Seconds-Long Preexposures to Pheromone from Rubber Septum or Polyethelene Tube Dispensers Alters Subsequent Behavioral Responses of Male *Grapholita molesta* (Lepidoptera: Tortricidae) in a Sustained-Flight Tunnel

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ABSTRACT Male Oriental fruit moths, *Grapholita molesta* (Busck), were briefly preexposed in a wind tunnel to plumes from a rubber septum lure releasing a three-component, optimally attractive pheromone blend for this species or to plumes generated by Isomate-M Rosso pheromone dispensers. The objectives were to determine how brief preexposures to low- and high-dosage pheromone dispensers affect initiation of anemotaxis, duration of sustained anemotactic flight, and peripheral sensitivity 15 min and 24 h after exposure. A greater proportion of *G. molesta* males took flight and successfully oriented toward a lure 15 min after briefly orienting in plumes generated by an identical lure compared with unexposed, naïve moths or control moths preexposed to clean air. In addition, the proportion of males contacting a lure or orienting to the lure without source contact 24 h after a preexposure was not significantly different from the proportions of naïve or control moths completing these behaviors. However, the mean duration of sustained flights of lure-preexposed male *G. molesta* in plumes generated by a lure was significantly shorter 15 min and 24 h after preexposure compared with that of naïve moths. The proportion of male *G. molesta* contacting lures 15 min and 24 h after preexposure to ropes was not statistically different from the proportions of naïve or control moths contacting the lure or orienting without source contact. However, as observed with moths preexposed to a lure, the mean duration of sustained flights of male *G. molesta* preexposed to an Isomate-M Rosso dispenser was significantly shorter than that of naïve moths 15 min and 24 h after preexposure. Mean durations of sustained flights of male *G. molesta* preexposed to a lure or rope were significantly longer after 24 h compared with 15 min after the exposure treatment, indicating that the effect of pheromone preexposure decayed over time. Electroantennograms recorded 15 min and 24 h after preexposures to lures or Isomate-M Rosso dispensers in the flight tunnel were indistinguishable from those recorded from unexposed moths. We suggest that false-plume following by naïve male *G. molesta* combined with decreases in duration of subsequent anemotactic orientations after previous bouts of false-plume following may explain why Isomate-M Rosso dispensers are effective in mating disruption experiments with *G. molesta.*

KEY WORDS mating disruption, pheromone preexposure, *Grapholita molesta*, Oriental fruit moth, sustained-flight tunnel

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THE ORIENTAL FRUIT MOTH, *Grapholita molesta* (Busck), is a major pest of stone fruit trees (Rosaceae) worldwide; it attacks shoots and feeds internally within fruits (Rothschild and Vickers 1991). Efforts to develop mating disruption as a means of controlling this pest began decades ago (Gentry et al. 1974, 1975, Rothschild 1975, Cardé et al. 1977, 1979). Promising results from some of these early studies led to the development of a commercial polyethylene dispenser (Isomate-M; Shin-Etsu Chemical Co., Tokyo, Japan) for releasing *G. molesta* pheromone into orchards (Vickers 1990). These and subsequent versions (Isomate-M 100 and M Rosso) of dispensers proved effective in numerous field trials (Pfeiffer and Killian 1988, Audemard et al. 1989, Rice and Kirsch 1990, Pree et al. 1994, Trimble et al. 2001, Atanassov et al. 2002, Trimble et al. 2004). Thus, *G. molesta* is a good subject for studying the factors underlying effective mating disruption.

Moth exposures to pheromone induce a range of responses. Various studies have documented peripheral adaptation or sensory fatigue in moths after intense exposure to their species-specific pheromones (Bartell and Roelofs 1973, Bartell and Lawrence

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1976a–c, Linn and Roelofs 1981, Sanders 1985, Stelinski et al. 2003a, b). Such studies have shown that prolonged exposure to pheromone decreases stereotyped behavioral responses by males, including wing fanning and rapid walking (Bartell and Roelofs 1973), captures in pheromone-baited traps in the field, and orientation to optimally attractive pheromone dispensers in wind tunnels (Rumbo and Vickers 1997, Daly and Figueredo 2000). In addition, peripheral adaptation of moth olfactory receptor neurons has been quantified electrophysiologically (e.g., Kuenen and Baker 1981, Baker et al. 1989, Marion-Poll and Tobin 1992, Schmitz et al. 1997, Stelinski et al. 2003a); major outcomes are decreased electroantennogram (EAG) responses or decreased spike frequencies from single sensillae during and after prolonged pheromonal stimulation.

