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Behaviors of Naïve Vs. Pheromone-Exposed Leafroller Moths in Plumes From High-Dosage Pheromone Dispensers in a Sustained-Flight Wind Tunnel: Implications for Mating Disruption of These Species

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Brief exposures of male Choristoneura rosaceana *and* Argyrotaenia velutinana *to the plumes generated by lures releasing 3-component pheromone blends specifically tuned for each species or by commercially distributed Isomate OBLR/PLR Plus pheromone "rope" dispensers induced markedly different subsequent behavioral responses to pheromone. A greater proportion of* C. rosaceana *males took flight and successfully oriented toward lures 24 h after preexposure to a lure, a rope, or the lure–rope combination in a sustained-flight wind tunnel compared to naïve moths. Flights were also longer for preexposed than naïve moths. Preexposed male* C. rosaceana *were not more likely to fly toward ropes 24 h after preexposure. By contrast, fewer male* A. velutinana *oriented to lures 24 h after preexposure than did naïve moths. Those preexposed* A. velutinana *successfully locking onto plumes from lures flew for significantly shorter intervals than did unexposed moths. Electroantennograms revealed no changes at the periphery 15 min and 24 h after preexposure. For* A. velutinana*, the long-lasting effect was decreased attraction to a lure and increased attraction to a rope. For* C. rosaceana*, pheromone preexposure increased responsiveness to its authentic blend. This behavioral evidence*

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is sufficient to explain why sexual communication of C. rosaceana *is more difficult to disrupt than that of* A. velutinana*. Furthermore, it suggests a more complete blend of pheromone may be necessary to disrupt the former species but not the latter when using rope dispensers.*

KEY WORDS: mating disruption; leafrollers; sustained-flight wind tunnel; *Choristoneura rosaceana*; *Argyrotaenia velutinana*.

INTRODUCTION

Much research has been aimed at understanding the physiological and behavioral mechanisms involved in mating disruption of moths by synthetic pheromone formulations (Sanders, 1985, 1996, 1998; Cardé *et al.*, 1997, 1998; Evenden *et al.*, 2000; and others). An understanding of the behavioral and physiological mechanisms underlying pheromone-based mating disruption is crucial to the successful development, deployment, and optimization of efficacious pheromone formulations, release devices, and deployment strategies (Cardé and Minks, 1995; Sanders, 1996). Although mating disruption through application of synthetic pheromone formulations has been successfully achieved and adopted for various moth species (Cardé and Minks, 1995), ideal levels of population control and accompanying crop protection have not always been attained for certain species under certain disruption regimes (Seabrook and Kipp, 1986; Deland *et al.*, 1994; Agnello *et al.*, 1996; Lawson *et al.*, 1996). It has been suggested that physiological variation in response to pheromone (i.e. varying degrees of peripheral adaptation) (Stelinski *et al.*, 2003a,b) along with differences in chemical characteristics of pheromones (degree of "stickiness" related to molecular chain length) (Gut *et al.*, 2004) may explain some of the variation in the degree of success achieved in controlling moth pests by mating disruption.

The major mechanisms thought to underlie mating disruption (Bartell, 1982; Cardé, 1990; Cardé and Minks, 1995; and others) are (1) camouflage of female-produced plumes amongst a constant background concentration of synthetic pheromone may impede successful anemotactic orientation, (2) false-plume-following of synthetic pheromone plumes by male moths may decrease the time available for finding authentic females, and (3) adaptation of peripheral receptors or habituation of the central nervous system (CNS) may impair or eliminate normal responses to pheromone. Other proposed mechanisms include advancement of the male's diel rhythm of response (Cardé *et al.*, 1998) and premature arrestment of male's response under high pheromone concentrations (Baker and Cardé, 1979). Cardé *et al.* (1997, 1998) caution that these mechanisms need not be mutually exclusive; rather they may act additively or synergistically.

A plausible scenario for mating disruption proposed by Cardé *et al.* (1998) and embellished upon here reasons that an initial bout of false-plume-following by a male moth may result in an eventual encounter with a high-dose releaser of synthetic pheromone (e.g., polyethelene tube dispensers, referred to as "ropes"). In the short term, such an encounter may cause that male's peripheral receptors to adapt, decreasing or eliminating his ability to perceive pheromone. Given the reversibility and short-lived nature of peripheral adaptation (Kuenen and Baker, 1981; Stelinski *et al.*, 2003a,b), it is plausible that an encounter with the rope dispenser may be more likely to decrease the male's responsiveness to pheromone *via* habituation. Such males may then preferentially orient to plumes emanating from high-dosage pheromone dispensers over the lower levels emitted by females. Further visits by preexposed and habituated males to dispensers such as ropes may compound a male's inability to find a female; e.g., greater levels of habituation, physiological exhaustion, and decreased fecundity with age.

