuable tool for crop management for nearly two decades

Table 2 Proportions of calling and copulation observed among females of four tortricid species in which females were pre-exposed to female sex pheromone and then caged with naïve males. Females were exposed for two or 24 hr at either low or high doses of female pheromone

[a]Denotes an effect (relative to the control of untreated females; $P<0.05$, log-likelihood ratio χ^2) of pre-exposure on calling or copulation

		Proportion of females exhibiting					
	Hours of exposure	Calling			Copulation		
		Control	Low Dose	High Dose	Control	Low Dose	High Dose
Grapholita molesta	2	0.74	0.76	0.64	0.68	0.66	0.40[a]
	24	0.78	0.82	0.74	0.72	0.34[a]	0.26[a]
Pandemis pyrusana	2	0.64	0.68	0.64	0.56	0.62	0.62
	24	0.70	0.68	0.60	0.60	0.42[a]	0.32[a]
Choristoneura rosaceana	2	0.88	0.78	0.80	0.82	0.74	0.76
	24	0.86	0.88	0.84	0.80	0.82	0.84
Cydia pomonella	2	0.78	0.86	0.84	0.74	0.82	0.84
	24	0.86	0.80	0.78	0.80	0.78	0.86

Table 3 Statistics for the effects of dosage, duration, and the interaction of dosage and duration, on proportion calling and copulation observed in four species of tortricid moths, in which females were pre-exposed to female sex pheromone and then caged with naïve males. X^2 statistics, degrees of freedom and the P-value are shown

	Statistics							
	Calling				Copulation			
	Grapholita molesta	*Pandemis pyrusana*	*Choristoneura rosaceana*	*Cydia pomonella*	*Grapholita molesta*	*Pandemis pyrusana*	*Choristoneura rosaceana*	*Cydia pomonella*
Dosage	X^2=2.682	X^2=0.915	X^2=0.956	X^2=0.140	X^2=26.259	X^2=2.437	X^2=0.251	X^2=2.014
	df=2	df=2	df=2	df=2	df=2	df=2	df=2	df=2
	P>0.05	P>0.05	P>0.05	P>0.05	P<0.001	P>0.05	P>0.05	P>0.05
Duration	X^2=1.692	X^2=0.019	X^2=0.722	X^2=0.088	X^2=5.669	X^2=7.437	X^2=0.944	X^2=0.077
	df=1	df=1	df=1	df=1	df=1	df=1	df=1	df=1
	P>0.05	P>0.05	P>0.05	P>0.05	P=0.017	P=0.008	P>0.05	P>0.05
Dosage*	X^2=0.153	X^2=0.561	X^2=1.2383	X^2=2.186	X^2=6.240	X^2=6.157	X^2=.993	X^2=0.748
Duration	df=2	df=2	df=2	df=2	df=2	df=2	df=2	df=2
	P>0.05	P>0.05	P>0.05	P>0.05	P=0.044	P=0.046	P>0.05	P>0.05

(Witzgall et al., 2011). It is likely to become increasingly prevalent in agro-ecosystems as knowledge of pheromone chemistry increases and technology for synthesizing and releasing pheromone blends becomes more available and affordable. Understanding the mechanisms of mating disruption is vital to its development, continued success, and sustainability. The current investigation explored the possibility of a new mechanism of mating disruption that specifically affects female moths. We tested the hypothesis that intense exposure to pheromone may affect the propensity of female moths to copulate.

Early work on female Lepidoptera exposed to their own pheromone proved that females of certain species are capable of autodetection, and that this may provide some information to females. Palanaswamy and Seabrook (1978) examined the behavioral responses of female *Choristoneura fumifera* in airstreams containing pheromone and found that females autodetected pheromone and, more importantly, were stimulated to oviposit. Based on similar experiments with *Trichoplusia ni* (Birch, 1977), Palanaswamy and Seabrook (1978) showed that *C. fumifera* females oviposit a minimum number of eggs before they disperse. These results indicated that female pheromone provides information to female moths that, in turn, initiates an adaptive behavioral response. Our experiments examined the behavior of females of four species of tortricid moths following exposure to pheromone. We found that, while the incidence of calling behavior was not reduced by pre-exposure to pheromone in any of the four species tested, the proportion of females that copulated following pheromone exposure was reduced in two of the four species. That prior exposure to pheromone resulted in reduced copulation proportions in *G. molesta* and *P. pyrusana*, suggests that, in these species at least, mating disruption using pheromone may simultaneously affect both male and female moths. Our results also indicated that the more intense (higher dosage) and prolonged the exposure to pheromone, the greater the likelihood of affecting female mating behaviors in these species. The combination of dosage and duration also appears to be a significant factor in mating disruption of male codling moths (Judd et al., 2005).