In contrast to decreases in behavioral or antennal responsiveness after pheromone exposure, other studies report increases in behavioral response in moths briefly preexposed to pheromone. For example, Sanders (1984, 1995) found that the percentage of male *Choristoneura fumiferana* successfully locking onto and flying to sources of pheromone off-blends dramatically increased after brief preexposures to calling females or a 95:5 blend of *E*:*Z*-11-tetradecenal. This effect was termed "priming" and thought to possibly increase false-plume following of males in mating disruption regimens using pheromone off-blends. Similarly, responses of *G. molesta* (Busck) to off-blends of pheromone (blends containing high %*E*) that normally elicited few completed flights from naïve moths, increased after minutes-long preexposures to *E*-8-dodecenyl acetate (Linn and Roelofs 1981).

Heightened behavioral responses to female-produced or optimally attractive synthetic sources of pheromone after preexposure to such chemicals are also known to be long-lasting. Specifically, Anderson et al. (2003) described a pronounced increase in behavioral response to presentations of the female sex pheromone in male *Spodoptera littoralis* having been briefly preexposed to a female-produced plume. This abnormally high responsiveness in exposed moths lasted up to 27 h and was not associated with a change in peripheral sensitivity. More recently, Stelinski et al. (2004a) described enhanced behavioral responses of *Choristoneura rosaceana* to pheromone sources in a wind tunnel after seconds- and minutes-long preexposures to pheromone dispensers, including Isomate OBLR/PLR polyethylene tubes intended for mating disruption. Not only did the proportion of male moths locking onto sources of pheromone increase after preexposure, but also the duration of sustained flights increased by up to four-fold. Interestingly, the same study revealed that the behavioral responses of *Argyrotaenia velutinana*, after identical exposures to those imposed on *C. rosaceana*, decreased for up to 24 after the preexposure treatment and the study revealed no evidence of "behavioral priming" for this species after pheromonal preexposure (Stelinski et al. 2004a). These authors suggested that the difference in behavioral responses between *C. rosaceana* and *A. velutinana* after brief preexposure to pheromone may explain why successful mating disruption has been more difficult to achieve in the former species compared with the latter as determined by field studies (Novak et al. 1978, Reissig et al. 1978, Novak and Roelofs 1985).

This study focused on describing the effects of brief preexposure to the currently marketed Isomate-M Rosso dispensers and optimally attractive, rubber septum lures, which approximate females, on the subsequent behavior of *G. molesta.* The overall goal was to gain insights into why mating disruption has proven so successful for this species. The specific objectives were to determine whether/how brief preexposures to low- and high-dosage pheromone dispensers affect initiation of anemotaxis, duration of sustained anemotactic flight, and peripheral sensitivity 15 min and 24 h after exposure.

Materials and Methods

Insects. *Grapholita molesta* were drawn from a 2-yr-old laboratory colony at Michigan State University (East Lansing, MI) originally collected as larvae from apple orchards in Southwest Michigan. Moths 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 were sorted by sex and emerged in 1-liter plastic cages containing 5% sucrose in plastic cups with cotton dental wicks protruding from their lids.

Chemicals and Release Devices. The behavioral responses of *G. molesta* were quantified in a sustained-flight tunnel using two types of pheromone dispensers. The first dispenser was formulated using red rubber septa loaded with a pheromone blend attractive to this species. The rubber septa were loaded with 3 μg of (Z)-8-dodecen-1-yl-acetate:(*E*)-8-dodecen-1-yl-acetate:(Z)-8-dodecen-1-ol in a 100: 6: 10 blend (Willis and Baker 1984). Pheromone blend solutions used to load rubber septa were prepared in high-performance liquid chromatography (HPLC) grade hexane and stored at −18°C. Henceforth, such rubber septum dispensers formulated specifically for this species will be referred to as "lures." The second type of dispenser was the Isomate-M Rosso polyethylene-tube 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. These polyethylene tube dispensers will be referred to as "ropes." All rope dispensers were aged for 2 wk in a laboratory fume hood before use in behavioral assays to allow dissipation of pheromone that might have built up on rope surfaces during shipping and freezer storage.