Responses by male moths to calling females or surrogate-female lures in the presence of background concentrations of pheromone have been quantified in laboratory wind tunnels. Prolonged preexposure (1–4 days) of *Choristoneura fumiferana* males to plumes of pheromone (95:5 *E:Z*-11-tetradecenal) emanating from rubber septa in a wind tunnel decreased male orientation by 27–95% in a dosage dependent manner; longer exposures were positively correlated with greater inhibition of orientation. A time-averaged atmospheric concentration of 20 ng/m^3 of pheromone was necessary to highly disrupt this species (Sanders, 1996). In further studies that employed a sustained-flight wind tunnel, the proportion of *C. fumiferana* males sustaining flights for 4 min or longer decreased from >50 to <10% as the concentration of background pheromone increased in the tunnel (Sanders, 1998). There was considerable variability in the responsiveness of males under simulated conditions of mating disruption (Sanders, 1998). The longest sustained flight recorded (53 min) was by a male orienting in a background concentration of ca. 20 pg/m^3 of the binary 95:5 blend of *E:Z*-11-tetradecenal. Sanders (1998) suggested that complete disruption of a population of *C. fumiferana* by this binary pheromone blend may be very hard to achieve given that a small proportion of males was able to lock onto female-produced plumes for prolonged durations despite high background concentrations of synthetic pheromone.

In similar wind tunnel investigations of the mechanisms of pheromone communication disruption using *C. rosaceana* as a study system, Evenden *et al.* (2000) documented that reduction of successful orientations to calling females was most pronounced in a background of the complete 4-component pheromone blend (Thomson *et al.*, 1991) rather than a less attractive 2-component formulation. In addition, pre-exposing *C. rosaceana* for 30 min

to pheromone 30 min prior to bioassay did not alter the proportion of males successfully contacting calling females. These authors concluded that disruption of *C. rosaceana* in their wind tunnel bioassay was due to a combination of peripheral adaptation, camouflage of the female-produced plume, and false-plume-following rather than by habituation.

Sanders' conclusions from work on *C. fumiferana* (Sanders, 1995) and that of Evenden *et al.* (2000) on *C. rosaceana* concur that sensory fatigue after preexposure to naturally produced and synthetic pheromone may be less important than false-plume-following in disrupting orientation of males to calling females. Interestingly, brief preexposures of male *C. fumiferana* to calling females dramatically increased the percentage of males successfully locking onto and flying to sources of pheromone off-blends. Similar results were obtained by pre-exposing males to a 95:5 blend of *E*:*Z*-11-tetradecenal (Sanders, 1984). This "priming" effect was thought possibly to increase false-plume-following of male moth in mating disruption regimes using off-blends.

Sanders' results (Sanders, 1984, 1995) are reminiscent of an earlier study by Linn and Roelofs (1981) documenting that minutes-long preexposures of male *Grapholita molesta* to *E*-8-dodecenyl acetate enhanced subsequent responses to selected off-blends of pheromone (blends containing high % *E*) that normally elicited few completed flights from naïve males. More recently, Anderson *et al.* (2003) found that male *Spodoptera littoralis* briefly preexposed to a female-produced plume responded more vigorously to subsequent presentations of the female sex-pheromone. This increased responsiveness to pheromone lasted up to 27 h and was not associated with a change in the sensitivity of sex-pheromone receptors. The authors suggested that the brief preexposure induced a change in the CNS that increased male responsiveness to the female's pheromone.

In electrophysiological studies aimed at understanding the mechanisms underlying the differences in susceptibility to mating disruption among two sympatric tortricid moths, *C. rosaceana* and *Argyrotaenia velutinana* (Stelinski *et al.*, 2003a), differences were found at the level of peripheral receptors. Specifically, the antennae of *C. rosaceana* exhibited a long-lasting adaptation (ca. 12.5 min) after 5-min exposure to pheromone, while the antennae of *A. velutinana* disadapted within 1 min after identical exposure. A consistent reduction in responsiveness to pheromone of 40–60% occurred for *C. rosaceana* after exposure to air-borne concentrations of pheromone ranging from 56 to below 1 ng/mL air (Stelinski *et al.*, 2003b).

The current study compared the behavioral responses of *C. rosaceana* and *A. velutinana* to high doses of pheromone delivered from rope dispensers in a sustained-flight wind tunnel to determine whether previously documented physiological differences between these species would result in measurable behavioral differences. The specific objectives were to (1)

compare the responses of *C. rosaceana* and *A. velutinana* to rope dispensers of pheromone used for mating disruption and lures used in monitoring traps in a sustained-flight wind tunnel and (2) determine whether/how brief preexposures to high-dose pheromone releasers modulate subsequent responses to those releasers 15 min and 24 h after exposure.

MATERIALS AND METHODS

Insect Colonies

C. rosaceana were drawn from a 5-year-old laboratory colony originally collected as first and second generation pupae from apple orchards in Southwestern Michigan. *A. velutinana* came from a long-established laboratory colony maintained at Geneva, NY by W. Roelofs. Both 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 5% sucrose in plastic cups with cotton dental wicks protruding from their lids.

Chemicals and Release Devices

The behavioral responses of *C. rosaceana* and *A. velutinana* were quantified in a sustained-flight wind tunnel using two types of pheromone dispensers. First, pheromone blends specific to each species were formulated in red rubber septa (Suterra, Bend, OR). The tuned lures in experiments with *A. velutinana* were rubber septa loaded with 0.93 mg (*Z*)- and 0.07 mg (*E*)-11-tetradecenyl acetates (93:7 ratio of *Z:E*) and 2.0 mg dodecyl acetate (Roelofs *et al.*, 1975). 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 blend solutions used to load rubber septa were prepared in HPLC grade hexane and stored at −18°C. Henceforth, such rubber septum dispensers formulated specifically for each species will be referred to as "lures." Second, we assayed the responses of both moth species to Isomate OBLR/PLR Plus polyethylene tube dispensers (Pacific Biocontrol, Vancouver, WA) containing 274 mg of 93.4% (*Z*)-11-tetradecenyl acetate, 5.1% (*E*)-11-tetradecenyl acetate, and 1.5% (*Z*)-9-tetradecenyl acetate. Such pheromone release devices are currently the dominant method of dispensing pheromone for mating disruption of leafroller pests in commercial orchards (Nagata, 1989; Agnello *et al.*, 1996; Knight *et al.*, 1998; Knight & Turner, 1999). All dispensers were

aged for 2 weeks in a laboratory fume hood prior to use in behavioral assays to allow dissipation of pheromone that might have built up on their surfaces during shipping and freezer storage. These polyethylene tube dispensers will be referred to as "ropes."