It is curious that *G. molesta* females were affected by pre-exposure dosage, whereas *P. pyrusana* females were not. The lack of effect on *P. pyrusana* females may indicate that they have a higher tolerance before exposure to sex pheromone modifies their behavior. This is supported by the result that *P. pyrusana* females required 24-hr exposure, to either low or high dosages of pheromone, before their behavior changed. This may indicate that *P. pyrusana* is better adapted for mating success at outbreak population levels when female pheromone is likely to be at higher concentrations. Alternatively, this difference in effect of dose and exposure between *G. molesta* and *P. pyrusana* females may be an artifact of differences in the physical properties of the pheromone components of these two species or differences in the effects of minor components on behavioral habituation. A possible adaptive explanation for the loss of female receptivity to mating in these species is that females perceive high levels of sex pheromone as an indicator of high competition that is unsuitable for reproductive success and thus may restrict their receptiveness to copulation.

An alternative interpretation for pre-exposure to pheromone affecting the behavior of female *G. molesta* and *P. pyrusana*, is that pre-exposed females may be unable to sense their conspecific male's aphrodisiac pheromone appropriately. Our EAG experiments indicated that adaptation

of antennal responses, following sex pheromone pre-exposure, occurred both in a species (*G. molesta*) requiring a male aphrodisiac as part of the courtship, as well as in a species (*C. rosaceana*) with a simpler courtship system. Of the four moth species investigated here, a specific male aphrodisiac pheromone has been identified only for *G. molesta*. Its aphrodisiac components include (*E*)-cinnamate, mullein, methyl jasmonate, and methyl-(*Z*)-epijasmonate (Nishida et al., 1982), the structures of which are quite distinct from those of the main female pheromone components, (*Z*)-8-dodecenyl acetate, (*E*)-8-dodecenyl acetate, and (*Z*)-8-dodecen-1-ol (Roelofs et al., 1969). Therefore, any sensory cross-adaptation would need to be broad. Cross-adaptation of neurons to odor stimuli has been shown in numerous studies, indicating that processing of different odor stimuli may share common physiological pathways (Daniel et al., 1994; Takeuchi et al., 2003; Gottfried et al., 2006; Anton et al., 2011). Trona et al. (2010) recently showed that structurally different sex pheromone and plant odor compounds interact in *C. pomonella* via an across-fiber coding pattern, with perception of these distinct chemicals relayed through projection neurons from ordinary glomeruli and from the macroglomerular complex. Further research is needed to determine the exact mechanism responsible for the reduced copulation of females following exposure to pheromone.

Copulation propensity of *G. molesta* and *P. pyrusana* females was affected by exposure to sex pheromone, while copulation appeared unaffected by the same treatment in *C. pomonella* and *C. rosaceana*. We find it interesting that this pattern is congruent with the respective susceptibilities of these species to pheromone mating disruption (reviewed in Gut et al., 2004). Reduction of female copulation propensity may be an additional mechanism of mating disruption that renders certain species more susceptible than others to this tactic. However, there are potential ecological consequences that need to be considered. Mating disruption could lead to destabilizing selection for utilization and attraction to abnormal pheromone blends (Evenden and Haynes, 2001). If altered mating success following exposure to pheromone is a result of an inability of females to perceive male aphrodisiacs, then females that are inherently less influenced by male aphrodisiacs during mate acceptance may be favored over females that are more strongly influenced and hence only accept males after detection of the aphrodisiac. Therefore, it is possible that, in an environment continuously treated with synthetic pheromone, selection would deemphasize the role of male aphrodisiacs in mating, and consequently reduce the efficacy of mating disruption.

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