Wind Tunnel. Behavioral assays were conducted in the Plexiglas sustained-flight tunnel detailed by Stelinski et al. (2004a). The working section of this wind tunnel measured 1.3 by 0.8 m in cross-section and 2.4 m long. It was housed in a temperature-controlled chamber maintained at 23°C and 50–70% RH. Light intensity was 700 lux inside the tunnel and was generated by two fluorescent bulbs (Philips model F96T12; 95 W) mounted 22 cm above the top of the wind

Table 1. Response of male *G. molesta* to lures or Isomate rope dispensers 15 min or 24 h after preexposure to clean air, a lure, or a rope dispenser

Moth type and pheromone source (time between exposure and testing)	N	Proportion of males exhibiting the indicated response[a]		
		No behavioral change	Orientation without source contact	Source contact
Naïve males				
Lure	105	0.21c[b]	0.18a	0.34b
Rope	96	0.72a	0.09b	0.00c
Preexposed orienters (15 min)				
Lure then lure	73	0.08c	0.19a	0.66a
Rope then lure	20	0.60ab	0.15a	0.20b
Rope then rope	9	0.78a	0.00b	0.00c
Preexposed nonorienters (15 min)				
Lure then lure	76	0.55b	0.05b	0.20b
Rope then lure	75	0.33bc	0.12ab	0.28b
Rope then rope	74	0.80a	0.00b	0.00c
Air then lure[3]	70	0.21c	0.13ab	0.37b
Preexposed orienters (24 h)				
Lure then lure	72	0.27c	0.21a	0.50ab
Rope then lure	14	0.57b	0.29a	0.14bc
Rope then rope	10	0.90a	0.00b	0.00c
Preexposed nonorienters (24 h)				
Lure then lure	67	0.20c	0.20a	0.39b
Rope then lure	68	0.43b	0.17a	0.30b
Rope then rope	67	0.82a	0.00b	0.00c
Air then lure[c]	90	0.22c	0.14ab	0.46b

[a] Proportions of moths wing-fanning only or flying out without anemotactic orientation are not shown.

[b] Numbers in the same column followed by the same letter are not significantly different (G^2 test of homogeneity, $P = 0.05$).

[c] Refers to control treatment.

tunnel. A variable speed, blower-type fan (5C090C; Dayton, Northbrook, IL) pushed air through the tunnel at 0.3 m/s. The pheromone plume emerging from the tunnel was expelled from the building through a roof-mounted stack.

Wind Tunnel Assays. The wind-tunnel assay procedures were a slight modification of those described by Stelinski et al. (2004a). Male *G. molesta*, 4–6 d old, were placed into cylindrical (17 cm long by 8 cm diameter) wire-mesh release cages 3 h before the end of a 16-h photophase. Each cage, containing one or five moths (depending on experiment), was placed into the wind tunnel for 1 h of acclimation before assays. Subsequently, bioassays ran for a maximum of 1.5 h terminating at 0.5 h before the end of the moths' normal photophase. At the upwind end of the tunnel, pheromone dispensers (lures or ropes) were placed 1 cm above a horizontal 7.5 by 12.5-cm yellow card (American Scholar, Bay Shore, NY) attached to a horizontally clamped 9-cm glass rod attached to a steel ring-stand. Pheromone was released 25 cm above the tunnel floor in stationary-floor experiments and 10 cm above the floor in moving-floor experiments. Wire-mesh release cages holding five male moths were placed at the down-wind end of the tunnel at a height matching that of the pheromone dispenser. In sustained-flight experiments, release cages were 10 cm long by 8 cm diameter and contained only one male moth.