Wind Tunnel General Description

Behavioral assays with male *C. rosaceana* and *A. velutinana* adults were conducted in a recently constructed Plexiglas sustained-flight wind tunnel patterned after that of Miller and Roelofs (1978). The rectangular wind tunnel measured 1.3 × 0.8 m in cross-section and 2.4-m long. It was housed in a temperature-controlled room maintained at 50–70% RH and 15–16°C to stimulate moth behavioral responsiveness to pheromone under light (Cardé *et al.*, 1975) and to simulate typical night-time summer temperatures in Michigan. Light intensity was 1800–2000 lux inside of the tunnel and was generated by eight fluorescent bulbs (Philips model F96T12, 95 W) mounted 22 cm above the top of the wind tunnel. A variable speed, blower-type fan (Dayton 5C090C, 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. The upwind fan pushed air first through both a Vari-Flow II filter for fine, particulate matter and a Vari-Pure high capacity, activated carbon filter; both filters were obtained from Airguard (Louisville, KY). Finally, air was pushed through a $1.3 \times 0.8 \times 0.2$ m hardwood frame attached tightly to the upwind end of the tunnel and enclosed with cloth dampening screens (20 mesh/cm) stretched tightly across each opening. The down-wind end of the tunnel was enclosed with wire-mesh screen.

A variable-speed continuous belt painted with alternating orange and white 0.15-m-wide transverse stripes was installed below the tunnel floor. The belt was driven in the same direction as the wind by an electric motor in experiments requiring sustained flights. Belt speed could be regulated by a rheostat that modulated voltage input into the motor. During sustained flights (see later), belt speed was adjusted in order to maintain moths near the tunnel's mid-point.

Wind Tunnel Assay Procedures

Male moths, 1–4-days old, were collected during the last 0.5 h of the photophase and placed into cylindrical (17-cm-long × 8-cm-diam.) wire-mesh release cages. The cages containing 1 or 5 moths (depending on experiment) were placed into the wind tunnel for 1 h of acclimation prior to

assays. Subsequently, bioassays ran for a maximum of 1.5 h terminating at 2 h into the moths' normal scotophase. At the upwind end of the tunnel, pheromone dispensers (lures or ropes) were placed 1 cm above a horizontal 7.5 × 12.5 cm yellow card 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 5 male moths of a given species 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 contained only one male moth.

Males were allowed 3 min to respond to an inserted pheromone dispenser in assays where the floor was held stationary and 2 min in sustained-flight assays employing the moving floor. 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 exhibiting no detectable behavioral change were recorded.

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 *A. velutinana* and *C. rosaceana* 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. We predicted that *A. velutinana* would be more adversely affected by the exposure than *C. rosaceana* given previously published reports that mating disruption is easier to achieve for *A. velutinana* than *C. rosaceana* (Stelinski *et al.*, 2003a and references within). Groups of 5 male moths of each species were released in plumes generated by (1) a rubber septum described above (standard lure used for monitoring each species); (2) a rope (standard pheromone dispenser used in mating disruption); and (3) a combination of a species-specific lure with a rope placed 5 cm directly above it. Moths were allowed 3 min to respond during all preexposure treatments. 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. 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. 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 prior to testing. The treatments were (1) preexposure to a lure followed by wind tunnel assay using a lure; (2) preexposure to a rope followed by wind tunnel assay using a lure; (3) preexposure to lure and rope combination followed by wind tunnel assay using a lure; (4) preexposure to a rope followed by wind tunnel assay using a rope; (5) 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 prior to 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, a rope, or a lure and rope combination.

To avoid bias due to 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 after what would have been the onset of normal scotophase.

Experiment 2

This experiment tested the effect of brief exposures of *A. velutinana* and *C. rosaceana* to pheromone plumes generated by high-release rope dispensers on the duration of sustained flights of male moths 24 h after initial exposure. Individual male moths of each species were released in plumes generated by a rope dispenser. As in Experiment 1, moths reaching the pheromone source or orienting to the source but not contacting it were segregated from those that either did nothing, wing-fanned only, or flew out of release cages without orienting to the pheromone. Recaptured moths were then placed in plumes generated by lures 24 h after the initial pheromone exposure. After moths locked onto the pheromone plume generated by a lure, the moving floor was activated to prolong flights. We recorded both the time it took moths to exit cages and to lock onto the plume, as well as the duration of sustained flight in the plume.

Electroantennogram Assays

Experiment 3

This experiment tested the hypothesis that briefly exposing *A. velutinana* and *C. rosaceana* to pheromone plumes generated by 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). EAG cartridges were made by pipetting various concentrations (2 μg–2 mg) of pheromone (96.1% (*Z*)-11-tetradecenyl acetate and 3.9% (*E*)-11-tetradecenyl acetate as determined by gas chomatography) in hexane (20 μL total solution) onto 1.4 × 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, sealed with Parafilm, and allowed to equilibrate for 24 h prior to use. Pheromone dosages were delivered alternately to both naïve and pheromone preexposed moths (15 min or 24 h after initial exposure) in ascending order of dosage ($N = 25$ per treatment). Four 1-mL puffs spaced 12 s apart were administered to each antenna at each dosage.