Males were allowed 5 min to respond to an inserted pheromone dispenser. This time interval was chosen because preliminary tests showed that if a *G. molesta* male did not respond by 5 min after it was placed into a pheromone plume, it was highly unlikely to respond at all. The behaviors recorded were: wing-fanning; nonanemotactic flight from the release cage; upwind anemotactic flight without touching the release device; upwind anemotactic flight followed by landing on the platform and touching the release device. Also, the numbers of individuals with no detectable behavioral change were recorded. Across all pheromonal treatments tested, there were few instances of significant differences between mean numbers of moths wing fanning only or exhibiting nonanemotactic flights out of release cages. Thus, for brevity, in Table 1, we only present the proportions of moths that oriented with and without source contact versus those exhibiting no behavioral change.

Release cages, ring stands, and glass rods were thoroughly washed with acetone after daily use. The interior of the wind tunnel was also briefly scrubbed with an acetone-soaked rag and immediately rinsed with water so as not to damage the Plexiglas. The exhaust fan ran for at least 4 h after assays were completed.

Experiment 1. This experiment tested the effect of brief exposures of *G. molesta* to pheromone plumes generated either by lures or rope dispensers on subsequent responsiveness of male moths to these pheromone sources either 15 min or 24 h after the initial exposure treatment. Groups of five male moths were released in plumes generated by a rubber septum described above or a rope. Groups of five moths were assayed at a time because preliminary tests showed that testing this number of moths was the most efficient way of collecting replicates without compromis-

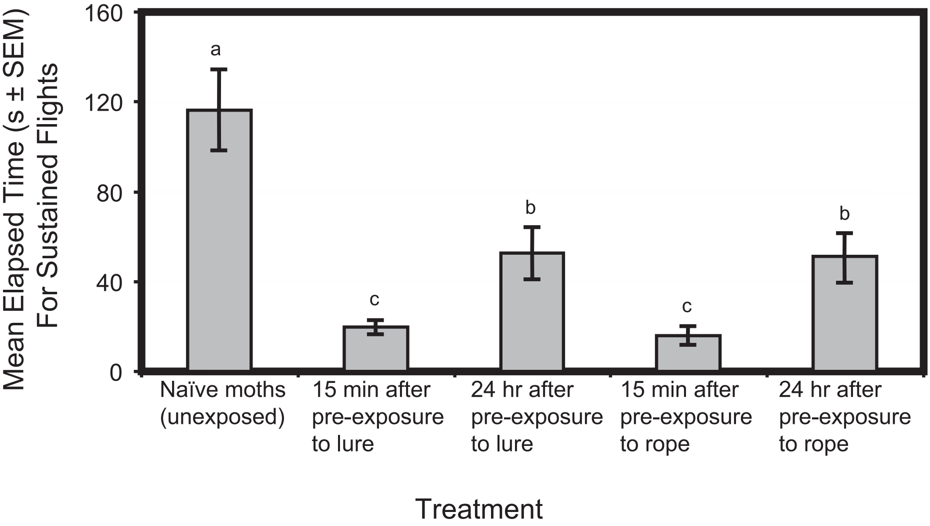

Fig. 1. Durations of lure-induced sustained flights of naïve and pheromone preexposed male *G. molesta* 15 min and 24 h after preexposure to a lure or rope. $N = 41$ per treatment. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

ing insect performance or data collection. Two or three observers conducted behavioral observations making it possible to keep track of several insects concurrently. Given that ≈30–66% of moths responded by contacting lures depending on treatment (see Table 1), there were no problems associated with multiple moths orienting concurrently and interfering with one another. In cases where there were multiple orienters from a single batch of five moths, moths took flight at different times throughout the 5-min preexposure treatment. Moths reaching or orienting to the pheromone source but not contacting it were segregated from those that either did nothing, wing-fanned, or flew out of release cages without orienting. Mean duration of moth orientations during the preexposure treatment was ~15 s. Thus, the duration of moth preexposure differed between moths that had oriented during preexposure versus those that did not; nonorienting moths received the full 5 min of exposure. Recaptured moths (both "orienters" and "nonorienters") were assayed in the wind tunnel again to each pheromone source either 15 min or 24 h after the initial pheromone preexposure. Care was taken to assay moths exactly after these two intervals. This was accomplished by placing moths into marked containers after the preexposure treatment, allowing investigators to keep track of when subsequent assays were to take place. Moths tested 24 h after the preexposure treatment were kept in an environmental chamber under the temperature and light-cycle conditions described above for the interval before testing. The combined initial preexposure treatments and subsequent assays were preexposure to a lure followed by wind-tunnel assay using a lure; preexposure to a rope followed by wind tunnel assay using a lure; preexposure to a rope followed by wind-tunnel assay using a rope; and preexposure to clean air followed by wind-tunnel assay using a lure. "Naïve" will refer to moths having no prior exposure to pheromone or the wind tunnel before assay. "Control" will refer to moths preexposed to moving air, but no pheromone in the wind tunnel. "Preexposed" will refer to moths preexposed to pheromone in the wind tunnel. This pheromone may have emanated from a lure or rope.

To avoid bias caused by possible slight variations between days, all treatment combinations were tested each day of testing. Treatment order was also randomized daily to equalize any effect of time before what would have been the onset of scotophase.

Experiment 2. This experiment tested the effect of brief exposures of *G. molesta* to pheromone plumes generated by lures or rope dispensers on the duration of sustained flights of male moths 15 min and 24 h after initial exposure. As in experiment 1, moths reaching the pheromone source or orienting to the source but not contacting it during preexposure were segregated from those either doing nothing, wing-fanning only, or flying out of release cages without orienting to the source. The response of recaptured moths to plumes generated by lures was assayed 15 min and 24 h after the initial pheromone exposure. After moths locked onto the pheromone plume generated by a lure, the moving floor was activated at a mean velocity of 0.28 m/s to prolong flights. We recorded the duration of sustained flight along the pheromone plume. For preexposed male *G. molesta*, mean durations of sustained flights after preexposure treatment did not differ statistically between those moths that oriented during preexposure versus those that did not (see Results). Therefore, for comparison between treated and control moths, mean flight durations of preexposed moths were combined whether or not they oriented during preexposure (see Fig. 1).

Experiment 3. This experiment also tested the effect of brief preexposures of male *G. molesta* to pheromone plumes generated by 3-μg lures as above on the duration of sustained flights of male moths 15 min after initial exposure. However, moths were assayed using lures of various loading dosages 15 min after the preexposure treatment. The hypothesis was that if preexposure treatments increased moth sensitivity 15 min later, then durations of sustained flights should increase as lure loading dosage decreased. Alternatively, if preexposure treatments reduced moth sensitivity, durations of sustained flights should increase as lure dosage increased. The combined initial preexposure treatments and subsequent assays were preexposure to a 3-μg lure followed by wind tunnel assay using a 9-μg lure; preexposure to a 3-μg followed by wind tunnel assay using a 3-μg lure; preexposure to a 3-μg lure followed by wind tunnel assay using a 1-μg lure; preexposure to a 3-μg lure followed by wind-tunnel assay using a 0.5-μg lure; preexposure to clean air followed by wind tunnel assay using a 3-μg lure; and preexposure to clean air followed by wind-tunnel assay using a 0.5-μg lure. All lures were loaded with the pheromone blend described under *Chemicals and Release Devices* and durations of sustained flights were measured as described under *Experiment 2.* All treatment combinations were tested each day of testing, and treatment order was randomized daily to equalize any effect of time before what would have been the onset of scotophase.

Electroantennogram Assays (Experiment 4). This experiment tested the hypothesis that briefly exposing *G. molesta* to pheromone plumes generated by lures or high-release rope dispensers affected EAG responses of moths 15 min or 24 h after initial exposure. The EAG system and test protocols were detailed by Stelinski et al. (2003a, b). EAG cartridges were made by pipetting various concentrations (2 μg to 20 mg) of pheromone in hexane (20 μl total solution) onto 1.4 by 0.5-cm strips of Whatman No. 1 filter paper. After 5 min in a fume hood for solvent evaporation, treated strips were inserted into disposable glass Pasteur pipettes. Preexposed *G. molesta* were mounted for EAG analysis either 15 min or 24 h after preexposure. Pheromone dosages were delivered alternately to both naïve and pheromone preexposed moths in ascending order of dosage ($N = 12$ per treatment). Four 1-ml puffs spaced 12 s apart were administered to each antenna at each dosage.