Statistical Analyses

For Experiments 1 and 2, a logistic model was used to measure the probability that a combination of the thee factors: pheromone delivery device (rubber septum or rope) × moth type (naïve, preexposed orienter, or preexposed nonorienter) × species (*C. rosaceana* or *A. velutinana*) would result in a particular behavioral category as defined previously using the Proc GENMOD procedure in SAS (SAS Institute, 1989). Subsequently, analyses of numbers of male moths responding were carried out using the G statistic (Sokal and Rolf, 1981). Proportions of moths responding within each behavioral category were compared both within each individual species under study (Tables I and II) and between species (Table III). In addition, for Experiment 2, data for sustained-flight duration and elapsed time to leave release cages were transformed to $\ln(x + 1)$ (which normalized the distributions) and then subjected to ANOVA; differences in pairs of means were separated using Tukey's multiple comparisons test (SAS Institute, 1989). For Experiment 3, data were subjected to ANOVA and differences in pairs of means were separated using Tukey's multiple comparisons test (SAS Institute, 1989). In all cases, the significance level was $\alpha < 0.05$.

RESULTS

Experiment 1

Responses of C. rosaceana

Compared to naïve or control (Air then Lure) males of the same age, significantly more *C. rosaceana* males contacted their respective lure 24 h

Table I. Response of Male *Choristoneura rosaceana* to Lures or Ropes 15 min or 24 h After Preexposure to Either Clean Air, a Lure, Rope, or Lure–Rope Combination

Moth type and pheromone dispenser	*N*	Proportion of males exhibiting the indicated response[a]		
		No behavioral change	Orientation without source contact	Source contact
Naïve males				
Lure	107	0.14bc[b]	0.30c	0.30bc
Rope	110	0.23bc	0.25cd	0.04d
L + R	123	0.13bcd	0.42b	0.04d
Preexposed orienters (15 min)				
Lure then lure	45	0.10cd	0.38bc	0.18c
Rope then lure	25	0.33a	0.25cd	0.25c
L + R then lure	39	0.19bc	0.38bc	0.00d
Preexposed nonorienters (15 min)				
Lure then lure	25	0.26ab	0.39bc	0.00d
Rope then lure	48	0.28ab	0.26c	0.09d
L + R then lure	44	0.33a	0.31c	0.10d
Preexposed orienters (24 h)				
Lure then lure	32	0.00d	0.52a	0.48ab
Rope then lure	25	0.00d	0.46ab	0.54a
L + R then lure	38	0.00d	0.57a	0.43abc
Rope then rope	27	0.37a	0.22cd	0.00d
Preexposed nonorienters (24 h)				
Lure then lure	29	0.00d	0.55a	0.31bc
Rope then lure	41	0.00d	0.37bc	0.59a
L + R then lure	31	0.03d	0.42b	0.45ab
Rope then rope	38	0.39a	0.13d	0.00d
Air then lure[c]	61	0.15bc	0.25cd	0.33bc

Note. L + R stands for lure + rope.
[a]Proportions of moths wing-fanning only or flying out without anemotactic orientation 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.

after brief preexposure to pheromone plumes generated by a rope dispenser (Table I). This result occurred irrespective of whether or not male *C. rosaceana* oriented to the rope during the preexposure treatment. Compared to naïve or control males, significantly more male *C. rosaceana* oriented to lures without contacting the source 24 h after brief preexposure to a lure, a rope, or a lure–rope combination (Table I). Again, this result generally held for both males that oriented during the preexposure procedure and those that did not. The only exception was for preexposed non-orienters after

Table II. Response of Male *Argyrotaenia velutinana* to Lures or Ropes 15 min or 24 h After Preexposure to Either Clean Air, a Lure, Rope, or Lure–Rope Combination

Moth type and pheromone dispenser	*N*	Proportion of males exhibiting the indicated response[a]		
		No behavioral change	Orientation without source contact	Source contact
Naïve males				
Lure	127	0.16cd[b]	0.35ab	0.24b
Rope	135	0.19c	0.42a	0.14cd
L + R	123	0.10d	0.44a	0.23b
Preexposed orienters (15 min)				
Lure then lure	38	0.05d	0.42a	0.16bcd
Rope then lure	29	0.31b	0.38ab	0.17bc
L + R then lure	28	0.21bc	0.50a	0.04de
Preexposed nonorienters (15 min)				
Lure then lure	25	0.47ab	0.26bc	0.00e
Rope then lure	25	0.63a	0.16c	0.11d
L + R then lure	28	0.55ab	0.09d	0.05de
Preexposed orienters (24 h)				
Lure then lure	42	0.07d	0.45a	0.17bc
Rope then lure	40	0.18c	0.43a	0.13cd
L + R then lure	47	0.32b	0.49a	0.11d
Rope then rope	35	0.03d	0.46a	0.40a
Preexposed nonorienters (24 h)				
Lure then lure	34	0.24bc	0.38ab	0.03e
Rope then lure	34	0.26bc	0.47a	0.03e
L + R then lure	24	0.79a	0.07d	0.00e
Rope then rope	29	0.52ab	0.10d	0.07de
Air then Lure[c]	53	0.15cd	0.28bc	0.34ab