Statistical Analyses. For experiment 1, a logistic model was used to measure the probability that a combination of the two factors: pheromone delivery device (rubber septum or rope) by moth type (naïve, preexposed orienter, or preexposed nonorienter) would result in a particular behavioral category as defined above using the Proc GENMOD procedure in SAS (SAS Institute 2000). Subsequently, analyses of numbers of male moths responding were carried out using the G statistic (Sokal and Rohlf 1981). For experiments 2 and 3, data for sustained-flight duration were transformed to $\ln(x + 1)$ (which normalized the distributions) and then subjected to analysis of variance (ANOVA); the significance of differences between means was tested using Tukey's multiple comparisons test (SAS Institute 2000). For experiment 4, data were subjected to ANOVA (SAS Institute 2000). In all cases, the significance level was $\alpha < 0.05$.

Results

Experiment 1. Compared with naïve or control males of the same age, a significantly greater proportion of male *G. molesta* contacted their respective lure 15 min after brief preexposure to pheromone plumes generated by such a lure (Table 1). This result occurred only for those *G. molesta* orienting to the lure during the preexposure treatment. The proportion of naïve or control males orienting to lures without contacting the source was not significantly different from the proportions of moths completing this behavior 15 min and 24 h after brief preexposure to a lure (Table 1). The proportion of moths contacting lures 15 min after preexposure to a lure during which moths did not orient was not significantly different from the proportion of naïve or control males completing this behavior (Table 1). The proportion of moths contacting lures and orienting without source contact 15 min and 24 h after preexposure to a lure during which moths did not orient was not significantly different from the proportion of naïve or control males completing these behaviors (Table 1).

Only 9% of naïve *G. molesta* oriented when placed into plumes generated by ropes and none contacted this dispenser (Table 1). The proportion of male *G. molesta* contacting or orienting to lures without source contact 15 min and 24 h after preexposure to ropes was not significantly different from the proportions of naïve or control moths exhibiting these behaviors (Table 1). No *G. molesta* oriented to ropes 15 min or 24 h after preexposure to ropes (Table 1).

Experiment 2. Naïve male *G. molesta* sustained flights to lures significantly longer than did moths of the same age but preexposed to a lure or rope plume 15 min or 24 h earlier (Fig. 1). Furthermore, mean durations of sustained flights of male *G. molesta* preexposed to a lure or rope were significantly longer 24 h after exposure compared with 15 min after exposure treatment indicating that the effect of exposure decayed over time (Fig. 1). Mean durations of sustained flights 15 min after preexposure were nearly identical irrespective of pheromone source (Fig. 1). Likewise, mean durations of sustained flights 24 h after preexposure were similar in moths preexposed to lures or ropes (Fig. 1).

Experiment 3. Male *G. molesta* exposed to clean air devoid of pheromone sustained flights to 3- and 0.5-μg lures significantly longer than did males preexposed to 3-μg lures, irrespective of the loading dosage of lures used during the assay 15 min after preexposure (Fig. 2). There were no significant differences in mean durations of sustained flights to the four assay dosages tested 15 min after preexposure to 3-μg lures, but the mean duration of sustained flights to 0.5-μg lures was slightly shorter compared with the other dosages

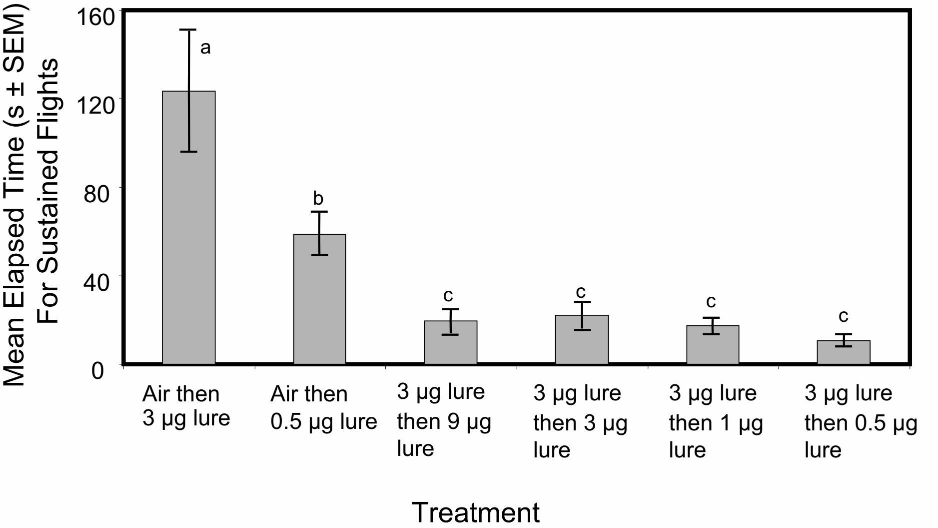

Fig. 2. Durations of sustained flights to lures of various loading dosages recorded from male *G. molesta* preexposed to clean air or a 3-μg pheromone lure 15 min prior. $N = 30$ per treatment. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

tested (Fig. 2). However, moths preexposed to clean air (control) sustained significantly shorter flights to 0.5-μg lures compared with 3-μg lures (Fig. 2).

Experiment 4. Mean EAG responses of naïve male *G. molesta* were not statistically different from those of moths assayed either 15 min or 24 h after preexposure to lures or rope dispensers (data not shown).

Discussion

The proportion of completed anemotactic flights to a rubber septum dispenser loaded with 3 μg of pheromone increased only in those *G. molesta* that had flown along a plume produced by such a dispenser 15 min before testing. Such increased responsiveness to pheromone after pheromone preexposure has been documented in flight tunnel studies for *C. fumiferana* (Sanders 1984), *C. rosaceana* (Stelinski et al. 2004a), and *S. littoralis* (Anderson et al. 2003). Furthermore, increased responsiveness to pheromone has been observed in *G. molesta* after preexposure. Specifically, Rumbo and Vickers (1997) found slightly increased responses of *G. molesta* to pheromone sources in a flight tunnel after 10 and 60 min (20 and 30 min of recovery, respectively) of preexposure in sealed containers with pheromone dispensers at release rates up to 320 times that of a single female moth (320 female equivalents). Diminution of responsiveness in the flight tunnel occurred for *G. molesta* only after the release rate of pheromone during preexposure was increased to 3,200 female equivalents (Rumbo and Vickers 1997).

In the various flight tunnel studies discussed above, the enhanced behavioral responses of moths after preexposure to their pheromone were detected as increases in the occurrence of various behaviors including wing fanning, take off, and the more complex anemotactic orientation with or without source contact. However, even the most complex overt behavior analyzed in the above studies, i.e., source contact, occurred after relatively brief flights in flight tunnels no longer than 2 m. The activation of a moving floor in our 2.4-m-long, sustained-flight tunnel revealed that, although a higher proportion of certain preexposed *G. molesta* responded and maintained brief anemotactic flights, the duration of orientation of all pheromone preexposed moths was considerably shorter compared with that of naïve, unexposed counterparts.

Preexposed male *G. molesta* were equally or in certain cases more apt to initiate anemotaxis; however, this was only for short periods (<15 s). Varying the loading dosage of lures in the assay after preexposure to 3-μg lures did not change the duration of sustained flights as we had postulated (Fig. 2). Our results suggest that preexposed *G. molesta* were not more sensitive to pheromone because there was no increase in durations of sustained flights to 1- and 0.5-μg lures, releasing less pheromone than a calling female (Baker et al. 1980, Figueredo and Baker 1992). Perhaps preexposed moths were more susceptible to desensitization (peripheral or central) after preexposure, which did not influence initiation of anemotaxis but prevented extended flights within the plume. Our data suggest this change in sensitivity took place in the central nervous system (CNS) because no changes were detected at the periphery both 15 min and 24 h after preexposure.