Note. L + R stands for lure + rope.
[a]Proportions of moths wing-fanning only or flying out without anemotactic orientation 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.

brief exposure to a rope. Nearly 100% of preexposed *C. rosaceana* locked onto plumes (oriented with or without source contact) generated by lures 24 h after preexposure compared to 58 or 60% for control or naïve moth, respectively. Significantly fewer male *C. rosaceana* oriented (with or without source contact) to ropes compared to lures before and 24 h after preexposure to such ropes (Table I). If they did not orient to those dispensers during the preexposure treatment, significantly fewer male *C. rosaceana* contacted lures 15 min after preexposure to a lure, rope, or the lure–rope combination

Table III. Comparison of the Response of *Choristoneura rosaceana* Vs. *Argyrotaenia velutinana* Males to Lures or Ropes 24 h After Preexposure to Either Clean Air, a Lure, Rope, or Lure–Rope Combination

Moth type and pheromone dispenser	*N*	Proportion of males exhibiting the indicated response[a]		
		No behavioral change	Orientation without source contact	Source contact
C. rosaceana				
Naïve males				
Lure	107	0.14de[b]	0.30c	0.30c
Rope	110	0.23cd	0.25cd	0.04e
L + R	123	0.13de	0.42bc	0.04e
Preexposed orienters (24 h)				
Lure then lure	32	0.00e	0.52a	0.48a
Rope then lure	25	0.00e	0.46b	0.54a
L + R then lure	38	0.00e	0.57a	0.43ab
Rope then rope	27	0.37bc	0.22d	0.00e
Preexposed nonorienters (24 h)				
Lure then lure	29	0.00e	0.55a	0.31c
Rope then lure	41	0.00e	0.37c	0.59a
L + R then lure	31	0.03e	0.42bc	0.45ab
Rope then rope	38	0.39b	0.13de	0.00e
Air then lure	61	0.15bc	0.25cd	0.33bc
A. velutinana				
Naïve males				
Lure	127	0.16d	0.35c	0.24cd
Rope	135	0.19d	0.42bc	0.14d
L + R	123	0.10e	0.44b	0.23cd
Preexposed orienters (24 h)				
Lure then lure	42	0.07e	0.45b	0.17d
Rope then lure	40	0.18d	0.43b	0.13de
L + R then lure	47	0.32bc	0.49ab	0.11e
Rope then rope	35	0.03e	0.46b	0.40b
Preexposed nonorienters (24 h)				
Lure then lure	34	0.24c	0.38bc	0.03e
Rope then lure	34	0.26c	0.47b	0.03e
L + R then lure	24	0.79a	0.07e	0.00e
Rope then rope	29	0.52b	0.10e	0.07e
Air then lure[c]	53	0.15cd	0.28c	0.34abc

Note. L + R stands for lure + rope.

[a] Proportions of moths wing-fanning only or flying out without anemotactic orientation 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.

(Table I). Statistically equal numbers of naïve and control male *C. rosaceana* contacted or oriented to lures (Table I).

Responses of A. velutinana

Of male *A. velutinana* orienting to the pheromone sources during preexposure treatments, significantly fewer contacted the lure compared to naïve moths 15 min after preexposure to the lure–rope combination and 24 h after preexposure to the rope and lure–rope combination (Table II). Of those male *A. velutinana* not orienting during the preexposure treatment, significantly fewer contacted the lure compared to naïve moths after preexposure to the lure, rope, and lure–rope combination 15 min and 24 h after preexposure (Table II). A significantly greater proportion of male *A. velutinana* contacted ropes 24 h after orienting to ropes compared to the proportion of naïve moth orienting to a lure, rope, or lure–rope combination (Table II). However, a statistically equal proportion of male *A. velutinana* contacted ropes 24 h after preexposure if they did not orient during the preexposure treatment compared to naïve moths (Table II). Of the male *A. velutinana* orienting to pheromone sources during preexposure, statistically equal proportions oriented (without source contact) to lures 15 min and 24 h after orienting to a lure, rope, or lure–rope combination compared with naïve moths. Of those male *A. velutinana* not orienting during the preexposure treatment to pheromone plumes, significantly fewer oriented (without source contact) to lures 15 min after preexposure to a rope or lure–rope combination and significantly fewer oriented to lures 24 h after preexposure to the lure–rope combination compared to naïve moths (Table II). Statistically equal numbers of naïve and control male *A. velutinana* contacted or oriented to lures (Table II).

Comparison of Responses Between Species

When comparing responses between species, statistically equal proportions of naïve males of both species either contacted or oriented without source contact to their respective lures (Table III). Compared to naïve *A. velutinana*, significantly fewer naïve *C. rosaceana* contacted ropes; however, statistically equal proportions of both species oriented to ropes without source contact. Compared to preexposed *A. velutinana*, a significantly greater proportion of *C. rosaceana* contacted lures 24 h after preexposure to a lure, rope or lure–rope combination, irrespective of whether or not they oriented during preexposure (Table III). Compared to preexposed *C. rosaceana*, significantly

more *A. velutinana* contacted ropes or oriented to ropes without source contact 24 h after orienting to ropes; no *C. rosaceana* contacted ropes 24 h after preexposure to ropes (Table III). Statistically equal proportions of clean-air preexposed (control) moths of both species contacted lures or oriented to lures without making contact 24 h after preexposure (Table III).