In addition to quantifying the effects of pheromone preexposure to plumes at concentrations approximating females, we also compared the effects of preexposures within plumes generated by an effective mating disruption dispenser for *G. molesta,* i.e., Isomate-M Rosso ropes (Trimble et al. 2004), which on average release pheromone at 0.06 mg/h (Sexton and Il'ichev 2001). We observed relatively few (9%) successful orientations to such high-release dispensers in our flight tunnel. Moreover, the proportions of male *G. molesta* exhibiting no detectable behavioral change when placed in plumes generated by Isomate-M Rosso dispensers were high, ranging from 72 to 90% (Table 1). However, in a companion study, where direct observations of Isomate-M Rosso dispensers were conducted in the field, the mean number of feral *G. molesta* approaching such dispensers per day was substantial and statistically equal to the mean number caught in traps baited with optimally attractive lures such as those used herein (Stelinski et al. 2004b). Willis and Baker (1984) found that *G. molesta* failed to progress upwind in flight tunnels when placed within a continuous and homogeneous cloud of pheromone. These authors suggested that arrestment of response in this situation may have been caused by the lack of "a necessary phasic stimulation to maintain state-switching" in the CNS rather than to receptor adaptation. In our study, it is conceivable that the high pheromone release rate from Isomate-M Rosso dispensers generated sufficiently large plumes to envelope moths within release cages to produce an effect similar to that described by Willis and Baker (1984). Alternatively, the general lack of behavioral response may have been caused by a rapid receptor adaptation given the overwhelmingly high release rate generated by these dispensers. Also, it is possible that *G. molesta* failed to orient to Isomate-M Rosso dispensers in the flight tunnel because the emission rate of pheromone from these dispensers exceeded the range of "behaviorally active" concentrations for normal upwind flight (Baker and Roelofs 1981).

It is important to note, however, that tortricids including *G. molesta* will orient along the edges of concentrated pheromone plumes or along clean air-pheromone boundaries generated within flight tunnels despite the fact that orientation is inhibited within homogeneous clouds of pheromone (Kennedy et al. 1981, Willis and Baker 1984). This may explain why numerous *G. molesta* were observed orienting to and closely approaching Isomate-M Rosso dispensers in the field (Stelinski et al. 2004b) but not in the current flight tunnel assays. This shows that direct field observations should be conducted before the conclusion is reached based solely on flight tunnel studies that moths do not approach high-release dispensers of pheromone.

Brief preexposures of *G. molesta* to Isomate-M Rosso dispensers did not decrease their ability to subsequently initiate anemotaxis to lures 15 min and 24 h later. However, preexposure to Isomate-M Rosso dispensers did reduce the durations of sustained flights to the same level measured after exposure to lures. Furthermore, durations of sustained flights increased over of time after preexposure to either a lure or rope (Fig. 1). These results suggest that orientations to Isomate-M Rosso dispensers by *G. molesta* might not reduce the propensity of such moths to initiate subsequent anemotactic orientations to other pheromone sources, including calling females. However, the distance and duration of anemotactic flight within pheromone plumes after previous exposure to an Isomate-M Rosso dispenser may be substantially reduced for at least 24 h. False-plume following of *G. molesta* to synthetic dispensers of pheromone has been previously implicated as an important mechanism mediating disruption of this species (Charlton and Cardé 1981). Thus, false-plume following by naïve male *G. molesta* combined with decreases in duration of subsequent anemotactic orientations after previous bouts of false-plume following may explain why Isomate-M Rosso dispensers have been so successful in disrupting orientation of *G. molesta* to traps in the field as observed by Trimble et al. (2004) and Stelinski et al. (2004b).

Figueredo and Baker (1992) suggested that lowered behavioral responsiveness of male *G. molesta* caused by habituation to the pheromone of conspecific females may be advantageous from an evolutionary perspective. It should prevent males from responding to plumes from distant females when closer females may be nearby. Thus, enhanced male orientation to closer rather than more distant calling females within high population densities could increase mating frequency. We extend this evolutionary hypothesis and propose that male *G. molesta* having previously oriented along plumes of pheromone from females or mating disruption dispensers do not lose their ability for initiating anemotaxis to subsequent identical presentations of pheromone. However, the durations of sustained flights in such plumes are substantially reduced after preexposure. Our data support the above hypothesis because the distance of male orientations after an initial experience with a female's plume may decrease and thus restrict the male's searching range. However, our findings also indicate that the ability of males to initiate anemotaxis within a putative restricted searching range may increase or remain unchanged. The overall effect would be to intensify local or patch search (Bell and Tobin 1982) at the expense of wide foraging. This phenomenon is common when foragers encounter highly rewarding resources or the cues closely associated with them (Bell and Tobin 1982, White et al. 1984). However, in the context of mating disruption using pheromones, males might exhibit restricted patch search when it is inappropriate for the actual distribution of authentic females.

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