Experiment 2

Provided they had been preexposed to ropes 24 h earlier, a significantly greater proportion of male *C. rosaceana* oriented to plumes generated by lures, irrespective of whether or not they oriented during preexposure compared to naïve males of the same age (Fig. 1A). Male *C. rosaceana* preexposed to ropes and not orienting during preexposure flew to lures for a

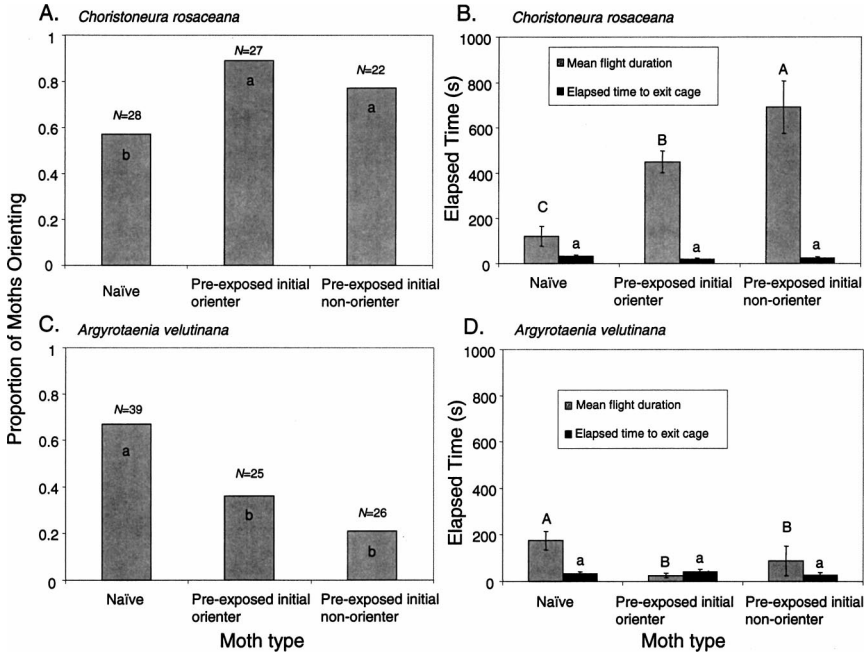

Fig. 1. A: Proportion of naïve and pheromone-rope preexposed *Choristoneura rosaceana* males that locked onto and oriented to lures in sustained-flight wind tunnel with moving floor 24 h after preexposure. B: Duration of sustained-flights of naïve and pheromone-rope preexposed *C. rosaceana* 24 h after preexposure. C: Proportion of naïve and pheromone-rope preexposed *Argyrotaenia velutinana* males that locked onto and oriented to lures in sustained-flight wind tunnel with moving floor 24 h after preexposure. D: Duration of sustained-flights of naïve and pheromone-rope preexposed *A. velutinana* 24 h after preexposure. Means within a given panel followed by the same letter and case of letter are not significantly different at a $\alpha < 0.05$.

significantly longer period than male *C. rosaceana* having oriented to ropes during preexposure 24 h earlier (Fig. 1B). All rope-preexposed male *C. rosaceana* (whether they oriented or not 24 h earlier) sustained flights to lures significantly longer than did naïve moths of the same age (Fig. 1B). There were no significant differences in the time required by preexposed and naïve male *C. rosaceana* to leave the release cages (Fig. 1B).

A significantly greater proportion of naïve male *A. velutinana* oriented upwind toward plumes generated by lures compared to those preexposed to ropes 24 h earlier (Fig. 1C). Naïve male *A. velutinana* sustained flights to lures significantly longer than did moths of the same age that had been preexposed to rope plumes 24 h earlier (Fig. 1D). There were no significant differences in the time required to leave the release cage between preexposed and naïve male *A. velutinana* (Fig. 1D).

Experiment 3

Mean EAG responses of naïve male *C. rosaceana* and *A. velutinana* were identical to those of moths assayed either 15 min or 24 h after preexposure to rope dispensers (Fig. 2A–D).

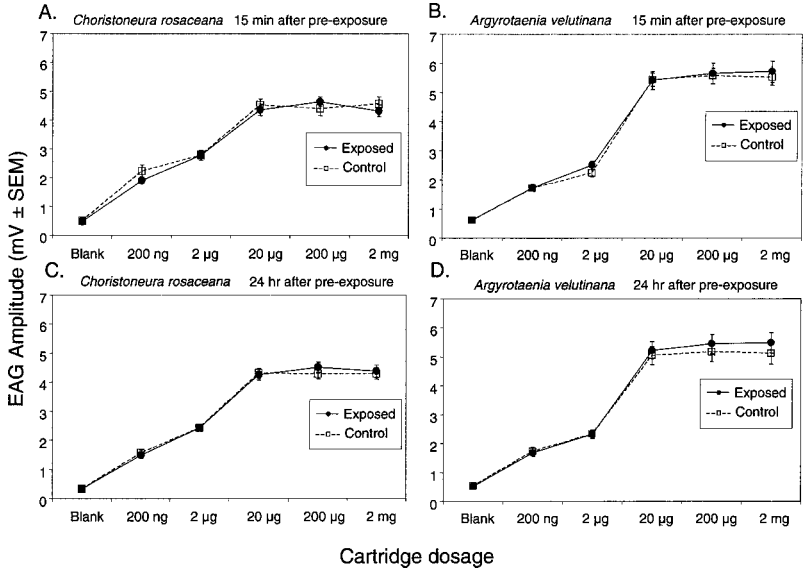

Fig. 2. EAG dosage-response relationships for naïve and pheromone-rope preexposed *Choristoneura rosaceana* (A: 15 min, C: 24 h after preexposure) and *Argyrotaenia velutinana* (B: 15 min, D: 24 h after preexposure) using live-insect antennal preparations.

DISCUSSION

Brief preexposures of male *C. rosaceana* and *A. velutinana* to plumes arising from rubber septa releasing pheromone blends specifically tuned for each species or by a commercial rope dispenser targeting both species induced markedly different effects on subsequent behavioral responses to pheromone. The clearest behavioral differences between the two species came 24 h after the preexposure when *C. rosaceana's* propensity for orienting toward lures containing a 3-component pheromone blend tuned for that species was heightened after brief preexposure to a lure, a rope dispenser, or a combination of a lure and rope. Under these conditions a greater proportion of *C. rosaceana* males successfully locked onto and progressed toward lures. Moreover, durations of sustained flights to lures were substantially prolonged relative to those of naïve, unexposed moths of the same age. But, such preexposed male *C. rosaceana* were not more likely to fly toward ropes 24 h after preexposure to ropes (Table I). By contrast, preexposed *A. velutinana*'s propensity for locking onto pheromone plumes generated by lures emitting a 3-component pheromone blend tuned for that species was greatly reduced 24 h after preexposure to a lure, a rope, or the lure–rope combination. Duration of anemotactic flights was also much reduced. This decreased propensity for orientation to lures was most dramatic in those individuals not orienting during the preexposure treatment. However, the proportion of male *A. velutinana* that contacted ropes 24 h after initially orienting to ropes was larger than that for naïve moths (Table II). Overall, nearly identical proportions of naïve male *C. rosaceana* and *A. velutinana* locked onto and progressed toward their respective lures; mean durations of sustained flights of naïve moths of the two species toward their respective lures were also nearly identical (Fig. 1B,D). Thus, naïve moths of both species behaved similarly in plumes of their respective lures; but, their behaviors diverged considerably 24 h after pheromone preexposure.

Numerous studies have investigated the effects of short and prolonged preexposures of moths to their species-specific synthetic pheromones and geometric isomers (Bartell and Roelofs, 1973; Bartell and Lawrence, 1976a,b,c; Linn and Roelofs, 1981; Sanders, 1985; Evenden *et al.*, 2000); however, few have focused on long-term effects of pheromone preexposure on subsequent behavioral responses (Figuerdo and Baker 1992, Anderson *et al.*, 2003). Figuerdo and Baker (1992) observed significantly decreased behavioral responses of male *G. molesta* to rubber septa releasing specifically tuned, multicomponent pheromone blends day(s) after preexposure to such septa. In contrast, Anderson *et al.* (2003) documented increased responsiveness of *S. littoralis* to female pheromone gland extracts and synthetically prepared lures 27 h after brief preexposure to female-produced plumes. A similar

response, however to off-blends, occurred in *C. fumiferana* after pheromone preexposure (Sanders, 1984, 1995).

Other recent investigations have focused on short-term intervals after preexposure of moths to pheromone. For example, Evenden *et al.* (2000) found no change in the ability for female plume-following by Western *C. rosaceana* males preexposed to pheromone for 1 h and then assayed in a wind tunnel 10–30 min after exposure. This short interval between exposure and assay chosen by Evenden *et al.* (2000) may not have permitted detection of the behavioral effects of pheromone preexposure uncovered by the current study, demonstrated by waiting 24 h to assay effects of pheromone preexposure, as well as using a sustained-flight tunnel to quantify durations of anemotactic flights.

The neurophysiological changes induced in both leafroller species by pheromone preexposure likely occurred in the CNS. No changes were detected at the periphery at both 15 min and 24 h after preexposure as evidenced by EAGs (Fig. 2A–D). For *A. velutinana*, the long-lasting effect of decreased attractiveness of a lure (lower release and more complete blend pheromone source) and increased attractiveness of a rope (higher release and less complete blend pheromone source) is consistent with an increase in respond threshold, requiring a higher concentration of stimulus to elicit normal responses.

For *C. rosaceana*, our data suggest a decreased response threshold perhaps combined with an increased ability for pheromone blend discrimination given the greater attractiveness of a lower-release, natural blend lure. Serotonin and octopamine enhance male responsiveness to pheromone and modulate the activity of neurons both in the CNS (Linn *et al.*, 1992; Linn, 1997) and the peripheral nervous system (Pophof, 2000, 2002; Stelinski *et al.*, 2003c). Alternatively, the behavioral changes documented herein may have resulted from some type of learning, like associative or aversive learning of *Drosophila* traceable to the mushroom bodies (Bell and Heisenberg, 1994; Yin *et al.*, 1994).

Our wind-tunnel data suggest that the induced behavioral modifications may have involved more than just changes in response thresholds. The propensity of preexposed *C. rosaceana* to contact lures had also increased as evidenced by the longer sustained-flight durations relative to naïve individuals. In addition, those male *C. rosaceana* not orienting during preexposure flew significantly longer than did those that oriented during preexposure (Fig. 1C). Perhaps initial non-orienters were exposed to more total pheromone than were their associated orienters. During preexposure, orienters usually left the release cage within 30 s and spent only ca. 10–30 s orienting to the source in a stationary-floor tunnel before landing on the tunnel floor or walls. In contrast, the majority of non-orienters spent a full

3 min in the release cages placed directly within the plume of pheromone. For *C. rosaceana*, longer duration of preexposure was positively correlated with longer sustained flights to lures 24 h after preexposure to ropes.

It is doubtful that the previously documented difference between *C. rosaceana* and *A. velutinana* in the duration of peripheral adaptation after prolonged exposure to pheromone (Stelinski *et al.*, 2003a) influenced the behaviors documented in this study. We had postulated that long-lasting peripheral adaptation of *C. rosaceana* might shield the CNS in this species during prolonged exposures to pheromone, while the short-lived peripheral adaptation of *A. velutinana* might permit marked impact of pheromone exposure on the CNS (Stelinski *et al.*, 2003a,b). The current study did not support such a difference. Rather, both species revealed effects at the level of the CNS, albeit differently. The relatively brief and discontinuous pheromone preexposure in the current study did not induce long-lasting adaptation in *C. rosaceana* as measured by EAGs (methods as above; $N = 10$ moth; data not shown).

Could this finding that a preexposure to pheromone can in some cases reduce and in other cases enhance subsequent responses to pheromone plumes explain some of the variation observed across moth species in their susceptibility to mating disruption? Western *C. rosaceana* are reported to be successfully controlled by mating disruption (Evenden *et al.*, 1999a,b). In contrast, eastern and midwestern populations of this pest are thought to be relatively difficult to control *via* mating disruption, perhaps requiring near-true blend formulations of pheromone for successful disruption (Novak *et al.*, 1978; Novak and Roelofs, 1985; Lawson *et al.*, 1996; Miller *et al.*, unpublished data). It is intriguing that pheromone preexposure caused Michigan *C. rosaceana* to improve at locking onto and progressing toward a lower release-rate and more complete blend pheromone emitter. The converse result was documented for *A. velutinana*, a species easily disrupted with only the main component of its pheromone blend (Novak *et al.*, 1978; Reissig *et al.*, 1978; Novak and Roelofs, 1985). Thus, the behavioral evidence uncovered in the current experiments seems sufficient to explain why *C. rosaceana* is more difficult to disrupt than *A. velutinana* and why a more complete blend of disruptant may be necessary to achieve success for the former species but not the latter. One day after pheromone preexposure, a lower-release and more complete blend lure was more attractive to *C. rosaceana*, while the converse was true for *A. velutinana*.

After having completed this study, we learned that the Isomate OBLR/PLR Plus rope dispensers contained a small, undeclared amount (1.5%) of (Z)-9-tetradecenyl acetate. Evenden *et al.* (1999c) showed that addition of >1.0% of (Z)-9-tetradecenyl acetate to 100 μg of the 4-component blend (Vakenti *et al.*, 1988), formulated in a rubber septum lures, reduced the

responsiveness of *C. rosaceana* compared with lures containing the pheromone alone. Therefore, the existence of this so-called "antagonist" in Isomate OBLR/PLR Plus rope dispensers may explain why responsiveness of naïve and pheromone-exposed *C. rosaceana* was lower than that of *A. velutinana*. If false-plume-following is an important mechanism mediating mating disruption of *C. rosaceana*, then perhaps the presence of this antagonist renders these dispensers less attractive to *C. rosaceana* and therefore less effective. Further work is needed to determine whether this component alone played a significant role in increasing responsiveness of *C. rosaceana* after preexposure.

Of the numerous published studies of the behavioral responses of moths to pheromone after pheromone preexposure, only a few have specifically focused on high-dosage dispenser technologies like ropes (Cardé *et al.*, 1997, 1998), currently the predominant dispenser for mating disruption. The explanations arising from most laboratory investigations of mating disruption are extrapolations from tests using low-release pheromone dispensers. Also, many have focused only on minutes-long effects of pheromone preexposure. Our results suggest that long-term effects of pheromone preexposure may be important and that commercially available pheromone ropes induce such changes. A critical question is whether leafrollers actually approach such rope dispensers under mating disruption in the field and remain in their vicinities sufficiently long to induce such behavioral modifications. We have gathered extensive field data that both *C. rosaceana* and *A. velutinana* do approach rope dispensers identical to those used in the current laboratory study, under standard application densities, and that behavioral responses to ropes in the field are very similar to those seen in the wind tunnel (Stelinski *et al.*, unpublished data). More attention needs to be focused on the sequence of events as these moths interact with pheromone dispensers in the field. The finding that appreciable numbers of moths can be observed approaching rope dispensers both in the laboratory and the field (Stelinski *et al.*, unpublished data) suggests that these experimental approaches are tractable. Moreover, these findings add to the growing body of evidence that false-plume-following is likely to be a major explanatory mechanism of pheromone disruption using rope dispensers.

Finally, the current data suggest that using a common rope dispenser to disrupt communication of both of these leafrollers may not be the optimal control tactic. The Isomate OBLR/PLR Plus pheromone rope dispenser appears to be well suited to disrupting sexual communication of *A. velutinana* but not for *C. rosaceana*. Our data suggest a better tactic for *C. rosaceana* would be to use a dispenser releasing a lowered rate of a blend closely matching the natural pheromone of this species. We are hopeful that efficacy for *C. rosaceana* can be optimized while maintaining acceptable cost.

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